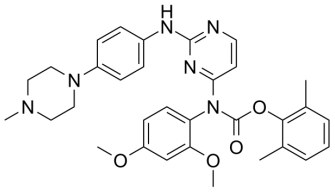
WH-4-023

Cat. No.:	HY-12299
CAS No.:	837422-57-8
Molecular Formula:	$C_{32}H_{36}N_6O_4$
Molecular Weight:	568.67
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 : 25 mg/mL (43.96 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.7585 mL	8.7924 mL	17.5849 mL
5 mM	0.3517 mL	1.7585 mL	3.5170 mL
10 mM	0.1758 mL	0.8792 mL	1.7585 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: ≥ 0.77 mg/mL (1.35 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 0.77 mg/mL (1.35 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

WH-4-023 is a potent and selective dual Lck/Src inhibitor with IC_{50} of 2 nM/6 nM for Lck and Src kinase respectively; little inhibition on p38α and KDR.

IC_{50} & Target

IC50: 2 nM (Lck), 6 nM (Src)[1]

In Vitro

WH-4-023 shows a similar potency increase on Lck as 2-substituted variants[1].

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

PROTOCOL

Kinase Assay [1]

The Lck HTRF kinase assay involves ATP-dependent phosphorylation of a biotinylated substrate peptide of gastrin in the presence or absence of inhibitor compound. The final concentration of gastrin is 1.2 μM. The final concentration of ATP is 0.5 μM (K_m app =0.6±0.1 μM), and the final concentration of Lck (a GST-kinase domain fusion (AA 225−509)) is 250 pM. Buffer conditions are as follows: 50 mM HEPES pH=7.5, 50 mM NaCl, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 2 mM DTT, 0.05% BSA. The assay is quenched and stopped with 160 μL of detection reagent. Detection reagents are as follows: Buffer made of 50 mM Tris, pH=7.5, 100 mM NaCl, 3 mM EDTA, 0.05% BSA, 0.1% Tween20. Prior to reading, Streptavidin allophycocyanin (SA-APC) is added at a final concentration in the assay of 0.0004 mg/mL, along with europilated anti-phosphotyrosine Ab (Eu-anti-PY) at a final conc of 0.025 nM. The assay plate is read in a Discovery fluorescence plate reader with excitation at 320 nm and emission at 615 and 655 nm[1].

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Cell Assay [1]

The purpose of this assay is to test the potency of T cell receptor (TCR; CD3) and CD28 signaling pathway inhibitors in human T cells. T cells are purified from human peripheral blood lymphocytes (hPBL) and preincubated with or without compound prior to stimulation with a combination of an anti-CD3 and an anti-CD28 antibody in 96-well tissue culture plates (1×10^5 T cells/well). Cells are cultured for ~20 h at 37°C in 5% CO_2 and then secreted IL-2 in the supernatants is quantified by cytokine ELISA. The cells remaining in the wells are then pulsed with ^{3}H-thymidine overnight to assess the T cell proliferative response. Cells are harvested onto glass fiber filters and ^{3}H-thymidine incorporation into DNA is analyzed by liquid scintillation counter. For comparison purposes, phorbol myristic acid (PMA) and calcium ionophore are used in combination to induce IL-2 secretion from purified T cells. Potential inhibitor compounds are tested for inhibition of this response as described above for anti-CD3 and -CD28 antibodies. Human whole-blood anti-CD3/CD28-induced IL-2 secretion assays are run in a similar fashion as described above using whole blood from normal volunteers diluted 50% in tissue culture medium prior to stimulation[1].

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

CUSTOMER VALIDATION

- Mol Cancer. 2022 Mar 18;21(1):77.
- Cell Mol Gastroenterol Hepatol. 2021;11(3):683-696.
- J Biol Chem. 2023 Nov 15:105462.
- Methods Mol Biol. 2023 Jun 24.

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REFERENCES

[1]. Martin MW, et al. Novel 2-aminopyrimidine carbamates as potent and orally active inhibitors of Lck: synthesis, SAR, and in vivo antiinflammatory activity. J Med Chem. 2006 Aug 10;49(16):4981-91.

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