Selective Inhibition of the Serotonin Transporter in the Treatment of Depression: Sertraline, Fluoxetine and Citalopram

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Discovery and development of the selective serotonin reuptake inhibitors mark a milestone in neuropharmacology. Drugs from this class alter the functioning of the serotonin system by the potentiation of serotonin through the negative allosteric modulation of its neuronal uptake by the human serotonin transporter. Selective serotonin reuptake inhibitors show few side effects compared to those caused by traditional antidepressants and they vary in the binding interactions formed during binding. Generally, their binding involves three specific regions of the drug structures, each participating in vital interactions, such as salt bridge formation and additional hydrophobic interactions with conserved residues in the central binding site of the target protein. Side effects, however, such as the initial lack of response to treatment, or drowsiness, nausea, and sexual dysfunction occasionally may arise. Additional binding studies, furthermore, highlighted the importance of enantioselectivity in the binding of these compounds, raising concerns about the beneficial application of racemate mixtures of some of these compounds. Therefore, additional characterisation of binding and further structural improvement of this class of drugs is necessary. The recently synthesized sertraline salts, and functional derivatives of fluoxetine and citalopram show promising results in delivering antidepressant activity as well as in effectively overcoming anorexigenic side-effects in rodent models. Hence, despite certain non-desired effects associated with selective serotonin reuptake inhibitor applications, this class of drugs is considered as first-line medication in the management of major depression, and is carrying an excellent potential for the development and refinement of the currently available and novel antidepressant therapies.

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Keywords: depression, serotonin transporter, sertraline, fluoxetine, citalopram

ABBREVIATIONS

$(SerH_2)_2[CuCl^4]$- anhydrous tetrachlorocuprate (II) salt, $(SerH_2)_2[CuCl^4]^{-1}/_2H_2O$- hydrated tetrachlorocuprate (II) salt, $(SerH_2)_2^{+}$ $[ZnCl_4]^{2-}$- sertralonium tertachlorozincate (II), 5-HT- serotonin, $5\text{-}HT_{2A}R$- serotonin 2A-subtype receptor, Å- angstrom, $^{-}$CCA- coumarin 3-carboxylate, CYP- Cytochrome P450, DA- dopamine, DAACS- mono- and dicarboxylic amino acid transporters, DAT- dopamine transporter, dDAT- *Drosophila melanogaster* dopamine transporter, EL- extracellular loops, GABA- gamma-aminobutyric acid, HCCA- coumarin 3-carboxylic acid, hSERT- human serotonin transporter, IL- intracellular loops, K_i- inhibitory constant, CNS- central nervous system, LeuT- bacterial leucine transporter, MAOIs- monoamine oxidase inhibitors, MDD- major depressive disorder, NA- noradrenalin, NaCCA- sodium coumarin 3-carboxylate, NDS- desmethylsertraline, NE- norepinephrine, NET- norepinephrine transporter, NSS- neurotransmitter:sodium symporters, pKA- acid-base dissociation constant , pm- picometer, SerH-CCA- sertraline salt, Ser-HCl- sertraline hydrochloride, SLC6A4- solute carrier family 6, member A4, SSRIs- selective serotonine reuptake inhibitors, $t_{1/2}$- half-life, TCAs- tricyclic antidepressants, TM- transmembrane, T_{max}- time of maximum concentration observed, TRIs- triple reuptake inhibitors.

BACKGROUND

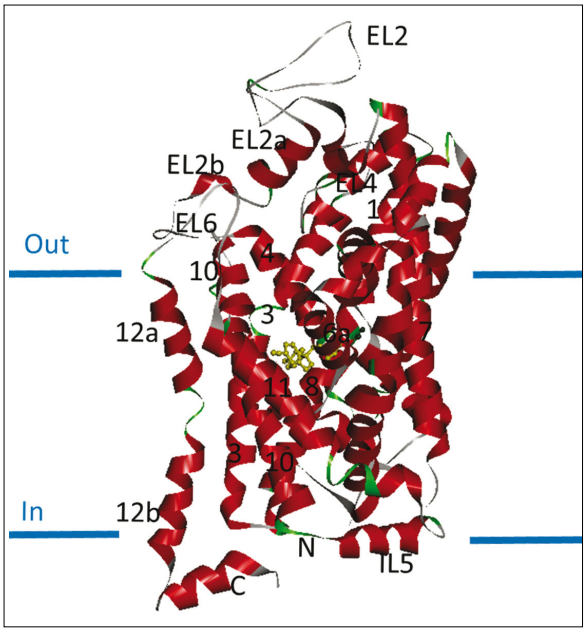
Depression is an illness characterized by multiple symptoms including anhedonia, depressed mood, suicidal thoughts, loss of appetite, and psychomotor retardation (Sharma H, Santra S, 2015). Major depressive disorder (MDD) is the most common type of mood disorders, affecting as much as 121 million people worldwide (Sharma H, Santra S, 2015; Butler and Meegan, 2008). Potential drug targets in the treatment of MDD have been identified and are being targeted by monoamine reuptake inhibitors, monoamine receptor agonists and antagonists, corticotrophin releasing hormone antagonists, neuropeptide targets (such as neuropeptide Y and neurokinin receptors), and N-methyl-D-aspartate receptor antagonists (Butler and Meegan, 2008). Medications modulating the functioning of the monoaminergic transmission may possess antidepressant activity. Unfortunately, however, a large proportion of patients with depression do not respond to initial pharmacotherapies, or show adverse effects, like drowsiness, nausea, or sexual dysfunction (Stevenson, 2018). Therefore, further strategies for the refinement and improvement of the currently used medications are necessary.

The purpose of this review is to summarise the present understanding of the binding of some of the first-line selective serotonine reuptake inhibitors (SSRIs) and their analogs to LeuT, a bacterial SERT homolog, as well as to the human serotonin transporter (hSERT) in the management of major depression.

STRUCTURE AND FUNCTION OF THE HSERT

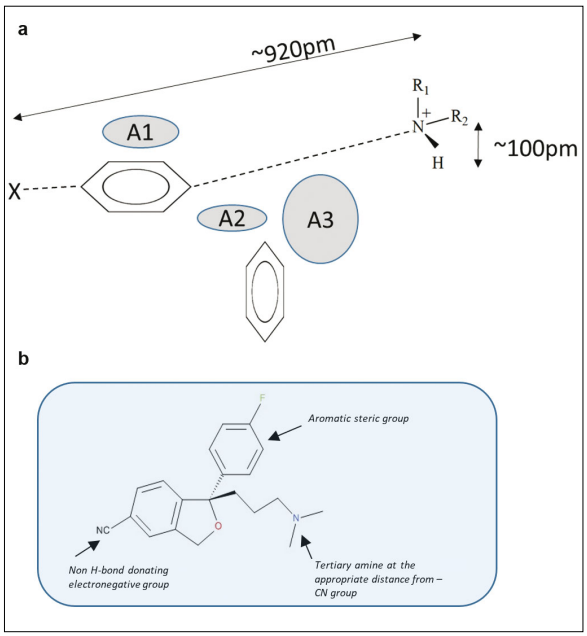
There are two major neuronal and glial neurotransmitter transport protein families; small molecule neurotransmitters like glycine, gamma-aminobutyric acid (GABA), dopamine (DA), norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) are transported by the *neurotransmitter:sodium symporters* (NSS), whereas large molecules, such as glutamate, are transported by the *mono- and dicarboxylic amino acid transporters* (DAACS) (Rudnick, 2006). The hSERT protein is an NSS family member, responsible for the selective reuptake of 5-HT from the synaptic cleft into the presynaptic nerve ending. It is mainly located in the cholesterol-rich lipid-raft domains of the cell membranes, with its N- and C-terminals protruding to the intracellular site, where these interact with proteins responsible for the protein`s localization and activity (Baudry et al., 2019). The extracellular surface of hSERT is composed of the extracellular loops (EL) 2, 4 and 6, where EL2 is looping around the extracellular surface, providing a solvent accessible surface of 3,376 $Å^2$ (Figure 1), and is predicted to contain two N-linked glycosylation sites (Asn208 and Asn217) and a conserved disulfide bridge between Cys200 and Cys209 (Coleman et al., 2016). The intracellular surface is capped by the intracellular loops (IL) 1, 5 and the C-terminal of the protein (Coleman et al., 2016). While alternatively exposing its substrate binding site on the cytoplasmic and extracellular surface, SERT binds a Na^+, a Cl^-, and a $5\text{-}HT^+$ on the extracellular site and releases them into the intracellular compartment, where a K^+ is bound to the intracellular active site and transported to the extracellular space (Rudnick, 2006). This activity provides an overall stoichiometry of 1:1:1:1 for the Na^+, Cl^-, $5\text{-}HT^+$, and K^+ ligand exchange. The hSERT protein is encoded by the *solute carrier family 6, member A4* (SLC6A4) gene which has been the most commonly studied target for psychiatric disease risk and medications (Baudry et al., 2019; Stevenson, 2018). Single nucleotide polymorphism (SNPs) such as the Pro339Leu exchange in the SLC6A4 gene, have previously been associated with psychiatric disorders

Figure 1. The structure of the hSERT protein



The structure was generated in Discovery Studio 2015 V16.1.0.15350 from the Protein Data Bank (PDB) structure 5I73 and further annotated in Microsoft Office, Power Point 2016 MSO (32 bit).

Figure 2. The pharmacophoric model of hSERT (a) and a representative example of a potent hSERT inhibitor, escitalopram (b)



Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

(Stevenson, 2018; Coleman et al., 2016). Further mutations, such as Ile425 in TM8, Lys201Asn in EL2 and Ser293Phe or Leu362Met in TM5 and 7 were reported to enhance the transport function of the protein, leading to an decreased serotonin concentration in the synaptic cleft, hence leading to depression like symptoms (Coleman et al., 2016).

Homology models of monoamine transporters, such as the bacterial (*Aquifex aeolicus*) SERT homolog leucine transporter (LeuT), provide essential insights into the structure-function relationship of NSS transporters (Yamashita et al., 2005). LeuT has an overall ~20-25 % sequence identity with the human monoamine transporters, and a ~50 % sequence identity for the residues within a 5 Å radius around the central binding site (Grouleff et al., 2015). Despite significant structural differences (e.g. length of the C- and N-terminals, phosphorylation sites, etc.) the structure of LeuT provides a valuable modality in the analysis of the molecular motions in the hSERT, as well as the interactions formed during SSRI binding.

PHARMACOPHORE MODEL OF THE HSERT

Potent SERT inhibitors must contain a positively charged amino group, where the protonated N atom is positioned at an optimal distance of ~610 picometer (pm) from the aromatic system, and at an optimal distance of ~920 pm from a negatively charged X group on the other end of the molecule (Figure 2) (Rupp et al., 1994). In this pharmacophoric model, the nitrogen atom lies approximately 100 pm above the aromatic system and is capable of forming intramolecular bonds, hence facilitating the stabilization of an active conformation (Butler and Meegan, 2008; Rupp et al., 1994). The electronegative region on the main ring (CN in escitalopram, Figure 2b) should not act as a hydrogen bond donor, as this can lead to a decreased inhibitory activity. Any substitution in the A1 region could results in the deviation of the tubular system (X-aromatic-nitrogen tubular system, Figure 2a), leading to the lack of inhibitory activity against the hSERT and the triggering of hallucinogenic processes through serotonin 2A-subtype receptor (5-HT_{2A}R) activation (Nichols, 2018; Butler and Meegan, 2008). Such structural differences have previously been highlighted in tryptamine derivatives, where the substitution of an oxygen moiety at the 4- and 5-positions (e.g. psilocin and 5-methoxytryptamine) led to 5-HT_{2A}R activation and subsequent hallucinogenic experiences (Nichols, 2018). Regions A2 and A3 of potent inhibitors, furthermore, should display *n*- and/or π-electrons, and aliphatic side-chains, respectively, together with an aromatic substitution positioned in a different

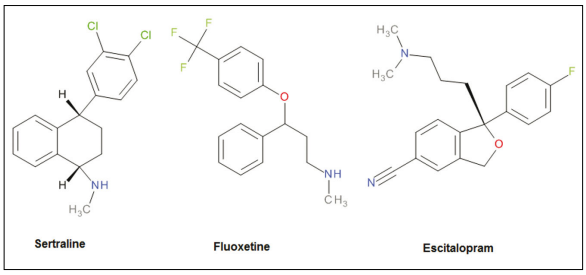
plane from the rest of the molecule and possibly perpendicular to each other (Butler and Meegan, 2008). This latter, although increases the lipophilicity of the molecule, it also contributes to higher affinity for the receptor, and together with the A2 and A3 regions is mainly responsible for the selectivity to the 5-HT and noradrenalin (NA) receptors (Rupp et al., 1994).

