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产品名称：5-(苯磺酰基)-N-(哌啶-4-基)-2-(三氟甲基)苯磺酰胺
产品别名：WAY 316606

生物活性：

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.
IC_{50} & 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 KD 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 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 IC50 for WAY-316606 is 0.5 μM[2].
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 EC50 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].
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

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (5.57 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.57 mM，饱和度未知) 的澄清溶液。

	以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 2.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.57 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.57 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
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.
实验参考：	
Cell Assay	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 [phenol red-free RPMI 1640 medium containing 5% fetal calf serum, 2 mM GlutaMAX-I, and 1% (v/v) penicillin-streptomycin] 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 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 I % (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 luciferase substrate. [1]
inase 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. Fluorescence changes are fitted to a quadratic equation to obtain apparent dissociation constants[2].
	[1]. Moore, WJ, et al. Modulation of Wnt Signaling Through Inhibition of Secreted Frizzled-Related Protein I

References	(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.