Discovery and Characterization of Orally Bioavailable, Potent, and Selective Small-Molecule AXL Kinase Inhibitors for Cancer Therapy

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OVERVIEW

- AXL receptor tyrosine kinase (AXL) is a transmembrane protein that is overexpressed in a variety of tumors and levels of which correlate with poor disease prognosis and therapy resistance in cancer patients.
- Arcus Biosciences has developed several series of novel small-molecule AXL kinase inhibitors.
- This poster describes the discovery of highly potent, selective, and orally bioavailable small-molecule AXL inhibitors via a structure-based drug design approach, which are characterized in multiple *in vitro* assays and *in vivo* studies.

AXL ACTIVATION AND INHIBITION

- AXL expression is correlated to proliferation, migration/invasion, and epithelial-mesenchymal transition (EMT) in cancer cells. Overexpression can cause drug resistance during cancer treatments and attenuate immune responses.[1,2]
- Activation and dimerization of AXL by its ligand, growth arrest specific protein 6 (GAS6), or ligand-independent dimerization facilitates AXL phosphorylation; GAS6-independent homodimerization, heterodimerization, or transcellular homophilic dimerization also leads to AXL phosphorylation.[1]
- AXL receptor signaling can be blocked using three main strategies: inhibition of dimerization through use of an antibody (Figure 1A), reduction of dimerization utilizing a GAS6 trap (Figure 1B), or blockade of receptor auto-phosphorylation with small molecule inhibitors of the intracellular kinase domain (Figures 1C).[3]

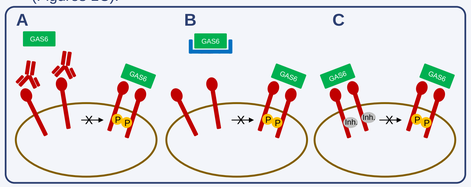


Figure 1. Selected strategies for inhibiting AXL phosphorylation.

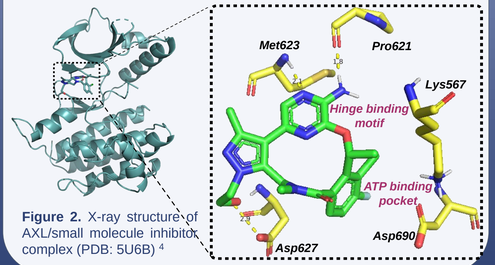


Figure 2. X-ray structure of AXL/small molecule inhibitor complex (PDB: 5U6B) [4]

INITIAL DESIGN, OPTIMIZATION, AND CHARACTERIZATION OF NOVEL AXL INHIBITORS

Drug Design and Optimization

The design of a novel AXL inhibitor commenced with interrogation of existing academic and commercial small molecules (Figure 2), followed by structure-based drug design, and finally SAR optimization of multiple assay readouts. Our goal was to discover an inhibitor which has excellent potency within a novel structural space, an excellent safety profile, and good pan-kinase selectivity. We specifically sought to avoid MERTK and TYRO3 inhibition to reduce any potential off-target effects.[5] A series of 1*H*-pyrazolo[3,4-*b*]pyridines were identified, culminating in the discovery of compound **1a** (Figure 3) and several additional lead AXL inhibitors.

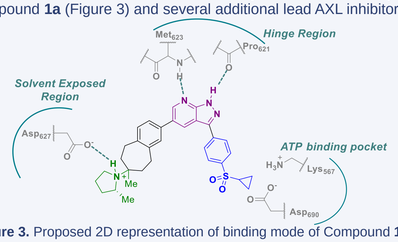
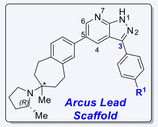


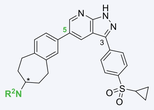
Figure 3. Proposed 2D representation of binding mode of Compound **1a**.



