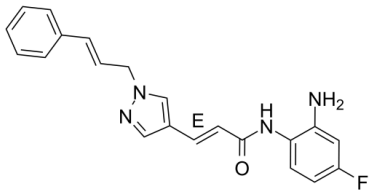
Data Sheet

Product Name:	RGFP966
Cat. No.:	CS-1628
CAS No.:	1357389-11-7
Molecular Formula:	C21H19FN4O
Molecular Weight:	362.40
Target:	HDAC
Pathway:	Cell Cycle/DNA Damage; Epigenetics
Solubility:	H2O : < 0.1 mg/mL (insoluble); DMSO : 50 mg/mL (137.97 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

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. IC50 & Target: IC50: 80 nM (HDAC3)[1] **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].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [2]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]. **Cell Assay:** RGFP966 is dissolved in DMSO and stored, and then diluted with appropriate medium before use[2].[2] 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:** RGFP966 is dissolved with 75% polyethylene glycol 200/25% sodium acetate (Mice)[3].[3][4]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.

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.

CAIndexNames:

2-Propenamide, N-(2-amino-4-fluorophenyl)-3-[1-(3-phenyl-2-propen-1-yl)-1H-pyrazol-4-yl]-, (2E)-

SMILES:

O=C(NC1=CC=C(F)C=C1N)/C=C/C2=CN(C/C=C/C3=CC=CC=C3)N=C2.[E]

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