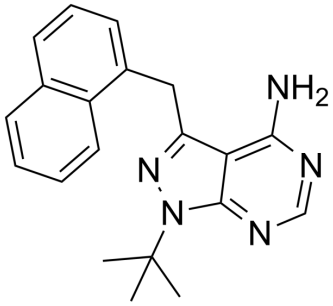
1-NM-PP1

Cat. No.:	HY-13942
CAS No.:	221244-14-0
Molecular Formula:	$C_{20}H_{21}N_5$
Molecular Weight:	331.41
Target:	Src
Pathway:	Protein Tyrosine Kinase/RTK
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 : 27.5 mg/mL (82.98 mM; Need ultrasonic and warming)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.0174 mL	15.0871 mL	30.1741 mL
	5 mM	0.6035 mL	3.0174 mL	6.0348 mL
	10 mM	0.3017 mL	1.5087 mL	3.0174 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 (7.54 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.54 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

1-NM-PP1, a cell-permeable PP1 analog, is a potent Src family kinases inhibitor with IC_{50}s of 4.3 nM and 3.2 nM for v-Src-as1 and c-Fyn-as1, respectively[1][2].

IC_{50} & Target

IC50: 4.3 nM (v-Src-as1), 3.2 nM (c-Fyn-as1), 28 μM (v-Src), 1 μM (c-Fyn), 3.4 μM (c-Abl), 29 μM (CDK2), 24 μM (CAMKII), 120 nM (cAbl-as2), 5 nM (CDK2-as1), 8 nM (CAMKII-as1)[2]

In Vitro

Cdk7 from $Cdk7^{as/as}$ or $Cdk7^{+/+}$ cells is immunoprecipitated and tested its kinase activity towards both a Pol II CTD-containing fusion protein (GST-CTD) and human Cdk2. Cdk7 recovered from the mutant, but not the wild-type, cells is inhibited by 1-NM-PP1 (1-NMPP1), with an IC_{50} of ~50 nM with either substrate. Replacement of wild-type Cdk7 with $Cdk7^{as/as}$ also rendered growth of HCT116 cells sensitive to 1-NM-PP1. In the absence of 1-NM-PP1, the wild-type and$Cdk7^{as/as}$

cells had population doubling times of ~17.9 and ~20.2 h, respectively, with similar cell-cycle distributions in asynchronous culture, indicating minimal impairment of Cdk7 function by the F91G mutation per se. The homozygous Cdk7$^{as/as}$ cells are sensitive to 1-NM-PP1, however, with an IC_{50} ~100 nM measured by cell viability (MTT) assays performed after 96 h of 1-NM-PP1 exposure. In contrast, wild-type HCT116 cells are resistant to 10 μM 1-NM-PP1. Addition of 10 μM 1-NM-PP1 retards G1/S progression by the mutant but not the wild-type cells. When added simultaneously with serum to the Cdk7$^{as/as}$ cells, 1-NM-PP1 prevents any progression into S phase in the next 15 h. After 24 h, there is evidence of progression into S-phase by a fraction of Cdk7$^{as/as}$ cells released from serum starvation directly into medium containing 1-NM-PP1, while a fraction remained in G1. The addition of 1-NM-PP1 3 h or 6 h after serum addition delays S-phase entry by ~7 h or by ~3 h, respectively[3].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]

Immunoblotting and immunoprecipitation, and kinase assays of immune complexes, are carried out. To measure Cdk1/cyclin B assembly, extracts (200 μg total protein) from cells in mitosis or G2 are pre-incubated with 2 μM 1-NM-PP1 or DMSO, then added 500 ng purified cyclin B1, amino-terminally tagged with hexahistidine and the Myc epitope, and an ATP-regenerating system. Where indicated, incubations are supplemented with 400 ng purified Csk1 or 600 ng wild-type or analog-sensitive, T-loop-phosphorylated Cdk7/cyclin H/Mat1 complex. After 90 min at room temperature, Myc-cyclin B and associated proteins are immunoprecipitated with anti-Myc antibodies and immune complexes are subjected to immunoblotting, with anti-Myc and anti-Cdk1 antibodies, and tested for histone H1 kinase activity[3].

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Cell Assay [1]

Wild-type or Cdk7$^{as/as}$ HCT116 cells are synchronized by incubation in serum-free medium for 48 h and released into medium containing 10% fetal calf serum. Synchronization with thymidine or nocodazole, and analysis of cell-cycle distribution by flow cytometry, are performed. Cell viability is measured by MTT assay[3].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell Biosci. 2021 May 21;11(1):93.
- Amyloid. 2019 Mar;26(1):24-33.
- Int J Parasitol. 2016 Jul;46(8):479-83.
- bioRxiv. 2023 Dec 19.
- Friedrich-Alexander University Erlangen-Nuremberg. Naturwissenschaftliche Fakultät. 2022 May.

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REFERENCES

[1]. Bishop A C, et al. Generation of Monospecific Nanomolar Tyrosine Kinase Inhibitors via a Chemical Genetic Approach. Journal of the American Chemical Society, 1999, 121(4):627-631.

[2]. Bishop AC, et al. A chemical switch for inhibitor-sensitive alleles of any protein kinase. Nature. 2000 Sep 21;407(6802):395-401.

[3]. Larochelle S, et al. Requirements for Cdk7 in the assembly of Cdk1/cyclin B and activation of Cdk2 revealed by chemical genetics in human cells. Mol Cell. 2007 Mar 23;25(6):839-50.

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

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