R¹	AXL IC_{50} (nM)	Selectivity Fold (MERTK / TYRO3)	Cell AXL K_D (nM)	Rat CL (L/h/kg)	Rat F% (%)	CYP IC_{50} 3A4 / 2D6 (μM)	hERG inhibition (10 μM)
1a	2.8	130x / 39x	9.4	1.6	8.9	4.6 / 5.0	85%
1b	0.95	104x / 92x	5.8	1.6	7.8	17 / 10	58%
1c[a]	1.8	65x / 130x	9.1	0.66	0.35	11 / 31	31%
1d	7.9	80x / 83x	50	0.28	11	5.9 / 4.0	57%
1e[a]	6.3	85x / 67x	8.5	1.9	5.4	28 / 14	25%
1f[a]	5.0	121x / 89x	18	1.6	11	13 / 20	56%

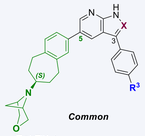
Table 1. Use of benzamide analogues resulted in improved CYP and hERG inhibition profiles compared to **1a**. However, this series suffered from low oral bioavailability due to their identification as P-gp substrates.(Efflux Ratio: **67.1** (**1c**); **31.6** (**1f**)). [a] Single diastereomer with (*S*,*R*) configuration on the C5 amine.

Drug Design and Optimization (cont.)



NR²	Cell AXL K_D (nM)	Rat F% / AUC_{last} (hr*ng/mL)
1a	9.4	8.9% / 103
2a	100	23% / 538
2b	21	20% / 307
2c	147	6.5% / 130
2d	120	n.d. / 151
2e	77	18% / 496
2f	75	7.0% / 117
2g	44	16% / 167
2h	40	18% / 463
2i	71	27% / 343
2j	126	39% / 354
2k	38	27% / 1027
2l	18	12% / 113
2m	40	n.d. / 146
2n	44	46% / 411
2o	43	17% / 211
2p	32	22% / 211

Table 2. Basicity modulation of the C5 amine (**NR²**) to improve oral bioavailability and retain potency. Compound **2k** showed the highest oral exposure in rat PO pharmacokinetic studies.



R³	Cell AXL K_D (nM)	Rat CL (L/h/kg)	Rat F% / AUC_{last} (hr*ng/mL)
3a (X = N)	15	0.28	23% / 1515
3b (X = CH)	8.7	1.0	27% / 450
3c (X = N)	6.8	0.14	30% / 3850
3d (X = N)	127	0.66	203% / 3130
3e (X = N)	27	0.49	31% / 1135
3f (X = N)	13	0.20	15% / 1348
3g (X = N)	66	0.90	112% / 2045
3h (X = CH)	38	1.0	40% / 502

Table 3. Further modification of C3 and hinge-binding central core with a variety of functional groups to find the right balance between AXL cellular potency, physicochemical properties, and pharmacokinetic profile. Compound **3c** was selected for additional characterization.

Characterization and Comparison of Compound 3c and Benchmark AXL Inhibitor

	Assay	Compound 3c	Bemcentinib[6] (BGB324, R428)
biochemical	hAXL HTRF IC_{50}	3.0 nM	5.2 nM
	mAXL HTRF IC_{50}	1.4 nM	2.7 nM
	Fold selectivity over hMERTK / hTYRO3 (enzyme IC_{50} over AXL IC_{50})	64x / 22x	42x / 33x
cellular	hAXL NanoBRET™ K_D	6.8 nM	50 nM
	pAXL ELISA IC_{50} (serum-free media)	16 nM	270 nM
	pAXL ELISA IC_{50} (100% serum)	73 nM	1,100 nM
	hERG (% inhibition at 10 μM)	35	96

Table 4. Characterization and comparison of optimized Arcus and benchmark AXL inhibitors. Kinase activity of AXL was determined using a HTRF KinEASE – TK kit (CisBio) in the presence of 700 μM ATP. Dose-dependent inhibition of AXL activation was determined using pAXL ELISA in HEK293T cells transiently transfected with Nanoluc-tagged AXL.

