SGC-CDKL5/GSK3-1: A Chemical Probe for CDKL5, GSK3α, and GSK3β

Version 1.0 (5th September 2023)

Web link for more details: https://www.thesgc.org/chemical-probes/SGC-CDKL5_GSK3

Overview

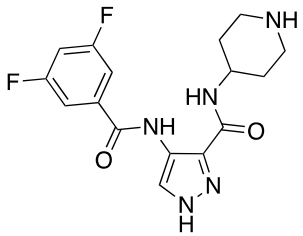
Cyclin-dependent kinase-like 5 (CDKL5) is an understudied, human serine/threonine kinase from the CMGC group of kinases and a member of the human CDKL family of kinases, which also includes CDKL1, CDKL2, CDKL3, and CDKL4. While CDKL5 is widely expressed in human tissues and cells, it is predominantly found in the brain. Accordingly, CDKL5 is important for neuronal proliferation, morphogenesis, maturation, differentiation, and survival. Despite its important role in brain function, the role of CDKL5 during neurodevelopment, its substrates, and the molecular mechanisms involved in its regulation are largely uncharacterized. Relevant to this probe, there is crosstalk between CDKL5 and GSK3α/β, such that in a *CDKL5* knockout mouse model increased activity of GSK3β is observed. Most CDKL5 literature focuses on its essential role in a rare and severe neurodevelopmental condition called CDKL5-deficiency disorder (CDD). More than 70 different point mutations in CDKL5 have been linked to CDD, most of which cause a loss of function phenotype in patients. There are no specific treatments nor a cure for CDD.

Summary

Chemical Probe Name	SGC-CDKL5/GSK3-1
Negative control compound	SGC-CDKL5/GSK3-1N
Target(s) (synonyms)	STK9, GSK3A, GSK3B
Recommended cell assay concentration	Use at concentration of ≤100 nM for SGC-CDKL5/GSK3-1 and SGC-CDKL5/GSK3-1N; use with control for best interpretation of data.
Suitability for *in* vivo use and recommended dose	SGC-CDKL5/GSK3-1 was not tested *in vivo*
Publications	10.1021/acschemneuro.3c00135; 10.1101/2023.02.09.527935
Orthogonal chemical probes	SGC-CAF382-1, SGC-GSK3-1
In vitro assay(s) used to characterise	Radiometric enzymatic and split luciferase binding assays
Cellular assay(s) for target-engagement	NanoBRET

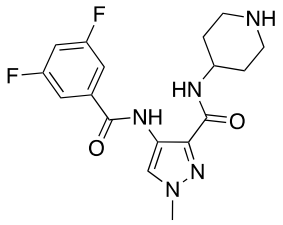
Chemical Probe & Negative Control Structures and Use

SGC-CDKL5/GSK3-1: Chemical Probe



SMILES: O=C(C1=NNC=C1NC(C2=CC(F)=CC(F)=C2)=O)NC3CCNCC3
InChiKey: NIHAFOURWLZLFN-UHFFFAOYSA-N
Molecular weight: 349.34
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-CDKL5/GSK3-1N: Negative Control



SMILES: O=C(C1=NN(C)C=C1NC(C2=CC(F)=CC(F)=C2)=O)NC3CCNCC3
InChiKey: ODZBDODDFLVDQZ-UHFFFAOYSA-N
Molecular weight: 363.67
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-CDKL5/GSK3-1 was profiled in the KINOMEscan assay against 403 wild-type kinases at 1 µM. Only 4 kinases showed PoC <10 giving an S_{10}(1 µM) = 0.01. When the PoC <35 fraction was examined, 11 kinases were included (S_{35}(1 µM) = 0.027). Potential off-targets within the S_{35}(1 µM) fraction were tested via biochemical enzymatic or binding assays plus NanoBRET

target engagement assays for CDKL5, GSK3α, GSK3β, CDK16, and CDK17. SGC-CDKL5/GSK3-1 binds to CDKL5, GSK3β, and GSK3α with PoC = 0.2, PoC = 0.5, and PoC = 14, respectively, in the corresponding DiscoverX assays. This chemical probe demonstrated a CDKL5 IC_{50} = 6.5 nM in the CDKL5 split luciferase assay (Luceome), a GSK3α IC_{50} = 4.0 nM, and a GSK3β IC_{50} = 9.0 nM in the respective GSK3α and GSK3β enzymatic assays (Eurofins). The closest off-target kinase based on enzymatic potency is CDK16 (IC_{50} = 590 nM, 65-fold selectivity window between GSK3β and CDK16 based on biochemical IC_{50} values). A similar selectivity window was observed between GSK3β and CDK16 in cells (GSK3β NanoBRET IC_{50} = 35 nM and CDK16 NanoBRET IC_{50} = 1900 nM).

Potency in Cells and Cellular Target Engagement:

SGC-CDKL5/GSK3-1 displayed an IC_{50} = 3.5 nM in the CDKL5 NanoBRET assay, an IC_{50} = 35 nM in the GSK3β NanoBRET assay, and an IC_{50} = 10 nM in the GSK3α NanoBRET assay, using HEK293 cells.

Our CDKL5/GSK3 chemical probe was found to promote motor neuron survival when iPSC-derived motor neurons were subjected to ER stress. Motor neuron viability was rescued at sub-micromolar concentrations. Since our GSK3 chemical probe (SGC-GSK3-1) was evaluated in parallel, we were able to conclude that CDKL5 inhibition is not detrimental to motor neuron survival.