M4K2234: ALK1/2 quality chemical probe

Version 1.0 (9th December 2021)

Web link for more details: https://www.thesgc.org/node/1649384

Overview

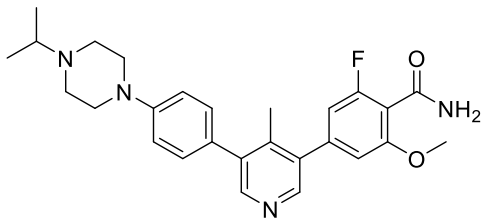
ALK1/2 are receptor serine/threonine protein kinases belonging to a group of tyrosine kinase-like kinases (TLK) and they are encoded by *ACVRL1* and *ACVR1* genes. They are composed of an extracellular domain, transmembrane domain, glycine-serine rich (GS) domain and kinase domain. ALK1/2, as well as 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	M4K2234
Negative control compound	M4K2234NC
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, low brain penetrance
Publications	
Orthogonal chemical probes	MU1700 (MU1700NC – neg. control)
In vitro assay(s) used to characterise	Radiometric assay and ^{33}PanQinase™
Cellular assay(s) for target-engagement	NanoBRET™

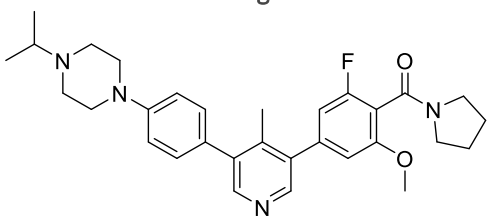
Chemical Probe & Negative Control Structures and Use

M4K2234 Chemical Probe



SMILES:
O=C(N)C1=C(OC)C=C(C2=C(C)C(C3=CC=C(N4CCN(C(C)C)CC4)C=C3)=CN=C2)C=C1F
InChiKey: RIWTUJFFSTVYPW-UHFFFAOYSA-N
Molecular weight: 462.567
Storage Stable as solid in the dark at -20°C.
Dissolution: It is possible to prepare 10 mM DMSO solution.

M4K2234NC Negative Control



SMILES:
O=C(N1CCCC1)C2=C(OC)C=C(C3=C(C)C(C4=CC=C(N5CCN(C(C)C)CC5)C=C4)=CN=C3)C=C2F
InChiKey: WFJOMSKCDAOJBT-UHFFFAOYSA-N
Molecular weight: 516.659
Storage: Stable as solid in the dark at -20°C.
Dissolution: It is possible to prepare 10 mM DMSO solution.

Chemical Probe Profile

***In vitro* Potency & Selectivity:**
M4K22334 was profiled against 375 kinases at 1 µM conc., showing only 1 off-target (TNIK) outside ALK1-7 subfamily at the threshold of 25 % residual activity. ALK1-6 and TNIK were then evaluated in radioenzymatic assay: IC_{50} (ALK1) = 7 nM, IC_{50} (ALK2) = 14 nM, IC_{50} (ALK3) = 168 nM, IC_{50} (ALK4) = 1660 nM, IC_{50} (ALK5) = 1950 nM, IC_{50} (ALK6) = 88 nM and IC_{50} (TNIK) = 41 nM.

Potency in Cells and Cellular Target Engagement:
Cellular potency was evaluated in NanoBRET assay for ALK1-6, providing following activities: IC_{50} (ALK1) = 83 nM, IC_{50} (ALK2) = 13 nM, IC_{50} (ALK3) = 526 nM, IC_{50} (ALK4) = 8424 nM, IC_{50} (ALK5) = 7932 nM, IC_{50} (ALK6) = 1628 nM. The cellular activity was also demonstrated using western blot assay where M4K2234 inhibits ALK2 catalysed phosphorylation of SMAD1/5/8.