Pan-Kinase Selectivity of Compound 3c

Compound **3c** was tested against a broad non-mutant kinase panel to determine potential off-target activity.[7] Using a qPCR-based competition binding experiment, compound **3c** showed <35% of control activity (indicating stronger binding) against 8 other kinase targets, excluding MERTK and TYRO3. Follow-up K_d values were determined through qPCR-based competition binding dose response.

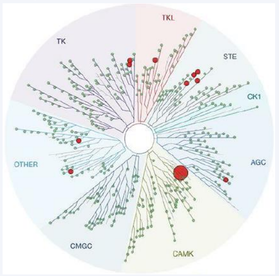


Figure 4. Kinome map (403 total) showing significant kinase/compound **3c** interactions in red.

Table 5. K_d values for compound **3c** with kinases showing <35% of control activity.

Kinase	K_d (nM)	$K_{d,kin}/K_{d,AXL}$
AXL	0.05	1
MERTK	3.6	72
TYRO3	-	>1000
BMPR1B	9.7	194
DRAK1	1.7	34
HPK1	23	460
MAP4K3	94	1880
MAP4K5	17	340
SGK	12	240
STK16	27	540
TNIK	18	360

PHARMACOKINETIC PROFILING

Compound **3c** exhibited a favorable *in vitro* pharmacokinetic profile with low intrinsic clearance in human hepatocytes (not shown), limited inhibition against a panel of CYP isoforms, no time-dependent CYP inhibition, and no CYP 3A4 induction. Compound **3c** was further characterized by low *in vivo* clearance, long half-life, and good oral bioavailability in rodents.

CYP Isoform	2C8	2C9	2C19	2D6	3A4
IC_{50} (μM)	40	40	19	37	20
TDI % activity loss (10 μM)	11	4	-	2	6

PXR Activation (% Ctrl)			
2 μM	-0.4%	10 μM	7.7%

Table 6. Compound **3c** was evaluated *in vitro* for its potential to inhibit major CYP isoforms, time-dependent inhibition, and PXR activation.

Rat PK Parameters				
CL (L/h/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	AUC_{last} (hr*ng/mL)	Bioavailability (%)
0.14	0.34	2.1	3850	30

Table 7. Summary of experimental PK parameters in rat. Rats were dosed 0.25 mg/kg IV in DMAC:ethanol:propylene glycol (~1:1:1); and 2 mg/kg PO in PEG400 with 2 equiv. aqueous HCl solution.

SUMMARY

- Through structure-based drug design and extensive SAR optimization, we have discovered a novel series of highly potent, selective, and orally bioavailable small-molecule AXL inhibitors, with compound **3c** as an exemplary member.
- As characterized in functional biochemical and cell-based assays, compound **3c** exhibits excellent potency and selectivity. Compared to a previous small-molecule AXL inhibitor, compound **3c** shows an improved profile.
- Compound **3c** shows an excellent pan-kinase selectivity profile, supportive of a very low probability for any off-target liabilities. The representative low K_d ratios are for DRAK1 (34-fold) and MERTK (72-fold), with all other kinases being >100-fold.
- Good pharmacokinetic properties of compound **3c** are observed in rodents, exhibiting low *in vivo* clearance, long half-life, and good oral bioavailability with a good safety profile (low DDI / cardiovascular risk).
- Compound **3c** and additional compounds in this series represent good progress towards orally bioavailable, potent and selective AXL inhibitors for the treatment of cancer.

REFERENCES AND NOTES

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2) Son, *et al. Front. Oncol.* **2021**, 11:756225.
3) Sang, *et al. Front. Oncol.* **2022**, 12:811247.
4) Gajwala, *et al. J. Biol. Chem.* **2017**, 292(38):15705.
5) Hamm, *et al. Arch. Toxicol.* **2022**, 96:613.
6) Data generated by Arcus. Compound purchased from Synnovator (Bemcentinib)
7) Analysis performed at Eurofins DiscoverX Corporation (San Diego, CA, USA).