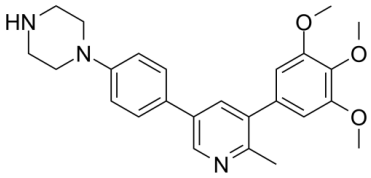
Data Sheet

Product Name:	LDN-214117
Cat. No.:	CS-3539
CAS No.:	1627503-67-6
Molecular Formula:	C25H29N3O3
Molecular Weight:	419.52
Target:	TGF-β Receptor
Pathway:	TGF-beta/Smad
Solubility:	DMSO : ≥ 100 mg/mL (238.37 mM)



BIOLOGICAL ACTIVITY:

LDN-214117 is a potent and selective ALK2 inhibitor with IC50 of 22 nM; > 100 fold selectivity for ALK5; also inhibits BMP6(IC50=100 nM). IC50 value: 22 nM(ALK2) [1] Target: ALK2 inhibitor LDN-214117 is a highly BMP selective compound, significantly biased toward ALK2 and its cognate ligands including BMP6 and also demonstrates a high degree of kinome selectivity and low cytotoxicity. LDN-214117 may be useful as highly selective probes of BMP-mediated cellular physiology that may provide a useful complement to the dorsomorphin class of compounds. Furthermore, this class of BMP inhibitors offers a structurally distinct template for the development of therapeutics for the treatment of BMP signaling-mediated diseases such as FOP.

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay(ALK2/ALK5) [1]: Purified recombinant ALK2 and ALK5 kinase proteins (Invitrogen), ATP (Sigma), ATP [γ-32P] (PerkinElmer), and dephosphorylated casein (Sigma) at final concentrations of 2.5 nM, 6 μM, 0.05 μCi μL.1, and 0.5 mg mL.1, respectively, were aliquoted in kinase buffer (Cell Signaling) containing 0.2% bovine serum albumin supplemented with 10 mM MnCl2 into 96-microwell plates, in combination with inhibitor compounds diluted at varying concentrations in kinase buffer (0.01 nM to 100 μM) in triplicate. In other experiments, purified recombinant ALK2 mutant kinase proteins34 were incubated with γ-32P and substrate under similar conditions, but in the presence of varying concentrations of unlabeled ATP, for the determination of Km and Vmax for each ALK2 mutant. Positive control samples lacking inhibitor compounds, and negative controls lacking recombinant kinase, were also measured in triplicate. The mixture was reacted at RT for 45 min, quenched with a final concentration of 2% phosphoric acid. The reaction mixture was transferred to 96-well P81 phosphocellulose filter plates (Millipore) and bound for 5 min. The plates were washed 20 times with 150 μL of 1% phosphoric acid solution per well by vacuum manifold. Plates were dried at RT for 1 h, sealed, and assayed with Microscint 20 scintillation fluid (PerkinElmer) using a Spectramax L luminometer (Molecular Devices) using the photon counting setting with an integration time of one second per well. Data was normalized to positive controls at 100% enzyme activity, with negative controls being subtracted as background. GraphPad (Prism software) was used for graphing and regression analysis by sigmoidal dose.response with variable Hill coefficient, or by Michaelis.Menten analysis for the determination of Km. Cell assay(Viability) [1]: HepG2 hepatocarcinoma cells were seeded in DMEM supplemented with 10% FBS at 25000 cells per well in tissue culture treated 96-well plates (Costar 3610; Corning). The cells were incubated for 2 h (37 °C and 5% CO2) and allowed to settle and attach. Compounds of interest or DMSO were diluted in DMEM and added at final compound concentrations of 1, 10, and 100 μM. Cells were incubated for 4 and 24 h, after which the media was discarded. Cells were lysed by adding 30 μL of passive lysis buffer (Promega) and shaken at RT for 15 min. Cell viability was determined by quantifying the ATP present in each well by adding 10 μL of Cell Titer Glo (Promega) per well and measuring the light output Spectramax L luminometer (Molecular Devices) with an integration time of one second per well. Data was normalized to 100% viability for cells receiving only DMSO without any concurrent compound.

References:

[1]. Mohedas AH, et al. Structure-activity relationship of 3,5-diaryl-2-aminopyridine ALK2 inhibitors reveals unaltered binding affinity for fibrodysplasia ossificans progressiva causing mutants. J Med Chem. 2014 Oct 9;57(19):7900-15.

CAIndexNames:

Piperazine, 1-[4-[6-methyl-5-(3,4,5-trimethoxyphenyl)-3-pyridinyl]phenyl]-

SMILES:

CC(C(C1=CC(OC)=C(OC)C(OC)=C1)=C2)=NC=C2C3=CC=C(N4CCNCC4)C=C3

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