

Development of Inhibitors of the Fibroblast Growth Factor Receptor (FGFR) Kinase Using a Fragment Based Approach

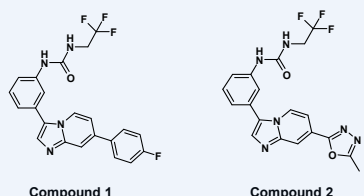
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INTRODUCTION

- 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. FGFR is a receptor tyrosine kinase which activates the extracellular signal-regulated kinase / mitogen-activated protein kinase and the protein kinase B / Akt pathways which promote cell growth and survival. Amplification, over-expression or activating mutations of fibroblast growth factor receptors have been associated with bladder tumours, multiple myeloma, hormone-refractory prostate cancer and breast cancer amongst others.
- Multiple lead series of FGFR inhibitors were developed using Astex's fragment based medicinal chemistry approach, Pyramid™, linked to high throughput X-ray crystallography. We describe here the profile of 2 compounds from one lead series that demonstrated activity against FGFR 1-4 with an IC₅₀ <100nM in an isolated kinase assay.
- In cell based assays these compounds inhibited FGFR1-4 kinase activity in BaF3 cell lines engineered to express the relevant kinase fusion proteins, and proliferation and survival of a panel of FGFR-dependent human tumour cell lines derived from several different tissues. The activity was >10 fold lower in cell lines lacking FGFR expression.
- In xenograft models in mice where aberrant FGF signalling underlies tumour pathology, tumour growth inhibition is observed at doses of 50mg/kg/day orally for up to 21 days. This efficacy was observed in multiple myeloma xenografts KMS11 and Jim1 and the endometrial xenograft AN3CA, in which aberrant FGFR signalling is involved in tumour pathology and significantly greater than that observed in models where FGFR signalling is not known to play a role. These data indicate that the mechanism of action is consistent with FGFR inhibition. The pharmacological profile in these models is distinct from other broader spectrum receptor tyrosine kinase inhibitors.

Figure 1. Structures of Compound 1 and Compound 2



- Multiple lead series were identified using a fragment-based approach.
- The structures of two examples from one of these series are represented above.
- The detailed identification of these compounds is described in the accompanying lecture #6304 - Fragment-based drug discovery of selective inhibitors of fibroblast growth factor receptor (FGFR) by Saxty et al.

RESULTS

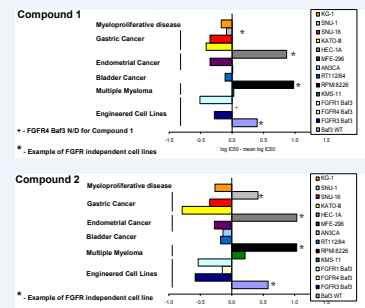
Figure 2. *In vitro* Selectivity Profile

Kinase	Cpd 1 (IC ₅₀ nM)	Cpd 2 (IC ₅₀ nM)
FGFR3	3	15
FGFR1	13	78
FGFR2	33	66
FGFR4	34	94
VEGFR1	13	440
VEGFR3	68	320
VEGFR2	100	380
PDGFRβ	290	2500
FK3	<100	60
EGFR	>10000	>10000
ErbB2	>10000	>10000

In a panel of >200 additional kinases tested IC₅₀ of >1000nM were obtained

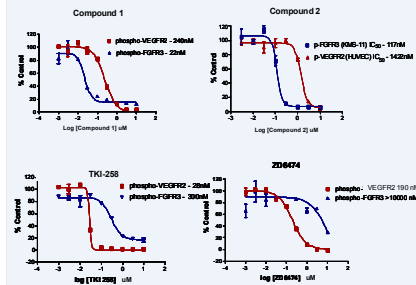
- Compound 1 and compound 2 were tested for kinase activity against a panel of >200 kinases in an *in vitro* assay format. In this assay format both compounds exhibited a 20-30 fold selectivity for FGFR over VEGFR2.
- Outside of the VEGFR family of kinases, FK3 was the only additional kinase inhibited at concentrations <100nM.

Figure 3. Antiproliferative Activity in FGFR-Dependent Cell Lines



- Cells were incubated with the indicated compounds for 72h prior to determination of cell number using Alamar Blue™ assay
- Cytotoxicity assays were performed in a panel of cell lines representing disease types known to be dependent upon FGFR isoforms for transformation or survival.
- Representative lines were also included that are transformed by different mechanisms.
- Values are represented as the fold log change from the mean log IC₅₀.
- For both compounds the most sensitive lines in this assay are those dependent upon FGFR signalling for survival.

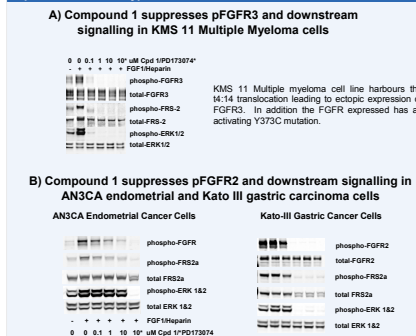
Figure 4. Compounds Exhibit Selectivity Over VEGFR2 in Cell Based Assays



Serum starved HUVEC cells (VEGFR2) or KMS-11 cells (FGFR3) were incubated with the indicated compounds for 30 minutes. HUVEC cells were stimulated by the addition of 100pg/ml VEGF₁₆₅ for 5 minutes and lysates made for the ELISA assay to monitor either pVEGFR2 or pFGFR3.

