

# NMDA receptor antagonist

BIII 277CL

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## Summary

BIII 277CL is a highly potent and selective antagonist of the NMDA receptor ( $K_i = 4.5$  nM) that can be used to test biological hypotheses.

## Chemical Structure

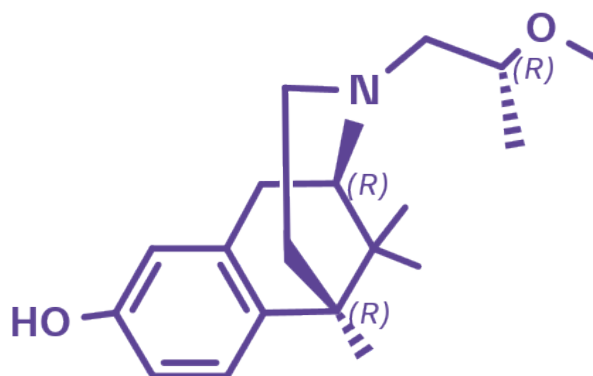


Figure 1: 2D structure of BIII 277CL

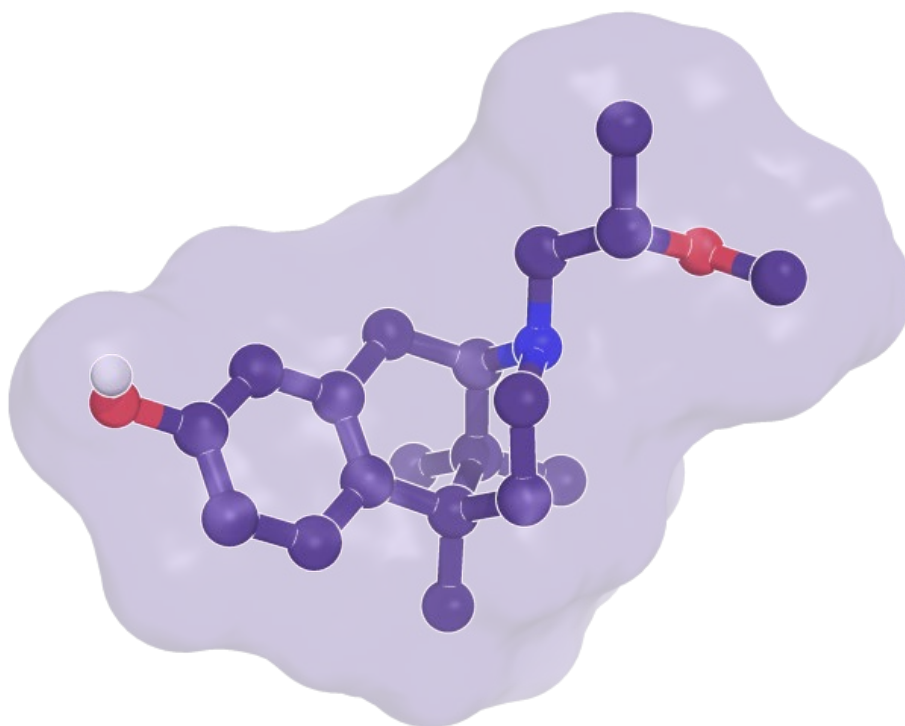


Figure 2: 3D structure of BIII 277CL

## Highlights

BIII 277CL is a selective high affinity blocker of the NMDA receptor ion channel ( $K_i = 4.5$  nM). This molecule is available for both *in vitro* and *in vivo* experiments. In mice, it showed significant neuroprotective effects. BIII 277CL showed dose-dependent reduction of the cortical infarct area in mice with focal cerebral ischemia and also prevented NMDA-induced lethality.

## Target information

The NMDA receptor is a subtype of excitatory amino acid receptor found in nerve cells, which allows cationic influx upon activation by glutamate and glycine.