CLASSICAL AND NEWLY DEVELOPED ANTIDEPRESSANTS

Currently, there are six main groups of antidepressant drugs, primarily targeting monoamine 5-HT and norepinephrine transporters. For decades, TCAs (tricyclic antidepressants) were the first-choice pharmacologic treatment for major depression, however, due to their non-specific effect on cholinergic, histaminergic, and α-adrenergic pathways and due to their low therapeutic index identified recently, their use is now very limited (Marks et al., 2008). Another class of early antidepressant drugs, the monoamine oxidase inhibitors (MAOIs), irreversibly inhibit monoamine catabolism, leading to increased drug-drug interactions, as well as cross-reaction with dietary tyramine (Marks et al., 2008). In the search for highly efficient antidepressant drugs with an improved safety profile, the considerable effort of the last three decades led to the development of the selective monoamine reuptake inhibitors, such as SSRIs. The binding of SSRIs completely blocks the functioning of the pump protein, resulting in an increased serotonin level in the central nervous system (CNS), leading to an elevated mood and emotional state (Tavoulari et al., 2009). The three main SSRIs, sertraline, fluoxetine, and citalopram (Figure 3) alter the functioning of the serotonin system by the potentiation of 5-HT through the negative allosteric modulation of its neuronal uptake by the hSERT (Vaswani et al., 2003). Sertraline, the second most potent inhibitor, is a naphthylamine derivative and it is the only SSRI to bind to the dopamine transporter (Vaswani et al., 2003). Fluoxetine`s bioavailability is <90 %, and it has the highest volume of distribution among the SSRIs, and its highest accumulation is in the lungs (Vaswani et al., 2003). Escitalopram has very high selectivity for inhibiting 5-HT reuptake as compared to the noradrenaline reuptake, and although its active form is the S(+)-enantiomer, it is sold as a racemate (Vaswani et al., 2003).

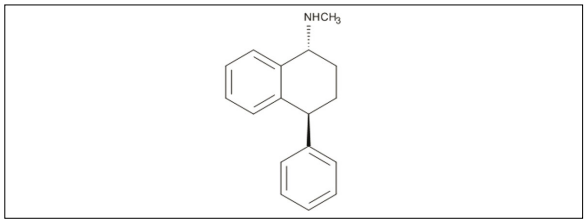
SSRIs are approved drugs for the treatment of major depression, anxiety disorders, pain disorders and premature ejaculation, and their use is cost-effective

Figure 3. Structures of sertraline, fluoxetine, and escitalopram



Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

Figure 4. Structure of sertraline`s lead compound, tametraline



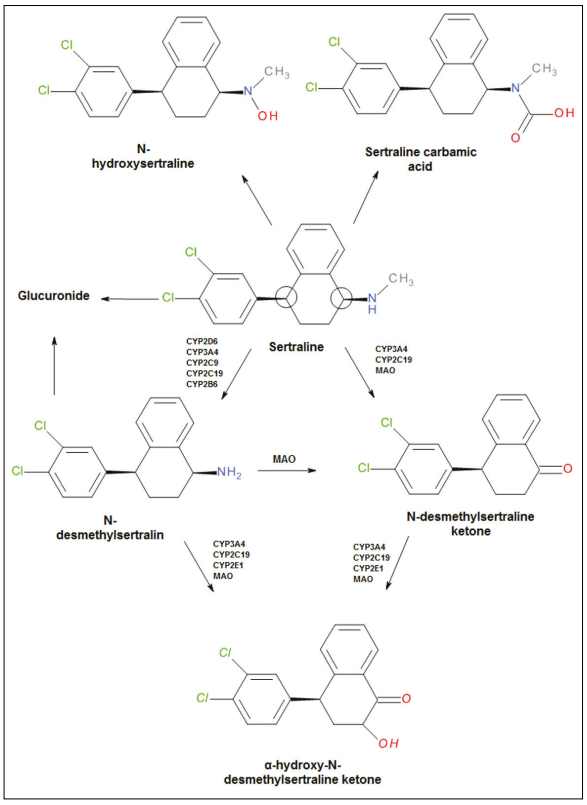
Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

without the need for laboratory monitoring. Although this group of drugs shows similar activity and improved tolerability and side effect profiles compared to those of TCAs and MAOIs, SSRIs do not show an increased efficacy or an improvement in the time of onset of the antidepressant response (Vaswani et al., 2003). Hence, further structural development and binding analysis of these first-line medications is necessary.

Sertraline and its derivatives

Sertraline (1S,4S)-4-(3,4-di-chlorophenyl)-1,2,3,4-tetrahydro-1-naphthyl(methyl)amine) was approved in 1991 for the treatment of major depression and in 2003 for the treatment of social anxiety disorder (Mandrioli et al., 2013; Welch, 1995). Its development started with the derivation of the lead compound tametraline (a norepinephrine reuptake inhibitor, Figure 4). Sertraline is a weak base (pKa=9.16) naphthylamine derivative with two chiral centers (Figure 5) and it easily penetrates through the blood-brain barrier due to its strong lipophilic characteristics and the lack of polar interactions (Mandrioli et al., 2013). It is sensitive to oxidation, absorbs slowly (T_{max} of 4-8 h), and it shows an elimination half-life ($t_{1/2}$) of 22-36h (Mandrioli et al., 2013). Sertraline is subject to an extensive first-pass metabolism into N-desmethylsertraline (NDS), a metabolite that reaches higher plasma concentration than its parent

