American Journal of Pharmacological Sciences, 2018, Vol. 6, No. 1, 25-32
Available online at http://pubs.sciepub.com/ajps/6/1/5
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DOI:10.12691/ajps-6-1-5

Molecular Docking and *In-Silico* ADME Prediction of Substituted (*E*)-4-Styryl-7,8-dihydroquinazolin-5(6*H*)-ones and 5-((*E*)-Styryl)pyrimidine[4,5-d]pyrimidine-2,4(1*H*,3*H*)-diones as Potential SERT Inhibitors and Antidepressants

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Received October 12, 2018; Revised November 16, 2018; Accepted November 29, 2018

Abstract A set of 66 compounds from three classes having either of the two nuclei, (*E*)-4-styryl-7,8-dihydroquinazolin-5(6*H*)-one (**1′-22′a** and **1′-22′b**) and 5-((E)-styryl)pyrimido[4,5-d]pyrimidine-2,4(1*H*,3*H*)-dione (**1′-22′c**) were docked with Serotonin reuptake transporter (SERT) using escitalopram as the reference compound for comparison. Five of the compounds (**18′b**, **19′a**, **15′c**, **19′c** and **6′a)** had binding energy lower than/equal to that of escitalopram (-8.8 kcal/mol) and were eliminated from the study. The remaining 61 compounds were assessed for druglikeness using Lipinski's rule of five which led to the elimination of one more compound (**19′b)**. From among the remaining 60 compounds, 31 having binding energy equal to/greater than -10 kcal/mol were submitted for ADME properties prediction on an online program (preADMET) and the analysis of the results, taking into consideration the compounds blood brain barrier penetration and predicted P-glycoprotein inhibition as the major criteria for elimination, 11 compounds were selected for synthesis and further study as antidepressant agents. None of the 5-((E)-styryl)pyrimido[4,5-d]pyrimidine-2,4(1*H*,3*H*)-dione made it to the final eleven compounds due to high polarity that limits their BBB penetration. From among the 11 selected for synthesis are 3 compounds also that have very good hepatic metabolism (CYP450 enzymes interactions) pharmacokinetic profiles, predicted. The compounds selected for synthesis preferentially bind to the allosteric site of the SERT.

Keywords: *serotonin, citalopram, quinazolinone, blood brain barrier, druglikeness, P-glycoprotein*

Cite This Article: Oyesakin Y.M., George D.E., Fadare R.Y., Idris A.Y., and Fadare O.A., "Molecular Docking and *In-Silico* ADME Prediction of Substituted (*E*)-4-Styryl-7,8-dihydroquinazolin-5(6*H*)-ones and 5-((*E*)-Styryl)pyrimidine[4,5-d]pyrimidine-2,4(1*H*,3*H*)-diones as Potential SERT Inhibitors and Antidepressants". *American Journal of Pharmacological Sciences*, vol. 6, no. 1 (2018): 25-32. doi: 10.12691/ajps-6-1-5.

1. Introduction

Depression is a common mental disorder characterized by persistent sadness and loss of interest in activities that people normally enjoy, accompanied by an inability to carry out daily activities, decrease in energy, feelings of guilt or low self-worth, and poor concentration for a long period of time. It is accompanied by symptoms of anxiety [1].

According to world health organization (WHO), more than 350 million people of all ages suffer from depression and every year approximately 844 thousand people die by suicide, which is the most severe consequence of uncontrolled depression and the second leading cause of death in 15-29 years old people [2]. Additionally, WHO predicts that depression will be the second leading cause of death by 2020 due to cardiovascular and stress-related complications, notwithstanding that it is a psychiatric condition [3,4]. Depression can be treated by various mechanisms to increase the synaptic concentration of monoamines. This finding led to the monoamine hypothesis of depression. The hypothesis was put forward by Coppen in 1967 about 50 years ago. Coppen proposed that the underlying pathophysiologic basis of depression is the depletion in levels of serotonin, norepinephrine, and/or dopamine (Figure 1) in the central nervous system [5,6,7]. Antidepressants can be divided into two main groups; the first-generation class, tricyclic antidepressants (TCA); and the second-generation class, selectively inhibiting sodium symporters (NSSs) classified as norepinephrine reuptake inhibitors (NRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), selective serotonin reuptake inhibitors (SSRIs) and other heterocyclics clinically employed as drug therapy. These drugs target the monoamine neurotransmitters in an attempt to increase the concentration of the neurotransmitters

in the synaptic cleft to activate the postsynaptic receptor [8].

