产品名称：**Salubrinal**
产品别名：**Salubrinal**

生物活性：

Description	Salubrinal is a cell-permeable and selective inhibitor of eIF2α dephosphorylation[1]. Salubrinal acts as a dual-specificity phosphatase 2 (Dusp2) inhibitor and suppresses inflammation in anti-collagen antibody-induced arthritis[2].
IC_{50} & Target	IC50: 1.7 μM (PP1)[1]
In Vitro	Salubrinal, a recently identified PP1 inhibitor capable to protect against endoplasmic reticulum (ER) stress in various model systems, strongly synergized with proteasome inhibitors to augment apoptotic death of different leukemic cell lines. Salubrinal preferentially seems to target the PP1/GADD34 complex, Salubrinal is of interest to examine whether the effect of Salubrinal could also be recapitulated by another inhibitor of this phosphatase. For this purpose cantharidin, wis selected, which is less toxic than okadaic acid, but which also blocks PP1 (IC_{50}=1.7 μM) activities[1].
In Vivo	Salubrinal is a synthetic chemical that inhibits de-phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α). Salubrinal significantly suppresses inflammation of the paws of CAIA mice. For instance, the clinical scores are 1.94±1.7 (placebo) and 0.31±0.6 (Salubrinal) on day 6; and 4.63±3.4 (placebo) and 1.09±1.6 (Salubrinal) on day 12. Consistent with the clinical scores, the thickening of the paws is also reduced in the Salubrinal-treated group. Furthermore, Salubrinal reduces the histological scores from 1.47±1.10 (N=16; placebo) to 0.59±0.64 (N=16; Salubrinal) (p=0.01)[2].

In Vitro:

DMSO : ≥ 50 mg/mL (**104.21 mM**)

H_2O : < 0.1 mg/mL (insoluble)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.0842 mL	10.4208 mL	20.8416 mL
	5 mM	0.4168 mL	2.0842 mL	4.1683 mL
	10 mM	0.2084 mL	1.0421 mL	2.0842 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。

Solvent&Solubility	***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: ≥ 2.5 mg/mL (5.21 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.21 mM，饱和度未知) 的澄清溶液。 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。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: 2.5 mg/mL (5.21 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (5.21 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 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。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.21 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.21 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。 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 玉米油中，混合均匀。
References	[1]. Drexler HC. Synergistic Apoptosis Induction in Leukemic Cells by the Phosphatase Inhibitor Salubrinal and Proteasome Inhibitors. PLoS One. 2009;4(1):e4161. [2]. Hamamura K, et al. Salubrinal acts as a Dusp2 inhibitor and suppresses inflammation in anti-collagen antibody-induced arthritis. Cell Signal. 2015 Apr;27(4):828-35.

实验参考：

Cell Assay	Cellular viability is assessed by the WST-1 colorimetric assay. Assays are performed on 96 well plates with 2×10^4 K562 cells/well in triplicate with Salubrinal concentrations ranging from 5-75 μM (total volume of 200 μL, 18 hrs). Untreated cells served as negative control sample[1].
Animal Administration	Mice[2] Using Balb/c female mice (~nine weeks old), CAIA is induced by intravenous injection of a 2 mg cocktail of ArthritoMAb antibodies on day 0 followed by intraperitoneal injection of 100 μg LPS on day 3. Mice are randomly divided into a placebo group and a Salubrinal-treated group. Salubrinal (2.0 mg/kg) is intravenously administered daily from day 0, while a solvent (49.5% PEG 400 and 0.5% Tween 80 in PBS) is administered to the placebo group.
	Phosphatase activities are determined on immunoprecipitates of the phosphatases. Briefly, 2×10^6 K562 cells are treated for 18 hr with Salubrinal (20 μM), PSI (10 nM), the combination of both drugs or okadaic acid (100 nM). After washing with PBS, cells are lysed for 15 min on ice either in PP1LB (for determination of PP1γ-activity; 20 mM Tris-HCl, pH 7.5, 1% Triton X-100, 10% glycerol, 132 mM NaCl, Roche complete protease inhibitor) or in RIPA (for PP2A), supplemented with Roche complete protease inhibitor). Cell lysates containing 500 μg (PP1γ) or 300 μg (PP2A) protein are

Kinase Assay	immunoprecipitated overnight at 4°C with 2-3 µg of the appropriate antibodies and then incubated with Protein A-Sepharose. Immunoprecipitates are washed three times in lysis buffer, followed by resuspension in phosphatase assay buffer (PP2A: 20 mM Tris-HCl, pH7.5, 0.1 mM $CaCl_2$; PP1γ: 50 mM Tris HCl pH 7.0, 0.2 mM $MnCl_2$, 0.1 mM $CaCl_2$, 125 µg/mL BSA, 0.05% Tween 20), supplemented with 100 µM 6,8-difluoro-4-methyl-umbelliferyl phosphate (DiFMUP). Precipitates are allowed to react with substrate for 1 hr at 37°C on an Eppendorf Thermoshaker, centrifuged and DiFMU fluorescence is measured on a BioTek Lambda Fluoro 320 microplate reader (360 nm_{ex}/460 nm_{em}). Phosphatase activities are given as percent change relative to the control (DMSO treated cells) [1]
References	[1]. Drexler HC. Synergistic Apoptosis Induction in Leukemic Cells by the Phosphatase Inhibitor Salubrinal and Proteasome Inhibitors. PLoS One. 2009;4(1):e4161. [2]. Hamamura K, et al. Salubrinal acts as a Dusp2 inhibitor and suppresses inflammation in anti-collagen antibody-induced arthritis. Cell Signal. 2015 Apr;27(4):828-35.