Diphenylterazine

Cat. No.: HY-111382

CAS No.: 344940-63-2

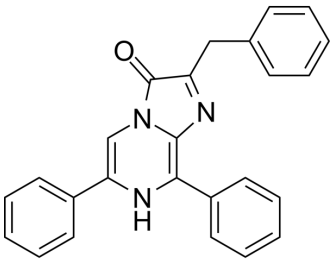
Molecular Formula: $C_{25}H_{19}N_3O$

Molecular Weight: 377.44

Target: Fluorescent Dye

Pathway: Others

Storage: -80°C, protect from light, stored under nitrogen



SOLVENT & SOLUBILITY

In Vitro

DMF : 11.11 mg/mL (29.44 mM; Need ultrasonic; DMSO can inactivate Diphenylterazine's activity)

H_2O : < 0.1 mg/mL (insoluble; DMSO can inactivate Diphenylterazine's activity)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.6494 mL	13.2471 mL	26.4943 mL
5 mM	0.5299 mL	2.6494 mL	5.2989 mL
10 mM	0.2649 mL	1.3247 mL	2.6494 mL

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

In Vivo

1. Add each solvent one by one: 50% PEG300 >> 50% saline
   Solubility: 2 mg/mL (5.30 mM); Suspended solution; Need ultrasonic
2. Add each solvent one by one: 10% DMF >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 1.11 mg/mL (2.94 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Diphenylterazine (DTZ) is a bioluminescence agent. Diphenylterazine alone yielded very little background, leading to excellent signal-to-background ratios[1].

In Vitro

Diphenylterazine elicits minimal cell toxicity at millimolar concentrations[1].
Diphenylterazine (DTZ) has high quantum yield, red-shifted emission, favorable in vivo pharmacokinetics and lacks cofactors required for light emission. Yeh AH (2023) used Diphenylterazine (DTZ) as the target substrate of luciferase, and the multinuclear transport factor NTF2-like superfamily as the target topology, to de novo design a small and stable protein scaffold to make the size and shape of the pocket suitable for Diphenylterazine. This method screens out designed luciferases with high selectivity and overcomes the limitations of natural proteins. The catalytic efficiency of the de novo designed luciferase for Diphenylterazine (kcat/Km = 10^6/M/s) is comparable to that of natural luciferase, but the substrate

	specificity is higher[2]. Notes: To make a stock solution for DTZ (Diphenylterazine), first, a premixture is prepared by dissolving 17.6 mg of L-ascorbic acid (HY-B0166) in 10 mL ethanol and 10 mL 1,2-propanediol; next, 1 mg of DTZ is dissolved in 88 µL of the premix, resulting in a 30 mM DTZ stock solution containing 5 mM L-ascorbic acid. The stock solution should be stable for a few months in -80°C freezers. It may also be aliquoted to 10-20 µL each at -80°C for the convenience of use. We want to note that this new formulation greatly enhances substrate stability, compared to the conventional acidic alcohol solution[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Diphenylterazine injections into untransfected BALB/c mice do not yield any background emission. The bioluminescence resulting from intraperitoneally injected Diphenylterazine displays extended kinetics[1]. DTZ (0.3 µmol/mouse (1.13 mg.ml^{-1}/100 ul/mouse); i.v.) treatment can track tumor growth in a xenograft NU/J mouse model [4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [1]	BALB/c mice are used and transfected with cells expressing teLuc, Antares, Antares2 and FLuc by injecting cells into the tail vein of BALB/c mice. After the diminishing of the FLuc bioluminescence, 0.3 µmol Diphenylterazine or furimazine is intraperitoneally injected. Mice are imaged with a 1-min exposure per frame over a course of 20 min. The images are processed using the Fiji image analysis software and the frames with highest signals in individual experiments are used for comparison[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Nature. 2023 Feb;614(7949):774-780.
- Nat Commun. 2022 Dec 17;13(1):7799.
- Adv Sci (Weinh). 2024 Jan 21:e2308750.
- Cancers (Basel). 2022, 14(19), 4735.
- J Biol Chem. 2021 Sep 30;101266.

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REFERENCES

[1]. Yeh AH, et al. De novo design of luciferases using deep learning. Nature. 2023 Feb;614(7949):774-780.

[2]. Yeh HW, et al. Red-shifted luciferase-luciferin pairs for enhanced bioluminescence imaging. Nat Methods. 2017 Oct;14(10):971-974.

[3]. Hsien-Wei Yeh, et al. ATP-Independent Bioluminescent Reporter Variants To Improve in Vivo Imaging. ACS Chem Biol. 2019 May 17;14(5):959-965.

[4]. Practical Notes for teLuc-DTZ and Antares2-DTZ (updated 07/29/2019).

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

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA