

## Product Data Sheet

Product Name: LY364947

Cat. No.: GC11604

### Chemical Properties

Cas. No. 396129-53-6

Chemical Name 4-(5-pyridin-2-yl-1H-pyrazol-4-yl)quinoline

SMILES C1=CC=C2C(=C1)C(=CC=N2)C3=C(NN=C3)C4=CC=CC=N4

Formula  $C_{17}H_{12}N_4$

M.Wt 272.3

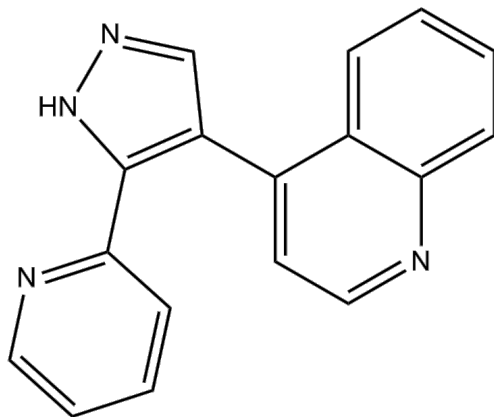
Solubility  $\geq 24.4\text{mg/mL}$  in DMSO

Storage Store at  $-20^{\circ}\text{C}$

General tips For obtaining a higher solubility, please warm the tube at  $37^{\circ}\text{C}$  and shake it in the ultrasonic bath for a while. Stock solution can be stored below  $-20^{\circ}\text{C}$  for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



### Protocol

#### Cell experiment [1]:

Cell lines human dermal lymphatic microvascular endothelial cells

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

## Product Data Sheet

---

HDLECs were cultured in EGM2-MV medium containing endothelial cell growth supplements with 5% fetal bovine serum. HDLECs were seeded at a density of  $2 \times 10^3$  cells/well in 96 well plates, and cells were treated or not with TGF- $\beta$  Preparation Method (1ng/mL) or LY364947 (3 $\mu$ M). After 24h incubation, Photographs of the cells were taken. Expression of Smad7 and PAI-1 mRNAs was determined by Real-time PCR. Cell numbers were determined by Water-Soluble Tetrazolium (WST) assay.

Reaction  
Conditions      3 $\mu$ M; 24h

Applications      LY364947 significantly inhibits TGF- $\beta$ 1-induced Smad2 phosphorylation, while promoting cord formation and cell migration.

### Animal experiment [2]:

Animal models      Male Sprague–Dawley rats

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

## Product Data Sheet

---

Preparation Method Male Sprague–Dawley rats weighing 220–240g were maintained in a room with constant temperature (22°C) and constant humidity (55%) under a 12h light/dark cycle with free access to regular rat chow and tap water. The animals were divided into two groups: NMDA+vehicle (dimethyl sulfoxide [DMSO]), NMDA+LY364947. Under general anesthesia with 50mg/kg pentobarbital sodium, 50nM LY364947, or DMSO mixed with 200nM NMDA in a total volume of 5µl was injected into the vitreous cavity of one eye. The same volume of vehicle (5µl) was injected into the vitreous cavity of the other eye as a control. Damage to the inner retinal layers and retinal blood vessels was assessed at 2 and 7 days after the injection. In some animals, the effects of NMDA or NMDA+LY364947 on the pericytes were examined because morphological change of pericytes is a measure of abnormality of blood vessels.

Dosage form 50nM; intravitreal injection; 7 days

Applications LY364947 almost completely prevented capillary degeneration and retinal vascular damage induced by NMDA.

### References:

- [1] Oka M, Iwata C, Suzuki HI, et al. Inhibition of endogenous TGF-beta signaling enhances lymphangiogenesis. *Blood*. 2008;111(9):4571-4579.
- [2] Ueda K, Nakahara T, Mori A, Sakamoto K, Ishii K. Protective effects of TGF-? inhibitors in a rat model of NMDA-induced retinal degeneration. *Eur J Pharmacol*. 2013;699(1-3):188-193.

### Background

LY364947 is a potent ATP-competitive inhibitor of TGFβR-I with IC<sub>50</sub> of 59nM<sup>[1]</sup>. TGFβRI is a serine/threonine kinase type I receptor of the TGF-β pathway that, upon activation, phosphorylates Smad2/3 to mediate fibrosis, immune suppression, and tumor progression<sup>[2]</sup>. LY364947 is widely used in research of TGF-β-related fibrosis, cancer, and immune-modulation by blocking the TGF-

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

## Product Data Sheet

---

$\beta$ /Smad signaling cascade<sup>[3][4]</sup>.

In vitro, treatment of human dermal lymphatic microvascular endothelial cells (HDLECs) with LY364947 (3 $\mu$ M; 24h) significantly induces the expression of Prox1 and LYVE-1 and inhibits TGF- $\beta$ 1-induced Smad2 phosphorylation, while promoting cord formation and cell migration<sup>[5]</sup>. Treatment of CML leukemia-initiating cells (LICs) with LY364947 (10 $\mu$ M; 2h) increased the nuclear export of Foxo3a, decreased the phosphorylation level of Smad2/3, and impaired cell colony forming ability<sup>[6]</sup>.

In vivo, LY364947 (5mg/kg; i.p.; 4 days) enhanced liver regeneration, increased cell proliferation (measured by PCNA, phosphorylated histone 3, p21) levels, recovered of CCl<sub>4</sub>-metabolizing enzyme CYP2E1 expression in hepatocytes, and improved liver function in a mouse model of acute liver injury induced by carbon tetrachloride (CCL<sub>4</sub>)<sup>[3]</sup>. LY364947 (50nM; intravitreal injection; 7 days) almost completely prevented capillary degeneration and retinal vascular damage and slightly attenuated the reduction in number of cells in the ganglion cell layer in a rat model of retinal degeneration induced by N-methyl-D-Aspartate (NMDA)<sup>[7]</sup>.

### References:

- [1] Peng SB, Yan L, Xia X, et al. Kinetic characterization of novel pyrazole TGF-beta receptor I kinase inhibitors and their blockade of the epithelial-mesenchymal transition. *Biochemistry*. 2005;44(7):2293-2304.
- [2] Hu X, Zuckerman KS. Transforming growth factor: signal transduction pathways, cell cycle mediation, and effects on hematopoiesis. *J Hematother Stem Cell Res*. 2001;10(1):67-74.
- [3] Tschernia NP, Gulley JL. Tumor in the Crossfire: Inhibiting TGF- $\beta$  to Enhance Cancer Immunotherapy. *BioDrugs*. 2022;36(2):153-180.
- [4] Karkampouna S, Goumans MJ, Ten Dijke P, Dooley S, Kruithof-de Julio M. Inhibition of TGF $\beta$  type I receptor activity facilitates liver regeneration upon acute CCl<sub>4</sub> intoxication in mice. *Arch Toxicol*. 2016;90(2):347-357.
- [5] Oka M, Iwata C, Suzuki HI, et al. Inhibition of endogenous TGF-beta signaling enhances lymphangiogenesis. *Blood*. 2008;111(9):4571-4579.
- [6] Naka K, Hoshii T, Muraguchi T, et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature*. 2010;463(7281):676-680.
- [7] Ueda K, Nakahara T, Mori A, Sakamoto K, Ishii K. Protective effects of TGF- $\beta$  inhibitors in a rat model of NMDA-induced retinal degeneration. *Eur J Pharmacol*. 2013;699(1-3):188-193.

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

## Product Data Sheet

---

***Caution: Product has not been fully validated for medical applications. For research use only.***

***Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: [tech@glpbio.com](mailto:tech@glpbio.com)***

***Address: 10292 Central Ave. #205, Montclair, CA, USA***