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### SZABO-SCANDIC HandelsgmbH

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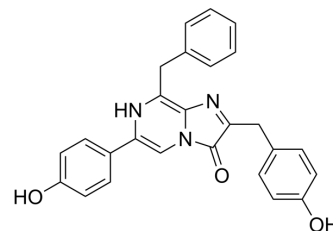
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## Coelenterazine

Cat. No.:	HY-18743
CAS No.:	55779-48-1
Molecular Formula:	C <sub>26</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub>
Molecular Weight:	423.46
Target:	Reactive Oxygen Species
Pathway:	Immunology/Inflammation; Metabolic Enzyme/Protease; NF-κB
Storage:	-20°C, protect from light, stored under nitrogen
* The compound is unstable in solutions, freshly prepared is recommended.	



### SOLVENT & SOLUBILITY

In Vitro	Ethanol : 1 mg/mL (2.36 mM; ultrasonic and warming and heat to 60°C; DMSO can inactivate Coelenterazine's activity)					
	Preparing Stock Solutions	<div><div>Solvent</div><div>Concentration</div></div>	Mass	1 mg	5 mg	10 mg
		1 mM		2.3615 mL	11.8075 mL	23.6150 mL
		5 mM		---	---	---
		10 mM		---	---	---
Please refer to the solubility information to select the appropriate solvent.						
In Vivo	1. Add each solvent one by one: 10% EtOH >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 0.2 mg/mL (0.47 mM); Clear solution					
	2. Add each solvent one by one: 10% EtOH >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 0.2 mg/mL (0.47 mM); Clear solution					
	3. Add each solvent one by one: 10% EtOH >> 90% Saline Solubility: ≥ 0.2 mg/mL (0.47 mM); Clear solution					

### BIOLOGICAL ACTIVITY

Description	Coelenterazine is a luminescent enzyme substrate for apoaquorin and Renilla luciferase. Renilla luciferase and substrate coelenterazine has been used as the bioluminescence donor in bioluminescence resonance energy transfer (BRET) to detect protein-protein interactions. Coelenterazine is a superoxide anion-sensitive chemiluminescent probe and it can also be used in chemiluminescent detection of peroxynitrite <sup>[1][2][3]</sup> .
In Vitro	HCT-8 control cells, transiently expressing Renilla luciferase (RLuc), showed low bioluminescence due to P-glycoprotein-mediated efflux transport of coelenterazine. By comparison, transiently expressing RLuc HCT-8 cells, wherein P-glycoprotein was down-regulated with shRNAi, showed high bioluminescence <sup>[3]</sup> . MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## In Vivo

The in vivo growth potential of HCC1806-RR was monitored by injecting animals with coelenterazine (2 mg/kg) i.v. and exposing them to a charged-coupled device (CCD) camera 5 minutes later. Rluc activity was detected as light emitted from the tumor cells and acquired as a pseudo-color image superimposed over a black and white photograph of the animal. All mice demonstrated very high Rluc activity at the primary site with the majority of mice simultaneously showing metastases to inguinal ILNs<sup>[4]</sup>.

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

## PROTOCOL

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

Cells are plated at a density of  $5 \times 10^4$  cells per well into 24-well plates and grown to 80-100% confluency. Just before imaging, media are changed to a colorless solution containing (in mM): 2.7 KCl, 139 NaCl, 8.1 Na<sub>2</sub>HPO<sub>4</sub>, 0.7 H<sub>2</sub>O, 1.5 KH<sub>2</sub>PO<sub>4</sub>, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub> and 5.5 d-glucose. Cells are preincubated for 15 min in the absence or presence of Pgp modulator, after which Coelenterazine (final concentration of 470 nM) is added directly to the cells<sup>[1]</sup>.

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

### Animal Administration <sup>[1]</sup>

Mice are anesthetized with metofane before tail vein injection of Coelenterazine (4 µg/g) formulated from an ethanol stock diluted in sodium phosphate buffer (50 mM). Bioluminescence imaging is performed on the in vivo imaging system at 2, 6, 8, and 11 min after injection. After imaging, animals are killed by cervical dislocation; tumors are then harvested and weighed <sup>[1]</sup>.

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

## CUSTOMER VALIDATION

- Prostate Cancer Prostatic Dis. 2022 Jan 24.
- Universidad de Zaragoza. 2022 Feb.

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## REFERENCES

- [1]. Markova SV, et al, Coelenterazine-dependent luciferases. Biochemistry (Mosc). 2015 Jun;80(6):714-32.
- [2]. Lucas M, et al. Coelenterazine is a superoxide anion-sensitive chemiluminescent probe: its usefulness in the assay of respiratory burst in neutrophils. Anal Biochem. 1992 Nov 1;206(2):273-7.
- [3]. Pichler A, et al. In vivo RNA interference-mediated ablation of MDR1 P-glycoprotein. Clin Cancer Res. 2005 Jun 15;11(12):4487-94.
- [4]. Volk-Draper LD, et al. Novel model for basaloid triple-negative breast cancer: behavior in vivo and response to therapy. Neoplasia. 2012 Oct;14(10):926-42.

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

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