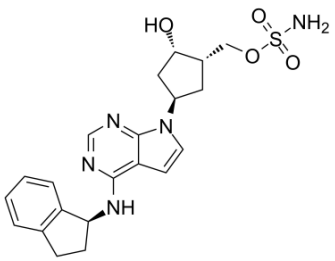
Pevonedistat

Cat. No.:	HY-70062
CAS No.:	905579-51-3
Molecular Formula:	$C_{21}H_{25}N_5O_4S$
Molecular Weight:	443.52
Target:	NEDD8-activating Enzyme
Pathway:	Metabolic Enzyme/Protease
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 50 mg/mL (112.73 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.2547 mL	11.2734 mL	22.5469 mL
	5 mM	0.4509 mL	2.2547 mL	4.5094 mL
	10 mM	0.2255 mL	1.1273 mL	2.2547 mL

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

In Vivo

1. Add each solvent one by one: **10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline**
   Solubility: ≥ 2.5 mg/mL (5.64 mM); Clear solution
2. Add each solvent one by one: **10% DMSO >> 90% (20% SBE-β-CD in saline)**
   Solubility: ≥ 2.5 mg/mL (5.64 mM); Clear solution
3. Add each solvent one by one: **10% DMSO >> 90% corn oil**
   Solubility: ≥ 2.5 mg/mL (5.64 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Pevonedistat (MLN4924) is a potent and selective **NEDD8-activating enzyme** (**NAE**) inhibitor with an **IC_{50}** of 4.7 nM[1].

IC_{50} & Target

IC50: 4.7 nM (NAE)[1]

In Vitro	Pevonedistat (MLN4924) is a potent inhibitor of NAE, and is selective relative to the closely related enzymes UAE, SAE, UBA6 and ATG7 (IC_{50}=1.5, 8.2, 1.8 and >10 μM, respectively) when evaluated in purified enzyme assays that monitor the formation of E2-UBL thioester reaction products. Pevonedistat (MLN4924) selectively inhibits NAE activity compared to the closely related ubiquitin-activating enzyme (UAE, also known as UBA1) and SUMO-activating enzyme (SAE; a heterodimer of SAE1 and UBA2 subunits), in purified enzyme and cellular assays. MLN4924 exhibits potent cytotoxic activity against a variety of human tumour-derived cell lines[1].
In Vivo	Pevonedistat (MLN4924) (sc, 10 mg/kg, 30 mg/kg, or 60 mg/kg) inhibits the NEDD8 pathway resulting in DNA damage in Mice bearing HCT-116 xenografts[1].Pevonedistat (sc, 120 mg/kg) and TNF-α (10 μg/kg) synergistically cause liver damage in SD rats[2].

PROTOCOL

Cell Assay [1]	HCT-116 cells grown in 6-well cell-culture dishes are treated with 0.1% DMSO (control) or 0.3 μM Pevonedistat (MLN4924) for 24 h. Whole cell extracts are prepared and analysed by immunoblotting. For analysis of the E2-UBL thioester levels, lysates are fractionated by non-reducing SDS-PAGE and immunoblotted with polyclonal antibodies to Ubc12, Ubc9 and Ubc10. For analysis of other proteins, lysates are fractionated by reducing SDS-PAGE and probed with primary antibodies as follows: mouse monoclonal antibodies to CDT1, p27, geminin, ubiquitin, securin/PTTG and p53 or rabbit polyclonal antibodies to NRF2, Cyclin B1 and GADD34[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1][2]	Mice[1] Mice bearing HCT-116 tumours of 300-500 mm^3 are administered a single Pevonedistat (MLN4924) dose (of 10, 30 or 60 mg/kg), and tumors are excised at various time-points over the subsequent 24 h period. The relative levels of NEDD8-cullin and NRF2 are estimated by quantitative immunoblot analysis using Alexa680-labelled anti-IgG as the secondary antibody. The statistical difference between the groups for NEDD8-cullin inhibition is determined using the Kruskal-Wallis test. For the analysis of CDT1 and phosphorylated CHK1 (Ser317) levels in tumour sections, formalin-fixed, paraffin-embedded tumour sections are stained with the relevant antibodies, amplified with HRP-labelled secondary antibodies and detected with the ChromoMap DAB Kit. Slides are counterstained with haematoxylin. Images are captured using an Eclipse E800 microscope and Retiga EXi colour digital camera and processed using Metamorph software. CDT1 and phosphorylated CHK1 levels are expressed as a function of the DAB signal area. Rats[2] Ten-week-old male Sprague-Dawley rats are used. Across two studies, a total of eight animals in each group are dosed with vehicle, TNF-α, Pevonedistat (MLN4924), or Pevonedistat (MLN4924)+TNF-α. Animals are first intravenously administered either vehicle (1×PBS) or 10 μg/kg TNF-α. One hour later, they are subcutaneously administered vehicle (20% sulfobutyl ether beta-cyclodextrin in 50 mM citrate buffer, pH 3.3) or 120 mg/kg Pevonedistat (MLN4924). Scheduled euthanasia occurred 24 h postdose. Unscheduled euthanasia is performed when animals exhibited moribund conditions. Serum is collected at necropsy and analyzed by Idexx Laboratories for serum chemistry markers of liver damage. Additionally, the livers from five animals in each group are removed, separated into two sections and either frozen at -80°C for subsequent protein analysis or fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4-6 μm, mounted on glass slides, stained with hematoxylin and eosin, and analyzed with an Olympus BX51 light microscope for histopathology assessment. Microscopic findings are recorded in concordance with the standardized nomenclature for classifying lesions within the livers of rats. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- **Cell**. 2019 Jul 11;178(2):330-345.e22.
- **Cancer Cell**. 2020 Mar 16;37(3):371-386.e12.
- **Nat Microbiol**. 2019 May;4(5):813-825.
- **Nat Plants**. 2019 Jan;5(1):34-40.
- **Nat Commun**. 2018 Sep 18;9(1):3801.

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REFERENCES

[1]. Soucy TA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009 Apr 9;458(7239):732-6.

[2]. F S Wolenski, et al. The NAE inhibitor pevonedistat (MLN4924) synergizes with TNF-α to activate apoptosis. Cell Death Discovery 1, Article number: 15034 (2015)

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