Original Article

THEORETICAL FINDING THROUGH MOLECULAR DYNAMICS OF NOVEL PIPERAZINE SULFONYL AMINE BASED NMDA-NR2B RECEPTOR ANTAGONIST

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Abstract

Utilizing molecular modelling approaches, a novel family of antagonists of the N-Methyl-D-Aspartate (NMDA) receptor subunit NR2B has been designed. In this study, we performed molecular dynamics (MD) simulations of protein–ligand complexes and determined how well MD simulations correlated the previously reported docking result. Our results suggest that appropriate candidate poses can be chosen from the many docking poses by using MD simulations with implicit solvents. The 25ns simulation suggests that the protein-ligand complexes are stable throughout the time scale. According to the MM/PBSA calculation the most stable Protein-ligand complexes in terms of binding free energies were found in cases with SE-C-13, SE-B-2, and SE-A-8. The designed compound will be subjected to synthetic accessibility analyses. Our results could provide theoretical guidance for the future development of new NMDA-NR2B Receptor Antagonist against Alzheimer.

Keywords: N-methyl d-aspartate (NMDA) receptor, piperazine sulfonyl amine derivatives, Molecular dynamics simulation, MM/PBSA binding energy.

Introduction

Ionotropic glutamate receptors are widely distributed in the vertebrate central nervous system and NMDA receptors are a sub type. This receptor plays leading roles in both physiological and pathological states of the CNS, like ischemic stroke, seizures, head trauma and pain.[1] NMDA (N-methyl D-aspartate) NR2B is the selective antagonist that binds to this receptor but not to other glutamate receptors. Activation of these receptors results in the opening of an ion channel that is nonselective to cations. Voltage-dependent activation is a specific property of this receptor, a result of ion channel block by extracellular magnesium ions. This permits voltage-dependent flow of sodium ions and little amounts of calcium ions into the cell and potassium out of the cell. It is believed that Ca^{2+} flow through NMDARs is essential for synaptic plasticity, a biological mechanism for memory and learning. The NMDA receptor differs from other receptors in 2 ways: 1st, it is ligand-gated and voltage-dependent; and 2nd, glutamate and glycine must be used in conjunction to activate it.[2]

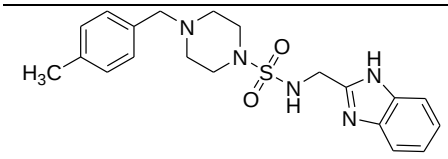
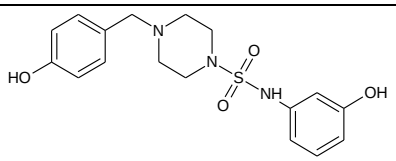
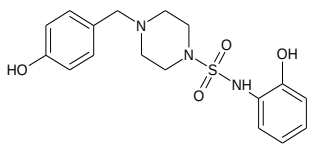
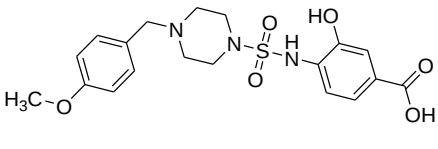
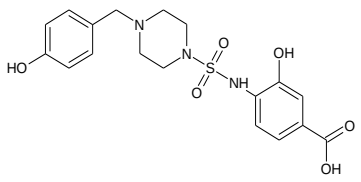
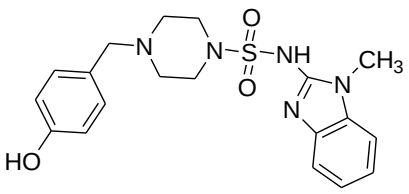
Many neuro-degenerative diseases, like Alzheimer's, Parkinson's, Huntington's disease, multiple sclerosis, Amyotrophic lateral sclerosis (ALS), and glaucoma, are caused by different mechanisms. But they may share a common pathway to neuronal injury because of the over stimulation of glutamate receptors, particularly of the NMDA NR2B sub type. A manifestation of excitotoxicity can also be seen in acute illnesses such stroke, CNS trauma, and epilepsy. As a result, N-methyl D-aspartate NR2B antagonists may be therapeutically useful for both acute and long-term neurological diseases that exhibit excessive NMDA receptor activity. N-methyl D-aspartate receptors are made up of different subunits: NR1, NR2 A, B, C, D, and, in few cases, NR3A or B subunits. These subunits, which make up the receptor, control the pharmacology and other aspects of the receptor-ion channel complex.

