An in-silico approach of assessments of human serotonin transporter inhibitory potential of various flavonoids for anti-depressants: molecular docking, MM-GBSA, molecular dynamics simulation studies

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Abstract

Depression is one of the typical CNS disorders. Millions of people suffer from depression, a chronic illness with economic consequences. Tricyclic antidepressants, selective dopamine reuptake inhibitors, selective norepinephrine reuptake inhibitors, and selective serotonin reuptake inhibitors are only few of the antidepressants that can used to treat depression. The main target of therapeutic activity is known to be the serotonin transporter (SERT) against depressants. In this article, various flavonoids were found with conditions of pharmacological activity and were designed by molecular docking, MM-GBSA and molecular dynamics (MD) Simulation studies for the treatment of depressants activity. The docking of ligands performed against depressant with protein of human serotonin transporter (SERT) PDB-ID:5I6X are performed by using Glide module, in silico ADMET screening by QikProp module, binding energy using Prime MMGB/SA module, MD simulation by Desmond module and atomic charges were derived by Jaguar module of Schrodinger suite 2021-1. Compounds with top binding affinity using extra precision in glide recorded as (-16.25) when compared to standard FDA approved drug Fluoxetine (-8.711) which were proposed for anti-depressant action. The residues PHE 335, TYR 95, ALA 96, PHE 341, VAL 501, TRP 103, TYR 175, ALA 169, GLY 338 of SERT Play a crucial role as binding pocket of ligands. The in-silico ADMET properties of the molecules were within the recommended values. The binding free energy was calculated using PRIME MM-GB/SA studies. Compound with top binding affinity of flavonoids was subjected to MD simulation at 100 ns to study the dynamic behavior of protein–ligand complex.

Introduction

CNS diseases have been associated with numerous neurotransmitter systems (Wang, Zhou et al. 2021). Depression is one of the typical CNS disorders. Millions of people suffer from depression, a chronic illness with tremendous economic consequences. One of the primary causes of illness or suicide among all ages, regardless of social background, is depression(Reeves, Ladner et al. 2010, Cates, Roberts et al. 2013), and it has been predicted that in the near future, mental disorders linked to depression would be the second greatest cause of death globally.

Tricyclic antidepressants, selective dopamine reuptake inhibitors, selective norepi-nephrine reuptake inhibitors, and SSRIs (selective serotonin reuptake inhibitors) are only a few of the antidepressants that can be used to treat depression (Xing, He et al. 2013). They exert against depression by interfering with the transporters that transport serotonergic (5-HT), dopaminergic (DA), and noradrenergic (NA) neurotransmitters across membranes, or by preventing monoamine oxidase from degrading these neurotransmit-ters(Olivares-Nazario, Fernández-Guasti et al. 2016). However, currently FDA-approved medications have some side effects and poor efficacy(Harmer, Duman et al. 2017). Therefore, a successful approach for the treatment of depression is the development of innovative antidepressants with high efficacy and low toxicity (Fournier, DeRubeis et al. 2010).

The main target of therapeutic activity is known to be the serotonin transporter (SERT) (Gabrielsen, Ravna et al. 2012). The most common form of treatment or medication for depression is antidepressant pills or

agents (Dessalew and Chemistry 2009). A monoamine transporter called the serotonin transporter (SERT) transports serotonin from the synaptic cleft into the presynaptic neuron, where it is crucial in the termination of serotonergic neurotransmission serotonin is typically reabsorbed by the nerve cells (also known as “reuptake”). It’s thought that SSRI's work by blocking (“inhibiting”) reuptake, meaning more serotonin is available to send additional impulses between nearby nerve cells.as shown in Fig. 1 (Muszynski, Scapozza et al.1999).

The concentration of synaptic serotonin regulates the uptake of serotonin into the presynaptic terminal, therefore any drug that can inhibit or block serotonin from entering the presynaptic terminal is thought to be a possible antidepressant (Olasupo, Uzairu et al. 2020). There are many antidepressant agents (drugs) as inhibitors of serotonin transporter (SERT) in clinical use, such as Citalopram (Celexa), Fluoxetine (Prozac), Sertraline (Zoloft), and others, but they all have varying degrees of side effects, such as allergic reactions, liver failure, constipation, and unresolved mechanisms of action in some cases (Bhat, Newman et al. 2019, Olasupo, Uzairu et al. 2020).

The pharmacological effects of flavonoids (Harborne and Williams 2000), which are naturally occurring polyphenols, have been extensively investigated. In the past few decades, numerous research have been carried out to study the antidepressant efficacy of natural chemical compounds, particularly flavonoids, which have diverse effects on the central nervous system(Bakoyiannis, Daskalopoulou et al. 2019). Some flavonoids have been reported to antidepression behaviour in rats in animal models, according to a number of preclinical investigations that have demonstrated their antidepressant poten-tial. The suggested underlying mechanisms for anti-depressant activity include increased expression levels of several neurotransmitters, neurotrophic factors, and neurogenesis in brain(Guan and Liu 2016).

Recently, many pharmaceutical firms have used computer-aided drug design (CADD) and cheminformatics research as important modern approaches in the processes of drug discovery, design, and development (Wang, Li et al. 2015). We have chosen a va-riety of phytoconstituents for their biological activities in the present study, which uses in silico and wet lab approaches to uncover active compounds for various biological activi-ties(Yang, Li et al. 2016). Therefore, in an effort to consolidate findings in current study using in silico methods to identify active compounds for a variety of biological activities from molecular docking analysis, pharmacokinetics study, and to examine binding in-teractions, we investigated pharmacokinetic properties and predicted flavonoids to search for novel inhibitors with better biochemical interactions and excellent pharmacological properties as potential anti-depressants. There are many different modules in Schrodinger Suite LLC, including Glide, Qikprop, Prime, Desmond, and others. In the current investigation, molecular docking was used to determine the binding modalities in order to identify inhibitors that target the human serotonin transporter. Additional MMGB-SA post docking minimization and molecular dynamics simulations were carried out to determine the patterns of natural chemical binding in the SERT active site (5I6X.pdb).

Materials and methods

Experimental procedures

We have confirmed the structures of more than 50 compounds have been reported in our previously published literature which have certain pharmacological effects for depression(Khan, Perviz et al. 2018, Ko, Kim et al. 2020, Pannu, Sharma et al. 2021). To identify compounds with a higher binding affinity than Fluoxetine, we used SP docking, resulting in the identification of 35molecules. Following the SP docking results,. Furthermore, we redocked these 25 compounds utilizing XP docking, and MM-GBSA analysis was further used to screen a total of 25 molecules with a good binding affinity. Induced-fit docking (IFD) was performed to identify the interactions between these 25 molecules and SERT receptor and performed density functional theory based quantum mechanics calculations. Based on the ligand-receptor interactions, and structure type (Figure), top binding affinity of ligand-receptor complexes were subjected to a molecular-dynamics (MD) simulation to assess their stability, and the ADME/T properties of these 25 molecules were evaluated.

Preparation of protein

The 3D x-ray crystal structure of ts3 human serotonin receptor with crystallized ligand (PDB ID: 5I6X) was retrieved from the RCSB protein data bank (RCSB PDB – 5I6X: X-ray structure of the ts3 human serotonin transporter complexed with paroxetine at the central site) with resolution of 3.14 Å. The above protein was prepared by protein preparation wizard module of Schrodinger suite 2021-1. Water molecules without hydrogen bonds are deleted. Missing chain atoms are added by using prime module of Schrodinger suite 2021-1. The possible ionization states were generated for the heteroatom present in the protein structure and the most stable state was chosen. Finally, a restrained minimization of the protein structure was carried out using OPLS4 force field to reorient side-chain hydroxyl groups and alleviate potential steric clashes. A grid box was generated at the centroid of active site for docking studies (Kalirajan, Pandiselvi et al. 2019).

