# 产品名称：Tubastatin-A
# 产品别名：Tubastatin A

## 生物活性：

**Description**

Tubastatin A is a potent and selective **HDAC6** inhibitor with **$IC_{50}$** of 15 nM in a cell-free assay, and is selective (1000-fold more) against all other isozymes except HDAC8 (57-fold more).

**$IC_{50}$ & Target**

| HDAC6 | HDAC8 | HDAC1 |
|---|---|---|
| 15 nM ($IC_{50}$) | 854 nM ($IC_{50}$) | 16400 nM ($IC_{50}$) |

**In Vitro**

Tubastatin A is substantially selective for all 11 HDAC isoforms and maintains over 1000-fold selectivity against all isoforms excluding HDAC8, where it has approximately 57-fold selectivity. In homocysteic acid (HCA) induced neurodegeneration assays, Tubastatin A displays dose-dependent protection against HCA-induced neuronal cell death starting at 5 μM with near complete protection at 10 μM[1]. At 100 ng/mL Tubastatin A increases Foxp3+ T-regulatory cells (Tregs) suppression of T cell proliferation in vitro[2]. Tubastatin A treatment in CC12 cells would lead to myotube formation impairment when alpha-tubulin is hyperacetylated early in the myogenic process; however, myotube elongation occurs when alpha-tubulin is hyeperacetylated in myotubes[3]. A recent study indicates that Tubastatin A treatment increases cell elasticity as revealed by atomic force microscopy (AFM) tests without exerting drastic changes to the actin microfilament or microtubule networks in mouse ovarian cancer cell lines, MOSE-E and MOSE-L[4].

**In Vivo**

Daily treatment of Tubastatin A at 0.5 mg/kg inhibits HDAC6 to promote Tregs suppressive activity in mouse models of inflammation and autoimmunity, including multiple forms of experimental colitis and fully major histocompatibility complex (MHC)-incompatible cardiac allograft rejection[2].

**Solvent&Solubility**

***In Vitro:***

**DMSO : ≥ 45 mg/mL (134.17 mM)**

* "≥" means soluble, but saturation unknown.

**Preparing Stock Solutions**

| Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|
| 1 mM | 2.9815 mL | 14.9076 mL | 29.8151 mL |
| 5 mM | 0.5963 mL | 2.9815 mL | 5.9630 mL |
| 10 mM | 0.2982 mL | 1.4908 mL | 2.9815 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: ≥ 0.94 mg/mL (2.80 mM); Clear solution

此方案可获得 ≥ 0.94 mg/mL (2.80 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 9.4 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；

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| | 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。<br><br>2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)<br>Solubility: ≥ 0.94 mg/mL (2.80 mM); Clear solution<br>此方案可获得 ≥ 0.94 mg/mL (2.80 mM，饱和度未知) 的澄清溶液。<br>以 1 mL 工作液为例，取 100 μL 9.4 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。<br><br>3.请依序添加每种溶剂： 10% DMSO →90% corn oil<br>Solubility: ≥ 0.94 mg/mL (2.80 mM); Clear solution<br>此方案可获得 ≥ 0.94 mg/mL (2.80 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。<br>以 1 mL 工作液为例，取 100 μL 9.4 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。 |
| **References** | [1]. Kyle V. Butler et al. Rational Design and Simple Chemistry Yield a Superior, Neuroprotective HDAC6 Inhibitor, Tubastatin A J. Am. Chem. Soc., 2010, 132 (31), pp 10842-10846<br>[2]. Kozyreva VK, et al. NEDD9 regulates actin dynamics through cortactin deacetylation in an AURKA/HDAC6-dependent manner. Mol Cancer Res. 2014 May;12(5):681-93.<br>[3]. de Zoeten EF, et al. Histone deacetylase 6 and heat shock protein 90 control the functions of Foxp3(+) T-regulatory cells. Mol Cell Biol. 2011 May;31(10):2066-78.<br>[4]. Brijmohan, Angela, et al. Role of HDAC6 in Transcription Factor EB Mediated Clearance of Misfolded Proteins in Chronic Kidney Disease. University of Toronto.Nov-2017.<br>[5]. Di Fulvio S, et al. Dysferlin interacts with histone deacetylase 6 and increases alpha-tubulin acetylation. PLoS One. 2011;6(12):e28563<br>[6]. Ketene AN, et al. Actin filaments play a primary role for structural integrity and viscoelastic response in cells. Integr Biol (Camb). 2012 May;4(5):540-9. |

