BAY 1816032: A Chemical Probe for BUB1

Version 1.0 (29th September 2022)

Web link for more details: https://www.sgc-ffm.uni-frankfurt.de/#!specificprobeoverview/BAY%201816032

Overview

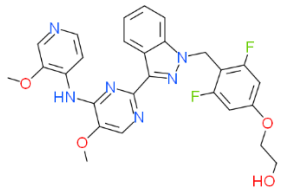
BUB1 is bound to kinetochores and has various functions during the cell cycle, mainly in the spindle assembly checkpoint (SAC) and chromosome alignment during metaphase. It is involved in centromere cohesion and attachment error correction. Inhibition of BUB1 sensitizes tumour cells toward paclitaxel and docetaxel, and toward ATR inhibitors and PARP inhibitors.

Summary

Chemical Probe Name	BAY 1816032
Negative control compound	BAY-283
Target(s) (synonyms)	BUB1 Mitotic Checkpoint Serine/Threonine Kinase, HBUB1
Recommended *in vitro* assay concentration	Use at concentration of 300 nM for BAY 1816032 and BAY-283; use with control for best interpretation of data
Suitability for *in* vivo use and recommended dose	Tested in mice, rat and dog at 0.5 to 5 mg/kg (po) and 0.5 to 1 mg/kg (iv); orally bioavailable.
Publications	PMID: 30429199
In vitro assay(s) used to characterise	Biochemical assay with low ATP; TR-FRET
Cellular assay(s) for target-engagement	Abrogation of histone H2A-Thr120 phosphorylation

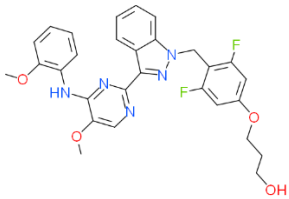
Chemical Probe & Negative Control Structures and Use

BAY 1816032 Chemical Probe



SMILES: COc1cnccc1Nc1c(cnc(c2c3ccccc3n(Cc3c(cc(cc3F)OCCO)F)n2)n1)OC
InChiKey: QVOGVAVHOLLLAZ-UHFFFAOYSA-N
Molecular weight: 534.18 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

BAY-283 Negative Control



SMILES: COc1ccccc1Nc1c(cnc(c2c3ccccc3n(Cc3c(cc(cc3F)OCCCO)F)n2)n1)OC
InChiKey: PYAFWWXPXHSNDM-UHFFFAOYSA-N
Molecular weight: 547.2 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

Chemical Probe Profile

In vitro Potency & Selectivity:

BAY 1816032 shows potent activity on human BUB1 with IC_{50} = 6.5 nM in a biochemical assay with low ATP, EC_{50} = 6.1 ± 2.5 nM at 10 µM ATP (TR-FRET), Kd = 2.1 nM (SPR), IC_{50} = 1 ± 0.5 nM (ePCA binding competition (equilibrium binding assay) and Kd = 3.3 nM (Kinome scan). Closest hits in a DiscoverX panel of 403 kinases at 1 µM are (Kd [nM]) STK10 (57; 17-fold), CDC42BPG (850), DDR1 (2300). Closest off-targets in a Eurofins-Panlabs Screen (89 targets at 10 µM) [% inh.] are human Adenosine transporter (71, IC_{50} = 370 nM), ADORA2A (67), GABAA Chloride Channel TBOB receptor (60), PTGS1 (54), sodium channel site 2 (53) and Phosphodiesterase PDE4 (52).

Potency in Cells and Cellular Target Engagement:

For the abrogation of histone H2A-Thr120 phosphorylation, a substrate of BUB1 kinase, in nocodazole arrested HeLa cells after one hour compound incubation an IC_{50} of 29 ± 23 nM was measured and for the formation of H2A-Thr120 the IC_{50} is 43 nM. BAY 1816032 inhibits tumor cell proliferation with a median IC_{50} of 1.4 µM.