Product Data Sheet

Product Name: Givinostat hydrochloride (ITF-2357 hydrochloride)
Cat. No.: GC33159

Chemical Properties

Cas. No.	199657-29-9		
SMILES	O=C(OCC1=CC=C2C=C(CN(CC)CC)C=CC2=C1)NC3=CC=C(C(NO)=O)C=C3.[H]Cl		
Formula	$C_{24}H_{28}ClN_3O_4$	M.Wt	457.95
Solubility	Soluble in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

Protocol

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Kinase experiment:

Recombinant human HDAC enzymes (HDAC1- HDAC11) are used. Activity of HDAC1, HDAC2, HDAC3, HDAC6, HDAC10 and HDAC11 is assayed using the Fluor de Lys deacetylase substrate. HDAC8 activity is assayed using Fluor de Lys Green deacetylase substrate. N-Trifluoroacetyl-L-lysine is used to assay activity of HDAC4, HDAC5, HDAC7 and HDAC9. Recombinant enzymes are preincubated with Givinostat (ITF2357) or ITF3056 at 30°C in a volume of 25 μL in wells of a microtiter plate. After a brief incubation, 25 μL of substrate is added, and the fluorescent signal is generated by the addition of 50 μL of developer containing 2 μM Trichostatin A. For each assay, the amount of enzyme, incubation times, assay buffer, and concentration of the substrates are optimized. Positive control for enzyme activity consisted of enzyme plus substrate without Givinostat or ITF3056. The fluorescence signal is detected using a Victor multilabel plate reader[1].

Cell experiment:

After the JS-1 cell line is cultured in DMEM with 10% fetal bovine serum for 24 h, 30 wells of JS-1 cells are divided into two groups. In the first group, the culture medium is replaced by complete medium with final Givinostat concentrations of 0 nM, 125 nM, 250 nM, 500 nM, and 1000 nM. In the second group, Givinostat (ITF-2357) of relevant concentrations is added concomitantly with 100 nM of LPS solution. Three replicates are performed for each group. After inoculation at 37°C and 5% CO2 for 24 h, each well (100 μL) is incubated with 10 μL of CCK-8 solution. The plates are incubated at 37 °C for 1 h and the absorbance is measured at 450 nm using a microplate reader[2].

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Animal experiment:

Mice[1] C57BL/6 mice are housed in the animal facility for at least 5 days before use. For the comparison study, Givinostat (ITF2357) at 10 mg/kg is administered orally, and Givinostat (ITF-2357) is injected intraperitoneally. One hour after administration of the compounds, the animals are treated intraperitoneally with LPS from Salmonella typhimurium at a dose of 2.5 mg/kg. 90 min after the LPS treatment, mice are sacrificed, and sera are collected and stored at -80°C until further analysis of cytokine productions.

References:

[1]. Li S, et al. Specific inhibition of histone deacetylase 8 reduces gene expression and production of proinflammatory cytokines in vitro and in vivo. J Biol Chem. 2015 Jan 23;290(4):2368-78.
[2]. Leoni F, et al. The histone deacetylase inhibitor ITF2357 reduces production of pro-inflammatory

cytokines in vitro and systemic inflammation in vivo. Mol Med. 2005 Jan-Dec;11(1-12):1-15.
[3]. Wang YG, et al. Givinostat inhibition of hepatic stellate cell proliferation and protein acetylation. World J Gastroenterol. 2015 Jul 21;21(27):8326-39.

Background

ITF 2357 inhibits class I and class II histone deacetylases (maize HDACs: HD2, HD-1B, and HD-1A with IC_{50}s = 7.5-16 nM) and reduces the production of several pro-inflammatory cytokines including TNFα, IL-1α, and IL-1β (IC_{50}s = 10-22 nM).[1] ITF 2357 also has activity against cells expressing janus kinase 2 (JAK2)^{V617F} (IC_{50}s = 1-10 nM), a mutated form of the JAK2 enzyme that is implicated in the pathophysiology of many myeloproliferative diseases, including polycythaemia vera.[2]

1.Leoni, F., Fossati, G., Lewis, E.C., et al.The histone deacetylase inhibitor ITF2357 reduces production of pro-inflammatory cytokines in vitro and systemic inflammation in vivoMol. Med.11(1-12)1-15(2005) 2.Guerini, V., Barbui, V., Spinelli, O., et al.The histone deacetylase inhibitor ITF2357 selectively targets cells bearing mutated

Product Data Sheet

JAK2V617FLeukemia22(4)740-747(2008)

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA