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## DATASHEET

Clozapine N-oxide (CNO) dihydrochloride (Water soluble)

### Product overview

<b>Name</b>	Clozapine N-oxide (CNO) dihydrochloride (Water soluble)
<b>Cat No</b>	HB6149
<b>Alternative names</b>	CNO dihydrochloride, CNO.2HCl
<b>Biological action</b>	Activator
<b>Purity</b>	>98%
<b>Customer comments</b>	<i>We have been using Clozapine N-oxide (CNO) dihydrochloride (CNO.2HCl) from Hello Bio. We are fully satisfied with this product: it is readily water soluble and stable. We have administered CNO.2HCl to animals via IP injection and intracranial microinjection; and we have observed CNO-induced cellular and behavioral effects fully consistent with its expected activation of DREADD receptors." <b>Verified customer, UTHSC</b></i>

*Great product. Great company and product. Better pricing & similar quality compared to the most of the other companies. We used CNO dihydrochloride for both i.p. and drinking water delivery, and it worked fine. Nice service. **Verified customer, University of Minnesota***

*Works as expected, and significantly cheaper than alternatives. Will use this for future CNO purchases **Verified customer, UCSF***

<b>Description</b>	Dihydrochloride salt of CNO - the prototypical DREADD activator
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### Images



### Biological Data

#### Biological description

#### Overview

Clozapine N-oxide dihydrochloride (CNO) is the dihydrochloride salt of CNO which is the prototypical chemical DREADDs activator. It is a metabolite of [clozapine](#). In rhesus macaques, CNO dihydrochloride shows improved bioavailability compared to CNO freebase with less conversion to clozapine

#### Uses

'Excitatory' ( $G_q$ -coupled) DREADDs:

CNO activates the excitatory  $G_q$ -coupled DREADDs: hM3Dq, hM1Dq and hM5Dq (pEC<sub>50</sub> values are 7.26 and 8.61 at hM3Dq and hM1Dq respectively).

The hM3Dq DREADD is typically used for enhancing neuronal firing and activity ( $G_q$  signaling in neuronal and non-neuronal cells).

#### 'Inhibitory' ( $G_i$ -coupled) DREADDs:

CNO also activates the inhibitory hM4Di and hM2Di  $G_i$ -coupled DREADDs ( $pEC_{50} = 6.89$  at hM4Di).

The hM4Di DREADD is the most commonly used inhibitory DREADD and is used for neuronal silencing.

#### $G_s$ and $\beta$ -arrestin coupled DREADDs:

CNO also activates the  $G_s$ -coupled DREADD (GsD) and the  $\beta$ -arrestin preferring DREADD: rM3Darr (Rq(R165L)).

Recent findings (Gomez et al 2017) suggest that systemically administered CNO does not readily cross the blood-brain-barrier in vivo, and converts to clozapine which activates DREADDs. Enzymatic and non-enzymatic reduction of CNO to clozapine has been shown in humans, rats, monkeys, guinea pigs and mice.

Care must therefore be taken in experimental design and proper controls should be incorporated, for example, the use of non-DREADD expressing animals may be appropriate (see Mahler and Aston-Jones (2018)).

Jendryka et al (2019) found that in mice, CNO does enter the brain and that unbound CNO is present in the brain at sufficient levels to activate DREADDs directly. Results suggested that CNO is a suitable DREADD agonist but requires between-subject controls for unspecific effects.

CNO has proved to be an effective actuator of muscarinic DREADDs and provided controls are in place, will continue to be an excellent tool. [Compound 21 \(DREADD agonist 21\)](#) represents an alternative to CNO for in vivo studies in which metabolic conversion of CNO to clozapine is an issue (Thompson et al 2019).

#### Administration

In the literature, CNO has been administered intraperitoneally (i.p.), subcutaneously, directly infused intracranially, via drinking water, osmotic mini-pump and recently via eye drops. See our [Technical review \(table 3\)](#) for example administration methods and doses.

#### Handling & stability

Our stability studies have shown that CNO dihydrochloride is easier to solubilize and handle and have found that this product does not precipitate in solution unlike the freebase form of CNO (which, due to its inherent chemical properties requires careful handling and has been shown in the literature to precipitate in aqueous solution under certain conditions). For more info please see [Stability of Water-Soluble DREADD ligands in Solution: A Technical Review](#) and our handling guidelines below.

#### Application notes

##### Other Non-CNO DREADD activators available:

- [Water soluble Compound 21 \(DREADD agonist 21\) hydrochloride](#) has minimal off-target activity and is indicated not to metabolize to clozapine. The freebase [Compound 21 \(DREADD agonist 21\)](#) is also available.
- [Perlapine hydrochloride \(water soluble\)](#) is a potent and selective hM3Dq DREADD agonist. [Perlapine freebase](#) is also available.
- [Salvinorin B \(SALB\)](#) Salvinorin B (SALB) potently activates the inhibitory KORD DREADD to induce neuronal inhibition.

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## Solubility & Handling

#### Storage instructions Solubility overview Handling

-20 °C (desiccate)

Soluble in water (100 mM). Always store solutions at -20 °C.

##### Storage of solid

- Store at -20 °C.
- Please note that the compound is a hygroscopic solid and contact with air may cause material to become sticky. Product performance should not be affected but we recommend storing the

material in a sealed jar.

#### Storage of solutions

- Make up solutions and use immediately.
- If storage of solutions is required, you should aliquot out the solution into tightly sealed vials and store at -20°C and store these for up to one month.
- Allow the product to equilibrate to RT for at least one hour before opening and using.

#### Storage of solutions at room temperature

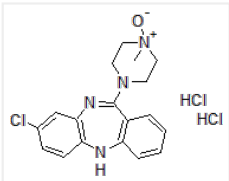
- We recommend only keeping solutions at room temperature (25°C) for a few days as our studies have shown that after 96 hours the purity of the compound in solution drops to ~95% and will continue to drop over time.

#### **Important**

This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

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## Chemical Data

<b>Chemical name</b>	8-Chloro-11-(4-methyl-4-oxido-1-piperaziny)-5H-dibenzo[b,e][1,4]diazepine dihydrochloride
<b>Molecular Weight</b>	415.74
<b>Chemical structure</b>	
<b>Molecular Formula</b>	C <sub>18</sub> H <sub>19</sub> ClN <sub>4</sub> O <sub>2</sub> ·2HCl
<b>CAS Number</b>	2250025-93-3
<b>PubChem identifier</b>	0
<b>SMILES</b>	<chem>C1C(C=C1)=CC2=C1NC(C=CC=C3)=C3C(N4CC[N+](C)([O-])CC4)=N2.Cl.Cl</chem>
<b>Source</b>	Synthetic
<b>InChi</b>	InChI=1S/C18H19ClN4O2.ClH/c1-23(24)10-8-22(9-11-23)18-14-4-2-3-5-15(14)20-16-7-6-13(19)12-17(16)21-18;;/h2-7,12,20H,8-11H2,1H3;2*1H
<b>InChiKey</b>	MBRGKRXDVKTUPT-UHFFFAOYSA-N
<b>Appearance</b>	Orange solid

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## References

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Wess et al (2013) Trends Pharmacol Sci. 34(7)

**PubMedID** [23769625](#)

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Arbruster et al (2007) Proc Natl Acad Sci U S A 104(12)

**PubMedID** [17360345](#)

### **Differential regulation of rat 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors after chronic treatment with clozapine, chlorpromazine and three putative atypical antipsychotic drugs.**

Kuoppamäki M *et al* (1995) Neuropsychopharmacology 13(2)

**PubMedID** [8597525](#)

### **Chemogenetics revealed: DREADD occupancy and activation via converted clozapine.**

Gomez et al (2017) Science 357(6350)

**PubMedID** [28774929](#)

### **CNO Evil? Considerations for the Use of DREADDs in Behavioral Neuroscience.**

Mahler and Aston-Jones (2018) Neuropsychopharmacology doi: 10.1038

**PubMedID** [29303143](#)

### **DREADD Agonist 21 Is an Effective Agonist for Muscarinic-Based DREADDs in Vitro and in Vivo**

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**A Comparative Study of the Pharmacokinetics of Clozapine N-Oxide and Clozapine N-Oxide Hydrochloride Salt in Rhesus Macaques.**

Allen et al (2019) J Pharmacol Exp Ther 368(2)

**PubMedID** [30523062](#)

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Jendryka et al (2019) Sci Rep. 9(1)

**PubMedID** [30872749](#)

**DREADDs: The Power of the Lock, the Weakness of the Key. Favoring the Pursuit of Specific Conditions Rather than Specific Ligands.**

Goutaudier et al (2019) eNeuro 6

**PubMedID** [31562177](#)

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