BAY1125976: A Chemical Probe for AKT1 and AKT2

Version 1.0 (29th October 2021)

Web link for more details: https://www.sgc-ffm.uni-frankfurt.de/#!specificprobeoverview/BAY1125976

Overview

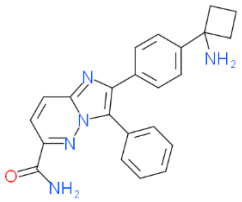
AKT kinases play a central role in numerous signalling pathways regulating e.g. proliferation and cell growth. Genetic lesions in PI3K/PTEN result in hyperactivated AKT associated with diverse types of human tumours and PIK3CA-related overgrowth syndromes. The activating mutation $AKT1^{E17K}$ acts as driver alteration in breast/gynaecologic cancers and Proteus syndrome. The multi-domain architecture enables complex regulation and modulation of the kinase activity.

Summary

Chemical Probe Name	BAY1125976
Negative control compound	BAY-940
Target(s) (synonyms)	AKT1 (AKT serine/threonine kinase 1, RAC, PKB, PRKBA); AKT2 (PKB Beta, PKBB, RAC-BETA, PKBBETA)
Recommended *in vitro* assay concentration	Use at concentration up to 1 µM for BAY1125976 and BAY-940; use with control and orthogonal probe for best interpretation of data
Suitability for *in* vivo use and recommended dose	BAY1125976 demonstrates potent anti-tumour efficacy in KPL-4 breast cancer nude mice model (Dose: 25 -50 mg/kg po QD)
Publications	PMID: 27699769, PMID: 31835495
Orthogonal chemical probes	Borussertib
In vitro assay(s) used to characterise	TR-FRET, SPR
Cellular assay(s) for target-engagement	Mechanistic cell assay: Reduction of AKT phosphorylation

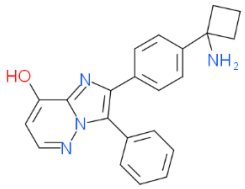
Chemical Probe & Negative Control Structures and Use

BAY1125976 Chemical Probe



SMILES: C1CC(C1)(c1ccc(cc1)c1c(c2ccccc2)n2c(ccc(C(N)=O)n2)n1)N
InChiKey: JBGYKRAZYDNCNV-UHFFFAOYSA-N
Molecular weight: 383.17 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-940 Negative Control



SMILES: C1CC(C1)(c1ccc(cc1)c1c(c2ccccc2)n2c(c(ccn2)O)n1)N
InChiKey: DSWFCCRSSVVRIR-UHFFFAOYSA-N
Molecular weight: 356.16 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:

BAY1125976 shows potent activity on AKT1/2 in TR-FRET assay with full-length protein (AKT1: IC_{50} = 5.2 nM at 10 µM ATP; 44 nM at 2 mM ATP; AKT2: IC_{50} = 18 nM at 10 µM ATP; 36 nM at 2 mM ATP) and SPR (active AKT1: Kd = 2.7 nM; inactive AKT1: Kd = 1.3 nM) but is inactive using truncated AKTs lacking the PH domain. It is selective against AKT3 (IC_{50} = 427 nM at 10 µM ATP, TR-FRET). The Millipore kinase panel (230 at 10 µM) is clean. Closest off-targets in the DiscoverX screen (468, Kd [nM]) are FLT(D835Y) (210), CKL1 (310), MKNK2 (330). There is no effect < 10 µM in the Ricerca Lead Profiler screen.

Potency in Cells and Cellular Target Engagement:

BAY1125976 reduces the basal levels of AKT phosphorylation in KPL-4 cells at T308 (phosphorylated exclusively by PI3K/PDK1) with IC_{50} = 0.9 nM. In the NanoBRET assay IC_{50} = 2.84 nM for AKT1 and 10.7 nM for AKT2.