Ligand preparation

The structures of the ligands were generated and subjected to LigPrep module of Schrodinger suite 2021. Structures were converted from 2D to 3D structures by including stereo chemical, ionization, tautomeric variations, as well as energy minimization and optimized for their geometry, desalted and corrected for their chiralities and missing hydrogen atoms. The ionization and tautomeric states were generated between pH of 7.0 ± 0.2 using Epik module. In the final stage of LigPrep, compounds were minimized using optimized potentials for liquid simulations 3 (OPLS4) force field in impact package of Schrodinger until a root mean square deviation of 1.8A0 was achieved. A single low en-ergy ring conformation per ligand was generated and the optimized ligands were used for docking analysis (Rajagopal, Byran et al. 2020).

Receptor Grid generation

The co-crystallized ligand was held in the protein structure of the protein ar-ranged from protein preparation wizard and it was utilized for the receptor lattice development. A Grid box was produced to

characterize the centroid of the dynamic site which is utilized for docking. A Grid box was generated (x = -32.918; y = -21.855 ; z = 1.682) at the centroid of active site keeping the Van der Waals scaling of 0.8 for the receptor with 0.15 as the partial charge cut-off (Bairi, Ramachandran et al. 2022, Ribaudo, Yun et al. 2023).

Molecular docking (Glide docking) studies

Using the Glide module of the Schrödinger suite 2021-1, the compounds were docked into the catalytic pocket of the serotonin transporter protein (PDB ID: 5I6X). The Glide score tool was used to choose the top docked molecules. Using the Glide ligand docking program, the beneficial contacts between the ligand molecules and the receptor were assessed. OPLS4 force field and standard precision (SP) mode and extra precision (XP)mode were both used for all docking calculations (Jupudi, Rajagopal et al. 2022). The docking procedure described above was carried out in flexible docking mode, generating conformations for each input ligand automatically. This approach penalizes, steric collisions while recognizing advantageous hydrophobic, hydrogen-bonding, and met-al-ligation inter-actions. The Glide Score scoring function was then used to reassess the poses that had been minimized. Active compound XP-Glide scores have been calculated and com-pared to the Glide scores of standard compounds including Selective serotonin reuptake inhibitors (SSRI) drug fluoxetine (Kalirajan, Sankar et al. 2017).

Binding free energy calculation by using prime/MMGBSA approach

The binding free energy of ligand-receptor complex and post docking energy minimization studies were performed using prime molecular mechanics-generalized born surface area (MM-GB/SA) of Schrodinger 2021 (Ylilauri, Pentikäinen et al. 2013). The energy for minimized XP docked pose of ligand receptor complex was calculated using the OPLS4 force field with distance from ligand $0A^0$ and generalized born/surface area (GB/SA) continuum VSGB 2.0 solvent model (Tuccinardi 2021)

In silico predicted ADMET properties for phytoconstituents

Prediction of physically and pharmacokinetically significant descriptors was performed by employing various tools such as QikProp module of Schrodinger suite. Properties like Molecular weight), SASA (Total solvent accessible surface area, Hydrogen bond acceptor and donor count, log P, Human oral absorption, log S, Molar volume, Dissociation constant (KD), No. of violations in lipinski's rule of five, Van der Waals Volume etc (Mohamed, Omar et al. 2023) .

Induced fit docking studies

Schrödinger suite 2021-1 developed the induced-fit docking (IFD) approach, which simulates the conformational changes based on by ligand interaction. In this method, the complex is used to generate the centroid of the residues by choosing the ligand from the protein. Each ligand is initially docked using the softening potential (van der Waals radius scaling). Then, sidechain prediction is done up to a certain distance from the ligand pose. The same set of residues and the ligand are then minimised for each protein-ligand complex posture. Consequently, the receptor structure in each pose indicates an induced fit

to the ligand structure and conformation (Liu, Yang et al. 2023). Finally, Glide XP is used to dock the ligand more precisely into the induced-fit receptor structure. Both the receptor and the ligand were subjected to a van der Waals scaling factor of 0.5 during the initial docking. The sidechains of the residues within 5 of the ligand were subjected to the Prime refining process. Each docked ligand was given a maximum of 20 positions to choose from when redocking in XP mode.

Molecular dynamics simulations

We performed a molecular dynamics simulation using the Desmond module of Schrodinger 2021-1, LLC, New York, NY, in order to examine the atomic-level binding behaviour of highly rated chemicals and comprehend the molecular interaction analysis. The complexes were solved using the TIP3P water model in an orthorhombic periodic boundary condition with a 10 A buffer between the box edges and the protein atoms. By adding 0.15M NaCl counter ions, the solvated system was made neutral. Following that, the system was minimized using the OPLS4 force field as default program. Using the smooth particle mesh Ewald approach, the long-range electrostatic interactions were computed with a 1e-09 tolerance. At a cut-off radius of 9.0 A, the short-range Van der Waals and Coulomb interactions were estimated. The MD simulation was run for a total of 100 ns with a time step of 2 fs in an isothermal-isobaric ensemble (NPT) at 300 K and 1 bar of pressure (Adcock and McCammon 2006). The Martyna-Tobias-Klein barostat technique and the Nose-Hoover chain thermostat were combined at 100 and 200 ps, respectively (Jupudi, Rajagopal et al. 2022). For bonded, short-range non-bonded, and long-range electrostatic forces, 2, 2, and 6 fs of a multiple time-step technique called RESPA (Reference System Propagator technique) were utilised, accordingly. Every 100ps, data was collected, and the generated trajectories were examined (Shivakumar, Williams et al. 2010).

QM/MM (Quantum Mechanics / Molecular Mechanics)-DFT Calculations

The earlier explained induced fit docking procedure produced the geometries used in the QM/MM computations. The ligands with the greatest docking scores and the protein with PDB-ID 5I6X were used in the QM/MM calculations. The Q site program was used to calculate QM/MM. This required incorporating the ligand as well as the interacted residues. The free ligand and its sidechains were chosen, and then the QM area was built. We chose the DFT-B3LP-D3 technique, which is dependent on electron density, for QM calculations utilising Jaguar and basis set 6-31G. The charge of the QM system was − 1(Mohamed, Omar et al. 2023). The OPLS-4 force field was applied to the MM region, which included the rest of the system and score was calculated (Yadav, Kumar et al. 2016).

Results and discussion

In-silico predicted ADMET properties

The in-silico ADMET properties for the 25 phytoconstituents were predicted using various software tools. Molecular weights of the compounds were observed in the range 254.242 to 610.568 $g.mol^{-1}$. Total

solvent accessible surface area (SASA), which is surface sum of polar atoms, was observed between 477.168 and 852.772 A0

ADMET features were predicted using the Schrödinger suite 2021 Qik prop module. Properties such as molecular weight, dipole, hydrogen bond donor, hydrogen bond acceptor, log P o/w, and Lipinski's rule of five are identified and mentioned in Table 1 below. According to Lipinski's rule of five, the molecule's molecular weight should be ≤ 500, the partition coefficient should be ≤ 5, and the number of hydrogen bond donors and acceptors should be ≤ 5 and ≤ 10, respectively (Rajagopal, Kalusalingam et al. 2023). All of these qualities, together with molecular flexibility, are thought to be important drivers of oral bioavailability. The globularicitrin ligand possesses a molecular weight of 610.524, a dipole moment of 10.222, an estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution is 6, and an estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution is 20.55. With fewer exceptions, the obtained ADMET attributes are within the suggested ranges.

