Huib32: A Chemical Probe for USP32

Version 1.0 (24th April 2025)

Web link for more details: https://www.sgc-ffm.uni-frankfurt.de/#!specificprobeoverview/Huib32

Overview

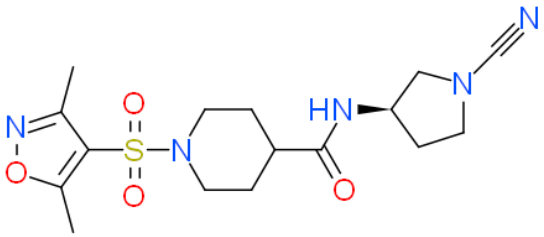
USP32 belongs to the family of ubiquitin-specific cysteine proteases and deubiquitinates Rab7, the main regulator of endocytosis and autophagy. It has been implicated in a range of cancers, including breast and lung cancer.

Summary

Chemical Probe Name	Huib32
Negative control compound	Huib32NC
Target(s) (synonyms)	USP32
Recommended *in vitro* assay concentration	Use at concentration of 5 µM for Huib32 and Huib32NC; use with control for best interpretation of data
Suitability for *in* vivo use and recommended dose	Not tested.
Publications	Preprint
In vitro assay(s) used to characterise	Activity assay using Ub-RhoMP
Cellular assay(s) for target-engagement	ABPP assay

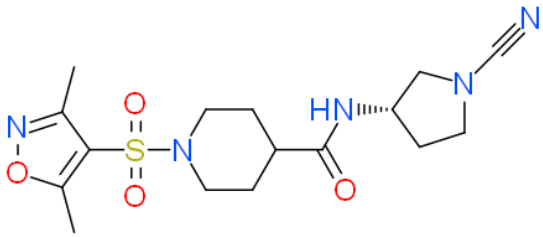
Chemical Probe & Negative Control Structures and Use

Huib32 Chemical Probe



SMILES: Cc1c(c(C)on1)S(N1CCC(CC1)C(N[C@@H]1CCN(C1)C#N)=O)(=O)=O
InChiKey: GAXWGHHQPWGBKQ-CQSZACIVSA-N
Molecular weight: 381.15 g/mol
Storage: As a dry powder or as DMSO stock solutions (10 mM) at -20 °C. DMSO stocks beyond 3-6 months or 2 freeze/thaw cycles should be tested for activity before use
Dissolution: Soluble in DMSO up to 10 mM; use only 1 freeze/thaw cycle per aliquot

Huib32NC Negative Control



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Chemical Probe Profile

In vitro Potency & Selectivity:

Huib32 is a selective fast reversible covalent inhibitor for the active form of USP32 with IC_{50} = 21.2 nM in an activity assay using Ub-RhoMP (k_{inact} = 0.00062 s-1, KI_{app}= 50.5 nM, k_{inact}/KI = 12,285 M-1s-1). No inhibition was found within the USP family and other DUBs in gel and proteomics-based ABPP (activity-based protein profiling) experiments in intact cells. 46 DUBs were tested in an Ub-Rho Fluorescent intensity assay (Ubiquigent) in vitro at 250 nM Huib32: only USP32 and weaker USP6 which has a 97% nucleotide similarity to the catalytic domain of USP32 were inhibited. A 1-step labelling pull-down proteomics experiment in MelJuSo cells (at 1 µM) revealed PCCA, MCCC1 and EEF2 as off-targets.

Potency in Cells and Cellular Target Engagement:

In an ABPP assay Huib32 inhibition was started at 0.1 µM with the maximum inhibition observed at 5-10 µM (EC_{50} < 0.1µM).