SGC-CDKL2/AAK1/BMP2K-1: A Chemical Probe for CDKL2, AAK1, and BMP2K

Version 1.1 (19th August 2024)

Web link for more details: https://www.thesgc.org/chemical-probes/sgc-cdkl2aak1bmp2k-1

Overview

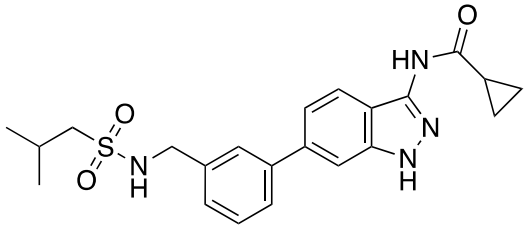
Cyclin-dependent kinase-like 2 (CDKL2) is an understudied, human serine/threonine kinase from the CMGC group of kinases. It is also a member of the human CDKL family of kinases, which includes CDKL1, CDKL3, CDKL4, and CDKL5. Enhanced tissue expression of CDKL2 is observed in the retina and testis. CDKL2 is also non-specifically expressed throughout the brain and in the kidneys and lungs. This kinase is cytoplasmic and localizes to the nucleoplasm and centrosome in cells. Animal and human cDNA clones support that at least four variants of this enzyme may exist, generated by alternative splicing. All isoforms identified from mouse, rabbit, and human cDNAs contain a common kinase domain and vary in their carboxy termini. Two major transcripts were found in the adult kidney, testis, brain, and lung of humans, and a single transcript has been found in the human fetal brain and kidney. Few publications have been published on CDKL2. While several studies have linked CDKL2 and tumorigenesis, how CDKL2 modulates oncogenic progression is not yet fully understood. As an example, CDKL2 was identified as a regulator of epithelial–mesenchymal transition (EMT), a process that is associated with increased migration, metastasis, and therapeutic resistance. The EMT process makes cells more stem cell-like. Beyond cancer, CDKL2 plays a role in development, supporting emotion, behavior control, and cognitive functions required to acquire contextual and spatial learning.

Summary

Chemical Probe Name	SGC-CDKL2/AAK1/BMP2K-1
Negative control compound	SGC-CDKL2/AAK1/BMP2K-1N
Target(s) (synonyms)	KKIAMRE, P56
Recommended cell assay concentration	Use at concentration of ≤1 µM for SGC-CDKL2/AAK1/BMP2K -1 and SGC-CDKL2/AAK1/BMP2K-1N; use with control and SGC-AAK1-1 for best interpretation of data.
Suitability for *in* vivo use and recommended dose	SGC-CDKL2/AAK1/BMP2K-1 was not tested *in vivo*
Publications	10.1021/acsmedchemlett.4c00219; 10.1101/2024.05.12.593776
Orthogonal chemical probes	
In vitro assay(s) used to characterise	Enzymatic and thermal shift assays
Cellular assay(s) for target-engagement	NanoBRET

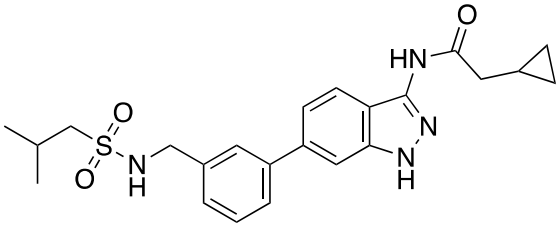
Chemical Probe & Negative Control Structures and Use

SGC-CDKL2/AAK1/BMP2K-1: Chemical Probe



SMILES: O=C(C1CC1)NC2=NNC3=C2C=CC(C4=CC=CC(CNS(CC(C)C)(=O)=O)=C4)=C3
InChiKey: IIKPYJZUOZRTIB-UHFFFAOYSA-N
Molecular weight: 426.54
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- CDKL2/AAK1/BMP2K-1N: Negative Control



SMILES: O=C(CC1CC1)NC2=NNC3=C2C=CC(C4=CC=CC(CNS(CC(C)C)(=O)=O)=C4)=C3
InChiKey: OZUHOOVGHGETRM-UHFFFAOYSA-N
Molecular weight: 440.56
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-CDKL2/AAK1/BMP2K-1 was profiled in the KINOMEscan assay against 403 wild-type kinases at 1 µM. Only 2 kinases, including one mutant kinase and CDKL2, showed PoC <10 giving an S_{10}(1 µM) = 0.002. When the PoC <40 fraction was examined, 10 wild-type kinases were included (S_{35}(1 µM) = 0.022). Potential off-targets within the S_{40}(1 µM) fraction were tested via biochemical enzymatic and/or NanoBRET target engagement assays. SGC-CDKL2/AAK1/BMP2K-1 binds to CDKL2, BMP2K, PIP5K1A, GRK4, PRP4, SRPK3, SRPK1, MEK5, RIOK1, and AAK1 with PoC values of 4.3, 17, 19, 23, 23, 27, 29, 30, 30, and 37, respectively, in the corresponding DiscoverX assays. This chemical probe demonstrated a CDKL2 IC_{50} = 43 nM in a CDKL2 enzymatic assay (Pabla lab). SGC-CDKL2/AAK1/BMP2K-1 also demonstrated a BMP2K IC_{50} = 320 nM and an AAK1 IC_{50} = 160 nM but was inactive (>10000 nM) in the remaining enzymatic assays (Eurofins). AAK1 and BMP2K are off-target kinases based on enzymatic potency.

Potency in Cells and Cellular Target Engagement:

SGC-CDKL2/AAK1/BMP2K-1 displayed an IC_{50} = 460 nM in the CDKL2 NanoBRET assay, an IC_{50} = 5700 nM in the BMP2K NanoBRET assay, and an IC_{50} = 2300 nM in the AAK1 NanoBRET assay, using HEK293 cells. In cells, SGC-CDKL2/AAK1/BMP2K-1 can be used at ≤1 µM to selectively inhibit CDKL2.

SGC-CDKL2/AAK1/BMP2K-1 was found to not be anti-proliferative when rat primary neurons and breast cancer cells were treated. This compound inhibits EB2 phosphorylation in rat primary neurons but was not found to consistently impact EMT in breast cancer cells.