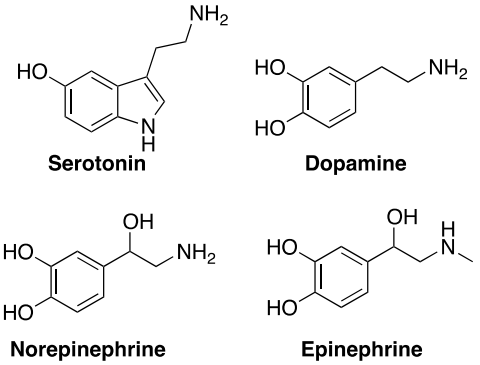


Figure 1. The monoamine neurotransmitters implicated in the monoamine hypothesis of depression (Coppen, 1967)

SSRIs work by inhibiting serotonin reuptake transporter (SERT) which leads to increase in the concentration of serotonin in the 5-HT receptor in an individual. Drugs that inhibit SERT (Figure 2) [9] are widely used for the treatment of many neuropsychiatric diseases such as anxiety [10], autism [11,12], depression [13], obsessive-compulsive disorder (OCD) [14,15]. Serotonin (5-hydroxytryptamine, 5-HT) transporter (SERT) belongs to Na+ / Cl– dependent solute carrier protein family which uses Na^+ and Cl^- ions as electrochemical gradients, is encoded by the SLC6 gene that includes transporters for neurotransmitters such as aminobutyric acid, norepinephrine, dopamine, and glycine [16]. The SERT regulates extracellular levels of 5-HT in the brain by transporting 5-HT into neurons and glial cells. It is also the target of illicit compounds like cocaine, ecstasy and amphetamines [17]. The serotonergic system plays an important role in a broad range of behavioral and physiological processes including cognition, mood, neuroendocrine function, memory, appetite and sleep as well as sexual behavior and anxiety [18, 19]. SERT is an integral membrane protein consisting of twelve putative trans-membranes (TMs) together with other monoamine neurotransmitters: Sodium symporters (NSSs) (eg. dopamine (DA) transporter (DAT), norepinephrine (NE) transporter (NET)) that control transmissions of ions and substrates, and terminate serotonergic signaling by uptake process of the released 5-HT into presynaptic nerve terminals [20]. Transport mechanism of NSS is proposed to be an alternate-access process [21,22,23]. In alternate-access process, centrally located substrate binding site (named S1), can be accessible from extracellular site (outward facing form) or from intracellular site (inward facing form) at a time [23,24,25]. Substrate occupies second substrate binding site, located in the extracellular vestibule (named S2), before displacement to the central site [26]. These two discrete binding sites exist in all three monoamine neurotransmitter transporters (MATs) (5-HT, DAT and NET) [27,28].

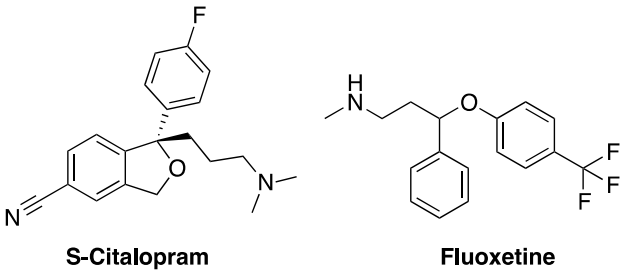
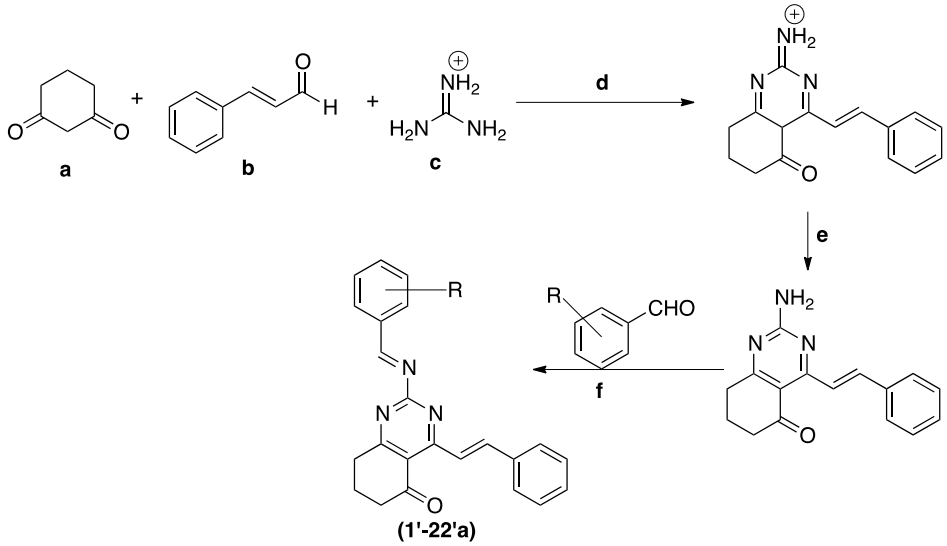


Figure 2. Structure of two SSRIs being used to manage depression clinically

The structural mechanism underlying SLC6 transporter function was largely unknown until 2005, when a high-resolution crystal structure of a bacterial homolog to mammalian SLC6 transporters, LeuT [29], provided the first structural insight into SLC6 transporter function. Since then, the LeuT structure has proven to be an excellent platform for constructing experimentally validated three-dimensional models of binding pockets for ions, substrate, and inhibitors in the human transporters [30-35]. As an important breakthrough; the crystal structure of human SERT (hSERT) has been resolved with both centrally-bound and allosterically-bound ligands at high resolutions in 2016 [36].



a = 1,3-cyclohexanedione; b = cinnamaldehyde; c = guanidinium hydrochloride; d = ethanol/H_2SO_4 e = sodium acetate; f = substituted aromatic aldehydes; R = 22 different substituents on the aromatic aldehyde, f.

