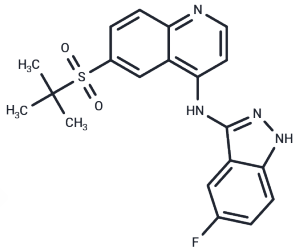
Data Sheet (Cat.No.T3537)

TargetMol

GSK583

Chemical Properties



CAS No. : 1346547-00-9

Formula: C20H19FN4O2S

Molecular Weight: 398.45

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	GSK583 is a highly effective and specific RIP2 kinase inhibitor (IC50: 5 nM).
Targets(IC50)	RIP kinase
In vitro	GSK583 possesses a comparable binding affinity for RIP3 kinase similarly to the RIP2 (RIP2/3 FP IC50: 5/16 nM) by an in-house FP binding assay configured. But GSK583 (10 μM) shows little or no inhibition of RIP3-dependent necroptotic cell death in a cellular assay. GSK583 effectively and dose-dependently inhibits MDP-stimulated TNFα production (IC50: 8 nM) in primary human monocytes. Following treatment with GSK583 at 1 μM, little inhibition of pro-inflammatory signaling is observed upon activation of cytokine receptors (IL-1R, TNFR) or Toll-like receptors (TLR2, TLR4, TLR7) but complete inhibition is observed upon activation of NOD1/2 receptors, which signal in a RIP2-dependent manner. Although it has excellent kinase selectivity, GSK583 does inhibit both the hERG channel and CYP3A4.
In vivo	GSK583 has moderate volumes of distribution, low clearance, and moderate oral bioavailability (in rat and mouse). Although GSK583 would not produce a human pharmacodynamic response within an acceptable dose range, the oral PK in mouse and rat supplies enough systemic exposure for use as a preclinical in vivo tool molecule in an acute inflammation challenge model.
Kinase Assay	A fluorescent polarization based binding assay is developed to quantitate interaction of novel test compounds at the ATP binding pocket of RIP2K by competition with a fluorescently labeled ATP competitive ligand. Full length FLAG His tagged RIP2K is purified from a baculovirus expression system and is used at a final assay concentration of twice the KD apparent. A fluorescent labeled ligand that is reversible and competitive with the inhibitors is used at a final assay concentration of 5 nM. Both the enzyme and ligand are prepared in solutions in 50 mM HEPES pH 7.5, 150 mM NaCl, 10 mM MgCl2, 1 mM DTT, and 1 mM CHAPS. Test compounds are prepared in 100% DMSO, and 100 nL is dispensed to individual wells of a multiwell plate. Next, 5 μL of RIP2K is added to the test compounds at twice the final assay concentration and incubated at room temperature for 10 min. Following the incubation, 5 μL of the fluorescent labeled ligand solution is added to each reaction at twice the final assay concentration and incubated at room temperature for at least 10 min. Finally, samples are read on an instrument capable of measuring fluorescent polarization. Test compound inhibition is expressed as percent

Kinase Assay	(%) inhibition of internal assay controls. For concentration response experiments, normalized data are fit using the following four parameter logistic equation: y = A + ((B-C))/(1+(10x)/(10C)D), where y is the % activity (% inhibition) at a specified compound concentration, A is the minimum % activity, B is the maximum % activity, C = log10(IC50), D = Hill slope, x = log10(compound concentration [M]), and pIC50 = (?C).
Cell Research	To assess cellular selectivity, monocytes are pretreated with inhibitor for 30 min, then stimulated for 6 h with ligands which selectively agonize NLRs NOD1, NOD2; Toll-like receptors TLR, TLR4, TLR7, or cytokine receptors IL-1R, TNFR. Release of pro-inflammatory cytokines, either TNFα (NOD2, TLR2, TLR4, IL1R) or IL-8 (NOD1, TLR7, TNFR), is measured by immunoassay. Percent inhibition and/or IC50 values are calculated. (Only for Reference)

Solubility Information

Solubility	DMSO: 45 mg/mL (112.94 mM),Sonication is recommended. H2O: < 1 mg/mL (insoluble or slightly soluble), Ethanol: 27 mg/mL (67.76 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (5.02 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.5097 mL	12.5486 mL	25.0973 mL
5 mM	0.5019 mL	2.5097 mL	5.0195 mL
10 mM	0.251 mL	1.2549 mL	2.5097 mL
50 mM	0.0502 mL	0.251 mL	0.5019 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Haile PA, et al. J Med Chem. 2016, 59(10):4867-80.