Evaluation of Compounds Developed against AXL Kinase

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INTRODUCTION

- Axl is a TAM family receptor tyrosine kinase and is expressed ubiquitously across various tissues and can be activated by its ligand, Gas 6, or ligand-independent mechanism.
- Axl regulates cell proliferation, survival, migration, invasion, inflammation and angiogenesis through the PI3K/Akt and Src/MAPK pathways.
- Axl is up-regulated and overactivated in a multitude of cancer types and correlated with tumor grade, prognosis, recurrence, metastasis and drug resistance.
- Axl has been validated as a therapeutic target for both solid and liquid tumors. Currently, none of FDA-approved kinase drugs were developed for the TAM family kinases.
- We characterized activity of two compounds from a novel chemical series using biochemical and cell-based assays.
- Selectivity of the compounds was evaluated against a panel of protein kinases from the major groups of the human kinome.
- Effect of these compounds on cell signaling pathways was elucidated, and their *in vivo* efficacy on tumor growth as monotherapy was assessed in A549 xenograft mouse model.

POTENCY AND SPECIFICITY

In biochemical assay, SLC-370 inhibits activity of Axl and Tyro3 by similar extent whereas SLC-391 preferentially targets Axl among the TAM family kinases. The IC_{50} values were determined using radiometric activity-based assays.

Code	MW	cLogP	AXL	TYRO3	MER
			IC_{50} (nM)		
SLC-370	<500	<2.6	5.2	5.0	26.6
SLC-391	<400	<1	9.6	42.3	63.5

PHARMACOKINETIC PROFILES

Results of the mouse PK studies indicated both SLC-370 and SLC-391 have desirable PK profiles with good bioavailability.

PK Parameters	SLC-370		SLC-391	
	IV	PO	IV	PO
C_{max} (μM)	—	1.37	—	0.52
T_{max} (h)	—	2.7	—	0.8
AUC_{Last} (μM*h)	1.65	11.01	0.46	2.34
$AUC_{0-\infty}$ (μM*h)	1.67	11.16	0.49	2.53
Clearance (L/h/Kg)	1.31	—	5.69	—
Vd (L/Kg)	8.74	—	21.64	—
Vd_{SS} (L/Kg)	4.77	—	33.28	—
Half Life (h)	4.62	3.46	2.64	6.18
Bioavailability (%F)	—	66.9	—	51.0

Dose and formulations: iv: 1 mg/kg; NMP:Ethanol:PEG200:NS (2:10:30:58). PO: 10 mg/kg; Tween 80:0.5% HPMC in water (2:98).

SELECTIVITY PROFILES

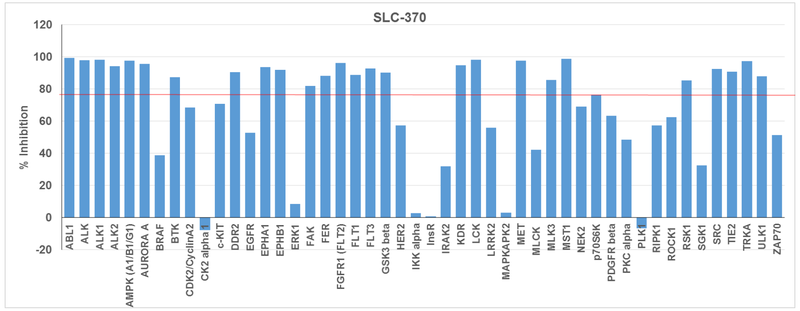


Figure 1. Among 48 protein kinases representing major groups of the human kinome, 14 kinases were significantly inhibited by SLC-391 at 1 μM by more than 75%, whereas 27 by SLC-370. Selectivity profiling was determined using radiometric activity-based assays.