Table 1
In-silico ADMET screening results of 25 molecules using Qikprop module

Compounds	Mol_MW	Dipole	SASA	DonorHB	AccptHB	Qplogpo/W	Rule of five
Chrysin	254.242	3.651	477.168	1	3	2.368	0
Naringenin	272.257	2.678	482.449	2	4	1.549	0
Naringin	580.541	1.697	852.772	7	19.3	-1.461	3
Miquelianin	478.365	10.534	663.403	6	13.05	-0.756	2
Vitexin	432.383	4.019	636.798	6	12.25	-0.97	1
Nobiletin	402.4	3.199	665.93	0	7	3.733	0
Fiestin	286.24	3.904	506.165	4	5.5	0.477	0
Fluoxetine Std	309.331	4.33	584.53	1	2.25	4.713	0
Apigenin	270.241	3.035	489.281	2	3.75	1.624	0
Baicalin	446.367	10.763	687.031	4	11.55	0.434	2
Cynaroside	448.382	10.846	701.542	6	13	-0.948	2
Hesperidin	610.568	3.45	826.23	7	20.05	-1.324	3
Isoquercetrin	464.382	6.747	664.702	7	13.75	-1.37	2
Isorhamnetin	316.267	1.061	535.065	3	5.25	1.236	0
Kamepferol 7,4 Dimethyl Ether- 3-O-Beta-D-Gluco-Pyranoside	476.436	9.123	700.547	4	13	0.666	1
Kamepferol	286.24	4.588	503.264	3	4.5	1.036	0
Luteolin	286.24	4.161	500.018	3	4.5	0.941	0
Myricetin	318.239	6.044	524.902	5	6	-0.303	1
Ombuin	330.293	7.444	558.725	2	5.25	2.049	0
Quercetin	302.24	4.899	514.002	4	5.25	0.362	0
Quercetrin	448.382	8.916	661.117	6	12.05	-0.569	2
Globularicitrin	610.524	10.222	742.594	6	20.55	-2.637	3
Scutellarin	462.366	9.773	699.151	5	12.3	-0.258	2
Wogonin	284.268	3.563	496.761	1	3.75	2.493	0

Compounds	Mol_MW	Dipole	SASA	DonorHB	AccptHB	Qplogpo/W	Rule of five
Hyperoside	464.382	10.588	659.143	7	13.75	-1.26	2

MolMW Molecular weight of the molecule with range of 130.0–725.0; Dipole Computed dipole moment of the molecule (1.0–12.5); SASA Total solvent accessible surface area (SASA) in square angstroms using a probe with a 1.4 Å radius (300.0–1000.0); Donor HB Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution. Values are with recommended ranges of (0.0–6.0); Accpt HB Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. Values are averages with recommended range of (2.0–20.0); QPlogP o/w Predicted octanol/water partition coefficient (–2.0–6); Rule Of Five Number of violations of Lipinski's rule of five [3]. The rules are: mol_MW < 500, QPlogP o/w < 5, donor HB ≤ 5, accpt HB ≤ 10. Compounds that satisfy these rules are considered druglike. (The "five" refers to the limits, which are multiples of 5). Range: Maximum is 4.

Molecular docking studies

The molecular docking studies of the designed ligands to protein active sites were per-formed by an advanced molecular docking program in Schrodinger suite-2021 for determining the binding affinities of the ligands (Pinzi and Rastelli 2019). The Phytoconstituents were docked into the serotonin transporter (5I6X) in order to ascertain their potential serotonin inhibition activity against a potential anti-depressant. The compounds (Figure s1) showed good affinity to the receptor when compared with standard bicyclic derivative of phenyl propyl amine (fluoxetine) with anti-depressant activity. The Glide scores of docking studies against SERT (PDB id 5I6X) are shown in Table 2. The obtained glide dock score is between − 16.25 and − 4.703 and the top score is for globularicitrin. It is clearly demonstrated that Compound has the highest G-score (-16.25) when compared to the standard

compounds which are proposed for depressant treatment such as fluoxetine (-8.711) The amino acid residues binding PHE 335,TYR 95,ALA 96,PHE 341,VAL 501,TRP 103,ILE 179,TYR 175 ALA 173,ILE 172,ALA 169 make hydrophobic interaction with ligand .The amino acid residues of GLY 442,GLY 338 ,GLY 498 ,GLY 100 make polar region. The lipophilic evidence of the aromatic moieties is which mostly causes the glide scores to increase. The Amino acid residues such as ASP 98 and GLU 493 form a negative charge around the ligand (Globularicitrin) and ARG104 forms a positive charge around the ligand. The discovered binding modes demonstrated the ligand created connections with various residues ILE 179 to VAL 501 surrounding the active pocket through hydrogen bonds, hydrophobic interactions and other mechanisms.

Table 2 Docking results of in-silico screening for the compounds based on their XP Gscores against human serotonin trans-porter (PDB ID: 5I6X)

ID	Compounds	Docking Score (kcal/mol) XP	XP Gscore (kcal/mol)	Glide Gscore (kcal/mol)	Glide Emodel (kcal/mol)
1.	Globularicitrin	-16.25	-16.285	-16.285	-45.13
2.	hesperidin	-12.63	-12.63	-12.63	-48.035
3.	scutellarin	-10.907	-10.913	-10.913	-69.79
4.	myricetin	-10.506	-10.553	-10.553	-63.508
5.	hyperoside	-10.319	-10.354	-10.354	-94.078
6.	naringin	-10.278	-10.278	-10.278	-71.376
7.	isoquercetrin	-10.062	-10.098	-10.098	-75.723
8.	fiestin	-10	-10.038	-10.038	-56.201
9.	isorhamnetin	-9.673	-9.712	-9.712	-55.058
10.	miquelianin	-9.655	-9.69	-9.69	-71.955
11.	luteolin	-9.519	-9.567	-9.567	-55.782
12.	quercetin	-9.153	-9.193	-9.193	-57.127
13.	naringenin	-8.95	-8.974	-8.974	-49.557
14.	kamepferol	-8.798	-8.837	-8.837	-53.522
15.	cynaroside	-8.766	-8.766	-8.766	-77.243
16.	fluoxetine std	-8.711	-8.712	-8.712	-63.424
17.	apigenin	-8.697	-8.745	-8.745	-57.11
18.	ombuin	-8.685	-8.696	-8.696	-59.111
19.	chrysin	-8.601	-8.653	-8.653	-46.906
20.	vitexin	-8.596	-8.639	-8.639	-84.08
21.	wogonin	-8.395	-8.439	-8.439	-58.769
22.	Kamepferol 7,4 Dimethyl ether-3-O-Beta-D-gluco-Pyranoside	-8.339	-8.342	-8.342	-63.417
23.	quercetrin	-8.175	-8.21	-8.21	-77.963
24.	Baicalin	-7.331	-7.34	-7.34	-67.73
25.	nobiletin	-4.703	-4.703	-4.703	-46.604

Binding free energy calculation by using MM-GBSA

Molecular docking was also evaluated with MM-GBSA free binding energy.which is related to post scoring approach for SERT Transporter (PDB ID: 5I6X) target. The accuracy of docking is confirmed by examining the lowest energy poses predicted by the scoring function. The Glide scores almost resemble the experimental binding mode as determined by X-ray crystallography. The Glide score and MM-GBSA free energy values are obtained by the docking of ligands into the binding pocket. The details of the MM-GBSA free binding energy for the compounds with standard shown in Table 3

Prime MMGBSA DG bind, the binding free energy, is calculated with the equation:

ΔG(bind) = Ecomplex(minimized) − Eligand(minimized) + E receptor (minimized)

Because of the significant negative values produced by all the test compounds in the MM/GBSA experiment, the energies that showed strong ligand binding in the binding pocket of 5I6X SERT transporter are van der Waals energy (MMGBA dG Bind vdW) and non-polar solv-ation (MMGBA dG Bind Lipo)(Friesner, Murphy et al. 2006). Other energies, such as covalent energy (MMGBA dG Bind Covalent) and electrostatic solvation (ΔGSolv), do not favor receptor binding. Moreover, greater negative values of (MMGBA dG Bind vdW and MMGBSA dG Bind Lipo) parameters demonstrate extraordinary hydrophobic interaction with 5I6X and ligands. According to the findings of the MM/GBSA research, the DG bind values for considerably active compounds were found to be in the range of 0.25 to − 44.99 kcal/mol.The OPLS4 force field and generalized-born/surface methods were used to compute the energy for the ligand receptor complex's minimised XP docked pose.continuum area (GB/SA) VSGB 2.0 solvent model and the energies are favorably contributing to the total binding energy (Rajagopal, Varakumar et al. 2021). Globularicitrin, which has the highest docking score, followed excellent DG bind values of − 44.99 kcal/mol with Prime energy − 35450.7 kcal/mol. By using MMGBSA scoring function, docking complex stability will be estimated. The Glide rating and the MM-GBSA free energy, which are more stable and are generated by docking ligands into the coupling pocket.

