

# Serine racemase interaction with N-methyl-D-aspartate receptors antagonist reveals potential alternative target of chronic pain treatment: Molecular docking study

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## ABSTRACT

Serine racemase (SR) catalyzes L-serine racemization to activate the N-methyl-D-aspartate receptor (NMDAR). NMDAR activation is associated with the progression of acute-to-chronic neuropathic pain. This study aimed to investigate NMDAR antagonist interactions with SR to obtain potential chronic pain target therapy. Several NMDAR antagonist drugs were obtained from the drug bank, and malonate was used as a control inhibitor. Ligands were prepared using the open babel feature on PyRx. The SR structure was obtained from Protein data bank (PDB) (3I6B) and then docked with ligands using the AutoDock Vina. Haloperidol had a lower binding affinity than malonate and other ligands. Ethanol had the highest binding affinity than other drugs but could bind to the Adenosine triphosphate (ATP)-binding domain. Haloperidol is bound to reface that function for reprotonation in racemization reaction to produce D-serine. Halothane bond with Arg135 residues aligned negatively charged substrates to be reprotonated properly by reface. Tramadol is bound to amino acid residues in the triple serine loop, which determines the direction of the SR reaction. Several NMDAR antagonists such as haloperidol, halothane, ethanol, and tramadol bind to SR in the specific binding site. It reveals that SR potentially becomes an alternative target for chronic pain treatment.

**Key words:** Chronic pain, N-methyl-D-aspartate receptor, serine racemase, treatment

## INTRODUCTION

Neuropathic pain is more difficult to treat than other types of chronic pain. This condition can reduce the patient's quality of life by affecting daily activity, loss of ability and

productivity to work, and increased costs associated with informal assistance by friends and families, which pose a considerable economic burden.<sup>[1,2]</sup> Neuropathic pain symptoms are complex, and it is challenging to determine an adequate treatment. Understanding the pathophysiology of neuropathic pain can help develop appropriate diagnostic and intervention procedures.<sup>[3]</sup>

Neuropathic pain involves central sensitization caused by the opening N-methyl-D-aspartate receptor (NMDAR),

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either in the peripheral or central nerves.<sup>[4]</sup> The NMDAR is activated by glutamate, co-agonists glycine, or D-serine. Glycine or D-serine binds to the GluN1 subunit, the obligatory subunit in NMDAR heteromers.<sup>[5]</sup> D-serine is more potent in activating NMDAR, ten times stronger than glycine.<sup>[6]</sup> Since activation of NMDA receptor regulated by binding of glutamate, co-agonist glycine, or D-serine, inhibition of its ligands-receptor binding could lower the activation of NMDAR.<sup>[7]</sup> This inhibition is used in several drugs that act as a noncompetitive antagonist of NMDAR.

D-serine is a product of L-serine racemization by serine racemase (SR).<sup>[8]</sup> The reduction of D-serine is associated with a decrease in pain symptoms.<sup>[9]</sup> Inhibition of SR activity may occur through interaction with noncompetitive antagonists of NMDA.<sup>[10]</sup> SR is found in astrocytes and fibroblasts, and Schwann cells of the sciatic nerve.<sup>[11]</sup> This inhibition of this enzyme might also help lower pain originating from the peripheral nerve.

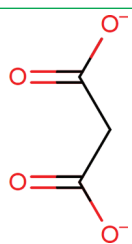
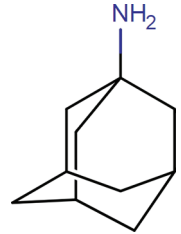
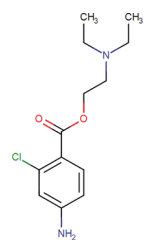
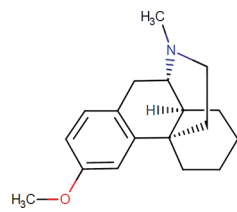
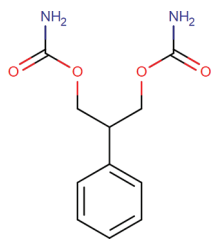
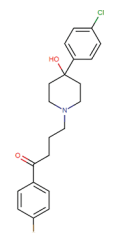
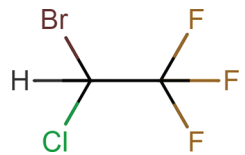
NMDAR hyperactivation due to an excess of D-serine plays a role in the emergence of various diseases, including neuropathic pain.<sup>[9]</sup> NMDA receptor antagonists are widely used to treat chronic pain.<sup>[12]</sup> A study reported that NMDA antagonist, i. e., (r, s)-ketamine, could reduce pain and lower D-serine blood concentration immediately after infusion in ketamine responder.<sup>[13]</sup> R-ketamine lowers the intracellular and extracellular D-serine while S-ketamine decreases only extracellular D-serine. A study by Laurido *et al.*<sup>[14]</sup> found that intrathecal injection of L-serine-O-sulfate and L-erythro-3-hydroxyaspartate could reduce D-serine by SR inhibition in mice. However, the study assessing SR as potential chronic pain target therapy is limited. Furthermore, this *in silico* study aims to investigate the interaction between NMDAR antagonist and SR, which can provide information about SR as potential chronic pain target therapy.