Figure 3. The proposed synthetic route for compounds selected for this study

Many researchers have used softwares like discovery studio to build homology models of SERT, GOLD docking program, structure based phamacophore models, internal coordinate mechanics ICM, four-point pharmacophore models, combinatoral support vector machine, flexible docking protocols and observed that most of the known inhibitors favor close gap between extracellular gate consisting of TYR 176 and PHE 335 [37-51]

2. Materials and Methods

Three classes of twenty two compounds each, designed to have either of the two nuclei, (E)-4-styryl-7,8-dihydroquinazolin -5(6*H*)-one and 5-((E)styryl) pyrimido[4,5-d]pyrimidine-2,4(1*H*,3*H*)-dione were generated (virtual, Figure 4, see Table 1 for R- groups) and docked with SERT in this study, using AutoDock Vina to estimate the binding energies. The results were compared with that of S-citalopram and fluoxetine docked with SERT under the same conditions.

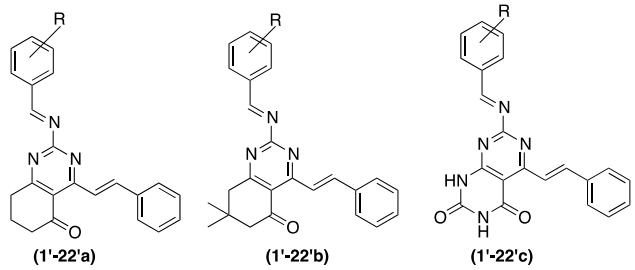


Figure 4. The general structures of the three classes of compounds used to generate the virtual library of 66 compounds (22 for each set, **a**, **b** & **d**). The **"b"** set was derived by replacing 1,3-cyclohexanedione with dimedone in the synthetic scheme while the **"c"** set was derived by replacing 1,3-cyclohexanedione with barbituric acid.

2.1. Protein Identification and Preparation

Crystal structure of human SERT complexed with S-citalopram was retrieved from the Protein Data Bank (PDB) with corresponding PDB ID 5I73 [36]. Pymol was used to remove impurities, water of crystallization and analyze the active site. MGL Tools 1.5.4 was used for the preparation of the protein for docking analysis. Bond orders were assigned, polar hydrogens and missing residues were added.

2.2. Ligand Preparation

The 3D structures of the compounds were prepared using chem3D and their energies were minimized with MM2 Force Field of Chem3D application interface and the ligands were saved as pdb files. MGL Tools 1.5.4 was used to generate the ligands' pdbqt files, setting the number of rotatable bonds to maximum.

2.3. Molecular Docking and *In-Silico* ADME Prediction

The protein and the ligands prepared above were used in molecular docking studies. MGL Tools 1.5.4 [50,51] was used to generate docking grid maps. The grid box of the macromolecule was assigned taking into consideration the binding site of citalopram to the SERT, in the downloaded PDB file. The dockings were performed using AutoDock Vina 1.1.2 which is a new generation of docking software from the Molecular Graphics Lab. Seven docking poses were generated for each ligand and they were viewed using pymol and the amino acids of the complexes within 4Å of the docked ligands and polar interactions were identified along with associated polar interactions. The binding energies of the compounds were compared with that of citalopram with SERT. All the Ligands were docked in the active site of the SERT and complexes with the high docking scores greater than that of citalopram were selected for further evaluation. Lipinski's rule and preADMET (an online program) were used to predict the druglikeness and pharmacokinetic properties of the compounds respectively. The ligands that were observed to be druggable and with favourable pharmacokinetics, were selected for synthesis and biological testing.

3. Results

In order to check the accuracy of the Vina docking program, we have docked the SERT co-crystallized ligand, S-citalopram into the SERT binding site.

The docking results were satisfactory when we analysed the root mean square deviation values (RMSD), which showed lower values (i.e RMSD < 3.0 Å).

Table 1. The list of the various substituents that R represents

S/N	R
1	H
2	*p*-OCH_3
3	2-hydroxy
4	3-hydroxy
5	3,4-dimethoxy
6	3,4,5-trimethoxy
7	*p*-dimethylamino
8*	1-naphthylaldehyde
9*	2-chloroquinoline-3-carbaldehyde
10	2-nitro
11*	Nicotinaldehyde
12	4-hydroxy
13	3,5-dimethoxy
14	3-methoxy-4-(methoxymethoxy)
15	4-hydroxy
16	4-sulfamate
17	3-hydroxy-4-methoxy
18	2,4,6-trimethoxy
19	3,5-dimethoxy-4-sulfamate
20	2,5-dimethoxy
21	3-nitro
22	2,4-dimethoxy

* represents aromatic aldehydes that are not substituted benzaldehydes.