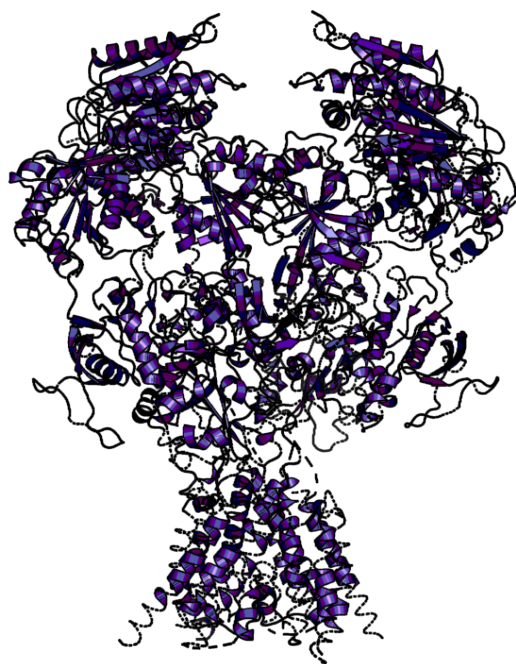
Structurally, the NMDA receptor–channel complex consists of a voltage-dependent  $\text{Na}^+/\text{Ca}^{2+}$  channel and at least 3 different regulatory domains: neurotransmitter recognition site (glutamate, aspartate, or NMDA binding site), strychnine-insensitive glycine site, and ion channel itself (can be blocked by  $\text{Mg}^{2+}$  or other blockers).

During ischemia, large amounts of glutamate and aspartate

(excitatory neurotransmitters) are released into the extracellular space, which can lead to neuronal deaths. This implicates the NMDA receptor–channel complex with pathophysiology of numerous neurological disorders, such as stroke, epilepsy, Alzheimer's disease, ALS, and others.

BIII 277CL is a high affinity blocker of the NMDA receptor ion channel with specificity over other binding sites of the NMDA receptor–channel complex. In addition, BIII 277CL did not exhibit significant affinities for other central neurotransmitter receptors.

BIII 277CL also antagonized NMDA-induced  $[^3\text{H}]$ noradrenaline release and NMDA-induced inhibition of protein synthesis in rat hippocampal slices.



**Figure 3: Structure of the NMDA receptor from *Xenopus laevis* (PDB code: 4TLM<sup>6</sup>)**

## ***In vitro* activity**

BIII 277CL is a high affinity blocker of the NMDA receptor ion channel ( $K_i = 4.5$  nM; [<sup>3</sup>H]MK-801 displacement assay in rat brain synaptosomal membrane) with specificity over other binding sites of the NMDA receptor–channel complex (glycine and NMDA binding sites). In addition, BIII 277CL did not exhibit significant affinities for other central neurotransmitter receptors.

PROBE NAME / NEGATIVE CONTROL	BIII 277CL	BI-25CL
MW [Da, free base] <sup>a</sup>	303.4	303.4
Displacement of [ <sup>3</sup> H]MK-801 $K_i$ [nM] <sup>b</sup> MK-801: dizocilpine	4.5	>50,000.0

<sup>a</sup> For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs

<sup>b</sup> Assay conditions see reference 1

## In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BIII 277CL	BI-25CL
logD @ pH 11	5.3	5.1
Solubility @ pH 7 [ $\mu\text{g/mL}$ ]	>38	>74
Solubility @ pH 5 [ $\mu\text{g/mL}$ ]	>38	>77
Caco-2 permeability AB @ pH 7.4 [ $\cdot 10^{-6} \text{ cm/s}$ ]	57	71
Caco-2 efflux ratio	0.9	0.7
MDCK permeability $P_{\text{appAB}}$ @ $1 \mu\text{M}$ [ $10^{-6} \text{ cm/s}$ ]	53	54
MDCK efflux ratio	0.9	0.9
Microsomal stability (human,mouse,rat) [% $Q_H$ ]	49 / 83 / >88	<23 / 87 / >88
Hepatocyte stability (human/mouse/rat) [% $Q_H$ ]	90 / 99 / 91	43 / 98 / 92
Plasma Protein Binding (human/mouse/rat) [%]	68.2 / 66.3 / 67.3	74.3 / 60.6 / 62.6
CYP 3A4 ( $\text{IC}_{50}$ ) [ $\mu\text{M}$ ]	>50	>50
CYP 2C8 ( $\text{IC}_{50}$ ) [ $\mu\text{M}$ ]	>50	>50
CYP 2C9 ( $\text{IC}_{50}$ ) [ $\mu\text{M}$ ]	>50	>50
CYP 2C19 ( $\text{IC}_{50}$ ) [ $\mu\text{M}$ ]	>50	18.5
CYP 2D6 ( $\text{IC}_{50}$ ) [ $\mu\text{M}$ ]	14.7	>50.0

## In vivo DMPK parameters

BIII 277CL	RAT
Clearance [% $Q_H$ ] <sup>a</sup>	220
Mean residence time after <i>i.v.</i> dose [h]	0.5
$V_{ss}$ [L/kg] <sup>a</sup>	4.8

<sup>a</sup> *i.v.* dose: 0.3 mg/kg

## In vivo pharmacology

In mice, BIII 277CL showed significant neuroprotective effects. The title compound prevented NMDA-induced lethality and caused disturbances in motor coordination ( $ID_{50} = 0.54$  mg/kg s.c. and  $ED_{50} = 0.47$  mg/kg s.c., respectively).