- Compound 1 and Compound 2 were significantly more potent inhibitors of pFGFR3 than pVEGFR2 in cell based systems. IC₅₀ for FGFR3 was 11-12 fold lower than for pVEGFR2 in both cases.
- TKI-258 (Novartis) and ZD6474 (AstraZeneca) are multi-targeted in nature, are more active vs VEGFR2 than FGFR3 and demonstrate selectivity in the opposite direction.

Figure 5. Inhibition of FGFR in Cell Lines Derived from Multiple FGFR-Dependent Disease Types

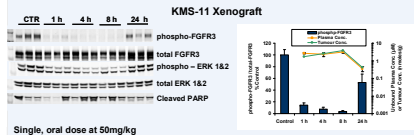


AN3CA endometrial carcinoma cell line harbour the K310R and N550K activating mutations in FGFR2. Kato III gastric carcinoma line overexpress a truncated, constitutively active form of FGFR2

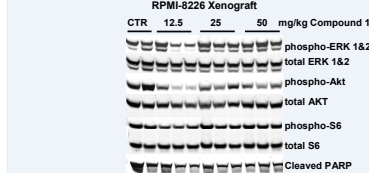
Cell lines were incubated with Compound 1 at the indicated concentrations for 30 mins. Where indicated 100pg/ml FGF-1 was added for a further 5 minutes before cell lysates were prepared and immunoblotted for pFGFR and downstream signalling. 10μM of the FGFR inhibitor PD173074 was included at control as a positive control in each case.

- Treatment of several different tumour cell lines with Compound 1 showed that phosphorylation of FGFR is readily inhibited in numerous cell types (Figure 5).
- Inhibition of downstream signalling through the MAPK pathway was also observed
- In certain cell lines the inhibition of MAPK signalling is more potently inhibited than others. In endometrial cells for example it is likely that FGFR2 signals predominantly through a pathway other than MAPK since this is little affected at concentrations shown to inhibit FGFR2
- The concentrations at which inhibition of the signalling pathway occurs were consistent with the IC₅₀s in a cytotoxicity assay.

Figure 6. Pharmacodynamics of Compound 1 in Multiple Myeloma Xenografts



Single, oral dose at 50mg/kg



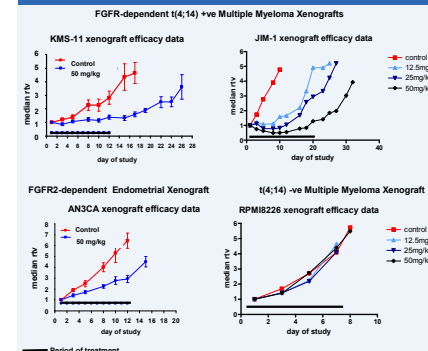
21 oral, daily doses at indicated concentration

Nude mice bearing KMS-11 xenografts were treated with a single dose of compound 1. Tumour and plasma samples were removed at subsequent times and processed to determine levels of phospho-signalling by immunoblotting and compound levels by LC-MS MS. Nude mice bearing RPMI-8226 xenografts had compound 1 administered daily for 21 days at the indicated doses. Tumours were removed at 4h following the final dose.

- In KMS-11 xenografts pFGFR3 is rapidly inhibited following dosing and the inhibitory effects were observed downstream in the MAPK and PKB pathways. Apoptosis is also induced within the tumour as indicated by the appearance of cleaved PARP.
- These tumour markers are inhibited in a way consistent with the levels of compound observed within plasma and tumour samples which remain >3x cell-based proliferation IC₅₀ for up to 24h.
- These data support the effectiveness of once daily dosing in an efficacy model.

- In the RPMI 8226 xenograft model that does not express any FGFR isoforms, no consistent modulation of MAPK pathway biomarkers was observed at doses up to 50mg/kg

Figure 8. Efficacy of Compound 1 in Xenograft Models



Tumour cells were injected s.c. into the flank of male nude mice. A treatment group consisted of 6-8 animals. Compound 1 was administered at the indicated doses once daily via the oral route.

- In the studies shown Compound 1 was administered once daily, at the doses and time period indicated.
- The volume of the tumour was calculated as an ellipsoid volume every 2 days.
- Compound 1 exhibits a dose dependent inhibition of the growth of the FGFR3 positive multiple myeloma models; Jim-1 and KMS-11.
- Consistent with the *in vitro* data presented growth inhibitory activity was also observed in the FGFR2-dependent endometrial xenograft AN3CA.
- Compound 1 is minimally active in the (4;14) negative myeloma models including RPMI 8226, when dosed in the same way.

CONCLUSIONS

- Two lead compounds were derived from a fragment based screen and characterised for FGFR inhibition in multiple cell based models.
- Both compounds exhibited effective inhibition of all 4 FGFR isoforms *in vitro* and in cell based models and demonstrated selectivity over the closely related kinase VEGFR2.
- Their selective nature was confirmed by an increased cytotoxic effect in cell line models representative of FGFR-dependent tumours over those transformed by other means, both *in vitro* and *in vivo*.
- The pre-clinical data presented here describes a first in class series of compounds that warrant further evaluation and may offer benefit to patients whose disease is dependent upon aberrant FGFR signalling.