From the result obtained from the molecular docking, the compounds that have binding energy greater than that of the citalopram were selected for druglikeness assessment and those that were found to be suitable were screened for their ADME properties with the main focus being blood brain barrier penetration. Most of the compounds have binding energy greater than that of citalopram. The compounds that have binding energy lower than or equal to that of the reference compound were eliminated (**18′b**, **19′a**, **15′c**, **19′c** and **6′a**) from the classes of compounds to be studied further due to their low binding energy while the remaining 61 compounds were assessed for druglikeness.

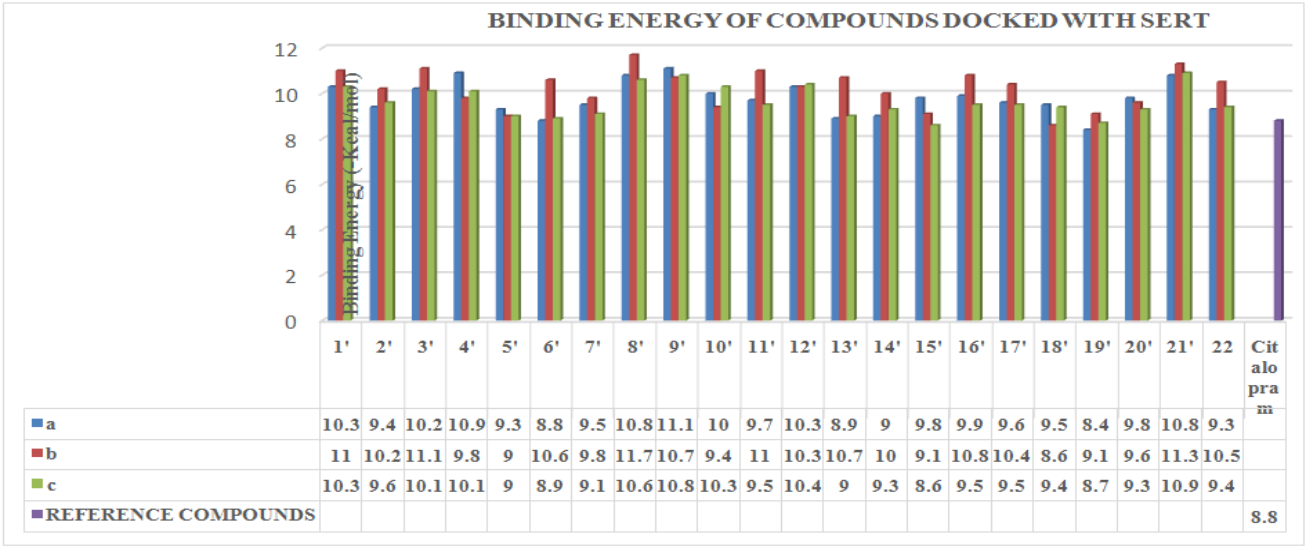
4. Discussion

Most of the compounds have binding energy greater than that of escitalopram (-8.8 kcal/mol) except for 5 compounds, **18′b** (-8.6 kcal/mol), **19′a** (-8.4 kcal/mol), **15′c** (-8.6 kcal/mol), **19′c** (-8.7 kcal/mol) and **6′a** (-8.8 kcal/mol) which have lower or the same binding energy with escitalopram (-8.8 kcal/mol, Figure 5). These five (5) compounds were eliminated from the study set of compounds due to their low binding energy and the remaining 61 compounds were assessed for druglikeness. Only one compound (**19′b**) failed the druglikeness assessment and was eliminated from the study set, reducing the number of compounds in the set to 60.

It is expedient that proposed CNS active agents possess the ability to cross the blood brain barrier. However, apart from crossing the lipophilic cell barrier, there is also the challenge of the drug efflux pump, P-glycoprotein (P-gp) which is an integral part of the BBB. The P-gp plays an important role in the bioavailability of CNS active agents in the brain [52]. Drug efflux by P-gp can prevent therapeutic brain concentrations of CNS active agents from being attained which results in treatment failure (Loscher & Potschka, 2005) [53]. Many antidepressants are substrates of P-gp (but not all) which determines to a large extent the distribution of antidepressants, that are its substrate, in the brain [52]. A study conducted by Karlsson *et al*., 2013, found that P-gp actively transports both the S- and R-enantiomers of citalopram and its two demethylated metabolites [54]. The propensity of escitalopram to be actively transported by P-gp may be responsible for over 30% of escitalopram treatment failure despite its clinical prevalence as an antidepressant. O'Brien *et al*., 2013 [55], suggested an adjunctive treatment with a P-gp inhibitor in a bid to increase escitalopram delivery to the brain by P-gp inhibition. The authors observed that escitalopram brain delivery was increased by P-gp inhibition using cyclosporine and verapamil resulting in enhanced antidepressant activity with three-fold increase in brain concentration. Ravikumar Reddy *et al*., 2016, also conducted a study which shows that natural flavonoids, silymarin and quercetin, improves the brain distribution of co-administered P-gp substrate drugs [56]. Therefore, taking into consideration the theory put forward by Pariante *et al*., 2004, that inhibition of P-gp may be involved in the mechanism of action of antidepressants [57], and the finding from Clark *et al*., 2009, that the P-gp inhibition by imipramine and desipramine was region specific after using various brain regions during *in-vivo* experiments as against previous authors who used whole brain for their assays and concluded that inhibition of P-gp was a potential mechanism of action for verapamil which facilitates the increase in the antidepressant drug concentration in the brain during treatment resistant depression [58], O'Brien *et al*., 2012b (a review) [52] and O'Brien *et al*., 2012a [59], hence, it is being proposed that in the design of new antidepressants, the ability of the proposed compounds to inhibit P-gp should be taken into consideration earlier such that there will not be need for co-administration of the antidepressant with a P-gp inhibitor as well as the tendency for the proposed compounds to serve as a P-gp substrate in this *in-silico* study that involves the screening of several compounds, with high binding affinity for SERT, by ADME prediction, focusing on BBB penetration and P-gp transporter association (inhibitor/substarte/non-substrate). Among the 60 compounds identified to have binding energy greater than/equal to that of escitalopram (-8.8 kcal/mol), a set of 31 compounds that have higher affinity for the SERT was selected. These 31 have binding energy greater than/equal to -10.0 kcal/mol. Their ADME properties were predicted *in-silico* using the online preADMET program. The ability to cross the lipophilic blood brain barrier was used as a benchmark for further screening (Figure 6) followed by their associations with P-gp. Essentially, the compounds to be selected for synthesis and further study are those that have the highest tendency to inhibit SERT, possess optimal physicochemical properties that allows them to cross the lipophilic BBB and are either P-gp inhibitors or non-substrates of P-gp. According to Ma *et al*., 2005 [60], using a classification of BBB penetration whereby compounds with high CNS absorption have values > 2.0, medium CNS absorption have values from 0.1-2.0 and those with low CNS absorption have values < 0.1 which are the values adopted for classification on the preADMET site. None of the "**c**"-class of compounds featured among those that have the potential to cross the lipophilic blood brain barrier and this may be ascribed to their higher polarity relative to the other set of compounds. This high polarity is due to the extra two -(NH)- groups in the six-membered ring that differentiates the three classes of compounds. The "**b**"-class of compounds having two methyl groups (which makes them more lipophilic) have among them the compounds with the highest BBB and most of them have high BBB. Among the 31 compounds with high binding energy, there was further classification based on their BBB penetration as follows; Compounds **6′b**, **13′b** and **14′b** have high BBB penetration, compounds **16′b**, **4′c** and **10′c** have low BBB penetration which is the category that escitalopram falls in, upon BBB penetration prediction on the preADMET site (escitalopram has BBB penetration value of 0.075). The other 25 compounds from among the 31 have medium BBB penetration (some close to 2.0), see Figure 6. Table 2 summarizes the BBB penetration, P-gp association and interactions with some CYP450 enzymes as a measure of hepatic metabolism for eleven compounds which have been selected for synthesis and further study. The first three compounds (**6′b, 13′b & 14′b**) in Table 2 have the best pharmacokinetic profile with regards to being

