SGC-PIKFYVE-1: A Chemical Probe for PIKfyve

Version 1.0 (11th October 2021)

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

Overview

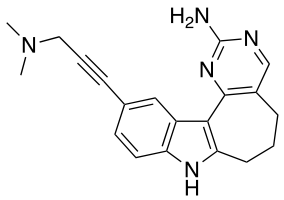
Phosphatidylinositol-3-phosphate 5-kinase (PIKfyve) is a ubiquitously expressed FYVE finger-containing phosphoinositide kinase that is categorized as a lipid kinase. This kinase plays roles in endosomal transport, membrane trafficking, retroviral budding, GLUT4 translocation, TLR signaling, and lysosomal function. A clinical stage PIKfyve inhibitor, apilimod, has been evaluated in clinical trials for Crohn's disease, rheumatoid arthritis, and COVID-19. PIKfyve inhibition has also been considered as a treatment for different cancers, including B-cell non-Hodgkin lymphoma, liver cancer, and myeloma.

Summary

Chemical Probe Name	SGC-PIKFYVE-1
Negative control compound	SGC-PIKFYVE-1N
Target(s) (synonyms)	PIKfyve
Recommended cell assay concentration	Use at concentration of 1 µM (and ≤ 5 µM) for SGC-PIKFYVE-1 and SGC-PIKFYVE-1N; use with control for best interpretation of data.
Suitability for *in* vivo use and recommended dose	SGC-PIKFYVE-1 was not tested *in vivo*
Publications	10.1021/acs.jmedchem.2c00697; 10.26434/chemrxiv-2022-bj274
Orthogonal chemical probes	Apilimod, YM201636
In vitro assay(s) used to characterise	Binding and radiometric enzymatic assays
Cellular assay(s) for target-engagement	NanoBRET

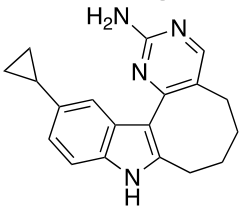
Chemical Probe & Negative Control Structures and Use

SGC-PIKFYVE-1: Chemical Probe



SMILES: CN(CC#CC1=CC2=C(NC3=C2C4=C(C=NC(N)=N4)CCC3)C=C1)C
InChiKey: DORZPJWJOBMQKC-UHFFFAOYSA-N
Molecular weight: 331.4230
Storage: Stable as a solid at room temperature. DMSO stock solutions (up to 10 mM) are stable at -20ºC.
Dissolution: Soluble in DMSO up to 10 mM

SGC-PIKFYVE-1N: Negative Control



SMILES: NC1=NC2=C(CCCCC3=C2C4=C(C=CC(C5CC5)=C4)N3)C=N1
InChiKey: XIVFOAKTTGQHLJ-UHFFFAOYSA-N
Molecular weight: 304.3970
Storage: Stable as a solid at room temperature. DMSO stock solutions (up to 10 mM) are stable at -20ºC.
Dissolution: Soluble in DMSO up to 10 mM

Chemical Probe Profile

In vitro Potency & Selectivity:

SGC-PIKFYVE-1 was profiled in the KINOMEscan assay against 403 wild-type kinases at 1 µM. Only 8 kinases showed PoC <10 giving an S_{10}(1 µM) = 0.02. When the PoC <35 fraction was examined, 20 kinases were included (S_{35}(1 µM) = 0.05). Potential off-targets within the S_{35}(1 µM) fraction as well as PIKfyve were tested using a NanoBRET target engagement assay and via biochemical enzymatic assays where an assay was available. SGC-PIKFYVE-1 binds to PIKfyve with a PoC = 0.1 in the PIKfyve DiscoverX assay and demonstrated an IC_{50} = 6.9 nM in the PIKfyve enzymatic assay (SignalChem). The closest off-target kinases based on enzymatic potencies are MYLK4 and MAP4K5 (IC_{50} = 66 nM and 89 nM, respectively). Affinity for these off-targets in cells is significantly different: MYLK4 NanoBRET IC_{50} = 270 nM and MAP4K5 NanoBRET IC_{50} >10000.

Potency in Cells and Cellular Target Engagement:

SGC-PIKFYVE-1 displayed an IC_{50} of 4.0 nM in the PIKfyve NanoBRET assay using HEK293T cells.

Our PIKfyve chemical probe disrupts multiple phases of the β-coronavirus lifecycle. SGC-PIKFYVE-1 potently inhibits β-coronavirus replication in MHV-NLuc reporter and SARS-CoV-2-nLuc assays. A deeper dive elucidated that SGC-PIKFYVE-1 effects both viral entry, specifically mediated by clathrin-mediated endocytosis, and a potential route of systemic viral spread.