Novel IRAK-4 inhibitors exhibit highly potent anti-proliferative activity in DLBCL cell lines with activating MYD88 L265P mutation

Wesley Roy Balasubramanian, Venkateshwar Rao Gummadi, Ravi Krishna Babu D, Sivapriya Marappan, Bhavesh Choudhary, Sreevalsam Gopinath, Kavitha Nellore, Shekar Chelur, Girish Daginakatte, Susanta Samajdar, Chetan Pandit, Murali Ramachandra.

Aurigene Discovery Technologies Ltd, Bangalore, India

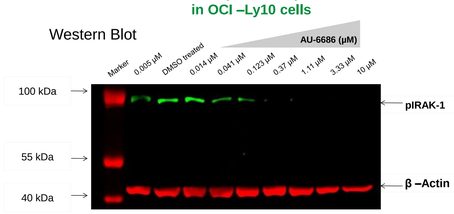
Abstract

Interleukin-1 Receptor Associated Kinase-4 (IRAK-4) is a serine/threonine protein kinase belonging to tyrosine like kinase (TLK) family. IRAK-4 is one of the important signalling components downstream of IL-1/Toll family of receptors (IL-1R, IL-18R, IL-33R, Toll-like receptors). Recent studies have reported occurrence of oncogenic mutations in MYD88 in 30% of activated B cell diffuse large B cell lymphomas (ABC DLBCL) and 90% of Waldenstrom's macroglobulinemia (WM). A significant proportion of ABC DLBCLs have a single amino acid substitution of proline for leucine at position 265 (L265P) in the TIR domain of MYD88 protein resulting in constitutive activation of MYD88 and enhanced activity of IRAK-4. Thus, IRAK-4 is an attractive therapeutic target for the treatment of B-cell lymphomas with activating *MYD88 L265P* mutation. We have recently designed, synthesized and characterized a series of ATP-competitive, bicyclic heterocycle small molecule compounds as IRAK-4 inhibitors. These novel compounds were profiled for their potency as IRAK-4 kinase inhibitors, kinase selectivity, and drug-like properties. Furthermore, selected compounds were tested in proliferation and pIRAK-1-based target inhibition assays using ABC-DLBCL cell lines with activating *MYD88 L265P* mutation,OCI-Ly10 and OCI-Ly3. Lead compounds exhibited potent inhibitory activity against IRAK-4 with single-digit nM IC_{50}s in biochemical assays and decreased pIRAK-1 levels in MYD88 mutant DLBCL cell lines, and potently inhibited the proliferation of DLBCL cell lines in culture. Lead compounds demonstrated potent *in vivo* antitumor activity in OCI-Ly10 DLBCL murine xenograft model, had excellent pharmacodynamic effect in an *in vivo* LPS induced Inflammation model, and resulted in potent activity in the rat Collagen-induced arthritis (CIA) model. In summary, a series of potent IRAK-4 inhibitors have been discovered and are being initially evaluated for treatment of B-cell lymphomas.

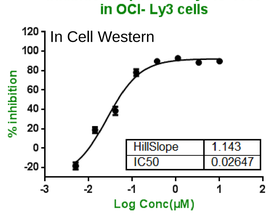
Cell Based Activity

Mechanistic Assay - p-IRAK-1 Inhibition in OCI-Ly10/3 cells: OCI-Ly10 cells were treated with compounds for 12 hr followed by lysate preparation. 50µg of total protein was loaded on gel for western blot analysis. Compound inhibition was normalized to DMSO control. OCI-Ly3 cells were seeded in Poly-D-Lysine coated plates and allowed to attach. Cells were treated with compounds for 12 hrs. followed by In cell Western (ICW) assay to measure pIRAK-1 levels. A representative compound AU-6686 inhibited phosphorylation of IRAK-1 in a dose dependent manner demonstrating potent target engagement.

Inhibition of pIRAK-1 by AU-6686 in OCI –Ly10 cells



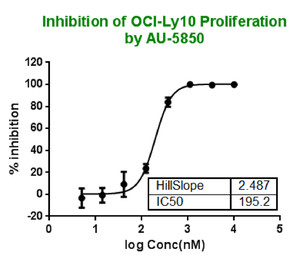
Inhibition of pIRAK-1 by AU-6686 in OCI- Ly3 cells



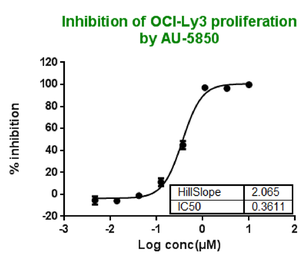
Functional Assay – Inhibition of Proliferation of OCI-Ly10 and OCI-Ly3 Cells

Cells were treated with compounds for 72 hrs. followed by addition of XTT to measure inhibition of proliferation

Inhibition of OCI-Ly10 Proliferation by AU-5850



Inhibition of OCI-Ly3 proliferation by AU-5850

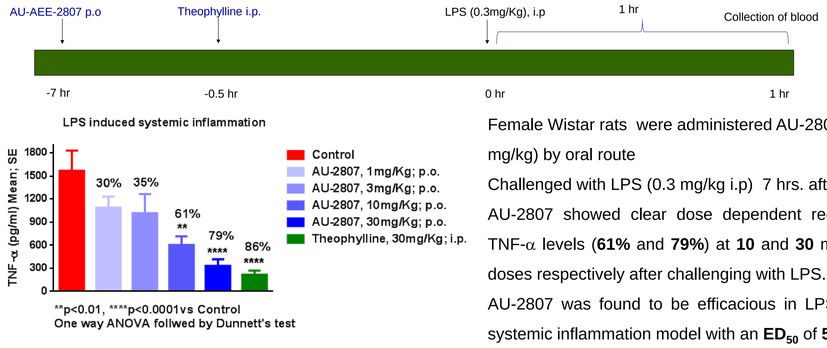


Profile of Representative Compounds

Parameters	AU-5850	AU-2807	AU-6686	AU-5792
hIRAK-4 cell free IC_{50} (nM)	6	7.5	3.5	3
LPS induced TNFα in hPBMC IC_{50}(nM)	ND	23.2	ND	12.5
OCI-Ly10 Proliferation IC_{50} (nM)	195	387 / 298	9	52
OCI-Ly3 Proliferation IC_{50} (nM)	361	53% @ 0.3 µM	31	132
pIRAK-1 inhibition in OCI-Ly10 IC_{50} (nM)	795	123/72.8	100	1510
Karpas 422 Proliferation IC_{50} (nM)	2096	4%@10µM	61	307
Ramos Proliferation IC_{50} (nM)	419	37%@1µM	14,16	347
MLM, HLM, RLM t1/2(min); Cl_{int} (µl/min/mg)	>90, <26, >90, <26, >90, <26	>90, <26, >90, <26, >90, <26	76, 30, 218.8, 10.6, 122, 18.9	>90, <26, >90, <26, >90, <26
PK	Mice PK	Rat PK	Mice PK	Mice PK
(IV) dose (mpk)	3	3	3	3
(IV) T ½ (hr)	3	5.4	5.97	1.4
(IV) AUC (ng.hr/mL)	1387	10631	2516	376
(IV) Cl (mL/hr/kg)	2181	286	1352	2667
(IV)Vd (mL/kg)	4276	1551	12942	4931
(PO) dose (mpk)	10	10	10	10
(PO) C max (ng/mL)	1245	427	1274	1598
(PO) AUC (ng.hr/mL)	3409	7306	6170	3054
%F	73.7	21	73.6	81.4

ND-not determined

In-vivo PK/PD Analysis in Inflammation Models

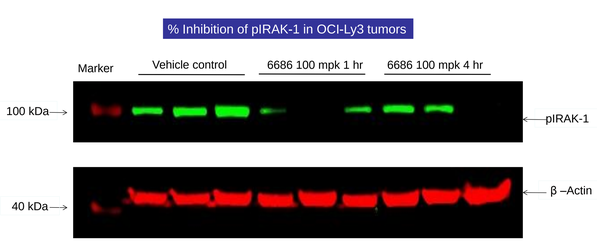


Female Wistar rats were administered AU-2807 (1 – 30 mg/kg) by oral route

Challenged with LPS (0.3 mg/kg i.p) 7 hrs. after dosing AU-2807 showed clear dose dependent reduction in TNF-α levels (**61%** and **79%**) at **10** and **30** mg/Kg p.o. doses respectively after challenging with LPS.

AU-2807 was found to be efficacious in LPS induced systemic inflammation model with an **ED_{50}** of **5.2** mg/Kg.

