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产品名称：CWHM-12
产品别名：CWHM-12

生物活性：

Description	CWHM-12 is a potent inhibitor of αV integrins with IC_{50}s of 0.2, 0.8, 1.5, and 1.8 nM for αvβ8, αvβ3, αvβ6, and αvβ1.
IC_{50} & Target	IC50: 0.2 nM (αvβ8), 0.8 nM (αvβ3), 1.5 nM (αvβ6), 1.8 nM (αvβ1), 61 nM (αvβ5)[1]
In Vitro	CWHM-12 (CWHM 12) also less potently inhibits αvβ5 (IC_{50}=61 nM) and αIIbβ3/α2β1/α10β1 (IC_{50}>5000 nM). CWHM-12 demonstrates high potency against all of the five possible β subunit binding partners (αvβ1, αvβ3, αvβ5, αvβ6 and αvβ8) in in vitro ligand-binding assays, with somewhat less potency against αvβ5 than against the other αv integrins[1].
In Vivo	Mice are treated with CCl_4 for 3 weeks to establish fibrotic disease and then treated with CWHM-12 (CWHM 12) or vehicle for the final 3 weeks of CCl_4. CWHM-12 significantly reduces liver fibrosis even after fibrotic disease have been established. Digital image quantitation demonstrates significantly reduced p-SMAD3 signaling in the livers of CWHM-12 treated mice compare to controls, demonstrating that the protection from CCl_4-induced hepatic fibrosis observed in CWHM-12 treated mice is due at least in part to a reduction in TGF-β activation by αv integrins. Besides, administration of CWHM-12 significantly inhibited progression of pulmonary fibrosis[1].
Solvent&Solubility	***In Vitro:*** **DMSO : 100 mg/mL** (**169.36 mM;** Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.6936 mL	8.4678 mL	16.9357 mL
	5 mM	0.3387 mL	1.6936 mL	3.3871 mL
	10 mM	0.1694 mL	0.8468 mL	1.6936 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 (4.23 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.23 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 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

	2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (4.23 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.23 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。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (4.23 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.23 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]. Henderson NC, et al. Targeting of αv integrin identifies a core molecular pathway that regulates fibrosis in several organs. Nat Med. 2013 Dec;19(12):1617-24.

实验参考：

Cell Assay	The stably transfected human 293 cells over-expressing human αvβ3 or αvβ5 are pre-incubated in HBSS buffer containing 200 μM $MnCl_2$ for 30 min at 37°C with 3-fold dilutions of compound (e.g., CWHM-12). Each sample is then added to triplicate wells of a 96-well plate which has been coated overnight at 4°C with a predetermined optimal concentration of purified vitronectin, washed, blocked by 1 hr incubation with BSA, and washed again. Cells are allowed to attach for 30 min at 37°C, and non-adherent cells are removed by washing. Remaining attached cells are measured by endogenous alkaline phosphatase activity using para-nitrophenyl phosphate and reading absorbance signal at 405 nM. The same procedure is used to measure adhesion of αvβ6-expressing human HT-29 cells to purified human latency associated peptide, and α5β1-expressing human K562 cells to human plasma fibronectin. In all cell-based assays, binding by the expected integrin is verified by testing activity of corresponding isotype-matched positive (function-blocking) and negative control antibodies[1].
Animal Administration	Mice[1] The mTmG (Td tomato/EGFP) and Ai14 (Rosa-CAG-LSL-tdTomato-WPRE) mice are used and crossed with *Pdgfrb*-Cre mice. Wild type C57/BL6 mice, $Itgav^{flox/flox}$ mice and $itgb8^{flox/flox}$ mice are used. Mice used for all experiments are 8-12 weeks old and are housed under specific pathogen-free conditions. For all studies CWHM-12 and CWHM-96 are solubilized in 50% DMSO (in sterile water) and dosed to 100 mg/kg/day. Drug or vehicle (50% DMSO) are delivered by implantable ALZET osmotic minipumps. For CCl_4-induced fibrosis, pumps are inserted subcutaneously either before the first dose of CCl_4 (prophylactic) or after 3 weeks of treatment (therapeutic) and livers are harvested after 6 weeks. For Bleomycin-induced fibrosis pumps are inserted 14 days after treatment with Bleomycin or saline and lungs are harvested at 28 days (therapeutic only).
	Functions of integrins αvβ1, αvβ8, α2β1 and α10β1 are measured using cell-free receptor-ligand interaction assays using purified recombinant human integrins. Ligands used are human fibronectin for αvβ1, human LAP for αvβ8, bovine collagen II for α2β1, and murine laminin I for α10β1. 96-well

Kinase Assay	plates are coated with the predetermined optimal concentration of ligand overnight, washed 3X with TBS+++ (25 mM Tris pH7.4, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1mM $CaCl_2$), and blocked with TBS+++/1%BSA. Purified integrin is diluted in TBS+++/0.1%BSA with or without compounds (e.g., CWHM-12), and the solution added to empty wells of the washed ligand-coated plate according to a standard template, with each sample repeated in triplicate. After incubation for 2 hr at room temperature, the plate is washed 3X with TBS+++. Biotin-labeled antibody against the αv subunit (αvβ1, αvβ8 assays) or β1 subunit (α2β1, α10β1 assays) is applied for 1 hr. The plate is washed 3X with TBS/0.1%BSA. Streptavidin-conjugated horseradish peroxidase is added to the wells, and the plate incubated for 20 min at room temperature. Following a 3X TBS+++ wash, bound integrin is detected using streptavidin-conjugated horseradish peroxidase and TMB substrate with absorbance measured at 650 nm. For assay of αIIbβ3 (IIbIIIa) function, plates are coated with the purified human integrin overnight, washed 3X with TBS+++, and blocked with TBS+++/1%BSA. Alexa Fluor647-labeled purified human fibrinogen is diluted in TBS+++/0.1%BSA with or without compounds, and the solutions are added to the integrin-coated plate. After 2 hr incubation, the plate is washed 3X with TBS+++, and bound ligand is detected by absorbance measured at 640/668nm. For all assays, concentration-response curves are constructed by non-linear regression analysis and IC_{50} values are calculated using GraphPad Prism software[1].
References	[1]. Henderson NC, et al. Targeting of αv integrin identifies a core molecular pathway that regulates fibrosis in several organs. Nat Med. 2013 Dec;19(12):1617-24.