Induced fit docking scoring

Induced-fit binding is the mechanism by which proteins shift their sidechains and backbone when they bind to a ligand. It can be difficult to assume a rigid receptor and precisely describe the binding process because of this variability. Consequently, induced-fit binding poses a difficult factor in drug design. Table 3 lists the IFD scores of the top binding ligands. Our findings show that each compound had IFD scores comparable to those of the prepared ligand, indicating good binding in an active pocket of SERT(Gani, Nurhan et al. 2021).

In fact, Compound (1-Globularicitrin ,2-Hesperidin,3-scutellarin, 4-myricetin,5-hyperoside) showed better IFD scores than the prepared ligand, indicating that these compounds have a higher likelihood of interacting well with the 5I6X SERT receptor.

DFT- QM/MM (Quantum Mechanics/Molecular Mechanics) Analysis

In this study, the amino acids TYR 176 and ASP 98 were selected for top 10 compounds the QM region, as they are common interaction sites for the tested compounds. Table 3 represents the results of the QM/MM binding ernergy calculations,. The compounds exhibit a range.The binding energies of the compounds were also calculated and ranged from − 596.273 to − 591.081 hartrees. The energy of Molecular mechanics suggest that the ligands are stable and may have potential as bioactive agents (Philipp and Friesner 1999). However, further experiments are necessary to confirm their biological activity of compounds. Due to the complexity and size of proteins, it is frequently necessary to use hybrid quantum mechanics/molecular mechanics (QM/MM) methods, which combine a QM treatment of the ligand with a treatment of the protein and the solvent(Gani, Nurhan et al. 2021). Quantum mechanics (QM) calculations can provide valuable energies into the electronic properties and interactions of protein-ligand complexes. The optimum pose for the molecule was determined in the present work using an induced-fit docking (IFD) method, after which QM/MM calculations were performed. With this technique, the protein's active site could be treated using the QM method while the rest of the protein was treated using the MM method. This method has the benefit of giving precise findings without requiring the intensive computing time needed to calculate the QM for a large number of atoms.

Table 3
Results of Prime Energy, MMGB-SA DG Bind, Induced Fit Docking and Quantum Mechanics Binding Energies

ID	Compounds	Prime Energy (kcal/mol)	MMGB-SA DG Bind	IFD Score	QM/MM Energy
1.	Globularicitrin	-35450.7	-44.99	-1788.82	-596.273
2.	hesperidin	-35245.2	-16.91	-1774.89	-591.63
3.	scutellarin	-35366.7	-19.88	-1779.25	-593.083
4.	myricetin	-35425.6	-33.4	-1781.83	-593.944
5.	hyperoside	-35394.5	-18.29	-1780.08	-593.36
6.	naringin	-35259.3	0.25	-1773.24	-591.081
7.	isoquercetrin	-35395.9	-20.77	-1779.89	-593.298
8.	fiestin	-35325.7	-25.64	-1776.32	-592.108
9.	isorhamnetin	-35413.9	-28.99	-1780.41	-593.469
10.	miquelianin	-35390.5	-10.07	-1779.22	-593.072
11.	luteolin	-35510	-34.84	-1785.07	-
12.	quercetin	-35423.8	-33.67	-1780.38	-
13.	naringenin	-35382.3	-22.67	-1778.09	-
14.	kamepferol	-35439.3	-24.51	-1780.8	-
15.	cynaroside	-35405.6	-16.21	-1779.05	-
16.	fluoxetine std	-35215.4	-39.01	-1769.48	-
17.	apigenin	-35530.1	-29.99	-1785.25	-
18.	ombuin	-35394.8	-43.64	-1778.44	-
19.	chrysin	-35486.2	-17.8	-1782.96	-
20.	vitexin	-35461.3	-11.08	-1781.7	-
21.	wogonin	-35426.3	-29	-1779.75	-
22.	Kamepferol 7,4 Dimethyl ether-3-O-Beta-D-gluco-Pyranoside	-35379.5	-38.37	-1777.32	-
23.	quercetrin	-35404.1	-20.39	-1778.42	-
24.	Baicalin	-35302.6	25.46	-1772.47	-
25.	nobiletin	-35275.7	-23.33	-1768.49	-

Molecular dynamics simulation analysis

To assess and examine the movements of atoms in a system over a given time period, an accurate method is molecular dynamics simulation. a number of refinements to calculations and predictions, docking still provides a still view of a compound's binding pose in the protein's binding site(Hollingsworth and Dror 2018). using the conventional Newtonian equation for motion as an introduction(Adcock and McCammon 2006).A 100ns molecular dynamics simulation for the docked pose of Globularicitrin/5I6X complex revealed that the RMSD of protein Ca atoms (Fig. 3a) were stabilized after ligand binding showing minute fluctuations from 1.8 to 2.9 A0. The ligand RMSD (Fig. 4a) exhibited Partial fluctuations with 1.6–2.8 A0 which then stabilized at 18-25ns with showing RMSD of 2.0 to 2.4 A0 till 100 ns. The RMSF (Fig. 3b) of protein looks stable except amino acids initial from 120 to 140 showed higher fluctuations till 3.5 A0which are present in loops then stabilized, finally the protein looks stable except amino acids from 420 to 520 showing higher fluctuation. The interaction time of each amino acid residues is given in (Fig. 3c). It could be noted that the interaction times of amino acid residues of TYR 95, ASP 98, ARG 104 were greater than all other amino acids. Amino acid SER 336 interaction was steady for the first 20 nanoseconds (ns), and then interaction was lost. Again, interaction occurs from 60 to 100 ns and no interaction found between LEU 337 and GLY 435.From the ligand interaction fraction (Fig. 3d) the compound were positioned in the active pocket by forming hydrogen bonding, hydrophobic and water bridged interactions with TYR 95, ASP 98, ARG104, ASN 177, SER 336, GLY 338, SER 438, SER 439, TRP 103, ALA 169, TYR 175, PHE 334, ILE 172, ALA 173 and TYR 176. From the 2D ligand interaction diagram (Fig. 3e) of the 100ns simulation, docked pose of Globularicitrin /5I6X complex revealed and exhibited strong hydrogen bonds with ASP 98 of 90%, ARG 104 of 94% and TYR 95 of 86%of total simulation trajectory. And hydrophobic bonds were observed TYR 95, ALA 169, TYR 175 in a stimulation trajectory and formed extensive water bridges interactions with ligand protein contacts ASN 177, PHE 334 and TRP 103. A 100 ns molecular dynamic simulation was conducted. Thus Globularicitrin, which has the best fit in the binding pocket in the binding site of protein 5I6X SERT Transporter

Conclusions

In conclusion, from the various flavonoids of (Globularicitrin to cymaroside), many compounds have significant binding affinity with human serotonin transporter. Molecular docking and binding free energy calculation studies were performed to find the possible binding modes of ligands and the influence of favorable and non-favorable interactions within the active pocket of serotonin SERT protein. MD simulation for the highly active inhibitor Globularicitrin in complex with protein 5I6X revealed that the stabilization of ligand was achieved due to the formation of uninterrupted hydrophobic-Bond interactions and ionic interactions. The results demonstrated for further modifications in induced fit binding analysis and quantum mechanics may help in improvement of inhibitory activity. The in-silico structuring strategy embraced in the present investigation helped for recognizing some lead molecules and furthermore ADMET evaluated pharmacokinetically passed and further determinations like in vitro and in vivo assessments. The results from the in-silico study exhibited that compounds ID of (1–15) may be

significantly active against serotonin for depressants with remedial possibilities and are probably going to be helpful after further refinement studies.

Declarations

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Author contribution Writing—original draft: S.N., L.B. and C.A.; Conceptualization: S.N. and S.J.; Supervision: M.S. and L.S.W; Investigation: S.D., S.J. and M.S.; Resources: S.D., M.S., and L.S.W.; Data curation: S.N., L.B., S.J. and C.A.; Writing—review and editing: S.N., C.A., L.B., S.J., S.D., M.S. and L.S.W. All authors have read and agreed to the published version of the manuscript.

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Figures

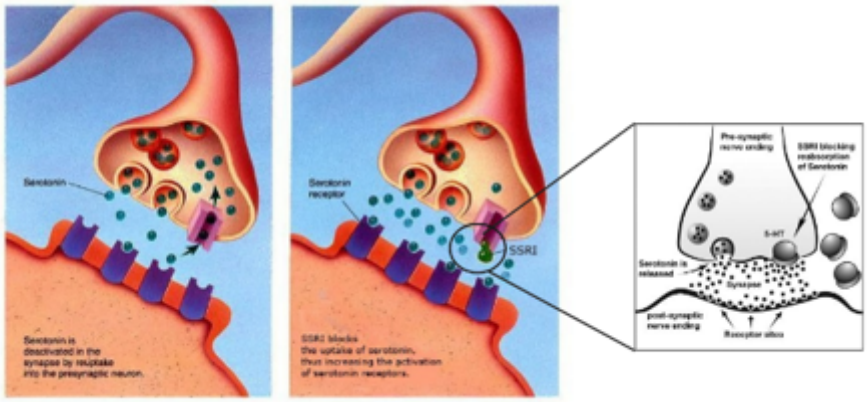

Figure 1

Represents how do SSRI's actually work ?

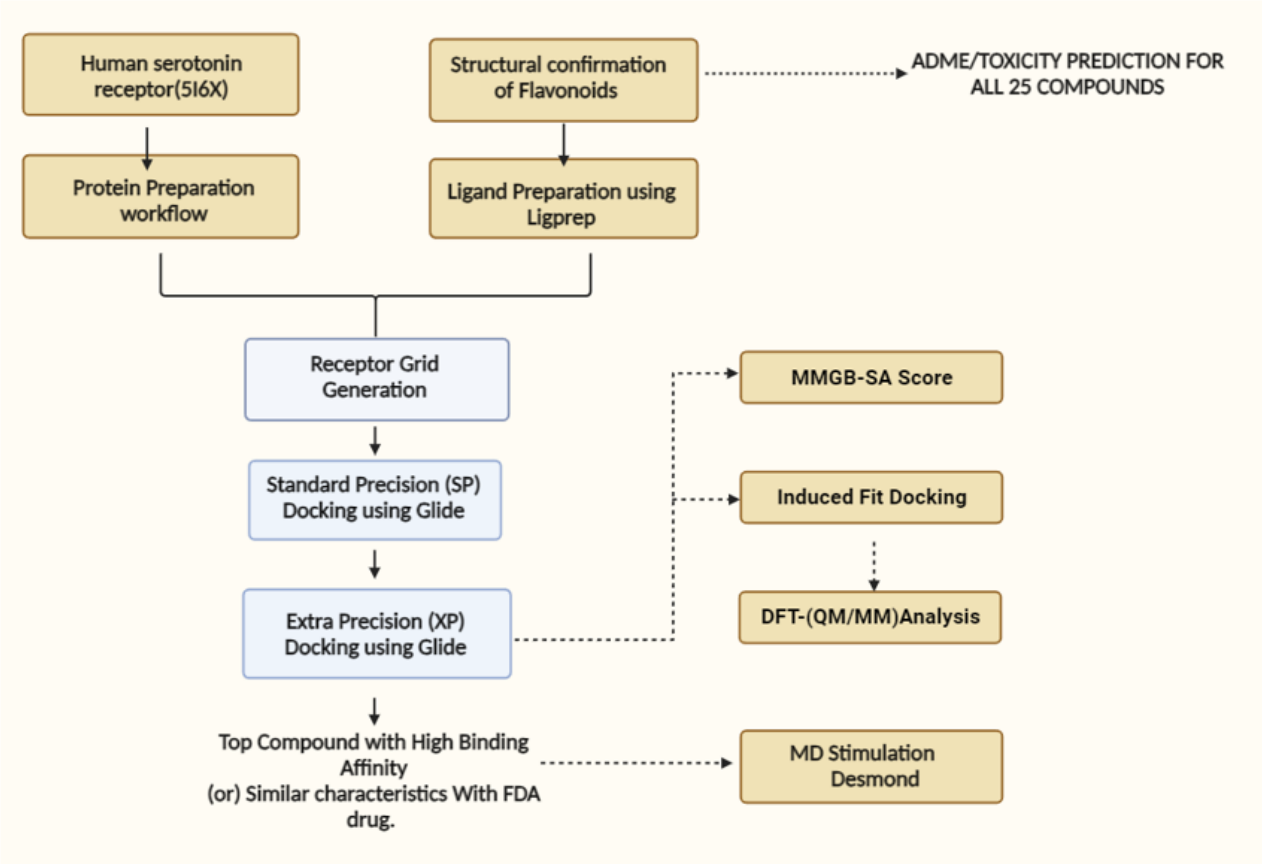

Figure 2

The flow chart of steps to identify the novel potential inhibitor for anti-depressants

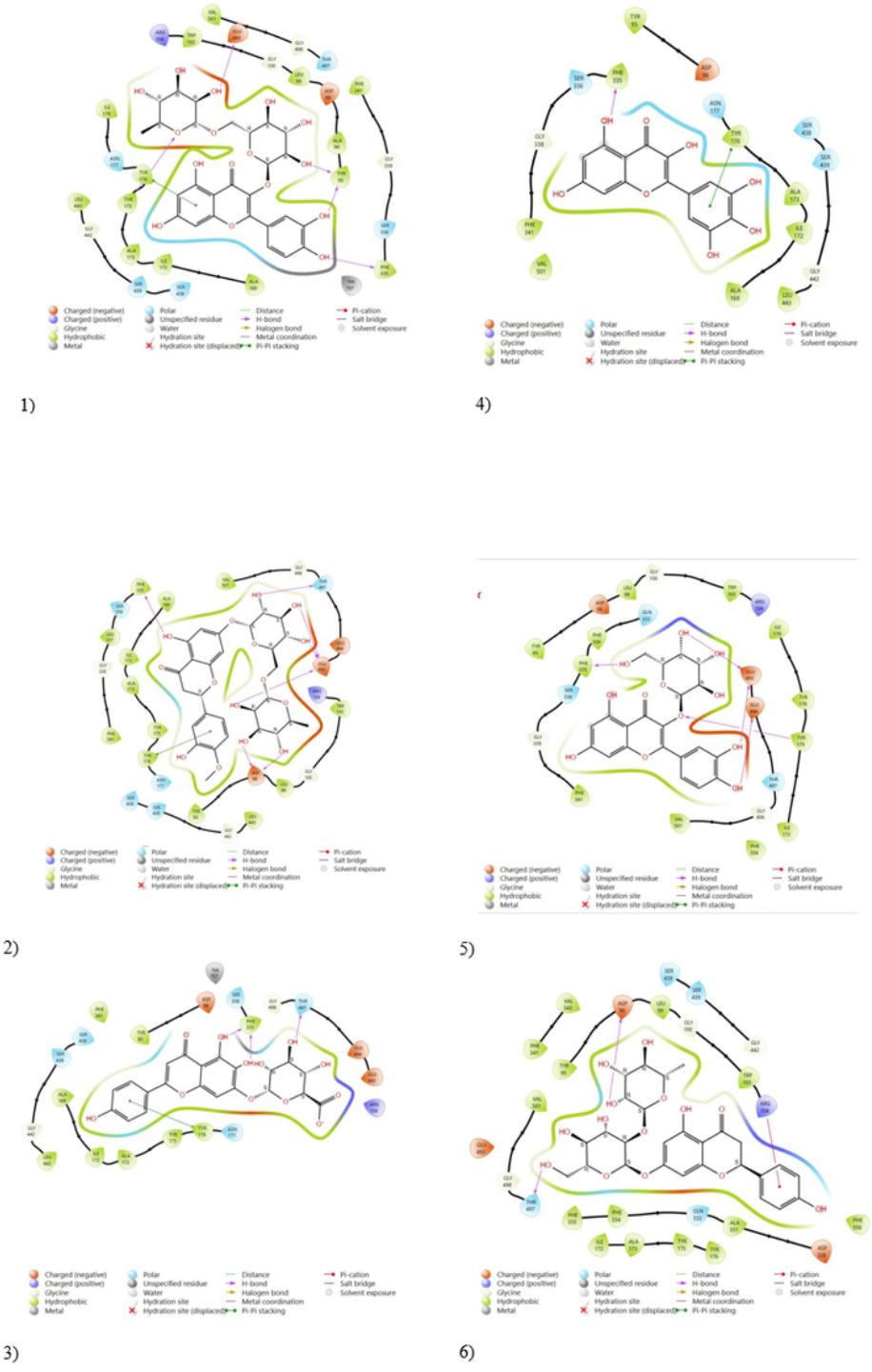

Figure 3

2D interaction diagram of top six compounds in the catalytic pocket of serotonin transporter (5I6X.pdb)

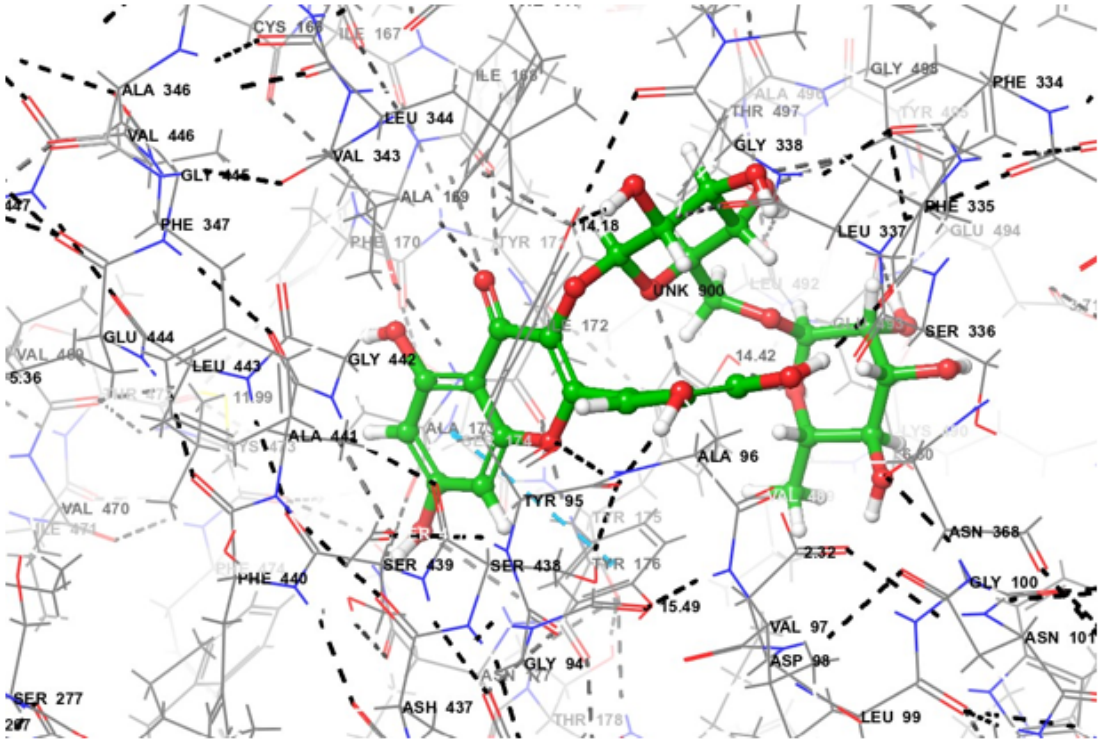

Figure 4

3D interaction diagram of top compound with good binding affinity and hydrogen bonding interation in the catalytic pocket of serotonin transporter (5I6X.pdb)

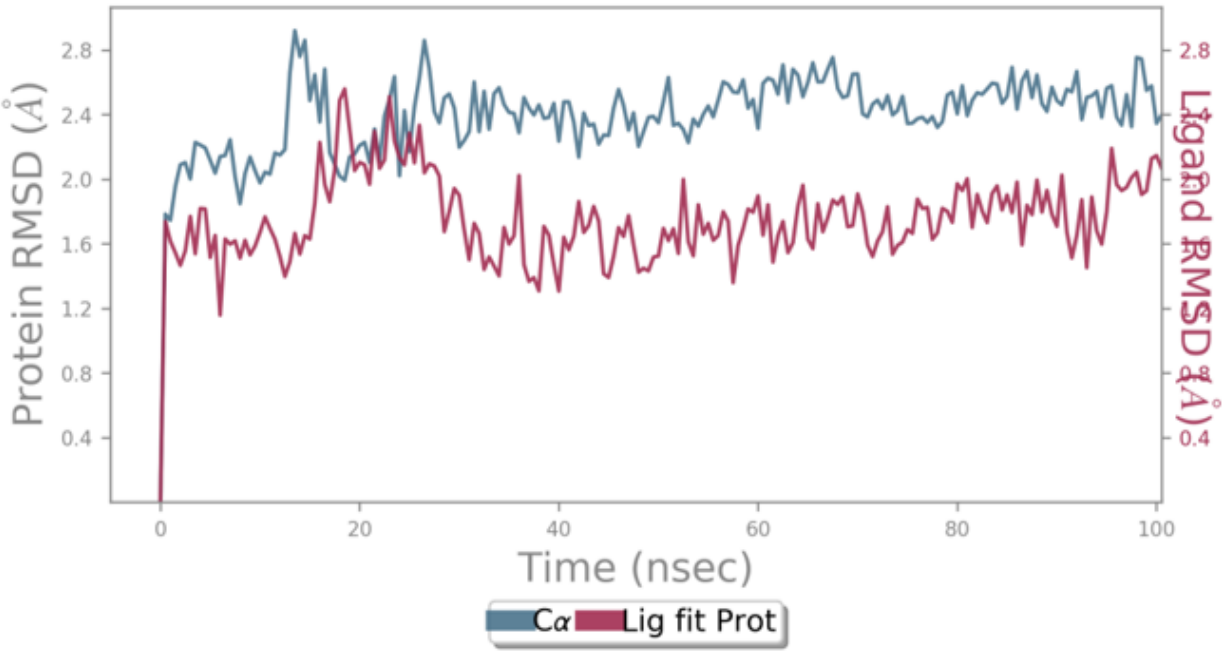

Figure 5

RMSD graph for the 100ns simulation trajectory analysis of protein ligand complex

