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**REVIEW ARTICLE**

# Recent Advances and Challenges of the Drugs Acting on Monoamine Transporters

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**Abstract:** ***Background*:** The human Monoamine Transporters (hMATs), primarily including hSERT, hNET and hDAT, are important targets for the treatment of depression and other behavioral disorders with more than the availability of 30 approved drugs.

***Objective*:** This paper is to review the recent progress in the binding mode and inhibitory mechanism of hMATs inhibitors with the central or allosteric binding sites, for the benefit of future hMATs inhibitor design and discovery. The Structure-Activity Relationship (SAR) and the selectivity for hit/lead compounds to hMATs that are evaluated by *in vitro* and *in vivo* experiments will be highlighted.

***Methods*:** PubMed and Web of Science databases were searched for protein-ligand interaction, novel inhibitors design and synthesis studies related to hMATs.

***Results*:** Literature data indicate that since the first crystal structure determinations of the homologous bacterial Leucine Transporter (LeuT) complexed with clomipramine, a sizable database of over 100 experimental structures or computational models has been accumulated that now defines a substantial degree of structural variability hMATs-ligands recognition. In the meanwhile, a number of novel hMATs inhibitors have been discovered by medicinal chemistry with significant help from computational models.

***Conclusion*:** The reported new compounds act on hMATs as well as the structures of the transporters complexed with diverse ligands by either experiment or computational modeling have shed light on the poly-pharmacology, multimodal and allosteric regulation of the drugs to transporters. All of the studies will greatly promote the Structure-Based Drug Design (SBDD) of structurally novel scaffolds with high activity and selectivity for hMATs.

**ARTICLE HISTORY**

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**Keywords:** Monoamine transporters, allosteric modulation, multi-target drug, common binding mode, drug selectivity, computational modeling, structure activity analysis.

## 1. INTRODUCTION

Monoamine Transporters (MATs) are the second-largest class of mammalian membrane-spanning protein belonging to the Solute Carrier 6 (SLC6) transporter family [1, 2]. There are three major classes ofMATs in human brains (hMATs), including hSERT (SLC6A4), hNET (SLC6A2) and hDAT (SLC6A3), which are responsible for the reuptake of the neurotransmitters: Serotonin (5-HT), Norepinephrine (NE); and Dopamine (DA), respectively, from extracellular space into neurons [1, 3-5]. Imbalance in neurotransmitter homeostasis is linked to a wide range of Central Nervous System (CNS) disorders, including depression, Attention Deficit Hyperactivity Disorder (ADHD), Parkinson's Disease (PD), anxiety and drug abuse [6-11]. Different classes of selective or nonselec-

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Feng Zhu Tel/Fax: 0571-88208444; 0571-88208444; Weiwei Xue Tel/Fax: 023-65678450; 023-65678450;
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tive therapeutic agents for regulating neurotransmission have been developed [12]; for example, the first-line medications currently prescribed for depression patients include the selective reuptake inhibitors of Serotonin (SSRIs), Norepinephrine (sNRIs), reuptake inhibitors of both Serotonin and Norepinephrine (SNRIs) and the multimodal inhibitors vilazodone and vortioxetine [13-15].

Currently, the approved drugs of hMATs are less than ideal considering their low remission rate, delayed onset of action, partial- or non-response and the associated risk of side effects [16-19]. There has been a continued need for the discovery of novel scaffolds for the further development of more potent and safety agents against CNS disorders based on the recent advances in both structural biology, computational modeling and medicinal chemistry [13-15, 20-24]. In the past, the hMATs lacks well resolved experimental structures of the protein complexed with ligands. This has severely hampered Structure-Based Drug Design (SBDD) efforts, such as Virtual Screening (VS), for novel drugs development based on the targets. Insights into the hMATs structures as well as the ligands binding have come from the cocrystalized complexes of the homologous *Aquifex aeolicus* Leucine Transporter (LeuT) [25, 26] and *Drosophila melanogaster* Dopamine Transporter (dDAT) [27-30]. The availability of these experimental structures prompted the modeling of the hMATs structures by various computational techniques [4, 8, 31, 32]. Computational modeling of ligands binding to hMATs has not only elucidated the binding (inhibitory) mechanism of substrates [33, 34] and diverse therapeutic agents [6, 35-42], but also provided the 3D structures for the discovery of novel inhibitors by SBDD methods and with some degree of success in some of them [43-47].

Recently, X-ray crystallographic structures of hSERT bound to diverse antidepressants were reported [48, 49]. Moreover, successful identification of an allosteric site in the extracellular vestibule of the hSERT structure sheds light on the understanding of the allosteric modulator sites in hNET and hDAT [48]. As for allosteric inhibitors of hMATs, the identified probe molecules suggested a more subtle mode of action, which might have an advantage over the central site inhibitors [50, 51]. Meanwhile, there are many pressing questions, such as the binding selectivity and druggabilities for the probe molecules and allosteric site, have not yet been clarified [48]. However, it is hoped that this will lead to new therapeutic opportunities for the discovery of medicines based on hMATs [52].

In this review, we focus on the recent advances in our understanding of the binding mode, inhibitory mechanism and structure-based development of drugs acting on both central and allosteric sites of human MATs and outline some important questions that remain unanswered. For other details, refer to previous reviews on this topic [4, 8, 12, 53-56].

## 2. STRUCTURE AND FUNCTION OF HUMAN MATS

The human MATs *i.e*. hSERT, hNET and hDAT, are large integral membrane proteins (more than 600 amino acids) composed of 12 Transmembrane domains (TM1 to TM12) connected by Intracellular and Extracellular loops (ILs and ELs) [1]. Since the first crystal structure determinations of the homologous LeuT, a sizable database of experimental structures or computational models has been reported. As a breakthrough in the understanding of the structure and function of the human MATs, the X-ray crystal structure of hSERT showed that the protein contains a 5 + 5 inverted-topological repeats, formed by TM1 to TM5 and TM6 to TM10 (Fig. **1**) [48, 49], is similar to the architecture of the human MATs homologues dDAT [27-29] and LeuT [25, 26]. A central site for substrate and drug binding was found approximately halfway across the membrane bilayer of the hSERT, and residues located at the edge of the central pocket form the site for $Na^+$ binding (Fig. **1**) [48, 49]. In addition to the central site, an allosteric site was identified residing at the periphery of the extracellular vestibule of hSERT [48].

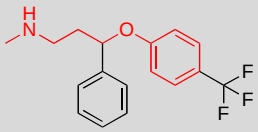
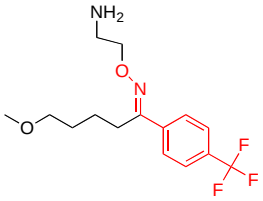
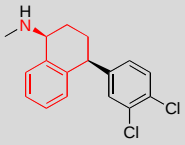
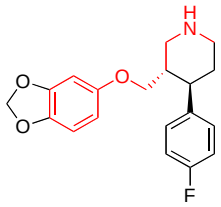
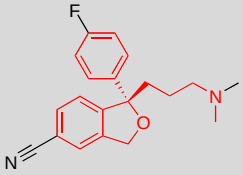
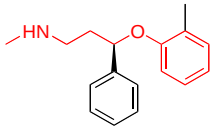
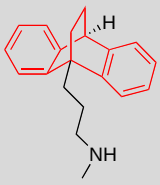
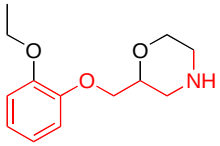
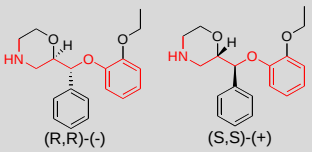
### 2.1. The Central Binding Site

X-ray crystal structures [25-29, 48, 49] and site mutagenesis experiments [6, 7, 57] have revealed that the central binding site of MATs is surrounded primarily of residues from TM1, TM3, TM6, TM8 and TM10. The substrates 5-HT, NE and DA bound in the central site are similar to each other in hSERT, hNET and hDAT [28, 34]. Drugs such as antidepressants SSRIs, SNRIs and TRIs lock the transporter in an outward-open conformation by lodging in the central binding site [26, 27, 29, 48, 49].

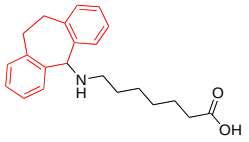
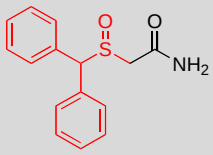
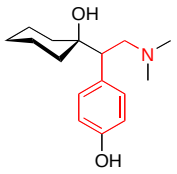
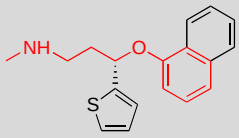
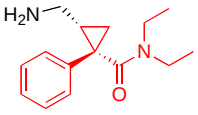
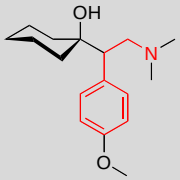
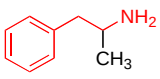
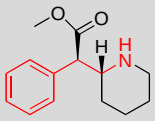
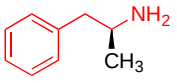
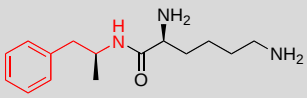
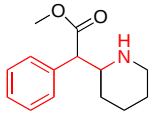
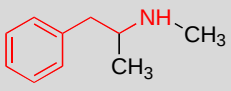
### 2.2. The Allosteric Binding Site

The allosteric effects of human MATs have been known for more than three decades through the Tri-

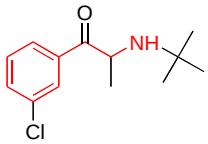
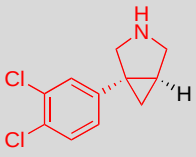
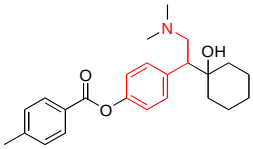
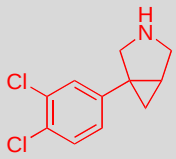
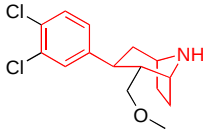
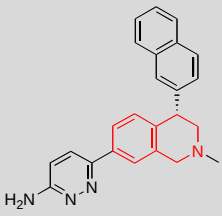
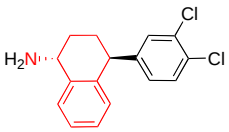
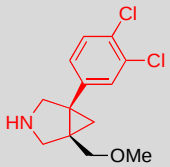
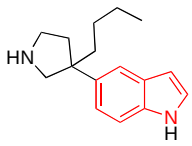
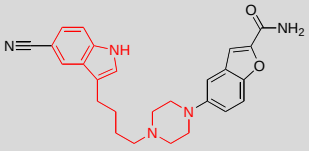
**Table 1. Structures, Binding Mechanism and Development information of hMATs inhibitors (the scaffolds of inhibitors are highlighted in red).**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 1 | Fluoxetine |  | SSRI | Approved | Hot spot residues for binding: Tyr95, Asp98, Ala169, Ile172, Ala173, Tyr176, Phe341, Ser438, Thr439, Gly442, Leu443. | [37] |
| 2 | Fluvoxamine |  | SSRI | Approved | Residues interacting with compounds: Tyr95, Asp98, Ile172, Tyr176, Phe341, Thr439, Phe335, Ser336, Thr497. | [49] |
| 3 | Sertraline |  | SSRI | Approved | Hot spot residues for binding: Tyr95, Asp98, Ala169, Ile172, Ala173, Tyr176, Phe341, Ser438, Thr439, Gly442, Leu443. | [37] |
| 4 | Paroxetine |  | SSRI | Approved | Residues interacting with compounds: Tyr95, Asp98, Ala169, Ile172, Ala173, Tyr176, Phe341, Thr439, Leu443, Phe335, Val501, Thr497. | [48] |
| 5 | Escitalopram |  | SSRI | Approved | Residues interacting with compounds: Tyr95, Asp98, Ala169, Ile172, Ala173, Tyr176, Phe341, Thr439, Leu443, Phe335, Val501, Thr497. | [48] |
| 6 | Atomoxetine |  | sNRI | Approved | Crucial residues for binding: Phe72, Asp75, Ala145, Val148, Gly149, Tyr152, Phe317, Phe323, Ser419, Ser420, Gly423. | [73] |
| 7 | Maprotiline |  | sNRI | Approved | Crucial residues for binding: Phe72, Asp75, Ala145, Val148, Gly149, Tyr152, Phe317, Phe323, Ser419, Ser420, Gly423. | [73] |
| 8 | Viloxazine |  | sNRI | Approved | Crucial residues for binding: Phe72, Asp75, Ala145, Val148, Gly149, Tyr152, Phe317, Phe323, Ser419, Ser420, Gly423. | [73] |
| 9 | Reboxetine |  (R,R)-(-) (S,S)-(+) | sNRI | Approved | Crucial residues for binding: Phe72, Asp75, Ala145, Val148, Gly149, Tyr152, Phe317, Phe323, Ser419, Ser420, Gly423. | [73] |

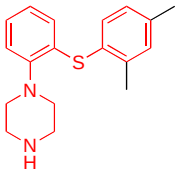
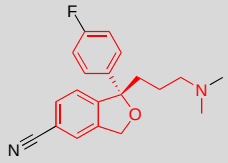
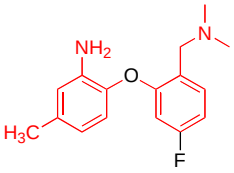
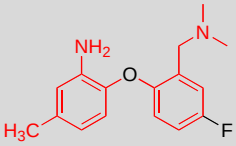
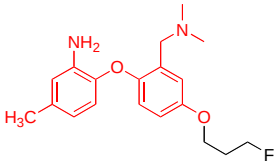
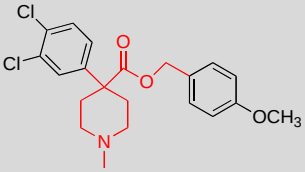
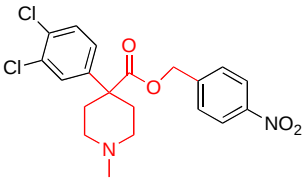
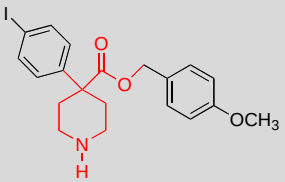
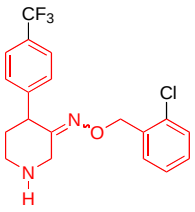
**(Table 1) contd….**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 10 | Amineptine |  | sDRI | Approved | N.A. | [83] |
| 11 | Modafinil |  | sDRI | Approved | Residues with high energy contribution: Phe76, Asp79, Ala77, Val152, Phe155, Tyr156, Phe320, Ser321, Phe326, Gly323, Ser422. | [64] |
| 12 | Desvenlafaxine |  | SNRI | Approved | Hot spot residues for binding in hSERT: Tyr95, Asp98, Ile172, Tyr176, Phe335, Phe341, Ser438, Thr439, Gly442. | [38] |
| 13 | Duloxetine |  | SNRI | Approved | Hot spot residues for binding in hSERT: Tyr95, Asp98, Ile172, Tyr176, Phe335, Phe341, Ser438, Thr439, Gly442. | [38] |
| 14 | Levomilnacipran |  | SNRI | Approved | Hot spot residues for binding in hSERT: Tyr95, Asp98, Ile172, Tyr176, Phe335, Phe341, Ser438, Thr439, Gly442. | [38] |
| 15 | Venlafaxine |  | SNRI | Approved | Hot spot residues for binding in hSERT: Tyr95, Asp98, Ile172, Tyr176, Phe335, Phe341, Ser438, Thr439, Gly442. | [38] |
| 16 | Amphetamine |  | NDRI | Approved | N.A. | [149] |
| 17 | Dexmethylphenidate |  | NDRI | Approved | Residues with high energy contribution: Phe76, Asp79, Ala77, Val152, Tyr156, Ser321, Phe320, Leu322, Gly323, Phe326, Ser422. | [64] |
| 18 | Dextroamphetamine |  | NDRI | Approved | Residues with high energy contribution: Phe76, Asp79, Ser149, Val152, Tyr156, Ser321, Phe320, Phe326, Ser422. | [64] |
| 19 | Lisdexamfetamine |  | NDRI | Approved | N.A. | [64] |
| 20 | Methylphenidate |  | NDRI | Approved | N.A. | [80] |
| 21 | Methamphetamine |  | NDRI | Approved | Residues interacting with compound in dDAT: Tyr124, Ser422, Ser421, Val120, Phe43, Phe319, Asp46, Asp121, Ser426. | [29, 80] |

