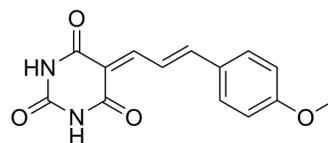


ML346

Cat. No.:	HY-18669		
CAS No.:	100872-83-1		
Molecular Formula:	C ₁₄ H ₁₂ N ₂ O ₄		
Molecular Weight:	272.26		
Target:	HSP		
Pathway:	Cell Cycle/DNA Damage; 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 : 12.5 mg/mL (45.91 mM; Need ultrasonic)				
		Solvent Concentration	Mass		
	Preparing Stock Solutions		1 mg	5 mg	10 mg
		1 mM	3.6730 mL	18.3648 mL	36.7296 mL
		5 mM	0.7346 mL	3.6730 mL	7.3459 mL
	10 mM	0.3673 mL	1.8365 mL	3.6730 mL	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 1.25 mg/mL (4.59 mM); Clear solution				

BIOLOGICAL ACTIVITY

Description	ML346 is an activator of Hsp70 expression and HSF-1 activity, with an EC ₅₀ of 4.6 μM for Hsp70. ML346 restores protein folding in conformational disease models, without significant cytotoxicity or lack of specificity. ML346 induces specific increases in genes and protein effectors of the heat shock response (HSR), including chaperones such as Hsp70, Hsp40, and Hsp27 ^[1] .
IC ₅₀ & Target	HSP70 4.6 μM (EC50, HeLa cells)
In Vitro	ML346 is an activator of Hsp70, with an EC ₅₀ of 4600 nM in HeLa cells. ML346 (10 μM) restores proteostasis, restores CFTR-mediated iodide conductance, and enhances the correct folding of proteins expressed in two different cellular compartments ^[1] . ML346 (Compound F1) induces multiple responses and strongly induces Hsp70, the oxidative stress response genes (HO1 and GCLM), and a 2.5-fold upregulation of BiP in WT MEF cells. ML346 (0.5-25 μM) exhibits

cytoprotective effects in cells after a 35 min severe heat shock, and also causes a two-fold protection from H₂O₂-induced apoptosis^[2].

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

In Vivo

ML346 suppress the aggregation of polyQ35 in a *C. elegans* model, suggesting the probe has efficacy in modifying protein aggregation and associated toxicity^[1].

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

PROTOCOL

Kinase Assay ^[2]

In brief, HeLa cells are incubated with either DMSO (negative control), the positive controls MG132 (10 μM) and lactacystin (6 μM) or the PRs A1, A3 and ML346 (F1) for 3 and 6 hours and then harvested. Cells are lysed in homogenization buffer (50 mM Tris-HCl, pH7.5, 250 mM sucrose, 5 mM MgCl₂, 2 mM ATP, 1 mM DTT, 0.5 mM EDTA, 0.025% digitonin) for 5 min on ice, and total protein concentration of whole cell extract is determined. 3 μg of whole cell extracts are combined with assay buffer (50 mM Tris-HCl, pH 7.5, 40 mM KCl, 5 mM MgCl₂, 0.5 mM ATP, 1 mM DTT, 0.05 mg/mL BSA) in a black 96-well plate and the reaction is initiated by the addition of a 2× (200 μM) fluorogenic peptide substrate Suc-LLVY-AMC. Fluorescence is measured every 10 min using a Synergy H4 multi-mode microplate reader^[2].

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

Cell Assay ^[2]

HeLa cells are plated at a density of 10,000 cells per well in black 96-well plates in 100 μL of DMEM supplemented with 10% FBS and 1% Pen/Strep/Neo. Plates are incubated for 16 hours at 37°C, 5% CO₂ and 95% relative humidity before compound addition. 1 μL of hit compounds (ML346) in DMSO or DMSO alone are added to the sample or control wells, respectively. Plates are then placed back in the incubator for 24 hours. After incubation, cells are washed 2× with 200 μL of PBS and 200 μL of a solution of 1 μg/mL of calcein AM is added to each well. Cells are then incubated for 45 min at 37°C, 5% CO₂ before fluorescence measurement using an Analyst GT multimode reader. Percent cytotoxicity is expressed relative to wells containing cells treated with DMSO only (100%)^[2].

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

CUSTOMER VALIDATION

- Clin Transl Med. 2023 Mar;13(3):e1229.
- Chem Biol Interact. 27 October 2022, 110240.
- RSC Med Chem. 31st October 2021.

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REFERENCES

[1]. Calamini B, et al. ML346: A Novel Modulator of Proteostasis for Protein Conformational Diseases. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD): National Center for Biotechnology Information (US); 2010-.2012 Dec 17.

[2]. Calamini B, et al. Small-molecule proteostasis regulators for protein conformational diseases. Nat Chem Biol. 2011 Dec 25;8(2):185-96.

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

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