

Product Data Sheet

Product Name: MLi-2
Cat. No.: GC30769

Chemical Properties

Cas. No. 1627091-47-7

SMILES C[C@@](C1)([H])O[C@](C)([H])CN1C2=CC(C3=NNC4=CC=C(OC5(C)CC5)C=C43)=NC=N2

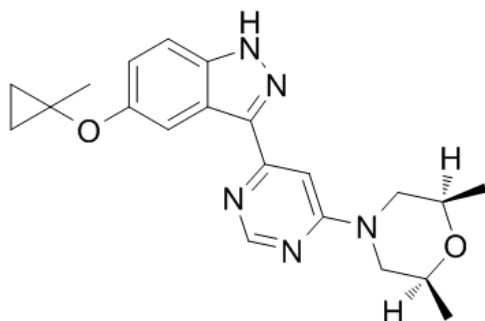
Formula $C_{21}H_{25}N_5O_2$ M.Wt 379.46

Solubility DMSO : ≥ 26 mg/mL (68.52 mM) Storage Store at -20°C

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 Primary microglia from wild-type and LRRK2 G2019S knock-in mice

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Preparation Method	Primary microglia were isolated from postnatal day 1–2 mouse brains and cultured for approximately 10 days. Cells were then treated with zymosan (200µg/mL) and/or MLi-2(1µM) for 24 hours to assess inflammatory and transcriptional responses.
Reaction Conditions	1µM MLi-2; 24 hours
Applications	MLi-2 significantly inhibited LRRK2 kinase activity, as shown by reduced phosphorylation at Ser935, and modulated gene expression profiles, particularly upregulating genes involved in ion transmembrane transport regulation and downregulating genes related to apoptotic processes in both wild-type and G2019S-LRRK2 microglia.
Animal experiment [2]:	
Animal models	LRRK2G2019S knock-in mice and wild-type littermates (C57BL/6 background)
Preparation Method	Mice were injected unilaterally with tau paired helical filaments (PHFs) derived from Alzheimer's disease brains into the hippocampus and overlying cortex at 3–4 months of age. One week prior to injection, mice were placed on a control diet or a diet containing the LRRK2 kinase inhibitor MLi-2 at doses of 8mg/kg or 45mg/kg. Mice were maintained on the diet for 6 months post-injection before sacrifice and brain tissue analysis.
Dosage form	8mg/kg/day or 45mg/kg/day; administered orally via diet.

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Applications

MLi-2 reduced total and phosphorylated LRRK2 (pS935) levels in kidney tissue, indicating target engagement. It induced enlargement of type II pneumocytes in the lungs, consistent with known LRRK2 inhibition-related morphological changes. In LRRK2G2019S mice, MLi-2 reversed the mutation-associated acceleration of tau pathology progression, particularly in cortical brain regions, without significantly affecting tau pathology in wild-type mice. These effects were more pronounced at 6 months post-injection and with the higher dose (45mg/kg/day), suggesting a dose- and time-dependent therapeutic effect in LRRK2 mutation carriers.

References:

- [1] Nazish I, Mamais A, Mallach A, et al. Differential LRRK2 Signalling and Gene Expression in WT-LRRK2 and G2019S-LRRK2 Mouse Microglia Treated with Zymosan and MLi2. *Cells*. 2023 Dec 26;13(1):53.
- [2] Lubben N, Brynildsen JK, Webb CM, et al. LRRK2 kinase inhibition reverses G2019S mutation-dependent effects on tau pathology progression. *Transl Neurodegener*. 2024 Mar 4;13(1):13.

Background

MLi-2 is a potent and selective inhibitor of leucine-rich repeat kinase 2 (LRRK2), with an IC_{50} value of 0.76nM [1]. MLi-2 exhibits excellent central nervous system penetration and serves as an important tool compound for studying LRRK2 function and the pathological mechanisms of Parkinson's disease (PD)[2-3]. Additionally, MLi-2 has been shown to alleviate DOPA-responsive dystonia [4].

