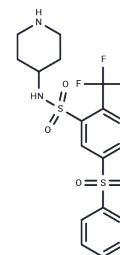


WAY 316606

## Chemical Properties

CAS No. : 915759-45-4  
 Formula: C<sub>18</sub>H<sub>19</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub>  
 Molecular Weight: 448.48  
 Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year  
 Actual storage temperature shall be subject to the COA.



## Biological Description

Description	WAY 316606 is an inhibitor of the secreted protein sFRP-1, an endogenous antagonist of the secreted glycoprotein [Wnt].
Targets(IC50)	Wnt/beta-catenin
In vitro	The EC <sub>50</sub> of WAY-316606 for Wnt-Luciferase Activity from U2-OS Cells is 0.65 μM[1]. WAY-316606 binds to the secreted frizzled-related protein (sFRP)-1 inhibitor with a KD of 0.08 μM and inhibits sFRP-1 with an EC <sub>50</sub> of 0.65 μM. WAY-316606 also binds to sFRP-2, albeit over 10 times weaker with a KD 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 <sub>50</sub> for WAY-316606 is 0.5 μM [2].
In vivo	WAY-316606 effectively promotes bone formation, as demonstrated in neonatal murine calvarial assays, with the capability to increase total bone area by up to 60% in a dose-dependent manner, achieving an EC <sub>50</sub> of approximately 1 nM. This compound exhibits favorable aqueous solubility, displays moderate to low inhibition of cytochrome P450 isozymes (3A4, 2D6, 2C9), and maintains good stability in both rat and human liver microsomes (t <sub>1/2</sub> >60 min for each species). Moreover, in female Sprague-Dawley rats, WAY-316606 shows high plasma clearance (77 mL/min/kg, surpassing hepatic blood flow) following a single intravenous bolus dose (2 mg/kg), leading to a swift reduction in plasma drug levels regardless of administration route [2].
Kinase Assay	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 [2].
Cell Research	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 <sup>6</sup> 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 [phenol red-free RPMI 1640 medium containing 5% fetal calf serum, 2 mM

Cell Research	GlutaMAX-I, and 1% (v/v) penicillin-streptomycin] to a final cell density of 1.5×10 <sup>5</sup> 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 <sup>4</sup> cells/well). The plates are incubated at 37°C inside a 5% CO <sub>2</sub> / 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 containing 10% fetal calf serum, 2 mM GlutaMAX-I, and 1% (v/v) penicillin-streptomycin. WAY-316606, or vehicle (typically DMSO), diluted in phenol red-free RPMI 1640 containing 2 mM GlutaMAX-I, and 1% (v/v) penicillin-streptomycin 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 a luciferase substrate.
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### Solubility Information

Solubility	DMSO: 125 mg/mL (278.72 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 10 mg/mL (22.3 mM),Solution. 10% DMSO+90% Saline: < 10 mg/mL (22.3 mM),Lower concentrations may be soluble, but exact solubility limit is unknown. <i>Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.</i>

### Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.2298 mL	11.1488 mL	22.2975 mL
5 mM	0.446 mL	2.2298 mL	4.4595 mL
10 mM	0.223 mL	1.1149 mL	2.2298 mL
50 mM	0.0446 mL	0.223 mL	0.446 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

### Reference

- 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.
- 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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