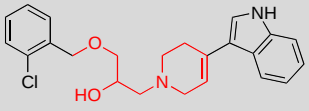
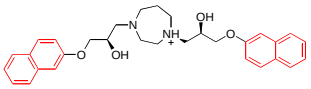
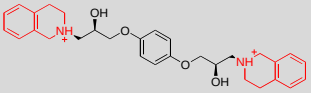
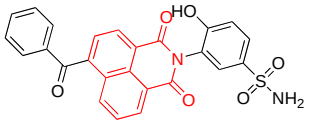
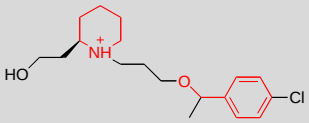
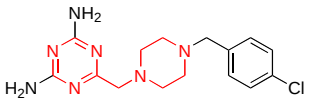
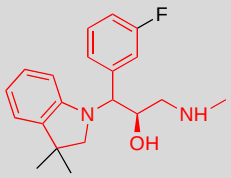
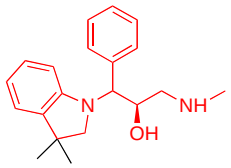
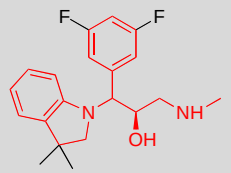
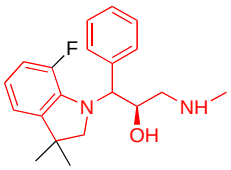
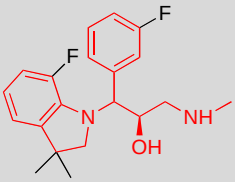
**(Table 1) contd….**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 22 | Bupropion |  | NDRI | Approved | Residues with high energy contribution: Phe76, Asp79, Ala77, Ser149, Val152, Tyr156, Ser321, Phe320, Phe326, Ser422, Ala480. | [64] |
| 23 | Amitifadine |  | TRI | Clinical Trial | Residues with high energy contribution in hSERT: Tyr95, Asp98, Ile172, Tyr176, Phe341, Ser438. | [98] |
| 24 | Ansofaxine |  | TRI | Clinical Trial | N.A. | [83] |
| 25 | DOV 216, 303 |  | TRI | Clinical Trial | N.A. | [83] |
| 26 | GSK372475 |  | TRI | Clinical Trial | N.A. | [83] |
| 27 | Liafensine |  | TRI | Clinical Trial | N.A. | [83] |
| 28 | SEP-225289 |  | TRI | Clinical Trial | N.A. | [83] |
| 29 | GSK1360707 |  | TRI | Clinical Trial | N.A. | [150] |
| 30 | RG-7166 |  | TRI | Clinical Trial | N.A. | [83] |
| 31 | Vilazodone |  | Multimodal Inhibitor | Approved | Residues interacting with compound in 5-$HT_{1A}$: D116, N386, Y390, T121, C120, S199, V117, T188, I189, T196, T200, A303, W358, F361, F362, M92, A93, W102, F112, I113. | [69] |

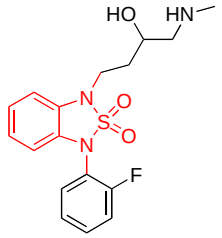
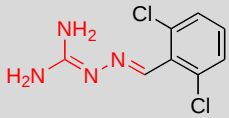
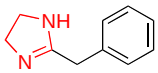
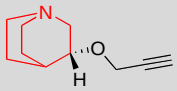
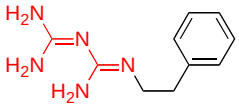
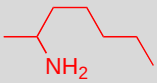
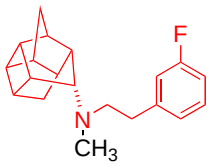
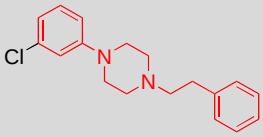
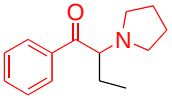
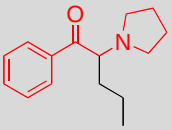
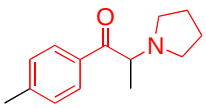
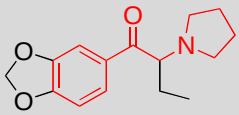
**(Table 1) contd….**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 32 | Vortioxetine |  | Multimodal Inhibitor | Approved | Residues interacting with compound: Ile168, Phe341, Phe335, Tyr95, Ala96, Asp98, Ser336, Tyr176, Thr439, Ile172, Gly442, Ala169. | [97] |
| 33 | Escitalopram |  | Allosteric Inhibitor | Approved | Residues interacting with compound: Glu494, Arg104, Gln332, Ala331, Phe556, Pro561. | [48] |
| 34 | Compound 1 |  | SSRI | Investiga-tive | A 3-fluoropropoxy or 4-fluoro-butoxy on the 5-position of compounds lead to high affinities for the SERT. | [110] |
| 35 | Compound 2 |  | SSRI | Investiga-tive | A 3-fluoropropoxy or 4-fluoro-butoxy on the 5-position of compounds lead to high affinities for the SERT. | [110] |
| 36 | Compound 3 |  | SSRI | Investiga-tive | A 3-fluoropropoxy or 4-fluoro-butoxy on the 5-position of compounds lead to high affinities for the SERT. | [110] |
| 37 | Compound 4 |  | SSRI | Investiga-tive | The meperidine benzyl ester scaffold and the normeperidine benzyl ester scaffold seem to be well. | [111] |
| 38 | Compound 5 |  | SSRI | Investiga-tive | The meperidine benzyl ester scaffold and the normeperidine benzyl ester scaffold seem to be well. | [111] |
| 39 | Compound 6 |  | SSRI | Investiga-tive | The meperidine benzyl ester scaffold and the normeperidine benzyl ester scaffold seem to be well. | [111] |
| 40 | Compound 7 |  | SSRI | Investiga-tive | The α and β aromatic moieties influence the potency of compounds for SERT. | [112] |

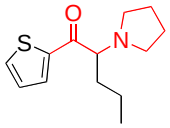
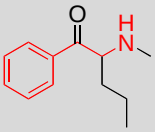
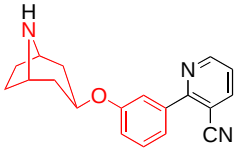
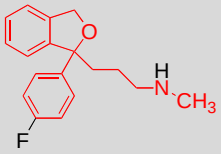
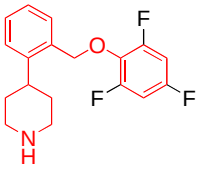
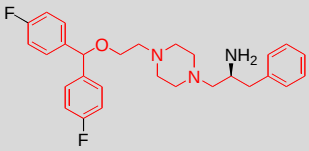
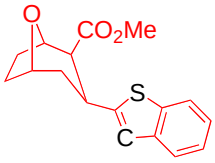
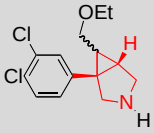
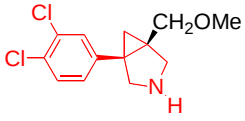
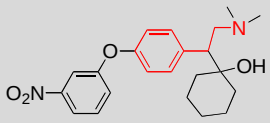
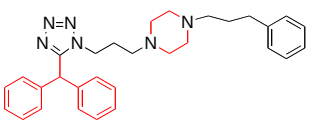
**(Table 1) contd….**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 41 | Compound 8 |  | SSRI | Investigative | N.A. | [45] |
| 42 | Compound 9 |  | SSRI | Investigative | Residues interacting with compound: Asp98, Glu493, Tyr176, Phe341, Thr497. | [95] |
| 43 | Compound 10 |  | SSRI | Investigative | Residues interacting with compound: Tyr95, Tyr175, Tyr176, Glu493. | [95] |
| 44 | Compound 11 |  | SSRI | Investigative | Residues interacting with compound: Glu493, Thr497, Tyr176, Phe335. | [95] |
| 45 | Compound 12 |  | SSRI | Investigative | Residues interacting with compound in molecular docking: Pro403, Tyr107, Arg104, Leu99, Ile179, Trp103. | [43] |
| 46 | Compound 13 |  | SSRI | Investigative | N.A. | [43] |
| 47 | Compound 14 |  | sNRI | Investigative | Small alkyl substitution at the C3 position of the indoline ring enhanced selectivity for the NET over the SERT. | [113] |
| 48 | Compound 15 |  | sNRI | Investigative | Small alkyl substitution at the C3 position of the indoline ring enhanced selectivity for the NET over the SERT. | [113] |
| 49 | Compound 16 |  | sNRI | Investigative | Small alkyl substitution at the C3 position of the indoline ring enhanced selectivity for the NET over the SERT. | [113] |
| 50 | Compound 17 |  | sNRI | Investigative | Small alkyl substitution at the C3 position of the indoline ring enhanced selectivity for the NET over the SERT. | [113] |
| 51 | Compound 18 |  | sNRI | Investigative | Small alkyl substitution at the C3 position of the indoline ring enhanced selectivity for the NET over the SERT. | [113] |

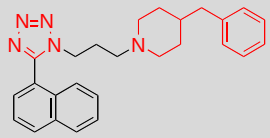
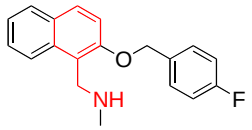
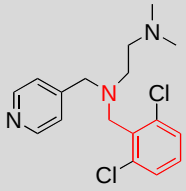
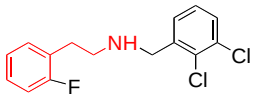
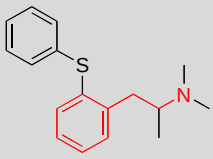
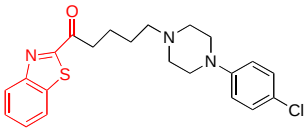
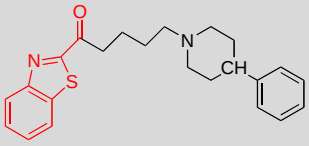
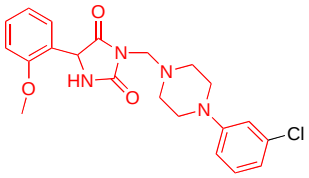
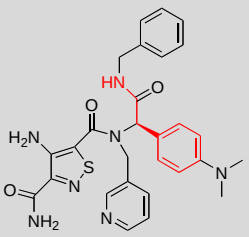
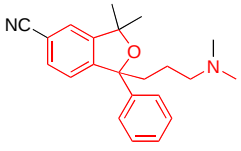
**(Table 1) contd….**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 52 | Compound 19 |  | sNRI | Investigative | N.A. | [114] |
| 53 | Compound 20 |  | sNRI | Investigative | Key residues in the molecular docking study: Phe72, Tyr152, and Phe317, Ala145, Asp75. | [44] |
| 54 | Compound 21 |  | sNRI | Investigative | Key residues in the molecular docking study: Phe72, Tyr152, and Phe317, Ala145, Asp75. | [44] |
| 55 | Compound 22 |  | sNRI | Investigative | Key residues in the molecular docking study: Phe72, Tyr152, and Phe317, Ala145, Asp75. | [44] |
| 56 | Compound 23 |  | sNRI | Investigative | Key residues in the molecular docking study: Phe72, Tyr152, and Phe317, Ala145, Asp75. | [44] |
| 57 | Compound 24 |  | sNRI | Investigative | Key residues in the molecular docking study: Phe72, Tyr152, and Phe317, Ala145, Asp75. | [44] |
| 58 | Compound 25 |  | sDRI | Investigative | The radiolabeling of compound with carbon-11 or fluorine-18 provided molecular probes for the *in vivo* imaging of the DAT using PET. | [117] |
| 59 | Compound 26 |  | sDRI | Investigative | High affinity for hDAT. | [118] |
| 60 | Compound 27 |  | sDRI | Investigative | Increasing α-pyrrolidinophenone substituent chain length increases potency at the hDAT and may increase abuse potential. | [120] |
| 61 | Compound 28 |  | sDRI | Investigative | Increasing α-pyrrolidinophenone substituent chain length increases potency at the hDAT and may increase abuse potential. | [120] |
| 62 | Compound 29 |  | sDRI | Investigative | Increasing α-pyrrolidinophenone substituent chain length increases potency at the hDAT and may increase abuse potential. | [120] |
| 63 | Compound 30 |  | sDRI | Investigative | Increasing α-pyrrolidinophenone substituent chain length increases potency at the hDAT and may increase abuse potential. | [120] |

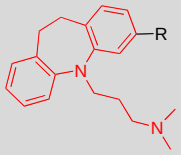
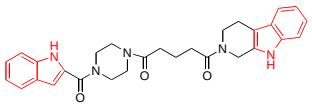
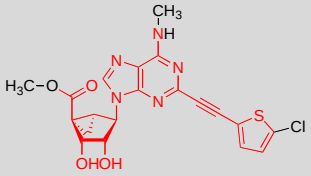
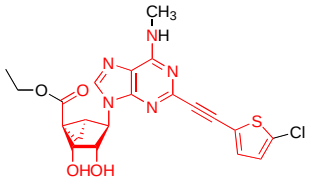
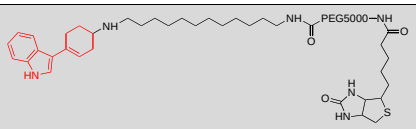
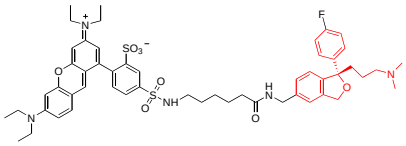
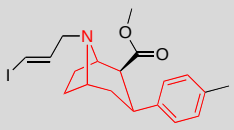
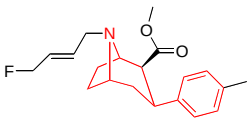
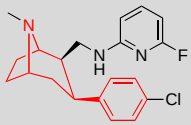
**(Table 1) contd….**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 64 | Compound 31 |  | sDRI | Investigative | Increasing α-pyrrolidinophenone substituent chain length increases potency at the hDAT and may increase abuse potential. | [120] |
| 65 | Compound 32 |  | sDRI | Investigative | Increasing α-pyrrolidinophenone substituent chain length increases potency at the hDAT and may increase abuse potential. | [120] |
| 66 | Compound 33 |  | sNRI | Investigative | N.A. | [123] |
| 67 | Compound 34 |  | sNRI | Investigative | High affinity for both SERT and NET. | [7] |
| 68 | Compound 35 |  | sNRI | Investigative | N.A. | [132] |
| 69 | Compound 36 |  | sDRI | Investigative | Same affinity for hSERT and hDAT. | [134] |
| 70 | Compound 37 |  | sDRI | Investigative | The most potent, but non-selective DAT and SERT inhibitor. | [135] |
| 71 | Compound 38 |  | TRI | Investigative | N.A. | [136] |
| 72 | Compound 39 |  | TRI | Investigative | N.A. | [137] |
| 73 | Compound 40 |  | TRI | Investigative | N.A. | [138] |
| 74 | Compound 41 |  | TRI | Investigative | The most potent compound with long linker and long distance between the piperazine and phenyl rings. | [139] |

**(Table 1) contd….**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 75 | Compound 42 |  | TRI | Investigative | The most potent 5-HT reuptake inhibitor with a 1-naphthyl substitution and long linker. | [140] |
| 76 | Compound 43 |  | TRI | Investigative | Scaffold differing from other reported MAT ligands. | [46] |
| 77 | Compound 44 |  | TRI | Investigative | Scaffold differing from other reported MAT ligands. | [46] |
| 78 | Compound 45 |  | TRI | Investigative | Scaffold differing from other reported MAT ligands. | [46] |
| 79 | Compound 46 |  | TRI | Investigative | Extending amine linker and lack of substituents are important for NET/DAT activity. | [46] |
| 80 | Compound 47 |  | Multimode Inhibitor | Investigative | Selective for SERT, and not DAT or NET. | [141] |
| 81 | Compound 48 |  | Multimode Inhibitor | Investigative | Potential to be further explored as dual-acting agent. | [141] |
| 82 | Compound 49 |  | Multimode Inhibitor | Investigative | Favorable antidepressant-like profile without affecting spontaneous locomotor activity. | [142] |
| 83 | Compound 50 |  | Allosteric Modulator | Investigative | Allosteric modulation of hSERT function. | [52] |
| 84 | Compound 51 |  | Allosteric Modulator | Investigative | Similar activity at S1 and S2 binding site. | [91] |

**(Table 1) contd….**

| No. | Name | Structure (scaffold colored in red) | Drug Class | Status | Binding Mechanism / Structure Characteristics | Refs. |
|---|---|---|---|---|---|---|
| 85 | Compound 52 |  | Allosteric Modulator | Investigative | N.A. | [143] |
| 86 | Compound 53 |  | Allosteric Modulator | Investigative | Residues interacting with compound: Glu494, Tyr175, Phe556, Phe334, Tyr176, Phe341, Asp328. | [95] |
| 87 | Compound 54 |  | Allosteric Modulator | Investigative | High binding affinities at hDAT and hNET. | [51] |
| 88 | Compound 55 |  | Allosteric Modulator | Investigative | More potent than cocaine at inhibiting DA uptake. | [51] |
| 89 | Compound 56 |  | Imaging Agent | Investigative | N.A. | [146] |
| 90 | Compound 57 |  | Imaging Agent | Investigative | High affinity binding for hSERT without any binding to hNET or hDAT. | [145] |
| 91 | Compound 58 |  | Imaging Agent | Investigative | N.A. | [117] |
| 92 | Compound 59 |  | Imaging Agent | Investigative | N.A. | [117] |
| 93 | Compound 60 |  | Imaging Agent | Investigative | N.A. | [147] |

**Abbreviations:** SSRI: Selective Reuptake Inhibitor of Serotonin; sNRI: Selective Reuptake Inhibitor of Norepinephrine; sDRI: Selective Dopamine Reuptake Inhibitor; NDRI: Norepinephrine and Dopamine Reuptake Inhibitor; SNRI: Serotonin and Norepinephrine Reuptake Inhibitor; TRI: Triple Reuptake Inhibitors; N.A.: Not Available.

