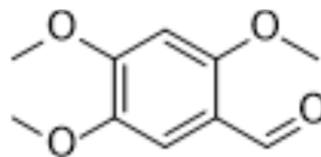


## Asaraldehyde

Cat. No.:	HY-100580
CAS No.:	4460-86-0
Molecular Formula:	C <sub>10</sub> H <sub>12</sub> O <sub>4</sub>
Molecular Weight:	196.2
Target:	COX
Pathway:	Immunology/Inflammation
Storage:	4°C, stored under nitrogen * In solvent : -80°C, 6 months; -20°C, 1 month (stored under nitrogen)



### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : 100 mg/mL (509.68 mM; Need ultrasonic)  
 Ethanol : 50 mg/mL (254.84 mM; Need ultrasonic)  
 H<sub>2</sub>O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	5.0968 mL	25.4842 mL	50.9684 mL
	5 mM	1.0194 mL	5.0968 mL	10.1937 mL
	10 mM	0.5097 mL	2.5484 mL	5.0968 mL

Please refer to the solubility information to select the appropriate solvent.

#### In Vivo

- Add each solvent one by one: 10% EtOH >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 3.75 mg/mL (19.11 mM); Clear solution
- Add each solvent one by one: 10% EtOH >> 90% corn oil  
Solubility: ≥ 3.75 mg/mL (19.11 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 2.5 mg/mL (12.74 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil  
Solubility: ≥ 2.5 mg/mL (12.74 mM); Clear solution

### BIOLOGICAL ACTIVITY

#### Description

Asarylaldehyde (Asaronaldehyde), a COX-2 inhibitor, significantly inhibits cyclooxygenase II (COX-2) activity with an IC<sub>50</sub> value of 100 µg/mL<sup>[1]</sup>.

#### IC<sub>50</sub> & Target

COX-2

## In Vitro

Asarylaldehyde (2,4,5-TMBA) is a natural COX-2 inhibitor, which isolated from carrot (*Daucus carota* L.) seeds significantly inhibits cyclooxygenase II (COX-2) activity at the concentration of 100 µg/mL compared to three commercial nonsteroidal anti-inflammatory drugs Aspirin, Ibuprofen, and Naproxen at their IC<sub>50</sub> values 180, 2.52, and 2.06 µg/mL, respectively. 2,4,5-TMBA, a natural inhibitor of cyclooxygenase-2, suppresses adipogenesis and promotes lipolysis in 3T3-L1 adipocytes. 2,4,5-Trimethoxybenzaldehyde (2,4,5-TMBA) present in plant roots, seeds, and leaves is reported to be a significant inhibitor of cyclooxygenase-2 (COX-2) activity at the concentration of 100 µg/mL. Because COX-2 is associated with differentiation of preadipocytes, the murine 3T3-L1 cells are cultured with 100 µg/mL of 2,4,5-TMBA during differentiation and after the cells are fully differentiated to study the effect of 2,4,5-TMBA on adipogenesis and lipolysis. Oil Red O staining and triglyceride assay revealed that 2,4,5-TMBA inhibited the formation of lipid droplets during differentiation; moreover, 2,4,5-TMBA down-regulated the protein levels of adipogenic signaling molecules and transcription factors MAP kinase kinase (MEK), extracellular signal-regulated kinase (ERK), CCAAT/enhancer binding protein (C/EBP)α, β, and δ, peroxisome proliferator-activated receptor (PPAR)γ, adipocyte determination and differentiation-dependent factor 1 (ADD1), and the rate-limiting enzyme for lipid synthesis acetyl-CoA carboxylase (ACC). In fully differentiated adipocytes, treatment with 2,4,5-TMBA for 72 h significantly decreased lipid accumulation by increasing the hydrolysis of triglyceride through suppression of perilipin A (lipid droplet coating protein) and up-regulation of hormone-sensitive lipase (HSL). When treated with 100 µg/mL of 2,4,5-TMBA for 24, 48, or 72 h, the viability of fully differentiated 3T3-L1 adipocytes is decreased by 8.35, 15.54, and 27.26%, respectively. When the preadipocytes are treated with 100 µg/mL of 2,4,5-TMBA for 24 h before differentiation medium is supplemented, the cell viability is decreased by 26.46%<sup>[1]</sup>. A COX-2 inhibitor 2,4,5-trimethoxybenzaldehyde (TMBA) is found to be the most abundant constituent, but is totally absent in its cultured broth and its natural host, *C. kanehirae* wood. 2,4,5-trimethoxybenzaldehyde (TMBA) is the major constituent in fruiting bodies<sup>[2]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## PROTOCOL

### Cell Assay<sup>[1]</sup>

3T3-L1 preadipocytes are seeded into 6-well plates at a concentration of 10<sup>5</sup>/well and cultured in DMEM supplemented with 10% bovine calf serum at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Two days after confluence, cells are cultured in FBS-containing DMEM (10%, v/v) with the addition of adipogenic factors (0.5 mM IBMX, 1 µM DEX, 5 µg/mL insulin) to induce differentiation (Day 0). Two days later (Day 2), the medium is changed to DMEM supplemented with 10% FBS and 5 µg/mL insulin for another two days. Afterward (Day 4), the medium is changed to DMEM supplemented with 10% FBS only. For the coculture study, 2,4,5-TMBA (0.1 g dissolved in 2 mL of DMSO) is added to the medium from Day 0 to Day 8 (final concentration 100 µg/mL). Control samples are prepared by adding isovolumetric DMSO to the culture medium. For the postculture study, 2,4,5-TMBA is added to the medium on Day 8 (when the cells are fully differentiated) at a final concentration of 100 µg/mL, followed by another 72 h culture. 3T3-L1 cells are seeded in 96-well plates at a concentration of 10<sup>4</sup>/well. Twenty-four hours after seeding, the cells are treated with 100 µg/mL of 2,4,5-TMBA for 24 h or for the whole 8-day differentiation period. Fully differentiated adipocytes are also treated with 100 µg/mL of 2,4,5-TMBA for 24-72 h to test the cytotoxicity. At the end of treatment, cells are cultured with MTT at a final concentration of 0.5 mg/mL for another 4 h. The purple MTT formazan is dissolved by DMSO and the absorbance at 570 nm is taken with a spectrophotometer. The absorbance is proportional to the viability of adipocytes<sup>[1]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## REFERENCES

- [1]. Wu MR, et al. 2,4,5-TMBA, a natural inhibitor of cyclooxygenase-2, suppresses adipogenesis and promotes lipolysis in 3T3-L1 adipocytes. *J Agric Food Chem*. 2012 Jul 25;60(29):7262-9.
- [2]. Chen CC, et al. Production of a COX-2 inhibitor, 2,4,5-trimethoxybenzaldehyde, with submerged cultured *Antrodia camphorata*. *Lett Appl Microbiol*. 2007 Apr;44(4):387-92.

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**Caution: Product has not been fully validated for medical applications. For research use only.**

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