## MATERIAL AND METHODS

### Ligand and protein data mining

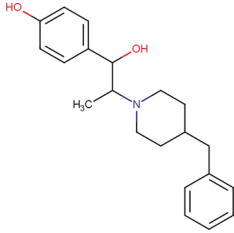
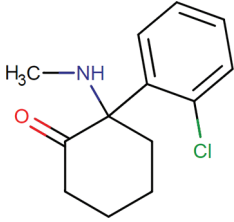
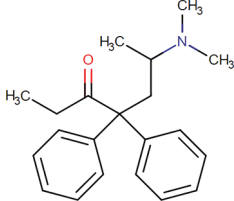
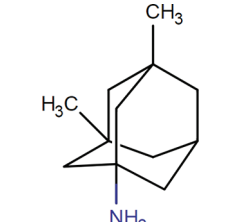
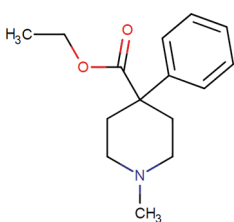
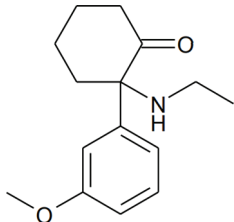
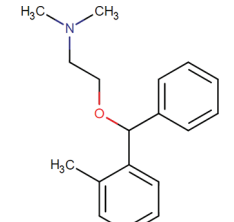
Ligands were searched using a drug bank ([www.drugbank.com](http://www.drugbank.com)) with the keyword NMDA receptor antagonist. Ligands 3D structure were downloaded from PubChem including L-serine (CID: 5951), malonate (CID: 9084), agmatine (CID: 199), amantadine (CID: 2130), chlorprocaine (CID: 8612), dextromethorphan (CID: 5360696), felbamate (CID: 3331), ethanol (702), haloperidol (CID: 3559), halothane (CID: 3562), ifenprodil (CID: 3689), ketamine (CID: 3821), methadone (CID: 4095), memantine (CID: 4054), meperidine (4058), methoxetamine (CID: 52911279), orphenadrine (CID: 4601), phencyclidine (CID: 6468), procaine (CID: 4914), and tramadol (CID 33741) [Table 1]. SR was used as a receptor and mined from PDB <http://www.rcsb.org/pdb> with ID 3l6B [Figure 1].

**Table 1: Two-dimensional structure of N-methyl-D-aspartate receptor antagonist**

Compound name	Structure 2D	Function
Malonate		Potent inhibitor of serine racemase, used as control
Amantadine		Antiparkinson, to treat extrapyramidal reaction and postherpetic neuralgia
Chlorprocaine		Local anesthetic, subarachnoid block, or spinal anesthesia
Dextromethorphan		For cough treatments, common cold and upper respiratory caused by allergies
Felbamate		Anticonvulsant in severe epilepsy
Haloperidol		To treat schizophrenia and psychoses
Halothane		General inhalation anesthetic

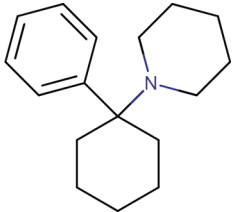
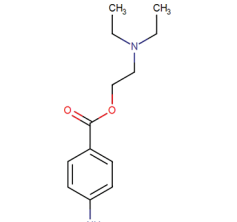
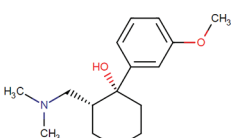
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Table 1: Contd...

Compound name	Structure 2D	Function
Ifenprodil		NMDAR noncompetitive antagonist, in the investigation to treat COVID-19
Ketamine		General anesthetic and NMDAR antagonist
Methadone		Opioid analgesic to treat severe pain
Memantine		Block glutamate neurotransmitters to prevent excessive neuron stimulation in Alzheimer's disease
Meperidine		To treat severe pain, i.e., neuropathic pain and cancer pain
Methoxetamine		Ketamine derivative, anesthetic, and analgesics
Orphenadrine		To relieve musculoskeletal pain

Contd...

Table 1: Contd...

Compound name	Structure 2D	Function
Phencyclidine		General anesthetic, an antagonist of NMDAR 3A
Procaine		Local anesthetic, peripheral, and spinal nerve blocks
Tramadol		Opioids to treat moderate-to-severe pain in adult

NMDAR: N-methyl-D-aspartate receptor

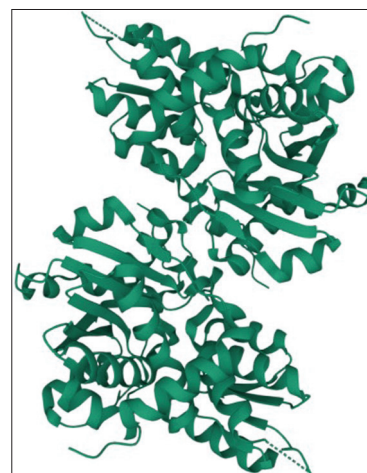


Figure 1: Serine racemase (PDB ID 316B)

### Molecular docking of ligand and receptor

Ligands were prepared using PyRx 0.8 to minimize the binding energy of the ligand, and SR was prepared using Discovery Studio v. 19 software to remove any ligand or water bound to the receptors. The receptors appear in dimers, and both had equivalent structures; thus, the monomeric were used for the calculations. Ligands and enzymes were docked and analyzed using AutoDock Vina. Docking is done by targeting several amino acid residues in SR based on known active sites and important binding domains,<sup>[15]</sup> including R-135, S-242, S-84, S-83, H-87, D-132, E-136, P-231, K-241, H-82, P-153, D-238, G-239, N-86, N-154, G-85, N-229, S-243, and H-152. Energy calculations are performed with each of these servers.

The docking results were visualized using PyMol and Discovery Studio.

## RESULTS

Ligand data mining was shown in Table 1, consisting of compound name, 2D structure, and its function in pain treatment. Protein data mining is shown in Figure 1.

Based on the docking result, malonate, a potent SR inhibitor, forms 10 hydrogen bonds with SR at the amino acid residue Ser83, Ser84 (3 bonds), Gly65, Asn86, His87, Arg135, Ser242, and Gly239 with a binding energy of  $-6$  kcal/mol [Table 2]. Docking between NMDAR antagonist and SR shows that haloperidol bind to Ser84 residue with the lowest binding energy than other antagonists ( $-7.3$  kcal/mol). Haloperidol forms five bond types: carbon-hydrogen bond, halogen; pi-anion; amide-pi; and pi-alkyl. Ethanol bonds with amino acid residues Ser84, Asn86, and His87 with a binding affinity of  $-3.3$  kcal/mol. Ethanol forms a hydrogen bond and is an unfavorable donor. Felbamate and ifenprodil did not share the same residues with control but had an equal binding affinity with malonate ( $-6$  kcal/mol). Halothane binds to the amino acid residue Arg135 ( $-4$  kcal/mol). Halothane forms three bonds: carbon-hydrogen bond, halogen, and alkyl. Other NMDAR antagonists include ketamine, amantadine, chlorprocaine, dextromethorphan,

meperidine, methoxetamine, orphenadrine, phencyclidine, methadone, tramadol, and procaine did not share the same amino acid residue with control. However, tramadol bind to the SR-specific binding site Asn154 [Table 3]. Based on the docking simulation, four NMDAR antagonists are known to form an interaction with SR. The two-dimension docking model is shown in Figure 2.

## DISCUSSION

Neuropathic pain involves a variety of pathological mechanisms in the central and peripheral nervous systems. Peripheral NMDAR interaction with D-serine plays a role in central sensitization, increasing synaptic neurons' excitability and efficacy in spinal pain pathways.<sup>[16]</sup> D-serine is made in presynaptic neurons and acts as an NMDAR co-agonist by binding to the glycine binding site. Basal D-serine levels are important in synaptic efficiency and long-term potentiation<sup>[17]</sup> and play a role in central sensitization in the occurrence of pain.<sup>[16]</sup> D-serine is produced from 3-phosphoglycerate to become L-serine in astroglia, then shuttled to presynaptic neurons containing SR.<sup>[18]</sup>

SR is allosterically activated by ATP and requires  $Mg^{2+}$  and  $Mn^{2+}$  cations. The amino acid residue Ser84 is a reface of SR. Another active site is on the si face, which is located on Ser56. L-serine that binds to the si face will undergo

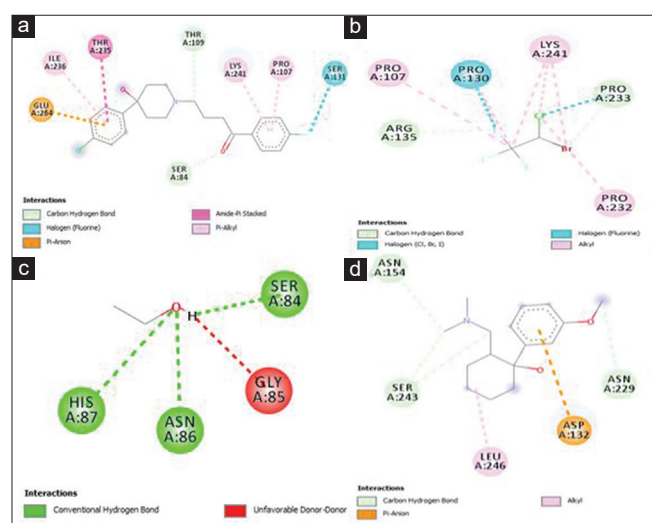
**Table 2: The binding affinity and amino acid residues of serine racemase and ligands**

Ligand	Binding affinity (kcal/mol)	Serine racemase amino acid residues	Bond
Malonate (control)	-6	Ser83; Asn86; His87; Gly85; Gly239; Ser84; Arg135; Ser242	Hydrogen bond
Amantadine	-4.4	Ser243; Asp132	Hydrogen bond
Chlorprocaine	-5.2	Leu230; Tyr231; Asn213; Asp215; Leu230; Leu230	Hydrogen bond; carbon-hydrogen bond; Pi-anion; alkyl; Pi-alkyl
Dextromethorphan	-5.9	Glu264; Pro111; 11236	Carbon-hydrogen bond; alkyl; Pi-alkyl
Ethanol	-3.1	Asn86; His87; Ser84	Hydrogen bond
Felbamate	-6	Thr109; Lys241; Pro130; Ppro233; Lys241	Hydrogen bond; carbon-hydrogen bond; Pi-alkyl
Haloperidol	-7.3	Ser84; Thr109; Ser131; Glu264; Thr235; Ile236; Pro107; Lys241; Ile236	Carbon-hydrogen bond; halogen; Pi-anion; amide-Pi; Pi-alkyl
Halothane	-4	Arg135; Pro233; Pro233; Pro130; Pro107; Pro130; Lys241; Pro233; Pro232; Pro233	Carbon-hydrogen bond; halogen; alkyl
Ifenprodil	-6	Thr235; Asp122; Glu264; Thr235; Ile236; Pro111; Ile236	Hydrogen bond; Oi-anion; amide Pi-stacked; alkyl; Pi-alkyl
Ketamine	-5.3	Glu136; Leu226; Pro228	Pi-anion; alkyl
Memantine	-5	Ser243; Leu226; Pro228	Hydrogen bond; Alkyl
Meperidine	-4.5	Asn213; Glu234; Tyr231; Tyr231	Hydrogen bond; carbon-hydrogen bond; Pi-Pi stacked
Methadone	-5	Gln4	Unfavorable bump
Methoxetamine	-5.1	Ser131; Leu230; Pro232	Hydrogen bond; Pi-donor hydrogen bond
Orphenadrine	-4.7	Tyr231; Tyr231; Tyr231; Tyr231; Pro233	Pi donor hydrogen; Pi-sigma; Pi-Pi stacked; Pi-alkyl
Phencyclidine	-4.7	Pro232; Tyr231	Carbon-hydrogen bond; Pi-alkyl
Tramadol	-5.3	Asn229; Ser243; Asn154; Asp132; Leu246	Carbon-hydrogen bond; Pi-anion; alkyl
Procaine	-5.4	Asp132; Thr109; Ser131; Asn299; Pro130; Ser131; Pro130; Pro233; Lys241	Hydrogen; carbon-hydrogen; amide Pi-stacked; Pi-alkyl

