INSILICO ANALYSIS OF SARS-COV-2 TARGET FOR SPIKE PROTEIN [RBD]

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Abstract:

Several changes to the coronavirus 2 linked to severe acute respiratory syndrome have increased the disease's transmission and death rates globally (COVID-19) (SARS-CoV-2). Despite the creation of multiple vaccines, a considerable portion of the global population continues to be at high risk for sickness. The 3D x-ray crystal structure of spike protein RBD with 2-acetamido-2-deoxy-Beta-D-glucopyranose derivative (6YZ5.pdb), H11-D4 complex with SARS-CoV-2 RBD, Macromolecule was retrieved from protein data bank and was then further prepared using the protein preparation wizard of Schrödinger suite 2020-1. In the present study have been performed to explore the binding modes of super natural compounds (SNP) against SARS-CoV-2 spike protein target by *in-silico* approach. The docking studies performed by Glide module, *In-silico* ADMET screening was performed by qik prop module and binding energy of ligand was calculated using prime Molecular Mechanics –Generalized

Born Surface area. From the result the SNP compounds. Molecular docking result SN00293542 –9.81, SN0021318 -9.07, SN00296151 –9.07. standard as a Ritonavir, - 7.48, Lopinavir - 6.94, Nelfinavir - 5.93. MMGBSA result like SN00293542 –54.44, SN0021318 - 40.96, SN00296151 –40.38. the docking results compounds exhibited similar mode of interaction with covid -19 and the residue SER- 459, LYS-458, ARG-457, GLU-465, ASP-467, SER- 469, GLN-474 play a crucial role binding with ligands. The Super Natural compounds are significantly active against Covid-19 which are useful for further drug development.

Key words – Coronavirus- 19, Molecular Docking, MMGBSA, Super Natural Compounds, Spike proteins.

INTRODUCTION

In December 2019, a new coronavirus produced the epidemic of novel coronavirus disease 2019 (COVID-19), which has now expanded globally and become a pandemic.(1) It was rapidly determined that a coronavirus later known as severe acute respiratory syndrome coronavirus 2 was responsible for the COVID-19 (SARS-CoV-2) It is the seventh coronavirus identified to infect people; the other four (229E, NL63, OC43, and HKU1) only induce mild cold symptoms. The fatality rates of the other three, SARS-CoV, MERS-CoV, and SARS-CoV-2, are 10%, 37%, and 5%, respectively, and are capable of producing severe symptoms as well as death.

SARS-CoV-2 is an RNA virus with a single stranded envelope.(2) Its whole genome, which is 29,881 bp long (GenBank no. MN908947) and encodes 9860 amino acids, has been characterised using an RNA-based metagenomics next-generation sequencing method (3). Both structural and non-structural proteins are expressed by gene fragments. The ORF area encodes non-structural proteins such as 3-chymotrypsin-like protease, papain-like protease, and RNA-dependent RNA polymerase, whereas the S, E, M, and N genes produce structural proteins.(4)

Numerous glycosylated S proteins are present on the surface of SARS-CoV-2 and bind to the angiotensin-converting enzyme 2 (ACE2) receptor to facilitate viral cell entry.(5) The type 2 TM serine protease TM protease serine 2 (TMPRSS2), which is found on the host cell membrane, activates the S protein when the S protein attaches to the receptor, facilitating virus entrance into the cell. Once within the cell, the viral RNA is released, the RNA genome's polyproteins are translated, and the replicas-transcriptase complex is assembled to

carry out the viral RNA genome's transcription and replication. In the host cell, viral RNA is reproduced, structural proteins are created, assembled, and packaged, and then viral particles are discharged.

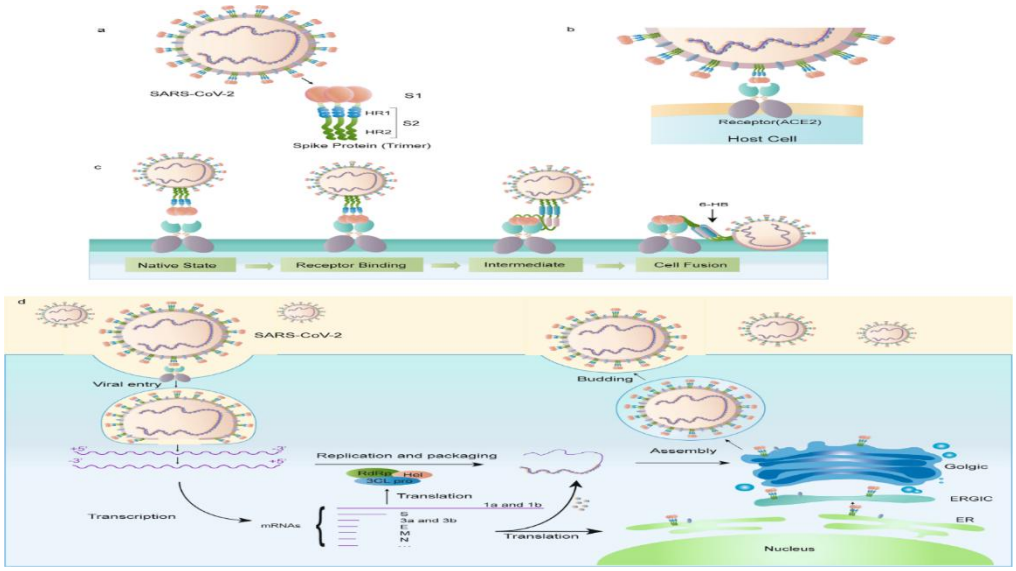

Figure:1 Life cycle of SARS-CoV- 2.

a. The schematic structure of the S protein. **b**. The S protein binds to the receptor ACE2. **c.**The binding and virus–cell fusion process mediated by the S protein. **d.**The life cycle of SARS-CoV-2 in host cells. These proteins serve as prospective targets for therapeutic therapy and are essential to the viral life cycle. For instance, experimental evidence has shown that ACE2-based peptide, 3CLpro inhibitor (3CLpro-1), and a new vinyl sulfone protease inhibitor are effective against SARS-CoV-2.(6) The S protein, which is shared by all human coronaviruses (HCoVs), is crucial for receptor recognition, viral attachment, and host cell invasion. Due to its crucial functions, it is one of the most important targets for COVID-19 vaccine and therapeutic studies. In this review, we discuss advancements in the understanding of the SARS-CoV-2 S protein and its drug target.

