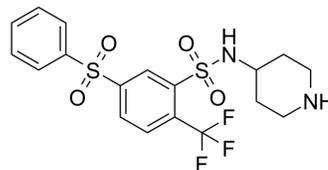


WAY 316606

Cat. No.:	HY-10858		
CAS No.:	915759-45-4		
Molecular Formula:	C ₁₈ H ₁₉ F ₃ N ₂ O ₄ S 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	6 months
		-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 100 mg/mL (222.98 mM)
 * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent		1 mg	5 mg	10 mg
	Concentration	Mass			
	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

- 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
- 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 IC₅₀ of 0.5 μM^[1].

IC₅₀ & Target

IC₅₀: 0.5 μM (sFRP-1)^[1]

In Vitro

The EC₅₀ 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 EC₅₀ 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 IC₅₀ 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 μL /well of PBS w/o calcium or magnesium and lysed with 50 μL /well of 1 \times 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

- 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.

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

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