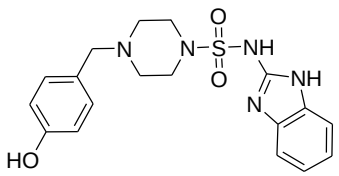
The search for new potent Anti Alzheimer's agent is a prime priority because of their extremely desired uses in medical practice. In this juncture, Alzheimer control is a universal health issue, signaling an ever-increasing demand for the discovery of new molecules with best activity and reduced side effects. For this aim, novel Piperazine Sulfonyl Amine Derivatives show potential as Anti Alzheimer's drugs and have been introduced to treat these types of diseases, reacting like selective antagonists of the NMDA receptor subunit NR2B and do not cause side effects associated with non-selective NMDA receptor antagonists.

The current research is conducted based on previous literature from our group[3] using three-dimensional quantitative structure activity relationships (3D-QSARs), a commonly used technology to discover new drugs, identifying high affinity ligands for a targeted protein[4-5]. The predictability, robustness and reliability of the constituted models have been studied with the help of Molecular dynamics for the 19 molecules (Table 1), as a crucial and decisive step to judge the prediction accuracy of the constructed model for a novel database[6-7]. In the second part, ADMET is predicted *in-silico* properties[8] of 19 molecules as an anti-Alzheimer's agent, with neuroprotective activity through its effects on N-Methyl-D-Aspartate (NMDA) receptors[9].

Molecular dynamics (MD) is a computational technique which simulates the dynamic behavior of molecular systems as a function of time, treating all the entities in the simulation box. In this study, MD simulations for protein–ligand complex structures predicted by computational docking were performed. We tested whether reasonable poses could be selected from MD simulations by comparing results of MD simulations for both reasonable docking poses and wrong poses. Molecular dynamics (MD) simulations[10-12] may help to elucidate the binding site and time dynamics of interacting amino acid residues and to extend the information obtained from previous docking studies.

Table 1: Designed Compound structure with code

Structure	Code	Structure	Code
	SE-C-13		SE-B-3
	SE-B-2		SE-D-8
	SE-B-8		SE-B-15



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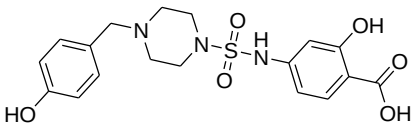

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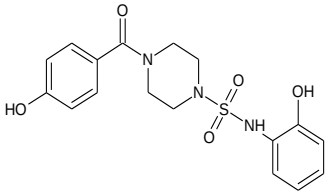

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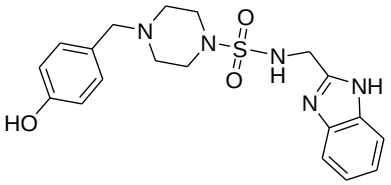

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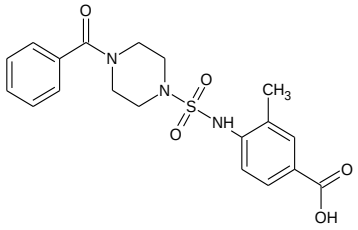

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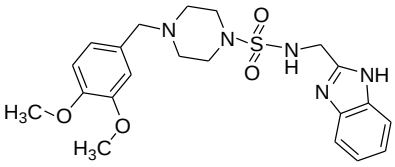

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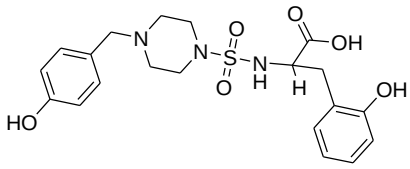

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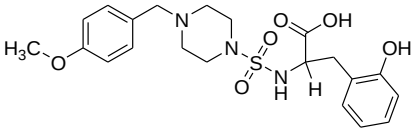

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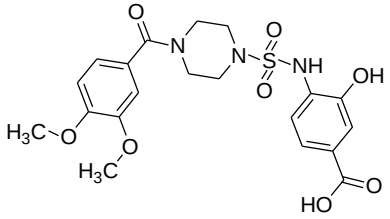
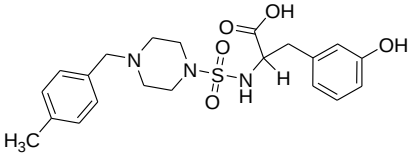
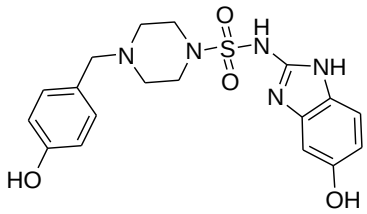


SE-A-8



SE-C-9



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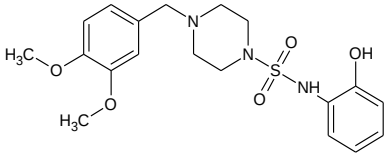

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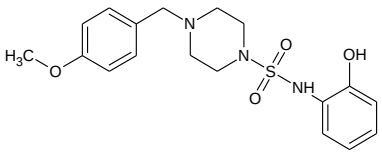


SE-D-2

Material and Methods

Molecular Dynamics Study

In this study, we performed MD simulations of protein–ligand complex structures predicted by computational docking. The best docked structures of series of 19 compounds which were earlier published (ref) as potent NMDA - receptors, considered for further molecular dynamics analysis using AMBER 22 package[13-14]. Molecular dynamics simulation provides the potential of a detailed description of the dynamic structure of ions and water at

molecular level. The GAUSSIAN 03 and RESP programs of AMBER 22 were used to generate all the parameters using values from the literature. The force-field that worked the best for our target NMDA receptor was ff19SB[15]. The Research Collaboratory for Structural Bioinformatics provided the NMDA receptor subunit NR2B structure (PDB ID: 3JPW)[16].

By substituting the solvent molecules with sodium ions counterions, the systems have been neutralized as needed. The systems' energy was initially minimized by setting less than 1000.0 kJ tolerance level and using the steepest descent technique, the systems were further energy-equilibrated. Next, the first stabilization for 200 ps at constant volume ensemble to achieve the temperature of 300 K using the leap-frog integrator. The systems were then put through a second equilibration stage at constant pressure (NPT) ensemble for 200 ps. The protein's backbone remained constrained during both equilibration phases to relax the solvent, ions, and side chains of the protein. The long-range electrostatic interactions were then controlled using the particle mesh Ewald (PME) approach. After that, by loosening the protein backbone, the manufacturing MD run for each system was carried out for 25 ns. Every 100 ps in production runs, the frames were gathered and saved for additional data analysis. The protein and ligand heavy atoms' RMSD were estimated using "least-square fitting" on the protein backbone atoms. Using the PTRAJ module, the RMSD of the protein-ligand complex was determined.

