GNE-2256: A Chemical Probe for IRAK4

Version 1.0 (20th October 2021)

Web link for more details: https://www.sgc-ffm.uni-frankfurt.de/#!specificprobeoverview/GNE-2256

Overview

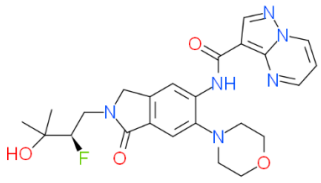
IRAK4 forms a critical node in multiple proinflammatory signaling pathways via the myddosome complex that transmits and amplifies the response of interleukin-1 receptor family and toll-like receptors to inflammatory cues. Upon activation, IRAK4 phosphorylates IRAK1 and triggers multiple signaling cascades leading to gene transcription and initiation of the inflammatory response with the release of cytokines such as Interferon alpha (IFNα). Secretion of IFNα leads to upregulation of several inflammatory proteins. The IFNα serum level is often enhanced in patients with systemic lupus erythematosus correlating with disease activity.

Summary

Chemical Probe Name	GNE-2256
Negative control compound	GNE-6689
Target(s) (synonyms)	IRAK4 (Interleukin 1 receptor associated kinase 4, NY-REN-64, LIMD67, REN64, IPD1)
Recommended *in vitro* assay concentration	Use with negative control GNE-6689 for best interpretation of data.
Suitability for *in* vivo use and recommended dose	Tested in mice, rat, dog and cynomolgus monkey at 1 mg/kg (po) and 1 mg/kg (iv). Shows pronounced and dose-dependent inhibition of TNFα at doses as low as 3 mg/kg in a TLR-challenge (toll-like receptor) mouse model.
Publications	PMID: 32184965, PMID: 32487715
Orthogonal chemical probes	
In vitro assay(s) used to characterise	FRET
Cellular assay(s) for target-engagement	NanoBRET, IL-6 human whole blood assay

Chemical Probe & Negative Control Structures and Use

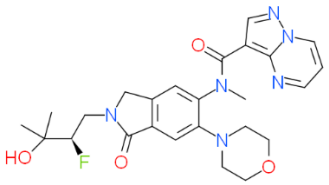
GNE-2256 Chemical Probe



SMILES:
CC(C)([C@@H](CN1Cc2cc(c(cc2C1=O)N1CCOCC1)NC(c1cnn2cccnc12)=O)F)O

InChiKey: AUYCSWFYKYVCLD-HXUWFJFHSA-N
Molecular weight: 482.21 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

GNE-6689 Negative Control



SMILES:
CC(C)([C@@H](CN1Cc2cc(c(cc2C1=O)N1CCOCC1)N(C)C(c1cnn2cccnc12)=O)F)O
InChiKey: JREVKKCSBRLATM-OAQYLSRUSA-N
Molecular weight: 496.22 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:

GNE-2256 shows potent activity on IRAK4 in the biochemical assay (FRET) (Ki = 1.4 nM). Closest off-targets in the kinase panel (221 at 1 µM; IC_{50} [nM]) are FLT3 (177), LRRK2 (198), NTRK2 (259), JAK1 (282), NTRK1 (313), JAK2 (486), MAP4K4 (680) and MINK1 (879). Off-targets with > 50% inhibition in the CEREP panel (36 at 1 µM) are TACR1, HTR2B and ACHE.

Potency in Cells and Cellular Target Engagement:

GNE-2256 is potent in the NanoBRET assay (IC_{50} = 3.3 nM), the IL-6 human whole blood assay (IC_{50} = 190 nM) and the IFNα human whole blood assay (IC_{50} = 290 nM).