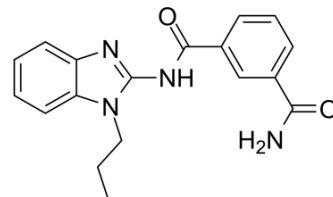


Takinib

Cat. No.:	HY-103490		
CAS No.:	1111556-37-6		
Molecular Formula:	C ₁₈ H ₁₈ N ₄ O 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 : 6.8 mg/mL (21.09 mM; Need ultrasonic and warming)					
	Preparing Stock Solutions	Solvent Concentration	Mass	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 IC₅₀ 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, GSK3, 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].

PROTOCOL

Kinase Assay ^[1]

Activity of purified TAK1-TAB1 protein is measured. In brief, TAK1-TAB1 (50 ng/well) is incubated with 5 μ M ATP containing radiolabeled [³²P]-ATP in the presence of 300 mM substrate peptide (RLGRDKYKTLRQIRQ) in a final volume of 40 μ L 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 μ L concentrated H₃PO₄. 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 TNF α . Plates at 0 h and 24 h following treatment are frozen at -80°C after removal of media. After 24 h, 100 μ L ddH₂O is added to each well and plates are refrozen. 1 μ L from Hoechst stock [1 mg/mL in 1:4 DMSO/H₂O] is dissolved in 1 mL of TNE buffer (10 mM Tris, 2 M NaCl, 1 mM Na₂EDTA) and 100 μ L 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 Aug 12;jbc.RA120.015283.
- 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 17;24(8):1029-1039.e7.

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

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