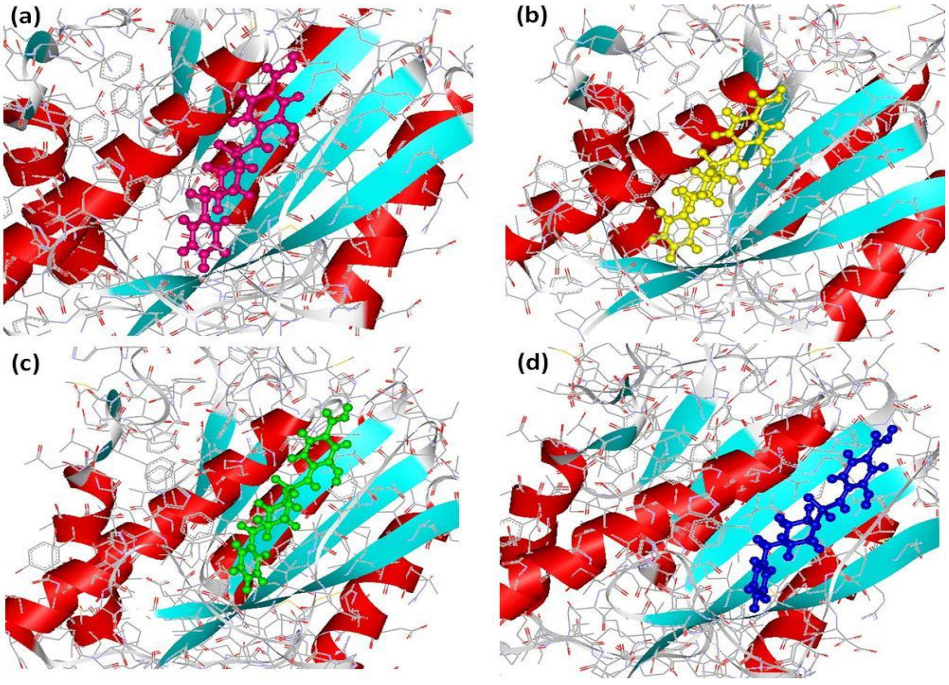
MM/PBSA Binding Free Energy Calculation

The calculation of the binding energy between a protein and a ligand is frequently done using the MM/PBSA method. To calculate the binding free energy (ΔG_{bind}), we ran a simulation of a single system protein-ligand complex under the assumption that the complex's conformation was the same in both its bound and unbound states. The Binding free energy (ΔG_{free}) of the MM/PBSA method can be express by the following equations:

$$\Delta G_{free} = \Delta\, G_{complex} - (G_{protein} + \Delta G_{ligand})$$

Here, the complex, protein, and ligand's binding free energies were expressed by $G_{complex}$, G_{ligand}, and $G_{protein}$, respectively. For the current study's estimation of the MM/PBSA binding free energy.

Figure 1: Time averaged 3D-structures of (a) SE-C-13, (b) SE-A-8, (c) SE-B2 and (d) SE-B-3 after 25ns simulation



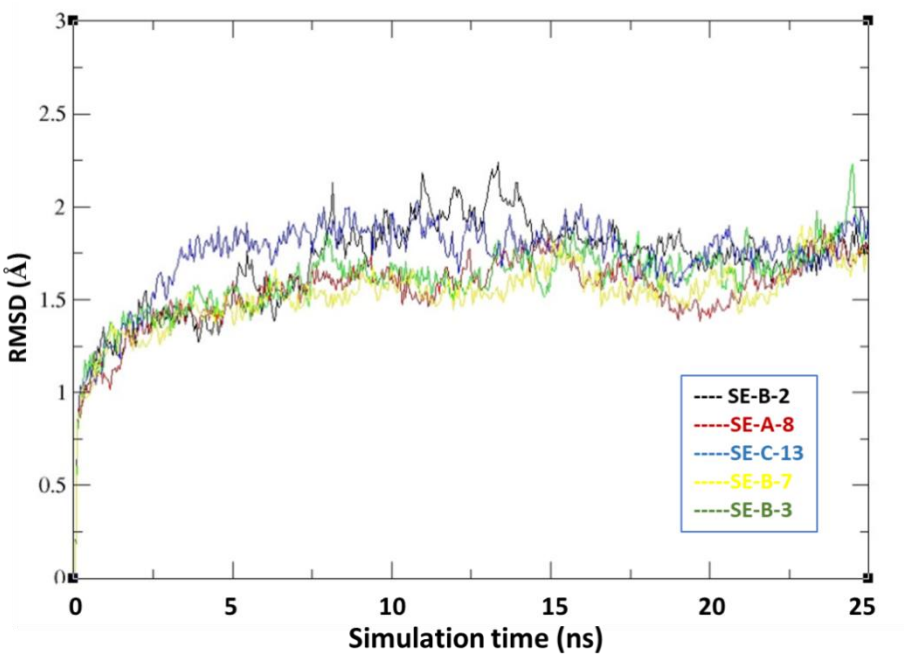
Result and Discussion

Molecular Docking Data Analysis

We have docked 19 molecules into the NMDA NR2B receptor's binding pocket. From the collection of all docked conformations, the protein-ligand complex conformation with the highest Binding Energy values and the lowest root mean square deviation (RMSD) was chosen. In molecular dynamics, the RMSD values for every molecule were less than 2 Å. We have selected the compounds SE-C-13, SE-A-8, SE-B2, SE-B-3 and SE-B-7 docked in NMDA NR2B receptor for detailed study through the trajectory obtained from 25 ns MD simulations.

After doing MD simulations of the aforementioned NMDA NR2B receptor-ligand complex, it was discovered that SE-C-13, SE-A-8, SE-B2, SE-B-3, and SE-B-7 more or less overlapping with the binding pose of the initially docked complexes and that no significant transition occurred during the simulation. Figure 1 depicts the intricate contacts of the receptor-ligand complexes at the 25th nanosecond relative to the crystal ligand, and Figure 2 depicts the pairwise root-mean-square deviations of these interactions. All five NMDA NR2B receptor-ligand complexes were found to be stable throughout the simulation trajectories, according to the RMSD plots. After the first 10-15 ns, there was a plateau in the trajectory, indicating that the systems had stabilized.

