

The use of LeuT as a receptor model for antidepressant development

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Abstrak

*Selective serotonin re-uptake inhibitors (SSRIs) merupakan golongan antidepresan yang cukup selektif terhadap serotonin re-uptake transporter (SERT). Sertraline dan fluoxetine merupakan golongan SSRI yang mempunyai afinitas kuat terhadap SERT serta mampu menghambat neurotransmitter recycling. Akan tetapi, kedua obat tersebut juga mempunyai afinitas terhadap norepinephrine and dopamine transporter (NAT dan DAT) yang menyebabkan terjadinya efek samping. Oleh karena itu, penelitian mengenai interaksi antara SSRI dan SERT sangat penting untuk mengetahui faktor penentu interaksi obat-reseptor. Penggunaan Leucine Transporter (LeuT), model SERT yang berasal dari bakteri *Aquifexaolicus*, bisa menjelaskan interaksi SSRI-SERT. Akan tetapi, karena perbedaan spesies asal reseptor, obat yang efektif pada LeuT belum tentu efektif pada SERT. Penggunaan LeuT bisa menjelaskan interaksi SSRI-SERT secara molekuler. Halogen binding pocket (HBP) merupakan faktor penentu dalam interaksi SSRI-SERT. LeuT memiliki ~25% sekuen yang sama dengan SERT pada manusia. Konservasi pada sekuen ini ditemukan pada sisi aktif reseptor dimana HBP berada. LeuT sebagai reseptor model mampu menggambarkan interaksi antara obat dan reseptor. Hasil penemuan tersebut sangat bermanfaat untuk pengembangan obat antidepresan yang lebih selektif. (*Health Science Indones 2010; 1: 51 - 57*)*

Kata kunci: LeuT, antidepresan, serotonin re-uptake transporter

Abstract

Selective serotonin re-uptake inhibitors (SSRIs) have been considered to be promising drugs in psychiatric practice because of their selectivity to serotonin re-uptake transporter (SERT). Sertraline and fluoxetine are considered to be effective SSRIs as their ability in binding SERT and inhibit neurotransmitter recycling. However, they also bind norepinephrine and dopamine transporter (NAT and DAT) that cause undesirable effects. Thus, the study of drug-receptor interaction between SSRIs and SERT is important to gauge the active site of the drugs. By using Leucine Transporter (LeuT) from *Aquifexaolicus* as a SERT model, the mode of SSRI-SERT interaction was revealed. Nonetheless, it is uncertain whether the drugs that bind to LeuT would be effective to human SERT because of the high evolutionary convergence between them. Halogen binding pocket (HBP) was found to be the key determinant in the drug-receptor interaction. LeuT has ~25% sequence similarity to human SERT with highly conserved amino acid sequences in the active site of these receptors, in which HBP is located. LeuT as the receptor model revealed the interaction between drugs and receptors. All these findings are very useful to develop more selective antidepressant medicines. (*Health Science Indones 2010; 1: 51 - 57*)

Key words: LeuT, antidepressant, serotonin re-uptake transporter

Structural studies of drugs and receptors interaction have disclosed the mechanism of action of the drugs. Molecular study is also useful in drug development to define a particular feature that plays a role in the drug and receptor interaction. By using this knowledge, a new drug could be directed towards a better efficacy and safety.

The discovery of selective serotonin re-uptake inhibitors (SSRIs) have emerged therapeutic advance in neuropsychopharmacology. SSRIs are effective antidepressants that have high selectivity to the receptors.¹ SSRIs act as antidepressant by slowing the removal of serotonin.² By maintaining serotonin balance in the brain, SSRIs help relieve the symptoms of depression.³

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Compared to tricyclic anti-depressant (TCA) and monoamine oxidase (MAO) inhibitors, SSRIs have a better selectivity as they only act on the neurotransmitter serotonin. Thus, they have fewer side effects than TCA and MAO inhibitors. The side effects of SSRIs may include dry mouth, nausea, nervousness, insomnia, drowsiness, weakness, uncontrollable shaking of a part of the body, loss of appetite, weight loss, headache, excessive sweating and sexual problems.⁴ Sertraline and fluoxetine are SSRIs drugs broadly used recently as the first line of treatment for depressive disorders.⁵

The effect of SSRIs is achieved by inhibition of the presynaptic plasma membrane serotonin reuptake transporter (SERT).^{1,6} SERT is located in the neural plasma membrane. Serotonin is mainly inactivated by a reuptake to the pre-synaptic plasma membrane through SERT. Thus, SERT plays an important role in pre-synaptic neurotransmission.² Consequently SERT is a suitable target for further anti-depressant development.⁷

X-ray crystallography is a very useful technique to demonstrate the interaction between drugs

and receptors. However, not every protein can be crystallized. In this case, human SERT is technically difficult to be crystallized.⁸ Therefore, many studies tried to find the most appropriate SERT model. To facilitate the study, a model protein derived from a bacterial cell (*Aquifexaeolicus*), named leucine transporter model (LeuT) has been used recently.¹

As LeuT protein is derived from bacteria; it raises questions regarding the effectiveness of the drug for a real application in human. Therefore, it is important to know the similarity of this model with human SERT, especially in their key determinant regions. The objective of this article is to review the key determinant of the drug-receptor interaction and to gauge the similarity between LeuT and SERT.

Structure and function analysis of LeuT-SSRI protein

A study utilized X-ray crystallography shows the interaction of a bacterial LeuT protein in complex with three different SSRIs (sertraline, *R*-fluoxetine and *S*-fluoxetine). The molecular structure were generated with PyMOL.¹

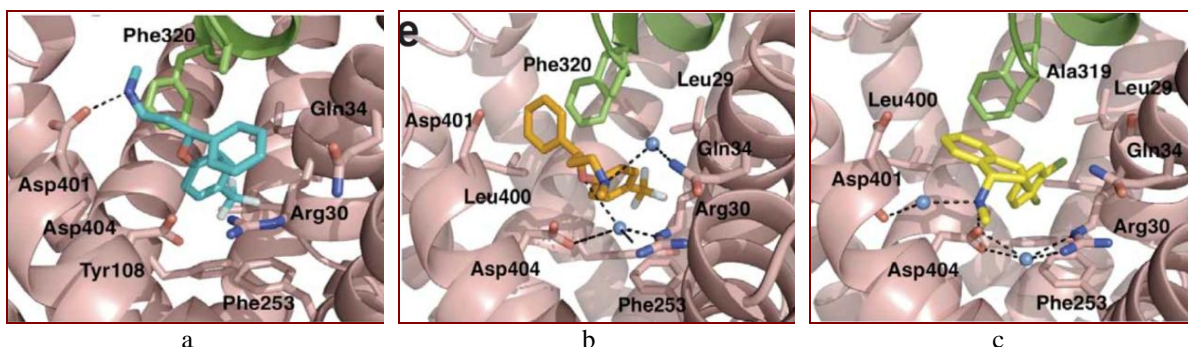


Figure 1. LeuT in complex with sertraline (a), *R*-fluoxetine (b) and *S*-fluoxetine (c).¹

Sertraline, *R*-fluoxetine and *S*-fluoxetine have same features in their interaction with LeuT. Based on the molecular complexes (Figure 1), it is shown that the drugs bound several similar amino acid residues although they do not share the same physical space.¹ Sertraline structure consists of two chlorine atoms attached to one of the aromatic rings, while fluoxetine structure has three fluoride atoms attached to one of the aromatic rings.⁹ Notably, the halogen atoms at

phenyl rings of SSRIs bind to LeuT at the same position.¹ These features suggest that SSRIs may also bind to human SERT in the same way at similar positions.

The location in which halogen atoms interact with LeuT molecule, named halogen-binding pocket (HBP), is the key determinant of the antidepressant drug receptors.¹ This result is supported by another study that shows the

importance of Cl⁻ in fluoxetine for affinity to SERT. The chlorine atoms affect the forming of appropriate conformation to achieve to most optimal binding to SERT.¹⁰ Therefore, the halogen atoms in SSRIs should be retained in further development of the drugs.

The amino tails of sertraline and R-fluoxetine point toward the cytoplasm and interact with Gln34 by binding a water molecule (Figure 1.a,b.). The complex of sertraline binds to LeuT was formed by interaction of two chlorine atoms on the aromatic ring insert to HBP within the LeuT molecule. Similarly, *R* and *S*-fluoxetine insert their fluoride atoms in the HBP (Figure 1.a, 1.b, 1.c.). The HBP is located in the EL4 hairpin loop and enclosed by Leu25, Gly26, Leu29, Arg30, Tyr108, Ile111 and Phe253. Four of these amino acid residues, which are Leu25, Gly26, Tyr108 and Phe253, interact with a substrate leucine at the opposite side of the polypeptide chain. The drug-binding site and the substrate-binding site share several amino acid residues although they interact at

the opposite side. The other part of the drugs might interact with LeuT in various ways, but the interaction of LeuT to halogen atoms remains unchanged.¹

Bioinformatics analysis of human SERT and LeuT from bacteria *Aquifexaeolicus*

Bioinformatics analysis was performed to observe homology between LeuT and human SERT. This approach utilized Pfam database and bioinformatics study from other related papers.

Global alignment of human SERT and LeuT

Since human SERT and LeuT from bacteria *Aquifexaeolicus* derived from different species with high evolutionary convergence, global alignment was chosen as the most suitable way to find their sequence similarity. The alignment (Figure 2) results in ~25% similarity between them. There are many gaps in the alignment. This probably caused by the different size of the gene encoding transporters.

Accession	Entry name	Protein names	Organism
<input type="checkbox"/> O67854	O67854_AQUAE	Transporter	snf (aq_2)
<input type="checkbox"/> Q5EE02	Q5EE02_HUMAN	Transporter	SLC6A4

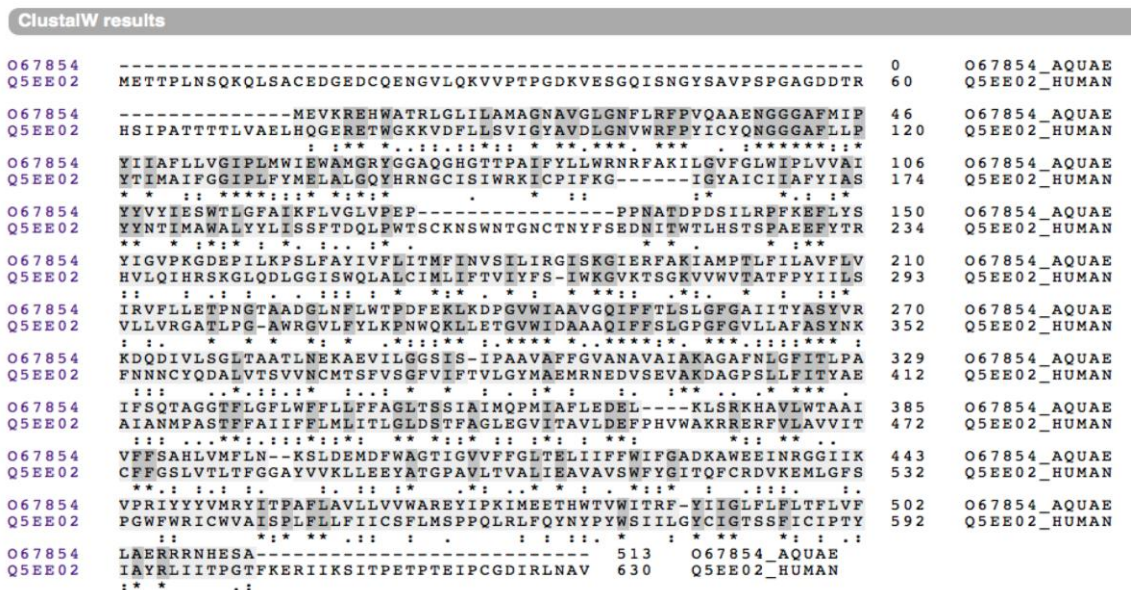



Figure 2. Alignment of human SERT and LeuT resulted from global alignment/Clustal W by using Pfam database.¹⁴

Protein domains of LeuT from Aquifex-aeolicus and human SERT in Pfam

Human SERT and LeuT domains were observed in UniProt database. From the result, it is shown

that human SERT have 5-HT transporter and sodium neurotransmitter symporter family (SNF) (Figure 3 and 4).


Sequence search results
[Show](#) the detailed description of this results page.
 We found **15** Pfam-A matches to your search sequence (**2** significant and **13** insignificant) . You did not choose to search for Pfam-B matches. The graphic below shows the arrangement of matches on your sequence:

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Significant Pfam-A Matches

[Show](#) or [hide](#) all alignments.

Pfam-A	Description	Entry type	Sequence		HMM		Bits score	E-value	Alignment mode	Predicted active sites	Show/hide alignment
			Start	End	From	To					
5HT_transporter	Serotonin (5-HT) neurotransmitter transporter, N-terminus	Family	23	64	1	42	100.5	5.9e-27	ls	n/a	Show
SNF	Sodium:neurotransmitter symporter family	Family	79	614	1	624	1210.8	0	ls	n/a	Show

Figure 3 Human SERT domains.¹⁴

Sequence search results
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Significant Pfam-A Matches

[Show](#) or [hide](#) all alignments.

Pfam-A	Description	Entry type	Sequence		HMM		Bits score	E-value	Alignment mode	Predicted active sites	Show/hide alignment
			Start	End	From	To					
SNF	Sodium:neurotransmitter symporter family	Family	5	68	1	64	64.4	1.1e-17	fs	n/a	Show
SNF	Sodium:neurotransmitter symporter family	Family	223	275	244	296	69.4	4.5e-19	fs	n/a	Show

Figure 4 LeuT domains.¹⁴

Both human SERT and LeuT have SNF domains although in LeuT it is smaller and also fragmented. It might be because LeuT has a smaller size of gene encoding the receptor compared to SERT. However, it could represent the possibility of the drugs to bind the similar domains.

The transport function of SERT is determined by the structure of an oligomeric protein with disulfide bridges between cysteine residues. It has 12 transmembrane α -helices (TMH) domains that transport serotonin together with sodium and chloride ions.^{2,12} It also has amino and carboxy termini and a large extracellular loop at which *N*-linked glycosylation sites were attached.¹³

A crystallography study showed that LeuT also has 12 TMH, which is similar to that of human SERT. Hence, LeuT has been used for model system to study human NSS (neurotransmitter sodium symporter).¹⁴

Conservation between SERT, LeuT, DAT and NET

SERT has certain similarities to other Neurotransmitter Sodium Symporter (NSS), which are Norepinephrine Transporter (NET) and Dopamine Transporter (DAT). Sequence alignment between SERT, LeuT,

DAT and NET in the previous study indicates conservation between them. Conservation of amino acid sequence in the halogen-binding pocket is important in maintaining the receptor functions. The only key difference in region EL4 is that SERT possesses a glycine residue at position 100 while NET and DAT possess alanine residue. According to the mutagenesis assay continued by binding assay, mutation at this glycine into alanine inhibited transport activity of SERT.¹ This indicates that glycine plays an important role in SERT specificity to SSRIs. In contrast, mutation of an alanine residue in NET and DAT to a glycine resulted in the increase of binding affinity to SSRIs. The result suggests that SERT is more specific to SSRIs compared to NET and DAT. Compared to NET and DAT, SSRIs have a better binding affinity to SSRIs.¹

Multiple sequence alignment between human NSS (SERT, NET and DAT) and LeuT that was conducted by another study showed evolutionary conservation between them. Those results were achieved by using ICM homology modeling.¹⁵ Based on this homology study combined with computational study, the structural models for the three NSS have been elucidated (Figure 5).