**实验参考：**

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| **Cell Assay** | Primary cortical neuron cultures are obtained from the cerebral cortex of fetal Sprague-Dawley rats (embryonic day 17) as described previously. All experiments are initiated 24 hours after plating. Under these conditions, the cells are not susceptible to glutamate-mediated excitotoxicity. For cytotoxicity studies, cells are rinsed with warm PBS and then placed in minimum essential medium containing 5.5 g/L glucose, 10% fetal calf serum, 2 mM L-glutamine, and 100 μM cystine. Oxidative stress is induced by the addition of the glutamate analogue homocysteate (HCA; 5 mM) to the media. HCA is diluted from 100-fold concentrated solutions that are adjusted to pH 7.5. In combination with HCA, neurons are treated with Tubastatin A at the indicated concentrations. Viability is assessed after 24 hours by MTT assay. [1] |
| | The effects of HDAC6 targeting in dextran sodium sulfate (DSS) and adoptive transfer models of colitis are evaluated, using 10 mice per group. Freshly prepared 4% (wt/vol) DSS (MP Biomedicals) is added daily for 5 days to the pH-balanced tap water of WT B6 mice. Mice are treated daily for 7 days with tubacin or niltubacin (0.5 mg/kg of body weight/day, i.p.), and colitis is assessed by daily monitoring of body weight, stool consistency, and fecal blood. Stool consistency is scored as 0 (hard), 2 (soft), or 4 (diarrhea), and fecal blood (Hemoccult) is scored as 0 (absent), 2 (occult), or 4 (gross). To assess prevention of colitis in a T cell-dependent model, $CD^{4+}$ CD45RBhi T cells |

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| **Animal Administration** | ($1\times10^6$) isolated from WT mice using magnetic beads (>95% cell purity, flow cytometry) are injected i.p. into B6/Rag1$^{-/-}$ mice plus $CD^{4+}$ $CD^{25+}$ Tregs ($1.25\times10^5$) isolated using magnetic beads from HDAC6$^{-/-}$ or WT mice (>90% Treg purity, flow cytometry) and mice are monitored biweekly for clinical evidence of colitis. To assess therapy of established T cell-dependent colitis, B6/Rag1$^{-/-}$ mice are injected i.p. with $CD^{4+}$ CD45RBhi cells ($1\times10^6$). Once colitis has developed, mice also receive $CD^{4+}$ $CD^{25+}$ Tregs ($5\times10^5$ cells) isolated as described above from HDAC6$^{-/-}$ or WT mice or treatment with HDAC6i (tubastatin A) or HSP90i (17-AAG). Mice are monitored for continued weight loss and stool consistency. At the cessation of the study, paraffin sections of colons stained with Alcian Blue or hematoxylin and eosin are graded histologically or evaluated by immunoperoxidase staining for $Foxp^{3+}$ Treg infiltration. [2] |
| **References** | [1]. Kyle V. Butler et al. Rational Design and Simple Chemistry Yield a Superior, Neuroprotective HDAC6 Inhibitor, Tubastatin A J. Am. Chem. Soc., 2010, 132 (31), pp 10842-10846<br>[2]. Kozyreva VK, et al. NEDD9 regulates actin dynamics through cortactin deacetylation in an AURKA/HDAC6-dependent manner. Mol Cancer Res. 2014 May;12(5):681-93.<br>[3]. de Zoeten EF, et al. Histone deacetylase 6 and heat shock protein 90 control the functions of Foxp3(+) T-regulatory cells. Mol Cell Biol. 2011 May;31(10):2066-78.<br>[4]. Brijmohan, Angela, et al. Role of HDAC6 in Transcription Factor EB Mediated Clearance of Misfolded Proteins in Chronic Kidney Disease. University of Toronto.Nov-2017.<br>[5]. Di Fulvio S, et al. Dysferlin interacts with histone deacetylase 6 and increases alpha-tubulin acetylation. PLoS One. 2011;6(12):e28563<br>[6]. Ketene AN, et al. Actin filaments play a primary role for structural integrity and viscoelastic response in cells. Integr Biol (Camb). 2012 May;4(5):540-9. |