

Introduction

Fragment based drug discovery¹ using the PyramidTM approach² has been applied to a large number of drug discovery targets at Astex, leading to multiple clinical candidates. We use biophysical screening of our proprietary fragment library (Figure 1 A) to identify initial fragment hits against the protein target. The use of a combination of X-ray crystallography, NMR, T_m and isothermal titration calorimetry (ITC) (Figure 1 B) allows detection of fragments with binding affinities (K_d) as low as the mM range. Despite having low affinity, these fragments typically form high quality interactions with the protein, giving high ligand efficiency (LE; defined as the free energy of binding in kcal/mol divided by the heavy atom count). Subsequently, in the fragments-to-leads stage (Figure 1 C) a detailed structural understanding of the binding interactions between the fragment and its target protein (utilizing X-ray crystallography or NMR) is critical. At Astex, great importance is placed on access to experimentally determined X-ray crystal structures of protein-ligand complexes for use in both the fragment screen and subsequent optimisation to nM potency compounds. The aim of PyramidTM is to identify multiple fragment starting points, allowing several lead series to be identified, often by synthesizing only small numbers of compounds. Since inappropriate physical properties are thought to be a major driver for attrition in drug development, we place great emphasis on tracking LE and logP during the optimisation process, to assess whether gains in potency are significant enough to justify increases in molecular size and lipophilicity. The case histories below (showing X-ray crystal structures) illustrate the optimisation of example fragment hits for three diverse oncology targets.

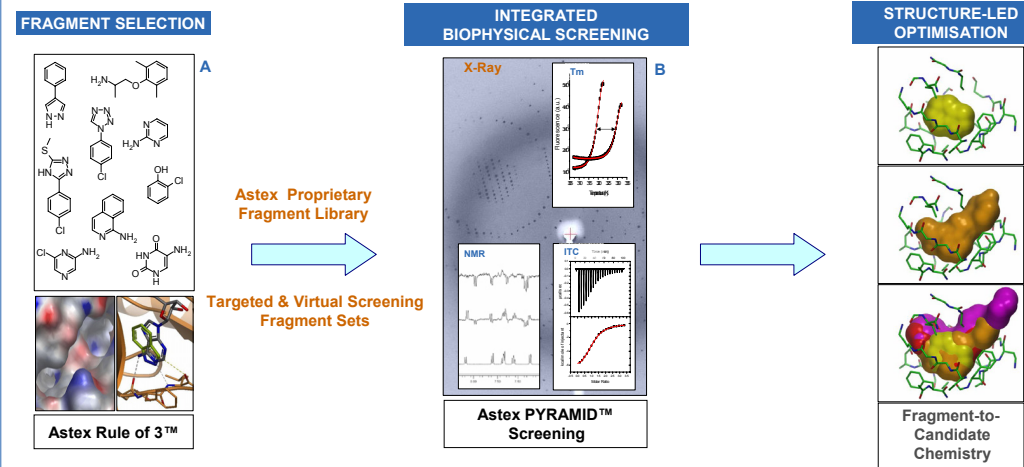


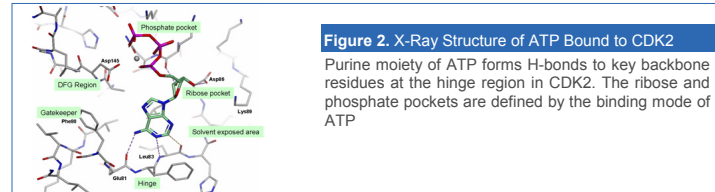
Figure 1. The Astex PyramidTM approach to fragment based drug discovery

References

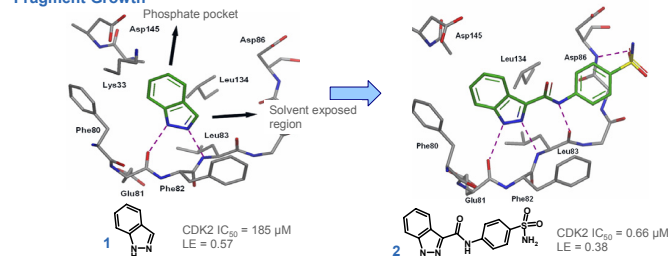
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2. T.G. Davies, R.L.M. van Montfort, G. Williams, H. Jhoti in *Methods and Principles in Medicinal Chemistry, Vol. 34: Fragment-Based Approaches in Drug Discovery*, W. Jahnke, D.A. Erlanson (eds.); 2006, Wiley-VCH, Weinheim.
3. (a) P.G. Wyatt et al.; *J. Med. Chem.* 2008, 51, 4986 - 4999. (b) M.S. Squires et al.; *Molecular Cancer Therapeutics* 2010, 9, 920 - 928.
4. C.W. Murray et al.; *J. Med. Chem.* 2010, 53, 5942-5955.
5. A.J. Woodhead et al.; *J. Med. Chem.* 2010, 53, 5956-5969.
6. C.S. Straub; *Current Topics in Medicinal Chemistry* 2011, 11, 291-316

Case History 1: CDK inhibitor AT7519

• Currently in Phase II: Solid tumours, Leukemias (MM, CLL, MCL)

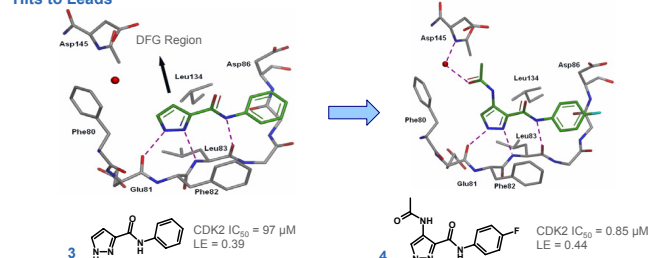


Fragment Growth



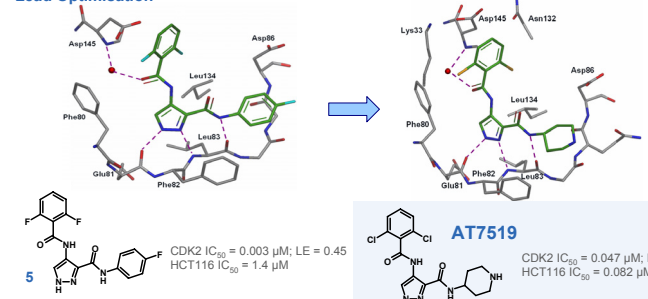
- Fragment 1 forms a bidentate interaction with the hinge, c.f. Figure 2
- Potency was improved by substitution towards solvent exposed region (e.g. compound 2). Note the slight change in orientation of indazole but key interactions to hinge maintained
- Amide linker forms an additional H-bond with hinge

Hits to Leads



- Simplification by changing indazole to pyrazole 3 retains LE; improves vector towards DFG loop
- Addition of an acetamide 4 improves potency and LE. It forms an intramolecular H-bond (ensuring planarity of the inhibitor) and a water-mediated H-bond to the backbone NH of Asp145

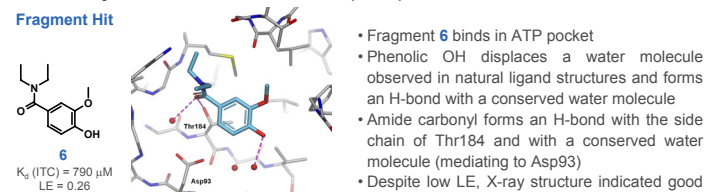
Lead Optimisation



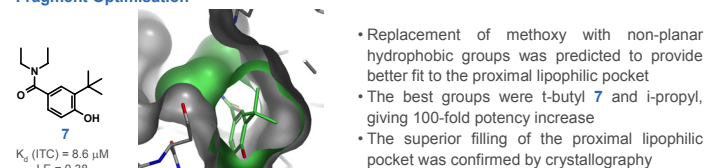
- Interaction between the (2,6-diF) Ph 5 and Asp145 leads to increase in potency
- Twisted conformation of the phenyl ring stabilised by di-ortho substitution
- Changing 4-fluorophenyl for piperidine improves physicochemical properties and cell permeability
- AT7519 is active in several xenograft models e.g. HCT116, A2780 & HT29, showing tumour regression with extended period for re-growth³

Case History 2: Hsp90 Inhibitor AT13387

• Currently in Phase II: Solid tumours (GIST)



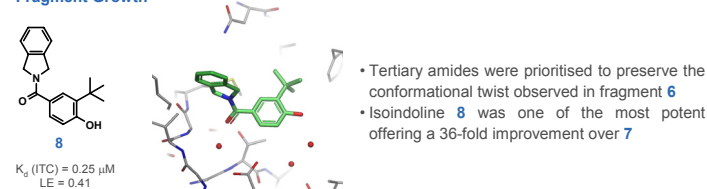
Fragment Optimisation



- Fragment 6 binds in ATP pocket
- Phenolic OH displaces a water molecule observed in natural ligand structures and forms an H-bond with a conserved water molecule
- Amide carbonyl forms an H-bond with the side chain of Thr184 and with a conserved water molecule (mediating to Asp93)
- Despite low LE, X-ray structure indicated good design ideas

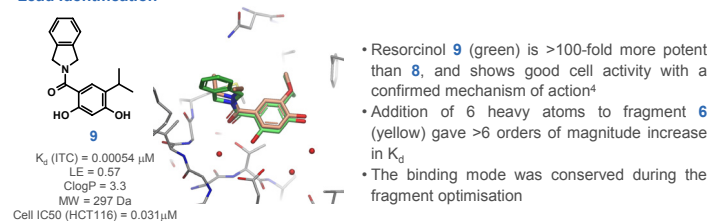
- Replacement of methoxy with non-planar hydrophobic groups was predicted to provide better fit to the proximal lipophilic pocket
- The best groups were t-butyl 7 and i-propyl, giving 100-fold potency increase
- The superior filling of the proximal lipophilic pocket was confirmed by crystallography

Fragment Growth



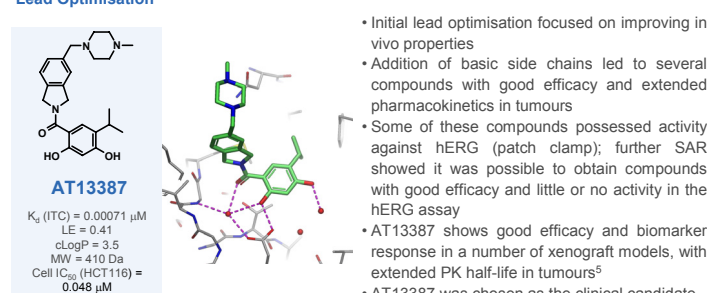
- Tertiary amides were prioritised to preserve the conformational twist observed in fragment 6
- Isoindoline 8 was one of the most potent offering a 36-fold improvement over 7

Lead Identification



- Resorcinol 9 (green) is >100-fold more potent than 8, and shows good cell activity with a confirmed mechanism of action⁴
- Addition of 6 heavy atoms to fragment 6 (yellow) gave >6 orders of magnitude increase in K_d
- The binding mode was conserved during the fragment optimisation

Lead Optimisation



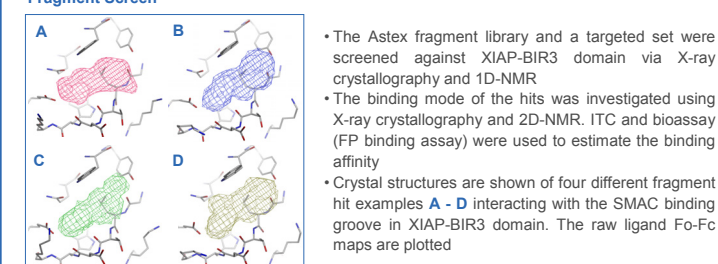
- Initial lead optimisation focused on improving in vivo properties
- Addition of basic side chains led to several compounds with good efficacy and extended pharmacokinetics in tumours
- Some of these compounds possessed activity against hERG (patch clamp); further SAR showed it was possible to obtain compounds with good efficacy and little or no activity in the hERG assay
- AT13387 shows good efficacy and biomarker response in a number of xenograft models, with extended PK half-life in tumours⁵
- AT13387 was chosen as the clinical candidate

Case History 3: XIAP/cIAP Inhibitor

• Currently in Lead Optimisation

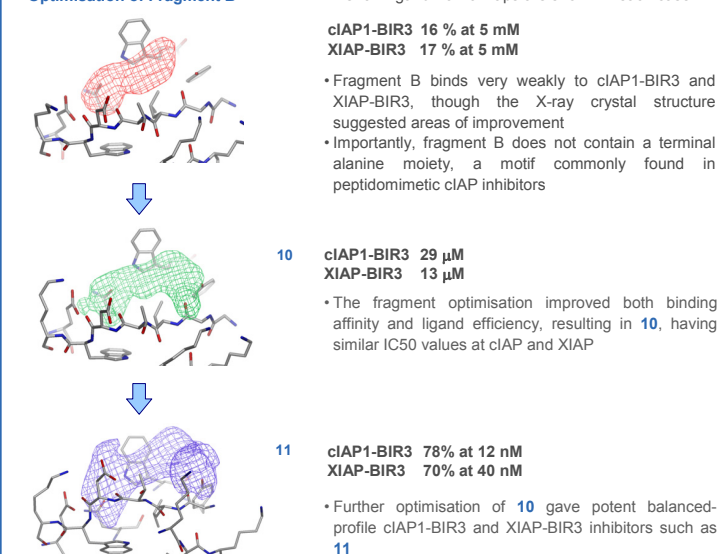
Background
Inhibitor of apoptosis (IAP) proteins⁶ are important regulators of cancer cell survival, possessing baculovirus IAP repeat (BIR) domains necessary for their anti-apoptotic activity. The N-terminus of a number of important apoptotic proteins (e.g. caspases) bind in the peptide-binding groove in the BIR domain. This protein-protein interaction contributes to oncogenesis and resistance to current therapies. Our target is a small molecule XIAP and cIAP BIR antagonist which can mimic the effects of SMAC (second mitochondrial activator of caspases), an endogenous inhibitor of the IAPs.

Fragment Screen



- The Astex fragment library and a targeted set were screened against XIAP-BIR3 domain via X-ray crystallography and 1D-NMR
- The binding mode of the hits was investigated using X-ray crystallography and 2D-NMR. ITC and bioassay (FP binding assay) were used to estimate the binding affinity
- Crystal structures are shown of four different fragment hit examples A - D interacting with the SMAC binding groove in XIAP-BIR3 domain. The raw ligand Fo-Fc maps are plotted

Optimisation of Fragment B



- Fragment B binds very weakly to cIAP1-BIR3 and XIAP-BIR3, though the X-ray crystal structure suggested areas of improvement
- Importantly, fragment B does not contain a terminal alanine moiety, a motif commonly found in peptidomimetic cIAP inhibitors

- The fragment optimisation improved both binding affinity and ligand efficiency, resulting in 10, having similar IC₅₀ values at cIAP and XIAP

Summary of Current Status

- Fragment B has been optimised to a series of non-alanine nM-potent dual XIAP and cIAP inhibitors
- Compound 11 showed antiproliferative activity in EVSA-T and MDA-MDB 231 cell lines with EC₅₀ values 36 nM and 190 nM respectively
- An engineered HEK293 cell line over-expressing flag-tagged XIAP and caspase-9 (HEK293-X-C9) was used in immunoprecipitation (I.P.) assays to demonstrate inhibition of the XIAP:caspase-9 interaction in cells. In this assay 11 was potent, having IC₅₀ 10 nM
- Oral bioavailability in rodents has been demonstrated for multiple series examples
- Efficacy has been demonstrated via the oral route of administration for series members in a mouse MDA-MDB 231 xenograft model