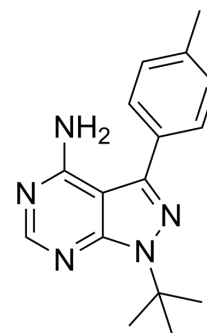


PP1

Cat. No.:	HY-13804		
CAS No.:	172889-26-8		
Molecular Formula:	C ₁₆ H ₁₉ N ₅		
Molecular Weight:	281.36		
Target:	Src; Apoptosis		
Pathway:	Protein Tyrosine Kinase/RTK; Apoptosis		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro	DMSO : 28 mg/mL (99.52 mM; Need ultrasonic)			
		Solvent Concentration	Mass	
			1 mg	5 mg
			10 mg	
Preparing Stock Solutions	1 mM	3.5542 mL	17.7708 mL	35.5417 mL
	5 mM	0.7108 mL	3.5542 mL	7.1083 mL
	10 mM	0.3554 mL	1.7771 mL	3.5542 mL
Please refer to the solubility information to select the appropriate solvent.				
In Vivo	<ol style="list-style-type: none"> Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 1.67 mg/mL (5.94 mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 1.67 mg/mL (5.94 mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 1.67 mg/mL (5.94 mM); Clear solution 			

BIOLOGICAL ACTIVITY

Description	PP1 is a potent, and Src family-selective tyrosine kinase inhibitor with IC ₅₀ of 5 and 6 nM for Lck and Fyn, respectively.
IC₅₀ & Target	IC ₅₀ : 5 nM (Lck), 6 nM (Fyn), 250 nM (EGFR), >50 μM (JAK2) ^[1]
In Vitro	PP1 inhibits Lck (IC ₅₀ =5 nM) and FynT (IC ₅₀ =6 nM) in vitro at concentrations significantly lower than those required to inhibit ZAP-70 (IC ₅₀ >100 μM), JAK2 (IC ₅₀ >50 μM), the EGFR kinase, and protein kinase A. PP1 inhibits whole cell tyrosine phosphorylation and proliferation in T cells stimulated with anti-CD3 and mitogens. PP1 selectively inhibits IL-2 gene

expression over GM-CSF and IL-2R gene induction in human T cells^[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[1]

Protein A-Sepharose beads (prepared as a 50% (w/v) suspension) are added to the antibody/lysate mixture at 250 µL/mL and allowed to incubate for 30 min at 4°C. The beads are then washed twice in 1 mL of lysis buffer and twice in 1 mL of kinase buffer (25 mM HEPES, 3 mM MnCl₂, 5 mM MgCl₂, and 100 µM sodium orthovanadate) and resuspended to 50% (w/v) in kinase buffer. Twenty-five microliters of the bead suspension is added to each well of the enolase-coated 96-well high protein binding plate together with an appropriate concentration of compound and [γ -³²P]ATP (25 µL/well of a 200 µCi/mL solution in kinase buffer). After incubation for 20 min at 20°C, 60 µL of boiling 2× solubilization buffer containing 10 mM ATP is added to the assay wells to terminate the reactions. Thirty microliters of the samples is removed from the wells, boiled for 5 min, and run on a 7.5% SDS-polyacrylamide gel. The gels are subsequently dried and exposed to Kodak X-AR film. For quantitation, films are scanned using a Molecular Dynamics laser scanner, and the optical density of the major substrate band, enolase p46, is determined. Concentrations of compound that causes 50% inhibition of enolase phosphorylation (IC₅₀) are determined from a plot of the density versus concentration of compound. In companion experiments for measuring the activity of compounds against Lck, the assay plate is washed with two wash cycles on a Skatron harvester using 50 mM EDTA, 1 mM ATP. Scintillation fluid (100 µL) is then added to the wells, and P incorporation is measured using a Pharmacia Biotech micro-β-counter. Concentrations of compound that causes 50% inhibition of enzyme activity (IC₅₀) are determined from a plot of the percent inhibition of enzyme activity versus concentration of compound^[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay ^[1]

Inhibition of anti-CD3-stimulated tyrosine phosphorylation in purified human peripheral blood T cells is measured as follows. All incubations are carried out at 37°C in an Eppendorf Thermomixer 5436 at a mixing setting of 11. Cells (1×10⁶ in 100 µL of RPMI 1640 medium) are incubated for 15 min with drug prior to a 6-min incubation with 1 µg of anti-CD3/mL (anti-leu4, 100 µg/mL). The final volume of the reaction is 115 µL. Reactions are terminated by the addition of 57.5 µL of 3× solubilization buffer incubated at 100°C prior to its addition. Samples are mixed, boiled for 5 min, and stored at -70°C. Western blots of these cell lysates, run on 10% SDS-polyacrylamide gels, are probed with a polyclonal anti-phosphotyrosine antibody, and immune complexes are detected with I-labeled protein A (ICN). For quantitation, films are scanned using a Molecular Dynamics laser scanner, and the optical densities of the major substrate band, p70, are quantitated in the presence of anti-CD3 (in the presence and absence of drug). Percent inhibition is calculated as follows: (1-(p70 optical density units in presence of drug/p70 units in absence of drug))×100. IC₅₀ equals the concentration of compound at which 50% inhibition is measured^[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Nat Commun. 2022 Sep 27;13(1):5675.
- J Thromb Haemost. 2021 Jan 27.
- Front Immunol. 2021 Nov 24;12:786602.
- J Cell Mol Med. 2019 Apr;23(4):2399-2409.
- BMC Cancer. 2022 Nov 24;22(1):1211.

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REFERENCES

[1]. Hanke JH, et al. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. J Biol Chem. 1996 Jan 12;271(2):695-701.

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

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