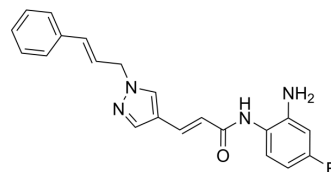


RGFP966

Cat. No.:	HY-13909		
CAS No.:	1357389-11-7		
Molecular Formula:	C ₂₁ H ₁₉ FN ₄ O		
Molecular Weight:	362.4		
Target:	HDAC		
Pathway:	Cell Cycle/DNA Damage; Epigenetics		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMF : 50 mg/mL (137.97 mM; Need ultrasonic)
DMSO : 50 mg/mL (137.97 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	2.7594 mL	13.7969 mL	27.5938 mL
	5 mM	0.5519 mL	2.7594 mL	5.5188 mL
	10 mM	0.2759 mL	1.3797 mL	2.7594 mL

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

In Vivo

- Add each solvent one by one: 50% PEG300 >> 50% saline
Solubility: 7.69 mg/mL (21.22 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
Solubility: ≥ 2.5 mg/mL (6.90 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil
Solubility: ≥ 2.5 mg/mL (6.90 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

RGFP966 is a highly selective HDAC3 inhibitor with an IC₅₀ of 80 nM and shows no inhibition to other HDACs at concentrations up to 15 μM. RGFP966 can penetrate the blood brain barrier (BBB).

IC₅₀ & Target

HDAC3
80 nM (IC₅₀)

In Vitro	<p>RGFP966 potently and selectively inhibits HDAC 3 with IC_{50} of 0.21 μM in RAW 264.7 macrophages, while HDACs 1 (IC_{50}=5.6 μM), 2 (9.7 μM) and 8 (>100 μM), indicating a good level of selectivity for HDAC 3. The mRNA levels of HDACs 1, 2 and 3 are not significantly affected by RGFP966 in RAW 264.7 macrophages, whereas the HDAC 1 and HDAC 2 protein levels are slightly, though significantly, reduced upon RGFP966 treatment. Moreover, RGFP966 significantly reduced the transcriptional activity of NF-κB p65, whereas NF-κB p65 acetylation and localization remain unaltered^[2].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
In Vivo	<p>RGFP966 (10 and 25 mg/kg) treatment significantly improves body weight, rotarod performance and several measures of motor function in the open field locomotor test^[3]. RGFP966 at a 10 mg/kg dose penetrates the blood-brain barrier into rat auditory cortex with typical pharmacokinetics, which together establish feasibility for the modulation of A1 plasticity due to action in the auditory cortex^[4].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

PROTOCOL

Kinase Assay ^[2]	<p>The respective human recombinant HDAC enzymes are incubated in absence and/or in presence of various concentrations RGFP966 and a pro-fluorogenic substrate at room temperature for 60 min. Next, the deacetylation reaction is stopped by the addition of the HDAC Stop Solution (6 mg/mL trypsin, 0.3 mM SAHA) in all wells and the plate is incubated at 37°C for 20 min. The release of the fluorescent 7-amino-4-methylcoumarin is monitored by measuring the fluorescence at λ_{em}=460 nm and λ_{ex}=390 nm using a Synergy H1 plate reader. The fluorescence value of the background wells is subtracted from the fluorescence of the positive control, blank and inhibitor wells. Nonlinear regression is used to fit the data to the log(inhibitor) vs. response curve using GraphPad Prism^[2].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Cell Assay ^[2]	<p>To investigate the influence of the HDAC 3-selective inhibitor RGFP966 on cell viability, RAW 264.7 macrophages, HBE cells and hASM cells are seeded in 96-well plates. To obtain identical cell density at the start of the experiments, RAW 264.7 macrophages are seeded at 25,000 cells/cm², HBE cells and hASM cells are seeded at 70% confluency (based on surface area) and are serum-starved for 24 h prior incubation with RGFP966. Shortly before incubation with RGFP966, the medium is replaced by 100 μL fresh (if appropriate serum free) culture medium. Incubations with LPS and IFNγ are performed as described for HDAC 1-3 downregulation by siRNA. After 20 h of incubation with RGFP966, 20 μL of CellTiter 96 AQ_{ueous} One Solution reagent is added to each well and incubated at 37°C for 1 h in the dark. The absorbance at 490 nm is measured using a Synergy H1 plate reader. LPS/IFNγ-stimulated cells without addition of RGFP966 are considered 100%^[2].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Animal Administration ^{[3][4]}	<p>Mice^[3]</p> <p>N171-82Q transgenic mice are housed and maintained on a normal 12-h light/dark cycle with lights on at 6:00 a.m and free access to food and water. Mice are administered RGFP966 (10 or 25 mg/kg) for 10 weeks by S.C. injection (3 injections/week) beginning at 8 weeks of age. RGFP966 is dissolved with 75% polyethylene glycol 200/25% sodium acetate (6.25 mM); control mice received an equal volume of drug vehicle. Body weights are recorded twice per week. Mice are sacrificed at 18 weeks of age, 6 h after the final injection by overdose with isofluorane anesthesia. Brains are removed, and striata and cortex dissected out for gene expression assays or intracardially perfused with 4% paraformaldehyde.</p> <p>Rats^[4]</p> <p>A total of thirty-three adult male Sprague Dawley rats (275-350 g) are used. Immediately following the daily training session, a posttraining systemic injection of either RGFP966 (10 mg/kg, s.c.) or vehicle (at a comparable volume to drug treatment) is delivered to each subject.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

CUSTOMER VALIDATION

- Cell Metab. 2021 Nov 20;S1550-4131(21)00532-5.
- Mol Cell. 2020 Oct 1;80(1):43-58.e7.
- Nucleic Acids Res. 2023 Mar 27;gkad212.
- Cell Death Differ. 2023 Jun 15.
- Theranostics. 2021 Mar 20;11(11):5605-5619.

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- [1]. Malvaez M, et al. HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. Proc Natl Acad Sci U S A. 2013 Feb 12;110(7):2647-52.
- [2]. Leus NG, et al. HDAC 3-selective inhibitor RGFP966 demonstrates anti-inflammatory properties in RAW 264.7 macrophages and mouse precision-cut lung slices by attenuating NF- κ B p65 transcriptional activity. Biochem Pharmacol. 2016 May 15;108:58-74.
- [3]. Jia H, et al. The Effects of Pharmacological Inhibition of Histone Deacetylase 3 (HDAC3) in Huntington's Disease Mice. PLoS One. 2016 Mar 31;11(3):e0152498.
- [4]. Bieszcza KM, et al. Histone Deacetylase Inhibition via RGFP966 Releases the Brakes on Sensory Cortical Plasticity and the Specificity of Memory Formation. J Neurosci. 2015 Sep 23;35(38):13124-32.

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