

Fragment Based Drug Discovery of Selective Inhibitors of Fibroblast Growth Factor Receptor (FGFR)

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G. Saxty¹, R. Akkari², P. Angibaud², J. Arts², P. Benderitter², V. Berdini¹, P. Bonnet², A. Cleasby¹, I. Csoka², W. Embrechts², E. Freyne², R. Gilissen², P. King², J. Lacrampe², E. J. Lewis¹, Y. Ligny², A. Madin¹, S. McClue², L. Mevellec², C. W. Murray¹, H. Newell³, M. Page², A. Papanikos², T. Perera², V. Poncelet², O. Querolle², D. C. Rees¹, S. J. Rich¹, B. Roux², S. M. Saalau-Bethell¹, E. Sement², Y. Simmonet², M. Squires¹, V. Tronel², G. A. Ward¹, M. Willems², B. Wroblowski², N.T. Thompson¹

¹Astex Therapeutics Ltd, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, UK. ²Janssen R&D, Turnhoutseweg 30, 2300 Beerse, Belgium. ³Northern Institute for Cancer Research, Paul O'Gorman Building, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK.

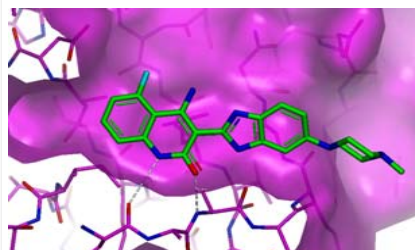
Aim

- The aim of this project was to identify an FGFR inhibitor with selectivity versus VEGFR2 and FLT3. The hypothesis is that this profile will treat aberrant FGFR dependent tumours and the clinical dose will not be limited by VEGFR2 and FLT3 activity.

Abstract

- Recent data in a number of tumour types has implicated Fibroblast Growth Factor (FGF) and Fibroblast Growth Factor receptor (FGFR) signalling as being key to the molecular pathology of cancer.
- A fragment screening campaign was conducted against the tyrosine kinase domain of FGFR1 to detect low molecular weight compounds that bound to the hinge region of the kinase. The screening produced several fragment inhibitors (molecular weight <250 Da) in the micromolar range and their binding modes were confirmed by X-ray crystallography.
- The poster will focus on the description of previously undescribed compounds bearing an imidazo[1,2-a]pyridine core scaffold which selectivity versus other protein kinases, for example FLT3, is obtained using the X-ray crystal structure and structure-based design. In summary we will illustrate how X-ray crystallography and fragment-based drug design (FBDD) can be used to discover compounds with activity in an FGFR driven xenograft model when dosed by the oral route.

Fig 1. FGFR1 Protein-Ligand Crystal Structure With TKI-258 (Novartis)

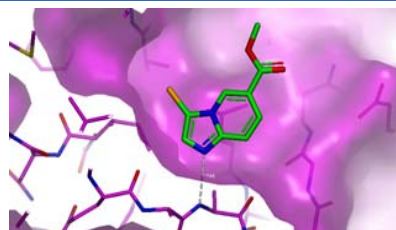


- The pan RTK inhibitor compound TKI-258 in a protein-ligand co-complex with FGFR1.
- TKI-258 IC50 (FGFR3) 0.009 μM IC50 (VEGFR2) 0.013 μM FLT3 0.001 μM*.
- Note the bicycle only contacts the protein in highly conserved parts of the kinase. This gives activity versus FGFR1, VEGFR2 and FLT3.

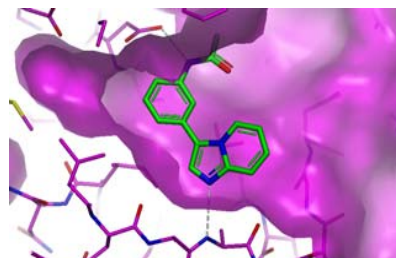
* Reference: Sang Hoon Lee et al., Clin Cancer Res. 2005,11 (10), 3688-3641

Results

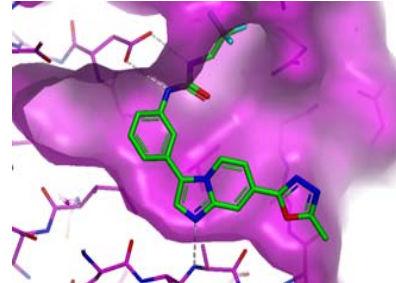
Fig. 2 Protein-Ligand X-ray Crystal Structures of Compound A, B & C in FGFR1



- Compound A, bound to the hinge of the kinase

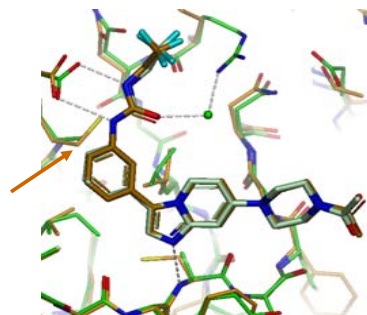


- Compound B, fragment grown to interact with aspartic acid residue



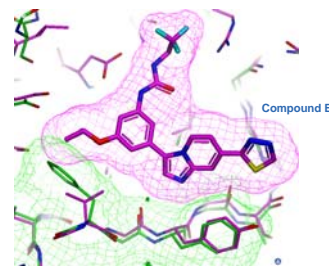
- Compound C, aspartic acid residue interacts with both urea hydrogen bond donors

Fig. 3 Structural Biology Rationale For Selectivity Versus VEGFR2 And FLT3



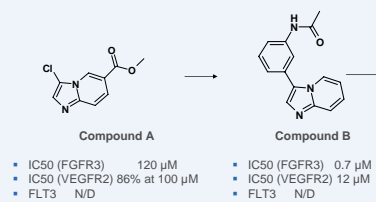
- The same binding pose is found in both FGFR1 (green) and VEGFR2 (orange).
- Note an analogue that has sufficient potency against VEGFR2 was required to afford the protein-ligand co-complex in VEGFR2.
- Selectivity profile of FGFR1/3 and VEGFR2 could be due to the Ala/Cys difference between the two proteins where the urea binds (shown with the orange arrow). There is also a water molecule (green) between the urea C=O and an arginine residue that has moved into the pocket for FGFR1.

- FGFR1 shares a 57.4% global identity and 72.6% global similarity to VEGFR2.

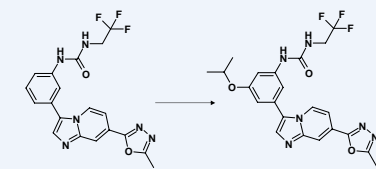


- The phenylalanine gatekeeper residue in FLT3 (in green, pdb code 1rj) differs to that of leucine in all the FGFR isoforms (magenta).
- The larger phenylalanine gatekeeper in FLT3 obscures the pocket causing a clash with the iPr-O motif of the ligand.

Fig. 4 Fragment to Lead Using Fragment Growth

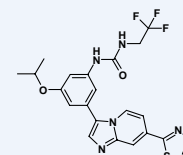


- Compound A: IC50 (FGFR3) 120 μM, IC50 (VEGFR2) 86% at 100 μM, FLT3 N/D
- Compound B: IC50 (FGFR3) 0.7 μM, IC50 (VEGFR2) 12 μM, FLT3 N/D



- Compound C: FGFR3 0.015 μM, VEGFR2 0.380 μM, FLT3 0.013 μM 1 Fold
- Compound D: FGFR3 0.015 μM, VEGFR2 0.630 μM, FLT3 >1.0 μM >100 Fold

	BaF3-FGFR3 -IL3 IC50 (μM)	BaF3-VEGFR2 -IL3 IC50 (μM)	BaF3-FLT3 -IL3 IC50 (μM)
TKI-258	0.5	0.7	0.05
Compound C	2.7	6.9	0.9
Compound D	2.4	7.7	>10
Compound E	0.4	NT	>10



- Compound E: FGFR3 0.003 μM, VEGFR2 0.092 μM

Fig 5. Pharmacodynamics of Compound D in KMS-11 Xenograft model

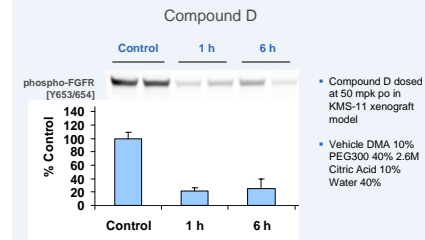
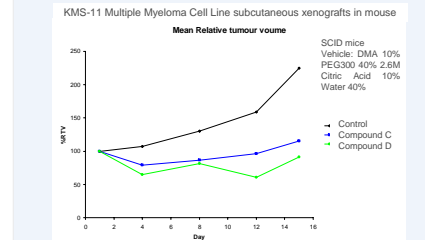


Fig. 6 Efficacy of Compound C and D in Xenograft Model



CONCLUSIONS

- A 120μM hit compound was elaborated, through a fragment growth approach, SBDD and lead optimisation to nanomolar Compound C with VEGFR2 selectivity.
- The undesired FLT3 activity was removed using a growth vector off the ligand to a residue that is different in FGFR versus FLT3.
- The selective nature was confirmed in an *in vitro* bioassay and in engineered BaF-TEL cell lines.
- The compound series also has PD activity and oral bioavailability in the FGFR dependent KMS11 xenograft model.
- The data presented here describe a series of selective FGFR inhibitors that, without the dose-limiting side effects associated with VEGFR2 and FLT3, may offer benefit to patients with tumours dependent on FGFR.