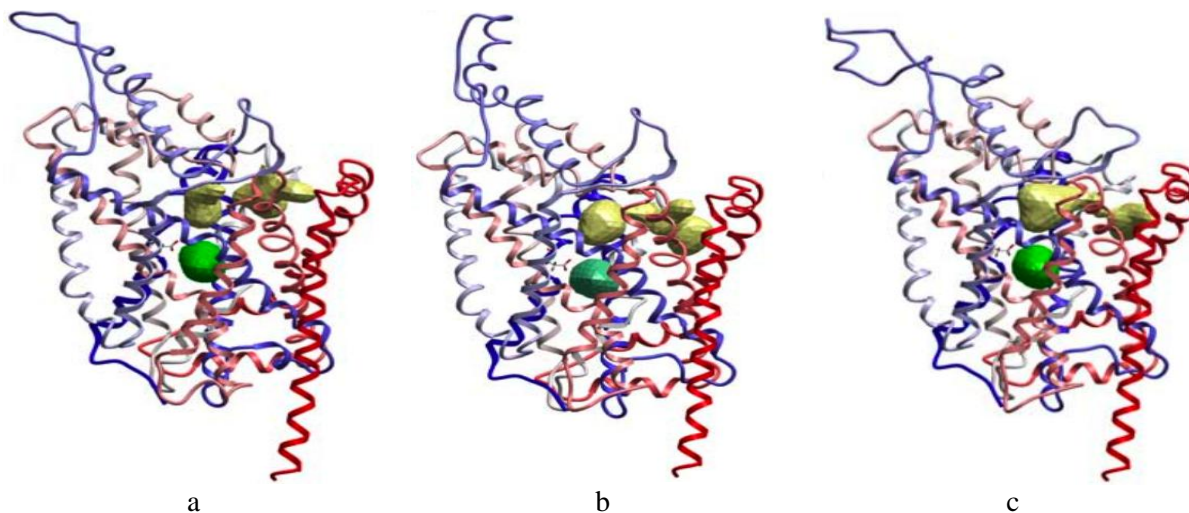


Figure 5. Structural model of human NSS utilized LeuT. a. SERT model, b. DAT model, c. NET model.¹⁵

Those structural model shows similarity of molecular structure between SERT, DAT and DET. They also have similar characteristic in their way forming a dipole moment that leads them being more negative towards the extracellular side.¹⁵ The previous study

elucidated sequence similarity of those receptors specifically in the EL4 hairpin loop position (Table 1).¹

Table 1. Sequence comparison of four NSS proteins in EL4 hairpin loop position¹

LeuT	SERT	NET	DAT
Leu25	Leu99	Leu76	Leu80
Gly26	Gly100	Ala71	Ala81
Leu29	Trp103	Trp80	Trp84
Arg30	Arg104	Arg81	Arg85
Tyr108	Tyr176	Tyr151	Tyr156
Ile111	Ile179	Ile155	Ile159
Phe253	Phe335	Phe317	Phe320

The only difference between amino acid sequence of LeuT and SERT in this region is tryptophan (Trp103), which is leucine (Leu29) in LeuT (Table 1). However, there is no other data regarding this feature. It is unknown whether this difference feature would affect the interaction and function of the receptors or not. Thus, it would be better if this feature were also observed to ascertain the similarity between LeuT and SERT.

A subsequent study found that homology of LeuT and SERT is optimal between transmembrane domains (TMDs) region. Also, it was found that the binding site for the substrates (either leucine or serotonin) as well as Na⁺ and Cl⁻ indicates similar kinetics and structural properties.¹⁶ Therefore, LeuT represents a reliable template to model SERT protein.

In conclusion, based on the alignment results, LeuT has low similarity to human SERT, which is only ~25%. But, the amino acid sequences are highly conserved in the active site of these receptors, in which the HBP is located. Mutagenesis and binding assay study proved that their conservation is important for the receptor function in transporting serotonin.

Additionally, LeuT has similar conformation with human SERT. All these suggest that LeuT is appropriate to represent human SERT in binding SSRIs.

The specificity of SERT bind to SSRIs is very important in defining the key feature that has to be retained in further development of antidepressants. HBP is the key determinant of SSRIs-SERT interaction. The halogen atoms play an important role in the drug-receptor binding. Thus, the halogen atoms should be retained in further antidepressant development.

As SSRIs have low binding affinity to NET and DAT and this interaction causes side effects, further study should be directed to eliminate this undesired interaction. Furthermore, a particular study regarding interaction of SSRIs and NET as well as DAT is required in order to know the key feature that should be eliminated to avoid their interaction.

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