Figure 2: All–atom RMSD plot for SE-C-13, SE-A-8, SE-B2, SE-B-3 and SE-B-7 docked in NMDA NR2B receptor.



Calculation of Binding Energy through MM/PBSA

The Binding Energy of the 19 compounds with NMDA NR2B receptor was evaluated by the MM/PBSA package of AMBER. The total Binding Energies of the 19 complexes include van der Waals, electrostatic, polar, and non-polar energy contributions and were tabulated in Table 2. The two compounds SE-C-13 and SE-B-which earlier showed best docking Scores[3] with NMDA NR2B receptor also exhibited a similar trend in binding energy estimation.

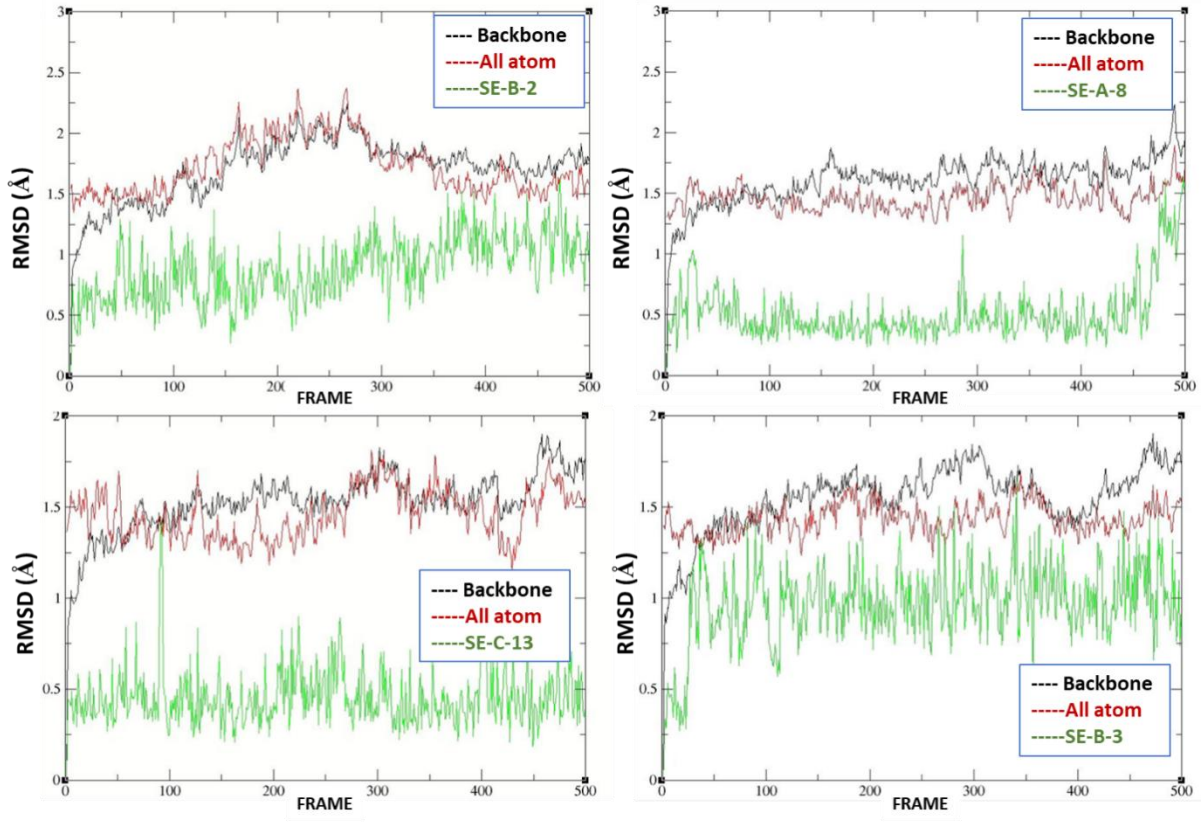
Table 2: MM/PBSA binding energy* computation of the compounds-NMDA NR2B receptor complex.

