

DATASHEET

Clozapine N-oxide (CNO) (freebase)

Product overview

Name	Clozapine N-oxide (CNO) (freebase)
Cat No	HB1807
Alternative names	CNO, CNO freebase
Biological action	Activator
Purity	>98%
Customer comments	Your <i>technical review</i> on CNO stability, solubility and use in the lab has been really helpful to my group. Verified customer

*Exactly as described - Clozapine N-oxide (CNO) (freebase) does exactly what it should. Great product and company. **Verified customer, Istituto Italiano di Tecnologia***
Prototypical DREADD activator. Clozapine metabolite.

Description

Images



Biological Data

Biological description

Overview

Clozapine N-oxide (CNO) is the prototypical chemical actuator for various DREADDs. It is a metabolite of the atypical antipsychotic **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' (G_i-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.

Water soluble Clozapine N-oxide (CNO) dihydrochloride is also available:

[Clozapine N-oxide \(CNO\) dihydrochloride](#) is water soluble and easier to solubilise and handle. Our stability studies have found that this product does not precipitate in aqueous solution unlike the freebase form of CNO (which due to its inherent chemical properties requires careful handling, has been shown in the literature to precipitate in solution under certain conditions and batch to batch variation in solubility can occur).

In rhesus macaques, CNO dihydrochloride shows improved bioavailability compared to CNO freebase with less conversion to clozapine

Stability Studies

For more info on the stability of CNO (freebase) and water-soluble CNO dihydrochloride, please see the following guides:

- [Clozapine N-Oxide \(freebase\) - a technical review on stability, solubility and use in the lab](#)
- [Stability of Water-Soluble DREADD ligands in Solution: A Technical Review](#)

Solubility & Handling

Storage instructions Solubility overview Handling

Room temperature (desiccate)

Soluble in DMSO (100mM).

Due to the inherent chemical properties of CNO, this product requires careful handling – our guidelines are detailed below.

Batch to batch variation in solubility can occur. Some batches are water soluble but ongoing solubility in aqueous solutions can be unpredictable.

CNO has been shown in the literature to precipitate in aqueous solution and following further solubility studies that we have undertaken, we have found that precipitation occurred in some concentrated (100mM) samples of CNO dissolved in water.

We therefore recommend using DMSO when working with CNO freebase. If you need to work with aqueous solutions, you should use [CNO dihydrochloride](#) which is easier to solubilize and handle than CNO freebase. Our stability studies have found that this product does not precipitate in solution.

Solubilizing in DMSO

CNO (freebase) is soluble in DMSO to 100 mM.

Storage of solutions in DMSO

1. Aliquot out the solution into tightly sealed vials for storage.
2. Storage of solutions should be at room temperature.
3. Please take care to ensure that your product is completely dissolved in your solution before use.

Solubilizing CNO (freebase) in aqueous solutions (e.g. water / saline)

We **do not** recommend using CNO freebase with aqueous solutions however, we provide these guidelines if you do:

When working with the compound in aqueous solutions, we recommend:

1. Make up solutions and use immediately. Do not store solutions.
2. Ensure you work in a dust free environment when preparing and handling solutions.
3. Ensure that the product and solvents are at ambient temperature before preparing solutions. This means that you should allow the product to equilibrate to RT for at least one hour before opening and using.
4. Please take care to ensure that your product is completely dissolved in your solution before use.

Stability

Our stability studies have shown that CNO in solution remains chemically stable (>99% purity by HPLC) for at least 4 weeks at room temperature.

Storage of solutions in aqueous solutions (e.g. water/saline)

We recommend that you do not store aqueous solutions of CNO. However, if this is necessary:

- Aliquot out the solution into tightly sealed vials for storage. Storage of solutions should be at room temperature.
- Always check that your product is completely dissolved before use; if precipitation is found follow the steps below.
- Do not store solutions at temperatures below room temperature (i.e. +4°C/ -20°C) - this is more likely to lead to precipitation of the compound.
- Preferably, solutions should be made in transparent, colorless vials so that any precipitation can be observed and remedied prior to use.

Precipitation

If you find precipitate in your solution, gently heat your solution in a water bath to approx 40°C and the compound should readily re-dissolve. Always take care to ensure that the compound is completely dissolved before use.

Water soluble alternatives:

CNO dihydrochloride is now available and is also water soluble. It is easier to solubilise and handle and unlike the freebase form of CNO, our stability studies have found that this product does not precipitate in solution.

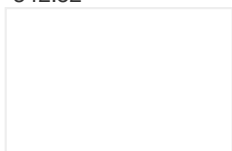
Important

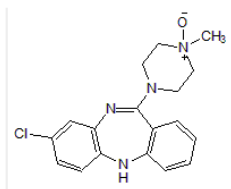
This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use.

Chemical Data

Chemical name
Molecular Weight
Chemical structure

8-Chloro-11-(4-methyl-4-oxido-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine
342.82





Molecular Formula	C ₁₈ H ₁₉ ClN ₄ O
CAS Number	34233-69-7
PubChem identifier	2819
SMILES	C[N+]1(CCN(CC1)C2=C3C=CC=CC3=NC4=C(N2)C=C(C=C4)Cl)[O-]
Source	Synthetic
InChi	InChI=1S/C18H19ClN4O/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/h2-7,12,21H,8-11H2,1H3
InChiKey	WYRDWWAASBTJLM-UHFFFAOYSA-N
MDL number	MFC00210190
Appearance	Yellow solid

References

Novel designer receptors to probe GPCR signaling and physiology.

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

PubMedID [23769625](#)

Evolving the lock to fit the key to create a family of G protein-coupled receptors potentially activated by an inert ligand.

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

PubMedID [17360345](#)

The role of M1 muscarinic receptor agonism of N-desmethylozapine in the unique clinical effects of clozapine.

Weiner et al (2004) Psychopharmacology (Berl). 177(1-2)

PubMedID [15258717](#)

A chemical-genetic approach to study G protein regulation of beta cell function in vivo.

Guettier et al (2009) Proc Natl Acad Sci U S A 106(45)

PubMedID [19858481](#)

Chemogenetics revealed: DREADD occupancy and activation via converted clozapine.

Gomez et al (2017) Science 357(6350)

PubMedID [28774929](#)

Chemogenetic inhibition of cells in the paramedian midbrain tegmentum increases locomotor activity in rats.

Wirtshafter and Stratford (2016) Brain Res 1632:

PubMedID [26707405](#)

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

Thompson et al (2018) ACS Pharmacol. Transl. Sci. 10.1021

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](#)

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

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](#)