**Table 3: Specific binding site of several compounds in serine racemase**

Compound	SR specific amino acid	Specific function
Haloperidol	Ser84	Reface of the SR that acts to reprotonate the $\alpha$ -carbon to form D-serine
Halothane	Arg135	Essential for negatively charged substrates to properly align for deprotonation
Ethanol	Asn86	The binding domain of ATP
Tramadol	Asn154	The triple serine loop plays a role in determining the direction of the reaction toward racemization or $\beta$ -elimination

ATP: Adenosine triphosphate, SR: Serine racemase

**Figure 2:** 2D Visualization of molecular docking between (a). Haloperidol and serine racemase, (b). Halothane and serine racemase, (c) ethanol and serine racemase, and (d) Tramadol and serine racemase. The interaction formed showed in colors

alpha deprotonation on external aldimine, producing carbanionic or quinonoid intermediate. Further protonation of quinonoid intermediates by reface will produce D-serine (racemization), while expulsion of the  $\beta$ -OH group, possibly followed by protonation, will produce enamine that is eventually released as pyruvate.<sup>[19]</sup> The  $\beta$ -elimination reaction is four times more favorable than the racemization reaction<sup>[20]</sup> maintaining the D-serine level in the tissue.

The study showed that haloperidol bind with the Ser84 residue, a reface of the SR, with the strongest binding energy than other compounds. The binding to Ser84 is thought to inhibit the reprotonation process required for the racemization reaction. The study of mutations in the Ser84 residue abolished the racemization process and increased  $\beta$ -elimination products while using L-threo-hydroxyaspartate and L-serine-O-sulfate as substrates.<sup>[20,21]</sup> Ethanol also binds to the Ser84 residue,

but ethanol has the weakest binding energy among other ligands.

Halothane binds to the Arg135 residue through fluorine which was negatively charged. The negatively charged substrate will bind to the Arg135 residue through a salt bridge to be aligned for deprotonation as in malonate.<sup>[20]</sup> These amino acid residues are essential for negatively charged substrates to align for deprotonation properly. Mutation of Ser84 to aspartate causes the formation of a salt bridge of Asp84 with Arg135, which prevents negatively charged substrates from being protonated to produce D-serine.<sup>[20]</sup>

Tramadol can bind to the amino acid residue Asp154, a triple serine loop consisting of residues His152, Pro153, Asn154, and Gln155. The triple serine loop plays a role in determining the direction of the reaction toward racemization or  $\beta$ -elimination. Mutation of amino acid residues in this region can lead to a tendency toward racemization, lowering the  $\beta$ -elimination yield.<sup>[22]</sup> Ethanol can bind to the Asn86 residue, which is the binding domain of ATP. ATP is essential in increasing the ability of the enzyme as a catalyst.<sup>[22]</sup> Mutations in the ATP binding domain decrease the catalytic ability of SR.<sup>[23]</sup> A study on Gln155 mutation to aspartate caused cells to favor racemization of L-ser to D-ser compared to  $\beta$ -elimination reaction.<sup>[24]</sup> Cell death after nerve injury involving activation of NMDAR and decreased GABA receptors causes the transition to chronic neuropathic pain and becomes difficult to treat.<sup>[25]</sup> These findings show that SR also potentially become a chronic pain treatment target besides NMDAR inhibition. Based on this study, further studies such as SR antagonists drug synthesis can be established in managing chronic neuropathic pain.

## CONCLUSION

SR potentially becomes an alternative target for neuropathic pain treatment. Some NMDAR antagonist drugs interfere with the SR racemization catalysis, such as haloperidol by binding to the reface, halothane by binding to amino acid residues that direct protonation, ethanol by binding to the ATP binding domain, and tramadol by binding to the "triple serine loop" residue which determines the direction of the reaction.

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## Conflicts of interest

There are no conflicts of interest.

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