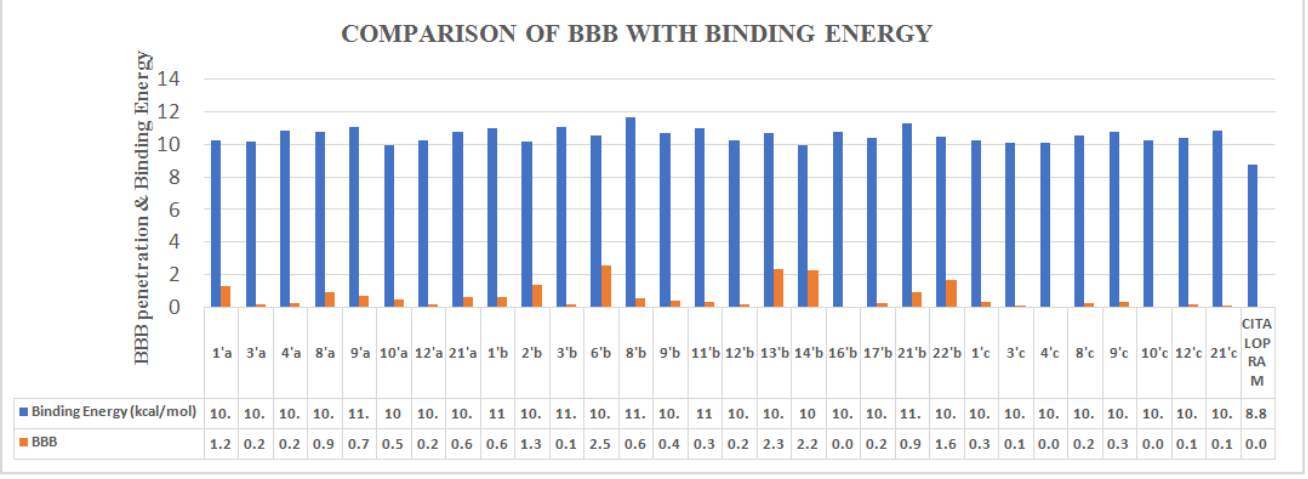
potential CNS active agents (antidepressants in this case). These three compounds are among those that have the highest tendency to inhibit SERT, have been predicted to have high BBB penetration and be possible P-gp inhibitors. All of the eleven compounds are predicted to have high plasma protein binding which may cause the compounds to have reduced distribution in the plasma but the high intestinal absorption may make up for the reduced plasma concentration by the high plasma protein binding. The cytochrome P450 enzyme, CYP3A4 is the one most implicated in the hepatic metabolism of most xenobiotics in the body and the three compounds (**6′b, 13′b & 14′b**) with the best pharmacokinetic profile for CNS bioavailability happen to be substrates of the CYP3A4 which might further reduce their bioavailability but their tendency to also inhibit CYP3A4 should offset the negative effect of CYP3A4 metabolism except for compound **13′b** which does not have the potential to inhibit the CYP3A4. Since this study is essentially a predictive *in-silico* study, it is hoped that a follow up study involving the synthesis and characterization of these proposed compounds would corroborate the predictions made so far in this attempt to develop new antidepressants.

The binding modes of the 11 compounds selected for synthesis from ADME and binding energy analysis are such that they have preference for the allosteric site (Figure 7). Some of the compounds (**2′b**, **6′b**, **13′b**, **14′b** and **22′b**) have extensions into the narrow gap between the two sites which consists of the extracellular gate and compound **14′b** reaching as far as the central site (Figure 8a). The interaction of these compounds with the protein is interesting and implies that these compounds can effectively block the passage between the allosteric and central site while occupying the cavity that is the allosteric site (Figure 8b). Most of the selected compounds have very strong interactions with ARG104, some having more than one polar interaction with ARG104 (Table 3). Table 3 summarizes the interactions of the selected compounds with the protein (residues within 4Å of the bound ligand) using colour coding to distinguish the residues from different sites. The red coloured residues represent the residues in the central site, the blue coloured residues represent the residues in the allosteric site, the orange coloured residues represent residues in the extracellular gate while the black residues represent other residues within 4Å of the ligands. The prevalence of blue coloured residues for the selected compounds is proof of their preference for the allosteric site.



	1'	2'	3'	4'	5'	6'	7'	8'	9'	10'	11'	12'	13'	14'	15'	16'	17'	18'	19'	20'	21'	22	Citalopram
a	10.3	9.4	10.2	10.9	9.3	8.8	9.5	10.8	11.1	10	9.7	10.3	8.9	9	9.8	9.9	9.6	9.5	8.4	9.8	10.8	9.3	
b	11	10.2	11.1	9.8	9	10.6	9.8	11.7	10.7	9.4	11	10.3	10.7	10	9.1	10.8	10.4	8.6	9.1	9.6	11.3	10.5	
c	10.3	9.6	10.1	10.1	9	8.9	9.1	10.6	10.8	10.3	9.5	10.4	9	9.3	8.6	9.5	9.5	9.4	8.7	9.3	10.9	9.4	
REFERENCE COMPOUNDS																							8.8

Figure 5. A bar chart showing the binding energy of the compounds docked with SERT and that of the reference compounds citalopram



	1'a	3'a	4'a	8'a	9'a	10'a	12'a	21'a	1'b	2'b	3'b	6'b	8'b	9'b	11'b	12'b	13'b	14'b	16'b	17'b	21'b	22'b	1'c	3'c	4'c	8'c	9'c	10'c	12'c	21'c	CITALOPRAM
Binding Energy (kcal/mol)	10.	10.	10.	10.	11.	10	10.	10.	11	10.	11.	10.	11.	10.	11	10.	10.	10	10.	10.	11.	10.	10.	10.	10.	10.	10.	10.	10.	10.	8.8
BBB	1.2	0.2	0.2	0.9	0.7	0.5	0.2	0.6	0.6	1.3	0.1	2.5	0.6	0.4	0.3	0.2	2.3	2.2	0.0	0.2	0.9	1.6	0.3	0.1	0.0	0.2	0.3	0.0	0.1	0.1	0.0

Figure 6: A bar chart showing the binding energy of the compounds that have the highest tendency to inhibit SERT, having binding energy ≥ 10.0 kcal/mol and comparing with their BBB penetration

Table 2. Table of predicted ADME properties for some selected compounds, showing values for BBB penetration, P-gp association, human intestinal absorption, plasma protein binding and CYP 450 enzymes associations as a measure of hepatic metabolism

Compounds	BBB	P-gp Inhibition	Plasma Protein Binding (PPB)	Human Intestinal Absorption (HIA)	CYP2C19 Inhibition	CYP2C9 Inhibition	CYP2D6 Inhibition	CYP2D6 Substrate	CYP3A4 Inhibition	CYP3A4 Substrate
6b	2.58	Inhibitor	91.076	98.039	Inhibitor	Inhibitor	Non	Non	Inhibitor	Substrate
13b	2.34	Inhibitor	92.845	97.632	Inhibitor	Inhibitor	Non	Non	Non	Substrate
14b	2.29	Inhibitor	90.592	98.039	Inhibitor	Inhibitor	Non	Non	Inhibitor	Substrate
22b	1.66	Inhibitor	91.886	97.632	Inhibitor	Inhibitor	Non	Non	Non	Substrate
2b	1.37	Inhibitor	92.975	97.432	Inhibitor	Inhibitor	Non	Non	Non	Weakly
1a	1.29	Inhibitor	93.650	97.369	Inhibitor	Inhibitor	Non	Non	Non	Weakly
8a	0.93	Inhibitor	96.232	97.795	Inhibitor	Inhibitor	Non	Non	Non	Weakly
9a	0.70	Inhibitor	93.836	97.770	Inhibitor	Inhibitor	Non	Non	Non	Weakly
21a	0.61	Inhibitor	93.447	99.412	Non	Inhibitor	Non	Non	Non	Weakly
1b	0.61	Inhibitor	94.140	97.418	Inhibitor	Inhibitor	Non	Non	Non	Weakly
8b	0.60	Inhibitor	98.336	97.896	Inhibitor	Inhibitor	Non	Non	Non	Weakly

Table 3. Table showing the residues within 4Å of the docked compounds with colour coded residues showing the orientation of the compounds within the protein

Compound (site bound to)	**Binding Energy (-kcal/mol)**	**Interactions**	
		Polar	**Key residues**
1′b (allosteric site)	11	ARG 104	ASP 98, ARG 104, 1LE 108, ALA 331, GLN 332, PHE 335, SER 336, GLY 498, PRO 499, LEU 502, ILE 552, PHE 556
2′b (allosteric site, but with extension into extracellular gate)	10.2	ARG 104 X 3	ASP 98, ARG 104, TYR 107, ILE 108, GLN 111, TYR 176, ALA 331, PHE 335, GLU 493, LEU 502, ILE 552, PHE 556
6′b (allosteric site but with extension into gate)	10.6	ARG 104 X 2	ASP 98, GLY 100, ARG 104, TYR 107, ILE 108, GLN 111, TYR 175, TYR 176, ALA 331, PHE 335, GLU 493, LEU 502, ILE 552, PHE 556
8′b (allosteric site)	11.7	ARG 104 X 2	ASP 98, ARG 104, TYR 107, ILE 108, GLN 111, TYR 175, TYR 176, ALA 331, PHE 335, GLU 493, GLY 498, PRO 499, LEU 502, PHE 556
13′b (allosteric site but with extension into gate)	10.7	ARG 104 X 2	ASP 98, GLY 100, ARG 104, TYR 107, ILE 108, GLN 111, TYR 175, TYR 176, ALA 331, PHE 335, SER 336, GLU 493, LEU 502, PHE 556
14′b (allosteric site but with extension into gate & central site)	10	-	TYR 95, ASP 98, GLY 100, ASN 101, ARG 104, ILE 108, ALA 331, GLN 332, PHE 335, SER 336, GLY 338, GLY 498, PRO 499, LEU 502, ILE 552, PHE 556
22′b (allosteric site but with extension into gate)	10.5	-	ASP 98, GLY 100, ASN 101, ARG 104, ILE 108, TYR 176, ALA 331, GLN 332, PHE 335, SER 336, GLY 498, PRO 499, LEU 502, ILE 552, PHE 556
1′a (allosteric site)	10.3	ARG 104	ASP 98, ARG 104, ILE 108, ALA 331, GLN 332, PHE 335, SER 336, GLY 498, PRO 499, LEU 502, ILE 552, PHE 556
8′a (allosteric site)	10.8	-	ARG 104, ILE 327, GLN 332, PHE 335, GLU 493, GLU 494, ILE 552, SER 555, PHE 556, PRO 561
9′a (allosteric site)	11.1	ARG 104 SER 555	ARG 104, TYR 107, ILE 108, ILE 327, PHE 335, GLU 493, ILE 552, SER 555, PHE 556, SER 559, PRO 561
21′a (allosteric site)	10.8	-	ALA 96, ASP 98, GLY 100, ASN 101, ARG 104, ILE 108, ALA 331, GLN 332, PHE 335, SER 336, GLY 498, PRO 499, LEU 502, ILE 552, PHE 556
CITALOPRAM in the allosteric site		GLN 332	ARG 104, ALA 331, GLN 332, PHE 335, GLU 493, GLU 494, PRO 499, LEU 502, ILE 552, PHE 556
CITALOPRAM in the central site		TYR 95	TYR 95, ALA 96, ASP 98, ALA 169, ILE 172, ALA 173, TYR 175, PHE 335, SER 336, GLY 338, PHE 341, GLY 442, LEU 443, THR 497, GLY 498, VAL 501
CITALOPRAM (DOCKED) in the central site	8.8	-	TYR 95, ILE 172, ALA 173, TYR 176, PHE 335, PHE 341, SER 438, GLY 442, LEU 443, GLU 493, VAL 501

