上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：RGFP966
产品别名：RGFP966

生物活性：

Description	RGFP966 is a highly selective HDAC3 inhibitor with an IC_{50} of 80 nM and shows no inhibition to other HDACs at concentrations up to 15 μM.
IC_{50} & Target	HDAC3
	80 nM (IC_{50})
In Vitro	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].
In Vivo	RGFP966 (10 and 25 mg/kg) treatment significantly improves body weight, rotarod performance and several measures of motor function in the open field locomoter 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].

Solvent&Solubility

In Vitro:

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

H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	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

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (6.90 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.90 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

	2.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (6.90 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.90 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[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-κB 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]. Bieszczad 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.

实验参考：

Cell Assay	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/cm^2, 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].
Animal Administration	Mice[3] 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. Rats[4] 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 RGPF966 (10 mg/kg, s.c.) or vehicle (at a comparable volume to drug treatment) is delivered to each subject.
	The respective human recombinant HDAC enzymes are incubated in absence and/or in presence of

Kinase Assay	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].
References	[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-κB 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]. Bieszczad 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.