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Resorufin benzyl ether

Cat. No.: HY-D0146

CAS No.: 87687-02-3

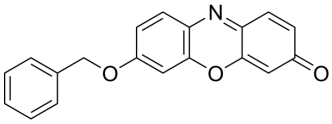
Molecular Formula: $C_{19}H_{13}NO_3$

Molecular Weight: 303.31

Target: Fluorescent Dye

Pathway: Others

Storage: Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description

Resorufin benzyl ether (BzRes), a fluorogenic enzyme substrate, can be used to detect CYP3A4 enzyme activity. Resorufin benzyl ether modified with a recognizing moiety boronate, can be used for $ONOO^-$ detection via a self-immolation mechanism. Ex/Em=530-570 nm/590 nm[1][2][3].

In Vitro

CYP3A4 activity assay[2]:

1. Preparation solution.

a. The standard stock solution containing 1 mM Resorufin benzyl ether as fluorogenic substrate. Dissolve 5 mg Resorufin benzyl ether in a mixture of 1 mL of 2%w/v Poloxamer 188 (HY-D1005A), 500 mL Dimethyl sulfoxide (HY-Y0320) and 3.5 mL Acetonitrile.

b. CYP3A4 enzyme solution should be freshly prepared. Dilute 5 mL of 1 mM enzyme stock solution with 995 mL of the buffer to obtain 5 nM enzyme solution.

2. Measurement of CYP3A4 activity

a. Conduct in a 96-well plate. Add 99 mL of buffer mixture solution, 1 mL of 1 mM Resorufin benzyl ether to each well. Adjust to the final concentration of 5 mM.

b. Transfer 100 mL of 5 nM enzyme solution prior the incubation at 37 ⊠ for 30 min.

c. Perform enzyme activity measurement with fluorescence detection at excitation wavelength, lex of 570 nm and emission wavelength, lem of 590 nm.

d. Factors influences in the assay of CYP3A4 activity. Such as buffer (phosphate, Tris-HCl buffer), concentration of buffer (50-200 mM) and incubation time (0-50 min).

CYP3A4 metabolism activity assay[3]:

1.Add CYP3A4 enzyme to final concentrations of 5 pmol/well.

2.Each reaction used 50 pM substrate and 200 mM potassium phosphate buffer.

3.Incubate with BzRes for 45 minutes prior to measuring metabolite fluorescence. The excitation filter (ex) is 530 nm and the emission filter (em) is 590 nm.

4.Assign the concentration designation of 100% to the full strength extract (diluted 1:4 in the final assay volume).

5.Run in duplicate for 1:3 serial dilutions of the 100% extract. Calculate mean and standard deviation of fluorescence values.

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

REFERENCES

[1]. Ji X, et al. Regulating the activity of boronate moiety to construct fluorescent probes for the detection of ONOO-in vitro and in vivo. Anal Methods. 2022 Dec 15;14(48):5027-5033.

[2]. Nuchtavorn N, et al. Paper-based sol-gel thin films immobilized cytochrome P450 for enzyme activity measurement. Anal Chim Acta. 2020 Feb 15;1098:86-93.

[3]. Yale SH, et al. Analysis of the inhibitory potential of Ginkgo biloba, Echinacea purpurea, and Serenoa repens on the metabolic activity of cytochrome P450 3A4, 2D6, and 2C9. J Altern Complement Med. 2005 Jun;11(3):433-9.

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