ML171

Cat. No.: HY-12805

CAS No.: 6631-94-3

Molecular Formula: $C_{14}H_{11}NOS$

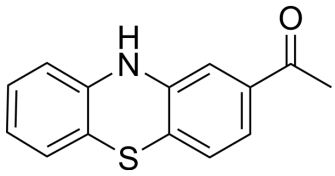
Molecular Weight: 241.31

Target: NADPH Oxidase

Pathway: Metabolic Enzyme/Protease

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	2 years
	-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 64 mg/mL (265.22 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	4.1440 mL	20.7202 mL	41.4405 mL
5 mM	0.8288 mL	4.1440 mL	8.2881 mL
10 mM	0.4144 mL	2.0720 mL	4.1440 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (10.36 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (10.36 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

ML171 (2-Acetylphenothiazine;2-APT) is a potent and selective NADPH oxidase 1 (Nox1) inhibitor that blocks Nox1-dependent ROS generation, with an IC_{50} of 0.25 μM in HEK293-Nox1 confirmatory assay.

IC_{50} & Target

NOX1

In Vitro

Nox1-dependent ROS generation has been shown to play a pivotal role in cell signaling, cell growth, angiogenesis, motility and blood pressure regulation. ML171 strongly blocks ROS generation in HT29 cells (IC_{50}=0.129 μM) and only increasing over-expression of Nox1 can overcome the blockage of ROS generation caused by ML171 treatment in HEK293 cell system reconstituted with all the components required Nox1-dependent ROS generation. ML171 efficiently blocks ROS production

measured by carboxy-H2-DCFDA staining as well as DPI used as a positive control. When ML171 is tested in HEK293-Nox1 reconstituted cell system, higher potency in blocking Nox1-dependent ROS generation is observed compared with the parental compound[1].

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

PROTOCOL

Cell Assay [1]

HT29 cells are cultured in 150 mm diameter plate and when 70-80% confluence is reached, cells are trypsinized, harvested in HBSS and counted. 4×10^4 cells are dispensed into individual wells in 30 µL final volume (384 well plates) by using a robotic liquid handler. Cells are treated for 60 min at 37°C with 50 nL of DPI, DMSO and library compounds (including ML171) which are automatically dispensed into individual wells from their respective assay plates. This will correspond to a final concentration of 10 µM DPI or library compounds (ML171), and 0.1% DMSO. 20 µL of a mixture containing 200 µM luminol plus 0.32 units of HRP (final concentration) is added. Luminescence is quantified using a 384-well plate luminometer. The data output consisting of the emission intensities for each well is imported into a spread-sheet program (such as Excel) for further processing. As designed, compounds that inhibit Nox1 activity will reduce cellular ROS production, leading to reduced probe-ROS interactions and reduced well luminescence. Compounds are considered 'hits' and further processed when light emission is blocked >75% 7 than DMSO wells (DMSO and DPI wells are set to 0% and 100% respectively). Compounds are tested in singlicate at a concentration of 10 µM[1].

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

CUSTOMER VALIDATION

- Redox Biol. 2020 Jul;34:101569.
- Int J Biol Macromol. 2021 Jul 23;S0141-8130(21)01587-7.
- Cell Biol Toxicol. 2022 Mar 18.
- Cells. 2021 Aug 12;10(8):2073.
- Biochem Pharmacol. 2023 Aug 8;115738.

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REFERENCES

[1]. Gianni D, et al. A novel and specific NADPH oxidase-1 (Nox1) small-molecule inhibitor blocks the formation of functional invadopodia in human colon cancer cells. ACS Chem Biol. 2010 Oct 15;5(10):981-93.

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