PP1

Cat. No.: HY-13804

CAS No.: 172889-26-8

Molecular Formula: $C_{16}H_{19}N_5$

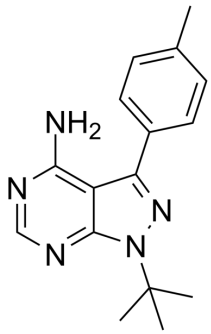
Molecular Weight: 281.36

Target: Src; Apoptosis

Pathway: Protein Tyrosine Kinase/RTK; Apoptosis

Storage:

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



SOLVENT & SOLUBILITY

In Vitro

DMSO : 28 mg/mL (99.52 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	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

1. 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
2. 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
3. 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.

IC_{50} & Target

IC50: 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 $MnCl_2$, 5 mM $MgCl_2$, 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-^{32}P]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 (IC_{50}) are determined from a plot of the percent inhibition of enzyme activity versus concentration of compound[1].

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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 (1×10^6 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. IC_{50} 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

- Front Immunol. 2021 Nov 24;12:786602.
- J Cell Mol Med. 2019 Apr;23(4):2399-2409.
- J Thromb Haemost. 2021 Jan 27.
- Patent. US20180263995A1.
- Harvard Medical School LINCS LIBRARY

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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.

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