Dibenzylfluorescein

Cat. No.: HY-116862

CAS No.: 97744-44-0

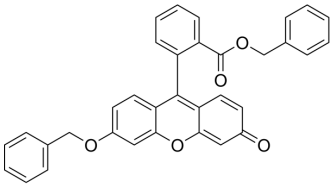
Molecular Formula: $C_{34}H_{24}O_5$

Molecular Weight: 512.55

Target: Cytochrome P450; Fluorescent Dye

Pathway: Metabolic Enzyme/Protease; Others

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



BIOLOGICAL ACTIVITY

Description

Dibenzylfluorescein (DBF) is a fluorogenic probe (Fluoresecent dye) that acts as a substrate for specific cytochrome P450 (CYP) isoforms, including CYP3A4, CYP2C8, CYP2C9, CYP2C19, and aromatase (CYP19). Dibenzylfluorescein is typically used near its K_m value of 0.87-1.9 μM (Ex=485nm⊠Em=535nm). Dibenzylfluorescein is used to detect changes in CYP catalytic activity caused by drugs or disease[1][2][3][4].

In Vitro

The protocol of P450-catalyzed metabolism of Dibenzylfluorescein and effect of base[3]:
Reaction Process: Dibenzylfluorescein is dealkylated by P450 to form a fluorescein benzyl ester, which is further hydrolyzed to fluorescein by NaOH (if present). Addition of 2 M NaOH causes also decomposition of Dibenzylfluorescein to fluorescein benzyl ether.
1. Incubation mixtures for CYP2C19 enzyme-catalyzed samples each 150 μL contains 0.1 M Tris-HCl buffer (pH 7.4), 10 μM Dibenzylfluorescein, 15 pmol of CYP2C19 enzyme, and 50 μL of NADPH-regenerating system.NADPH-regenerating system contains 1.13 mM NADP, 12.5 mM isocitric acid, 56.33 mM KCl, 187.5 mM Tris-HCl, pH 7.4, 12.5 mM MgCl2, 0.0125 mM MnCl2, and 0.075 U/ml isocitrate dehydrogenase.
2. The samples were incubated for 30-60 min at 37°C. The reactions were terminated by rapid cooling to 4°C and after centrifugation, the supernatants were analyzed by LC-MS.
3. Pure Dibenzylfluorescein, fluorescein benzyl ester, fluorescein benzyl ether, and fluorescein (all 10 μM) were used as standards and were analyzed in the absence and presence of 2 M NaOH.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Stresser DM., et al. Substrate-dependent modulation of CYP3A4 catalytic activity: Analysis of 27 test compounds with four fluorometric substrates. Drug Metabolism and Disposition 28(12), 1440-1448 (2000).

[2]. Donato MT., et al. Fluorescence-based assays for screening nine cytochrome P450 (P450) activities in intact cells expressing individual human P450 enzymes. Drug Metab. Dispos. 32(7), 699-706 (2004).

[3]. Salminen KA, et al. Simple, direct, and informative method for the assessment of CYP2C19 enzyme inactivation kinetics. Drug Metabolism and Disposition 39(3), 412-418 (2011).

[4]. Moutinho D, et al. Altered human CYP3A4 activity caused by Antley-Bixler syndrome-related variants of NADPH-cytochrome P450 oxidoreductase measured in a robust in vitro system. Drug Metabolism and Disposition 40(4), 754-760 (2012).

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

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