

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

Overview

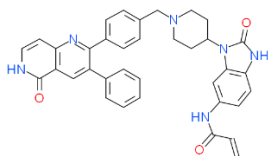
AKT kinases play a central role in numerous signalling pathways regulating e.g. proliferation and cell growth. Genetic lesions in PI3K/PTEN result in hyperactivated AKT associated with diverse types of human tumours and PIK3CA-related overgrowth syndromes. The activating mutation AKT1^{E17K} acts as 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	Borussertib
Negative control compound	RL2578
Target(s) (synonyms)	AKT1 (AKT serine/threonine kinase 1, RAC, PKB, PRKBA); AKT2 (PKB Beta, PKBB, RAC-BETA, PKBBETA)
Recommended <i>in vitro</i> assay concentration	Use at conc. of 1 μ M for Borussertib and RL2578; might be tested up to a conc. of 10 μ M.; use with control and orthogonal probe for best interpretation of data
Suitability for <i>in vivo</i> use and recommended dose	Tested in mice with 2 mg/kg i.v., 20 mg/kg oral gavage and 20 mg/kg, i.p.
Publications	PMID: 30996949 , PMID: 30858154
Orthogonal chemical probes	BAY1125976
<i>In vitro</i> assay(s) used to characterise	Homogeneous Time Resolved Fluorescence (HTRF)
Cellular assay(s) for target-engagement	NanoBRET, CellTiter-Glo luminescent cell viability assay (CTG)

Chemical Probe & Negative Control Structures and Use

Borussertib Chemical Probe



SMILES: C=CC(Nc1ccc2c(c1)N(C1CCN(CC1)Cc1ccc(cc1)c1c(cc3C(NC=Cc3n1)=O)c1cccc1)C(N2)=O)=O

InChiKey: HXBRBOYWXLHDC-UHFFFAOYSA-N

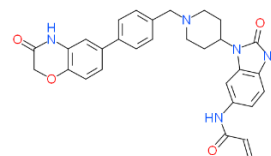
Molecular weight: 596.25 g/mol

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

RL2578 Negative Control



SMILES: C=CC(Nc1ccc2c(c1)N(C1CCN(CC1)Cc1ccc(cc1)c1ccc3c(c1)NC(CO3)=O)C(N2)=O)=O

InChiKey: DHRJWFCEMBINAK-UHFFFAOYSA-N

Molecular weight: 523.22 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:

Borussertib shows potent activity in the HTRF assay (AKT1: IC₅₀ = 0.8 nM; AKT2: IC₅₀ = 59 nM). It is selective against AKT3 (IC₅₀ = 650 nM (HTRF); IC₅₀ = 4.3 μ M (NanoBRET)). The SelectScreen® Kinase Profiling (100 at 1 μ M) is clean.

Potency in Cells and Cellular Target Engagement:

Borussertib is potent in the NanoBRET assay with IC₅₀ = 21 nM for AKT1 and 68 nM for AKT2 and also in the CellTiter-Glo luminescent cell viability assay (CTG) (EC₅₀ = 5 nM (ZR-75-1, breast), 50 nM (T-47D, breast), 190 nM (AN3-CA, endometrium), 370 (BT-474, breast), 280 (MCF-7, breast)).