GSK484 hydrochloride

Cat. No.: HY-100514

CAS No.: 1652591-81-5

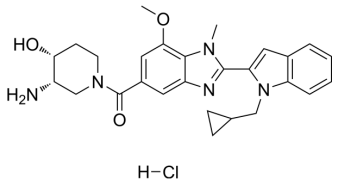
Molecular Formula: $C_{27}H_{32}ClN_5O_3$

Molecular Weight: 510.03

Target: Protein Arginine Deiminase

Pathway: Epigenetics

Storage: 4°C, stored under nitrogen, away from moisture
* In solvent : -80°C, 1 year; -20°C, 6 months (stored under nitrogen, away from moisture)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 125 mg/mL (245.08 mM; Need ultrasonic)
H_2O : 50 mg/mL (98.03 mM; Need ultrasonic)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9607 mL	9.8033 mL	19.6067 mL
	5 mM	0.3921 mL	1.9607 mL	3.9213 mL
	10 mM	0.1961 mL	0.9803 mL	1.9607 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: Saline
   Solubility: 100 mg/mL (196.07 mM); Clear solution; Need ultrasonic
2. Add each solvent one by one: PBS
   Solubility: 50 mg/mL (98.03 mM); Clear solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (4.08 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (4.08 mM); Clear solution
5. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (4.08 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

GSK484 hydrochloride is a selective and reversible peptidylarginine deiminase 4 (PAD4) inhibitor. GSK484 hydrochloride demonstrates high affinity binding to PAD4 with IC_{50}s of 50 nM in the absence of Calcium. In the presence of 2 mM Calcium, notably lower potency (250 nM) is observed.

IC_{50} & Target	IC50: 50 nM (PAD4, in the absence of Calcium), 250 nM (PAD4, in the presence of 2 mM Calcium)[1]
In Vitro	GSK484 demonstrates high affinity binding to the low-calcium form of PAD4 with IC_{50}s of 50 nM and 250 nM in the absence of Calcium (0 mM) and Calcium (2 mM), respectively. GSK484 also inhibits PAD4 citrullination (at 0.2 mM Calcium) of benzoyl-arginine ethyl ester (BAEE) substrate in a concentration-dependent manner, as detected using an NH_3 release assay[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	To address whether PAD4 inhibition can suppress cancer-associated kidney injury, MMTV-PyMT mice are treated with the PAD4 inhibitor GSK484 at 4 mg/kg daily for one week. This dose suppress the elevated number of neutrophils undergoing NETosis in peripheral blood in mice with cancer. In parallel, the total protein level in urine from MMTV-PyMT mice is significantly reduced compared with untreated tumor-bearing mice, further supporting an improved functional status of the kidneys after GSK484 treatment. Administration of GSK484 at a dose of 4 mg/kg daily during one week reverts signs of kidney dysfunction in tumor-bearing mice to the same extent as DNase I treatment, without any detectable signs of toxicity [2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	PAD4 is serially diluted in the presence of 10 nM GSK215 in assay buffer (100 mM HEPES, pH 8, 50 mM NaCl, 5% glycerol, 1 mM CHAPS, 1 mM DTT) at varying concentrations of calcium (0, 0.2, 2 and 10 mM). Following incubation for 50 min, apparent K_ds for each calcium concentration are determined using a single site saturation curve. For IC_{50} determination, test compounds (e.g., GSK484) are serially diluted in DMSO (1% final assay concentration) and tested at the same range of calcium concentrations in the presence of PAD4 (at the calculated K_d for each calcium condition) and 10 nM GSK215 in the same assay buffer and volume. Reactions are incubated for 50 min after which IC_{50} values are calculated using a four-parameter logistic equation[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	HEK293 cells stably expressing N-terminal FLAG-tagged PAD1, PAD2, PAD3 or PAD4 are engineered by retroviral transduction. Cells are grown in 15 cm diameter plates to subconfluency in DMEM supplemented with 10% Foetal Bovine Serum, harvested by centrifugation and washed once in PBS/2 mM EGTA. Cells are lysed in 50 mM Tris-Cl, pH 7.4, 1.5 mM $MgCl_2$, 5% glycerol, 150 mM NaCl, 25 mM NaF, 1 mM Na_3VO_4, 0.4% NP40, 1 mM DTT with protease inhibitors. Lysates are pre-incubated for 20 min at 4°C with DMSO alone (2%), 100 µM of GSK199, GSK484, GSK106 or 200 µM Cl-amidine. Citrullination reactions are performed for 30 min at 37°C in the presence of 2 mM calcium. Extracts are loaded on to gels, proteins separated by SDS-PAGE and transferred to PVDF membranes. Citrullinated proteins are then chemically modified and detected using anti-modified citrulline antibody. FLAG-PAD constructs are detected using anti-FLAG antibody[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2]	Mice[2] The study includes two transgenic mouse models, the MMTV-PyMT mouse model for mammary carcinoma (FVB/n background) and the RIP1-Tag2 mouse model for pancreatic neuroendocrine carcinoma (C57BL/6 background). Mice are treated daily by intra-peritoneal injections of the PAD4 inhibitor GSK484 (4 mg/kg). GSK484 is dissolved in 99.9% ethanol at a concentration of 25 mg/mL to generate a stock solution and further diluted 1:50 in 0.9% NaCl shortly before injection of 200 µL/mouse[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cancer Cell. 2021 Mar 8;39(3):423-437.e7.
- Immunity. 2023 Nov 18:S1074-7613(23)00483-1.

- Immunity. 2020 May 19;52(5):856-871.e8.
- Cell Mol Immunol. 2024 Mar 12.
- Nat Commun. 2023 Feb 16;14(1):872.

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REFERENCES

[1]. Lewis HD, et al. Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. Nat Chem Biol. 2015 Mar;11(3):189-91.

[2]. Cedervall J, et al. Pharmacological targeting of peptidylarginine deiminase 4 prevents cancer-associated kidney injury in mice. Oncoimmunology. 2017 Apr 20;6(8):e1320009.

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