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

Version 1.0 (5th September 2023)

Web link for more details: https://www.thesgc.org/chemical-probes/SGC-GSK3-1

Overview

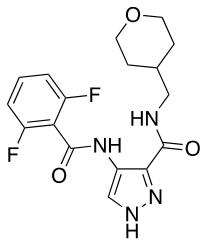
Glycogen synthase kinase-3α (GSK3α) and glycogen synthase kinase-3β (GSK3β) are well studied human serine/threonine kinases from the CMGC group of kinases. With more than 100 known substrates, these isozymes have been identified as regulators of a variety of key biological pathways and, accordingly, linked to numerous diseases, including type 2 diabetes, Alzheimer's disease, inflammation, obesity, and cancer. Both are cytoplasmic and widely expressed in all human tissues, but GSK3β demonstrates higher abundance in the CNS and testis. When used at an appropriate concentration (<500 nM) in cells, SGC-GSK3-1 is exquisitely selective for GSK3α or GSK3β. Potent and selective, structurally divergent GSK3 inhibitors have been described, which can be used alongside our chemical probe set to characterize the impacts of GSK3 inhibition on downstream biology.

Summary

Chemical Probe Name	SGC-GSK3-1
Negative control compound	SGC-CDKL5/GSK3-1N
Target(s) (synonyms)	GSK3A, GSK3B
Recommended cell assay concentration	Use at concentration of <500 nM for SGC-GSK3-1 and SGC-CDKL5/GSK3-1N; use with control for best interpretation of data.
Suitability for *in* vivo use and recommended dose	SGC-GSK3-1 was not tested *in vivo*
Publications	10.1021/acschemneuro.3c00135; 10.1101/2023.02.09.527935
Orthogonal chemical probes	
In vitro assay(s) used to characterise	Radiometric enzymatic and split luciferase binding assays
Cellular assay(s) for target-engagement	NanoBRET

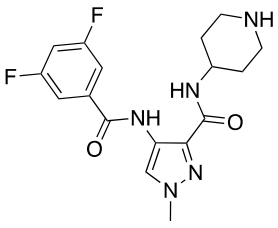
Chemical Probe & Negative Control Structures and Use

SGC-GSK3-1: Chemical Probe



SMILES: O=C(C1=NNC=C1NC(C2=C(F)C=CC=C2F)=O)NCC3CCOCC3
InChiKey: OCQCKUTZHBQNEI-UHFFFAOYSA-N
Molecular weight: 364.35
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-GSK3-1 was profiled in the KINOMEscan assay against 403 wild-type kinases at 1 µM. Only 5 kinases showed PoC <10 giving an S_{10}(1 µM) = 0.012. When the PoC <35 fraction was examined, 15 kinases were included (S_{35}(1 µM) = 0.037). 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, and DYRK1B. SGC-GSK3-1 binds to GSK3β and GSK3α with PoC = 0 and PoC = 0.2, respectively, in the corresponding DiscoverX assays. This chemical probe demonstrated a GSK3α IC_{50} = 1.0 nM and a GSK3β IC_{50} = 2.0 nM in the respective GSK3α and GSK3β enzymatic assays (Eurofins). The closest off-target kinase based on

enzymatic potency is DYRK1B (IC_{50} = 47 nM, 23-fold selectivity window between GSK3β and DYRK1B based on biochemical IC_{50} values). An enhanced selectivity window (200-fold) was observed between GSK3β and DYRK1B in cells (GSK3β NanoBRET IC_{50} = 12 nM and DYRK1B NanoBRET IC_{50} = 2400 nM).

Potency in Cells and Cellular Target Engagement:

SGC-GSK3-1 displayed an IC_{50} = 12 nM in the GSK3β NanoBRET assay and an IC_{50} = 4.6 nM in the GSK3α NanoBRET assay, using HEK293 cells.

Our 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.