产品名称：**NQDI-1**
产品别名：**NQDI-1**

生物活性：

Description	NQDI-1 inhibits apoptosis signal-regulating kinase 1 (**ASK1**) with a **K_i** of 500 nM and an **IC_{50}** of 3 μM.
IC_{50} & Target	ASK1
	3 μM (IC_{50})
In Vitro	The selectivity of NQDI-1 is evaluated in vitro on four serine/threonine protein kinases (protein kinase CK2 (CK2), c-Jun N-terminal kinase 3 (JNK3), Rho-associated protein kinase 1 (Rock1), and Aurora A) and three tyrosine protein kinases (FGFR1, hHGFR, and endothelial TEK tyrosine kinase (Tie2)). The results show that NQDI-1 is a selective inhibitor of ASK1. The activity of FGFR1 protein kinase is inhibited by NQDI-1 (residual activity of 44%). NQDI-1 inhibits ASK1 with a Ki of 500 nM. Inhibition of ASK1 by NQDI-1 is competitive with respect to the phosphodonor substrate ATP[1].
In Vivo	250 nmol NQDI-1 in DMSO is intracerebroventricularly injected following brain insult. Western blotting is performed to determine the expression of ASK1 in the sham, Hypoxia-ischemia (HI), DMSO and NQDI-1 groups and indicate that NQDI-1 markedly inhibits the expression of ASK1 in the brain cortex, compared with the HI and DMSO group. Furthermore, immunofluorescence staining also indicates that the expression of ASK1 is inhibited by NQDI-1 in the brain cortex. The expression of downstream targets of ASK1 is also determined in the present study. The expression levels of p-JNK, p-c-Jun, p53 and caspase 3 are significantly decreased by NQDI-1, compared with the HI and DMSO groups. Low expression of p-JNK in the brain cortex is also observed by immunofluorescence in the NQDI-1-treated group[2].
Solvent&Solubility	***In Vitro:***
	DMSO : 10 mg/mL (**31.32 mM;** Need ultrasonic and warming)
	H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	3.1318 mL	15.6588 mL	31.3175 mL
5 mM	0.6264 mL	3.1318 mL	6.2635 mL
10 mM	0.3132 mL	1.5659 mL	3.1318 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: 0.71 mg/mL (2.22 mM); Suspended solution; Need ultrasonic

此方案可获得 0.71 mg/mL (2.22 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。

以 1 mL 工作液为例，取 100 μL 7.1 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。

	2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: 0.71 mg/mL (2.22 mM); Suspended solution; Need ultrasonic 此方案可获得 0.71 mg/mL (2.22 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 7.1 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。
References	[1]. Volynets GP, et al. Identification of 3H-naphtho[1,2,3-de]quinoline-2,7-diones as inhibitors of apoptosis signal-regulating kinase 1 (ASK1). J Med Chem. 2011 Apr 28;54(8):2680-6. [2]. Hao H, et al. NQDI-1, an inhibitor of ASK1 attenuates acute perinatal hypoxic-ischemic cerebral injury by modulating cell death. Mol Med Rep. 2016 Jun;13(6):4585-92.

实验参考：

Animal Administration	Rats[2] A total of 12 female Sprague-Dawley rats with litters of mixed gender pups are used. The mothers are housed at 25°C under a 12-h light/dark cycle, with ad libitum access to food and water, until the pups are 7-days-old. The HI model is established. The pups are anesthetized with 2.5% halothane and are intracerebroventricularly infused with DMSO or 250 nmol NQDI-1, dissolved in DMSO into the right cerebral hemisphere 30 min prior to HI using a 30-gauge needle with a 5 μL Hamilton syringe (infusion rate, 1 μL/min).
Kinase Assay	Enzyme activity of human protein kinases ASK1, Aurora A, ROCK1, HGFR, FGFR1, Tie2, JNK3, and CK2 is determined using in vitro kinase assay (γ-32P-ATP method). Each reaction mixture contains 6 μL of buffer solution (25 mM MOPS, pH 7.2, 2.5 mM EGTA, 2.5 mM EDTA, 0.5 mM DTT, 0.25 mg/mL BSA, 20 mM β-glycerophosphate), 3 μL of substrate solution (MBP, Long S6 kinase substrate peptide, KKKSPGEYVNIEFG, IGF-IRtide (12-527), TK substrate 2, JNK3tide, or RRRDDDSDDD for each kinase, respectively) (5.0 μg/μL), 0.3 μL of enzyme (protein kinase catalytic subunit, 0.1 μg/μL≈32 mU/μL), and 10.25 μL of H2O. The reaction mixture (total volume of 19 μL) is quickly added to 1.5 mL tubes at room temperature. The stock solutions of inhibitors (e.g., NQDI-1) are prepared in DMSO, and the concentration of inhibitor is 1 mM. The concentration of DMSO in the reaction does not exceed 3%. Then 1 μL of inhibitor solution in DMSO is added to each tube and mixed by pipetting. ATP solution is prepared separately. For each sample 0.05 mCi γ-[P32]ATP is taken (specific activity of 100 μCi/μM). The total concentration of labeled and unlabeled ATP is 100 μM. The reaction is started with addition of ATP solution (150 μM ATP, 30 mM MgCl2, 15 mM MOPS, pH 7.2). The time of reaction is 20 min at 30°C. The reaction is stopped by adding 20 μL of 0.5 M orthophosphoric acid. Then the reaction mixture is loaded on the 20 mm filter disks of the cellulose phosphate paper. Filters are washed three times with 0.075 M orthophosphoric acid at room temperature and dried. For detection of products, dried filters are counted by Tri-Carb 2800-TR liquid scintillation analyzer. Then 1 μL of DMSO is added to the reaction volume instead of the inhibitor stock solution for a positive control[1].
References	[1]. Volynets GP, et al. Identification of 3H-naphtho[1,2,3-de]quinoline-2,7-diones as inhibitors of apoptosis signal-regulating kinase 1 (ASK1). J Med Chem. 2011 Apr 28;54(8):2680-6. [2]. Hao H, et al. NQDI-1, an inhibitor of ASK1 attenuates acute perinatal hypoxic-ischemic cerebral injury by modulating cell death. Mol Med Rep. 2016 Jun;13(6):4585-92.