In vitro, treatment with MLi-2 (100nM) for 2 hours in SH-SY5Y and MEF cells expressing either wild-type or 4xSA (S910A/S935A/S955A/S973A) mutant LRRK2 significantly inhibited LRRK2 kinase activity, as evidenced by a marked reduction in the phosphorylation levels of Rab8a (T72) and Rab10 (T73), as well as decreased autophosphorylation of LRRK2 at residues S910, S935, S955, and S973[5]. MLi-2 (1 μ M) treatment for 24 hours in primary microglia derived from wild-type and G2019S-LRRK2 mice significantly reduced LRRK2 phosphorylation at Ser935, indicating effective inhibition of LRRK2 kinase activity. RNA-Seq analysis revealed that MLi-2 treatment primarily upregulated genes associated with the regulation of ion transmembrane transport and downregulated genes involved in

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the positive regulation of apoptosis^[6].

In vivo, MLi-2 (8 or 45mg/kg/day) was administered via diet to wild-type and LRRK2 G2019S knock-in mice injected with Alzheimer's disease-derived tau fibrils. Treatment began one week prior to tau injection and continued for 6 months. MLi-2 significantly reduced tau pathology progression in cortical regions of G2019S mutant mice, with the effect being more pronounced at the 45mg/kg/day dose. MLi-2 also reversed the altered direction of pathology spread caused by the G2019S mutation.

Additionally, long-term MLi-2 treatment led to enlargement of pneumocytes in the lungs^[7]. MLi-2 (10mg/kg) was administered via intraperitoneal injection to 12-month-old LRRK2 G2019S knock-in (KI) mice to assess its effects on dopamine transporter (DAT) function. Although MLi-2 significantly inhibited LRRK2 kinase activity (as shown by a 75% reduction in pSer1292 LRRK2 levels), MLi-2 failed to reverse the abnormally elevated DAT uptake activity (V_{max}) observed in G2019S KI mice^[8].

References:

- [1] Fell MJ, Mirescu C, Basu K, et al. MLi-2, a Potent, Selective, and Centrally Active Compound for Exploring the Therapeutic Potential and Safety of LRRK2 Kinase Inhibition. *J Pharmacol Exp Ther*. 2015 Dec;355(3):397-409.
- [2] Hatcher JM, Zwirek M, Sarhan AR, et al. Development of a highly potent and selective degrader of LRRK2. *Bioorg Med Chem Lett*. 2023 Oct 1;94:129449.
- [3] Tengberg JF, Russo F, Benned-Jensen T, et al. LRRK2 and RAB8A regulate cell death after lysosomal damage in macrophages through cholesterol-related pathways. *Neurobiol Dis*. 2024 Nov;202:106728.
- [4] Roman KM, Dinasarapu AR, Cherian S, et al. Striatal cell-type-specific molecular signatures reveal potential therapeutic targets in a model of dystonia. *Neurobiol Dis*. 2025 Aug;212:106981.
- [5] Kania E, Long JS, McEwan DG, et al. LRRK2 phosphorylation status and kinase activity regulate (macro)autophagy in a Rab8a/Rab10-dependent manner. *Cell Death Dis*. 2023 Jul 15;14(7):436.
- [6] Nazish I, Mamais A, Mallach A, et al. Differential LRRK2 Signalling and Gene Expression in WT-LRRK2 and G2019S-LRRK2 Mouse Microglia Treated with Zymosan and MLi2. *Cells*. 2023 Dec 26;13(1):53.
- [7] Lubben N, Brynildsen JK, Webb CM, et al. LRRK2 kinase inhibition reverses G2019S mutation-dependent effects on tau pathology progression. *Transl Neurodegener*. 2024 Mar 4;13(1):13.
- [8] Domenicale C, Mercatelli D, Albanese F, et al. Dopamine Transporter, PhosphoSerine129 α -Synuclein and α -Synuclein Levels in Aged LRRK2 G2019S Knock-In and Knock-Out Mice. *Biomedicines*. 2022 Apr 12;10(4):881.

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