MU1700: ALK1/2 quality chemical probe

Version 1.0 (9th December 2021)

Web link for more details: https://www.thesgc.org/node/1649385

Overview

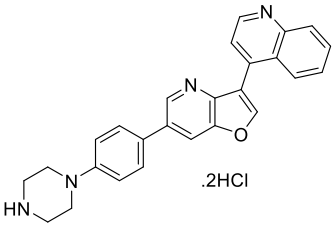
ALK1/2 are receptor serine/threonine protein kinases belonging to a group of tyrosine kinase-like kinases (TLK) and they are encoded by *ACVRL1* and *ACVR1* genes. They are composed of an extracellular domain, transmembrane domain, glycine-serine rich (GS) domain and kinase domain. ALK1/2, as well as others related kinases ALK3-7, are so-called transforming growth factor β (TGF-β) type I receptors. ALK1-7 form hetero-tetrameric complexes with TGF-β type II receptors, that are stabilized by ligands from TGF-β superfamily. Upon formation of hetero-tetrameric complex and ligand binding, the constitutively active TGF-β type II receptors phosphorylate ALK1-7 on several Ser/Thr residues of the GS domain, which leads to the stabilization of the kinase domain in the active state. ALKs mediate SMAD independent as well as SMAD depended signaling pathways. ALK2 has emerged in the literature as a promising therapeutic target for treatment of fibrodysplasia ossificans progressiva, diffuse intrinsic pontine glioma and, more recently, also multiple sclerosis.

Summary

Chemical Probe Name	MU1700
Negative control compound	MU1700NC
Target(s) (synonyms)	ALK1 (ACVRL1), ALK2 (ACVR1)
Recommended cell assay concentration	Highest recommended conc. for cellular assays is 1 µM.
Suitability for *in* vivo use and recommended dose	Suitable for in vivo testing, remarkably high brain penetrance
Publications	
Orthogonal chemical probes	M4K2234 (M4K2234NC – neg. control)
***In vitro* assay(s) used to characterise**	Radiometric assay and ^{33}PanQinase™
Cellular assay(s) for target-engagement	NanoBRET™

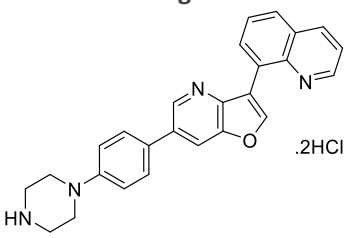
Chemical Probe & Negative Control Structures and Use

MU1700 Chemical Probe



SMILES:
C1(C=CC=C2)=C2N=CC=C1C3=COC4=C3N=CC(C5=CC=C(C=C5)N6CCNCC6)=C4
InChiKey: FFLJVBPCONQSQW-UHFFFAOYSA-N
Molecular weight: 406.488
Storage Stable as solid in the dark at -20°C.
Dissolution: For better solubility, we commend to formulate MU1700 and MU1700NC as a salt (e.g. .2HCl).

MU1700NC Negative Control



SMILES:
C1(N=CC=C2)=C2C=CC=C1C3=COC4=C3N=CC(C5=CC=C(C=C5)N6CCNCC6)=C4
InChiKey: KNGDSLDIOAICSE-UHFFFAOYSA-N
Molecular weight: 406.488
Storage: Stable as solid in the dark at -20°C.
Dissolution: For better solubility, we commend to formulate MU1700 and MU1700NC as a salt (e.g. .2HCl).

Chemical Probe Profile

In vitro Potency & Selectivity:

MU1700 shows potent activity on ALK1/2 of 13nM and 6 nM in radio-enzymatic assay. The Reaction biology kinase panel (370 kinases at 1 µM conc.) doesn't show any off-targets outside ALK1-7 subfamily at threshold of 25 % residual activity. The closest off-targets, based on in the Reaction biology kinome screen, were evaluated in biochemical assay: ALK3 (IC_{50} = 425 nM), ALK6 (IC_{50} = 41 nM), DDR1 (IC_{50} = 501 nM), FLT3 (IC_{50} = 751 nM), KHS/MAR4K5 (IC_{50} = 539 nM).

Potency in Cells and Cellular Target Engagement:

In the NanoBRET assay IC_{50} = 27 nM for ALK1 and 225 nM for ALK2. The cellular activity was also demonstrated using western blot assay where MU1700 inhibits ALK2 catalysed phosphorylation of SMAD1/5/8.