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Discovery and Characterization of Potent and Selective AXL Receptor Tyrosine Kinase Inhibitors

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Introduction

- AXL is a transmembrane protein that is overexpressed in numerous cancers and has been implicated as a driver of resistance to chemo- and immunotherapies.[1,2]
- High AXL expression in tumors is generally correlated with tumor growth and poor prognosis in cancer patients, making it an attractive target for cancer therapeutics.
- AXL can be activated extracellularly by its ligand, growth arrest specific protein 6 (GAS6), or through a ligand-independent pathway triggering receptor dimerization and autophosphorylation.
- Activation of AXL initiates signaling cascades responsible for promoting cancer cell proliferation and survival and an immunosuppressive microenvironment.
- This poster describes the discovery, SAR-driven optimization, and characterization of a novel and potent class of small-molecule AXL inhibitors at Arcus Biosciences.

Inhibition of AXL

AXL receptor signaling can be blocked by three main strategies (Figure 1), aimed at either disruption of AXL dimerization or direct inhibition of phosphorylation.[3] Small-molecule inhibition of AXL is an attractive method to pursue given that it directly inhibits the AXL signaling pathway and does not depend on the state of AXL dimerization.

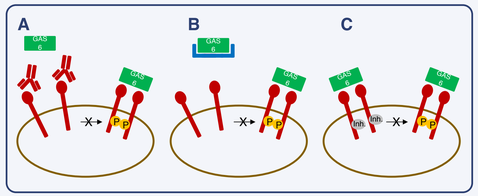


Figure 1. Three main strategies for inhibiting AXL activation. A) Use of blocking antibody to prevent dimerization. B) Utilizing GAS6 trap (blue) to limit dimerization. C) Small molecule inhibition of kinase domain to prevent auto-phosphorylation.

Methods

Kinase activity of AXL, MERTK, and TYRO3 were tested using HTRF KinEASE – TK kit (CisBio) in the presence of 30 or 700 μM ATP. Inhibitor engagement with 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 h followed by 3 h Gas6 (1 μg/mL) induction. Assay was carried out in either a serum-free or 100% human serum medium.

Initial Design and Optimization

Our initial approach to inhibitor design was a combination of structure-based design from an available literature x-ray co-crystal structure and pharmacophore mapping, using inspiration from known inhibitor structures. Following this strategy, we quickly identified a double-digit nanomolar compound to use as a starting point for SAR-driven optimization of AXL biochemical and cellular engagement.

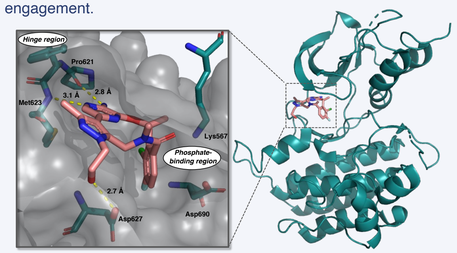


Figure 2. X-ray structure of AXL with bound small molecule inhibitor (Compound 1) (PDB: 5U6B).[4] Binding is facilitated by hydrogen bonding interactions to hinge residues (Met623 and Pro621) and Asp627.

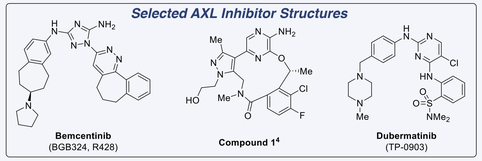


Figure 3. Selected commercial and literature small molecule AXL inhibitors provided inspiration for pharmacophore mapping approach.

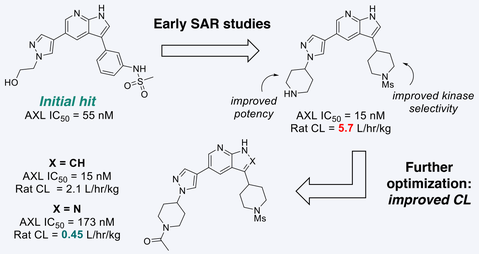
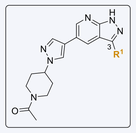


Figure 4. After identifying an initial potent hit, early SAR studies led to a compound with improved potency and selectivity against in-house kinases. The rat clearance was improved by first removing the basic amine (no impact on potency) and switching from an azaindole to azaindazole core. Low clearance came at the expense of potency, so further optimization was required. (AXL HTFR assay in the presence of 30 μM ATP.)

Inhibitor Structure-Activity Relationships



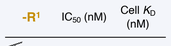
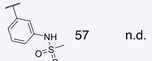
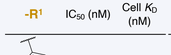
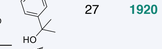
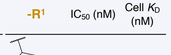
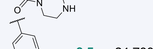
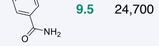
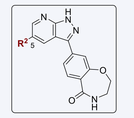
-R¹	IC50 (nM)	Cell K_D (nM)
	57	n.d.
	59	5900
	37	7670
	27	1920
	8.1	707
	1.6	2670
	46	22,500
	9.5	24,700
	29	12,500
	7.3	5960

Table 1. Modification of the C3 sidechain (R¹) was explored to improve cellular potency. Extensive studies identified certain benzamide and phenyl dimethyl carbinol substituents improved biochemical and cellular potency; however, improvements to cellular potency were limited. (AXL HTFR assay in the presence of 30 μM ATP.)



