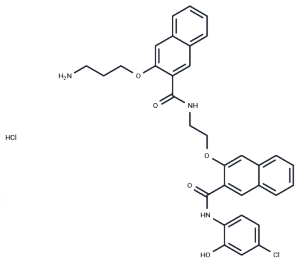
Data Sheet (Cat.No.T5318)

TargetMol

666-15

Chemical Properties



CAS No. : 1433286-70-4

Formula: C33H31Cl2N3O5

Molecular Weight: 620.52

Storage: Store at low temperature
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	666-15 is a selective CREB inhibitor with an IC50 of 81 nM. 666-15 can effectively inhibit the growth of breast cancer.
Targets(IC50)	Epigenetic Reader Domain
In vitro	**METHODS**: 666-15 was used to treat OA-like chondrocytes at a concentration of 62.5 to 500nM, and the viability of 666-15 on OA-like aged guinea pig chondrocytes was studied. **RESULTS** p-CREB1 protein levels were significantly reduced and cell viability of aged guinea pig chondrocytes was significantly increased. [1] **METHODS**: To investigate the biological effects of CREB inhibition, SySa cells were incubated with increasing concentrations (0.13-5 μM) of 666-15 and subjected to MTT assay. **RESULTS** 666-15 significantly inhibited SySa cell viability in a dose-dependent manner (IC50: 0.36-1.72 μM). [2]
In vivo	**METHODS**: Mice were randomly assigned to different groups, including normal group, sham operation group, ACLT+solvent control group, and two different doses of 666-15 treatment groups (ACLT+666-15 low-dose group, 5 mg/kg; ACLT+666-15 high-dose group, 10 mg/kg, intraperitoneal injection, once a week for six weeks). **RESULTS** 666-15 treatment can alleviate joint degeneration, reduce the levels of cartilage degradation markers such as CTX-II, reduce synovial inflammation, and has no effect on the body weight of mice, indicating its safety. [1]
Cell Research	HEK 293T cells in a 10 cm plate were transfected with pCRE-RLuc (6 μg) with Lipofectamine2000 following the manufacturer's instructions. Three hours after transfection, the cells were collected and replated into 96-well plates at ~10?000 cells/well. The cells were allowed to attach to the bottom of the plates overnight. The cells were then treated with different concentrations of different compounds for 30 min when forskolin (10 μM) was added to each well. The cells were incubated for further 5 h before cell lysis using 1× 30 μL Renilla luciferase lysis buffer. An amount of 5 μL of the lysate was combined with 30 μL of benzyl-coelenterazine solution in PBS (pH 7.4, 10 μg/mL). The protein concentration in each well was determined. The Renilla luciferase activity was normalized to protein content in each well and expressed as relative luciferase unit/μg protein (RLU/μg protein). The IC50 was derived from nonlinear

Cell Research	regression analysis of the RLU/μg protein-concentration curve [1].
Animal Research	Each 6- to 8-week old BALB/c nude mouse was inoculated subcutaneously at the right flank with MDA-MB-468 cells (5 × 10^6) in 0.1 mL of HBSS with Matrigel (1:1) for tumor development. When the tumor volume reached approximately 100 mm3, the mice were randomized to be treated with either vehicle or 666-15 at 10 mg/kg. 3i was dissolved in 1% N-methylpyrrolidone (NMP), 5% Tween-80 in H2O. The dosing solution was prepared weekly. The mice were treated once a day for 5 consecutive days a week, and the treatment lasted for 5 weeks. During the treatment, the tumor size and body weight were measured 2-3 times a week. The tumors were measured in two dimensions using a digital caliper, and the volume was expressed in mm3 using the formula V = 0.5ab2, where a and b represent the long and short diameters of the tumor, respectively. The tumor volume was normalized to the initial tumor volume at the time of the first treatment [1].

Solubility Information

Solubility	DMSO: 60 mg/mL (96.69 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: 2 mg/mL (3.22 mM),Sonication is recommended. *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.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.6116 mL	8.0578 mL	16.1155 mL
5 mM	0.3223 mL	1.6116 mL	3.2231 mL
10 mM	0.1612 mL	0.8058 mL	1.6116 mL
50 mM	0.0322 mL	0.1612 mL	0.3223 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

Wang Y, et al. The CREB1 inhibitor 666-15 maintains cartilage homeostasis and mitigates osteoarthritis progression. Bone Joint Res. 2024 Jan 2;13(1):4-18.
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Cyra M, et al. SS18-SSX drives CREB activation in synovial sarcoma. Cell Oncol (Dordr). 2022 Jun;45(3):399-413.
Zheng Y, Ding W, Zhang T, et al. Antimony-induced astrocyte activation via mitogen-activated protein kinase activation-dependent CREB phosphorylation. Toxicology Letters. 2021
Zou L, Hong D, Li K, et al. Salt-inducible kinase 2 (SIK2) inhibitor ARN-3236 attenuates bleomycin-induced pulmonary fibrosis in mice. BMC Pulmonary Medicine. 2022, 22(1): 1-11
Xie F,et al. Identification of a Potent Inhibitor of CREB-Mediated Gene Transcription with Efficacious in Vivo Anticancer Activity. J Med Chem. 2015 Jun 25;58(12):5075-87.
Qin Y, et al. Interfering MSN-NONO complex-activated CREB signaling serves as a therapeutic strategy for triple-negative breast cancer. Sci Adv. 2020 Feb 19;6(8):eaaw9960.
Zhi Y, Lu C, Zhu G, et al. Positive regulation of the CREB phosphorylation via JNK-dependent pathway prevents antimony-induced neuronal apoptosis in PC12 cell and mice brain. NeuroToxicology. 2020 Dec;81:101-108