



上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话: 021-61312973 传真: 021-55068248
网址: www.shyuanye.com
邮箱: shyysw@sina.com

产品名称:

4-(2,3-DIHYDRO-1,4-BENZODIOXIN-6-YL)-2,4-DIHYDRO-5-[(5-NITRO-2-THIAZOLYL)THIO]-3H-1,2,4-TRIAZOL-3-ONE

产品别名: **BI-78D3**

| 生物活性: | | | | | |
|---|--|--------------------------------|-----------|------------|------------|
| Description | BI-78D3 functions as a substrate competitive inhibitor of JNK, inhibit the JNK kinase activity (IC50=280 nM). | | | | |
| IC50 & Target | JNK | | | | |
| | 280 nM (IC50) | | | | |
| In Vitro | BI-78D3, dose-dependently inhibits the phosphorylation of JNK substrates both in vitro and in cell. BI-78D3 is able to compete with the D-domain of JIP1 (amino acids 153-163; pepJIP1) for JNK1 binding (IC50=500 nM). Using the same in vitro LanthaScreen kinase assay and the same ATF2 substrate, BI-78D3 is found to be 100-fold less active vs. p38α, a member of the MAPK family with high structural similarity to JNK, and completely inactive against mTOR and PI3-kinase (α-isoform), both unrelated protein kinases. Furthermore, Lineweaver-Burk analysis clearly indicates that BI-78D3 is competitive with ATF2 for binding to JNK1 with an apparent Ki value of 200 nM. In an attempt to profile the properties of BI-78D3 in the context of a complex cellular milieu, the cell-based LanthaScreen kinase assay is used. In this assay BI-78D3 is able to inhibit TNF-α stimulated phosphorylation of c-Jun in cell (EC50=12.4 μM)[1]. | | | | |
| In Vivo | The link between ConA-induced liver failure, TNF receptor signaling, and JNK function has been established by studies employing JNK1 ^{-/-} and JNK2 ^{-/-} mice. For this analysis, insulin insensitive mice are injected only once with 25 mg/kg BI-78D3, 30 min before insulin injection. The effect of insulin on blood glucose levels is then measured. BI-78D3 results in a statistically significant reduction in blood glucose levels as compared with the vehicle control. Thus, the ability of BI-78D3 to abrogate ConA-induced liver damage and restore insulin sensitivity is consistent with its proposed function as an effective JNK inhibitor. Liquid chromatography/mass spectrometry bio-availability analysis demonstrates that BI-78D3 has favorable microsome and plasma stability (T1/2=54 min)[1]. | | | | |
| | In Vitro: DMSO : 100 mg/mL (263.59 mM; Need ultrasonic) | | | | |
| | Preparing Stock Solutions | Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
| | | 1 mM | 2.6359 mL | 13.1797 mL | 26.3595 mL |
| | | 5 mM | 0.5272 mL | 2.6359 mL | 5.2719 mL |
| | | 10 mM | 0.2636 mL | 1.3180 mL | 2.6359 mL |
| <p>*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。</p> <p>储备液的保存方式和期限 -80℃, 6 months; -20℃, 1 month。 -80℃ 储存时，请在 6 个月内使用， -20℃ 储存时，请在 1 个月内使用。</p> <p>In Vivo:</p> <p>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。</p> | | | | | |



上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话: 021-61312973 传真: 021-55068248
网址: www.shyuanye.com
邮箱: shyysw@sina.com

| | |
|-------------------------------|---|
| Solvent&Solubility | <p>——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</p> <p>1.请依序添加每种溶剂: 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline</p> <p>Solubility: ≥ 2.5 mg/mL (6.59 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (6.59 mM, 饱和度未知) 的澄清溶液。</p> <p>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。</p> <p>2.请依序添加每种溶剂: 10% DMSO→ 90% (20% SBE-β-CD in saline)</p> <p>Solubility: ≥ 2.5 mg/mL (6.59 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (6.59 mM, 饱和度未知) 的澄清溶液。</p> <p>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。</p> |
| References | [1]. Stebbins JL et al. Identification of a new JNK inhibitor targeting the JNK-JIP interaction site. Proc Natl Acad Sci U S A, 2008 Oct 28, 105(43):16809-13. |
| 实验参考: | |
| Cell Assay | <p>Mice^[1]</p> <p>ConA and BI-78D3 is injected i.v. at 10 mg/kg into 6 to 8 weeks old male BL/6 mice. For partial hepatectomy, mice are anesthetized with isoflurane and subjected to midventral laparotomy followed by removal of the left lateral and median lobes. Animals are killed, blood is collected by cardiac puncture, and livers are surgically removed. Serum is separated and analyzed for alanine-aminotransferase levels^[1].</p> <p>Eleven-week-old male BKS.Cg-+Lepr^{db}/+Lepr^{db}/OlaHsd db/db mice are randomized based on blood glucose levels acclimated three days before drug dosing. Blood glucose is read by using a hand-held glucose meter (Mice are fasted 6 h before i.p. (i.p.) administration of 25 mg/kg BI-78D3. Thirty minutes after test article administration, Bovine Insulin (I-0516 at 0.75 mg/kg) is administered via i.p. injection. Blood samples are taken at designated time points and blood glucose levels are measured as described. Food is returned three hours after test article administration^[1].</p> |
| Kinase Assay | <p>The cell based kinase assays for c-Jun and ATF2 phosphorylation carry out by using the LanthaScreen c-Jun (1-79) HeLa and LanthaScreen ATF2 (19-106) A549 cell lines which stably express GFP-c-Jun 1-79 and GFP-ATF2 19-106, respectively. Phosphorylation is determined by measuring the time resolved FRET (TR-FRET) between a terbium labeled phospho-specific antibody and the GFP-fusion protein. The cells are plated in white tissue culture treated 384 well plates at a density of 10,000 cell per well in 32 μL assay medium (supplemented with 1% charcoal/dextran-treated FBS, 100 U/mL Penicillin and 100 μg/mL Streptomycin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 25 mM Hepes, pH 7.3, and lacking phenol red). After overnight incubation, cells are pretreated for 60 min with BI-78D3 (0.001, 0.01, 0.1, 1, 10, and 100 μM) followed by 30 min of stimulation with 2 ng/mL of TNF-α that stimulates both JNK and p38. The medium is then removed by aspiration and the cells are lysed by adding 20 μL of lysis buffer (20</p> |



上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话: 021-61312973 传真: 021-55068248
网址: www.shyuanye.com
邮箱: shyysw@sina.com

| | |
|-------------------|---|
| | <p>mM Tris•HCl, pH 7.6, 5 mM EDTA, 1% Nonidet P-40 substitute, 5 mM NaF, 150 mM NaCl, and 1:100 protease and phosphatase inhibitor mix, SIGMA P8340 and P2850, respectively). The lysis buffer includes 2 nM of the terbium-labeled anti-pc-Jun (pSer73) or anti-pATF2 (pThr71) detection antibodies. After allowing the assay to equilibrate for 1 h at room temperature, TR-FRET emission ratios are determined on a BMG Pherastar fluorescence plate reader (excitation at 340 nm, emission 520 nm and 490 nm; 100 μs lag time, 200 μs integration time, emission ratio=Em520/Em490)[1].</p> |
| References | <p>[1]. Stebbins JL et al. Identification of a new JNK inhibitor targeting the JNK-JIP interaction site. Proc Natl Acad Sci U S A, 2008 Oct 28, 105(43):16809-13.</p> |



源叶生物