-R²	IC50 (nM)	Cell K_D (nM)
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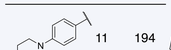
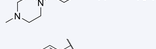
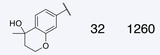
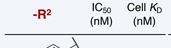
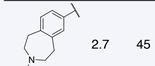
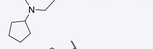
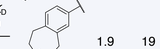
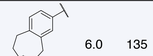
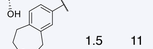
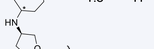
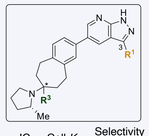
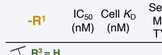
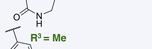
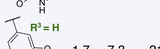
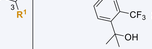
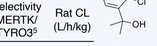
	32	1260
	27	921
	5.0	104
	2.7	45
	1.9	19
	1.0	6.1
	1.5	5.8
	1.8	2.3
	6.0	135
	1.5	11
	3.0	48
	8.8	440
	30	682

Table 2. Modification of the C5 sidechain (R²) was explored to further improve cellular potency. Fused 6,7-ring systems with a basic amine significantly improved cellular potency. Reduction of amine basicity decreased potency. (*1:1 mixture of isomers; AXL HTFR assay in the presence of 700 μM ATP.)

Inhibitor SAR (cont.)



-R¹	IC50 (nM)	Cell K_D (nM)	Selectivity MERTK/ TYRO3[5]	Rat CL (L/h/kg)
	1.0	6.1	12x / 4x	7.7
	1.8	2.3	9x / 3x	7.8
	1.7	7.3	21x / 2x	11
	1.4	8.3	17x / 5x	9.3
	1.7	6.8	16x / 1x	6.4
	8.0	31	43x / 12x	2.4
	19	48	47x / 12x	2.3
	4.2	17	168x / 48x	3.3
	5.2	9.5	16x / 14x	n.d.
	8.1	32	43x / 7x	5.7
	9.6	38	79x / 13x	2.8
	2.8	9.4	130x / 39x	1.6

Compound A

Table 3. Further optimization of the C3 sidechain (R¹) was aimed at improving MERTK/TYRO3 selectivity, to mitigate potential off-target effects,[6] and *in vivo* pharmacokinetics. Compound A provided the best combination of potency, selectivity, and pharmacokinetics and was further characterized. (*1:1 mixture of isomers; AXL HTFR assay in the presence of 700 μM ATP.)

Characterization and Comparison to Benchmark AXL Inhibitor

	Assay	Compound A	Bemcentinib[7]
biochemical	hAXL HTRF IC_{50}	2.8 nM	5.2 nM
	mAXL HTRF IC_{50}	0.95 nM	2.7 nM
	Fold selectivity over hMERTK / hTYRO3 (enzyme IC_{50} over AXL IC_{50})	130x / 39x	42x / 33x
cellular	AXL PathHunter IC_{50}	36 nM	340 nM
	AXL PathHunter IC_{50} (100% serum)	719 nM	2270 nM
ADME	CL_{int} (μL/min/10^6 cells) (r / h hepatocytes)	3.8 / 2.6	4.8 / < 2.7
	Rat PK Parameters: CL (L/hr/kg) / Vss (L/kg) / $t_{1/2}$ (h) / %F	1.6 / 3.3 / 1.9 / 8.9	1.3 / 12 / 6.9 / 5.7
	CYP inhibition IC_{50} (μM) : 2C8 / 2C9 / 2C19 / 2D6 / 3A4	40 / 40 / 9.2 / 5.0 / 4.6	5.9 / 40 / 9.8 / 10 / 9.9

In Vivo Efficacy

Figure 5. Significant efficacy is observed with AXL inhibition in combination with Osimertinib initially and post-relapse.

Summary

- High AXL expression is associated with poor prognosis and tyrosine kinase inhibitor/immunotherapy resistance in several cancer types, making it an attractive target for new therapies.
- Utilizing an initial strategy of structure-based design and pharmacophore mapping, we quickly identified a potent starting point for SAR optimization.
- Modification of the C5 sidechain resulted in significant improvement in cellular potency.
- Modification of the core and C3 sidechain improved *in vivo* rat clearance.
- Compound A has been characterized in functional biochemical and cell-based assays, and demonstrates good progress towards identifying an orally bioavailable, potent, and selective AXL inhibitor.
- Significant anti-tumor activity is observed with Compound A in combination with targeted therapy and upon acquired resistance to a tyrosine kinase inhibitor in xenograft models.
- Arcus has identified a novel and potent class of small-molecule AXL inhibitors suitable for further preclinical development.

References and Notes

1. Zhu, *et al. Mol. Cancer* **2019**, 18:153.
2. Son, *et al. Front. Oncol.* **2021**, 11:756225.
3. Sang, *et al. Front. Oncol.* **2022**, 12:811247.
4. Gajiwala, *et al. J. Biol. Chem.* **2017**, 292(38):15705.
5. MERTK or TYRO3 HTRF IC_{50} divided by AXL HTRF IC_{50} (700 μM ATP).
6. Hamm, *et al. Arch. Toxicol.* **2022**, 96:613.
7. Data generated by Arcus. Compound purchased from Synnovator.