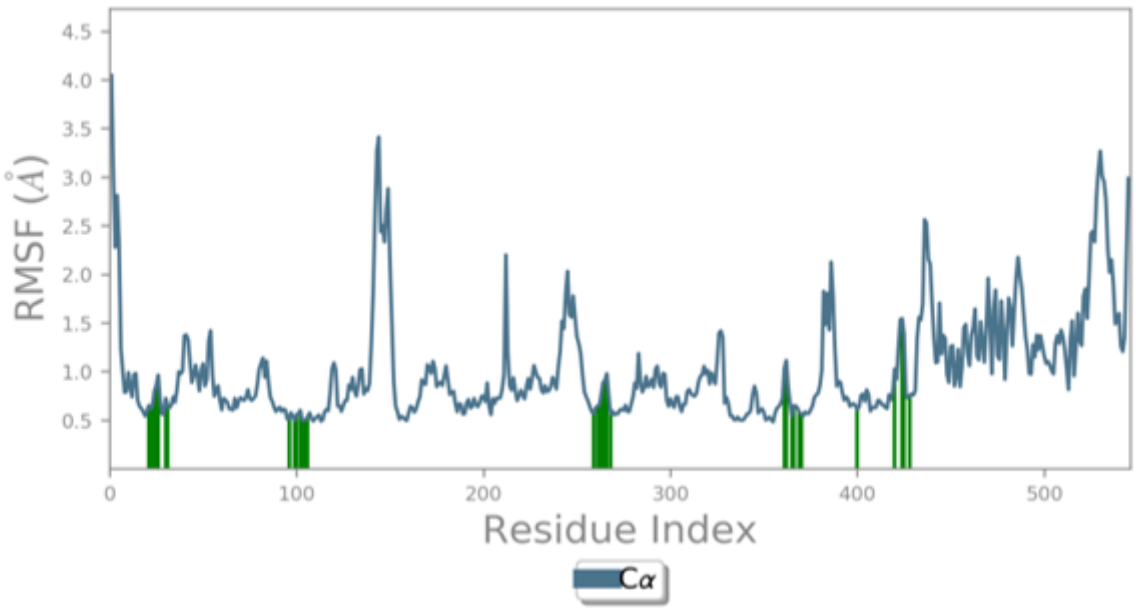

Figure 6

RMSF graph for the 100ns simulation trajectory analysis of protein ligand complex

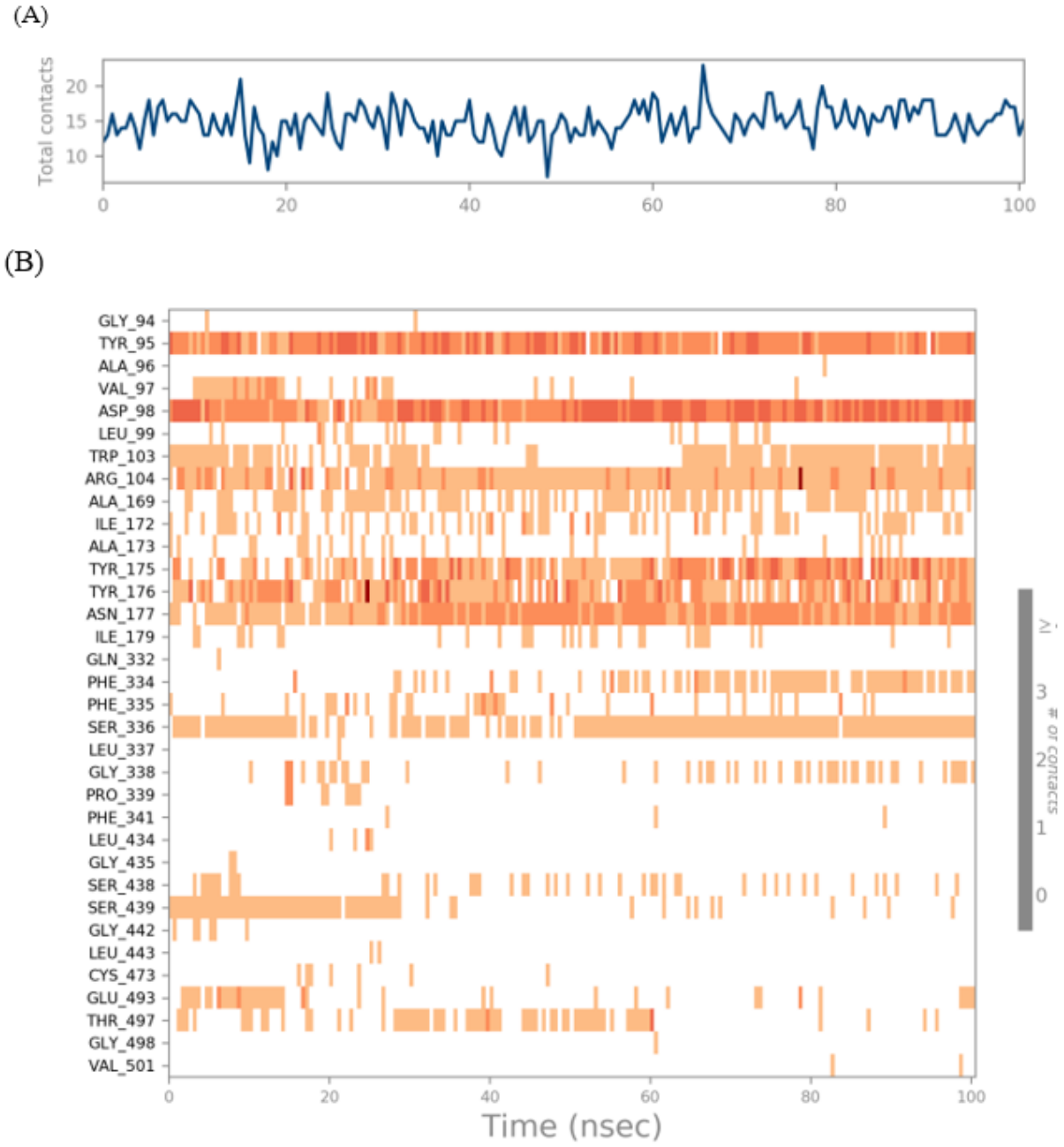

Figure 7

(A) &(B) Interaction time of each amino acid residues

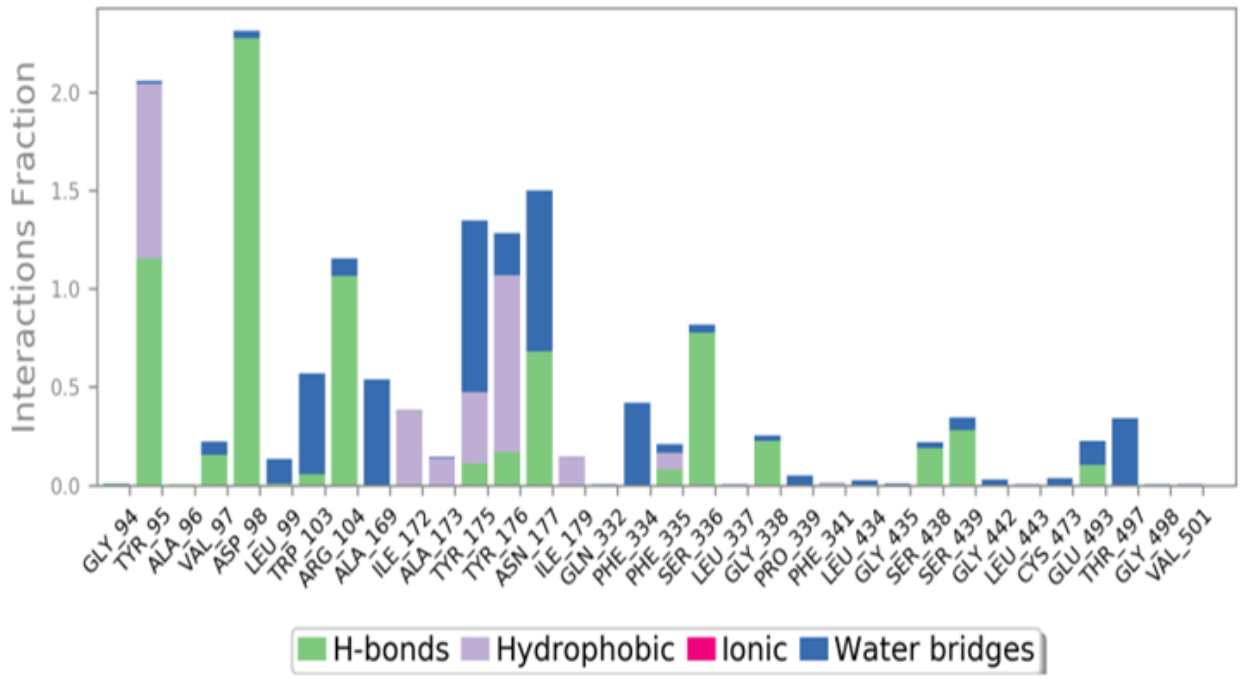

Figure 8

Interaction fraction of amino acids of protein-ligand complexes

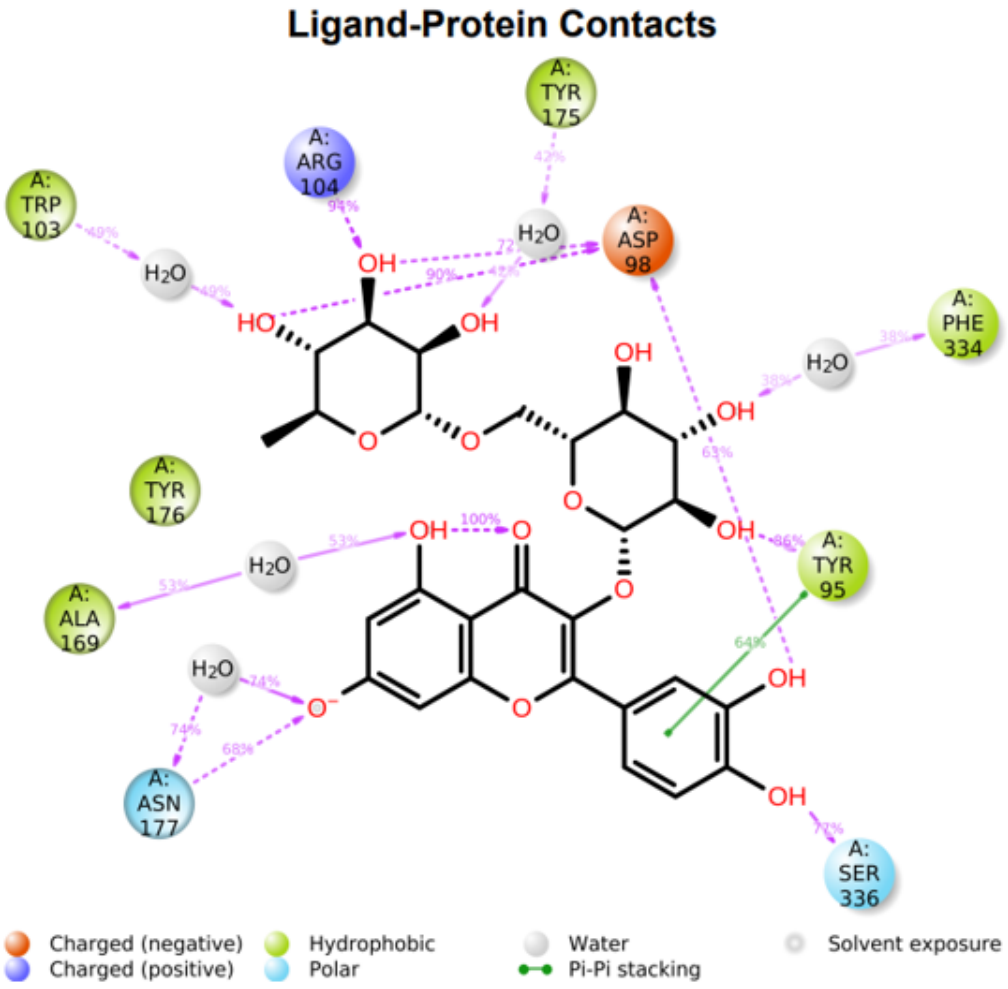

Figure 9

2D interaction diagram for the 100ns simulation trajectory of protein- ligand complex

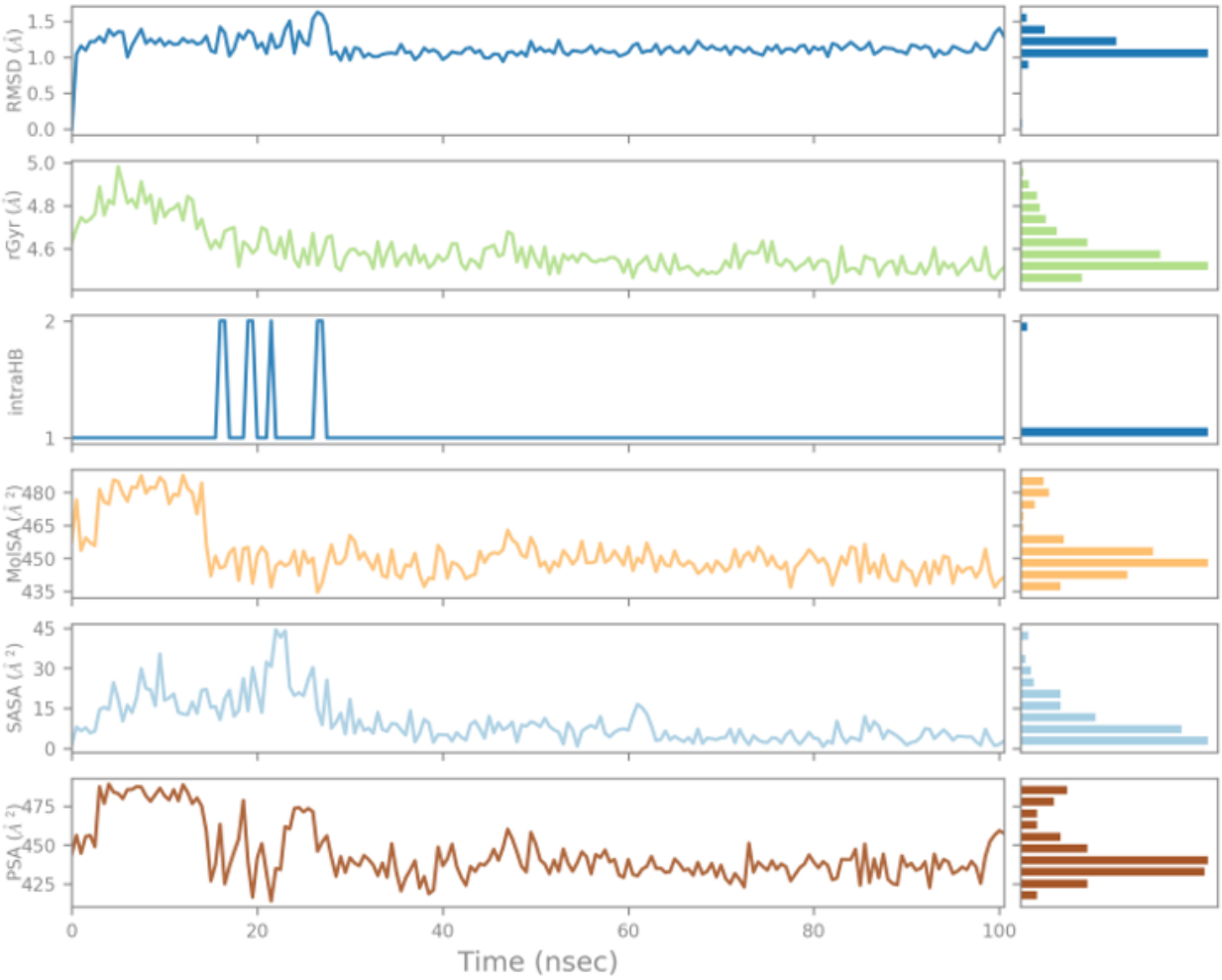

Figure 10

Ligand properties of characteristics such as ligand RMSD, radius of gyration (rGyr), intramolecular hydrogen bonds (intraHB), molecular surface area (MolSA), solvent accessible surface area (SASA), and polar surface area (PSA). The ligand and protein root-mean-square fluctuation in complex with 5I6X during 100 ns MD simulation.