SGC-PI5P4Kγ/MYLK4-1: A Chemical Probe for PI5P4Kγ and MYLK4

Version 1.0 (11th October 2021)

Web link for more details: https://www.thesgc.org/chemical-probes/SGC-PI5P4Kgamma_MYLK4-1

Overview

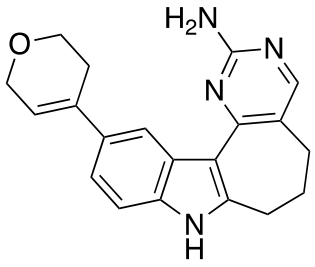
Phosphatidylinositol-5-phosphate 4-kinase gamma (PI5P4Kγ), which phosphorylates phosphatidylinositol-5-monophosphate (PI(5)P), and myosin light chain kinase family member 4 (MYLK4). PI5P4Kγ is an understudied human lipid kinase with described roles in inflammation, T cell activation, autophagy regulation, immunity, heart failure, and multiple cancers. MYLK4 is also a poorly characterized human kinase. To provide a high-quality chemical tool that would enable additional characterization of these proteins, we designed and evaluated a potent, selective, and cell-active inhibitor of human PI5P4Kγ and MYLK4. Based on our results in orthogonal assay formats reliant on competition with an ATP-competitive reagent, ATP titration studies, and published co-crystal structures with structurally related compounds, we hypothesize that our probe binds in the ATP active site of PI5P4Kγ.

Summary

Chemical Probe Name	SGC-PI5P4Kγ/MYLK4-1
Negative control compound	SGC-PI5P4Kγ/MYLK4-1N
Target(s) (synonyms)	PI5P4Kγ and MYLK4
Recommended cell assay concentration	Use at concentration of 1 µM (and ≤ 5 µM) for SGC-PI5P4Kγ/MYLK4-1 and SGC-PI5P4Kγ/MYLK4-1-1N; use with control for best interpretation of data.
Suitability for *in* vivo use and recommended dose	SGC-PI5P4Kγ/MYLK4-1 was not tested *in vivo*
Publications	10.1016/j.crchbi.2022.100036; 10.1101/2022.09.08.507203
Orthogonal chemical probes	
In vitro assay(s) used to characterise	Binding and radiometric enzymatic assays
Cellular assay(s) for target-engagement	NanoBRET

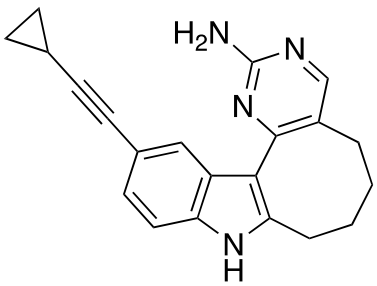
Chemical Probe & Negative Control Structures and Use

SGC-PI5P4Kγ/MYLK4-1: Chemical Probe



SMILES: NC1=NC2=C(CCCC3=C2C4=C(C=CC(C5=CCOCC5)=C4)N3)C=N1
InChiKey: VGFHPNRWHOQLEB-UHFFFAOYSA-N
Molecular weight: 332.41
Storage: Stable as a solid at room temperature. DMSO stock solutions (up to 10 mM) are stable at -20°C.
Dissolution: Soluble in DMSO up to 10 mM

SGC-PI5P4Kγ/MYLK4-1N: Negative Control



SMILES: NC1=NC2=C(CCCCC3=C2C4=C(C=CC(C#CC5CC5)=C4)N3)C=N1
InChiKey: ZPQLOIBNJAOMDO-UHFFFAOYSA-N
Molecular weight: 328.42
Storage: Stable as a solid at room temperature. DMSO stock solutions (up to 10 mM) are stable at -20°C.
Dissolution: Soluble in DMSO up to 10 mM

Chemical Probe Profile

In vitro Potency & Selectivity:

SGC-PI5P4Kγ/MYLK4-1 was profiled in the DiscoverX *scan*MAX assay against 403 wild-type kinases at 1 µM. Only 7 kinases showed PoC <10 giving an S_{10}(1 µM) = 0.017. When the PoC <35 fraction was examined, 10 kinases were included (S_{35}(1 µM) = 0.025). Potential off-targets within the S_{35}(1 µM) fraction were tested via biochemical enzymatic or binding assays plus

NanoBRET target engagement assays for PIKfyve, MYLK4, PI5P4Kγ, DYRK1A, and CLK2. SGC-PI5P4Kγ/MYLK4-1 binds to PI5P4Kγ with PoC = 1.7 in the PI5P4Kγ DiscoverX binding assay and demonstrated K_d = 19 nM in the same PI5P4Kγ DiscoverX binding assay (Eurofins DiscoverX). PIKfyve, MYLK4, DYRK1A, and CLK2 are off-target kinases that demonstrate enzymatic IC_{50} values within 30-fold of the PI5P4Kγ K_d value: PIKfyve IC_{50} = 12 nM, MYLK4 IC_{50} = 12 nM, CLK2 IC_{50} = 370 nM, and DYRK1A IC_{50} = 520 nM. No other S_{35}(1 μM) kinases demonstrated IC_{50} values within 30-fold of the PI5P4Kγ K_d value.

Potency in Cells and Cellular Target Engagement:

SGC-PI5P4Kγ/MYLK4-1 displayed an IC_{50} = 67 nM in the PI5P4Kγ NanoBRET assay, IC_{50} = 14 nM in the MYLK4 NanoBRET assay, IC_{50} = 450 nM in the PIKfyve NanoBRET assay, IC_{50} = 3200 nM in the CLK2 NanoBRET assay, and IC_{50} > 10000 nM in the DYRK1A NanoBRET assay using HEK293 cells.

Our PI5P4Kγ/MYLK4 chemical probe was found to increase mTORC1 signaling in MCF-7 cells. Phosphorylation of p70-S6K was increased in a dose-response manner 48h after treatment. The negative control did not impact mTORC1 signaling. No associated toxicity was observed due to probe or negative control treatment.