Roflumilast

Cat. No.: HY-15455

CAS No.: 162401-32-3

Molecular Formula: $C_{17}H_{14}Cl_2F_2N_2O_3$

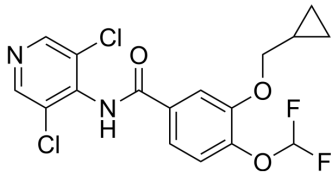
Molecular Weight: 403.21

Target: Phosphodiesterase (PDE); RSV

Pathway: Metabolic Enzyme/Protease; Anti-infection

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	2 years
	-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 50 mg/mL (124.00 mM)

H_2O : < 0.1 mg/mL (ultrasonic) (insoluble)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.4801 mL	12.4005 mL	24.8010 mL
5 mM	0.4960 mL	2.4801 mL	4.9602 mL
10 mM	0.2480 mL	1.2400 mL	2.4801 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.20 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Roflumilast (APTA-2217) is a selective PDE4 inhibitor with IC_{50}s of 0.7, 0.9, 0.7, and 0.2 nM for PDE4A1, PDEA4, PDEB1, and PDEB2, respectively, without affecting PDE1, PDE2, PDE3 or PDE5 isoenzymes from various cells.

IC_{50} & Target

PDE4

In Vitro

Roflumilast does not affect PDE enzymes apart from PDE4, and is a subnanomolar inhibitor of most PDE4 splicing variants tested. It showed no PDE4 subtype selectivity apart from PDE4C (4C1, IC_{50}=3 nM; 4C2, IC_{50}=4.3 nM), which is inhibited with a slightly lower potency[2]. Roflumilast is a potent and selective PDE4 inhibitor. Roflumilast is a monoselective PDE4 inhibitor since it does not affect other PDE isoenzymes, including PDE1, PDE2, PDE3, and PDE5 up to 10,000-fold higher concentrations. Roflumilast inhibits human neutrophil functions. Roflumilast inhibits TNFα synthesis in monocyte-derived

	dendritic cells. Rolfumilast inhibits proliferation and cytokine synthesis in $CD4^+$ T cells. Proliferation is inhibited to a maximum of about 60% by Roflumilast with a potency (IC_{30}) of 7 nM[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Animal studies with Roflumilast demonstrated that it reduced the accumulation of neutrophils in bronchoalveolar lavage fluid following short-term exposure of guinea pigs, mice or rats to tobacco smoke, and following exposure of rats to a combination of tobacco smoke and bacterial lipopolysaccharide, and abolished the lung parenchymal influx of inflammatory cells seen in rats exposed to tobacco smoke for 7 months[2]. Roflumilast blocks COPD progression in $pIgR^{?/?}$ mice. For these studies, 9-month-old WT or $pIgR^{?/?}$ mice are treated daily by oral gavage with 100 μg of Roflumilast (5 μg/g) or vehicle (4% methylcellulose, 1.3% PEG400) for 3 months and lungs are harvested at 12 months of age. Unlike $pIgR^{?/?}$ mice treated with vehicle, mice treated with Roflumilast had no progression of small airway wall remodelling after starting treatment. Strikingly, 12-month-old $pIgR^{?/?}$ mice treated with Roflumilast had reduced indices of emphysema compared with 9-month-old $pIgR^{?/?}$ mice, indicating that Roflumilast not only blocks progression of emphysema in this model but apparently facilitates some resolution of the emphysematous destruction of lung parenchyma[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [3]	PDE activity is determined with some modifications. The assay mixture contain 50 mM Tris (pH 7.4), 5 mM $MgCl_2$, 0.5 μM cAMP or cGMP, and [^{3}H]cAMP or [^{3}H]cGMP (about 30,000 cpm/assay), the indicated concentration of the inhibitor and an aliquot of the enzyme solution at a final assay volume of 200 μL. Stock solutions of the compounds are diluted 1:100 (v/v) in the Tris buffer mentioned above; appropriate dilutions are prepared in 1% (v/v) DMSO/Tris buffer, which are diluted 1:2 (v/v) in the assays to obtain the desired final concentrations of the inhibitors at a DMSO concentration of 0.5% (v/v). DMSO itself affected none of the PDE activities. After preincubation for 5 min at 37°C, the reaction is started by the addition of substrate (cAMP or cGMP) and the assays are incubated for further 15 min at 37°C. Then 50 μL of 0.2 N HCl is added to stop the reaction and the assays are left on ice for about 10 min. Following incubation with 25 μg of 5′-nucleotidase (Crotalus atrox snake venom) for 10 min at 37°C, the assays are loaded on QAE Sephadex A-25 (1 mL of bed volume in Poly-Prep chromatography columns). The columns are eluted with 2 mL of 30 mM ammonium formate (pH 6.0) and the eluate is counted for radioactivity. Results are corrected for blank values (measured in the presence of denatured protein) that are below 5% of total radioactivity. The amount of cyclic nucleotides hydrolyzed did not exceed 30% of the original substrate concentration [3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [4]	Mice[4] WT or $pIgR^{-/-}$ mice are used. For studies using Roflumilast, 200 μL of 0.5 mg/mL suspension of Roflumilast or vehicle (4% methylcellulose, 1.3% PEG400 and 5 μg drug per g animal weight) is administered by oral gavage once daily, 5 days a week for the duration of treatment. Mice are treated daily by oral gavage with 100 μg of Roflumilast (5 μg/g) or vehicle (4% methylcellulose, 1.3% PEG400) for 3 months and lungs are harvested at 12 months of age. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Inflamm Res. 2020 Dec;69(12):1191-1199.
- Ceram Int. 30 September 2021.
- J Dermatol Sci. 2023 Apr 3.
- BMC Neurosci. 2023 Jul 31;24(1):39.
- Int Urol Nephrol. 2019 Feb;51(2):253-260.

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[1]. Hatzelmann A, et al. The preclinical pharmacology of roflumilast--a selective, oral phosphodiesterase 4 inhibitor in development for chronic obstructive pulmonary disease. Pulm Pharmacol Ther. 2010 Aug;23(4):235-56.

[2]. Rabe KF. Update on roflumilast, a phosphodiesterase 4 inhibitor for the treatment of chronic obstructive pulmonary disease. Br J Pharmacol. 2011 May;163(1):53-67.

[3]. Hatzelmann A, et al. Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhibitor roflumilast in vitro. J Pharmacol Exp Ther. 2001 Apr;297(1):267-79.

[4]. Richmond BW, et al. Airway bacteria drive a progressive COPD-like phenotype in mice with polymeric immunoglobulin receptor deficiency. Nat Commun. 2016 Apr 5;7:11240.

[5]. Ding H, et al. Treatment of obesity-associated overactive bladder by the phosphodiesterase type-4 inhibitor roflumilast. Int Urol Nephrol. 2017 Jul 29.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA