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Design, synthesis, and evaluation of novel inhibitors for wild-type human serine racemase

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ABSTRACT

Most of the endogenous free D-serine (about 90%) in the brain is produced by serine racemase (SR). D-Serine in the brain is involved in neurodegenerative disorders and epileptic states as an endogenous co-agonist of the NMDA-type glutamate receptor. Thus, SR inhibitors are expected to be novel therapeutic candidates for the treatment of these disorders. In this study, we solved the crystal structure of wild-type SR, and tried to identify a new inhibitor of SR by *in silico* screening using the structural information. As a result, we identified two hit compounds by their *in vitro* evaluations using wild-type SR.

Based on the structure of the more potent hit compound **1**, we synthesized 15 derivatives and evaluated their inhibitory activities against wild-type SR. Among them, the compound **9C** showed relatively high inhibitory potency for wild-type SR. Compound **9C** was a more potent inhibitor than compound **24**, which was synthesized by our group based upon the structural information of the mutant-type SR.

Serine racemase (SR) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme that catalyzes both L-serine to D-serine and also the elimination of water from L-serine, generating pyruvate and ammonia.[1] A significant amount of free D-serine is found in forebrain regions.[2] Most of the endogenous D-serine (about 90%) in the brain is produced by SR as revealed in Srr-knockout (Srr-KO) mouse studies.[3–5] D-Serine is a co-agonist of the *N*-methyl D-aspartate-type glutamate receptors (NMDARs). Full activation of the NMDARs requires endogenous agonist L-glutamate and co-agonist D-serine or glycine. NMDARs are involved in many physiological functions such as neurotransmission in the central nervous system, higher brain functions of learning and memory, synaptic plasticity, and neuronal network formation.[6,7] Over activation of the NMDARs is involved in neuronal cell damage observed in many neurodegenerative disorders and epileptic states.[8] This neuronal cell damage is attenuated in Srr-KO mice.[3,9] Thus, SR inhibitors are expected to be novel therapeutic candidates for the treatment of these disorders.

Many efforts, including those of our group, to develop SR inhibitors have resulted in the generation of some relatively low-affinity SR inhibitors,[10–15] thus, further development of novel SR inhibitors is desired. In our previous *in silico* screening of the candidate compounds of human SR inhibitor, we used the previously reported crystal structure of malonate-bound form of human SR with C2D and C6D mutations and the homology-modeled structure of ligand-free form of human SR with C2D and C6D mutations, which was derived from the X-ray crystal structure of ligand-free rat SR.[16] However, enzymatic activity of the mutant-type SR and the effect of inhibitors on mutant-type SR are significantly different from those of wild-type SR. Thus, we expressed and purified recombinant wild-type SR and solved the crystal structure of wild-type SR. Based on the obtained structural information of the crystal structure of wild-type SR, we tried to identify new compounds by *in silico* screening, the procedure of which is described in Supplementary material. We purchased 9 candidates of possible SR inhibitors, and identified 2 hit compounds (Fig. 1) by their *in vitro* evaluations using wild-type SR as shown in Table 1.

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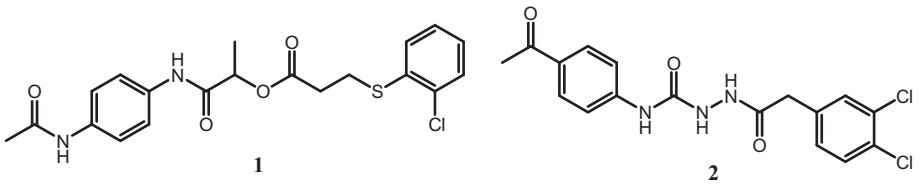


Fig. 1. Structure of two hit compounds for wild-type SR.

Table 1
Results of *in vitro* assay using wild-type SR. The activity of compounds (1 mM) was evaluated with the percentage of the D-serine production and compared with vehicle (DMSO).