Cyclic Antidepressant (TCA) imipramine which binds to hSERT and acts as a non-competitive inhibitor of the transporter [58]. In recent years, compelling evidence from dissociation rates of probe compound (such as escitalopram and rigid adenine nucleoside derivatives) from transporter proteins suggests that hSERT, hNET and hDAT possess one or more allosteric binding sites [50, 59-61]. However, for the first time, the structure information regarding the presence of the allosteric site in human MATs came from the X-ray crystal structure of LeuT, which was distinct from the central site located within the extracellular vestibule of the transporter [25]. More recently, the existence of the allosteric site on hSERT was identified by using X-ray

crystallography [48]. The co-crystal structure showed that antidepressant escitalopram can bind to the allosteric site defined by residues in TM1b, TM6a, TM10, TM11, EL4 and EL6 [48]. The discovery of the allosteric site in hSERT makes it feasible to further explore and understand the allosteric regulation mechanism of hNET and hDAT.

## 3. DRUGS TARGETING HUMAN MATS

Drugs that target hSERT, hNET and hDAT are used to treat depression, ADHD and other conditions [13]. Typical antidepressants of this class include SSRIs and SNRIs, which increase the concentration of 5-HT and/or NE in the synaptic cleft to treat depression [37, 62]. More than 10 SSRIs and (or) SNRIs have been approved by the U.S. Food and Drug Administration (FDA) for depression treatment [63]. ADHD drugs targeting human MATs include sNRIs and NDRIs, which play a therapeutic role primarily by modulating catecholaminergic signaling in the prefrontal cortex of the brain [64]. Until now, 9 ADHD drugs have been approved by the U.S. FDA [63]. However, these medications are less than ideal considering their low remission rate [16], delayed onset of action [18], partial- or non-response [17] and associated side effect [19]. Major side effects include sexual dysfunction, suicide, gastrointestinal or cardiovascular side effects, and discontinuation symptoms [65-68]. Recently, several promising inhibitors with novel mechanism have been approved by the U.S. FDA or in the clinical trials. According to their mechanism of action, the newly reported inhibitors can be classified into three groups: Triple Reuptake Inhibitors (TRIs) [19]; multimodal inhibitors [62]; and allosteric inhibitors [52]. They showed greater potency, safety and rapid onset of effects with few/absence of drug interactions [19, 53, 62, 69]. All of the approved drugs targeting human MATs will be reviewed in the following sections.

### 3.1. Single-target Inhibitors

#### *3.1.1. SSRIs*

SSRIs including fluoxetine, fluvoxamine, sertraline, paroxetine and escitalopram (Tables **1** and **2**) are the first-line and the most prescribed class of antidepressants [70]. Fluvoxamine, an SSRI approved by the U.S. FDA to treat obsessive-compulsive disorder, is sometimes used to treat depression [71]. All SSRIs work in a similar way [49] and generally can cause similar side effects [65].

#### *3.1.2. sNRIs*

sNRIs are psychostimulant which are commonly used for mood and behavioral disorders [72]. Currently, 4 sNRIs (atomoxetine, maprotiline, reboxetine and viloxazine) have been approved and marketed by either the U.S. FDA or the European Medicines Agency (Tables **1** and **2**) [73] for treating ADHD [74] and depression [75]. Amongst these 4 sNRIs, reboxetine is a racemic mixture of (R, R)- and (S, S)- enantiomers. (S, S)-reboxetine showed 130-fold higher affinity to hNET than (R, R)-reboxetine, and was reported as the predominant influence on reboxetine's steady state pharmacological property [76].

#### *3.1.3. Selective Dopamine Reuptake Inhibitors (sDRIs)*

sDRIs drugs are most often utilized for the treatment of ADHD, wakefulness disorders like narcolepsy, and in some cases to help treat obesity [77]. Due to poor tolerability and potential for abuse, many DRIs have been withdrawn from global markets and clinical development [78]. At present, sDRIs used in clinical practice including amineptine and modafinil (Tables **1** and **2**) [77].

### 3.2. Dual-target inhibitors

#### *3.2.1. SNRIs*

SNRIs are a class of antidepressants that have properties making them one of the best therapeutic choices. They are characterized by a mixed action on both major neuroamines of depression, 5-HT and NE. At present, 4 SNRIs (desvenlafaxine, duloxetine, levomilnacipran, and venlafaxine) [38] approved by the U.S. FDA used in clinical practice (Tables **1** and **2**). SNRIs show a good efficacy in the treatment of depression, slightly higher than SSRIs [79].

#### *3.2.2. NDRIs*

NDRIs are mainly used for anti-ADHD agents in clinical practice [77]. In total, 7 NDRIs (amphetamine, dexmethylphenidate, dextroamphetamine, lisdexamfetamine, methylphenidate, methamphetamine and bupropion) (Tables **1** and **2**) had been approved or in clinical trial for treating ADHD [80]. Among these drugs, lisdexamfetamine was an inactive prodrug of dextroamphetamine [81]. Dextroamphetamine and dexmethylphenidate were D-enantiomers of amphetamine and methylphenidate, respectively.

**Table 2. The Binding Affinities and Side Effects of the studied hMATs inhibitors.**

| No. | Name | Cell Line Studied | Binding Affinities / Uptake Potency (nM) | Positive Control (nM) | Side effects | Refs. |
|---|---|---|---|---|---|---|
| 1 | Fluoxetine | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=1/660/4180 | N.A. | Sexual dysfunction, suicide | [65-67, 151] |
| 2 | Fluvoxamine | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=11/1119/16790 | N.A. | Sexual dysfunction, gastrointestinal side effects | [65, 151, 152] |
| 3 | Sertraline | hSERT/hNET/hDAT expressed on HEK-293 | $IC_{50}$=0.4/420-817/22-25 | N.A. | Sexual dysfunction, discontinuation symptoms, suicide | [67, 151, 153, 154] |
| 4 | Paroxetine | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=0.34/156/268 | N.A. | Sexual dysfunction, suicide | [66, 67, 151] |
| 5 | Escitalopram | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=0.8-1.1/7800/27400 | N.A. | Sexual dysfunction, discontinuation symptoms, suicide | [65, 67, 155] |
| 6 | Atomoxetine | SERT/NET/DAT | $K_i$=77/5/1451 | N.A. | GI disturbances, Cardiovascular side effects | [68] |
| 7 | Maprotiline | hSERT/hNET/hDAT expressed on HEK-293 | $K_D$=5800/11/1000 | N.A. | Anticholinergic side effects | [153] |
| 8 | Viloxazine | hSERT/hNET/hDAT expressed on HEK-293 | $K_D$=17300/155/>10000 | N.A. | Anticonvulsant properties, increased libido | [153, 156, 157] |
| 9 | Reboxetine | SERT/NET/DAT | $K_i$=273.5/13.4/>10000 | N.A. | N.A. | |
| 10 | Amineptine | SERT/NET/DAT | $IC_{50}$>100000/3560/3330 | N.A. | Dermatological side effects, abuse and dependence | [158, 159] |
| 11 | Modafinil | hDAT expressed on COS-7 cell | $IC_{50}$=13000 | N.A. | Enhancement of attention, executive functions and learning | [160, 161] |
| 12 | Desven-lafaxine | hSERT/hNET expressed on HEK-293 | $K_i$=40.2/588.4 | N.A. | N.A. | [162] |
| 13 | Duloxetine | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=0.8/7.5/240 | N.A. | Somnolence, dizziness, liver derangement | [163, 164] |
| 14 | Levomil-nacipran | hSERT/hNET | $K_i$=11/91 | N.A. | Nausea, dizziness, sweating, constipation, insomnia, increased heart rate and blood pressure, urinary hesitancy | [165] |
| 15 | Venlafaxine | hSERT/hNET expressed on JAR cell/MDCK-Net6 cell | $IC_{50}$=27/535 | Fluoxetine: 9.4/N.A. Desipramine: N.A./3.4 | Nausea, headache, insomnia, drowsiness, dry mouth, constipation, sexual dysfunction | [166, 167] |
| 16 | Amphetamine | hSERT/hNET/hDAT expressed on HEK-293 | $IC_{50}$>10000/94/1300 | N.A. | Physical, Cardiovascular side effects | [168, 169] |

**(Table 2) contd….**

| No. | Name | Cell Line Studied | Binding Affinities / Uptake Potency (nM) | Positive Control (nM) | Side Effects | Refs. |
|---|---|---|---|---|---|---|
| 17 | Dexmethyl-phenidate | NET/DAT | $K_i$=206/161 | N.A. | Appetite loss, anxiety/nervousness, nausea, headaches | [170, 171] |
| 18 | Dextroam-phetamine | SERT/NET/DAT | $K_i$=3830/39/34 | N.A. | Cardiovascular side effects | [169] |
| 19 | Lisdexamfe-tamine | N.A. | N.A. | N.A. | Insomnia | [172] |
| 20 | Methylpheni-date | SERT/NET/DAT | $K_i$>1000/788/121 | N.A. | Impair athletic performance | [170, 173, 174] |
| 21 | Metham-phetamine | SERT/NET/DAT | $K_i$=2137/48/1571 | N.A. | Lose teeth | [175] |
| 22 | Bupropion | DAT | $K_i$=526 | N.A. | Epileptic seizures | [176] |
| 23 | Amitifadine | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=99/262/213 | N.A. | N.A. | [177] |
| 24 | Ansofaxine | SERT/NET/DAT | $IC_{50}$=723/763/491 | N.A. | N.A. | [178] |
| 25 | DOV 216,303 | hSERT/hNET/hDAT expressed on HEK-293 | $IC_{50}$=14/20/78 | N.A. | N.A. | [179] |
| 26 | GSK372475 | SERT/NET/DAT | $IC_{50}$=10/2/10 | N.A. | Dry mouth, headache, insomnia, and nausea | [83, 180] |
| 27 | Liafensine | SERT/NET/DAT | $IC_{50}$=1.1/8.8/5.7 | N.A. | Potential for abuse | [83] |
| 28 | SEP-225289 | SERT/NET/DAT | $IC_{50}$=14/4/2 | N.A. | Insomnia, decreased appetite, dry mouth, anxiety, nausea | [83, 181] |
| 29 | GSK1360707 | hSERT/hNET/hDAT expressed on LLCPK cell | $pK_i$=9.2/8.1/8.0 nM | DOV 21,947: 6.85/6.83/7.10 DOV 102,677: 7.08/7.23/7.42 | N.A. | [137] |
| 30 | RG-7166 | SERT/NET/DAT | $K_i$=16/9/90 | N.A. | N.A. | [83] |
| 31 | Vilazodone | 5-HT/5-$HT_{1A}$ in rat | $IC_{50}$=0.2/0.5 | N.A. | N.A. | [182] |
| 32 | Vortioxetine | SERT/NET/DAT in rat synaptosomes | $IC_{50}$=5.3/140/890 | N.A. | N.A. | [183] |
| 33 | Escitalopram | SERT expressed on COS-7 cell: S1/S2 binding site | $IC_{50}$=10/5800 | 0.01/5.8uM | N.A. | [91] |
| 34 | Compound 1 | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=7/503/960 | MADAM: 1.65/N.A./N.A. | N.A. | [110] |
| 35 | Compound 2 | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=8/1245/503 | MADAM: 1.65/N.A./N.A. | N.A. | [110] |
| 36 | Compound 3 | hSERT/hNET/hDAT expressed on HEK-293 | $K_i$=7/632/6976 | MADAM: 1.65/N.A./ N.A. | N.A. | [110] |

**(Table 2) contd….**

| No. | Name | Cell Line Studied | Binding Affinities / Uptake Potency (nM) | Positive Control (nM) | Side Effects | Refs. |
|---|---|---|---|---|---|---|
| 37 | Compound 4 | SERT/NET/DAT in rat brain tissue | $K_i$=1.7/300/2792 | Fluoxetine: 2/473/784 Paroxetine: 0.05/98/59 | N.A. | [111] |
| 38 | Compound 5 | SERT/NET/DAT in rat brain tissue | $K_i$=1.0/333/1530 | Fluoxetine 2/473/784 Paroxetine: 0.05/98/59 | N.A. | [111] |
| 39 | Compound 6 | SERT/NET/DAT in rat brain tissue | $K_i$=0.6/2731/2925 | Fluoxetine: 2/473/784 Paroxetine: 0.05/98/59 | N.A. | [111] |
| 40 | Compound 7 | SERT/NET in rabbit cortical membrane-sand/DAT in rabbit striatal membranes | $K_i$=10.28/10000/ >100000 | Fluoxetine: 5.80/609/4000 Paroxetine: 0.31/80/769 | N.A. | [112] |
| 41 | Compound 8 | hSERT-HEK293, hNET-HEK293, hDAT-N2A | $K_i$=37/>10000/2129 | N.A. | N.A. | [45] |
| 42 | Compound 9 | hSERT | N.A. | N.A. | N.A. | [95] |
| 43 | Compound 10 | hSERT | N.A. | N.A. | N.A. | [95] |
| 44 | Compound 11 | hSERT | N.A. | N.A. | N.A. | [95] |
| 45 | Compound 12 | hSERT expressed on HEK-293 cell | $K_i$=38000 | N.A. | N.A. | [43] |
| 46 | Compound 13 | hSERT expressed on HEK-293 cell | $K_i$=17000 | N.A. | N.A. | [43] |
| 47 | Compound 14 | hSERT/hNET/hDAT expressed on JAR/MDCK-Net6/CHO cell | $IC_{50}$=4519/6.3/0 | Reboxetine: 242/3.2/N.A. | N.A. | [113] |
| 48 | Compound 15 | hSERT/hNET/hDAT expressed on JAR/MDCK-Net6/CHO cell | $IC_{50}$=2871/5.7/18 | Reboxetine: 242/3.2/N.A. | N.A. | [113] |
| 49 | Compound 16 | hSERT/hNET/hDAT expressed on JAR/MDCK-Net6/CHO cell | $IC_{50}$=5293/6.5/4 | Reboxetine: 242/3.2/N.A. | N.A. | [113] |
| 50 | Compound 17 | hSERT/hNET/hDAT expressed on JAR/MDCK-Net6/CHO cell | $IC_{50}$=1099/2.7/7 | Reboxetine: 242/3.2/N.A. | N.A. | [113] |
| 51 | Compound 18 | hSERT/hNET/hDAT expressed on JAR/MDCK-Net6/CHO cell | $IC_{50}$=1614/5.5/2 | Reboxetine: 242/3.2/N.A. | N.A. | [113] |

**(Table 2) contd….**

| No. | Name | Cell Line Studied | Binding Affinities / Uptake Potency (nM) | Positive Control (nM) | Side Effects | Refs. |
|---|---|---|---|---|---|---|
| 52 | Compound 19 | hNET expressed on MDCK-Net6 cell | $IC_{50}$=1.2 | N.A. | N.A. | [114] |
| 53 | Compound 20 | hNET expressed on HEK-293 cell | $IC_{50}$=69600 | N.A. | N.A. | [44] |
| 54 | Compound 21 | hNET expressed on HEK-293 cell | $IC_{50}$=68000 | N.A. | N.A. | [44] |
| 55 | Compound 22 | hNET expressed on HEK-293 cell | $IC_{50}$=43200 | N.A. | N.A. | [44] |
| 56 | Compound 23 | hNET expressed on HEK-293 cell | $IC_{50}$=10900 | N.A. | N.A. | [44] |
| 57 | Compound 24 | hNET expressed on HEK-293 cell | $IC_{50}$=3700 | N.A. | N.A. | [44] |
| 58 | Compound 25 | SERT/NET/DAT expressed on human embryonic kidney cell | $K_i$=N.A./N.A./1.2 | N.A. | N.A. | [117] |
| 59 | Compound 26 | SERT/NET | $K_i$=802/1107/0.04 | UMB38: >10000/>10000/9148 | N.A. | [118] |
| 60 | Compound 27 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=67000/143/78 | N.A. | Abuse potential | [120] |
| 61 | Compound 28 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=57000/46/19.7 | N.A. | Abuse potential | [120] |
| 62 | Compound 29 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=10340/1070/566 | N.A. | Abuse potential | [120] |
| 63 | Compound 30 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=3100/146/57 | N.A. | N.A. | [120] |
| 64 | Compound 31 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=242000/175/342.8 | N.A. | N.A. | [120] |
| 65 | Compound 32 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=9000/239/176.2 | N.A. | Abuse potential | [120] |
| 66 | Compound 33 | SERT expressed on HEK-293; NET expressed on MDCK; DAT expressed on HEK-293 | $IC_{50}$=101/23/>10000 | N.A. | N.A. | [123] |
| 67 | Compound 34 | SERT/NET expressed on COS-7 cell | $K_i$=32/44 | [$^{125}$I]β-CIT: 3.9/13.1 | N.A. | [7] |
| 68 | Compound 35 | SERT/NET expressed on HEK-293; DAT expressed on CHO-K1 | $pK_i$=8.5/8.8/6.7 | N.A. | N.A. | [132] |
| 69 | Compound 36 | SERT/DAT | $K_i$=22/20 | N.A. | N.A. | [134] |
| 70 | Compound 37 | SERT/DAT | $IC_{50}$=10/9 | N.A. | N.A. | [135] |

**(Table 2) contd….**

| No. | Name | Cell Line Studied | Binding Affinities / Uptake Potency (nM) | Positive Control (nM) | Side Effects | Refs. |
|---|---|---|---|---|---|---|
| 71 | Compound 38 | SERT/NET/DAT in rat native tissues | $pK_i$=9.17/9.87/7.87 | DOV 21,947: 7.70/7.20/6.90 DOV 102,677: 6.70/6.10/6.70 | N.A. | [136] |
| 72 | Compound 39 | SERT/NET/DAT in rat | $pK_i$=8.98/7.92/7.92 | DOV 21,947: 6.85/6.83/7.10 DOV 102,677: 7.08/7.23/7.42 | N.A. | [137] |
| 73 | Compound 40 | SERT/NET/DAT in rat brain | $IC_{50}$=720/1570/3970 | Imipramine | N.A. | [138] |
| 74 | Compound 41 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=158.7/99/97.5 | Venlafaxine HCl: 200/2550/N.A. Bupropion: N.A./3240/1200 GBR12909: N.A./110/43 | N.A. | [139] |
| 75 | Compound 42 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=310/2510/6000 | Venlafaxine HCl: 200/2550/N.A. Bupropion: N.A./3240/1200 GBR12909: N.A./110/40 | N.A. | [140] |
| 76 | Compound 43 | hSERT expressed on HEK; hNET/hDAT expressed on N2A | $K_i$=1029/613/3058 | N.A. | N.A. | [46] |
| 77 | Compound 44 | hSERT expressed on HEK; hNET/hDAT expressed on N3A | $K_i$=668/323/>20000 | N.A. | N.A. | [46] |
| 78 | Compound 45 | hSERT expressed on HEK; hNET/hDAT expressed on N4A | $K_i$>20000/841/15740 | N.A. | N.A. | [46] |
| 79 | Compound 46 | hSERT expressed on HEK; hNET/hDAT expressed on N5A | $K_i$=12600/215/780 | N.A. | N.A. | [46] |
| 80 | Compound 47 | SERT/NET/DAT/5-$TH_{1A}$/$5HT_{2C}$ | $K_i$=81/>10000/4742/28.3/N.A. | Prozac: 1.1/N.A./N.A./N.A./72 Vilazodone: 0.5/N.A./N.A./0.3/N.A. | N.A. | [141] |

**(Table 2) contd….**

| No. | Name | Cell Line Studied | Binding Affinities / Uptake Potency (nM) | Positive Control (nM) | Side Effects | Refs. |
|---|---|---|---|---|---|---|
| 81 | Compound 48 | SERT/NET/DAT/ 5-$TH_{1A}$/$5HT_{2C}$ | $K_i$=64/49.0/N.A./7.3/37 38 | Prozac: 1.1/N.A./N.A./N. A./72 Vilazodone: 0.5/N.A./N.A./0.3 /N.A. | N.A. | [141] |
| 82 | Compound 49 | SERT/5-$TH_{1A}$/5-$TH_{2A}$ in rat brain | $K_i$=167/38/89 | N.A. | N.A. | [142] |
| 83 | Compound 50 | SERT expressed on COS-7: S2 binding site | $IC_{50}$=13900 | N.A. | N.A. | [52] |
| 84 | Compound 51 | SERT expressed on COS7: S1/S2 binding site | $IC_{50}$=10000/12000 | Escitalopram: 10/5800 | N.A. | [91] |
| 85 | Compound 52 | SERT | N.A. | N.A. | N.A. | [143] |
| 86 | Compound 53 | hSERT | N.A. | N.A. | N.A. | [95] |
| 87 | Compound 54 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=10000/3380/127 | N.A. | N.A. | [51] |
| 88 | Compound 55 | hSERT/hNET/hDAT expressed on HEK-293 cell | $IC_{50}$=10000/3980/107 | N.A. | N.A. | [51] |
| 89 | Compound 56 | hSERT | N.A. | N.A. | N.A. | [146] |
| 90 | Compound 57 | hSERT expressed on COS-7 cell | $K_i$=34 | Citalopram: 7.4 | N.A. | [145] |
| 91 | Compound 58 | SERT/NET/DAT ex- pressed on human em- bryonic kidney cell | $IC_{50}$=991/1728/0.37 | N.A. | N.A. | [117] |
| 92 | Compound 59 | SERT/NET/DAT ex- pressed on human em- bryonic kidney cell | $IC_{50}$>1000/>1000/2.4 | N.A. | N.A. | [117] |
| 93 | Compound 60 | SERT/NET/DAT ex- pressed on LLC-PK1 cell | $K_i$=20.6/66.2/4.1 | β-CCT: N.A./N.A./5.1 β-CBT: N.A./N.A./3.5 β-CFT: 181/653/14.7 | N.A. | [147] |

N.A.: Not Available.

### 3.3. Triple Reuptake Inhibitors (TRIs)

TRIs, which simultaneously inhibit the reuptakes of 5-HT, NE and DA, have been developed recently for MDD treatment [82]. However, none of TRIs have been approved for clinical use. So far, a number of TRIs (amitifadine, ansofaxine, DOV 216,303, GSK372475, liafensine, SEP-225289, GSK1360707, RG-7166) (Tables **1** and **2**) [83] have advanced to clinical trial. Among these, amitifadine (also known as DOV-21,947 or EB-1010) is the only one which has ever been clinically tested in Phase 3 for treating depression [19, 84]. Because the increase of dopaminergic neurotransmission can alleviate the persistence of anhedonia (a core treatment-resistant symptom of depression), TRIs are found to produce higher efficacy than first-line antidepressants such as SSRIs and SNRIs [82, 85, 86].

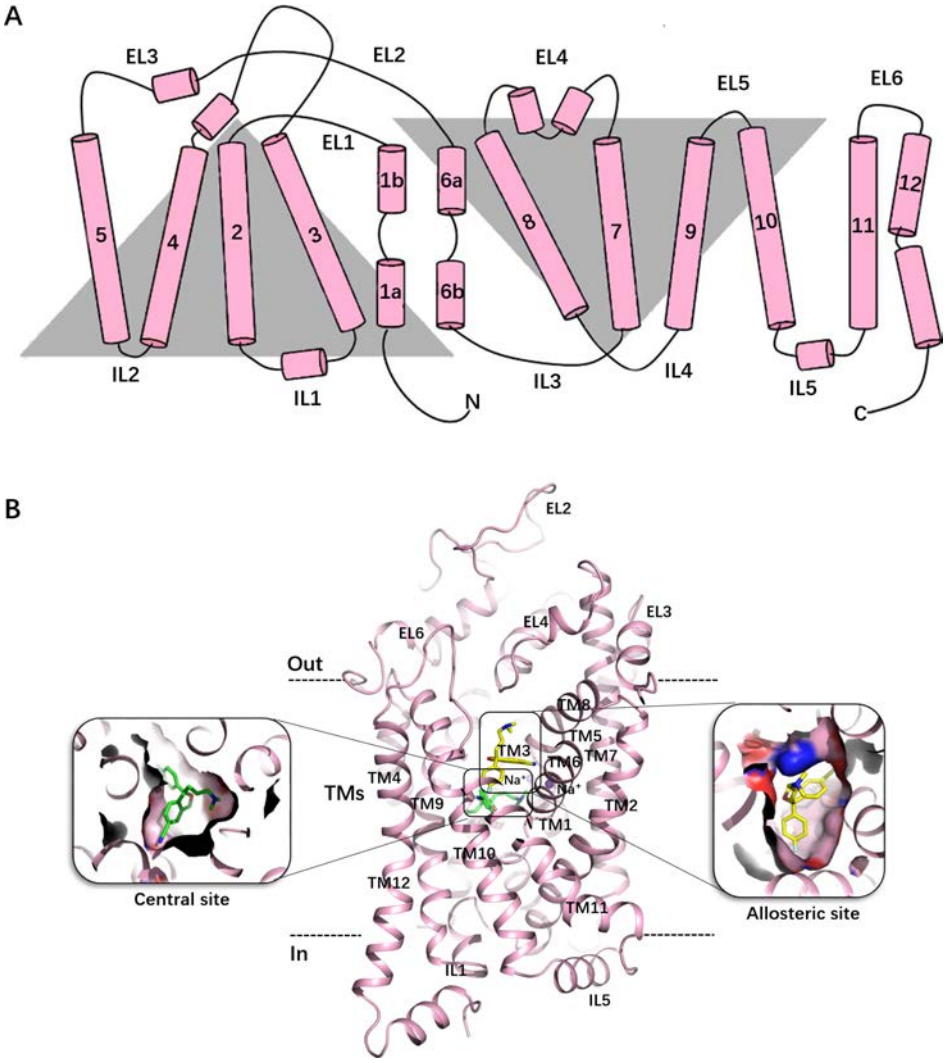



**Fig. (1).** The topology of and the structure of hSERT: (**A**) Schematic view of the topology of the 12TMs in hSERT. TM1-5 and TM6-10 are related by a pseudo two-fold rotation as indicated by tshe gray triangles; (**B**) X-ray crystal structure of human serotonin transporter complexed with escitalopram at the central and allosteric sites (PDB ID: 5I73 [80]). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

### 3.4. Multimodal Inhibitors

Serotonin reuptake inhibition and 5-$HT_{1A}$R partial agonism (SPARI), also known as multimodal inhibitors, have been expected to yield enhanced drug efficacy and rapid onset of action for treating depression [56, 62]. Evidences demonstrate that the pre- and postsynaptic 5-$HT_{1A}$ receptors play opposite roles in depression, greater activation of postsynaptic 5-$HT_{1A}$ will relieve and improve the depression symptoms by regulating the concentration of serotonin [69]. To date, 2 SPARIs (vilazodone [14] and vortioxetine [15]) (Tables **1** and **2**) have been approved by the FDA for the treatment of MDD, and hundreds of active SPARIs of diverse scaffolds have been found *in vitro*. Vortioxetine showed multimodal activities by broadly binding to the serotonin reuptake transporter and 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_3$, 5-$HT_7$, and 5-$HT_{1D}$ receptors [87-89]. Compared with vortioxetine, vilazodone and its analogues were much more selective to the Serotonin Transporter (SERT) and 5-$HT_{1A}$R [14].

### 3.5. Allosteric Inhibitors

The allosteric inhibition of MATs was known as antidepressants such as SSRIs are capable of binding to the central and allosteric sites of hSERT simultaneously when at high concentration, while the allosteric inhibitors always bind to the central site with higher affinity [90]. So far, escitalopram is the most potent allosteric inhibitor reported in published papers (Tables **1** and **2**) [91]. It has been suggested that the fast onset of action and high efficacy of escitalopram is due to its

allosteric action at the transporter [50]. However, the lack of probe compounds with high binding selectivity and affinity for the allosteric site limits the pharmacological implications of hMATs allosteric inhibition and the allosteric binding site in the transporters need to be further elucidated [91].

## 4. BINDING MODE AND MECHANISM OF HUMAN MATS INHIBITORS

Human MATs are fruitful targets for Central Nervous System (CNS) drug discovery [92]. In recent decades, with the elucidation of the X-ray crystal structures for the MAT homolog LeuT [25, 26] and dDAT [27-29] complexed with ligands as well as the more recently released X-ray crystal structures of hSERT (Table **3**) [48, 49], the structure-based drug design of novel potent MATs reuptake or allosteric inhibitors has come true [93-95].

Based on the X-ray crystal structures of LeuT [25, 26] and dDAT [27-29], the closely related homolog of the human MATs proteins, the homology models of hSERT, hNER and hDAT were generated (Table **4**), and the information of several types of human MATs inhibitors binding with the transporters were obtained by molecular docking and MD simulations studies [4, 8, 96]. The computational models unravel the binding mode and inhibitory mechanism of ligand and indicate the essential aspects determining drugs binding selectivity and affinity, which will facilitate drug discovery procedure targeting MATs.

### 4.1. Binding Mode

#### *4.1.1. The Common Binding Mode of Reuptake Inhibitors*

Human MATs are responsible for the synaptic reuptake of neurotransmitters 5-HT, NE and DA that terminates a neurotransmission event [1, 3, 4]. X-ray crystal structures of LeuBAT [26], dDAT [27, 28] and hSERT [48, 49] in complex with substrate (leucine and dopamine) and diverse antidepressants (SSRIs, sNRIs, SNRIs and TCAs) reveal that inhibitors lock the transporters in an outward-facing open conformation by competitively binding to the central substrate binding site surround by TM1, TM3, TM6, TM8 and TM10. The LeuBAT is a LeuT variant engineered to harbor hSERT or hNET-like pharmacology by mutating key residues around the central site [26]. Moreover, all of those co-crystallographic structures show that the chemically diverse antidepressants have the similar binding mode [26, 48, 49].

Meanwhile, using the LeuT structure as a template (with ~ 20% sequence identity to the human MATs), computational models have been widely used to investigate the substrates [33, 34] and drugs [6, 35, 36, 40] binding to human MATs. A common binding mode for the three substrates (5-HT, NE and DA) was proposed by homology modeling, Induced Fit Docking (IFD), MD simulation and mutation experiments [34]. Results showed that the ammonium group of substrates form an ionic interaction with a highly conserved Aspartate (Asp98 in hSERT; Asp75 in hNET; and Asp79 in hDAT). The 6-position of 5-HT and the para-hydroxyl groups of NE and DA contact with Ala173 in hSERT, Gly149 in hNET and Gly153 in hDAT through hydrophobic [34]. With similar computational protocols, the mode of SSRIs (fluoxetine [6], escitalopram [35] and sDRI mazindol [40]) binding to corresponding targets were also predicted. It is suggested that the amino group of fluoxetine was found to have a key role for high-affinity binding in hSERT, while TM10 residues in hSERT have less important role in inhibitor binding than that in hDAT [6]. Residues Tyr95 and Asn444 play a key role in forming stable interaction in escitalopram-hSERT complex [35].

From 2013 to 2016, the X-ray crystal structures of the dDAT, which has greater than 50% sequence identity with hSERT, hNER or hDAT, bound to its substrate (dopamine) [29], the antidepressants (nortriptyline, nisoxetine and reboxetine) [27, 28] and psychostimulants (D-amphetamine, methamphetamine, and cocaine) [29] were successively released. The breakthrough has greatly promoted the exploration of all classes of antidepressants including SSRIs, sNRIs, SNRIs, NDRIs as well as multimode inhibitors binding in hSERT, hNER or hDAT [32, 37, 38, 64, 73, 80, 96-101].

For single target drugs, the binding mode shared by the 4 approved SSRIs (fluoxetine, sertraline, paroxetine and escitalopram) were identified by hierarchically clustering MD based per-residue free energies. The binding mode was composed of collective interactions between 3 chemical groups in SSRIs and 11 hot spot residues in hSERT (Fig. **2A**) [37]. In similar, 4 approved sNRIs (atomoxetine, maprotiline, reboxetine and viloxazine) were docked into hNET for MD simulation and a binding mode defined by interactions between 3 chemical moieties in sNRIs and 11 residues in hNET was identified (Fig. **2B**) [73].

**Table 3. Crystal structures of hMATs and homologues.**

| No. | Crystal Structure | PDB code (Co-Crystalized Ligand) | Year | Refs. |
|---|---|---|---|---|
| 1 | LeuT | 2A65. | 2005 | [184] |
| 2 | LeuT | 2Q6H (L-leucin, clomipramine), 2Q72 (L-leucin, imipramine), 2QEI (L-leucin, clomipramine), 2QB4 (L-leucin, desipramine), 2QJU (L-leucin, desipramine). | 2007 | [185] |
| 3 | LeuT | 3F3A (tryptophan), 3F3C (4-fluorophenylalanine), 3F48 (alanine), 3F4I (selenomethionine), 3F4J (glycine), 3F3E (L-leucine), 3F3D (L-methionine). | 2008 | [186] |
| 4 | LeuT | 3GWV (leucine, R-fluoxetine), 3GWW (leucine, S-fluoxetine), 3GJD (leucine, octylglucoside), 3GJC (leucine, octylglucoside), 3GWU (leucine, sertraline). | 2009 | [187] |
| 5 | LeuT | 3MPN (leucine). | 2010 | [188] |
| 6 | LeuT | 3QS4 (L-tryptophan). | 2011 | [189] |
| 7 | LeuT | 3TU0 (alanine). | 2012 | [190] |
| 8 | LeuT | 3USP (L-leucine). | 2012 | [191] |
| 9 | LeuT | 3USL (L-selenomethionine), 4FXZ (L-leucine). | 2012 | [191] |
| 10 | LeuT | 4HMK (leucine). | 2013 | [192] |
| 11 | LeuT | 4MM4 (paroxetine), 4MMF (mazindol), 4MMD (S-duloxetine), 4MMC (desvenlafaxine), 4MMB (sertraline), 4MMA (clomipramine), 4MM9 (fluvoxamine), 4MM8 (R-fluoxetine). | 2013 | [26] |
| 12 | LeuT | 5JAF. | 2016 | [193] |
| 13 | dDAT | 4M48 (nortriptyline). | 2013 | [27] |
| 14 | dDAT | 4XPF (RTI-55). | 2015 | [29] |
| 15 | dDAT | 4XPT (3,4-dichlorophenethylamine). | 2015 | [29] |
| 16 | dDAT | 4XPH. | 2015 | [29] |
| 17 | dDAT | 4XPG (β-CFT). | 2015 | [29] |
| 18 | dDAT | 4XPB (cocaine). | 2015 | [29] |
| 19 | dDAT | 4XP9 (D-amphetamine). | 2015 | [29] |
| 20 | dDAT | 4XP1 (dopamine). | 2015 | [29] |
| 21 | dDAT | 4XNU (nisoxetine). | 2015 | [28] |
| 22 | dDAT | 4XNX (reboxetine). | 2015 | [28] |
| 23 | hSERT | 5I6X (paroxetine). | 2016 | [48] |
| 24 | hSERT | 5I75 (escitalopram, Br-citalopram). | 2016 | [48] |
| 25 | hSERT | 5I74 (Br-citalopram). | 2016 | [48] |
| 26 | hSERT | 5I73 (escitalopram). | 2016 | [48] |
| 27 | hSERT | 5I71 (escitalopram). | 2016 | [48] |
| 28 | hSERT | 6AWN (paroxetine), 6AWQ (serotonin), 6AWP (fluvoxamine), 6AWO (fluoxetine). | 2018 | [49] |

**Table 4. Reported Computational models of hMATs.**

| No. | Target | Drug | Template | PDB code | Computational methods | Year | Refs. |
|---|---|---|---|---|---|---|---|
| 1 | hDAT | Dopamine, amphetamine. | LeuT | 2A65 | MOE | 2008 | [194] |
| 2 | hSERT | Serotonin, serotonin analogues. | LeuT | 2A65 | MODELLER | 2008 | [33] |
| 3 | hDAT, hSERT, hNET | Dopamine, serotonin, norepinephrine. | LeuT | 2A65 | MODELLER | 2008 | [195] |
| 4 | hDAT | Dopamine, cocaine, CFT. | LeuT | 2A65 | MODELLER | 2008 | [196] |
| 5 | hSERT | Serotonin. | LeuT | 2A65 | Rosetta | 2009 | [197] |
| 6 | hDAT | Dopamine. | LeuT | 2A65 | MODELLER | 2009 | [198] |
| 7 | hSERT | Imipramine, clomipramine, amitriptyline, citalopram. | LeuT | 2A65 | MODELLER | 2009 | [199] |
| 8 | hSERT, hDAT, hNET | Cocaine, clomipramine. | LeuT | 2A65 | ICM | 2009 | [200] |
| 9 | hSERT | Sertraline, R-fluoxetine, S-fluoxetine. | LeuT | 2A65 | Nest | 2009 | [201] |
| 10 | hSERT | Imipramine, fluoxetine, paroxetine, cocaine. | LeuT | 2A65 | MODELLER | 2009 | [202] |
| 11 | hDAT | Cocaine, dopamine. | LeuT | 2A65 | InsightII | 2009 | [203] |
| 12 | hSERT | (S)-citalopram. | LeuT | 2A65, 3F3A | MODELLER | 2010 | [204] |
| 13 | hDAT | Dopamine. | LeuT | 2A65 | MOE | 2010 | [205] |
| 14 | hSERT | Imipramine, desipramine, short imipramine, clomipramine, 3-cyanoimipramine. | LeuT | 2A65 | MODELLER | 2010 | [206] |
| 15 | hSERT | Citalopram. | LeuT | 2A65 | MODELLER | 2010 | [106] |
| 16 | hDAT | Bivalent phenethylamines. | LeuT | 2QJU | MODELLER, MOE | 2010 | [207] |
| 17 | hSERT | Serotonin. | LeuT | 2A65 | MOE | 2010 | [208] |
| 18 | hSERT | Serotonin, 3,4-methylenedioxymethamphetamine. | LeuT | 2A65 | Rosetta | 2010 | [209] |
| 19 | hSERT | Serotonin. | LeuT | 2A65 | * | 2010 | [210] |
| 20 | hSERT | Serotonin. | LeuT | 2A65 | MODELLER | 2010 | [211] |
| 21 | hDAT | Dopamine, cocaine, CFT, BZT and JHW007. | LeuT | 2A65 | MODELLER | 2011 | [212] |
| 22 | hSERT | Imipramine, clomipramine, desipramine, carbamazepine, dihydrocarbamazepine, amitriptyline. | LeuT | 3F3A | MODELLER, SWISS MODEL | 2010 | [213] |
| 23 | hSERT | Serotonin, tryptophan. | LeuT | 2A65 | MODELLER | 2011 | [214] |
| 24 | hSERT, hDAT, hNET | Paroxetine, fluoxetine, 4-(aryl)piperidin-3-one O-4-benzyl oxime hydrochlorides. | LeuT | 2A65 | ICM | 2011 | [112] |
| 25 | hDAT | Dopamine. | LeuT | 2A65 | MODELLER | 2011 | [215] |
| 26 | hNET | RTI-113. | hDAT (constructed by LeuT ) | - | InsightII | 2010 | [216] |
| 27 | hSERT | Serotonin, tryptamine derivatives. | LeuT | 2A65 | ICM | 2012 | [217] |

**(Table 4) contd….**

| No. | Target | Drug | Template | PDB code | Computational methods | Year | Refs. |
|---|---|---|---|---|---|---|---|
| 28 | hSERT, hNET | Citalopram, talopram. | LeuT | 2A65, 3F3A | MODELLER | 2011 | [7] |
| 29 | hSERT | SSRI ligands. | LeuT | 2A65 | Discovery Studio, MODELLER | 2011 | [43] |
| 30 | hNET | Prescription drugs. | LeuT | 2A65 | MODELLER | 2011 | [44] |
| 31 | hDAT | Ligands from ENAMINE virtual screening collection. | LeuT | 2A65 | MOE | 2011 | [45] |
| 32 | hSERT | MI-17, SSA-426. | LeuT | 2A65 | Discovery Studio | 2011 | [45] |
| 33 | hSERT | Serotonin. | LeuT | 2A65 | MODELLER | 2011 | [218] |
| 34 | hSERT | 58 inhibitors. | LeuT | 2A65, 2Q72 | ICM | 2012 | [219] |
| 35 | hSERT | Nitroquipazine analogues. | LeuT | 2A65, 3F3A | ICM | 2012 | [220] |
| 36 | hNET | Nisoxetine. | LeuT | 2A65 | MODELLER | 2012 | [221] |
| 37 | hDAT | Cocaine, cocaine analogs. | LeuT | 2QJU | MODELLER | 2012 | [222] |
| 38 | hSERT | (S)-citalopram, clomipramine. | LeuT | 2A65 | MODELLER | 2012 | [223] |
| 39 | hSERT, hDAT | 1-phenyl-piperazine, 1-(3-hydroxyphenyl)-piperazine. | LeuT | 2A65 | MODELLER | 2012 | [36] |
| 40 | hDAT | Dopamine, amphetamine. | LeuT | 2A65 | MOE | 2012 | [224] |
| 41 | hDAT, hNET, hSERT | Dopamine, norepinephrine, serotonin. | LeuT | 2A65 | MODELLER | 2013 | [34] |
| 42 | hDAT | N.A. | LeuT | 2A65 | MODELLER | 2013 | [225] |
| 43 | hSERT | 3 million small molecules. | LeuT | 2QJU | MODELLER | 2013 | [52] |
| 44 | hSERT | Cocaine, noribogaine, Serotonin. | LeuT | 2A65 | MODLLER | 2013 | [226] |
| 45 | hSERT | Ligands from the NCI database. | LeuT | 2A65 | Discovery Studio | 2013 | [148] |
| 46 | hSERT, hDAT | Phenylethylamines, (S)-fenfluramine. | LeuT | 2A65 | MODELLER | 2013 | [105] |
| 47 | hSERT | Quinine, cinchonidine. | LeuT | 2A65, 3F3A | Rosetta | 2014 | [227] |
| 48 | hSERT, hDAT | Mazindol. | LeuT | 2A65 | MODELLER | 2014 | [40] |
| 49 | hSERT | 4-(4-(dimethylamino)-phenyl)-1-methylpyridinium, 1-butyl-4-[4-(1-dimethylamino)phenyl]-pyridinium bromide, 1-methyl-4-[4-(1-piperidinyl)phenyl]-pyridinium. | LeuT | 2A65 | MODELLER | 2014 | [228] |
| 50 | hSERT | Modafinil analogues. | LeuT | 2A65 | MODELLER | 2014 | [229] |
| 51 | hSERT, hNET | Fluoxetine. | LeuT | 2A65 | MODELLER | 2014 | [6] |
| 52 | hSERT | Ligands from 5 databases. | LeuT | 3F3A | ICM | 2014 | [230] |
| 53 | hSERT | SSRI, TCA drugs. | LeuT | 2A65 | Discovery Studio, MODELLER | 2014 | [46] |

**(Table 4) contd….**

| No. | Target | Drug | Template | PDB code | Computational methods | Year | Refs. |
|---|---|---|---|---|---|---|---|
| 54 | hDAT | RTI 82. | LeuT | 2A65, 3F3A | MODELLER | 2014 | [231] |
| 55 | hSERT, hDAT | Methcathinone analogues. | dDAT | 4M48 | MODELLER | 2015 | [232] |
| 56 | hDAT | Dopamine, amphetamine, orphenadrine. | dDAT | 4M48 | MODELLER | 2015 | [233] |
| 57 | hSERT | Vortioxetine. | LeuT | 2A65 | MODELLER | 2015 | [97] |
| 58 | hSERT | Vortioxetine. | dDAT | 4M48 | MODELLER | 2015 | [97] |
| 59 | hSERT | 1849 molecules from MDDR and ChEMBL database. | dDAT | 4M48 | SWISS-MODEL | 2015 | [102] |
| 60 | hDAT | Dopamine. | dDAT | 4M48 | MODELLER | 2015 | [234] |
| 61 | hSERT | Fluoxetine, sertraline, paroxetine and escitalopram. | dDAT | 4M48 | SWISS-MODEL | 2016 | [37] |
| 62 | hSERT | Paroxetine. | dDAT | 4XP4 | MODELLER | 2016 | [235] |
| 63 | hNET | Atomoxetine, maprotiline, reboxetine, viloxazine, nisoxetine, talopram. | dDAT | 4XNX | SWISS-MODEL | 2016 | [73] |
| 64 | hSERT | 5HT-PEG4-5HT. | dDAT | 4M48 | MODELLER | 2016 | [236] |
| 65 | hDAT | Ligands from Sigma-Aldrich catalog. | LeuT | 2A65 | MOE | 2016 | [237] |
| 66 | hSERT, hDAT, hNET | N.A. | dDAT | 4M48 | SWISS-MODEL | 2016 | [238] |
| 67 | hSERT, hDAT, hNET | N.A. | dDAT | 4XPA | SWISS-MODEL | 2016 | [238] |
| 68 | hDAT | Atomoxetine, dexmethylphenidate, dextroamphetamine, dextromethamphetamine, R-bupropion, R-modafinil. | dDAT | 4XNU, 4XP6, 4XP9, 4XPH, 4XNX | SWISS-MODEL | 2017 | [64] |
| 69 | hNET | Atomoxetine, dexmethylphenidate, dextroamphetamine, dextromethamphetamine, LY2216684, reboxetine, viloxazine. | dDAT | 4XNU, 4XP6, 4XP9, 4XPH, 4XNX | SWISS-MODEL | 2017 | [80] |
| 70 | hDAT | Dexmethylphenidate, dextroamphetamine, dextromethamphetamine, R-bupropion. | dDAT | 4XNU, 4XP6, 4XP9, 4XPH, 4XNX | SWISS-MODEL | 2017 | [80] |
| 71 | hDAT, hNET | Desvenlafaxine, duloxetine, levomilnacipran, venlafaxine. | dDAT | 4M48 | SWISS-MODEL | 2018 | [38] |
| 72 | hDAT | Amphetamine, modafinil, cocaine. | dDAT | 4M48 | Discovery Studio | 2018 | [239] |
| 73 | hDAT, hNET | Nisoxetine, clobenpropit. | dDAT | 4XNU | MODELLER | 2018 | [240] |
| 74 | hSERT, hDAT, hNET | Amitifadine. | dDAT | 4M48 | SWISS-MODEL | 2018 | [98] |
| 75 | hSERT | Escitalopram. | dDAT | 4M48 | SWISS-MODEL | 2017 | [103] |

*The name of the database used for Virtual Screening not mentioned in the reference paper.

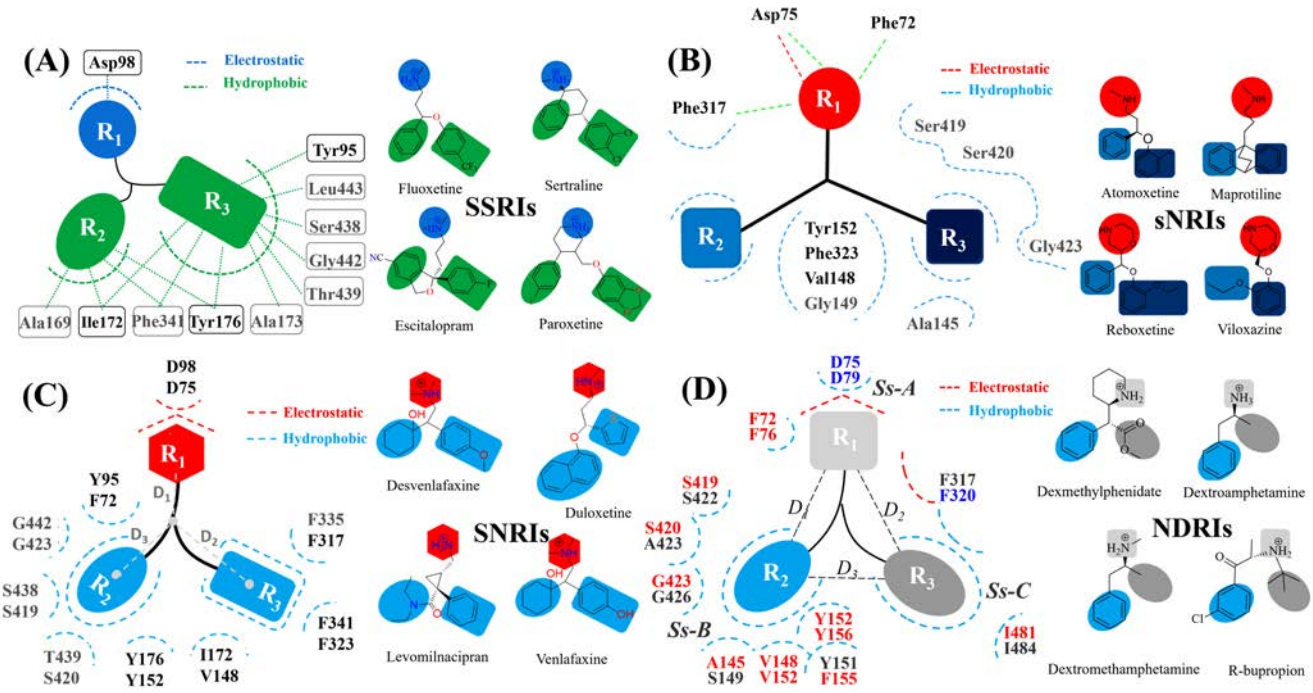



**Fig. (2).** The computationally identified common binding mode of drugs in hSERT, hNET and hDAT for: (**A**) SSRIs; (**B**) sNRIs; (**C**) SNRIs; and (**D**) NDRIs, respectively. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

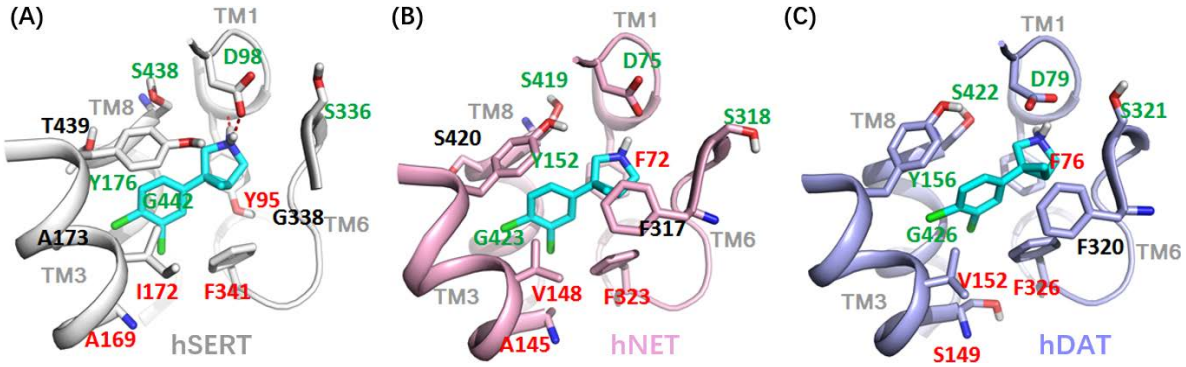



**Fig. (3).** Predicted binding modes of amitifadine in the central site of: (**A**) hSERT; (**B**) hNET; and (**C**) hDAT. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

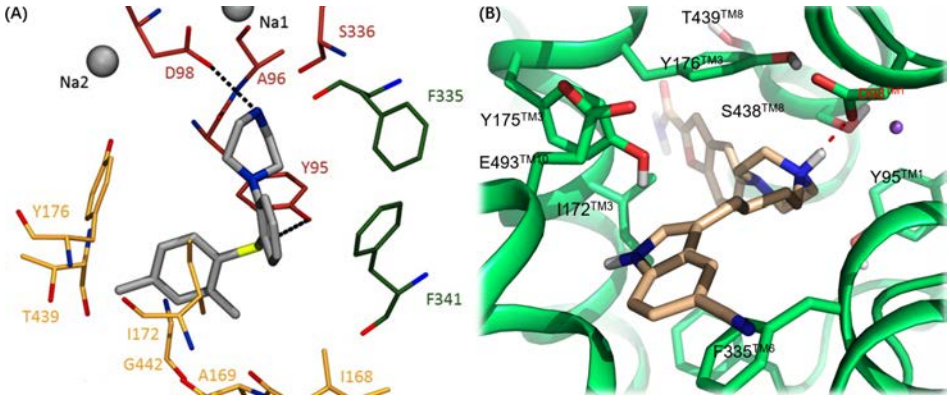



**Fig. (4).** The predicted binding modes of: (**A**) Vortioxetine; and (**B**) Vilazodone, in the central site of hSERT. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

For dual target drugs, the common binding modes shared by all approved SNRIs (desvenlafaxine, duloxetine, levomilnacipran, and venlafaxine) and NDRIs (dexmethylphenidate, dextroamphetamine, dextromethamphetamine and R-bupropion) in hSERT/hNET (Fig. **2C**) [38] and hNET/hDAT (Fig. **2D**) [80] were identified, respectively. In addition, residues Phe335, Leu337, Gly338, and Val343 located at the TM6 of (the corresponding residues Phe317, Leu319, Gly320, and Val325 in hNET) were discovered accounting for SNRIs dual-acting inhibition [38].

