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Discovery and Characterization of Potent and Selective AXL Receptor Tyrosine Kinase Inhibitors for Cancer Therapy

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OVERVIEW

- AXL receptor tyrosine kinase (AXL) is a transmembrane protein which is overexpressed in a variety of cancers and has been implicated in the development of resistance to chemotherapy and immunotherapy.
- Arcus Biosciences is developing novel and selective AXL receptor tyrosine kinase inhibitors as potential anti-cancer drugs.
- This poster describes the discovery and characterization of two novel inhibitors, developed through study of earlier literature compounds. These compounds are characterized by various *in vitro* assays and show good potency and selectivity toward AXL in relation to similar kinases. Initial pharmacokinetic profiles are promising for future *in vivo* efficacy studies.

AXL BIOLOGY & DOWNSTREAM EFFECTS

- Binding of Gas6 to AXL on the tumor cell surface induces AXL autophosphorylation.[1]
- AXL activation induces PI3K-AKT pathway signaling, resulting in upregulation of genes associated with EMT[2] & DNA damage repair[3] leading to therapeutic resistance to multiple agents.

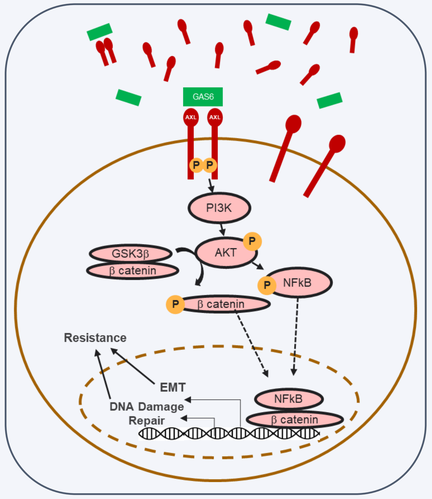

Figure 1. Gas6 induced AXL Signaling. Binding of Gas6 to AXL leads to AXL autophosphorylation and signaling through the PI3K-AKT pathway, inducing genes that drive EMT & DNA damage repair

INITIAL DESIGN, OPTIMIZATION, AND CHARACTERIZATION OF ARCUS AXL INHIBITORS

Structural Biology and Rationale of AXL Receptor Tyrosine Kinase Inhibition

Upon GAS6-induced AXL dimerization, tyrosine residues throughout the intracellular domain are phosphorylated by action of the intracellular kinase domain, resulting in various biological effects.[4] Dimerization can be prevented extracellularly through use of an antibody or AXL 'decoy receptor' (Figure 2). Alternatively, phosphorylation is mitigated through small molecule inhibition of the intracellular kinase domain (Figures 2C and 3)

We embarked upon the design of novel AXL inhibitors, utilizing a pharmacophore mapping approach based on existing commercial and academic molecules. Aside from pan-kinase selectivity, we specifically sought to avoid MERTK and TYRO3 inhibition to reduce any unwanted off-target effects.[5] Due to the high homology of these catalytic domains, design of AXL-selective inhibitors is challenging.[6]

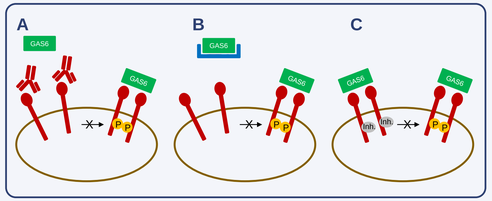

Figure 2. Selected strategies for inhibiting AXL autophosphorylation. a) Blocking antibody. b) AXL 'decoy receptor' to sequester GAS6. c) Small molecule inhibition of kinase domain.