Ligand	**ΔE_{vdw}**	**ΔE_{ele}**	**ΔG_{Gas}**	**ΔG_{Free}**
SE - C - 13	-33.78 ± 2.91	-27.13 ± 4.44	-60.91 ± 5.19	-32.19 ± 3.64
SE - B - 2	-33.33 ± 2.60	-24.79 ± 6.72	-58.12 ± 5.95	-29.30 ± 3.77
SE - B - 3	-29.19 ± 1.98	94.77 ± 10.57	65.58 ± 10.00	-17.17 ± 2.50
SE - B - 12	-18.91 ± 2.46	-10.83 ± 5.77	-24.74 ± 6.02	-16.29 ± 3.46
SE - G - 2	-13.78 ± 2.91	-22.13 ± 4.44	-45.91 ± 5.19	-22.19 ± 3.74
SE - F - 8	-16.53 ± 3.08	-23.37 ± 8.03	-39.90 ± 8.55	-14.99 ± 6.28
SE - B - 11	-22.29 ± 1.98	82.67 ± 9.24	45.48 ± 9.24	-15.12 ± 3.70
SE - A - 8	-29.91 ± 2.46	-15.83 ± 5.77	-45.74 ± 6.02	-30.29 ± 3.46
SE - B - 14	-17.81 ± 3.46	-9.73 ± 5.77	-14.74 ± 5.02	-15.29 ± 2.46
SE - D - 2	-12.29 ± 2.98	54.67 ± 7.24	32.22 ± 7.22	-12.11 ± 2.70
SE - B - 8	-27.19 ± 1.98	85.77 ± 10.24	55.58 ± 10.24	-18.17 ± 2.70
SE - D - 8	-15.72 ± 2.46	-15.83 ± 2.77	-18.74 ± 7.02	-11.29 ± 5.46
SE - B - 15	-08.78 ± 3.91	-12.13 ± 5.44	-25.91 ± 6.19	-18.19 ± 5.74
SE - B - 7	-26.53 ± 3.08	-33.37 ± 9.03	-59.90 ± 9.55	-24.99 ± 7.28
SE - B - 13	-14.53 ± 5.08	-18.37 ± 7.13	-27.90 ± 9.55	-12.23 ± 7.28
SE - E - 13	-20.19 ± 1.95	65.62 ± 7.12	22.48 ± 10.24	-13.32 ± 3.92
SE - D - 11	-11.71 ± 2.46	-7.73 ± 4.22	-15.82 ± 4.02	-12.30 ± 2.76
SE - C - 9	-14.62 ± 2.56	-14.93 ± 3.87	-17.84 ± 6.02	-10.29 ± 2.46
SE - E - 2	-12.22 ± 1.98	55.77 ± 12.24	32.25 ± 09.24	-11.17 ± 3.70

*All the energies are calculated in Kcal/mol

Significant contributions from van der Waals interactions were found as -33.78, -33.33, -29.19, -29.91 and -26.53 Kcal/mol in SE-C-13, SE-B-2, SE-B-3, SE-A-8 and SE-B-7 respectively. Besides van der Waals interactions, the other thermodynamic parameters like electrostatic, polar, and non-polar energy, SASA energies contribute significantly, and we get the effective binding free energies in the same trend as -32.19, -29.30, -17.17, -30.29 and -24.09 Kcal/mol respectively for the above-mentioned SE-C-13, SE-B-2, SE-B-3, SE-A-8 and SE-B-7 compounds with NMDA receptors.

Figure 3: Detail RMSD plots of (a) SE-C-13, (b) SE-A-8, (c) SE-B2 and (d) SE-B-3 during 25ns simulation.



Conclusion

With the performed molecular dynamics and trajectory analyzed MM/PBSA binding energy calculations we have shown improve the potency of prescreened NMDA receptors. MD simulation was performed to estimate the critical interactions with the binding site residues of SE-C-13, SE-B-2, SE-B-3, SE-A-8, and SE-B-7 with other potent compounds, to elucidate the difference in their biological activity. The calculated Binding free energy indicated that several amino acids in the binding pocket provided key binding energy contributing residues. The results showed that SE-C-13, SE-B-2, and SE-A-8 have good potential to become the NMDA receptors. Our finding might be helpful in the future designing of Piperazine Sulfonyl Amine based NMDA-NR2B Receptor with better pharmacokinetic properties.

Conflict of Interest

The authors declared no conflict of interest.

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