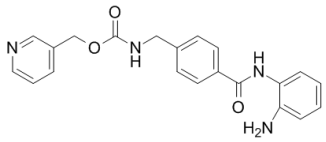
Data Sheet

Product Name:	Entinostat
Cat. No.:	CS-0511
CAS No.:	209783-80-2
Molecular Formula:	$C_{21}H_{20}N_4O_3$
Molecular Weight:	376.41
Target:	Autophagy; HDAC
Pathway:	Autophagy; Cell Cycle/DNA Damage; Epigenetics
Solubility:	DMSO : ≥ 300 mg/mL (797.00 mM)



BIOLOGICAL ACTIVITY:

Entinostat is an oral and selective class I **HDAC** inhibitor, with **IC50**s of 243 nM, 453 nM, and 248 nM for **HDAC1**, **HDAC2**, and **HDAC3**, r espectively.
IC50 & Target: IC50: 243 nM (HDAC1), 453 nM (HDAC2), 248 nM (HDAC3)[1]
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].
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].

PROTOCOL (Extracted from published papers and Only for reference)

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]. **Cell Assay:** Entinostat (MS-275) is dissolved in DMSO and stored, and then diluted with appropriate media before use[1].[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]. **Animal Administration:** Entinostat (MS-27-275) is dissolved in 0.05 N HCl, 0.1% Tween 80 (Mice)[3].;Entinostat (MS-275) is suspended in PBS (rats)[4].[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.

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.

Tel: 732-484-9848 Fax: 888-484-5008 E-mail: sales@ChemScene.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA