Takinib

Cat. No.: HY-103490

CAS No.: 1111556-37-6

Molecular Formula: $C_{18}H_{18}N_4O_2$

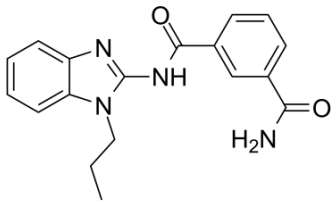
Molecular Weight: 322.36

Target: MAP3K; Apoptosis

Pathway: MAPK/ERK Pathway; Apoptosis

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 50 mg/mL (155.11 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.1021 mL	15.5106 mL	31.0212 mL
	5 mM	0.6204 mL	3.1021 mL	6.2042 mL
	10 mM	0.3102 mL	1.5511 mL	3.1021 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: 2.5 mg/mL (7.76 mM); Suspended solution; Need ultrasonic
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: 2.5 mg/mL (7.76 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

Takinib is a potent and selective TAK1 inhibitor with an IC_{50} of 9.5 nM, which inhibits autophosphorylated and non-phosphorylated TAK1 that binds within the ATP-binding pocket and inhibits by slowing down the rate-limiting step of TAK1 activation.

IC_{50} & Target

TAK1 9.5 nM (IC_{50})	IRAK4 120 nM (IC_{50})	IRAK1 390 nM (IC_{50})	GCK 430 nM (IC_{50})
CLK2 430 nM (IC_{50})	MINK1 1.9 μM (IC_{50})		

In Vitro	At 10 mM, Takinib shows significant inhibitory activity (<10% enzyme activity after exposure) on six serine/threonine kinases, including TAK1, IRAK4, IRAK1, GCK, CLK2, and MINK1[1]. Analysis reveals that increasing concentrations of Takinib leads to a decrease in V_{max} while maintaining K_M. When the enzyme is activated with 5 mM ATP for 3 hr, the same V_{max} is reached for 0, 10, 50, and 100 nM Takinib, and K_M increases for these concentrations, which implies that Takinib is an ATP-competitive inhibitor if TAK1 is ATP activated. Importantly, results show that Takinib inhibits the function of both activated and un-activated TAK1 with identical potency[1]. TNF-α stimulation in the presence of Takinib induces caspase activity in MDA-MB-231 cells in a dose-dependent manner, whereas unstimulated cells do not upregulate caspase activity. Takinib reduces phosphorylation significantly but does not influence total protein levels. Takinib inhibits phosphorylation of IKK, MAPK 8/9, and c-Jun in a dose-dependent manner. Takinib shows an almost complete inhibition of IL-6 secretion at micromolar concentrations following 24 hr of treatment in the presence of TNF-α[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Activity of purified TAK1-TAB1 protein is measured. In brief, TAK1-TAB1 (50 ng/well) is incubated with 5 μM ATP containing radiolabeled [^{32}P]-ATP in the presence of 300 mM substrate peptide (RLGRDKYKTLRQIRQ) in a final volume of 40 μL in the presence of buffer (containing 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% β-Mercaptoethanol, 10 mM magnesium acetate, 0.5 mM MnCl) and indicated compounds. The reaction is let go for 10 min and stopped with 10 μL concentrated H_3PO_4. The remaining activity is measured using a scintillation counter. Dose-response curves are repeated 3 times. For kinetic mechanistic studies, experiments are repeated two times and averaged[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	MDA-MB-231 cells (1,000 cells/well) are seeded in a 96-well plate with 10% FBS, 5% Pen/Strep, 4 g/L glucose DMEM medium. After 24h, cells are serum starved with 1% FBS, 5% Pen/Strep, 4 g/L glucose DMEM medium for 4h. Cells are treated with titrations of Takinib in the presence or absence of 30 ng/mL TNFα. Plates at 0 h and 24 h following treatment are frozen at -80°C after removal of media. After 24 h, 100 μL ddH_2O is added to each well and plates are refrozen. 1 μL from Hoechst stock [1 mg/mL in 1:4 DMSO/H_2O] is dissolved in 1 mL of TNE buffer (10 mM Tris, 2 M NaCl, 1 mM Na_2EDTA) and 100 μL of this solution is added to each well. The fluorescence is determined at 355/460 nm[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- ACS Cent Sci. 2018 Aug 22;4(8):982-995.
- J Cell Biol. 2018 Aug 6;217(8):2727-2742.
- J Am Heart Assoc. 2018 Dec 4;7(23):e03677.
- J Biol Chem. 2020 Oct 16;295(42):14325-14342.
- Mol Biol Cell. 2018 Oct 1;29(20):2470-2480.

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REFERENCES

[1]. Totzke J, et al. Takinib, a Selective TAK1 Inhibitor, Broadens the Therapeutic Efficacy of TNF-α Inhibition for Cancer and Autoimmune Disease. Cell Chem Biol. 2017 Aug 17;24(8):1029-1039.

[2]. Totzke J, et al. Takinib, a Selective TAK1 Inhibitor, Broadens the Therapeutic Efficacy of TNF-α Inhibition for Cancer and Autoimmune Disease. Cell Chem Biol. 2017 Aug

Caution: Product has not been fully validated for medical applications. For research use only.

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