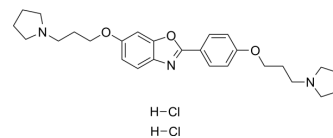


## E6446 dihydrochloride

<b>Cat. No.:</b>	HY-12756A		
<b>CAS No.:</b>	1345675-25-3		
<b>Molecular Formula:</b>	C <sub>27</sub> H <sub>37</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>3</sub>		
<b>Molecular Weight:</b>	522.51		
<b>Target:</b>	Toll-like Receptor (TLR); Stearoyl-CoA Desaturase (SCD)		
<b>Pathway:</b>	Immunology/Inflammation; Metabolic Enzyme/Protease		
<b>Storage:</b>	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : 7.6 mg/mL (14.55 mM; Need ultrasonic and warming)

Concentration	Solvent	Mass		
		1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM	1.9138 mL	9.5692 mL	19.1384 mL
	5 mM	0.3828 mL	1.9138 mL	3.8277 mL
	10 mM	0.1914 mL	0.9569 mL	1.9138 mL

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

### BIOLOGICAL ACTIVITY

#### Description

E6446 dihydrochloride is a potent and orally active TLR7 and TLR9 antagonist, used in the research of deleterious inflammatory responses. E6446 dihydrochloride is also a potent SCD1 inhibitor (KD: 4.61 μM), significantly inhibiting adipogenic differentiation and hepatic lipogenesis through SCD1-ATF3 signaling. E6446 dihydrochloride also improves liver pathology in high-fat diet (HFD)-fed mice and may be useful in the study of non-alcoholic fatty liver disease (NAFLD)<sup>[1][2][3]</sup>.

#### IC<sub>50</sub> & Target

TLR7	TLR9
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#### In Vitro

E6446 dihydrochloride is a potent and orally active TLR7 and TLR9 inhibitor. E6446 potently suppresses DNA stimulation of HEK:TLR9 cells, with an IC<sub>50</sub> value of 10 nM, but is significantly less effective at suppressing LPS endotoxin stimulation of HEK:TLR4 cells or R848 stimulation of HEK:TLR7 cells. E6446 potently inhibits IL-6 production induced by CpG2216 but is ineffective against induction by the TLR3 ligand poly inosine-cytosine. The ability of E6446 to inhibit TLR7 is ligand dependent, E6446 is a potent inhibitor of IL-6 induction by RNA but a relatively poor inhibitor of IL-6 induction by the small molecule imidazoquinoline ligand R-848. E6446 suppresses TLR9-DNA interaction in vitro, with an IC<sub>50</sub> in the 1 to 10 μM range<sup>[1]</sup>. E6446 (0.01-0.03 μM) specifically inhibits TLR9 activation with CpG ODN 2006, and blocks TLR7/8 activated by the imidazoquinoline compound R848 at 2-8 μM. E6446 reduces 50% of TLR4 activation at 30 μM, and shows IC<sub>50</sub>s of 0.01 μM

and 0.23  $\mu\text{M}$  in HEK-TLR9 cells stimulated with oligo 2006 and in human PBMCs stimulated with oligo 2216, respectively<sup>[2]</sup>. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### In Vivo

E6446 (20 mg/kg, p.o.) almost completely inhibits CpG1668-induced IL-6 production, and dose-dependently suppresses the development of ANA (anti-nuclear antibodies) in mice at 20 and 60 mg/kg<sup>[1]</sup>. E6446 (20, 60 mg/kg, p.o.) dose-dependently inhibits TLR9 signaling in mice. E6446 (60, 120 mg/kg, p.o.) prevents hyperresponsiveness of TLRs and LPS-induced septic shock in rodent malaria, diminishes TLR responsiveness during acute malaria, suppresses activation of both TLR7 and TLR9<sup>[2]</sup>.

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

## PROTOCOL

#### Cell Assay<sup>[1]</sup>

E6446 is assayed for the suppression of BALB/c mouse spleen interleukin-6 (IL-6) production in response to stimulation by oligonucleotide CpG1668. E6446 is added to dissociated splenocytes ( $5 \times 10^5$  per well in complete RPMI/10% fetal bovine serum in a 96-well plate) before addition of TLR agonists. Cells are stimulated for 72 hours, and supernatants are removed for ELISA analysis of IL-6. Mouse bone marrow-derived dendritic cells (BMDCs) are generated by culturing BALB/c marrow cells in RPMI containing 100 ng/mL Flt3 ligand for 7 days. Cells ( $1 \times 10^5$ ) in 50  $\mu\text{L}$  are assayed for IL-6 production after overnight or 24-hour stimulation with various TLR ligands. For studies using human peripheral blood mononuclear cells, Ficoll-separated mononuclear cells are isolated from healthy volunteer donors, washed, and plated with stimulatory oligonucleotide CpG2216 in complete RPMI for 72 hours. Interferon in supernatant is quantified by ELISA<sup>[1]</sup>.

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

#### Animal Administration<sup>[1]</sup>

Mice<sup>[1]</sup>

MRL/lpr mice are dosed orally five times a week with 20 or 60 mg/kg E6446 or 60 mg/kg hydroxychloroquine beginning at 5 weeks of age. Cytoxan is administered at 50 mg/kg i.p. every 10 days. A serum sample is taken immediately before the beginning of treatment to monitor changes in autoreactive antibodies. Subsequently, serum samples are collected approximately monthly and analyzed for anti-dsDNA by ELISA after 1:500 dilution. Body weights and urine samples are taken at the same interval, and proteinuria is assessed by ChemStrips. Anti-nuclear antibodies (ANA) are assessed using commercially available HEp2 slide kits, with serum diluted to 1:100 in kit buffer. ANA scores are read blinded<sup>[1]</sup>.

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

## CUSTOMER VALIDATION

- Cell Commun Signal. 2023 Sep 30;21(1):268.

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## REFERENCES

[1]. Lamphier M, et al. Novel small molecule inhibitors of TLR7 and TLR9: mechanism of action and efficacy in vivo. *Mol Pharmacol*. 2014 Mar;85(3):429-40.

[2]. Franklin BS, et al. Therapeutic targeting of nucleic acid-sensing Toll-like receptors prevents experimental cerebral malaria. *Proc Natl Acad Sci U S A*. 2011 Mar 1;108(9):3689-94.

[3]. Wang W, et al. Identification of novel SCD1 inhibitor alleviates nonalcoholic fatty liver disease: critical role of liver-adipose axis. *Cell Commun Signal*. 2023 Sep 30;21(1):268.

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

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