The common binding mode of reuptake inhibitors in hSERT, hNET and hDAT from the publications can be summarized as three main interaction features (Fig. **2**): i) R1 with amino group form salt bridge interactions with conserved Asp98 in hSERT (Asp75 in hNET and Asp79 in hDAT); ii) R2 interact with Ala169, Ile172, Tyr176 and Phe341 (Ala145, Val142, Tyr152 and Phe323 in hNET and Ser149, Val148, Tyr156 and Phe326 in hDAT) via hydrophobic contacts; (iii) R3 mainly contact hydrophobic hydrophobically with Tyr95, Ser438, Thr439 and Gly442 (Phe72, Ser419, Ser420 and Gly423 in hNET and Phe76, Ser422, Ala423 and Gly426 in hDAT). The identified common binding mode could provide valuable information for the identification of privileged drug-like scaffolds with improved drug efficacy.

For reuptake inhibitors with a novel mode of action, computational modeling methods have been used to investigate the binding mode of TRI amitifadine and SPARI vortioxetine. Amitifadine, the only TRI ever clinically tested in Phase 3 for treating depression, simultaneously interacting with hSERT, hNET and hDAT. The common features for amitifadine binding to three human MATs were identified through quantitatively analyzing the amitifadine-MATs interaction mode (Fig. **3**) [39].

Fig. (**4**) shows the binding mode of the novel multimodal antidepressants vortioxetine and vilazodone with hSERT. Andersen *et al.* determined the functional relevant orientation of vortioxetine within the central binding site of hSERT by combining comparative modeling with mutational analysis and characterization of drug analogs binding to selected point mutants (Fig. **4A**) [97]. Wang *et al.* reported the binding of vilazodone in hSERT by docking [102]. In the current review, were added to the binding of vilazodone to hSERT by coupling docking and MD simulation. In Fig. (**4B**), it is obvious that the salt bridge between the positively charged nitrogen of vilazodone and the carboxyl of D98 in hSERT played a pivotal role for ligand in recognizing the S1 binding site. The other two moieties of vilazodone including the indole and arypiperazine mainly participated in the hydrophobic interaction with the residues in the binding pocket of hSERT.

#### *4.1.2 Allosteric Inhibitors*

As for hMATs drugs, only the binding mode of the escitalopram in the hSERT allosteric site has been explored by crystallographic experiment [48]. The reported X-ray structures of hSERT cocrystalized escitalopram bind to the allosteric site made up of TM1b, TM6a, TM10, TM11, EL4 and EL6 (Fig. **5**). To quantitatively evaluate the binding of escitalopram in the novel allosteric site of hSERT, MD simulation and per-residue energy binding free energy calculation study were added in this review. Fig. (**5A**) shows the representative snapshot of the escitalopram-hSERT complex from MD simulation. The calculated energy contribution of key residues is depicted in Fig. (**5B**). As shown, 12 residues in hSERT play an important role in escitalopram binding. Among them, the energy contribution of Phe335, Arg104, Glu494, Gln332 and Ala331 was more than 1 kcal/mol, and Glu494 formed a hydrogen bond with the ammonium group of escitalopram (Fig. **5A**). Therefore, the amino group of escitalopram may play a key role in inhibitor recognition in the allosteric site of hSERT. However, compared to the binding mode of escitalopram in the central binding site, the hydrophobic properties of the allosteric site are less than that of the central site, which can be further reflected by the identified key residues contributing to drug binding. Moreover, the allosteric site located in the extracellular vestibule of the transporter has much more plasticity, leading to the design of high affinity and selective inhibitors as a great challenge.

### 4.2. Inhibitory Mechanism

Based on the binding mode of drugs in the hMATs, further investigations of the detailed inhibitory mechanism have promoted the understanding of the hMATs drugs selectivity, enantiomers binding as well as the addiction (Fig. **6**).

#### *4.2.1. Drug Selectivity*

Sequence alignment (Fig. **7A**) shows that hSERT, hNET and hDAT share greater than 50% sequence identity between each other, and their 3D structures share a particular similar molecular architecture (Fig. **7B**) [103]. In particular, the TM1, 3, 6, 8 and 10 regions that primarily contribute to the central binding site of the transporters showed that 62% (hSERT and hNET), 57% (hSERT and hDAT) and 85% (hNET and

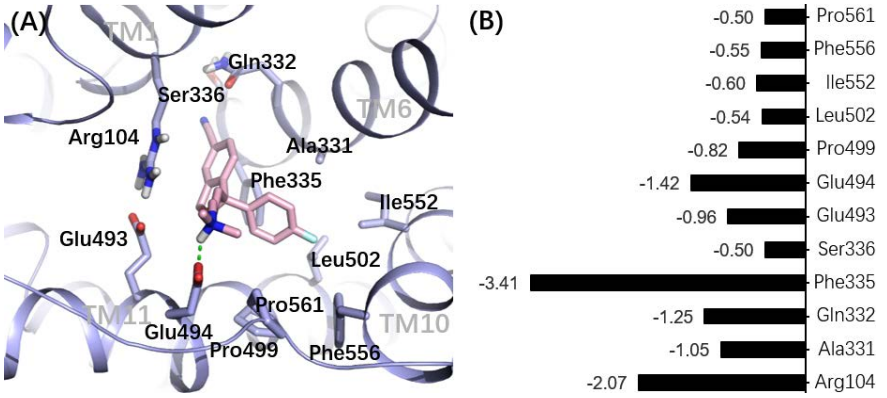



**Fig. (5). Quantitative evaluation of the escitalopram binding in hSERT allosteric site.** (**A**) The representative snapshot of the escitalopram-hSERT complex from MD simulation; (**B**) The calculated key residues energy contribution of escitalopram binding in hSERT allosteric site. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

hDAT) are conserved (Fig. **7C**) [103]. To design small molecules with the appropriate selective inhibition of MATs, which influences the efficacy and tolerability of drug candidates [7, 53], the understanding of the physicochemical basis underlying the binding selectivity of inhibitors to hSERT, hNET and hDAT has been explored by several experimental and computational studies [7, 38, 80, 96, 98, 104].

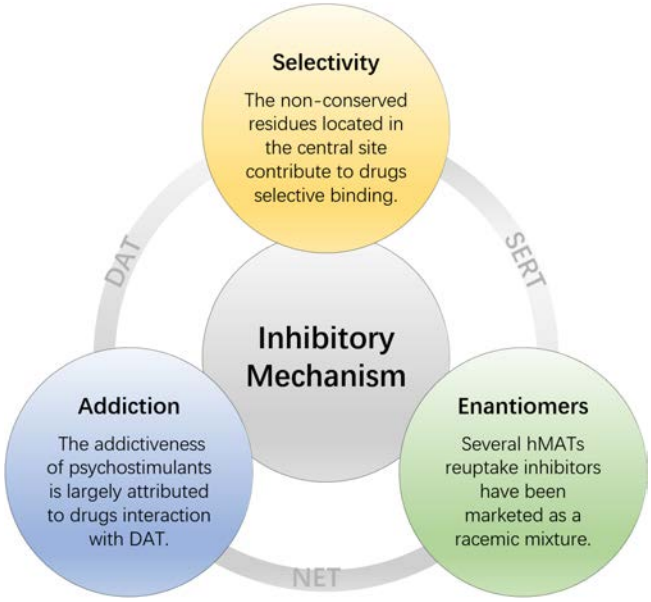



**Fig. (6). The schematic diagram of the inhibitory mechanism underlying drug selectivity, enantiomers and addictiveness in hMATs.** (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Mutational analysis of nonconserved hSERT and hNET residues show that the selectivity of SSRI escitalopram and sNRI talopram is determined by amino acid differences in the central binding site of the transporters [7]. Comparison of the X-ray crystal structures of hSERT and dDAT suggesting that the more open character of the central site in hSERT versus hDAT plays a role in selectivity [96].

Based on the crystal structures, computational models of SSRIs, sNRIs, SNRIs, NDRIs, TRIs in hSERT, hNET and hDAT have discovered the residues may contribute to the binding selectivity [38, 80, 98, 105]. For hSERT and hNET, it is found that residues lining TM3 and TM8 (Ile172, Ser438, Thr439, and Leu443 in hSERT; Val148, Ser419, Ser420, and Met424 in hNET) contribute more to the binding of SSRIs and sNRI than that of SNRIs in hSERT or hNET (Fig. **8A** and **8B**) [38]. In addition, residues Phe335, Leu337, Gly338, and Val343 located at the TM6 of hSERT (the corresponding residues Phe317, Leu319, Gly320, and Val325 in hNET) were found the determinants accounting for the dual-acting inhibition mechanism (Fig. **8A** and **8B**), which is consistent with the depth of SNRIs aromatic rings stretching into hydrophobic pockets [38]. For hSERT and hDAT, MD simulation combined with mutagenesis studies show that the nonconserved residue (Ile172 in hSERT, Val152 in hDAT) is the driving factor for hDAT-over-hSERT selectivity to cathinone [104]. For hNET and hDAT, comparing to sNRIs (contain ethoxy-, methoxy- and methyl-substituted phenyl group), the reduction in hydrophobic property of the functional group in NDRIs (phenyl group) leads to significantly decreased interaction with the subsite site in hNET and hDAT, which is the key physicochemical property for NDRIs drugs selectivity [80]. Using TRI amitifadine as a probe molecule,

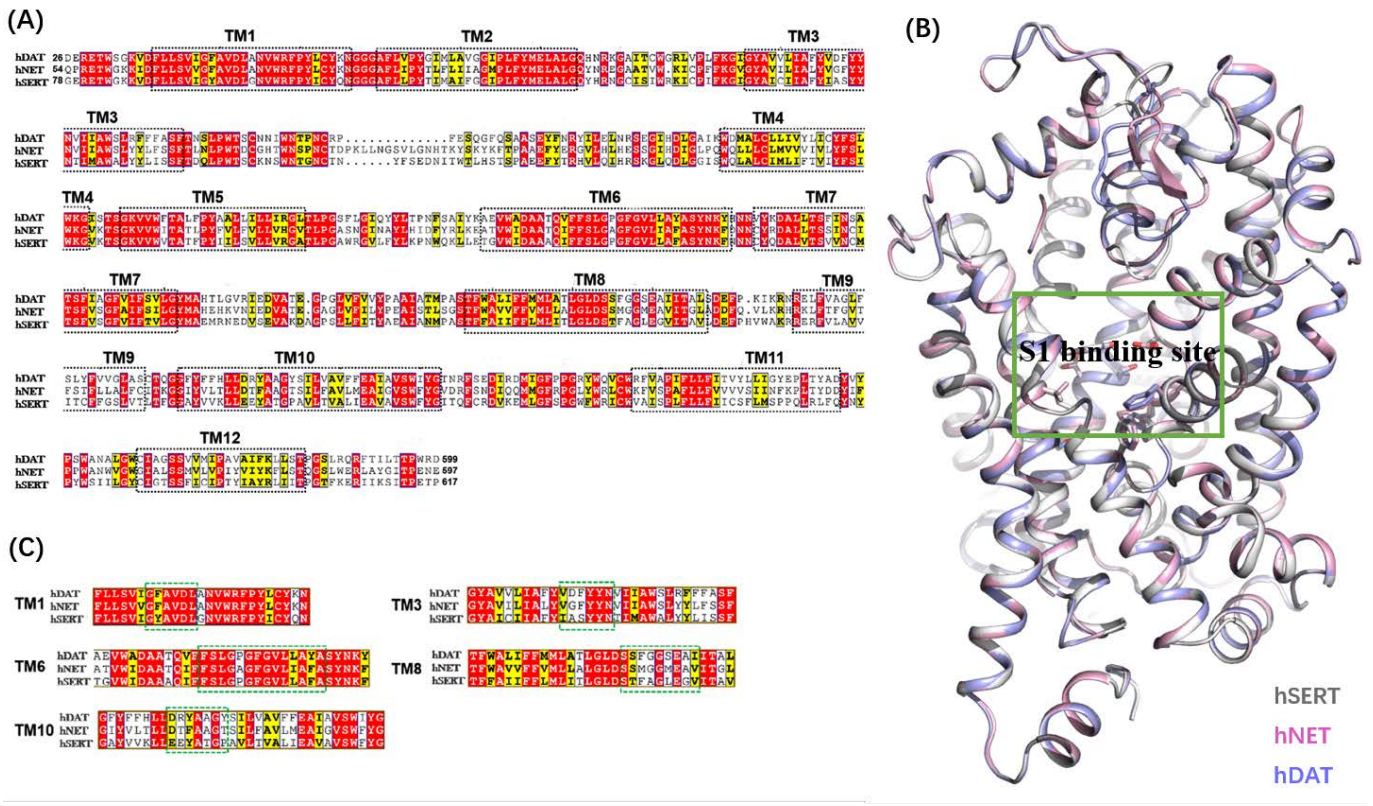



**Fig. (7). Sequence and structure alignments of the three hMATs.** (**A**) Sequence alignment of hSERT (from Glu78 to Pro617), hNET (from Gln54 to Glu597) and dDAT (from Glu26 to Asp599). The twelve Transmembrane (TM1 to TM12) alpha helices are labelled with the black dotted box. The red shadow periods refer to the identical residues, the yellow shadow periods refer to the conservative substitutions; (**B**) Superimposition of the homology models of hSERT, hNET and hDAT using X-ray crystal structure of dDAT as the template; (**C**) Sequence alignment of the regions of TM1, 3, 6, 8 and 10. Residues that primarily contribute to the S1 binding site of hSERT, hNET and hDAT were labelled with green dotted box. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

