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Novel and Selective Axl Inhibitors

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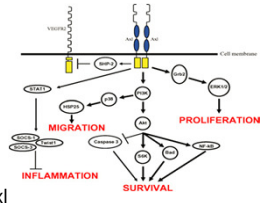
Introduction

- Axl is a member of the TAM tyrosine kinase family and is activated by binding of the ligand growth arrest specific 6 (Gas6). Axl activates the downstream PI3K/AKT and MAPK pathways.
- Axl is overexpressed in glioblastoma, melanoma, erythroid, leukemias, osteosarcoma uterine, colon, prostate, thyroid, ovarian, and liver cancers.
- Axl overexpression correlates with tumor metastasis and invasiveness in a number of tumor types, including renal cell carcinoma, glioblastoma, and breast, gastric, lung, and prostate cancers.
- Axl expression is upregulated in drug-resistant cancer cells.
- RNAi-mediated inhibition of Axl expression reduced the growth of MDA-MB-231 human breast carcinoma cells in a xenograft model.
- Therefore, Axl is a potential target for the development of therapeutic agents for cancer therapy.



- This work reports the discovery of a novel series of potent and selective Axl inhibitors which demonstrated nM potency against Axl and good selectivity against Tyro3, Mer and other selected panel of kinases. Many of these new compounds inhibited the phosphorylation of Akt at Ser-473 in several cancer cell lines in a dose dependent manner. The compounds demonstrated inhibition of cell proliferation using thymidine incorporation assay as well as colony formation of cancer cells at sub-μM concentrations. Furthermore, the pharmaceutical properties of these compounds are desirable for further development. These new Axl inhibitors represent a new approach for cancer therapy.

Experimental

- Western blot assay: Serum starved human non-small cell lung carcinoma A549 cells were treated with compounds of various doses for 1 h followed by 5 nM Gas6 for 15 min. The resulting total cell lysates were subject to Western blot analyses with anti-pAkt [Ser-473] antibody. Intensity of the target signals was detected by enhanced chemiluminescence (ECL) and was quantified by ImageLab.
- Thymidine incorporation assay: Cells seeded in 96-well Isoplates were treated with compounds in the presence of 10% FBS for 48 h followed by the addition of 1 μCi of [^{3}H] thymidine for 24 h. Fifty microliters of 50% TCA was added into each well and incubate at 4°C for additional 2 h. Plates were washed with water and air dried. Incorporation of [^{3}H] thymidine was measured in MicroTriLux.
- Colony formation assay: Cells in growth medium were mixed with 0.7% agar and plated onto 0.5% base agar. After 6-day incubation at 37°C to allow for colony formation, compounds were added into the plates. The medium was replaced with appropriate concentration of compound twice a week. The size and number of colonies were then recorded on Day 20 after initiation of compound treatment.

Results and Discussions

Effect of Axl siRNA knockdown in A549 cells:

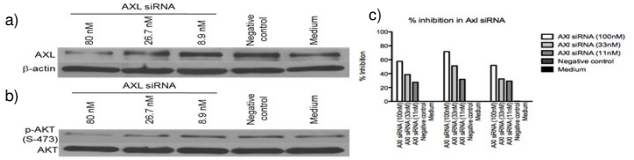


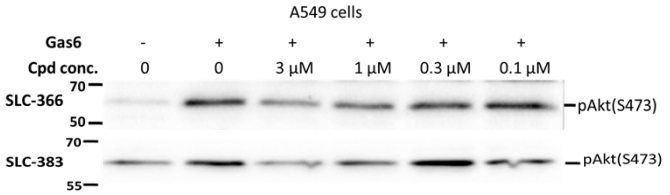
Figure 1. Effect of Axl siRNA knowckdown in A549 cells on: a) Axl protein expression; b) p-Akt (Ser-473) level; and c) inhibition of cell proliferation determined by thymidine incorporation assay. A dose dependent inhibition of Axl protein expression, p-Akt level and the cell proliferation was observed.

