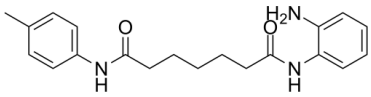
Data Sheet

Product Name:	Pimelic Diphenylamide 106
Cat. No.:	CS-5229
CAS No.:	937039-45-7
Molecular Formula:	$C_{20}H_{25}N_3O_2$
Molecular Weight:	339.43
Target:	HDAC
Pathway:	Cell Cycle/DNA Damage; Epigenetics
Solubility:	DMSO : 125 mg/mL (368.26 mM; ultrasonic and warming and heat to 60°C)



BIOLOGICAL ACTIVITY:

Pimelic Diphenylamide 106 is a slow, tight-binding inhibitor of class I HDAC (HDAC 1, 2, and 3, with IC50 values of 150 nM , 760nM, and 370 nM, respectively), demonstrating no activity against class II HDACs. IC50 value: 150 nM (HDAC1), 370 nM (HDAC3), 760nM(HDAC2) Target: HDAC in vitro: Pimelic Diphenylamide 106 has preference toward HDAC3 with Ki of 14 nM, 15 times lower than the Ki for HDAC1. Pimelic Diphenylamide 106 exhibits weaker inhibitory activities against HDAC 8 with IC50 of 5 μM after a 3-h preincubation with HDAC8.

PROTOCOL (Extracted from published papers and Only for reference)

Enzyme assay [2] Recombinant human HDAC1, HDAC 2, HDAC3/NcoR2, and HDAC8 were expressed in baculovirus. The class II HDACs 4 and 5 were tagged with the Flag epitope from their respective cDNAs, expressed in HEK293t cells, and purified on Flag-M2 affinity resin. The trifunctional probes, Pimelic diphenylamide 106 and other HDAC inhibitors were assayed with the BioMol AK500 kit to determine IC50 values with recombinant HDACs or with a HeLa nuclear extract. Since our previous studies indicated a slow on-rate for 106, these IC50 measurements include a prolonged incubation time (1 h) to insure that the inhibitor-enzyme complex came to equilibrium before initiation of the enzyme assay. Samples were processed as described by BioMol and read with a 96-well fluorescence plate reader. For class I HDACs, the synthetic substrate acetyl-Lys(Ac)-AMC was used and deacetylated lysine-AMC was released by trypsin treatment and free flourogenic 4-methylcoumarin-7-amide (MCA) was generated. The flourogenic MCA could then be read with an excitation wavelenghth of 370 nm and emission wavelength of 460 nm. Assays for class II HDACs were done using acetyl-Lys(trifluoroacetyl)-AMC under the same conditions. A semilogarithmic plot of the data was analyzed with Kaleidagraph software to obtain the IC50 value. Ki values were determined from enzyme progression curves, performed at various inhibitor concentrations. Cell assay [1] A lymphoblastoid cell line derived from a Friedreich ataxia patient (GM15850) were cultured in RPMI 1640 media with 10% fetal bovine serum and 10 mM HEPES, at 37°C in 5% CO2. After the 5th split, the cells were treated with inhibitor Pimelic diphenylamide 106 (2 μM) or SAHA (2 μM) for 24 h, in culture medium. The treatment concentrations were determined based on growth inhibition through an MTS cell proliferation assay. These concentrations correspond approximately to the EC10 for Pimelic diphenylamide 106, and 2 μM SAHA is near its EC50 for blocking cell proliferation. 24 h after treatment, the cells were washed twice with Hanks' balanced salts buffer to remove the inhibitor. A portion of the cell population was then harvested immediately after washing, and referred to as a time 0 point (24 h treatment point), and the rest of the cells were then re-cultured in cell culture media without added inhibitor. The re-cultured cells and controls were then harvested every hour for a total of 7 h. The cells were washed twice before lysis with a low salt lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 0.5% Triton X-100, and 1× protease inhibitors) for 30 min, and followed by a 15-s sonication pulse at 3 watts. The cell lysates were then denatured with LDS loading buffer and run on 4-12% SDS gradient polyacrylamide gels.

References:

[1]. Chou CJ, et al. Pimelic diphenylamide 106 is a slow, tight-binding inhibitor of class I histone deacetylases. J Biol Chem. 2008 Dec 19;283(51):35402-35409.

[2]. Xu C, et al. Chemical probes identify a role for histone deacetylase 3 in Friedreich's ataxia gene silencing. Chem Biol. 2009 Sep 25;16(9):980-989.

CAIndexNames:

Heptanediamide, N1-(2-aminophenyl)-N7-(4-methylphenyl)-

SMILES:

O=C(NC1=CC=CC=C1N)CCCCCC(NC2=CC=C(C)C=C2)=O

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