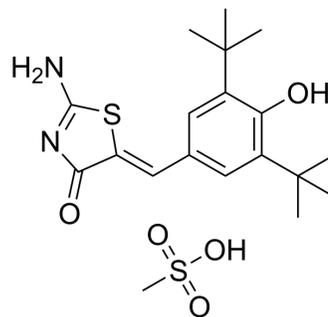


## Darbufelone mesylate

<b>Cat. No.:</b>	HY-101438A
<b>CAS No.:</b>	139340-56-0
<b>Molecular Formula:</b>	C <sub>19</sub> H <sub>28</sub> N <sub>2</sub> O <sub>5</sub> S <sub>2</sub>
<b>Molecular Weight:</b>	428.57
<b>Target:</b>	Prostaglandin Receptor; Leukotriene Receptor
<b>Pathway:</b>	GPCR/G Protein
<b>Storage:</b>	4°C, sealed storage, away from moisture and light * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light)



### SOLVENT & SOLUBILITY

<b>In Vitro</b>	DMSO : 110 mg/mL (256.67 mM; Need ultrasonic)			
	H <sub>2</sub> O : < 0.1 mg/mL (ultrasonic;warming;heat to 60°C) (insoluble)			
		<b>Solvent</b>	<b>Mass</b>	
		<b>Concentration</b>	<b>1 mg</b>	<b>5 mg</b>
<b>Preparing Stock Solutions</b>	<b>1 mM</b>	2.3333 mL	11.6667 mL	23.3334 mL
	<b>5 mM</b>	0.4667 mL	2.3333 mL	4.6667 mL
	<b>10 mM</b>	0.2333 mL	1.1667 mL	2.3333 mL
Please refer to the solubility information to select the appropriate solvent.				
<b>In Vivo</b>	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: 5.5 mg/mL (12.83 mM); Suspended solution; Need ultrasonic			

### BIOLOGICAL ACTIVITY

<b>Description</b>	Darbufelone mesylate (CI-1004 mesylate) is a dual inhibitor of cellular PGF <sub>2α</sub> and LTB <sub>4</sub> production. Darbufelone potently inhibits PGHS-2 (IC <sub>50</sub> = 0.19 μM) but is much less potent with PGHS-1 (IC <sub>50</sub> = 20 μM).			
<b>IC<sub>50</sub> &amp; Target</b>	PGF <sub>2α</sub>	LTB <sub>4</sub>	PGHS-2 0.19 μM (IC <sub>50</sub> )	PGHS-1 20 μM (IC <sub>50</sub> )
<b>In Vitro</b>	Darbufelone is a noncompetitive inhibitor of PGHS-2 (K <sub>i</sub> =10±5 μM). Darbufelone quenches the fluorescence of PGHS-2 at 325 nm (lambda(ex)=280 nm) with K <sub>d</sub> =0.98±0.03 μM <sup>[1]</sup> . To test the putative anti-proliferative effect of Darbufelone, A549, H520 and H460 cell lines are used, which are established from three distinct pathological subtypes of NSCLC (adenocarcinoma, squamous and large cell lung cancer respectively). Increasing concentrations of Darbufelone, ranging from 5 to 60 μM, are tested for 72 h. The cell growth inhibition of these three cell lines gradually increases with higher drug concentration. The IC <sub>50</sub> of A549 and H520 are 20±3.6 and 21±1.8 μM, respectively, while the H460 has much lower IC <sub>50</sub> (15±2.7 μM) <sup>[2]</sup> .			

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

#### In Vivo

Darbufelone is a dual inhibitor of cellular PGF2R and LTB4 production. Darbufelone is orally active and nonulcerogenic in animal models of inflammation and arthritis<sup>[1]</sup>. When mice are treated with Darbufelone at dosage of 80 mg/kg/day, the tumor volumes decrease in a time-dependent manner. In contrast, lower dose of Darbufelone (20 or 40 mg/kg/day) does not show any significant inhibition of tumor weight. At necropsy, the tumor weight in mice treated with Darbufelone (80 mg/kg/day) is reduced by 30.2% in comparison with control group<sup>[2]</sup>.

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## PROTOCOL

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

The effect of Darbufelone on the cyclooxygenase activity of PGHS-2 is determined in assays with no enzyme-inhibitor preincubation. HoloPGHS-2 (30 nM final concentration) is added to reaction mixtures that contain 20 mM Tris-HCl buffer (pH 7.4), 100 μM TMPD, and varying levels of Arachidonic acid (0-60 μM) and Darbufelone (0-30 μM). The cyclooxygenase activity is measured by monitoring the oxidation of TMPD at 610 nm using a microplate reader<sup>[1]</sup>.

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#### Cell Assay <sup>[2]</sup>

A549 (CCL-185, lung adenocarcinoma cancer cell line), NCI-H520 (HTB-182, lung squamous cancer cell line) and NCI-H460 (HTB-177, lung large cell cancer cell line) cells are cultured in RPMI-1640, supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL Penicillin G, and 100 μg/mL Streptomycin. Cells are grown at 37°C in a humidified atmosphere of 95% air and 5% CO<sub>2</sub> and routinely passaged using 0.25% trypsin-EDTA. The effect of Darbufelone on human lung carcinoma cell viability is determined by MTT reduction assay. In brief, tumor cells growing in log-phase are trypsinized and seeded at 5×10<sup>3</sup> cells per well into 96-well plates and allowed to attach overnight. Medium in each well is replaced with fresh medium or medium containing various concentrations of Darbufelone (5-60 μM) in at least triplicate wells. Cells are cultured to another 72 h. After treatment, 1/10 volume of MTT solution (5 mg/mL) is added to each well, and the plate is incubated at 37°C for another 4 h. Two hundred microliters of DMSO is added to each well to solubilize the MTT-formazan product after removal of the medium. Absorbance at 595 nm is measured with a multi-well spectrophotometer. Growth inhibition is calculated as a percentage of the untreated controls<sup>[2]</sup>.

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#### Animal Administration <sup>[2]</sup>

Mice<sup>[2]</sup>

C57Bl/6 male mice at 4-5 weeks are used. These mice are housed in air-conditioned quarters and are provided food and water ad libitum. On the day 0, Lewis Lung Carcinoma cells (1×10<sup>6</sup>) are implanted into the left armpit of C57Bl/6 mice. The mice are randomly divided into four treatment groups of ten animals each. The day after inoculation (day 1), control group is treated with CMC-Na, and other groups are administered Darbufelone by gavage at doses of 20, 40, and 80 mg/kg/day. The treatment is continued till the end of the study. On day 14, animals are killed, and tumors are excised, weighed, and fixed in formalin for the further histochemical analysis.

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## REFERENCES

[1]. Johnson AR, et al. Slow-binding inhibition of human prostaglandin endoperoxide synthase-2 with darbufelone, an isoform-selective antiinflammatory di-tert-butyl phenol. *Biochemistry*. 2001 Jun 26;40(25):7736-45.

[2]. Ye X, et al. Darbufelone, a novel anti-inflammatory drug, induces growth inhibition of lung cancer cells both in vitro and in vivo. *Cancer Chemother Pharmacol*. 2010 Jul;66(2):277-85.

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

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