

Ebelin lactone as the most promising neuroprotective compound from *Bacopa monnieri* extract targeting microtubule affinity regulation kinase-4 involved in Alzheimer's disease: A Computational Study

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Abstract

Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder in which amyloid-beta (A β) and tau tangles are vital in causing neurodegeneration. Only five FDA-approved drugs are available in the market which manages the symptoms. In this study, 52 novel phytochemicals were selected from the medicinal plant *Bacopa monnieri*, a medicinal plant with neuroprotective compounds.

Objective

The main aim of this study is to find the most promising compound inhibiting microtubule affinity regulation kinase 4 (MARK 4), which is involved in AD.

Methods

First, an ADMET analysis was conducted, and the selected compounds were molecularly docked against the MARK4-associated protein (5ES1). Based on the top five binding affinities, a molecular dynamics simulation was performed.

Results

Cucurbitacin E, oroxindin, ebelin lactone, cucurbitacin B, and bacosine showed binding affinity of more than -10.0 kcal/mol, and molecular dynamics (MD) simulations of these molecules in complex with MARK4 was performed. Subsequent trajectory analysis for structural changes and end-state MMGBSA binding energy were performed for Cucurbitacin E, oroxindin, ebelin lactone, cucurbitacin B and bacosine. The MD simulation and MMGBSA calculations showed stable interactions between the screened molecules and MARK4.

Conclusion

This computational study predicted ebelin lactone to be the most promising compound from *Bacopa monnieri* that can be further developed as a drug to treat AD after pre-clinical and clinical studies.

INTRODUCTION

Alzheimer's disease (AD) is a leading progressive neurodegenerative disorder that causes memory deficits, personality alterations, and cognitive and language deficits. There is no reliable or conclusive test to identify a person with AD, and practitioners depend on all the diagnostic information, such as records, clinical examinations, brain imaging, and blood tests. [1, 2, 3]. In the first stage of AD, episodic and spatial memory deficits are detected, and in the late stages of the disorder, long- and remote (factual) memory deficits are present. According to the Alzheimer's Association, by 2030, 5.4 million will be affected by AD. Tau and A β pathology plays a significant role in the disease's pathogenesis. Numerous research has focused on either amyloid or tau pathology [4, 5, 6, 7]. Currently, only five medications are available to treat AD, of which four are cholinesterase inhibitors and memantine which inhibits glutamate production. Patients with mild to moderate AD are given cholinesterase inhibitors, whereas memantine is used in the mild to extreme phases of AD [8, 9, 10]. This study considers a medicinal plant, "Brahmi", and Microtubule affinity regulation kinase 4 (MARK4). *Bacopa monnieri* is a small perennial herbaceous plant generally referred to as "Brahmi" which belongs to a member of the Scrophulariaceae family [11]. It has antioxidant, antipyretic, antidiabetic, antiarthritic, antihypertensive, anticancer properties and healing properties that have been used in Ayurvedic medicine for the last 3,000 years [12]. It is used as a conventional medicine to cure various nervous problems, as a dietary aid to enhance comprehension, memory, and attention, as well as to reduce anxiety and treat skin disorders, asthma and epilepsy [13, 14, 15, 16]. Bacopa flower also helps in the regeneration of damaged neurons, neuronal growth, and synaptic activity restoration, as well as improving brain function [17, 18, 19]. MARK4 belongs to a serine/threonine kinase family with a molecular mass of 82.5 kDa and 752 amino acid residues responsible for phosphorylating microtubule-binding proteins. Studies show that MARK4 overexpression is associated with tau protein phosphorylation in AD. Therefore, MARK4 and its isoforms are considered vital targets for AD [20, 21]. The aim of current research is to identify the most promising neuroprotective compound from *Bacopa monnieri* extract, which can be further used for pre-clinical and clinical studies of AD. First, we collected 52 novel compounds (Supplementary 1) isolated from *Bacopa monnieri* from the literature [22], then performed ADME and toxicity analysis. We tailored the list to 32 that meet Lipinski's "rule of five". Due to the lack of structures, 25 were chosen for molecular docking. The five compounds, i.e., Cucurbitacin E, oroxindin, ebelin lactone, cucurbitacin B and bacosine, show the highest binding affinity, and we proceed with molecular dynamics simulation with end-state MMGBSA binding energy. During molecular dynamics simulation and MMGBSA, all compounds showed stable interactions between the screened molecules and MARK4. Finally, based on all the results, we concluded that ebelin lactone is considered the most promising neuroprotective compound from *Bacopa monnieri*. However, further in vitro, in vivo and clinical studies are needed.

