JA310: A Chemical Probe for MST3/4

Version 1.0 (6th November 2023)

Web link for more details: https://www.thesgc.org/chemical-probes/JA310

Overview

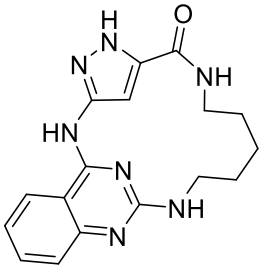
Mammalian STE20-like protein kinase 3 and -4 (MST3/4) are part of the MST family, which comprises three additional members, namely MST1, MST2 and YSK1. The MSTs are involved in cell proliferation, cell migration and cell polarity. For MST3 it has been shown that it can phosphorylate STK38L and stimulate its kinase activity; for MST4 ATG4B has been demonstrated as a substrate and thereby regulates the autophagic activity.

Summary

Chemical Probe Name	JA310
Negative control compound	JA262
Target(s) (synonyms)	MST3 (Mammalian STE20-like protein kinase 3)/ STK24 (Serine/threonine-protein kinase 24) MST4 (Mammalian STE20-like protein kinase 4)/ STK26 (Serine/threonine-protein kinase 26)
Recommended cell assay concentration	Use at concentration of 1 µM (and ≤ 10 µM) for JA310 and JA262; use with control for best interpretation of data.
Suitability for *in* vivo use and recommended dose	JA310 was not tested in vivo.
Publications	https://doi.org/10.1101/2023.10.20.563248
Orthogonal chemical probes	
In vitro assay(s) used to characterise	DSF, ITC, Radiometric inhibition assay
Cellular assay(s) for target-engagement	NanoBRET™

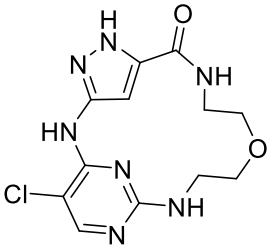
Chemical Probe & Negative Control Structures and Use

JA310 Chemical Probe



SMILES: O=C(NCCCCCNC1=NC2=C(C=CC=C3)C3=N1)C4=CC(N2)=NN4
InChiKey: XBWKVZHZUZNMJE-UHFFFAOYSA-N
Molecular weight: 337.39
Storage Stable as solid in the dark at -20°C. NB making aliquots rather than freeze-thawing is recommended
Dissolution: Soluble in DMSO up to 10 mM; use only 1 freeze/thaw cycle per aliquot

JA262 Negative Control



SMILES: O=C(NCCOCCNC1=NC2=C(Cl)C=N1)C3=CC(N2)=NN3
InChiKey: SXVMAWWNAHHYKH-UHFFFAOYSA-N
Molecular weight: 323.74
Storage: Stable as solid in the dark at -20°C. NB making aliquots rather than freeze-thawing is recommended
Dissolution: Soluble in DMSO up to 10 mM; use only 1 freeze/thaw cycle per aliquot

Chemical Probe Profile

In vitro Potency & Selectivity:

The binding of JA310 towards MST3/4 was determined by DSF (7.5 K/7.4 K) and confirmed by ITC with an K_D of 116 nM on MST4. In the radiometric assay ^{33}PanQinase™ Activity Assay (ReactionBiology) JA310 has a mean residual activity of 13.5 respectively 18.0 % for MST3/4 at 1µM. In the selectivity profiling from Reaction Biology against 340 wild-type kinases the closest off-targets were LIMK1/2 with 36.6% and 38.2% mean residual activity at 1µM.

Potency in Cells and Cellular Target Engagement:

In NanoBRET assay using HEK293T cells JA310 displayed an EC_{50} of 106 nM in intact cells respectively 76 nM in the lysed cells on MST3. For MST4 JA310 had an EC_{50} of 1431 nM in intact cells and an EC_{50} of 362 nM in the lysed cells. The closest off-targets LIMK1/2 had EC_{50} values of 5.67/1.78 µM in the NanoBret assay.

JA310 showed no effect on cell viability over 48 h at 1 µM in Human colorectal carcinoma cells (HCT116). At the highest concentration tested (10 µM), the normalized cell count decreased in a time-dependent manner to less than 30% after 72 h.