Hello Bio, Inc. 304 Wall St., Princeton, NJ 08540 USA

T. 609-683-7500 F. 609-228-4994

customercare-usa@hellobio.com



DATASHEET

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

Product overview

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

Cat No HB6149

Alternative names CNO dihydrochloride, CNO.2HCl

Biological action Activator Purity >98%

Customer comments 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." Verified customer, UTHSC

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

Description Dihydrochloride salt of CNO - the prototypical DREADD activator

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 (pEC₅₀ 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' (Gi- coupled) DREADDs:

CNO also activates the inhibitory hM4Di and hM2Di G_i-coupled DREADDs (pEC₅₀ = 6.89 at hM4Di).

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

Gs and β-arrestin coupled DREADDs:

CNO also activates the G_s - coupled DREADD (GsD) and the β -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 & stabliity

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.

Solubility & Handling

Solubility overview Storage instructions Handling Soluble in water (100 mM). Always store solutions at -20 °C. -20 °C (desiccate) Storage of solid

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

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.

Shipping Conditions Important

Stable for ambient temperature shipping. Follow storage instructions on receipt.

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

for human or veterinary use

Chemical Data

Chemical name Molecular Weight **Chemical structure** 8-Chloro-11-(4-methyl-4-oxido-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine dihydrochloride

415.74

C₁₈H₁₉CIN₄O.2HCI

Molecular Formula **CAS Number PubChem identifier**

2250025-93-3

SMILES

Source

InChl=1S/C18H19CIN4O.2CIH/c1-23(24)10-8-22(9-11-23)18-14-4-2-3-5-15(14)20-16-7-6-13(19)12-InChi

17(16)21-18;;/h2-7,12,20H,8-11H2,1H3;2*1H

InChiKey **Appearance** MBRGKRXDVKTUPT-UHFFFAOYSA-N

Orange solid

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)

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Differential regulation of rat 5-HT2A and 5-HT2C receptors after chronic treatment with clozapine, chlorpromazine and three putative atypical antipsychotic drugs.

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Chemogenetics revealed: DREADD occupancy and activation via converted clozapine.

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

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Allen et al (2019) J Pharmacol Exp Ther 368(2) **PubMedID**30523062

Pharmacokinetic and pharmacodynamic actions of clozapine-N-oxide, clozapine, and compound 21 in DREADD-based chemogenetics in mice.

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