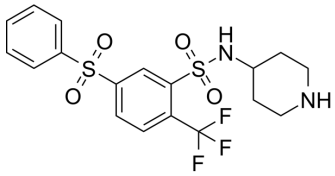
WAY 316606

Cat. No.:	HY-10858
CAS No.:	915759-45-4
Molecular Formula:	$C_{18}H_{19}F_3N_2O_4S_2$
Molecular Weight:	448.48
Target:	sFRP-1
Pathway:	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 : ≥ 100 mg/mL (222.98 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.2298 mL	11.1488 mL	22.2975 mL
5 mM	0.4460 mL	2.2298 mL	4.4595 mL
10 mM	0.2230 mL	1.1149 mL	2.2298 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.57 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.57 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

WAY 316606 is an inhibitor of the secreted protein sFRP-1, an endogenous antagonist of the secreted glycoprotein Wnt. The affinity of WAY-316606 for sFRP-1 is determined using the FP binding assay with IC_{50} of 0.5 μM[1].

IC_{50} & Target

IC50: 0.5 μM (sFRP-1)[1]

In Vitro

The EC_{50} of WAY-316606 for Wnt-Luciferase Activity from U2-OS Cells is 0.65 μM[1]. WAY-316606 binds to secreted frizzled-related protein (sFRP)-1 inhibitor with a K_D of 0.08 μM and inhibits sFRP-1 with an EC_{50} of 0.65 μM. WAY-316606 also binds to sFRP-2, albeit over 10 times weaker with a K_D of 1 μM. Using a fluorescence polarization binding assay that employs a fluorescent probe compound and purified human sFRP-1 protein in a competitive-binding format, the IC_{50} for WAY-316606 is

	0.5 μM[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	WAY-316606 increases bone formation when tested in a neonatal murine calvarial assay. WAY-316606 increases total bone area up to 60% in a dose-dependent manner with an EC_{50} of about 1 nM. WAY-316606 has good aqueous solubility, moderate to low inhibition of cytochrome p450 isozymes (3A4, 2D6, 2C9) and good stability in rat and human liver microsomes ($t_{1/2}$>60 min in each species). In female Sprague-Dawley rats, WAY-316606 exhibits high plasma clearance (77 mL/min/kg, greater than hepatic blood flow) following a single intravenous bolus dose (2 mg/kg), which results in a rapid decline of drug exposure in the plasma despite the route of administration[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [2]	WAY-316606 binding to purified sFRP is determined by spectroscopy methods. The sFRP-1 or -2 stock solutions are diluted to 1 μM in a buffered solution and the initial fluorescence is measured. Increasing concentrations of WAY-316606 (0 to 50 μM) are added to the protein in the cuvette and incubated for 5 min prior to assessing fluorescence intensity using a Fluoromax-2 fluorometer. In control experiments, the DMSO (vehicle control)-matched buffer solution is used. Fluorescence spectra are scanned in the ratio mode (S/R, signal/reference) to compensate for variations in lamp output as a function of wavelength. Fluorescence changes are fitted to a quadratic equation to obtain apparent dissociation constants[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	U2OS bone cells are infected with recombinant adenovirus 5 (Ad5)−WNT3 at a multiplicity of infection (MOI) of 2, followed by infection with Ad5-sFRP-1 and Ad5-16xTCF-luciferase, each at an MOI of 10. Four hours after infection, the cells are frozen in sterile cryogenic vials at a cell density of 9×10^6 cells/mL and stored in a -150°C freezer. For the assay, a vial of frozen cells is thawed, and the cells are resuspended in plating medium to a final cell density of 1.5×10^5 cells/mL. The resuspended cells are then plated in 96-well tissue culture treated plates at a volume of 100 μL of cell suspension/well (i.e., 1.5×10^4 cells/well). The plates are incubated at 37°C inside a 5% CO_2/ 95% humidified air incubator for 5 h or until the cells have attached and started to spread. Prior to the addition of WAY-316606, the medium is replaced with 50 μL/well of phenol red-free RPMI 1640. WAY-316606, or vehicle (typically DMSO), diluted in phenol red-free RPMI 1640 are then added to the wells in replicates of 4 wells/dilution and the plates are incubated at 37°C overnight. Dose−response experiments are performed with the compounds in 2-fold serial dilutions from 10000−4.9 nM. After the overnight incubation, the cells are washed twice with 150 uL/well of PBS w/o calcium or magnesium and lysed with 50 μL/well of 1× cell culture lysis reagent on a shaker at room temperature for 30 min. Aliquots of the cell lysates (30 μL) are transferred to 96-well luminometer plates, and the luciferase activity is measured in a MicroLumat PLUS luminometer using 100 μL/well of luciferase substrate. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Commun Biol. 2023 Aug 29;6(1):884.
- Front Pharmacol. 2021 Sep 2;12:724147.
- Int J Dev Neurosci. 2018 May;66:24-32.

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REFERENCES

[1]. Moore, WJ, et al. Modulation of Wnt Signaling Through Inhibition of Secreted Frizzled-Related Protein I (sFRP-1) with N-Substituted Piperidinyl Diphenylsulfonyl Sulfonamides. Journal of Medicinal Chemistry (2009), 52(1), 105-116.

[2]. Bodine PV, et al. A small molecule inhibitor of the Wnt antagonist secreted frizzled-related protein-1 stimulates bone formation. Bone. 2009 Jun;44(6):1063-8.

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