Figure 5. The metabolic pathway of sertraline

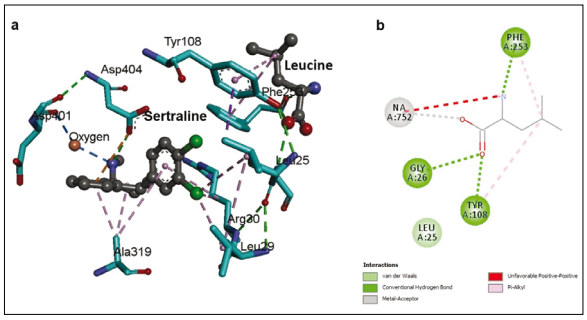


CYP= Cytochrome P450
MAO= Monoamine oxidase
Black circles indicate chiral centers in sertraline.
Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

compound and shows a $t_{1/2}$ of 62-104h. Nonetheless, due to its low pharmacological activity, NDS does not contribute to the clinical effect of sertraline (Mandrioli et al., 2013). Despite that multiple Cytochrome P450 (CYP) enzymes can demethylate sertraline *in vitro*, the primary metabolic pathway responsible for sertraline metabolism *in vivo* is related to the CYP2C19 enzyme (Figure 5) (Hicks et al., 2013; Mandrioli et al., 2013). Hence, sertraline metabolism is very much dependent on the variation in the CYP2C19 genetic background (Hicks et al., 2013). Three main CYP2C19 variants (CYP2C19*1, CYP2C19*2, and CYP2C19*17) affecting general drug metabolism have recently been reported to influence the oral clearance of sertraline and possibly causing side effects, hence calling for precautions in patients carrying these variants of the CYP2C19 gene (Hicks et al., 2013).

Sertraline binds to the extracellular vestibule of the bacterial LeuT protein, with the two chlorine atoms being inserted into the binding pocket formed by the Leu25, Gly26, Leu29, Arg30, Tyr108, Ile111, and Phe253 residues, with additional *van der* Waals

Figure 6. The binding of sertraline and leucine to the bacterial LeuT

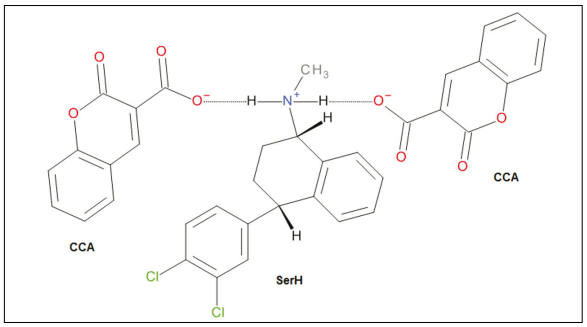


a. The binding of sertraline and leucine to the bacterial LeuT.
b. 2D representation of the common amino acid residues involved in the binding of both sertraline and leucine.
Images were generated in Discovery Studio 2015 V16.1.0.15350 from the Protein Data Bank (PDB) structure 3GWU.

interactions formed with Leu29, Tyr108 and Phe253 (Figure 6a) (Zhou et al., 2009). The di-chlorophenyl ring is rotated by 180° compared to the unbound form of the drug and is approximately perpendicular to the tetrahydronaphthalene ring, which interacts with the residues Leu400, Asp401 and Thr409 on the TM10, Arg30 and Gln34 on the TM1, and Ala319 of the EL4 hairpin loop (Zhou et al., 2009). The amine nitrogen of sertraline forms a salt bridge with the carboxyl group of Asp404, and interacts with the oxygen of Asp401 via a water molecule (Zhou et al., 2009). The four halogen-binding amino acids Leu25, Gly26, Tyr108, and Phe253 also interact with leucine at the opposite of the polypeptide chain (Figure 6a,b) (Zhou et al., 2009).

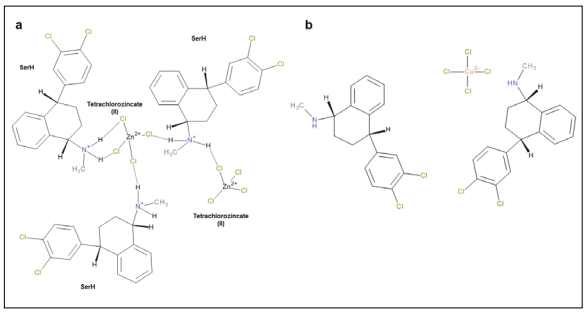
Recently reported sertraline salts formed with acetate, L-lactate and L-aspartate showed improved bioavailability compared to the parent compound sertraline (Escudero et al., 2016). The ionic sertraline salt (SerH-CCA) synthesized from the cationic sertraline and the anionic form of coumarin 3-carboxylic acid (HCCA), coumarin 3-carboxylate ($^{-}$CCA), has been shown to induce antidepressant activity in rodents, when compared to the control saline solution, to sodium coumarin 3-carboxylate (NaCCA) and the commercially available sertraline hydrochloride (Ser-HCl) (Escudero et al., 2016). FTIR and X-ray diffraction methods enforced the stabilizing function of the H bonds between the amine group of sertraline (cation) and the carboxylate group of the CCA (anion) in the structure of the salt (Figure 7). An antimicrobial activity of the SerH-CCA complex and a strong binding to BSA (bovine serum albumin) was furthermore detected, possibly resulting in better pharmacokinetic properties (Escudero et al.,

Figure 7. Structure of the SerH-CCA ion pair



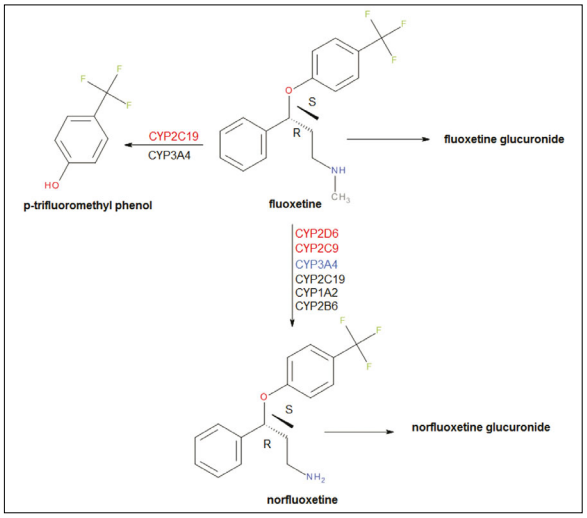
CCA= 2-Oxo-2H-1-benzopyran-3-carboxylate
SerH= sertraline in its protonated form
Dashed lines indicate intermolecular hydrogen bonds.
Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit) from Escudero et al. (2016).

Figure 8. The structures of $(SerH_2)^2[ZnCl_4]$ (a) and $(SerH_2)^2[CuCl_4]$ (b)



SerH= sertraline in its protonated form.
Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit) from Escudero et al. (2017) (a) and Martini et al. (2017) (b).

Figure 9. The metabolism of fluoxetine



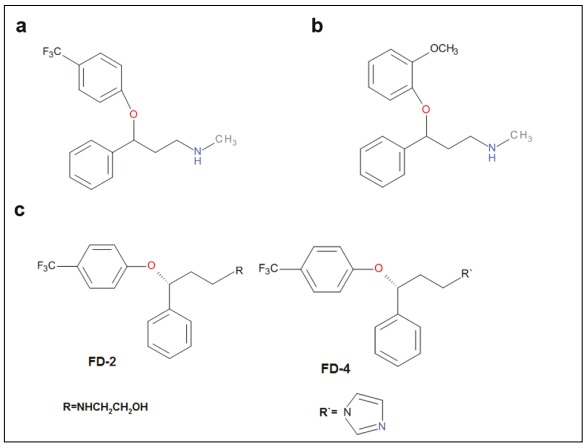
CYP= Cytochrome P450
Red, blue and black colors refer to the major, moderate and minor metabolic pathways, respectively.
Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

2016). Additional structural improvement of sertraline through the inclusion of a Zn^{++} ion resulted in the salt $(SerH_2)_2^+$ $[ZnCl_4]^{2-}$ (sertralonium tertachlorozincate (II), Figure 8a) (Escudero et al., 2017). The $(SerH_2)^{2+}$ $[ZnCl_4]^{2-}$ salt includes a pair of sertraline molecules in their protonated form (cationic moiety, $SerH^{2+}$), with the phenyl and di-chlorophenyl groups assuming planar position. When the $(SerH_2)_2[ZnCl_4]$ was administered tor rodents, once a day at the doses 13 mg/Kg and 26 mg/Kg for 14 days, a significantly lower immobility time, with a concomitant increase in swimming and climbing behaviour was found (Escudero et al., 2017). Additionally, treatment at both doses showed promising results in the management of anorexic side effects experienced from other antidepressants (Escudero et al., 2017). Further hydrated and anhydrous forms of the tetrachlorocuprate (II) salts (sertraline based copper complex, $(SerH_2)_2[CuCl_4]^{\cdot 1}/_2H_2O$ and $(SerH_2)_2[CuCl^4]$, Figure 8b) were synthesized and showed dose dependent antidepressant activity and efficient suppression of anorexigenic side-effects in rodents (Martini et al., 2017).

Fluoxetine and its derivatives

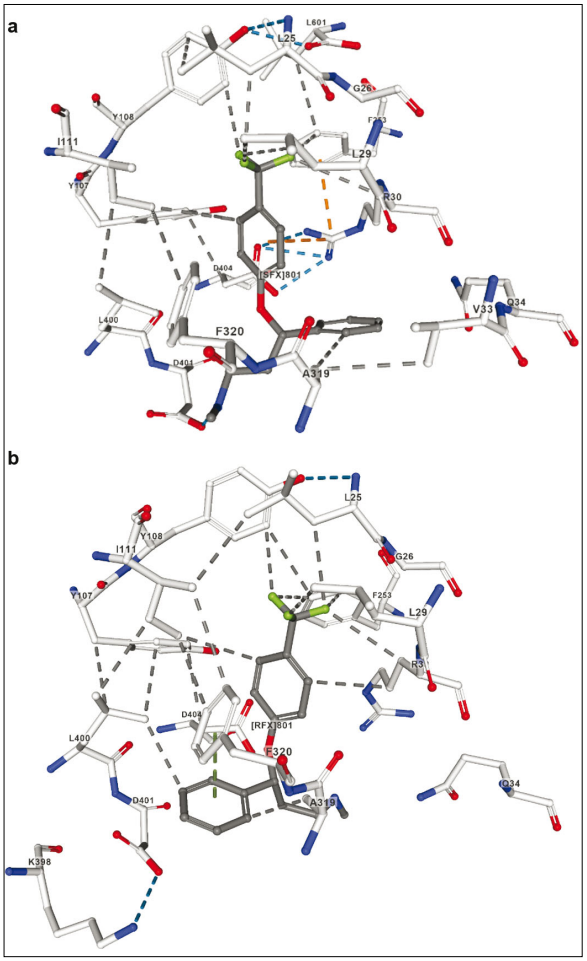
Fluoxetine is sold as a racemic mixture, with both enantiomers displaying potent inhibitory activity against 5-HT re-uptake in synaptosomal preparation (K_i= 21 nM and 16 nM for *R*-fluoxetine and *S*-fluoxetine, respectively) (Wong et al., 1995). The parent compound in the development of fluoxetine, N-methyl-phenoxyphenylpropylamine, is a secondary amine with moderate SERT inhibitory activity (K_i=102 nM), which, through the introduction of a trifluoromethyl group at the para-position of the phenoxy-ring, led to an increased inhibitory activity and selectivity towards 5-HT re-uptake, but diminished potency towards NE re-uptake (Wong et al., 1995). Major metabolites of the two enantiomers are their demethylated forms, *S*- and *R*-norfluoxetine (Figure 9). Fluoxetine has the largest volume of distribution of all SSRIs, it is completely absorbed following oral administration, and has a high CNS-penetration (Wenthur et al., 2014). It has low plasma protein binding and a long half-life that requires a relatively long time (1-22 months) to achieve steady state (Wenthur et al., 2014). Fluoxetine is subject to an extensive hepatic metabolism by cytochrome P450 enzymes, resulting in an excretion as either the parent compound (fluoxetine), N-desmethylfluoxetine (norfluoxetine), or as glucuronides of these (Figure 9) (Wenthur et al., 2014).

