PFI-6: A Chemical Probe for human MLLT1/3

Version 1.0 (2nd February 2021)

Web link for more details: https://www.thesgc.org/chemical-probes/PFI-6

Overview

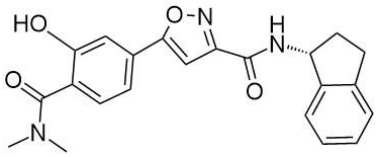
The small YEATS protein domain is found in four human proteins, including MLLT1 and MLLT3, and is an epigenetic reader of acetyl-lysine histone marks. MLLT1 has been found to be a driver of acute myeloid leukaemia. A collaboration between Pfizer and the SGC has resulted in the discovery of PFI-6, a potent disrupter of protein:protein interactions involving the YEATS domains of MLLT1/3.

Summary

Chemical Probe Name	PFI-6
Negative control compound	PFI-6N
Target(s) (synonyms)	MLLT1/MLLT3 (ENL;LTG19;YEATS1 / AF-9;YEATS3)
Recommended cell assay concentration	10 µM for PFI-6 and PFI-6N; use with negative control and orthogonal probe for best interpretation of data
Suitability for *in* vivo use and recommended dose	This chemical probe is not suitable for *in vivo* use
Publications	None at time of writing
Orthogonal chemical probes	NVS-MLLT-1
In vitro assay(s) used to characterise	HTRF, DSF, BLI, ITC
Cellular assay(s) for target-engagement	NanoBRET, FRAP

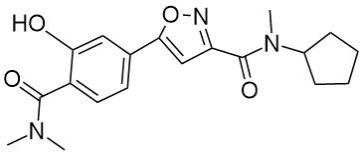
Chemical Probe & Negative Control Structures and Use

PFI-6 Chemical Probe



SMILES: O=C(N(C)C)C1=C(O)C=C(C2=CC(C(N[C@@H]3CCC4=C3C=CC=C4)=O)=NO2)C=C1
InChiKey: IXWUILRSNIQHDM-QGZVFWFLSA-N
Molecular weight: 391.43
Storage: As a dry powder or as DMSO stock solutions (10mM) 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 10mM; use only 1 freeze/thaw cycle per aliquot

PFI-6N *Negative* Control



SMILES: O=C(N(C)C)C1=C(O)C=C(C2=CC(C(N(C)C3CCCC3)=O)=NO2)C=C1
InChiKey: CKEICVFLYGXFOP-UHFFFAOYSA-N
Molecular weight: 357.41
Storage: As a dry powder or as DMSO stock solutions (10mM) 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 10mM; use only 1 freeze/thaw cycle per aliquot

Chemical Probe Profile

In vitro Potency & Selectivity:

PFI-6 shows potent activity on MLLT1/3. PFI-6 is universally inactive in the SGC panel of 48 Bromodomains, and showed no activity in an Invitrogen panel of 40 kinases (screening conducted at 10µM). Additionally, PFI-6 shows no activity in a panel of 25 PDEs, ion channels and GPCRs >50µM. The negative control, PFI-6N, is not active against MLLT1 >30µM, MLLT3 >30µM, YEATS2 >30µM and YEATS4 >30µM.

	MLLT1	MLLT3	*YEATS2*	*YEATS4*
HTRF IC_{50} (µM)	0.14	0.16	>40	>40
BLI K_d (µM)	0.11	0.11	n.d.	n.d.
ITC K_d (µM)	0.082	0.076	n.d.	n.d.
DSF (Tm Shift) (K)	3.61	5.13	0.00	-0.02

Potency in Cells and Cellular Target Engagement:

In a NanoBRET cellular target engagement assay, PFI-6 displays potent binding, with an average IC_{50} value of 0.76 µM (±0.1). In comparison, PFI-6N shows no binding properties (up to 30 µM). Further in cell validation using a Fluorescence Recovery After Photobleaching (FRAP) assay has been used to confirm target inhibition by PFI-6.