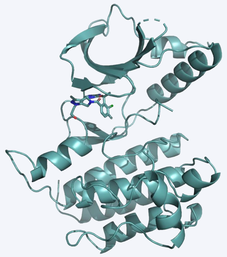

Figure 3. X-ray structure of AXL/small molecule inhibitor complex (PDB: 5U6B).[6]

Characterization and Comparison of Optimized Arcus and Benchmark AXL Inhibitors

Assay	Compound A	Compound B	Bemcentinib[7]	Dubermatinib[7]	Sitravatinib[7]
hAXL HTRF IC_{50} (biochemical, nM)	2.8	1.8	5.2	77	0.98
mAXL HTRF IC_{50} (biochemical, nM)	0.95	0.90	2.7	--	--
hMERTK / hTYRO3 HTRF selectivity (biochemical, enzyme IC_{50} over AXL IC_{50})	130x / 39x	65x / 130x	42x / 33x	14x / 9x	10x / 2x
hAXL NanoBRET™ K_D (cellular, nM)	13	9.1	135	1710	12
hAXL PathHunter® IC_{50} (cellular, nM)	36	15	340	1110	11
hAXL PathHunter® IC_{50} (cellular, nM) 100% serum	719	776	2270	2930	162

Table 1. Characterization and comparison of optimized Arcus and benchmark AXL inhibitors. Kinase activity of AXL, MERTK and TYRO3 were tested using HTRF KinEASE – TK kit (CisBio) in the presence of 700 µM ATP. Inhibitor engagement to intracellularly expressed AXL kinase domain was detected using AXL NanoBRET™ TE intracellular kinase assay (Promega) with transiently transfected HEK293 cells. Compound activity in inhibiting SH2 domain translocation to phosphorylated AXL cytoplasmic domain upon AXL activation was tested using PathHunter® U2OS AXL functional assay (Eurofins DiscoverX). Cells were preincubated with inhibitors for 1 hr followed by 3 hrs Gas6 (1 µg/mL) induction. Assay was carried out in either a serum-free or 100% human serum medium.

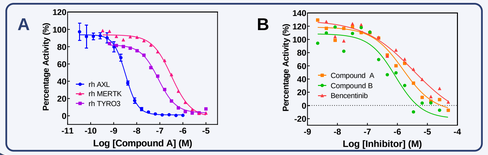

Figure 4. A) Inhibition of AXL, MERTK, and TYRO3 by Compound A in the HTRF biochemical assay**. B)** Inhibition of AXL by Compound A, Compound B, and Bemcentinib in the AXL PathHunter® cellular assay with 100% serum.

Pan-Kinase Selectivity of Arcus AXL Inhibitors

In addition to being selective against MERTK and TYRO3, Compound B was tested against a broad non-mutant kinase panel to determine any other potential off-target effects. Using a qPCR-based competition binding experiment, Compound B showed <20% of control activity (indicating stronger binding) against 10 other kinase targets, excluding MERTK and TYRO3. Follow-up K_d values were determined through qPCR-based competition binding dose response.

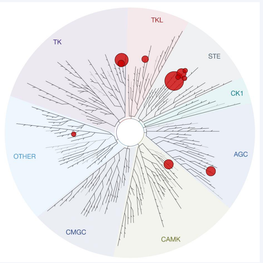

Figure 5. Kinome map (403 total) showing significant kinase/Compound B interactions as red circles.

Kinase	K_d	Kinase	K_d
BMPR1B	8.4 nM (221x)	MAP4K5	1.7 nM (44x)
DRAK1	4.5 nM (118x)	SGK	2.3 nM (61x)
HPK1	4.5 nM (118x)	SLK	84 nM (2210x)
MAP4K2	13 nM (342x)	STK16	13 nM (342x)
MAP4K3	4.7 nM (123x)	TNIK	14 nM (368x)

Table 2. K_d values for selected kinases with Compound B. Selectivity values in parentheses defined as kinase-inhibitor K_d over by AXL-inhibitor K_d (0.038 nM).

AXL Engagement with Gas6 Results in Decreased Soluble AXL and Is Rescued by AXL Inhibition

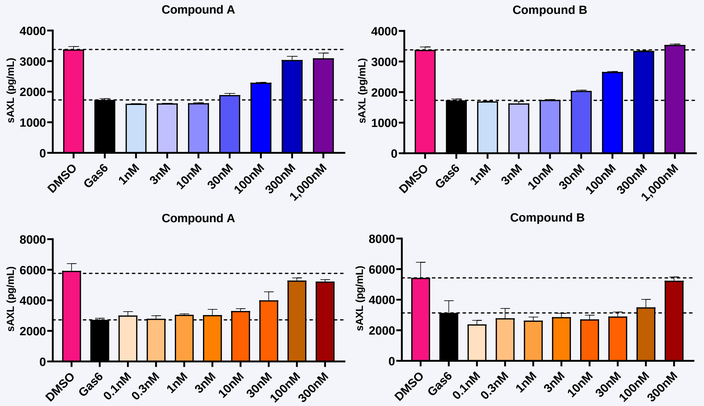

Figure 6. Prototype AXL inhibitors Compound A and Compound B rescue soluble AXL levels that are reduced by Gas6. A549 human lung carcinoma cells (top) and 4T1 murine breast cancer cells (bottom) were treated with 1 – 1,000 nM (A549) or 0.1 – 300 nM (4T1) of Compound A or Compound B for 1 h. 200 ng/mL hGas6 (A549 cells) or 500 ng/mL mGas6 (4T1) was added 1 h after addition of AXL inhibitors and cells were cultured for 72 h. Supernatants were collected and soluble AXL levels were determined by ELISA.

PHARMACOKINETICS and SAFETY

Pharmacokinetic and Safety Characterization

Both Compound A and Compound B exhibit favorable *in vitro* pharmacokinetic profiles with low intrinsic clearance in both rat and human hepatocytes (Table 3). When dosed in rat models, Compound A and Compound B show moderate to low clearance (Table 4). Additionally, Compound B exhibits a good safety profile, with acceptable CYP isoform and hERG inhibition (Table 5) as well as no time-dependent CYP inhibition (data not shown).

Hepatocyte Stability

CL_{int} (µL/min/10^6 cells)

Compound	Mouse	Rat	Dog	Human
Compound A	--	3.8	--	2.6
Compound B	13.8	<0.7	<0.7	3.1

Table 3. Summary of hepatocyte stability in various species.

In Vivo Rat Pharmacokinetics

Compound	CL (L/h/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)
Compound A	1.6	3.3	1.9
Compound B	0.66	2.2	4.8

Table 4. Summary of experimental rat PK parameters. Rats were dosed 0.25 mg/kg IV in DMAC:Ethanol:PG (ca. 1:1:1).

CYP Isoform and hERG Inhibition of Compound B

IC_{50} 2C8 (µM)	IC_{50} 2C9 (µM)	IC_{50} 2C19 (µM)	IC_{50} 2D6 (µM)	IC_{50} 3A4 (µM)	hERG inh. at 10 µM
>40	>40	38	31	11	31%

Table 5. *In vitro* evaluation of Compound B for potential to inhibit major human drug metabolizing enzymes of the cytochrome P450 family and hERG.

SUMMARY

- Two unique prototype AXL inhibitors have been discovered and characterized in functional biochemical and cell-based assays. When taken together, these compounds show improved potency and selectivity compared to previous small-molecule AXL inhibitors (Table 1 and Figure 4).
- Compound B shows a good pan-kinase selectivity profile, suggesting minimal off-target effects within this inhibitor class. The lowest selectivity ratio is for MAP4K5, at 44-fold (Figure 5 and Table 2).
- A decrease in soluble AXL levels is observed upon addition of GAS6 in both lung and breast cancer cells. Addition of either Compounds A or B resulted in an increase of soluble AXL levels to pre-GAS6 concentrations (Figure 6).
- Both Arcus inhibitors show promising pharmacokinetic profiles with low intrinsic and *in vivo* clearances. Compound B shows a good safety profile, with minimal CYP and hERG liabilities (Tables 3-5).

REFERENCES

1) Linger, *et al. Adv. Cancer Res.* **2008**, 100:35.
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4) Zhu, *et al. Mol. Cancer* **2019**, 18:153.
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7) Data generated in-house. Compounds purchased from Synnovator (Bemcentinib), Advanced ChemBlocks (Dubermatinib), and Cayman Chemical (Sitravatinib).