METHODS

1. Toxicity screening and ADME analysis

Each of the SMILE's substrings from each phytocompound in the SwissADME online tool (<http://www.swissadme.ch/>) and pkCSM (<http://biosig.unimelb.edu.au/pkcsml/>) webserver have been used to evaluate the physicochemical characteristics, compute ADMET parameters, and estimate drug-like nature, pharmacokinetic properties, and medicinal chemistry friendliness. The 52 compounds studied in this research were evaluated for their ADMET profiles to seek small molecule or drug-like characteristics (Lipinski/Ghose/Veber/Egan/Muegge).

2. Molecular Docking of 25 compounds of Bacopa monnieri with AutoDock 4.2.6

Molecular docking studies were performed with AutoDock 4.2.6. First, PDB structures were converted into PDBQT format, and Gasteiger charges were added to the ligands. Then MARK4 associated protein (5ES1) PDB file was loaded in AutoDock MGL Tools. Subsequently, polar hydrogens and Kollman charges were added and saved in PDBQT format, which is required for molecular docking.

A grid box with x, y, and z directions was generated to cover the active sites of the target protein, keeping enough space for the rotational and translational movement in the ligands and keeping the grid spacing at 0.375 Å, and flexible docking was performed. The outputs were analyzed and visualized in AutoDock 4.2.6.

3. Molecular Dynamics Simulation of 5 compounds of *Bacopa monnieri* with Desmond

Desmond, a software from Schrodinger LLC, was used to perform molecular dynamics for 100 nanoseconds on five chosen complexes [23]. Structural investigations of the protein-ligand complex were carried out as the initial stage in creating the molecular dynamics model. Studies of molecular connectivity may be used to estimate the binding status of ligands in static environments. Newton's classical equation of motion is used in molecular dynamics simulation to figure out how atoms move over time, while the substrate attachment makes a static picture of how a single molecule binds to the protein's active site [24]. The binding status of the ligands in the physiological environment was predicted using MD simulations [25, 26]. Each ligand-protein complex was pretreated, optimized, and minimized using Maestro's Protein Preparation Wizard. The System Builder application was used to create each system. TIP3P (Transferable Intermolecular Interaction Potential 3 Points) was chosen as the orthorhombic solvent model because of its simplicity. Simulations were conducted using the OPLS 2005 force field [27]. 0.15 M sodium chloride (NaCl) was used to replicate the physiological settings. The NPT ensemble, with a temperature of 300 K and a pressure of 1 atm, was applied throughout the simulation. The generated trajectories were examined for structural changes in the protein structure brought on by ligand binding during the simulation. These analyses included root mean square deviation (RMSD), root mean square fluctuation (RMSF), the radius of gyration (Rg), secondary structure changes, solvent accessible surface area (SASA), and ligand torsion profile

4. Molecular Mechanics Generalized Born Surface Area (MMGBSA) calculations

The binding free energies (ΔG_{bind}) of the ligand-bound protein complexes were computed by employing the molecular mechanics generalized born surface area (MMGBSA) methodology (Schrodinger suite, LLC, New York, NY, 2017-4). Binding free energies were calculated using OPLS 2005 force field, VSGB solvent model and rotamer search methods [28]. After performing MD, ten ns was used to select the MD trajectory frame. The total free binding energy is calculated using the below-mentioned formula:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}})$$

ΔG_{bind} = binding free energy, G_{complex} = free energy of the complex, G_{protein} = free energy of the target protein, and G_{ligand} = free energy of the ligand.

RESULTS

1. Phytochemical retrieval and ADME analysis

An extensive literature search was conducted to obtain the plant's available compounds. A list of compounds from the Brahmi (*Bacopa monnieri*) plant was discovered in the literature database. The Pharmacokinetics analysis of these compounds is listed in Table 1.

Table 1
Pharmacokinetic properties of the screened ligands from *Bacopa monnieri*

SI.NO	Compound Name	COMPOUNDS CID	Molecular weight	G.I. Absorption	BBB Permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	C
1.	LOLIOLIDE	CID 100332	196.24 g/mol	High	Yes	No	No	No	No	No	N
2.	BACOSTEROL	CID 101389368	414.71 g/mol	Low	No	No	No	No	No	No	N
3.	ASIATIC ACID	CID 119034	488.70 g/mol	High	No	Yes	No	No	No	No	N
4.	EBELIN LACTONE	CID 15559069	454.68 g/mol	Low	No	No	No	No	Yes	No	N
5.	STIGMASTANOL	CID 241572	416.72 g/mol	Low	No	No	No	No	No	No	N
6.	OROXINDIN	CID 3084961	460.39 g/mol	Low	No	Yes	No	No	No	No	N
7.	3,4 Dimethoxyeinnamic Acid morpholine	CID 3981577	277.32 g/mol	High	Yes	No	No	Yes	No	No	N
8.	QUERCETIN	CID 5280343	302.24 g/mol	High	No	No	Yes	No	No	Yes	Y
9.	APIGENIN	CID 5280443	270.24 g/mol	High	No	No	Yes	No	No	Yes	Y
10.	LUTEOLIN	CID 5280445	286.24 g/mol	High	No	No	Yes	No	No	Yes	Y
11.	STIGMASTEROL	CID 5280794	412.69 g/mol	Low	No	No	No	No	Yes	No	N
12.	CUCURBITACIN A	CID 5281315	574.70 g/mol	Low	No	No	No	No	No	No	Y
13.	CUCURBITACIN B	CID 5281316	558.70 g/mol	Low	No	Yes	No	No	No	No	Y
14.	CUCURBITACIN C	CID 5281317	560.72 g/mol	Low	No	Yes	No	No	No	No	Y
15.	CUCURBITACIN D	CID 5281318	516.67 g/mol	No	Yes	No	No	No	No	No	Y
16.	CUCURBITACIN E	CID_5281319	556.69 g/mol	Low	No	Yes	No	No	No	No	Y
17.	WOGONIN	CID 5281703	284.26 g/mol	High	No	No	Yes	No	Yes	Yes	Y
18.	ASCORBIC ACID	CID 54670067	176.12 g/mol	High	No	No	No	No	No	No	N
19.	MANNITOL	CID 6251	182.17 g/mol	Low	No	No	No	No	No	No	N

Sl.NO	Compound Name	COMPOUNDS CID	Molecular weight	G.I. Absorption	BBB Permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	C
20.	URSOLIC ACID	CID 64945	456.70 g/mol	Low	No	No	No	No	No	No	↗
21.	BETULINIC ACID	CID 64971	456.70 g/mol	Low	No	No	No	No	Yes	No	↗
22.	BACOSINE	CID 71312547	456.70 g/mol	Low	No	No	No	No	Yes	No	↗
23.	MADECASSIC ACID	CID 73412	504.70 g/mol	High	No	Yes	No	No	No	No	↗
24.	NICOTINE	CID 89594	162.23 g/mol	High	Yes	No	No	No	No	No	↗
25.	ROSAVIN	CID 9823887	428.43 g/mol	No	No	No	No	No	No	No	↗

2. Molecular docking analysis

The binding affinity of 25 compounds of *Bacopa monnieri* on the target protein MARK4 is shown in Table 2. The top 5 binding affinity compounds lead to molecular dynamics simulation.

Table 2
Docking scores of the 25 compounds of *Bacopa monnieri* on the target protein MARK4

S.NO	Compound Id	Binding affinity (kcal/mol)
1.	CID 5281319	-10.5
2.	CID 3084961	-10.5
3.	CID 15559069	-10.5
4.	CID 5281316	-10.4
5.	CID 71312547	-10.3
6.	CID 5281318	-10.2
7.	CID 73412	-10.2
8.	CID 5281315	-9.9
9.	CID 5281317	-9.9
10.	CID 5280794	-9.8
11.	CID 5280443	-9.8
12.	CID 9823887	-9.8
13.	CID 64945	-9.8
14.	CID 119034	-9.7
15.	CID 241572	-9.6
16.	CID 5280343	-9.6
17.	CID 64971	-9.4
18.	CID 5280445	-9.4
19.	CID 5281703	-8.8
20.	CID 101389368	-8.8
21.	CID 3981577	-7.8
22.	CID 100332	-7.6
23.	CID 54670067	-6.4
24.	CID 6251	-5.8
25.	CID 89594	-6.4

3. Molecular Dynamics Simulation

We considered the top 5 compounds for molecular dynamics simulation based on binding affinity and interaction with the active site residues. The overall assessment of the MD trajectories was done to identify the stability of the complexes. The protein-ligand contact summary with the interactions maintained over more than 30% of the entire trajectory is shown in Fig. 1. The RMSD of the C-alpha of the protein was calculated for all five complexes, as shown in Fig. 2. In all the complex systems, the RMSD of the C-alpha of protein remained stable and converged to the maximum RMSD of 3.2 Å. At the same time, the ligands fluctuated in their RMSDs, with most fluctuations observed in the case of 5281319, 15559069, and 71312547 molecules to adjust to the energetically most favoured pose within the active site of the protein.

In contrast, the other two molecules (3084961 and 5281316) were relatively stable regarding the observed fluctuations. Overall, the RMSD of the ligands ranged from 3.5 Å to 6 Å compared to their initial docked pose and finally attained a stable state by the end of the simulation time. RMSF (Root Mean Square Fluctuations) of the ligand and protein atoms were calculated to estimate the internal fluctuations of the system. The RMSF of the atoms and residues of the ligand and protein, respectively, are the time-averaged fluctuations of the square deviation of the heavy atoms and residues for the initial reference frame calculated over the entire simulation period. In Fig. 3a and Fig. 3b, most of the fluctuations were observed in the heavy atoms of the ligands, which are involved in the interaction with the protein active site residues.