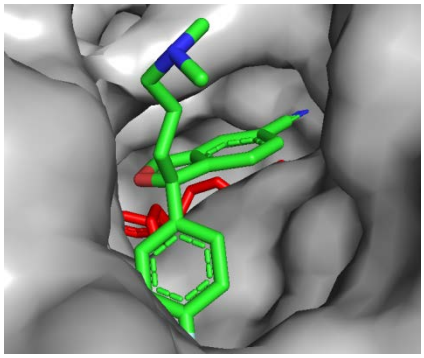


Figure 7. Escitaloprams lodged in the central and allosteric site of the SERT. The green coloured escitalopram is in the allosteric site while the red coloured escitalopram is embedded in the central site and both citaloprams are separated by the extracellular gate

It is important to note that the allosteric site is extremely malleable and physically changes shape in response to ligands [61] and the authors, Coleman *et al*., 2016 [61] are of the opinion that this plasticity could be exploited in future drug design work. If indeed these compounds have the predicted strong polar interaction with ARG104 within the lining of the allosteric site, it may contribute to its mechanism of inhibition of the SERT. It will, therefore, be interesting to know whether the plasticity of this site contributes to the affinity of these compounds for the allosteric site in a future study and whether the strong binding of the compounds (by virtue of their high binding energies and interaction with ARG104) and tendency to occlude the site will prevent serotonin reuptake and by extension cause the desired antidepressant effect.

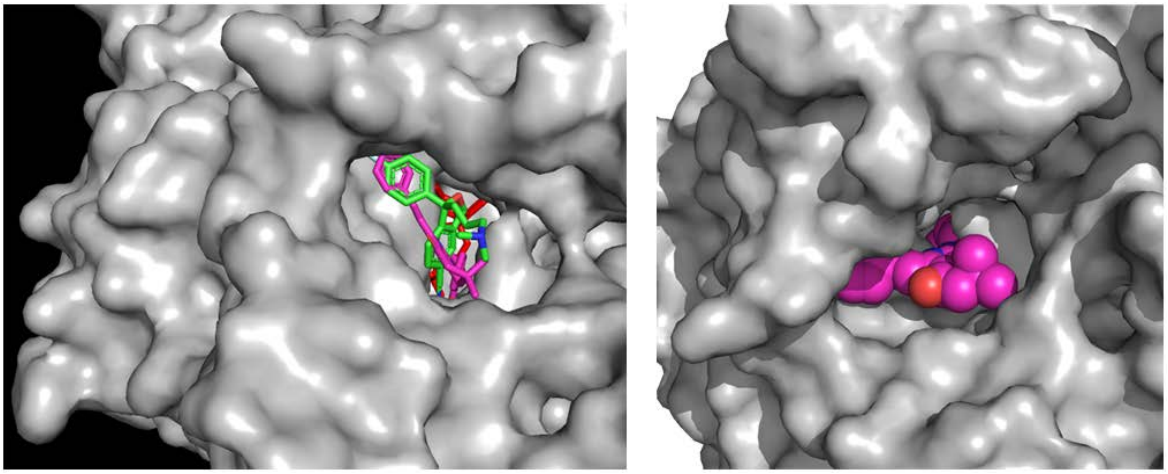


Figure 8. (a) Compound **14′b** (pink) bound to SERT upon molecular docking having most of it in the allosteric site, superimposed with escitalopram (green) in the allosteric site and a bit of it extending into the central site through the extracellular gate. (b) Binding pose of compound 14b rendered as spheres depicting the complete blockade of the central site by virtue of its occupation of the allosteric site and extracellular gate

5. Conclusion

This study has investigated a class of virtual compounds as possible SERT inhibitors and antidepressants using computational techniques. A set of 66 compounds have been screened using binding energy, drug-likeness assessment and ADME prediction to prune down to 11 compounds having high inhibitory potential for the SERT and favourable CNS pharmacokinetic profile in terms of ability to cross the lipophilic blood brain barrier and P-gp inhibition in order to facilitate high concentration in the brain. These 11 compounds have therefore been proposed for synthesis, characterization, *in-vitro* and *in-vivo* antidepressant study using animal models.

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