Summary of SAR:

Code	AXL	TYRO3	MER	Code	AXL	TYRO3	MER
	IC_{50} (nM)				IC_{50} (nM)		
SLC-320	12.3	391.6	221.9	SLC-362	97.3	278.3	66.0
SLC-321	14.2	191	123.8	SLC-377	129.2	6369	66.3
SLC-234	15.9	>1000	176.2	SLC-388	156.5	1217	460
SLC-344	21.8	502	181.0	SLC-214	271.3	46.5	194.1
SLC-366	26.4	>1000	>10000	SLC-264	276.1	50.7	358.9
SLC-128	60.8	1007	144.3	SLC-125	348.4	>1000	>1000
SLC-368	65.2	>1000	>10000	SLC-229	375.2	>1000	>1000
SLC-359	85.6	2379	29.9	SLC-383	458.3	>1000	3516
SLC-233	86.6	503.8	235.5	SLC-153	649.9	80.4	379.3
SLC-369	90.9	323.6	>1000	SLC-205	692.6	57.9	330.3
SLC-376	93.5	>1000	>1000	SLC-114	935.2	328.3	>1000
SLC-256	95.9	>1000	>10000	SLC-168	1072	86.3	389.9

- >100 analogues have been synthesized and SAR trend has emerged.
- The key structural information has been obtained on modulating the selectivity for Axl and for Tyro3.

Summary of cell-based assay data and ADME properties:



Code	pAkt Inhibition	Proliferation	Solubility	HLM	MLM	PAMPA	3A4	2C9	2D6	2C19
	IC_{50} (μM)	IC_{50} (μM)	(μM)	(% Remaining, 30')		($LogP_{app}$)	(% Inhibition at 10 μM)			
SLC-128	TBD	>10000	91.12	56	33	-5.43	6	12	16	11
SLC-366	2.1	2.86	TBD							
SLC-368	TBD	>10000	21.78	75	103	-6.66	5	3	7	0
SLC-376	TBD	>10000	78.34	ND			-31	7	9	16
SLC-383	0.96	>10000	2.03	12	12	-6.38	-64	11	21	16

Selectivity profiles:

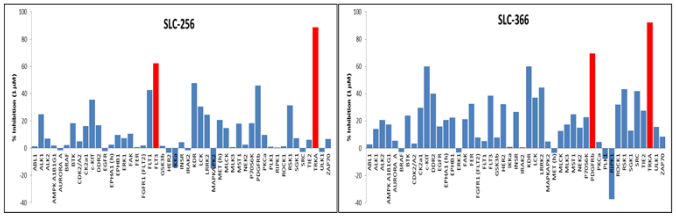
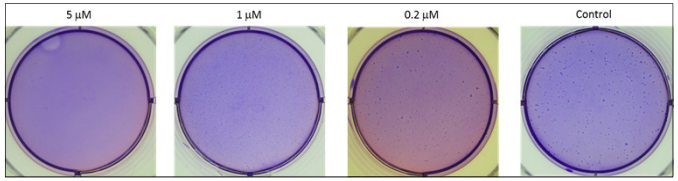


Figure 2. Selectivity profiles of selected compounds. These compounds are selective against Axl. Among the 47 kinases tested, only very few off-target kinases were inhibited to >60% at 1 μM.

Inhibition of colony formation of A549 cells:

SLC-229:



SLC-366:

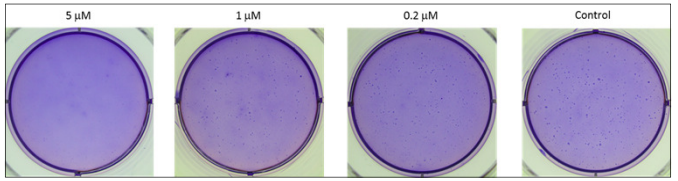


Figure 3. Inhibition of colony formation of A549 cells by compounds. Both compounds have effectively reduced the colony formation even at 0.2 μM. At 5 μM, The formation of colonies was completely inhibited.

Summary

- A series of potent and selective Axl inhibitors was discovered.
- Some compounds have demonstrated sub-micromolar potency in inhibiting pAkt levels in cancer cells.
- These compounds also effectively inhibited cell proliferation and colony formation of cancer cells.
- Many compounds possess the desirable properties for further studies.
- Targeting Axl kinase may provide a new strategy for effective cancer therapy.

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