SGC-CAF382-1: A Chemical Probe for CDKL5, CDK9, CDK16, CDK17, and CDK18

Version 1.0 (5th September 2021)

Web link for more details: https://www.thesgc.org/chemical-probes/SGC-CAF382-1

Overview

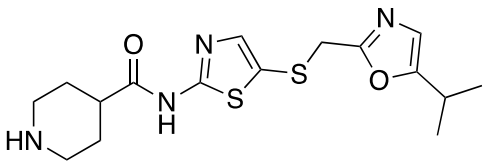
Cyclin-dependent kinase-like 5 (CDKL5) is an understudied, human serine/threonine kinase from the CMGC group of kinases. It is also a member of the human CDKL family of kinases, which includes CDKL1, CDKL2, CDKL3, and CDKL4. While CDKL5 is found in most human tissues and cells, it is predominantly expressed in the brain. Accordingly, CDKL5 is important for neuronal proliferation, maturation, differentiation, morphogenesis, and survival. Despite its central role in brain function, the role of CDKL5 during neurodevelopment, its substrate proteins, and the mechanisms involved in its regulation remain largely uncharacterized. Knowing that there is crosstalk between CDKL5 and GSK3α/β, such that *CDKL5* knockout mice display increased activity of GSK3β, this probe was designed to be devoid of GSK3 inhibition. 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. This tool was developed to model CDD and better understand where intervention might be possible to help CDD patients.

Summary

Chemical Probe Name	SGC-CAF382-1
Negative control compound	SGC-CAF268-1N
Target(s) (synonyms)	STK9, PITALRE, PCTAIRE, PCTK1, PCTAIRE2, PCTK2, PCTAIRE3, PCTK3
Recommended cell assay concentration	Use at concentration of ≤100 nM for SGC-CAF382-1 and SGC-CAF268-1N; use with control for best interpretation of data.
Suitability for *in* vivo use and recommended dose	SGC-CAF382-1 was only tested *in vivo* in mice at a concentration of 2.29 mg/kg; more profiling is required to determine the best dose
Publications	10.7554/eLife.88206; 10.1101/2023.04.24.538049
Orthogonal chemical probes	SGC-CDKL5/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-CAF382-1: Chemical Probe

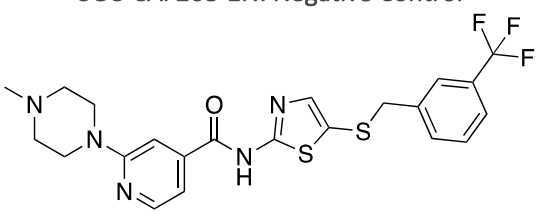


SGC-CAF382-1

SMILES: O=C(NC1=NC=C(S1)SCC2=NC=C(O2)C(C)C)C3CCNCC3

InChiKey: MCLDWKVRXDHDEI-UHFFFAOYSA-N
Molecular weight: 366.50
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-CAF268-1N: Negative Control



SGC-CAF268-1N

SMILES:O=C(C1=CC=NC(N2CCN(CC2)C)=C1)NC3=NC=C(S3)SCC4=CC=CC(C(F)(F)F)=C4
InChiKey: RUYKDMMLESXEOA-UHFFFAOYSA-N
Molecular weight: 493.57
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-CAF382-1 was profiled in the KINOMEscan 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) = 0.024). Potential off-targets within the S_{35}(1 µM) fraction and GSK3β (PoC = 51) were tested via biochemical enzymatic or binding assays plus NanoBRET target engagement assays for CDKL5, GSK3α, GSK3β, CDK9, CDK16, CDK17, and CDK18. SGC-CAF382-1 binds to CDKL5, GSK3α, GSK3β, CDK9, CDK16, CDK17, and CDK18 with PoC values of 0.7, 0.7, 51, 8.3, 5.5, 1.3, and 17, respectively, in the corresponding DiscoverX assays. This chemical probe demonstrated a CDKL5 IC_{50} = 6.7 nM in the CDKL5 split luciferase assay (Luceome). SGC-CAF382-1 also demonstrated a GSK3α IC_{50} = 470 nM, a GSK3β IC_{50} = 2200 nM, a CDK9 IC_{50} = 20 nM, a CDK16 IC_{50} =62 nM, a CDK17 IC_{50} = 89 nM, and a CDK18 IC_{50} = 100 nM in the respective enzymatic assays (Eurofins). The closest off-target kinase based on enzymatic potency is CDK7 (IC_{50} = 300 nM, 44-fold selectivity window between CDKL5 and CDK7 based on biochemical IC_{50} values).

Potency in Cells and Cellular Target Engagement:

SGC-CDKL5/GSK3-1 displayed an IC_{50} = 10 nM in the CDKL5 NanoBRET assay, an IC_{50} = 2400 nM in the GSK3β NanoBRET assay, an IC_{50} = 1500 nM in the GSK3α NanoBRET assay, an IC_{50} = 280 nM in the CDK9 NanoBRET assay, an IC_{50} = 390 nM in the CDK16 NanoBRET assay, an IC_{50} = 240 nM in the CDK17 NanoBRET assay, and an IC_{50} = 260 nM in the CDK18 NanoBRET assay, using HEK293 cells.

SGC-CAF382-1 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. In rat hippocampal brain slices, this chemical probe was found to dose-dependently and selectively reduce postsynaptic function of AMPA-type glutamate receptors and decrease hippocampal LTP.