

## Product Data Sheet

Product Name: Coelenterazine

Cat. No.: GC17680

### Chemical Properties

Cas. No. 55779-48-1

Chemical Name 8-benzyl-2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl)imidazo[1,2-a]pyrazin-3(7H)-one

SMILES OC1=CC=C(CC(C2=O)=NC3=C(NC(C4=CC=C(O)C=C4)=CN32)CC5=CC=CC=C5)C=C1

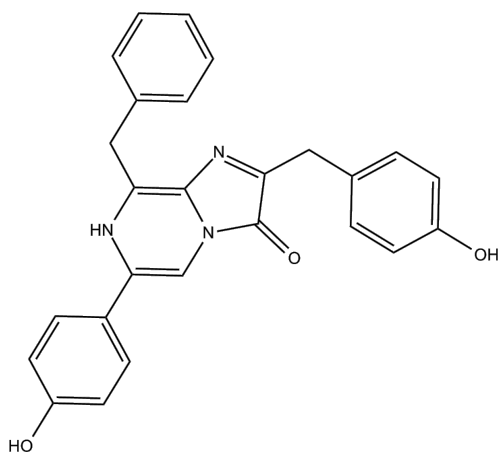
Formula  $C_{26}H_{21}N_3O_3$  M.Wt 423.46

Solubility Ethanol : 2 mg/mL (4.72 mM; Need ultrasonic; DMSO can inactivate Coelenterazine's activity) Storage -20°C, protect from light, stored under nitrogen, unstable in solution, ready to use.

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure



### Protocol

Protocol for Coelenterazine use<sup>[1-3]</sup>

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

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This protocol provides only a guide. Please modify according to specific needs.

### Preparation of staining solution:

- (1) Dissolve Coelenterazine in methanol to prepare a 4.7mM stock solution. Note: Aliquot unused stock solution and store at -20°C or -80°C protected from light. Avoid repeated freeze-thaw cycles.
- (2) Dilute the stock solution with 0.1M potassium phosphate buffer to prepare a 0.4μM to 2.5μM working solution. Note: Adjust the working concentration according to actual conditions. Prepare fresh before each use.

### Mitochondrial reactive oxygen species detection (using rat heart mitochondria as an example):

- (1) Isolate rat heart mitochondria or submitochondrial particles (SMP). Adjust protein concentration to approximately 5mg/ml.
- (2) Add 1 mg of mitochondria or SMP suspension to 2.5μM Coelenterazine and 2mg/ml NADH. Immediately measure cumulative luminescence signal within 15 seconds.

### Cell staining:

- (1) Culture cells expressing Renilla luciferase. Wash cells once with colorless solution.
- (2) Add 0.4μM Coelenterazine. Incubate immediately at 37°C protected from light for 6 hours.
- (3) Acquire images using a bioluminescence imaging system.

### In vivo mouse imaging:

- (1) Inject 2mg/kg Coelenterazine via tail vein into mice bearing xenograft tumors stably expressing Renilla luciferase.
- (2) Allow Coelenterazine to circulate in vivo for approximately 5 minutes. Use a bioluminescence imaging system to capture images of orthotopic tumors and metastases. After sacrifice, homogenize major organs. Mix 50μL Coelenterazine substrate with 10μL tissue lysate. Quantify using a luminometer.

### Precautions:

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- (1) Animal experiments must comply with animal welfare guidelines.
- (2) For your safety and health, please wear a lab coat and disposable gloves during operation.

### References:

- [1] Volk-Draper LD, Rajput S, Hall KL, et al. Novel model for basaloid triple-negative breast cancer: behavior in vivo and response to therapy. *Neoplasia*. 2012 Oct;14(10):926-42.
- [2] Raha S, Myint AT, Johnstone L, et al. Control of oxygen free radical formation from mitochondrial complex I: roles for protein kinase A and pyruvate dehydrogenase kinase. *Free Radical Biology & Medicine*. 2002;32(5):421-30.
- [3] Pichler A, Zelcer N, Prior JL, et al. In vivo RNA interference-mediated ablation of MDR1 P-glycoprotein. *Clin Cancer Res*. 2005 Jun 15;11(12):4487-94.

### Background

Coelenterazine is a natural, cell-permeable marine luciferin. Coelenterazine is a substrate for multiple luciferases or photoproteins. Coelenterazine is a probe for calcium ion and reactive oxygen species detection (Ex/Em=429/466nm). Coelenterazine can be used in reporter gene assays, in vivo bioluminescence imaging, Ca<sup>2+</sup> detection, BRET and oxidative stress-related studies<sup>[1-4]</sup>.

### References:

- [1] Markova SV, Vysotski ES. Coelenterazine-dependent luciferases. *Biochemistry (Mosc)*. 2015 Jun;80(6):714-32.
- [2] Jiang T, Song J, Zhang Y. Coelenterazine-Type Bioluminescence-Induced Optical Probes for Sensing and Controlling Biological Processes. *Int J Mol Sci*. 2023 Mar 7;24(6):5074.
- [3] Krasitskaya VV, Bashmakova EE, Frank LA. Coelenterazine-Dependent Luciferases as a Powerful Analytical Tool for Research and Biomedical Applications. *Int J Mol Sci*. 2020 Oct 10;21(20):7465.
- [4] Jiang T, Du L, Li M. Lighting up bioluminescence with coelenterazine: strategies and applications. *Photochem Photobiol Sci*. 2016 Apr;15(4):466-80.

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