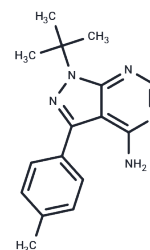


## PP1

## Chemical Properties

CAS No. :	172889-26-8
Formula:	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub>
Molecular Weight:	281.36
Appearance:	no data available
Storage:	Powder: -20°C for 3 years   In solvent: -80°C for 1 year



## Biological Description

Description	PP1 (AGL 1872), a specific and effective Src inhibitor, is with IC <sub>50</sub> for Lck/Fyn is 5 nM/ 6 nM, respectively.
Targets(IC <sub>50</sub> )	Apoptosis,EGFR,Bcr-Abl,c-Kit,Src
In vitro	PP1 is a nano-molar inhibitor of Lck and FynT, inhibits anti-CD3-induced protein-tyrosine kinase activity in T cells (IC <sub>50</sub> , 0.5 μM), demonstrates selectivity for Lck and FynT over ZAP-70, and preferentially inhibits T cell receptor-dependent anti-CD3-induced T cell proliferation (IC <sub>50</sub> , 0.5 μM) over non-T cell receptor-dependent phorbol 12-myristate 13-acetate/interleukin-2 (IL-2)-induced T cell proliferation. PP1 (1 μM) selectively inhibits the induction of the IL-2 gene, but not the granulocyte-macrophage colony-stimulating factor or IL-2 receptor genes. PP1 also inhibits Src (IC <sub>50</sub> , 170 nM) and Hck (IC <sub>50</sub> , 20 nM). PP1 is 50-100-fold less active in the inhibition of A-431 epidermal growth factor receptor autophosphorylation (IC <sub>50</sub> , 0.25 μM). [1] PP1 also inhibits Kit and Bcr-Abl tyrosine kinases with IC <sub>50</sub> of ~75 nM and 1 μM, respectively. PP1 completely abrogates the proliferation of M07e cells in response to SCF with IC <sub>50</sub> of 0.5-1 μM. PP1 (1 μM) inhibits SCF-induced c-Kit autophosphorylation in intact cells and blocks the activation of mitogen-activated protein kinase and Akt. PP1 inhibits the activity of mutant constitutively active forms of c-Kit (D814V and D814Y) found in mast cell disorders, and triggers apoptosis in the rat basophilic leukemia cell line RBL-2H3 that expresses mutant c-Kit. PP1 reduces the constitutive activation of signal transducer and activators of transcription 5 and mitogen-activated protein kinase and triggers apoptosis in FDCP1 cells expressing Bcr-Abl. [2]
Kinase Assay	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 <sub>2</sub> , 5 mM MgCl <sub>2</sub> , 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- <sup>32</sup> 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 (IC<sub>50</sub>) 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  $\mu$ L) is then added to the wells, and P incorporation is measured using a Pharmacia Biotech micro- $\beta$ -counter. Concentrations of compound that causes 50% inhibition of enzyme activity (IC<sub>50</sub>) are determined from a plot of the percent inhibition of enzyme activity versus concentration of compound[1].

## Cell Research

PP1 is dissolved in DMSO and stored, and then diluted with appropriate medium before use[2]. 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 $\times$ 10<sup>6</sup> in 100  $\mu$ L of RPMI 1640 medium) are incubated for 15 min with drug prior to a 6-min incubation with 1  $\mu$ g of anti-CD3/mL (anti-leu4, 100  $\mu$ g/mL). The final volume of the reaction is 115  $\mu$ L. Reactions are terminated by the addition of 57.5  $\mu$ L of 3 $\times$  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:  $(1 - (\text{p70 optical density units in presence of drug} / \text{p70 units in absence of drug})) \times 100$ . IC<sub>50</sub> equals the concentration of compound at which 50% inhibition is measured[1].

## Solubility Information

## Solubility

H<sub>2</sub>O: < 1 mg/mL (insoluble or slightly soluble),  
DMSO: 4 mg/mL (14.22 mM), Sonication is recommended.  
Ethanol: < 1 mg/mL (insoluble or slightly soluble),  
(< 1 mg/mL refers to the product slightly soluble or insoluble)

## Preparing Stock Solutions

	1mg	5mg	10mg
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
50 mM	0.0711 mL	0.3554 mL	0.7108 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.

### Reference

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