Figure 10. The structures of fluoxetine (a) and nisoxetine (b) and the fluoxetine analogs FD2 and FD4 (c)



Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

Figure 11. The binding of S-fluoxetine (a) and R-fluoxetine (b) to LeuT



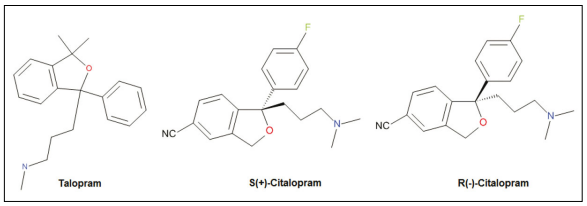
SFX801= S-fluoxetine
RFX801= R-fluoxetine
Image from the RSCB PDB (rcsb.org) of PDB 3GWW (a) and 3GWV (b) (Zhou et al., 2009)

Binding studies of fluoxetine and nisoxetine (Figure 10) analogues confirmed that *ortho*-substitution of the phenoxy ring is important in the selectivity to NET, whereas *para*-substitution leads to selectivity for SERT (Andersen et al., 2014). Extending the aminoalkyl chain of fluoxetine also increases selectivity over NET, and it was found that R and S enantiomers of fluoxetine have a different magnitude of selectivity to SERT over NET, suggesting that stereochemistry is an important determinant for selectivity in the binding of fluoxetine (Andersen et al., 2014).

Molecular docking studies confirmed that fluoxetine binds to the S1 pocket of hSERT, with its CF_3-substituted phenyl ring pointing towards S_2 (Andersen et al., 2014). Interestingly, both *R*-fluoxetine and *S*-fluoxetine bind to the halogen binding pocket of the LeuT, and interact with the same amino acids (Leu25, Gly26, Leu29, Arg30, and Tyr108) as sertraline (Zhou et al., 2009). Nonetheless, in the binding of *R*-fluoxetine, the Ala319/Phe320 and Leu400/Asp401 of LeuT surround the phenyl ring, whereas the amine tail of *R*-fluoxetine interacts with Gly34, and points to cytoplasmic direction (Figure 11a) (Zhou et al., 2009). In contrast, during the binding of *S*-fluoxetine, although the same amino acid residues (Leu25, Gly26, Leu29, Arg30, Tyr108, and Phe253) interact with the drug and are being shared between the drug binding site and the substrate (leucine) binding site, the rest of the drug is reversed in the binding pocket: the phenyl ring interacts with the gate of the protein, the amine tail is pointing towards the extracellular space, and the amine N forms a salt bridge with Asp401 (Figure 11b) (Zhou et al., 2009). Additionally, whereas the methylphenoxy ring of R-fluoxetine shows a 46˚ rotation at the O5-O6 bond, in *S*-fluoxetine there is a less defined rotation of this ring (19˚, data not shown), possibly due to the differences in the chirality of the two molecules (Zhou et al., 2009).

Recently, fluoxetine analogs with the substitution of the N-methyl amine group of fluoxetine have been synthesized (Yoon et al., 2009). The two analogues, FD-2 ((R)-N-ethanol-3-(4-trifluorophenoxy)-3-phenylpropaneamine) and the FD-4 (N-(R)-3-trifluorophenoxy-3-phenylpropane-imidazole) (Figure 10c,d) were found to be effective dopamine re-uptake inhibitors (Yoon et al., 2009). FD-2 was found to be three times more potent than FD-4 in inhibiting dopamine reuptake through hDAT (Yoon et al., 2009).

Figure 12. The structures of the lead talopram, and the two enantiomers of citalopram

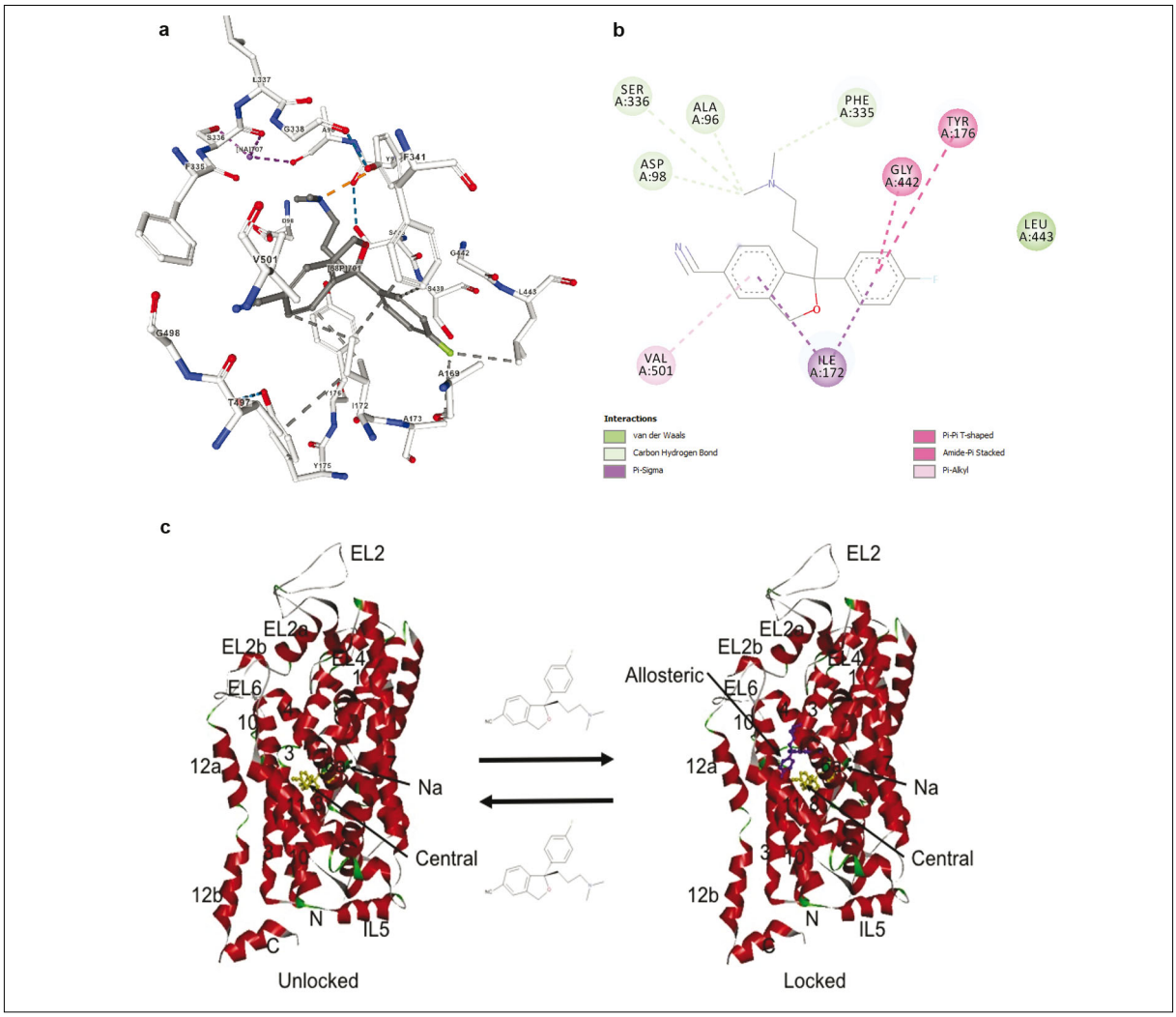


Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

Citalopram and its derivatives

Citalopram (Figure 12) was first synthesized in 1971 from the highly selective NE reuptake inhibitor talopram (Figure 12), a compound synthesized through a series of reactions and ring closure strategies from phthalide (Boges and Sanchez, 2012). The pharmacological activity of citalopram is primarily attributable to the S enantiomer, while the activity of the R enantiomer is reportedly 30-40 times weaker both *in vitro* and *in vivo* (Sánchez et al., 2004). The binding of escitalopram to the central binding site (S1) of the thermostable 3 mutation bearing hSERT (ts3 hSERT) has recently been reported by Coleman et al. (2016). In this model, the amine group of escitalopram

Figure 13. The binding of escitalopram to the hSERT (a, b) and the allosteric modulation of its binding (c)

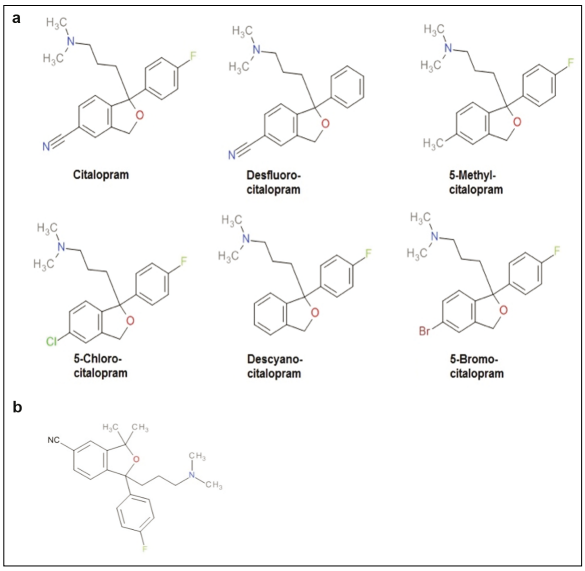


a,b. Escitalopram binding to the central binding site of the hSERT. 3D image (a) from the RSCB PDB (rcsb.org) of PDB 5I73 (Coleman et al, 2016). 2D image (b) was generated in Discovery Studio V16.1.0.15350 from the Protein Data Bank (PDB) structure 5I73.
c. Allosteric binding slows down the dissociation from the primary binding site. Image was generated in Discovery Studio V16.1.0.15350 from the PDB structure 5I73 and further annotated in Microsoft Office, Power Point 2016 MSO (32 bit).

interacts with the carboxylate of Asp98, whereas the fluorophenyl group forms a hydrophobic interaction with Tyr176 (Figure 13a,b) (Coleman et al., 2016). The Phe341 of the ts3 hSERT forms an aromatic interaction with the escitalopram cyanophthalane, with the fluorine of the drug being inserted into the binding pocket formed by the residues Ser439, Leu443, Ala169 and Ala173 (Coleman et al., 2016). Indeed, when the residues Tyr95, Asp98, Ile172, Asn177, Phe341, and Ser438 were altered, and [^{3}H]-5-HT uptake was measured, a 10- to 400-fold shift in the activity was found, suggesting that the above residues are vital determinants in the escitalopram binding to the hSERT (Andersen et al., 2010). Additionally, the alteration of the aromatic moiety in the Tyr95 (Y95V, Y95Q, Y95A) negatively influenced the Ki values (up to 436-fold increase), suggesting that the aromatic group of tyrosine is also crucial in the bindign of escitalopram (Andersen et al., 2010). Interestingly, an allosteric binding site (S2) in the hSERT is reportedly functioning as a modulatory unit of the primary binding site, and has been shown to have similar affinity to both *R*- and escitalopram binding (Sánchez et al., 2004). When *R*-citalopram is bound to S2, however, the off-rate of escitalopram bound to S1 is significantly affected due to S2 obstructing the ligand exit from the central binding site (Figure 13c). This latter could be the cause of the pharmacological and clinical differences observed between citalopram (racemate) and escitalopram treatments (Coleman et al., 2016; Sánchez et al., 2004).

Derivatives of escitalopram targeting the cyanophthalane and the fluorophenyl moieties (Figure 14a), and inhibitory activity measurements have recently been reported (Andersen et al., 2010). Results showed that the residues Ile172, Ala173, and Asn177 are responsible for anchoring the fluorophenyl group of citalopram and are also responsible for orienting the cyanophthalane group towards Val343 (Andersen et al., 2010). Larsen et al. (2016) designed citalopram derivatives and conducted SAR studies to investigate critical molecular determinants of the allosteric potency of citalopram. The Group found that the cyano-group in citalopram derivatives is essential for their binding to the allosteric binding site in the hSERT (Larsen et al., 2016). Allosteric potency was also increased by fluorine substituent and phthalane dimethyl groups, although N-methyl substituents were minor determinants in this respect (Larsen et al., 2016). The compound with the highest allosteric potency and lowest orthosteric affinity reported was dimethyl citalopram (Figure 14b), with a

Figure 14. The structure of escitalopram and its analogs (a) and the most potent dimethyl citalopram (b)



Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

two-fold increase in the allosteric potency compared to the orthosteric affinity (Larsen et al., 2016). The tested compounds were all racemic mixtures. To date, investigation of the enantioselectivity of these compounds has not been reported.