EFFECT ON THE AXL PATHWAY

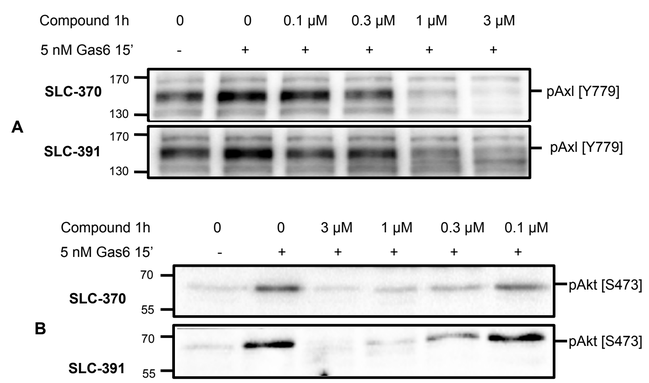


Figure 2. SLC-370 and SLC-391 exhibit dose-dependent inhibitory activity towards both Axl itself and its downstream signaling protein Akt1 in A549 NSCLC cell line, as evidenced by the reduction in their respective activating phosphorylation events induced by Gas 6. The IC_{50} values derived from immunoblotting of pAkt [S473] are 0.21 μM for SLC-370 and 0.29 μM for SLC-391.

EFFICACY STUDY

Inhibition of Tumor Growth in vivo:

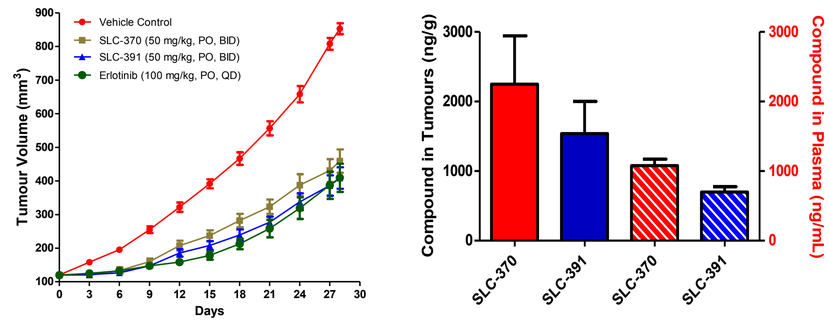
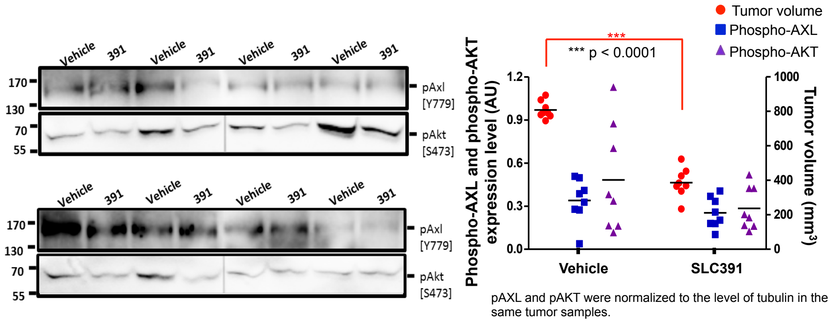


Figure 3. In an A549 xenograft mouse model study with 28-day dosing, SLC-370 and SLC-391 suppressed tumor growth by 46 and 52%, respectively, similar to the effect of Erlotinib. No significant weight loss was observed with either compound throughout the study. Significant amount of compound was detected in the tumor samples (6 h post last dosing).



pAXL and pAKT were normalized to the level of tubulin in the same tumor samples.

Figure 4. Correlation between downstream signaling proteins (Axl/Akt1) and tumor volume in SLC-391-treated A549 mouse xenograft model at the end of the 28 day-dosing period (6 h post last dosing).

SUMMARY

- Two compounds from a novel chemical scaffold have been identified with single-digit nanomolar IC_{50} towards the TAM family kinases, based on the SAR studies of the initial hits from a library screen.
- The compounds differ in their specificity towards the TAM family members and the selectivity in the human kinome.
- Both compounds exhibit inhibitory activity towards the Axl-mediated pathway as expected, which was recapitulated in an A549 xenograft mouse model.
- Both compounds have desirable PK profiles with good bioavailability and reasonable half-life.
- The compounds suppressed tumour growth significantly in an A549 xenograft mouse model without noticeable toxicity.
- *In vivo* efficacy studies in different tumor models and in combination with other anticancer agents are currently under way.