Furthermore, intraperitoneal or subcutaneous application of BIII 277CL to mice dose-dependently reduced the cortical infarct area from focal cerebral ischemia by unilateral occlusion of the middle cerebral artery.

## Negative control

In addition, we offer a structurally closely related BI-25CL, which showed no marked affinity at the NMDA receptor ion channel ( $K_i = 50$   $\mu$ M; [ $^3$ H] MK-801 displacement assay in rat brain synaptosomal membrane) to be used as a control compound in *in vitro* studies.

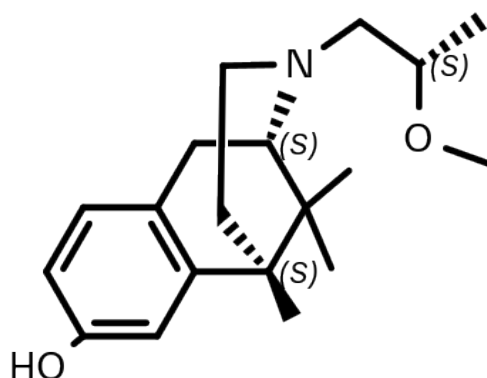


Figure 4: BI-25CL which serves as a negative control

## Selectivity

BIII 277CL displayed only weak affinities for  $\sigma$ - and  $\mu$ -opiate binding sites ([ $^3$ H]DTG, [ $^3$ H]dihydromorphine, [ $^3$ H]naloxone displacement assays in rat brain synaptosomal membrane) and 200-fold selectivity toward the channel site of the NMDA receptor–channel complex with no marked affinities up to 10  $\mu$ M for the glycine site and the NMDA binding site of the NMDA receptor–channel complex.

Additionally, BIII 277CL did not exhibit affinities up to 100  $\mu$ M for the dopamine  $D_1$  and  $D_2$  receptors ([ $^3$ H]SCH 23390 and [ $^3$ H]spiroperidol displacement assay).

SELECTIVITY DATA AVAILABLE	BIII 277CL	BI-25CL
SafetyScreen44™ with kind support of  eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

## Reference molecule(s)

For an overview of NMDA receptor antagonists please refer to the corresponding [Wikipedia article](#) and references therein.

## Supplementary data

Selectivity data can be downloaded free of charge from [opnMe](#).

## References

1. Grauert M., Bechtel W. D., Ensinger H. A., Merz H., Carter A. J. Synthesis and structure-activity relationships of 6,7-benzomorphan derivatives as antagonists of the NMDA receptor-channel complex *J Med Chem* **1997**, 40(18), 2922–2930. [DOI: 10.1021/jm970131j](#), [PubMed: 9288174](#).
2. Carter A. J., Bechtel W. D., Grauert M., Harrison P., Merz H., Stransky W. BIII 277 CL is a potent and specific ion-channel blocker of the NMDA receptor-channel complex *J Pharmacol Exp Ther* **1995**, 275(3), 1382–1389, [PubMed: 8531106](#).
3. Grauert M., Rho J. M., Subramaniam S., Rogawski M. A. N-methyl-D-aspartate receptor channel block by the enantiomeric 6,7-benzomorphans BIII 277 CL and BIII 281 CL *J Pharmacol Exp Ther* **1998**, 285(2), 767–776, [PubMed: 9580625](#).
4. Pschorn U., Carter A. J. The influence of repeated doses, route and time of administration on the neuroprotective effects of BIII 277 CL in a rat model of focal cerebral ischemia *J Stroke Cerebrovasc Dis* **1996**, 6(2), 93–99. [DOI: 10.1016/s1052-3057\(96\)80010-3](#), [PubMed: 17894976](#).
5. Carter A. J. The importance of voltage-dependent sodium channels in cerebral ischaemia *Amino Acids* **1998**, 14(1-3), 159–169. [DOI: 10.1007/BF01345257](#), [PubMed: 9871456](#).



6. Lee C.-H., Lü W., Michel J. C., Goehring A., Du J., Song X., Gouaux E. Nmda receptor structures reveal subunit arrangement and pore architecture Nature 2014, 511(7508), 191–197. [DOI: 10.1038/nature13548](https://doi.org/10.1038/nature13548), [Pubmed:25008524](https://pubmed.ncbi.nlm.nih.gov/25008524/).