

Product Data Sheet

Product Name: BML-284 (Wnt agonist 1)

Cat. No.: GC17764

Chemical Properties

Cas. No. 853220-52-7

Chemical Name (Z)-1-(benzo[d][1,3]dioxol-5-yl)-N-(2-imino-6-(3-methoxyphenyl)-2,3-dihydropyrimidin-4(1H)-ylidene)methanamine hydrochloride

SMILES COC1=CC=CC(C(=N2C(=NC(=C2)N=C3C=CC(=C(C(=O)O4)C=C3)=C1.Cl

Formula $C_{19}H_{19}ClN_4O_3$

M.Wt 386.83

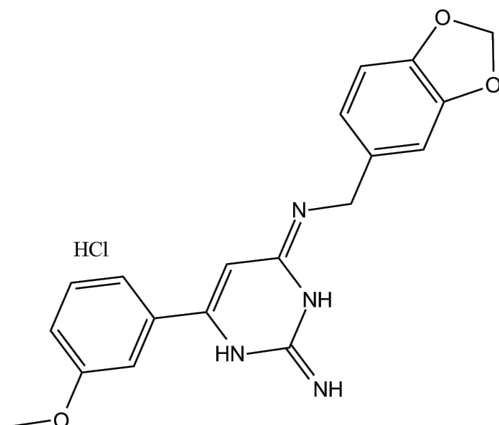
Solubility $\geq 38.7\text{mg/mL}$ in DMSO

Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Cell experiment [1]:

Cell lines HeLa cells

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HeLa cells were seeded in 96-well plates at a density of 5000 cells/well and kept in an incubator with 5% CO₂ at 37°C overnight. Subsequently, the cells were treated with the BML-284 at the indicated concentrations (0.001, 0.01, 0.1, 1, and 10µM) for 72h. Paclitaxel was used as the positive control. MTT powder was dissolved in physiological saline to obtain a 5mg/ml solution; then, 20µl MTT solution was added to each well and incubated at 37°C for 2h. The absorbance of each well was determined at 570nm.

Reaction Conditions 0.001, 0.01, 0.1, 1, and 10µM; 72h

Applications BML-284 treatment inhibited the cell viability of HeLa cells in a dose-dependent manner.

Animal experiment [2]:

Animal models Male Sprague-Dawley rats

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Preparation Method	Seventy-two male Sprague-Dawley rats (8 weeks of age, 220±20g) were housed individually in cages maintained at 23±1°C with a 12-h light/dark cycle and ad libitum access to food and water. After 1 week of acclimatization, isoproterenol (ISO) was dissolved in normal saline and injected subcutaneously into the rats at a dose of 5mg/kg/day for 14 consecutive days to establish a rat model of myocardial fibrosis (MF). Rats were randomly divided into four groups: normal control group (NC group), ISO group, TP+ISO group, and BML-284+TP+ISO group. Rats in the ISO, TP+ISO, and BML-284+TP+ISO groups were injected subcutaneously with ISO (5mg/kg/day), and rats in the NC group were injected subcutaneously with the same volume of normal saline. Rats in TP+ISO group were injected subcutaneously with TP at a concentration of 150µg/kg. In the BML-284+TP+ISO group of rats, a daily intravenous injection of 500µl of BML-284 (2mg/ml) was administered, along with subcutaneous injections of TP (µg/kg) and ISO (5mg/kg) over a period of 14 days. Rat hearts were removed for analysis.
Dosage form	500µl (2mg/ml); once a day for 14 days; i.v.
Applications	BML-284 antagonized the protective effect of TP against MF by activating the Wnt/β-catenin pathway and aggravated cardiac dysfunction in rats.

References:

- [1] Zhang C, Yan W, Liu Y, et al. Structure-based design and synthesis of BML284 derivatives: A novel class of colchicine-site noncovalent tubulin degradation agents[J]. European Journal of Medicinal Chemistry, 2024, 268: 116265.
- [2] Zhang Y, Lu F. Molecular mechanism of triptolide in myocardial fibrosis through the Wnt/β-catenin signaling pathway[J]. Scandinavian Cardiovascular Journal, 2024, 58(1): 2295785.

Background

BML-284 (Wnt agonist 1) is a potent agonist of Wnt signaling, with an EC₅₀ value of 0.7µM for inducing β-catenin- and TCF-dependent transcriptional activity^[1]. BML-284 binds to the colchicine-

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site and forms a low barrier hydrogen bond with E198 of β -tubulin, to mediate $\alpha\beta$ -tubulin degradation through the ubiquitin-proteasome pathway^[2]. BML-284 has been widely used as a model compound to synthesize a series of photosensitive azo derivatives that undergo reversible cis-trans isomerization upon visible light irradiation, with the cis-isomer capable of activating Wnt signaling ^[3].

In vitro, BML-284 treatment for 72 hours significantly inhibited the proliferation of HeLa, HCT116, MCF-7 and K562 cells with IC₅₀ values of 0.09 μ M, 0.52 μ M, 0.69 μ M and 0.10 μ M, respectively ^[4]. Treatment with 1 μ M BML-284 for 24 hours promoted invasion and inhibited apoptosis of A549 cells transfected with pcDNA-AOC4P^[5]. Pre-incubation of human aortic vascular smooth muscle cells (HA-VSMCs) with 10 μ M BML-284 for 24 hours activated the Wnt signaling pathway, increased the protein expression of Wnt3a and β -catenin, and reversed the protective effects of si-SOX2 on calcification, osteogenic/contractile protein expression, and autophagy activity^[6].

In vivo, BML-284 treatment (5 μ l; 0.5 μ g/eye) via injection into the conjunctiva adjacent to the limbus for 48 hours promoted corneal epithelial healing and corneal nerve repair in diabetic mice^[7]. BML-284 treatment via daily intravenous injection at a dose of 500 μ l (2mg/ml) for 14 days antagonized the protective effect of Triptolide (TP) against myocardial fibrosis (MF) by activating the Wnt/ β -catenin pathway, and aggravated cardiac dysfunction in rats^[8].

References:

- [1] Liu J, Wu X, Mitchell B, et al. A small-molecule agonist of the Wnt signaling pathway[J]. *Angewandte Chemie International Edition*, 2005, 44(13): 1987-1990.
- [2] Yang J, Li Y, Qiu Q, et al. Small molecules promote selective denaturation and degradation of tubulin heterodimers through a low-barrier hydrogen bond[J]. *Journal of Medicinal Chemistry*, 2022, 65(13): 9159-9173.
- [3] Ahmad S, Hashim P K, Imajo M, et al. Photoswitchable agonists for visible-light activation of the Wnt signaling pathway[J]. *Organic & Biomolecular Chemistry*, 2025, 23(17): 4240-4245.
- [4] Zhang C, Yan W, Liu Y, et al. Structure-based design and synthesis of BML284 derivatives: A novel class of colchicine-site noncovalent tubulin degradation agents[J]. *European Journal of Medicinal Chemistry*, 2024, 268: 116265.
- [5] Li F, Rong T, Cao G, et al. AOC4P suppresses viability and invasion and induces apoptosis in NSCLC cells by inhibiting the Wnt/ β -catenin pathway[J]. *Chemico-Biological Interactions*, 2020, 325: 109110.

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[6] Xiang H, Hong J, Zhang Y, et al. Inhibition of SOX2 mitigates vascular calcification mediated by autophagy through Wnt signaling pathway[J]. Egyptian Journal of Medical Human Genetics, 2025, 26(1): 189.

[7] Yang S, Zhang Y, Zhang Z, et al. Insulin promotes corneal nerve repair and wound healing in type 1 diabetic mice by enhancing Wnt/ β -catenin signaling[J]. The American Journal of Pathology, 2020, 190(11): 2237-2250.

[8] Zhang Y, Lu F. Molecular mechanism of triptolide in myocardial fibrosis through the Wnt/ β -catenin signaling pathway[J]. Scandinavian Cardiovascular Journal, 2024, 58(1): 2295785.

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