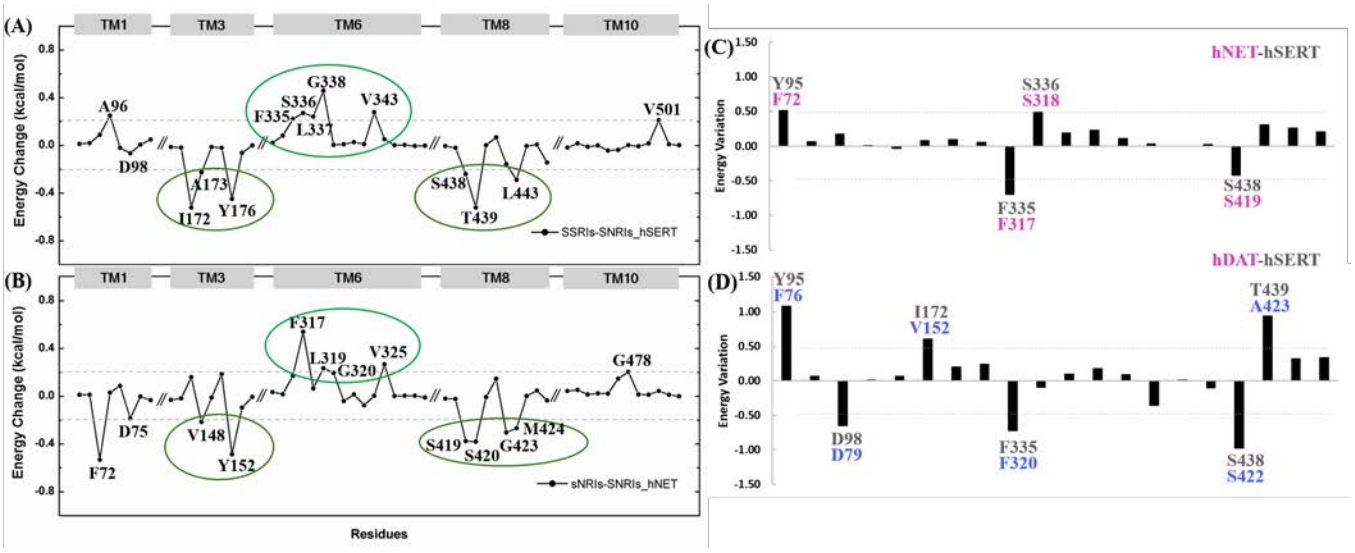



**Fig. (8). Plots of the mean energy contribution changes of residues:** (**A**) between SSRIs and SNRIs in hSERT; and (**B**) between sNRIs; and SNRIs in hNET. Plots of the energy contribution changes of residues in the central site between: (**C**) hSERT and hNET; and (**D**) hSERT and hDAT, for amitifadine binding. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

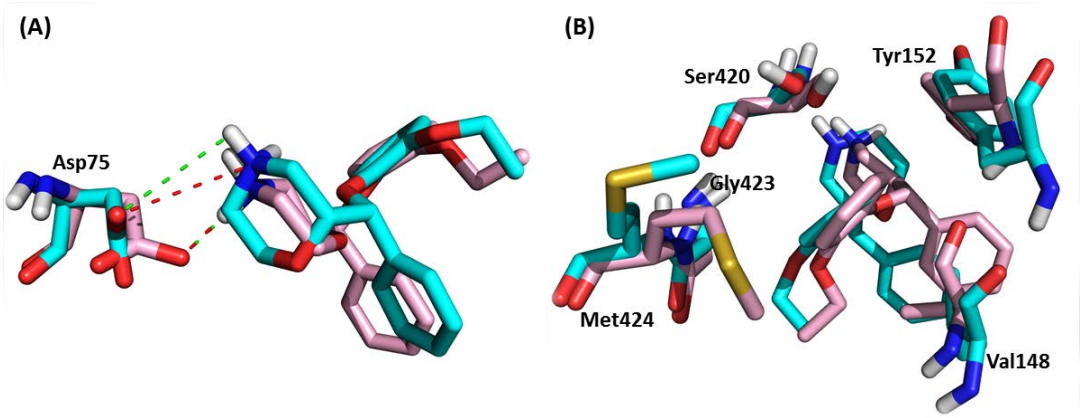



**Fig. (9). Superimposition of and conformation variation between (R, R)-reboxetine (in cyan) and (S, S)-reboxetine (in light pink) together with their corresponding interacting residues (in corresponding color).** The conformation changes of reboxetine and residues Asp75, Val148, Tyr152, Ser420, Gly423 and Met424 were displayed in (**A**) and (**B**) from different views. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

per-residue energy contribution and cross *in silico* mutagenesis discovered the variation in the inhibition ratio of amitifadine between hSERT and two other MATs (hNET and hDAT) was to mainly come from non-conserved residues (Y95, I172 and T439 in hNET and Y95, I172, A169 and T439 in hDAT) (Fig. **8C** and **8D**) [98].

#### *4.2.2. Enantiomers Binding*

There are several MATs reuptake inhibitors have been marketed as a racemic mixture such as citalopram and reboxetine. Usually, there is a great deal of difference between the two enantiomers affinity to target [76]. It is reported that the two enantiomers of citalopram bind in the central pocket with opposite orientations of their aromatic group. The fluorine atom escitalopram is located near Ala173 and Thr439 and the cyano group is in close proximity of Phe341, while these contacts are found to be reversed for the other enantiomer [106]. For reboxetine, binding modes of its enantiomers with hNET were compared, 6 key residues (Asp75, Val148, Tyr152, Ser420, Gly423 and Met424) favoring the binding of (S, S)-reboxetine over that of (R, R)-reboxetine were discovered (Fig. **9**) [73].

#### *4.2.3. Drug Addiction*

According to statistic, six out of nine approved anti-ADHD drugs were psychostimulants and one of the major concerns about psychostimulants was their highly addictive profile (with great abuse potential) [64]. It is known that the addictiveness of psychostimulants is largely attributed to their interaction with DAT [107-109]. Multiple computational methods were integrated to differentiate binding modes between approved psychostimulants and ADHD drugs of little addictiveness. The energy contribution variation of 8 hDAT residues (Ala77, Val152, Gly153, Phe155, Phe320, Phe326, Asp421 and Ala480) between addictive and nonaddictive drugs was observed, and a reduction in hydrophobicity of drugs 2 functional groups was identified as the indicator of drugs addictiveness (Fig. **10**) [64]. The finding agreed well with the physicochemical properties of 8 officially reported controlled substances [64]. In addition, it is hypothesized that cocaine, methylphenidate and related cocaine binding site ligands are DAT "inverse agonists", which is the major contributor to their pharmacological actions [109].

## 5. DEVELOPMENT OF NOVEL MATS INHIBITORS

### 5.1. Reuptake Inhibitors

#### *5.1.1. SSRIs*

In 2010, three diphenyloxide derivatives (compounds **1**-**3**) (Tables **1** and **2**) were reported by Mavel *et al.* as novel SSRIs [110]. Compounds **1**-**3** exhibited high affinity ($7 < K_i < 8$ nM) for hSERT over the other human MATs hNET and hDAT ($K_i > 500$ nM). It is indicated that adding a fluorine atom to 4- and 5-position gave **1** and **2** high affinity and selectivity for the hSERT. In addition, *in vitro* evaluation showed that the hSERT binding site seemed to be more able to accept fluoro alkoxy group at the 5-position, leading to the substitution by a 3-fluoropropoxy (**3**) with high affinity and selectivity for the hSERT. Through synthesizing a series of benzyl ester derivatives of meperidine and normeperidine, Gu *et al.* identified **4**-**6** (Tables **1** and **2**) with low nanomolar binding affinities for the

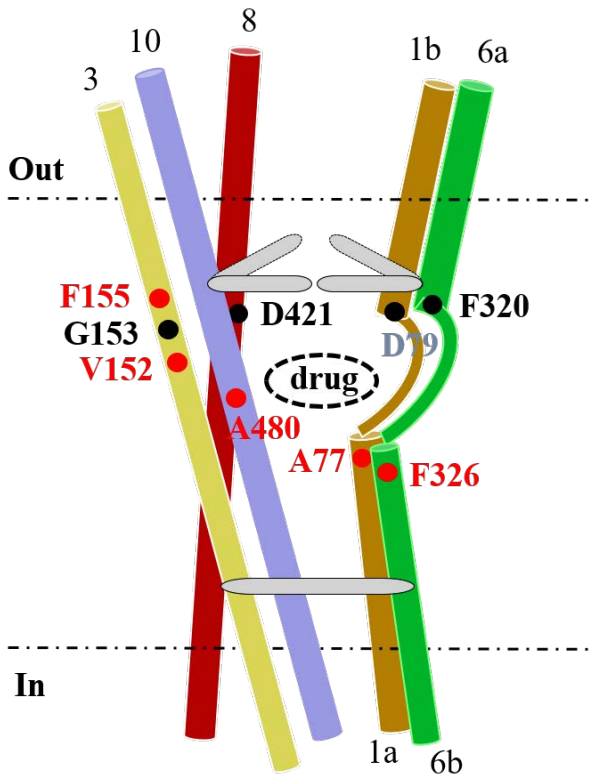



**Fig. (10). Side view of ADHD drugs binding site surrounded by 8 residues of significant energy fold changes between addictive and non-addictive drugs**. Residues preferentially binding non-addictive drugs were represented in red and residues favoring binding of addictive drugs were shown in black. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

hSERT and good hSERT selectivity [111]. For the compounds **4** and **5**, the affinity ($K_i$ values < 2 nM) and selectivity (hNET/hSERT > 1500 and hDAT/hSERT > 1500) was very similar to fluoxetine. The 4-(4-iodophenyl) normeperidine 4-methoxybenzyl ester **6** was the most potent ($K_i$ = 0.6 nM) and selective compound for hSERT (hNET/hSERT = 4600 and hDAT/hSERT = 4900). Moreover, the selective of **6** to hSERT exceeded that of fluoxetine and paroxetine. Results show that the meperidine benzyl ester scaffold and the normeperidine benzyl ester scaffold seem to be well suited for the development of new compounds that display high potency and selectivity for the hSERT.

Nencetti *et al.* synthesized and evaluated the binding affinity of 4-(aryl)piperidin-3-one O-4-benzyl oxime hydrochlorides of both E and Z configuration for hSERT, hNET and hDAT [112]. The compound **7** (Tables **1** and **2**) of E configuration possessing an affinity for hSERT ($K_i$ = 10.28 nM) in the same range as fluoxetine and an excellent hSERT selectivity (hNET/hSERT = 972 and hDAT/hSERT > 9000), higher than that of fluoxetine and paroxetine (Tables **1** and **2**). Molecular docking study using paroxetine binding in the human MATs homology models as a reference showed that the E configuration compound and the absence of bulky substituents in para position of the phenyls seem to be determinant for the affinity towards hSERT.

In addition to pure medicinal chemistry approach, computational tools such as docking and pharmacophore model are another important way to identify active compounds to hMATs. Table **5** provides an overview of published virtual screening studies to identify MATs inhibitors. Through docking-based VS and molecular hybridization, Nolan *et al.* identified DJLDU-3-79 (**8**) (Tables **1** and **2**) as hSERT inhibitor with affinity of $K_i$ = 284 nM [45]. DJLDU-3-79 is a molecular hybrid of MI-17 and SSA-426 (a dual hSERT/5-$HT_{1A}$R antagonist) and displayed good hSERT selectivity (hNET/hSERT > 200 and hDAT/hSERT = 50). Based on the recently released hSERT crystal structure [48], Erol *et al.* screened a database contain approximately 260,000 small molecules by molecular docking and top-ranked hit compounds with Otava ID: 7118020138 (**9**); 7117171303 (**10**); and 118671819 (**11**) (Tables **1** and **2**), were proposed as hSERT inhibitors [95]. The high binding affinities of the identified compounds were further assessed and were confirmed by MD simulation-based MM/GBSA binding free energy calculations. In addition to docking-based VS, the pharmacophore-based VS studied were reported to discover novel hSERT inhibitors [43, 46]. Manepalli *et al.* identified SM-10 (**12**) and SM-11 (**13**) (Tables **1** and **2**) as new hSERT selective inhibitors [43]. The binding affinities ($K_i$) of SM-10 (**12**) and SM-11 (**13**) to hSERT were 38 and 17 nM, respectively.

### *5.1.2. sNRIs*

In 2010, Vu *et al.* discovered 1-(Indolin-1-yl)-1-phenyl-3-propan-2-olamines as novel sNRIs scaffold [113]. SAR analysis demonstrated that the substitutions of 3,3-dimethyl group on the indoline ring (**14-18**) (Tables **1** and **2**) leads to potent ($IC_{50}$ = 2.7-6.5 nM) and selective hNET inhibition over both hSERT and hDAT ($IC_{50}$ > 1 μM). Additional *in vivo* evaluation was performed for **17** suggested the potential efficacy of the compound in alleviating vasomotor symptoms as well as attenuating acute and neuropathic pain.

**Table 5. Reported Virtual Screening studies to identify MATs inhibitors.**

| No. | Method | Program | Database | Number of active compounds | Year | Ref. |
|---|---|---|---|---|---|---|
| 1 | Ligand-based pharmacophore | Catalyst | NCI2000 | 12,570 for DAT, 3250 for NET, 240 for SERT | 2008 | [241] |
| 2 | Docking, structure-based pharmacophore | MOE | Sigma-Aldrich catalog | 1 | 2010 | [94] |
| 3 | Docking, structure-based pharmacophore | MOE | ZINC | 2 | 2011 | [43] |
| 4 | Docking | DOCK | KEGG DRUG | 18 | 2011 | [44] |
| 5 | Docking | MOE | ENAMINE | 10 | 2011 | [45] |
| 6 | Docking, structure-based pharmacophore | GOLD | * | 1 | 2013 | [52] |
| 7 | Docking, structure-based pharmacophore | Discovery Studio | NCI | 2 | 2013 | [148] |
| 8 | Docking, ligand-based pharmacophore | Jchem,Discovery Studio, ICM | Asinex, ChemBridge, ChemDiv, Enamine, Life Chemicals | 74 | 2014 | [230] |
| 9 | Docking, structure-based pharmacophore | MOE | PubChem | 19 | 2014 | [46] |
| 10 | Docking, support vector machines | AutoDock | MDDR, PubChem, ChEMBL | 91 | 2015 | [102] |
| 11 | Docking, structure-based pharmacophore | MOE | Sigma-Aldrich catalog | 1 | 2016 | [237] |
| 12 | Docking | Maestro, GOLD | Otava Chemical | 3 for central binding site, 1 for allosteric binding site | 2017 | [95] |
| 13 | Docking, ligand-based pharmacophore | AutoDock, DUD-E website, FLAP, Discovery Studio | CHEMBL, Specs | 6 | 2018 | [239] |

*The name of the Database used for Virtual Screening not mentioned in the reference paper.

WYE-103231 (**19**) (Tables **1** and **2**), the representative compound of 4-[3-aryl-2,2-dioxido-2,1,3-benzothiadiazol-1(3H)-yl]-1-(methylamino)butan-2-ols), is a novel sNRIs discovered by O'Neill *et al.* from a virtual screening hit [114]. Compound **20** had high hNET potency ($IC_{50}$=1.2 nM) and excellent selectivity over hSERT and hDAT (hSERT/hNET >1600 and hDAT/hNET > 600). Moreover, **19** had a good pharmacokinetic profile and demonstrated oral efficacy in rat models of ovariectomized-induced thermoregulatory dysfunction and morphine dependent flush as well as the hot plate and Spinal Nerve Ligation (SNL) models of acute and neuropathic pain.

Through VS, 6,436 drugs from the Kyoto Encyclopedia of Genes and Genomes (KEGG DRUG) database against the NET model, Schlessinger *et al.* identified 10 of the 18 high-scoring drugs tested experimentally were to be hNET inhibitors [44]. Further analysis discovered five novel NRIs (**20**-**24**) (Tables **1** and **2**) including guanabenz (**20**, $IC_{50}$ = 69.6 μM), tolazolin (**21**, $IC_{50}$ = 68.0 μM), talsaclidine (**22**, $IC_{50}$ = 43.2 μM), phenformin (**23**, $IC_{50}$ = 10.9 μM) and tuaminoheptane (**24**, $IC_{50}$ = 3.7 μM). Result suggested the efficacy of several sympathetic (tuaminoheptane) and antidepressant (tranylcypromine) drugs, as well as side effects of diabetes (phenformin) and Alzheimer's (talsaclidine) drugs may be rationalized.

### *5.1.3. sDRIs*

Trishomocubane [115] and phenylpiperazines [116] scaffolds have been developed as sigma (σ) receptor ligands in several studies. The first trishomocubane derived sDRI (**25**) (Tables **1** and **2**) was identified by Banister *et al.* in 2011 [117]. Compound **25** exhibited high affinity for hDAT ($K_i$ = 1.2 nM). The selectivity over the human MATs hSERT and hNET is greater

than 8300-fold, and it has a moderate affinity for $\sigma_1$ or $\sigma_2$ receptors. In addition, the radiolabeling of **25** with carbon-11 or fluorine-18 could provide molecular probes for the *in vivo* imaging of the hDAT using PET, which will be reviewed in Section 5.4. For phenylpiperazines scaffold, Motel *et al.* discovered that a 1-(3-chlorophenyl)-4-phenethylpiperazine (**26**) (Tables **1** and **2**) had high affinity for hDAT ($K_i$ = 0.04 nM) and displayed the greatest selectivity over hSERT and hNET ($K_i$ > 800 nM) [118].

Cathinone analogs are usually act as substrates or inhibitors of human MATs [119]. Several comprehensive (Q)SAR analysis of the substituted cathinones with hSERT, hNER and hDAT [119-122]. Eshleman *et al.* found that **27**-**32** (Tables **1** and **2**) had high affinity (< 1 μM) and selectivity for hDAT [120]. However, it was discovered that compounds α-pyrrolidinophenones (**27**, **28**) and pentedrone (**32**) with higher potency at hDAT than at hSERT have a high likelihood of abuse in an intracranial self-stimulation procedure in rats [119, 120]. In addition, increasing α-pyrrolidinophenone substituent chain length increases potency at the hDAT and may increase abuse potential [120].

### *5.1.4. SNRIs*

Starting from a sNRI, Angus *et al.* identified sNRI **33** (Tables **1** and **2**) exhibited the best balance potency of hSERT ($IC_{50}$ = 101 nM) and hNET ($IC_{50}$ = 23 nM) [123]. During the optimisation process, the authors found that incorporation of a nitrile in the meta-position of the A-ring allowed them to increase the hSERT inhibition and without introducing hDAT inhibition ($IC_{50}$ > 10,000 nM). Further preclinical evaluation indicated that compound **33** is a useful tool for evaluating the hNET > hSERT >> hDAT profile in neuropathic pain models.

To explore the molecular determinants for selective recognition of antidepressants in the hSERT and hNET, Andersen *et al.* performed SAR study of escitalopram (SSRI) scaffold and identified **34** (Tables **1** and **2**) [7]. Compound **34** is a SNRI with high affinity for both hSERT ($K_i$ = 32 nM) and hNET ($K_i$ = 44 nM). The results suggested that by subtle perturbations of the same chemical scaffold, the selectivity ratio between hSERT and hNET can be controlled.

TD-9855 (**35**) (Tables **1** and **2**) was a novel and potent inhibitor of hSERT ($K_i$ = 32 nM) and hNET ($K_i$ = 32 nM) characterized by *in vitro* pharmacology analysis [124-131]. Further preclinical to clinical translation of CNS transporter occupancy of TD-9855 performed by Smith *et al.* reveal the long pharmacokinetic half-life (30-40 h) allowed for sequential assessment of SERT and NET occupancy. At doses of greater than 4 mg, the projected steadystate NET occupancy higher than 75%, and the plasma $EC_{50}$ for NET was estimated to be 1.21 ng/mL. For SERT, after a single oral dose of 20 mg, the occupancy was 25(±8)% at a plasma level of 6.35 ng/mL [132]. As a result, the translational approach established TD-9855 as a CNS-penetrant, NET-selective SNRI suitable for further investigation in patients with CNS disorders.

### *5.1.5. SDRIs*

Based on sDRIs 1-[2-[bis(4-fluorophenyl)methoxy] ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909) and 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine (GBR 12935) [133], Hsin *et al.* synthesized a series of novel derivatives in positions C2 and C3 of the phenylpropyl side chain [134]. Through evaluating their affinities binding to hSERT and hDAT, the role of amino, fluoro, hydroxyl, methoxyl, methyl, methylene, and oxo substituents on affinity for the hSERT and the hDAT was investigated. In the C2 series, the amino-substituted derivative **36** (Tables **1** and **2**) showed essentially the same affinity for hSERT ($K_i$ = 22 nM) and hDAT ($K_i$ = 20 nM), which might be a lead compound for the discovery of potent able to simultaneously block the hSERT and the hDAT. To develop a comprehensive picture about the substituent role in the inhibition effect of hSERT and the hDAT, 4 novel series of oxabicyclo[3.2.1]octenes containing aryl substituents at the 3-position was developed by Torun *et al.* [135]. In all series, **37** (Tables **1** and **2**) was the most potent and dual-action hSERT ($IC_{50}$ = 10 nM) and hDAT ($IC_{50}$ = 9 nM) inhibitor.

### *5.1.6. TRIs*

Clinical evidence reveals that TRIs elevate DA in addition to 5-HT and NE demonstrate greater efficacy [83], with the reversal of anhedonia and improved tolerability, and the desired profile for a "ideal" TRI having relative affinities in the order of hSERT ≥ hNET > hDAT [82].

Micheli *et al.* developed 1-(Aryl)-6-[alkoxyalkyl]-3-azabicyclo[3.1.0]hexanes and 6-(Aryl)-6-[alkoxyalkyl]-3-azabicyclo[3.1.0]hexanes as a new series of TRIs with high *in vitro* potency and selectivity at SERT, NET, and DAT [136]. *In vivo* experiments identified **38** (Tables **1** and **2**) has an appropriate developability profile with $pK_i$ = 9.17/9.87/7.87 on rat SERT, NET, and DAT, respectively. Further concurrent locomotor activity measurement and quantification of 5-HT, NE and

DA brain levels were performed by HPLC analysis and electrochemical detection and results demonstrate that the acute administration of **38** produced a slow onset/long lasting increase in the extracellular levels of all monoamines in medial prefrontal cortex (MPC) in rats, in agreement with the TRIs profile. In addition, a new class of 6-(3,4-dichlorophenyl)-1-[(methyloxy)methyl]-3-azabicyclo[4.1.0]heptanes with high *in vitro* potency and selectivity at SERT, NET, and DAT were reported by Micheli *et al.* [137]. *In vivo* microdialysis experiments in different animal models identified **39** (Tables **1** and **2**) has an appropriate profile $pK_i$ = 8.98/7.92/7.92 on rat SERT, NET, and DAT. Meanwhile, **39** inhibited monoamine uptake with $pIC_{50}$ = 8.4/8.7/8.1 for hSERT, hNET and hDAT using LLCPK cells.

LPM580153 (**40**) (Tables **1** and **2**), 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl) ethyl] phenyl 3-nitrophenyl ether, is a novel TRI derivative from venlafaxine by Zhang *et al.* [138]. The mechanism of action was explored using neurotransmitter uptake assay and a corticosterone-induced cell injury model. The effects ($IC_{50}$ values) of **40** on the uptake of 5-HT, NE and DA were for 0.72 μM,1.57 μM and 3.97μM, respectively. In addition, assessment of **40** on animal models of depression indicate that the robust antidepressant-like effects of the compound.

The scaffolds of 1,4-disubstituted piperazines and piperidines were designed and synthesized by Paudel *et al.* as novel TRIs [139]. Among the reported compounds, 1-(4-(5-benzhydryl-1H-tetrazol-1-yl)butyl)-4-(3-phenylpropyl)piperazine (**41**) (Tables **1** and **2**) was the most advanced inhibitor. It is notable that **41** has a longer linker and a longer distance between the piperazine and phenyl rings. *In vitro* evaluation indicated that **41** was able to inhibit monoamine neurotransmitter reuptake ($IC_{50}$) of 158.7 nM for 5-HT, 99 nM for NE and 97.5 nM for DA.

Based on molecular docking, Paudel *et al.* designed and synthesized a series of benzylpiperidine-tetrazole compounds [140]. *In vitro* evaluation indicated that **42** (Tables **1** and **2**) inhibit monoamine neurotransmitter reuptake ($IC_{50}$) of 0.31 nM for 5-HT, 2.51 nM for NE and 6 nM for DA. SAR and docking analysis of the study revealed that compounds containing three-carbon units in the linker showed potent 5-HT, NE and DA reuptake inhibition. Pharmacophore-based VS of the PubChem database by Nolan *et al.* discovered TN-01 (**43**), TN-05 (**44**), TN-06 (**45**) and TN-13 (**46**) (Tables **1** and **2**) as novel inhibitors of human MATs [46]. Further *in vivo* characterization of them revealed TN-01 (**43**), TN-06 (**45**) and TN-13 (**46**) have antidepressant-like activity in a rodent model.

### 5.2. Multimode Inhibitors

To search for new dual-acting agents as potential antidepressants, benzothiazoles scaffold was designed and evaluated for discover probe molecules for the $5HT_{1A}$ receptor and SERT by Zhu *et al.* [141]. Among the reported compounds, **47** ($K_i$ = 28.3 and 81) and **48** ($K_i$ = 7.3 and 64) (Tables **1** and **2**) exhibited moderate binding affinities at both the $5HT_{1A}$ receptor and the SERT, respectively.

Computer Aided Drug Design (CADD) strategy was also used to obtain compounds with dual 5-$HT_{1A}$ receptor and SERT affinity [142]. Starting from the imidazolidine-2,4-dione derivatives, Czopek *et al.* designed 5-arylimidazolidine-2,4-dione derivatives with 4-(3-chlorophenyl)piperazinylmethyl moiety as the most promising compounds. Furthermore, the forced swim test in mice identified **49** (Tables **1** and **2**) exhibiting a favorable antidepressant-like profile without affecting spontaneous locomotor activity. Compound **49** possessed significant affinities ($K_i$) for both 5-$HT_{1A}$ receptor (38 nM) and SERT (0.17 μM). It is noted that the effective antidepressant doses of **49** (20 mg/kg) did not stimulate the spontaneous locomotor activity, indicating that the compound has specific antidepressant-like activity.

### 5.3. Allosteric Inhibitors

Through integrating computational simulation and CADD methods, Kortagere *et al.* identified an allosteric modulator ATM7 (**50**) (Tables **1** and **2**) of the hSERT [52]. Starting from the structural model of hSERT based on LeuT, MD simulation was performed to characterize the allosteric site. The identified allosteric site was further used to VS a database of 3 million small molecules. The obtained screening results were subjected to functional transport assays. Kortagere *et al.* identified **50** which increased the reuptake of 5-HT. Results demonstrated that **50** acts through a novel mechanism that involves allosteric modulation of SERT function.

Larsen *et al.* performed a systematic SAR study based on the citalopram and talopram scaffolds [91]. Fourteen citalopram or talopram analogous were obtained and their binding affinities for hSERT central site (S1) and allosteric site (S2) were evaluated through transiently expressing SERT on COS7 cells. Results showed that 8 compounds containing the cyano-group had a significant allosteric activity ($t_{1/2}$ for [$^3$H]escitalo-

pram dissociation > 500 min). Among the 8 compounds, **51** (Tables **1** and **2**) with similar activity at S1 ($IC_{50}$ = 10 μM) and S2 ($IC_{50}$= 12 μM) was identified as a novel probe molecule to guide the future synthesis of compounds bearing high selectivity and high affinity towards the allosteric binding site.

Based on molecular docking and Steered Molecular Dynamics (SMD) information, several 3-linked imipramines analogs (**52**) (Tables **1** and **2**) were reported by Brinkø *et al*. [143]. The SAR analysis showed that the most potent were always the shortest compounds. In addition, mutations (W103A and I179C) around the allosteric site were found to affect the larger compounds, while the smaller compounds were mostly unaffected. This study sheds light on the design and development of a new generation of improved antidepressants that fully exploit both binding sites.

Using the recently released hSERT crystal structure [48], Erol *et al*. screened a database contain approximately 260,000 small molecules by molecular docking and top-ranked hit compound with Otava ID: 6248262 (**53**) (Tables **1** and **2**) was proposed as hSERT allosteric inhibitor [95]. The high binding affinities of the identified compounds were further assessed and were confirmed by MD simulation based MM/GBSA binding free energy calculations. In addition, Topiol *et al*. reported the evaluation of citalopram analogs selectivity for both S1 and S2 site in hSERT [144]. The SAR analysis identified eleven analogs with a higher affinity and selectivity than benchmark R-Citalopram for the S2 versus the S1 site. Computational modeling was used to explain the SAR based on the recently released X-ray structures of hSERT containing the S1 and S2 sites.

In addition to hSERT, Janowsky *et al*. reported a new class of rigid adenine nucleoside derivatives as novel allosteric modulators of the hNET and hDAT [51, 59]. MRS7292 (**54**) and MRS7232 (**55**) displayed high binding affinities at hDAT ($EC_{50}$ = 35 nM) and hNET ($EC_{50}$ = 35 nM), and MRS7232 (**55**) was more potent than cocaine at inhibiting DA uptake ($IC_{50}$ = 107 nM) [51]. The combination of binding enhancement and inhibition of DA uptake suggests possible allosteric binding with respect to cocaine analogs [59, 60].

### 5.4. Imaging Agents

Several compounds were designed and synthesized as imaging agents for human MATs in living cells [117, 145-147]. In 2011, Tomlinson *et al*. reported the synthesis of hSERT-selective compound amenable to conjugation to quantum dots via a biotin-streptavidin binding interaction [146]. As a result, seven compounds were identified as fluorescent probes for live cell imaging of membrane-bound hSERT. Among the seven compounds, IDT374 (**56**) exhibited the highest potency for interactions with hSERT. On the basis of escitalopram, Kumar *et al*. reported the novel rhodamine-labeled compound **57** demonstrated high affinity binding for hSERT ($K_i$ = 34 nM) without any binding to hNET or hDAT, which suggested compound **57** as a novel tool for studying hSERT expression and distribution in living cells [145]. The carbon-11 labeled **58** and **59** reported by Banister *et al*., were used as the popular molecular probes for *in vivo* imaging of the hDAT by Positron Emission Tomography (PET) [117]. Liu *et al*. synthesized a series of *N*-fluoropyridyl derivatives of tropane [147]. Evaluation of the compounds identified **60** as the highest binding affinity to DAT ($K_i$ = 4.1 nM), and selectivity for hDAT over hSERT (5-fold) and hNET (16-fold). After radiolabeling with Fluorine-18 ($^{18}$F), the [$^{18}$F]-**60** may be useful as a potential radioligand for imaging DATs with PET.

## CONCLUSION AND FUTURE PERSPECTIVES

Over 30 approved MATs drugs are available in the market for the treatment of depression and other behavioral disorders, although there are some side effects. Clinical Phase 3 trials of TRI amitifadine [19, 84] and approval of multimodal antidepressants vilazodone and vortioxetine [14, 15] have shown that hMATs are still the worthwhile targets and thus there is a continuous need for the development of novel drugs with rapid onset and better tolerance profiles.

For central inhibitors, optimization of the lead compound to higher affinity and better selectivity at hSERT, hNET and hDAT is one of the top challenges. For example, an effective therapeutic response without addictive and other liabilities of TRIs is based on the optimal ratio of triple reuptake inhibition, and this has not been fully understood [83]. In recent years, a number of X-ray crystal structures [25-29, 48, 49] and computational models [6, 35-40, 148] of MATs complexed with diverse antidepressants or substrates were determined and this has opened new perspectives for research on this challenging topic. From complexed structures, it is known that chemically diverse antidepressants have the similar binding mode, which the pharmacophores interact with subsites A, B and C in the primary binding pocket (Fig. **2**) [26, 48, 49]. The nonconserved residues [38, 80, 98, 104] and the conformation opening states [96] of the hMATs central

binding sites, were identified as molecular parameters determining drug selectivity.

For allosteric inhibitors, the considerable plasticity of the binding site is a great challenge to design novel scaffold drugs. The more recently reported hSERT crystal structure successfully captured escitalopram binding to a secondary site located at the periphery of the extracellular vestibule of the transporter [48]. Escitalopram has an allosteric effect that can modulate hSERT activity by altering the kinetics of ligand dissociation from the central site. In addition to hSERT, a new class of rigid adenine nucleoside derivatives were reported as novel allosteric modulators of the hNET and hDAT [51, 59].

For multimodal antidepressant drugs, although the functional relevant orientation of vortioxetine within the central binding site of hSERT was determined by combining comparative modeling with mutational analysis [97], the binding mode of vilazodone to the 5-HT1A receptor has been revealed by computational method [69], but there is no crystal structure or computational model of vilazodone in hSERT was reported, and the binding of vilazodone to hSERT was predicted by combine docking and MD simulation in this review, the detailed binding mode of drugs in different kinds of targets (the transport and receptor) is still not well understood.

Despite the challenges as mentioned above, there are still many opportunities. This review summarized the structure information, activity and selectivity characteristics, binding modes and side effects of the approved, in clinical studies or developed compounds. The information will play an important role in the structure-based discovery of novel chemotypes and chemical fragments with high activity and selectivity to the central site. Meanwhile, considering the possibility that allosteric modulator sites constitute a shared mechanism in hMATs, the discovered allosteric site in hSERT could eventually open a whole new area of drug research targeting the allosteric regulation of hMATs. In addition, crystallographic experiment or computational method were hoped to elucidate the detailed mechanism of combined 5-HT reuptake inhibition with agonism, partial agonism and antagonism of receptors at the atomic level. It is expected that in the near future, the number of MATs modulators in clinical trials will grow, and hopefully at least some of them will progress to FDA approval for effective and safe CNS disorders treatment.

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| hMATS | = | Human Monoamine Transporters |
| SERT | = | Serotonin Transporter |
| NET | = | Norepinephrine Transporter |
| DAT | = | Dopamine Transporter |
| 5-HT | = | Serotonin |
| NE | = | Norepinephrine |
| DA | = | Dopamine |
| CNS | = | Central Nervous System |
| MDD | = | Major Depression Disorders |
| ADHD | = | Attention Deficit Hyperactivity Disorder |
| PD | = | Parkinson's Disease |
| SSRIs | = | Selective Reuptake Inhibitors of Serotonin |
| sNRIS | = | Norepinephrine |
| SNRIs | = | Reuptake Inhibitors of Both Serotonin and Norepinephrine |
| TRIs | = | Triple Reuptake Inhibitors |
| SPARI | = | Serotonin Reuptake Inhibition and 5-HT1AR Partial Agonism |
| LeuT | = | Leucine Transporter |
| TCA | = | Tricyclic Antidepressant |
| CADD | = | Computer Aided Drug Design |
| MD | = | Molecular Dynamics |
| SMD | = | Steered Molecular Dynamics |
| SAR | = | Structure-Activity Relationship |
| SBDD | = | Structure-Based Drug Design |
| VS | = | Virtual Screening |

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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