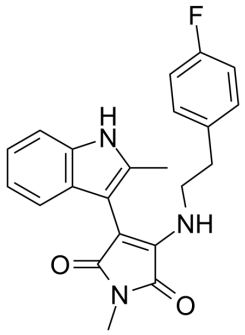
IM-12

Cat. No.:	HY-12292
CAS No.:	1129669-05-1
Molecular Formula:	$C_{22}H_{20}FN_3O_2$
Molecular Weight:	377
Target:	GSK-3
Pathway:	PI3K/Akt/mTOR; Stem Cell/Wnt
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 : ≥ 54.9 mg/mL (145.62 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.6525 mL	13.2626 mL	26.5252 mL
5 mM	0.5305 mL	2.6525 mL	5.3050 mL
10 mM	0.2653 mL	1.3263 mL	2.6525 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 (6.63 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

IM-12 is an inhibitor of GSK-3β, with an IC_{50} of 53 nM, and also enhances Wnt signalling.

IC_{50} & Target

GSK-3β
53 nM (IC_{50})

In Vitro

IM-12 inhibits GSK-3β in ReNcell VM cells, with I_{50} of 3.8 μM. IM-12 (3 μM) enhances the β-catenin amount, with no further effect at lower or higher concentration. IM-12 (3 μM) also attenuates the proliferation of ReNCell VM cells. IM-12 increases TCF-activity of ReNcell VM[1].

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

PROTOCOL

Kinase Assay [1]	Cells are lysed in RIPA buffer, supplemented with protease and phosphatase inhibitors and centrifuged for 5 min at 15,000 rpm. Immunoprecipitation of GSK-3β is performed with a specific mouse monoclonal anti GSK-3β [G8] antibody with 5 μg/sample for 2 h at 4°C. The bound protein is precipitated with Protein A/G-Plus agarose-beads (10 μL beads per sample). GSK-3β kinase activity is measured in a reaction mixture containing final concentrations of: 4 mM MOPS pH 7.2; 0.4 mM EDTA; 1 mM EGTA; 2.5 mM β-glycerophosphate; 4 mM $MgCl_2$; 40 μM BSA; 0.05 mM DTT. 10 μg/sample pGS-2 peptide substrate is used[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	To measure viable cells, 50-100 μL of cell suspension is analyzed using CASY technology with the appropriate program. ReNcell VM cells are seeded at a defined cell number and proliferated for 24 h. Then the medium is changed to proliferation medium with added substances at indicated concentrations. The cell number is determined every 24 h. Cells are exposed to the added drugs during the whole experiment, whereas the media is changed every 24 h[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- J Cell Physiol. 2019 Jul;234(7):10698-10708.
- Carcinogenesis. 2023 Jan 16;bgad003.
- Mol Med Rep. 2022 Oct;26(4):306.
- Methods Mol Biol. 2023 Jun 24.

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REFERENCES

[1]. Schmole AC, et al. Novel indolylmaleimide acts as GSK-3beta inhibitor in human neural progenitor cells. Bioorg Med Chem. 2010 Sep 15;18(18):6785-95.

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