A generalized binding of SSRIs

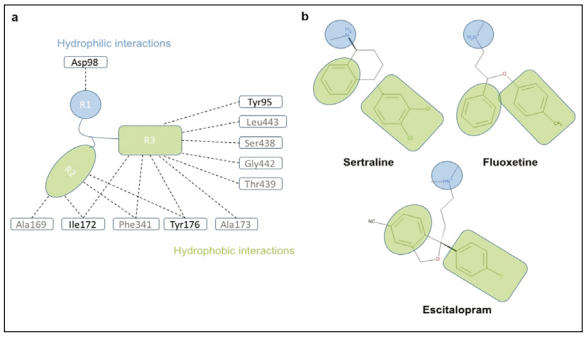
Based on the Drosophila melanogaster dopamine transporter (dDAT) structure, Xue et al. (2016) designed a hSERT model for the investigation of the binding modes of the three SSRIs, fluoxetine, sertraline, and escitalopram. A generalized binding mode of SSRI-hSERT was presented, with some highlighted hydrophobic and hydrophilic interactions involving three characteristic moieties present in these drugs (see R1, R2, and R3 on Figure 15a,b).

In general, a salt-bridge and hydrogen bond interaction between the R1 moiety of the three drugs and Asp98 are formed. R2 interacts with Ala169, Ile172, Tyr176 and Phe341 through hydrophobic interactions, whereas R3 forms hydrophobic interactions with Tyr95, Ala173, Ser438, Thr439, Gly442 and Leu443, and also with Ile172, Tyr176 and Phe341 (Xue et al., 2016).

CONCLUSIONS AND FUTURE PERSPECTIVES

SSRIs have become a beneficial class of drugs from the treatment of depression. Despite their improved side

Figure 15. Generalized binding interactions of sertraline, fluoxetine, and escitalopram



Residues with strong and relatively strong contributions are shown in black and grey (a), respectively. Moieties significant for binding interactions are shown in blue and green (b). Structures were generated in BioviaDraw, 2016, V16.1 9 (32 bit).

effect profile compared to those of TCAs and MAOIs, SSRIs show some limitations, such as a delayed onset in the antidepressant effect (4-6 weeks), only about 65 % of patients respond to treatment, and occasionally exhibit sexual dysfunction side effects (Sharma H, Santra S, 2015; Marks et al., 2008). Hence, novel drug structures with improved pharmacokinetic and side effect profiles are in demand. Recently synthesized sertraline salts showed enhanced antidepressant activities and, in some cases, were also able to overcome anorexic symptoms apparent in depressed patients. New derivatives of escitalopram helped to map the allosteric binding site in the hSERT and also led to the design of new citalopram analogs, such as dimethyl-citalopram, a potent allosteric inhibitor of hSERT. Binding studies of fluoxetine and hSERT provided useful information on the key determinants in the fluoxetine binding and led to the design of further fluoxetine and nisoxetine derivatives, such as FD2 and FD4 that proved to be efficient in DA reuptake inhibition in the treatment of cocaine addiction.

Despite the popularity of SSRIs, a new group of drugs called triple reuptake inhibitors (TRIs) have recently been in the center of attention. TRIs simultaneously inhibit the reuptake of serotonin, noradrenaline and dopamine through the competitive inhibition of SERT, NET, and DAT, respectively, (Sharma H, Santra S, 2015). Despite that the structural variation amongst monoamine transporters represents a significant challenge in the design of small molecule drugs that simultaneously inhibit all three transporters and show improved bioavailability and safety profile, TRIs may be suitable for strategies where potency ratios are varied for the benefit of different clinical outcomes (Sharma H, Santra S, 2015). As such, one potency ratio could be useful in the treatment of a symptom, whereas another potency ratio could be useful in the treatment of another one (Sharma H, Santra S, 2015). TRI development is an active field of research where further clinical studies are ongoing in the hope to reveal whether this class of drugs has broader advantages over SSRIs regarding side effects, efficacy, and early onset (Sharma H, Santra S, 2015). Meanwhile, SSRIs, carrying an improved efficacy profile with well-tolerated side effects, remain some of the most commonly applied drugs in the world for the treatment of major depressive disorders.

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A szerotonintranszporter szelektív gátlása a depresszió kezelésében: szertralin, fluoxetin és citalopram

A szelektív szerotoninvisszavétel-gátlók felfedezése és alkalmazása a neuropszichofarmakológia egyik meghatározó mérföldkövének tekinthető. Ezen kis molekulájú gyógyszerek reverzibilisen kötődnek a humán szerotonintranszporterhez, és gátoljak a szerotonin neurotranszmitter visszavételét a szinaptikus résből. A csoportba tartozó molekulák a target centrális ligandkötőhelyén fejtik ki hátasukat, a csoport egyes tagjai változó interakciókban vesznek reszt. Működésük során a szelektív szerotoninvisszavétel-gátlók három jelentősebb régiója specifikus hidrogénhíd- illetve további hidrofób interakciókat létesít bizonyos konzervált aminosavakkal. Alkalmazásukkor kései terápiás válasz, vagy akár bizonyos mellékhátasok mint szédülés, hányinger és szexuális működési zavarok jelentkeznek, habár a tradicionális antidepresszánsokkal szemben a szelektív szerotoninvisszavétel-gátlók így is előnyösebb mellékhatásprofillal bírnak. Új kutatások továbbá rávilágítottak az enantiomer-specifikus különbségekre az egyes gyógyszerek működése esetén, felvetve bizonyos kérdéseket a racémvegyületek terápiás alkalmazásával kapcsolatosan, további strukturális fejlesztések igényét hangsúlyozva. Nemrégiben előállított szertralinsók, illetve fluoxetin- és citalopramanalógok biztató eredményeket produkáltak rágcsáló modellrendszerben, ahol fokozott antidepresszív hatás illetve anorexiás tünetek enyhítése volt tapasztalható. Így, a mellékhatások ellenére, a szelektív szerotoninvisszavétel-gátlók vezető pozíciót értek el az antidepresszánsok között, és mivel jelentős mennyiségű eredmény áll rendelkezésünkre a működésükkel kapcsolatban, az alkalmazásuk megfontolandó az új antidepresszáns hatású molekulák fejlesztésében.

Kulcsszavak: depresszió, szerotonintranszporter, szertralin, fluoxetin, citalopram