# WH-4-023

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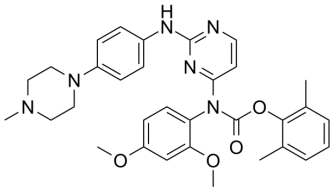
|---|---|
| **Cat. No.:** | HY-12299 |
| **CAS No.:** | 837422-57-8 |
| **Molecular Formula:** | $C_{32}H_{36}N_6O_4$ |
| **Molecular Weight:** | 568.67 |
| **Target:** | Src |
| **Pathway:** | Protein Tyrosine Kinase/RTK |
| **Storage:** | Powder -20°C 3 years<br>4°C 2 years<br>In solvent -80°C 2 years<br>-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 ($1\times10^5$ 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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