In contrast, for the protein molecule, the highest fluctuation was observed in the N-terminal region and the loop regions marked by the sharply increased RMSF peaks in Fig. 3e. The fluctuations in the active site residues were well below 2 Å in all the cases. The dynamic protein-ligand contacts analysis over the simulated trajectories was done, as shown in Fig. 4 and Supplementary 2. The hydrophobic interactions and water bridges were most prominent, followed by a few hydrogen bonds for 3084961 and 15559069 ligands. For the other ligands, the significant interactions observed were of water bridges followed by hydrogen bonds. 3084961 and 71312547 also showed considerable ionic interaction with the target protein residues. The maximum number of contacts of the ligand molecules with the protein was observed, which is shown in Fig. 5 to be 12 in the case of ligands 3084961 (4a) and 5281319(4c), while upto nine

contacts for 15559069 (4d) and 71312547 (4e) and up to 8 contacts with 5281316 (4b). The maximum number of active site residues of the target protein interacted for a significant fraction of the time of the simulation with 3084961 (oroxindin) followed by 15559069 (Ebelin-Lactone).

In contrast, the least interactions of the active site residues (Ile 62; Lys 64; Val 70, Ala 83, Lys 85; Glu 133, Tyr 134, Ala 135, Gly 138, Glu 182, Asn 183, and Asp 196) were observed for the other ligand molecules. The total secondary structure elements (SSE), like alpha-helices (red) and beta-strands (blue), were monitored for each residue of the protein in complex with the respective ligands throughout the simulation time. Most of the protein is stabilized by the helices (29.62 to 30.41%) followed by the beta-sheets (13.22 to 14.39%), with the overall total SSE ranging from 42.51 to 44.81% for all the protein-ligand complexes shown in supplementary 3. The SASA of the target protein in complex with the ligand molecules was also calculated and shown in Fig. 5. The SASA ranged from 1500 Å² to 1700 Å² with some fluctuations in the values depending on the conformations mapped by the protein and the ligand molecules over the simulation time. The Ligand torsion profile and properties of all five compounds are included in supplementary Figs. 4 and 5.

Molecular Mechanics Generalized Born and Surface Area (MMGBSA) calculations.

In the end, the Molecular Mechanics Generalized Born and Surface Area (MMGBSA) calculations for the first and last frame for all the complex trajectories are shown in Fig. 6. The binding energy values were consistent for the initial and final frame, ranging from 0.021 to 7.37 kcal/mol. For ligand 5281316, a significant reduction in the binding affinity by 35.57 kcal/mol was observed. The highest binding affinity was shown by 3084961, followed by 15559069, 5281319, and 71312547, and the lowest affinity by 5281316. Based on the MMGBSA binding energy scores, all the complexes are stable with the binding affinity order mentioned above.

DISCUSSION

The medicinal plants also had anticancer, anti-inflammatory, antioxidant, and neuroprotective properties. In this study, we considered the well-known medicinal plant "*Bacopa monnieri*", which has many natural active compounds, including alkaloids and saponins with therapeutic potential. To find the most promising inhibitor from *Bacopa monnieri* extract which can target MARK4 protein which is highly overexpressed in AD first, the interaction of the *Bacopa monnieri* compounds with MARK4 was assessed through molecular docking followed by subsequent molecular dynamics simulations of the top 5 neuroprotective compounds based on binding affinity and validation of the docking results was done through MMGBSA binding energy calculations. Based on these analyses, Ebelin Lactone (CID-15559069) maintained constant and stable interactions with the active site residues of MARK4 over the entire simulation trajectory. Hence, ebelin lactone is the most promising neuroprotective compound from *Bacopa monnieri*, which targets MARK4 and can be used for further *in-vitro*, *in-vivo*, pre-clinical, and clinical studies of AD. Previous studies conducted by Shamsi et al.; 2020 showed that donepezil, rivastigmine tartrate, and acetylcholine inhibitors bind with the active site of the MARK4 [29]. Recently, a study conducted by Waseem. et al.; 2021 found out that irisin; (a protein that plays a vital role in memory dysfunction in AD) binds with MARK4 and maintains stability [30]. Most recent data indicates that ganoderic acid A and ganoderenic acid B are the most promising molecules derived from *Ganoderma lucidium* that bind with MARK4 [31]. Previous research supports our study, and ebelin lactone is considered the most promising neuroprotective compound from the *Bacopa monnieri* extract, which binds with MARK4. However, our findings need to be supported through *in vitro* and *in vivo* studies before moving toward clinical studies.