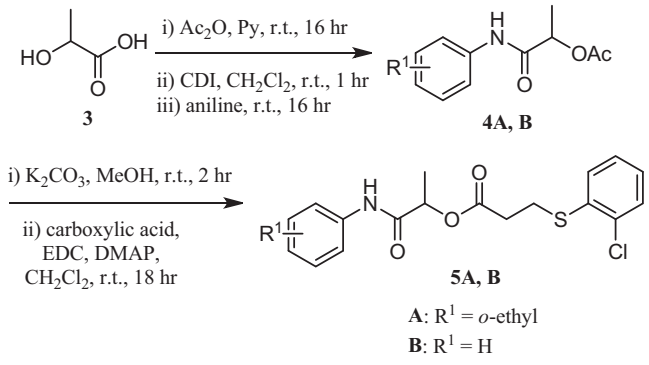
Compound	Activity [%]
1	49
2	56

Several efforts to discover novel SR inhibitors based on the structure of mutant-type mouse[10,13,14a] or rat[14b] SR have been reported. The investigations of the activities and properties of wild-type human SR,[14c–e] and a few efforts to discover novel SR inhibitors with the wild-type human SR[12,14f] have also been reported. However, this approach with the wild-type human SR still has remained as difficult target. In this study, we report the X-ray crystal structure of ligand-free (apo) form of wild-type human SR, and development of novel inhibitors of human SR based on the X-ray structure of the apo human wild-type SR for the first time.

The X-ray crystal structure of human wild-type SR was determined at 1.8 Å resolution. The wild-type structure is an open form: helix 5, helix 6, stand 3, and strand 4 in the small subdomain are moved away from the large subdomain (PDB code 5X2L). The positions of the strand 4, the helix 6, and the loop between them in the human wild-type SR structure are different from those in the homology-modeled structure of ligand-free form of human SR with C2D and C6D mutations in our previous study. It was suggested that both *R*- and *S*-isomers of **1** could bind to SR in almost the same fashion, but the binding affinity of the *R*-isomer would be somewhat stronger than that of the *S*-isomer, because the binding free energy (ΔG_{bind}) of the *R*-isomer was estimated to be about 0.9 kcal/mol more stable than that of the *S*-isomer. Fig. 2 shows the interaction model of the *R*-isomer of **1** with wild-type SR.

According to the interaction model, the acetoamide group in the left benzene ring offers the hydrogen donor to Glu283, and the central two carbonyl groups provide hydrogen acceptors to His87 and Ser242, respectively. To verify the importance of acetoamide substituent on the left benzene ring, we first prepared two derivatives (**5A**, **B**) as shown in Scheme 1. After acetylation of **3**, the resulting carboxylic acid was converted to amides **4A**, **B**, which was transformed into **5A**, **B** in two steps. Both **5A** and **5B** showed no inhibitory effects on *in vitro* evaluations (Table 2).

Next, we examined the effect of the substituent on the right benzene ring. Condensation of aniline **6** with **7** provided amide **8**, which was converted to eight derivatives **9A**–**H**. Some of the *des*-methyl derivatives **9A**–**H** showed good inhibitory effects on



Scheme 1. Synthesis of derivatives **5A**, **B**.

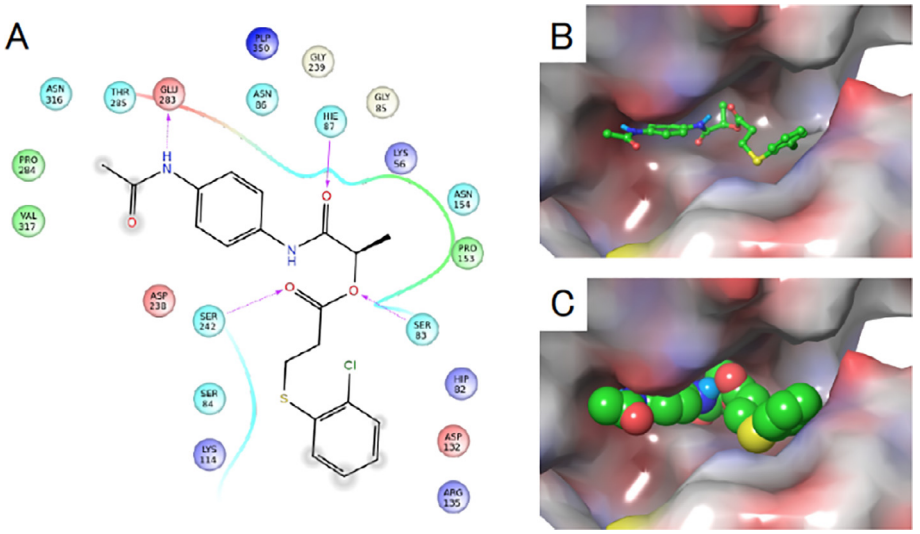


Fig. 2. Interaction model of **1** with wild-type SR. (A) Interaction diagram. (B) Compound **1** is shown in stick display. (C) Compound **1** is shown in space-filling display.