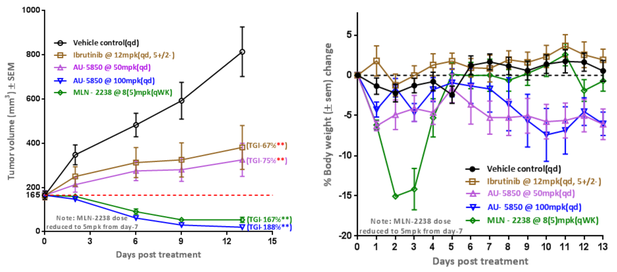
In-vivo PK/PD Analysis in DLBCL Xenograft Models



AU-6686 treatment (100 mpk) resulted in 84% and 70% reduction in pIRAK-1 levels at 1hr and 4hr time points respectively

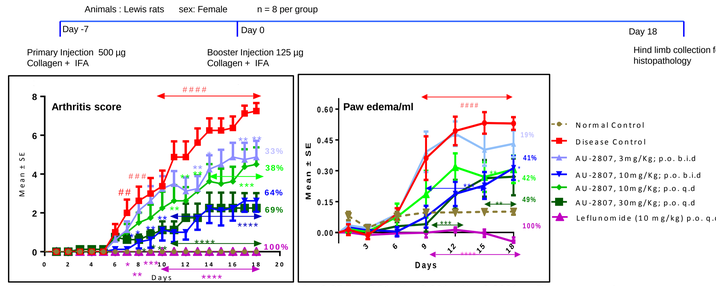
- Male SCID Beige mice 11-12 weeks old were injected with OCI Ly3 cells (10 X 10^6 cell in 200 µl (1:1 Media and Matrigel)
- Tumor bearing SCID Beige mice were treated orally with AU-6686 at 100mpk (qd)
- Tumor samples were isolated at 1hr and 4 hrs. post treatment and analyzed for pIRAK-1 modulation
- AU-6686 treatment demonstrated good PD marker modulation in OCI-Ly3 tumor model

Efficacy in OCI-Ly10 Xenograft Model



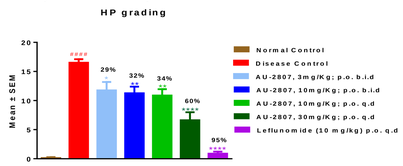
NOD-SCID mice bearing OCI-Ly10 tumors were treated orally once daily with AU-5850 at the indicated doses for two weeks. AU-5850 treatment resulted in 75% tumor growth inhibition at 50 mg/kg dose & tumor regression at 100 mg/kg dose. AU-5850 was well tolerated at both the doses with no major changes in body weight. MLN-2238, a proteasome inhibitor, was used as positive control.

Efficacy in Rat CIA Model



##p<0.01,###p<0.001,####p<0.0001 vs Normal control
Two way ANOVA followed by Bonferroni test

*p<0.05,**p<0.01,***p<0.001,****p<0.0001 vs Disease control
Two way ANOVA followed by Bonferroni test



####p<0.0001 vs Normal Control; t-test
*p<0.05,**p<0.01,****p<0.0001 vs Disease control
One way ANOVA follwed by Dunnett's test

- Disease control animals showed significant increase in arthritis score (day 7 onwards) and paw edema (day 9 onwards) compared to normal controls.
- AU-2807 at 3 mg/kg b.i.d. (day 13, 14, 17&18) 10 mg/kg b.i.d,(day 7 onwards), 10 mg/kg qd (day 11 onwards) & 30 mg/kg qd (day 8 onwards) and leflunomide (day 7 onwards) showed significant reduction in arthritis score compared to disease control.
- AU-2807 at 10 mg/kg b.i.d,(day 9 onwards), 10 mg/kg qd (day 15 onwards) & 30 mg/kg qd (day 9 onwards) and leflunomide (day 9 onwards) showed significant reduction in paw edema compared to disease control.
- Reduction in clinical score correlates well with improvement in histopathological changes

AU-2807 treatment shows superior efficacy (oral dosing, 69% inhibition) compared to reference compound (bid IP dosing, 39% inhibition) at same dose of 30 mpk.

In Vitro Tox Profile of AU-2807

- No rat hepatocyte cytotoxicity (EC50 >5 µM)
- Negative in Ames test (tested with TA98, TA100, TA102, TA1535 & TA1537)
- No significant hERG activity

Conclusion

- Potent IRAK-4 inhibitors from multiple chemically distinct series identified
- Excellent potency in both biochemical and cell based assays
- Good PK profile with oral administration
- Good PK/PD correlation established in LPS-induced rat systemic inflammation model.
- Efficacy demonstrated with oral dosing in disease models of inflammation (CIA) and DLBCL (OCI-Ly3 xenograft model)
- *In-vivo* 14 day tox study in progress for select compounds

Contact : wesley_r@aurigene.com