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Produktinformation

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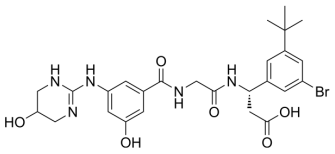
CWHM-12

Cat. No.:	HY-18644
CAS No.:	1564286-55-0
Molecular Formula:	$C_{26}H_{32}BrN_5O_6$
Molecular Weight:	590.47
Target:	Integrin
Pathway:	Cytoskeleton

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 (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

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (3.52 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (3.52 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (3.52 mM); Clear solution

BIOLOGICAL ACTIVITY

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].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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].

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PROTOCOL

Kinase Assay [1]

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 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].

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Cell Assay [1]

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] .

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Animal Administration [1]

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).

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CUSTOMER VALIDATION

- Cell. 2021 Sep 16;184(19):4919-4938.e22.
- Gut. 2021 Jan 19;gutjnl-2020-323719.
- Glia. 2022 Sep 15.
- Cells. 2022, 11(2), 237.
- Sci Rep. 2021 Mar 15;11(1):5885.

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REFERENCES

[1]. Basta J, Robbins L, Stout L, Prinsen MJ, Griggs DW, Rauchman M. Pharmacologic inhibition of RGD-binding integrins ameliorates fibrosis and improves function following kidney injury. Physiol Rep. 2020;8(7):e14329.

[2]. 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.

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