Entinostat

Cat. No.: HY-12163

CAS No.: 209783-80-2

Molecular Formula: $C_{21}H_{20}N_4O_3$

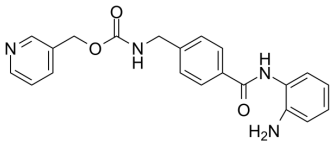
Molecular Weight: 376.41

Target: HDAC; Autophagy; Apoptosis

Pathway: Cell Cycle/DNA Damage; Epigenetics; Autophagy; Apoptosis

Storage:

Powder	-20°C	3 years
In solvent	-80°C	1 year
	-20°C	6 months



SOLVENT & SOLUBILITY

In Vitro

DMSO : 50 mg/mL (132.83 mM; ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	2.6567 mL	13.2834 mL	26.5668 mL
	5 mM	0.5313 mL	2.6567 mL	5.3134 mL
	10 mM	0.2657 mL	1.3283 mL	2.6567 mL

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

In Vivo

1. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: ≥ 2.5 mg/mL (6.64 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (5.53 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (5.53 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (5.53 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Entinostat is an oral and selective class I HDAC inhibitor, with IC_{50}s of 243 nM, 453 nM, and 248 nM for HDAC1, HDAC2, and HDAC3, respectively.

IC_{50} & Target			
	HDAC1	HDAC3	HDAC2
	243 nM (IC_{50})	248 nM (IC_{50})	453 nM (IC_{50})

In Vitro	Binding affinity of Entinostat (MS-275) against HDAC1 and HDAC2 is 282 nM and 156 nM, respectively[1]. Effects of the HDAC inhibitor Entinostat (MS-275) have been examined in human leukemia and lymphoma cells (U937, HL-60, K562, and Jurkat) as well as in primary acute myelogenous leukemia blasts in relation to differentiation and apoptosis. MS-275 displays dose-dependent effects in each of the cell lines. When administered at a low concentration (e.g., 1 μM), MS-275 exhibits potent antiproliferative activity, inducing p21CIP1/WAF1-mediated growth arrest and expression of differentiation markers (CD11b) in U937 cells. Entinostat (MS-275) potently induces cell death, triggering apoptosis in ~70% of cells at 48 h[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Entinostat (MS-27-275) at 49 mg/kg shows marked antitumor effects against KB-3-1, 4-1St, and St-4 tumor lines, and a moderate effect against Capan-1 tumor. Entinostat at 24.5 mg/kg and 12.3 mg/kg also shows significant effects against these tumors. In addition, oral administration of Entinostat apparently increases the level of histone acetylation in HT-29 tumor xenografts 4-24 h after the administration[3]. MS-275 administration (3.5 mg/kg i.p.) to Experimental autoimmune neuritis (EAN) rats once daily from the appearance of first neurological signs greatly reduces the severity and duration of EAN and attenuated local accumulation of macrophages, T cells and B cells, anddemyelination of sciatic nerves. In addition, MS-275 treatment increases proportion of infiltrated Foxp3$^+$ cells and anti-inflammatory M2 macrophages in sciatic nerves of EAN rats[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Biochemical assays of HDAC activity are carried out by Nanosyn in a reaction volume of 10 μL in 384-well microplates. A standard enzymatic reaction contains 5 μL of 2× HDAC inhibitor (e.g., Entinostat), 4 μL of 2.5× enzyme, and 1 μL of 10× substrate in assay buffer (100 mM HEPES, pH 7.5, 25 mM KCl, 0.1% BSA, 0.01% Triton X-100, 1% DMSO). Final concentration of all HDACs in the enzymatic assays is between 0.5 and 5 nM. A final substrate concentration of 1 μM FAM-RHKK(Ac)-NH_2 or FAM-RHKK(trifluoroacetyl)-NH_2 is used in all assays and found to be below the determined $K_{m,app}$ for each enzyme[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	SH-SY5Y cells are maintained under normal culture conditions in a humidified incubator at 37°C with 5% CO_2 and are split twice weekly. Cells are plated in black 384-well plates at 2500 cells/well in 20-μL volume of DMEM/F-12 culture media supplemented with 10% FBS and permitted to adhere overnight. The following day, HDAC inhibitors (e.g., Entinostat) are serially diluted in 100% DMSO, and this series is subsequently cross-diluted into culture media. 5 μL of compound (e.g., Entinostat) diluted in media is added to the appropriate well of the cell plate to afford the indicated final concentration of inhibitor (e.g., Entinostat) with a final 0.1% DMSO. Treated cells are incubated under normal tissue culture conditions for 6, 24, 48, 72, or 96 h prior to quantitation of cellular ATP levels as measured using CellTiter-Glo reagents. Similarly, after 6 h of incubation with HDAC inhibitors (e.g., Entinostat), media from separate cell plates are aspirated, and cells are washed once with media containing no inhibitors. 25 μL of media supplemented with 10% FBS and 0.1% DMSO (no inhibitors) is added back to the cells, and cellular ATP levels are determined using CellTiter-Glo after 24, 48, 72, or 96 h of incubation. Luminescence is measured at each time point using an Envision Instrument with a 0.1 s count time[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [3][4]	Mice[3] A2780 cells (9×10^6) are suspended in PBS and are injected subcutaneously into the flank of nude mouse. For the other tumor lines, KB-3-1, HCT-15, 4-1St, Calu-3, St-4, Capan-1, and HT-29, tumors are passaged several times before starting in vivo antitumor testing, and a tumor lump (2-3 mm in diameter) is transplanted subcutaneously into the flank of a nude mouse by using a trocar needle. Treatment (four or five mice in each experimental group) with the drugs is started after the tumors are confirmed to have grown in the body (tumor size, 20-100 mm^3). Entinostat is administered orally once daily 5 days per week for 4 weeks. Tumor length and width are monitored twice weekly, and tumor volume is calculated. Rats[4] Male Lewis rats (8-10 weeks, 170-200 g) are housed under a 12-h light/dark cycle with free access to food and water. For therapeutic treatment, EAN rats receive i.p. injection of MS-275 (3.5 mg/kg) daily from day 10 to day 14 (six rats/group). For injection, MS-275 is suspended in phosphate buffered saline (PBS) and the same volume (1 mL) of PBS is given to control rats.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell. 2019 Mar 7;176(6):1447-1460.e14.
- Cell Metab. 2022 Feb 7;34(3):424-440.e7.
- Mol Cell. 2023 Nov 20:S1097-2765(23)00914-0.
- Clin Cancer Res. 2023 Sep 19.
- Clin Cancer Res. 2020 Apr 15;26(8):2011-2021.

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REFERENCES

[1]. Lauffer BE, et al. Histone deacetylase (HDAC) inhibitor kinetic rate constants correlate with cellular histone acetylation but not transcription and cell viability. J Biol Chem. 2013 Sep 13;288(37):26926-43.

[2]. Rosato RR, et al. The histone deacetylase inhibitor MS-275 promotes differentiation or apoptosis in human leukemia cells through a process regulated by generation of reactive oxygen species and induction of p21CIP1/WAF1 1. Cancer Res. 2003 Jul 1;63(13):36

[3]. Saito A, et al. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. Proc Natl Acad Sci U S A, 1999, 96(8), 4592-4597.

[4]. Zhang ZY, et al. MS-275, an histone deacetylase inhibitor, reduces the inflammatory reaction in rat experimental autoimmune neuritis. Neurosci, 2010, 169, 370-377.

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