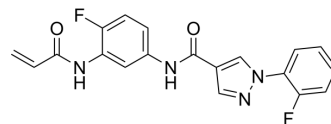


EN6

Cat. No.:	HY-128892		
CAS No.:	1808714-73-9		
Molecular Formula:	C ₁₉ H ₁₄ F ₂ N ₄ O ₂		
Molecular Weight:	368.34		
Target:	Autophagy		
Pathway:	Autophagy		
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 : 5 mg/mL (13.57 mM; Need ultrasonic)
 Ethanol : 1.11 mg/mL (3.01 mM; ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	2.7149 mL	13.5744 mL	27.1488 mL
	5 mM	0.5430 mL	2.7149 mL	5.4298 mL
	10 mM	0.2715 mL	1.3574 mL	2.7149 mL

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

In Vivo

1. Add each solvent one by one: 50% PEG300 >> 50% saline
 Solubility: 10 mg/mL (27.15 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

EN6 is a small-molecule in vivo autophagy activator that covalently targets cysteine 277 in the ATP6V1A subunit of the lysosomal v-ATPase. EN6-mediated modification of ATP6V1A uncouples v-ATPase from Rag, leading to inhibition of mTORC1 signalling, increased lysosomal acidification, and activation of autophagy. EN6 also scavenges TDP-43 aggregates (causative agents of frontotemporal dementia) in a lysosome-dependent manner^[1].

In Vitro

EN6 (50 µM; 1, 4, 8 h) increases the levels of LC3BII, and triggers formation of LC3 puncta in a time- and dose-dependent manner, in HEK293A cells^[1].
 EN6 leads to significant increases in the number autophagosomes and autolysosomes in HEK293A cells^[1].
 EN6 (25 µM; 1 h) blocks mTORC1 lysosomal localization and activation in HEK293A cells^[1].
 EN6 (50 µM; 4 h) activates the v-ATPase and lysosome acidification in HEK293A cells^[1].
 EN6 (25 µM; 7 h) promotes autophagic clearance of protein aggregates in IPTG-inducible GFP-TDP43 U2OS osteosarcoma

cell line model^[1].

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

Western Blot Analysis^[1]

Cell Line:	HEK293A cells
Concentration:	50 μ M
Incubation Time:	1, 4, 8 h
Result:	Time- and dose-dependently triggered formation of LC3 puncta and increased the levels of LC3BII.

Western Blot Analysis^[1]

Cell Line:	HEK293A cells
Concentration:	25 μ M
Incubation Time:	1 h
Result:	Led to complete inactivation of mTORC1 signaling, as shown by reduced levels of phosphorylated canonical substrates, S6 kinase 1 (S6K1), 4EBP1, and ULK1.

Immunofluorescence^[1]

Cell Line:	HEK293A cells
Concentration:	50 μ M
Incubation Time:	4 h
Result:	Led to significantly increased acidification of the lysosome in HEK293A cells, and this heightened acidification was blocked by BafA1.

Immunofluorescence^[1]

Cell Line:	IPTG-inducible GFP-TDP43 U2OS osteosarcoma cell line model
Concentration:	25 μ M
Incubation Time:	7 h
Result:	Reduced IPTG-induced TDP43 aggregates by 75 %.

In Vivo

EN6 (50 mg/kg; i.p.; single) inhibits mTORC1 and activates autophagy in vivo^[1].

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

Animal Model:	Six-week-old male C57BL/6 mice ^[1] .
Dosage:	50 mg/kg
Administration:	Intraperitoneal injection; single
Result:	Significantly inhibited mTORC1 signaling in both skeletal muscle and heart, as demonstrated by reduced phosphorylation of S6, 4EBP1 and ULK1. Strongly activated autophagy as shown by heightened LC3BII levels and reduced p62

levels.

CUSTOMER VALIDATION

- Proc Natl Acad Sci U S A. 2022 Oct 25;119(43):e2207280119.
- Neurochem Res. 2022 Oct 15.

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REFERENCES

[1]. Chung CY, et al. Covalent targeting of the vacuolar H⁺-ATPase activates autophagy via mTORC1 inhibition. Nat Chem Biol. 2019 Aug;15(8):776-785.

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

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