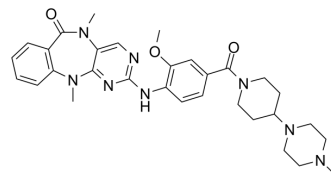


LRRK2-IN-1

Cat. No.:	HY-10875		
CAS No.:	1234480-84-2		
Molecular Formula:	C ₃₁ H ₃₈ N ₈ O ₃		
Molecular Weight:	570.69		
Target:	LRRK2; Apoptosis		
Pathway:	Autophagy; Apoptosis		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro	DMSO : 30 mg/mL (52.57 mM; Need ultrasonic)				
		Solvent Concentration	Mass 1 mg	5 mg	10 mg
	Preparing Stock Solutions	1 mM	1.7523 mL	8.7613 mL	17.5226 mL
		5 mM	0.3505 mL	1.7523 mL	3.5045 mL
10 mM		0.1752 mL	0.8761 mL	1.7523 mL	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	<ol style="list-style-type: none"> Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.5 mg/mL (4.38 mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (4.38 mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (4.38 mM); Clear solution 				

BIOLOGICAL ACTIVITY

Description	LRRK2-IN-1 is a potent and selective LRRK2 inhibitor with IC ₅₀ of 6 nM and 13 nM for LRRK2 (G2019S) and LRRK2 (WT), respectively.
IC₅₀ & Target	IC ₅₀ : 13 nM (WT), 6 nM (G2019S)
In Vitro	Wild-type and G2019S transduction results in 2.5 fold higher TR-FRET signal which can be inhibited by LRRK2-IN-1 in a dose-dependent manner with IC ₅₀ values of 0.08 μM and 0.03 μM, respectively ^[1] . LRRK2-IN-1 possessed an IC ₅₀ of 45 nM for

inhibition of DCLK2 and exhibits an IC₅₀ of greater than 1 μM when evaluated in biochemical assays for AURKB, CHEK2, MKNK2, MYLK, NUA1, and PLK1. LRRK2-IN-1 is confirmed to inhibit MAPK7 with an EC₅₀ of 160 nM. LRRK2-IN-1 induces a dose dependent inhibition of Ser910 and Ser935 phosphorylation accompanied by loss of 14-3-3 binding to both wild type LRRK2 and LRRK2[G2019S] stably transfected into HEK293 cells^[2]. LRRK2-IN-1 is moderately cytotoxic with IC₅₀ of 49.3 μM in HepG2 cells. LRRK2-IN-1 exhibits genotoxicity in the presence and absence of S9 at 15.6 and 3.9 μM, respectively^[3]. LRRK2-IN-1 inhibits proliferation, migration, and induces cell death with hallmarks of apoptosis of HCT116 and AsPC-1 cells^[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

LRRK2-IN-1 (100 mg/kg, i.p.) induces dephosphorylation of LRRK2 in the kidney of the mice^[2]. Peritumoral injection of LRRK2-IN-1 (100 mg/kg) results in a significant decrease in tumor volume and weight of AsPC-1 tumor xenografts^[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[2]

Active GST-LRRK2 (1326-2527), GST-LRRK2 [G2019S] (1326-2527), GST-LRRK2 [A2016T] (1326-2527) and GST-LRRK2 [A2016T+G2019S] (1326-2527) enzyme is purified with glutathione sepharose from HEK293 cell lysate 36 h following transient transfection of the appropriate cDNA constructs. Peptide kinase assays, performed in duplicate, are set up in a total volume of 40 μL containing 0.5 μg LRRK2 kinase (which at approximately 10% purity gives a final concentration of 8 nM) in 50 mM Tris/HCl, pH 7.5, 0.1 mM EGTA, 10 mM MgCl₂, 20 μM Nictide, 0.1 μM [γ-³²P]ATP (500 cpm/pmol) and the indicated concentrations of inhibitor dissolved in DMSO. After incubation for 15 min at 30°C, reactions are terminated by spotting 35 μL of the reaction mix onto P81 phosphocellulose paper and immersion in 50 mM phosphoric acid. Samples are washed extensively and the incorporation of [γ-³²P]ATP into Nictide is quantified by Cerenkov counting. IC₅₀ values are calculated with GraphPad Prism using non-linear regression analysis. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay ^[4]

Cells (10⁴ cells per well) are seeded into a 96-well tissue culture plate in triplicate. The cells are cultured in the presence of LRRK2-IN-1 with DMSO as a vehicle at 0, 0.31, 0.63, 1, 2, and 5, 10, and 20 μM. 48 h post treatment, 10 μL of TACS MTT Reagent is added to each well and the cells are incubated at 37°C until dark crystalline precipitate become visible in the cells. 100 μL of 266 mM NH₄OH in DMSO is then added to the wells and placed on a plate shaker at low speed for 1 minute. After shaking, the plate is allowed to incubate for 10 minutes protected from light and the OD550 for each well is read using a microplate reader. The results are averaged and calculated as a percentage of the DMSO (vehicle) control +/- the standard error of the mean. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration ^[2]

LRRK2-IN-1 is dissolved in Captisol and administered by intraperitoneal injection into wild type male C57BL/6 mice at a dose of 100 mg/kg. Control mice are injected with an equal volume of Captisol. At 1 and 2 h time points, mice are extingushed by cervical dislocation and kidney and brain tissue rapidly dissected and snap-frozen in liquid nitrogen. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Mol Pharm. 2018 Aug 6;15(8):3252-3259.
- Anticancer Res. 2018 Nov;38(11):6225-6230.
- Harvard Medical School LINCS LIBRARY

See more customer validations on www.MedChemExpress.com

REFERENCES

-
- [1]. Hermanson SB, et al. Screening for Novel LRRK2 Inhibitors Using a High-Throughput TR-FRET Cellular Assay for LRRK2 Ser935 Phosphorylation. PLoS One. 2012;7(8):e43580. Epub 2012 Aug 28.
- [2]. Deng, Xianming., et al. Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. Nature Chemical Biology (2011), 7(4), 203-205.
- [3]. Koshibu K, et al. Alternative to LRRK2-IN-1 for Pharmacological Studies of Parkinson's Disease. Pharmacology. 2015;96(5-6):240-7.
- [4]. Weygant N, et al. Small molecule kinase inhibitor LRRK2-IN-1 demonstrates potent activity against colorectal and pancreatic cancer through inhibition of doublecortin-like kinase 1. Mol Cancer. 2014 May 6;13:103.
-

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

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA