

Product Data Sheet

Product Name: ML334
Cat. No.: GC38819

Chemical Properties

Cas. No. 1432500-66-7

SMILES O=C(C1=CC=CC=C1C2=O)N2C[C@@H]3C4=CC=CC=C4CCN3C([C@H]5[C@H](CC(C5)C(O)=O)=O

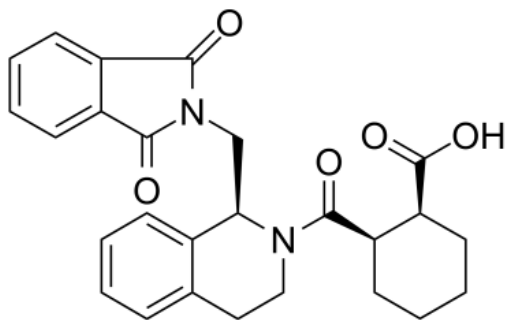
Formula $C_{26}H_{26}N_2O_5$ M.Wt 446.5

Solubility DMSO: 44.65 mg/mL (100.00 mM); Storage Store at -20°C, stored under nitrogen
Ethanol: 44.65 mg/mL (100.00 mM)

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

Cell experiment [1]:

Cell lines HEK293 cells

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

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Preparation Method Cells were treated with ML334 for 3 hours.

Reaction
Conditions 100 μ M; 3 hours

Applications ML334 enhances the expression and nuclear translocation of nuclear factor (erythroid-derived 2)-like 2 (NRF2) in HEK293 cells.

Competitive SPR assay

[2]:

Animal models

A biotinylated 16mer peptide from the Keap1 binding region of Nrf2 was captured by streptavidin on a CM5 chip surface (300 RU). A solution containing 40 nM of the Kelch domain of Keap1 protein, preincubated with ML334, was injected over the immobilized Nrf2 peptide surface. The assay was conducted at 25°C with a flow rate of 50 μ L/min, allowing for a 1-minute association time and Preparation Method a 3-minute dissociation time. The sensor chip surface was regenerated with 1M NaCl at a flow rate of 100 μ L/min for 1 minute, followed by two consecutive 1-minute washes with the running buffer at the same flow rate. The binding signal of Keap1 to Nrf2 was recorded. The concentration of unbound Keap1 was calculated using its standard curve, and the fraction of bound Keap1 was plotted against the concentration of the compounds.

Dosage form

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Applications In a competitive surface plasmon resonance (SPR) assay, ML334 binds to Keap1 with a K_D of 1 μ M.

References:

[1]:Wen X, Thorne G, et.al. Activation of NRF2 Signaling in HEK293 Cells by a First-in-Class Direct KEAP1-NRF2 Inhibitor. J Biochem Mol Toxicol. 2015 Jun;29(6):261-6. doi: 10.1002/jbt.21693. Epub 2015 Feb 12. PMID: 25683455; PMCID: PMC4713195.

[2]:Wang L, Lewis T, et.al.The identification and characterization of non-reactive inhibitor of Keap1-Nrf2 interaction through HTS using a fluorescence polarization assay. 2012 Dec 17 [updated 2013 Sep 16]. In: Probe Reports from the NIH Molecular Libraries Program [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2010—. PMID: 24260785.

Background

ML334 is a potent, cell-permeable activator of NRF2. It achieved through the inhibition of the Keap1-NRF2 protein-protein interaction. It demonstrates a binding affinity to Keap1 with a dissociation constant (K_D) of 1 μ M in competitive surface plasmon resonance (SPR) assays. Furthermore, ML334 competes with an Nrf2 peptide, exhibiting an IC_{50} of 1.6 μ M in fluorescence polarization (FP) assays^[1-3].

ML334(100 μ M; 3 hours) induces the expression and nuclear translocation of nuclear factor (erythroid-derived 2)-like 2 (NRF2) in HEK293 cells. Meanwhile, ML334 treatment induced the expression of HO-1 and TRX1 proteins in HEK293 cells ^[4].ML334-induced (50 μ M; 48 hours) overexpression of Nrf2 enhances the antifibrotic effect of panaxatriol saponin (PTS) in cardiac fibroblasts^[5].

[1]. Hu L, Magesh S, Chen L, Wang L, Lewis TA, Chen Y, Khodier C, Inoyama D, Beamer LJ, Emge TJ, Shen J, Kerrigan JE, Kong AN, Dandapani S, Palmer M, Schreiber SL, Munoz B. et.al. Discovery of a small-molecule inhibitor and cellular probe of Keap1-Nrf2 protein-protein interaction. Bioorg Med Chem Lett. 2013 May 15;23(10):3039-43. doi: 10.1016/j.bmcl.2013.03.013. Epub 2013 Mar 14. PMID: 23562243; PMCID: PMC3648997.

[2]. Shen J, Magesh S, et.al. Enantiomeric characterization and structure elucidation of LH601A using

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vibrational circular dichroism spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 2018 Mar 5;192:312-317. doi: 10.1016/j.saa.2017.11.033. Epub 2017 Nov 21. PMID: 29172127; PMCID: PMC10544735.

[3]. Wang L, Lewis T. et,al. The identification and characterization of non-reactive inhibitor of Keap1-Nrf2 interaction through HTS using a fluorescence polarization assay. 2012 Dec 17 [updated 2013 Sep 16]. In: Probe Reports from the NIH Molecular Libraries Program [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2010—. PMID: 24260785.

[4]. Wen X, Thorne G, et,al. Activation of NRF2 Signaling in HEK293 Cells by a First-in-Class Direct KEAP1-NRF2 Inhibitor. J Biochem Mol Toxicol. 2015 Jun;29(6):261-6. doi: 10.1002/jbt.21693. Epub 2015 Feb 12. PMID: 25683455; PMCID: PMC4713195.

[5]. Yao H, He Q, et,al. Panaxatriol saponin ameliorates myocardial infarction-induced cardiac fibrosis by targeting Keap1/Nrf2 to regulate oxidative stress and inhibit cardiac-fibroblast activation and proliferation. Free Radic Biol Med. 2022 Sep;190:264-275. doi: 10.1016/j.freeradbiomed.2022.08.016. Epub 2022 Aug 14. Erratum in: Free Radic Biol Med. 2023 Jun;202:34. PMID: 35977659.

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