CONCLUSION

This investigation aimed to identify the most promising neuroprotective compound which targets MARK4 involved in AD. Our structure-based drug design strategy identified ebelin lactone as a promising neuroprotective compound possessing potential interaction potential with MARK4. These outcomes were validated by molecular docking followed by molecular dynamics and end-state binding energy estimations. To establish the therapeutic potential of ebelin lactone in managing and treating AD, pre-clinical and clinical studies are required.

Abbreviations

AD - Alzheimer's disease

MARK4 - Microtubule affinity regulation kinase 4

PD - Parkinson's Disease

MMGBSA - Molecular Mechanics Generalized Born Surface Area

PCA - principal component analysis

FEL - free energy landscape

RMSD - root mean square deviation

RMSF - root mean square fluctuation

Rg - radius of gyration

SASA - secondary structure changes, solvent-accessible surface area

Declarations

Acknowledgments

Not applicable.

Conflicts of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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No funding was received for this study.

Data Availability Statement

The data generated during this study are included in this article.

Ethical Approval

Not applicable

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Figures

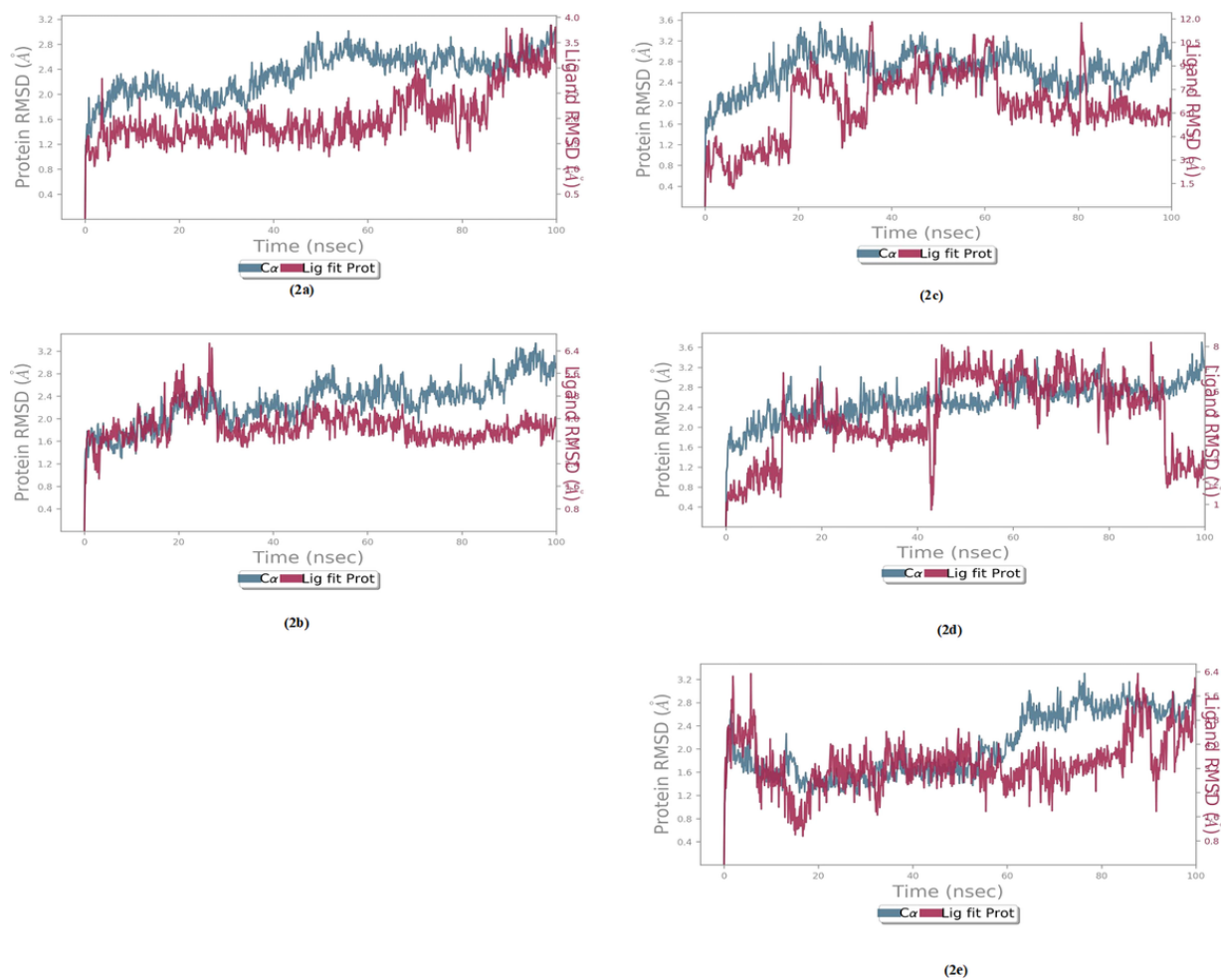


Figure 2

RMSD analysis of MD simulation trajectory of 5es1 in complex with (2a) 3084961, (2b) 5281316, (2c) 5281319, (2d) 15559069 and (2e) 71312547

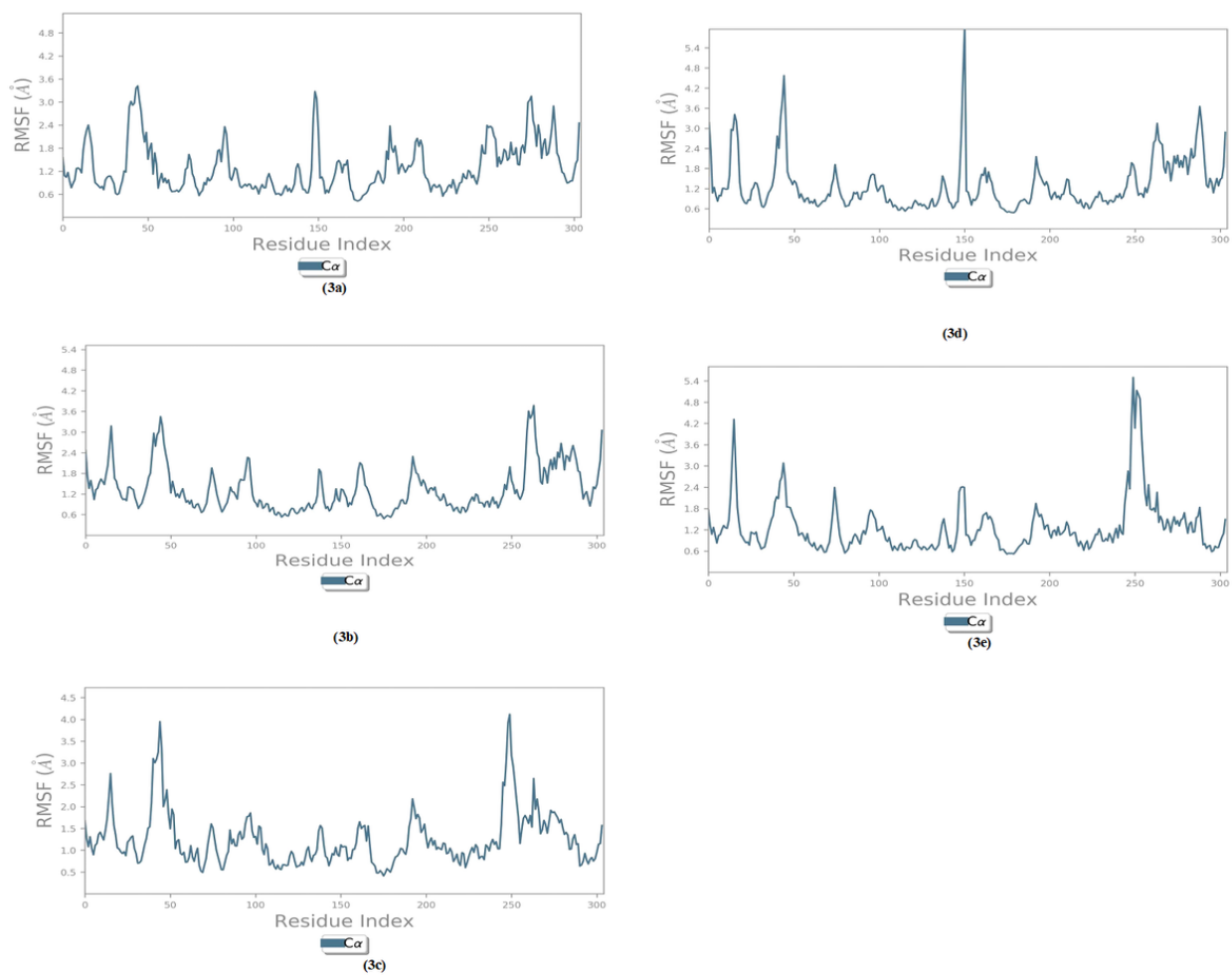


Figure 3

C-alphas RMSF of protein (5es1) in complex with (3a) 3084961, (3b) 5281316, (3c) 5281319, (3d) 15559069 and (3e) 71312547

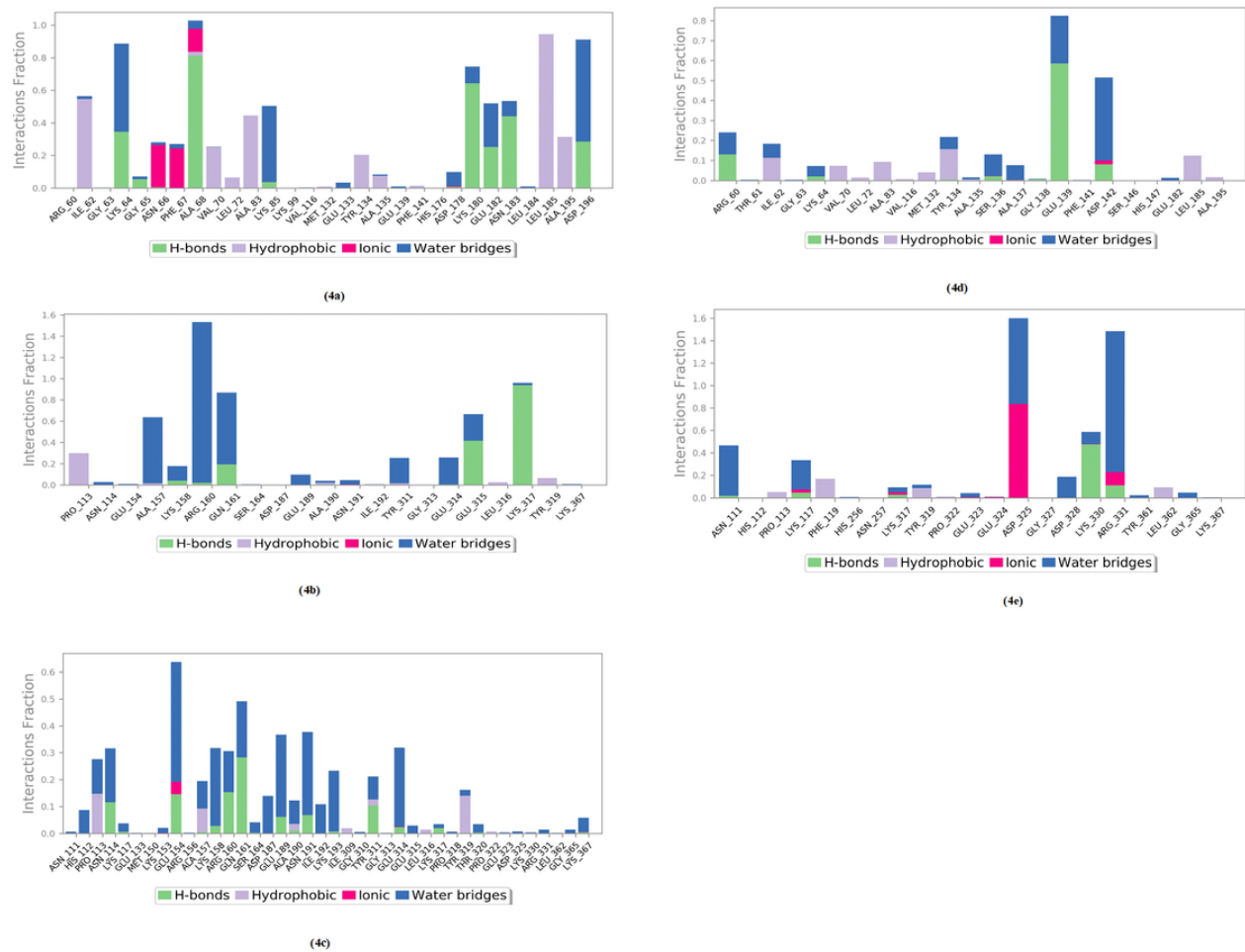


Figure 4

Protein-ligand contacts histogram of 5es1 in complex with (4a) 3084961, (4b) 5281316, (4c) 5281319, (4d) 15559069 and (4e) 71312547

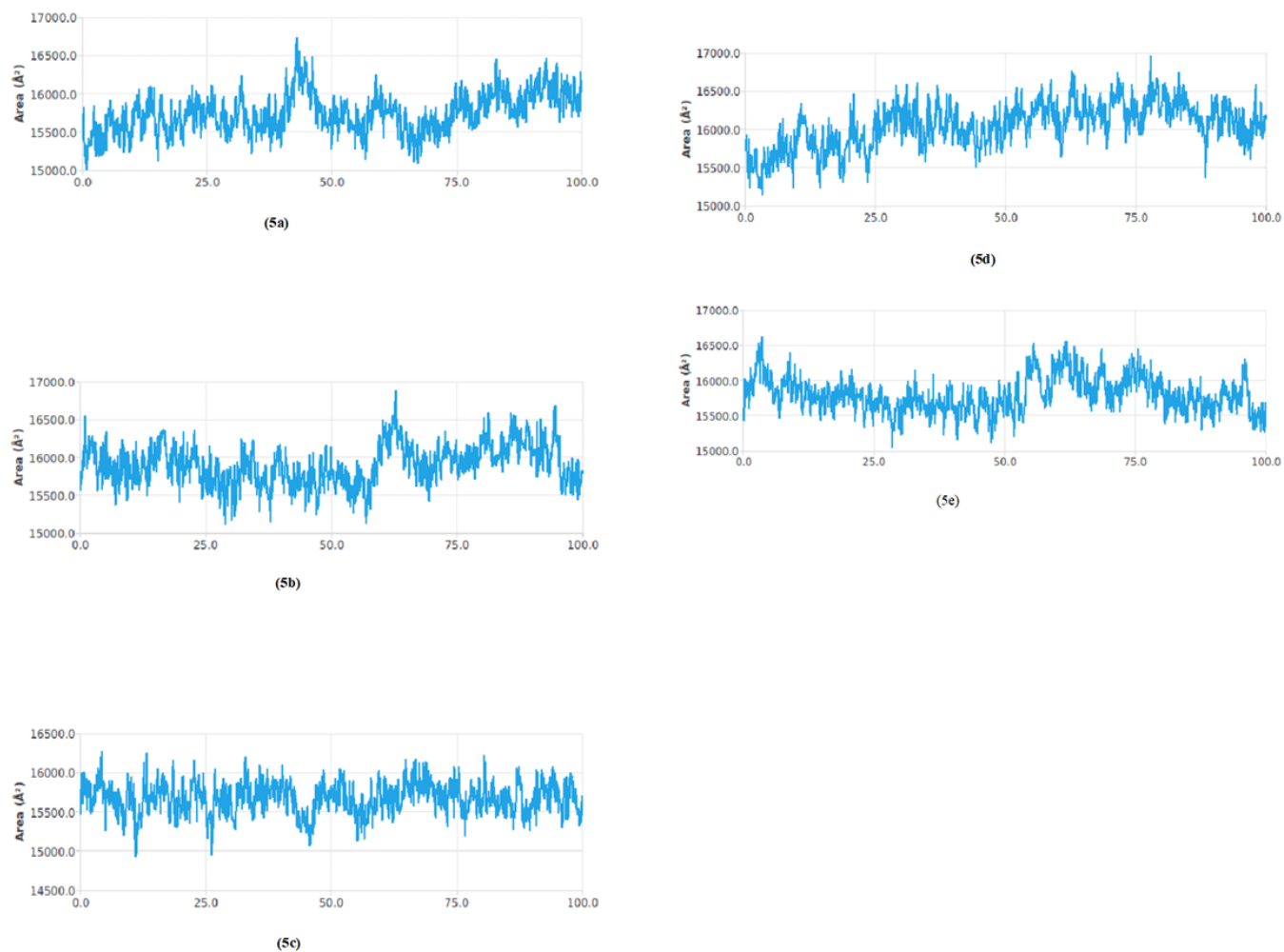


Figure 5

SASA of (5a) 5es1 and 3084961, (5b) 5es1 and 5281316, (5c) 5es1 and 5281319, (5d) 5es1 and 15559069 and (5e) 5es1 and 7131254

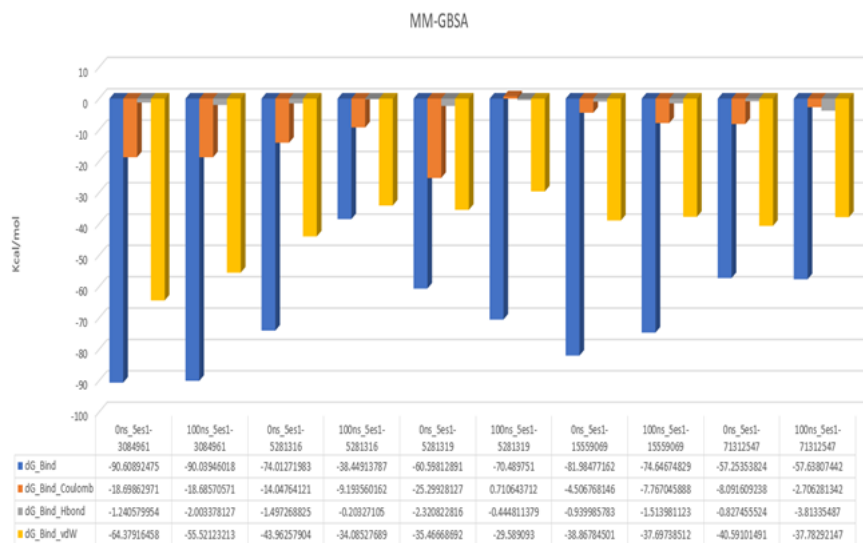


Figure 6

MMGBSA analysis of initial and final frame of 5es1 with the ligands 3084961, 5281316, 5281319, 15559069 and 71312547