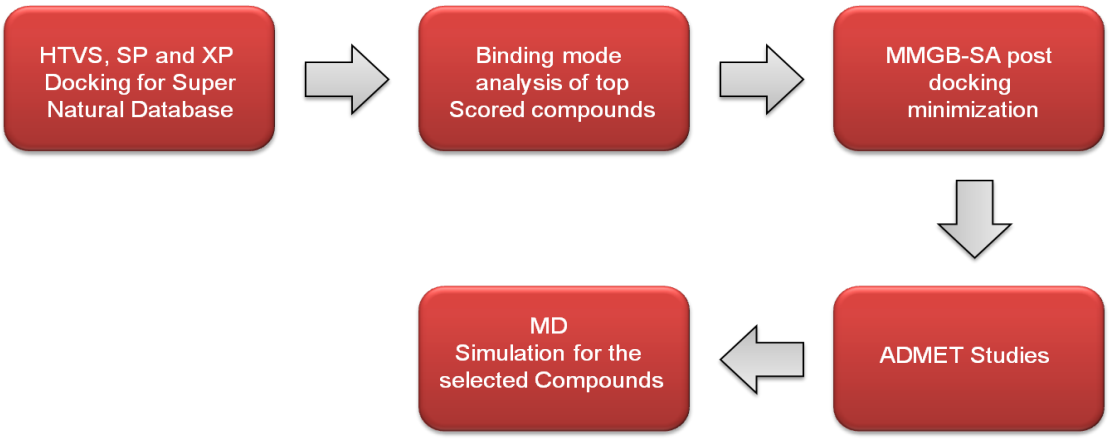

Figure 2: Flow chart for the identification of omicron variant

MATERIAL AND METHODS:

High-throughput virtual screening and Molecular docking

The 3D x-ray crystal structure of spike protein RBD with 2-acetamido-2-deoxy-beta-D-glucopyranose derivative (6YZ5.pdb), H11-D4 complex with SARS-CoV-2 RBD, Classification as a Viral protein, no mutation, Resolution – 1.8A, Macromolecule (Nano body – H11-D4 , Spike glycoprotein)was retrieved from protein data bank and was then further prepared using the protein preparation wizard of Schrödinger suite 2020-1. The protein was prepared by eliminating crystal waters and bond orders adjusted with addition of hydrogen. At pH 7.0, Prime was used to add missing side chains and loops, and then protonation and tautomeric states for acidic and basic residues were generated. The OPLS3e (Optimized Potentials for Liquid Simulations) molecular force field was used to minimize the protein, with the RMSD of crystallographic heavy atoms kept at 0.30. Å Grid box was generated (x = 52.16; y = 30.59; z = -0.62) 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. The docking protocol was validated using re-docking of Co-crystal ligand followed by calculating the RMSD difference between initial energy minimized pose and XP docked poses of Co-crystal ligand. The overlay of energy minimized initial pose and XP docked pose with RMSD difference was illustrated in The 3D conformers of 3, 25, 000 natural compounds were obtained from Super Natural Database. A virtual workflow was initiated with preparation of ligands using Ligprep and prefilter option to exclude redundant ligands. The ligands were sequentially docked into the catalytic pocket of Spike protein RBD (6YZ5.pdb) using HTVS, (High Throughput Virtual Screening), SP (standard precision) and XP (extra precision)

modes keeping default parameters. The Best docking pose was selected based on glide g-score, glide energy values and hydrogen bond analysis.(7)

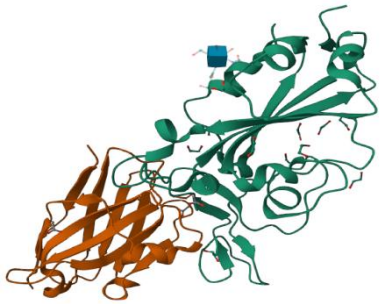

Figure 3: Structure of 6YZ5 – H11-D4 Complex with SARS CoV-2 Spike RBD

MMGB-SA free energy calculation studies

The contributions of enthalpy and entropy related components towards binding of ligand-protein complex was calculated using Prime MMGB-SA approach (8) which integrates Generalized-Born/Surface Area (GB/SA) continuum solvent model (9). The contributions from molecular mechanics' energies, polar solvation and a nonpolar solvation terms were estimated (kcal/mol) using equation: (10–12)

ΔG_{bind} = Calculated binding free energy of complex

$G_{complex}$ = Binding free energy of minimized complex

$G_{protein}$ = Binding free energy of receptor

G_{ligand} = Binding free energy of unbound ligand

Result and Discussion:

The results are summarized in covid-19 inhibitory of the compounds obtained from Super natural database and Protein revealed from RCSB PDB 6YZ5, The 3D X- ray crystal structure structure of spike protein RBD, with 2-acetamido-2-deoxy-Beta-D-nglucopyranose derivative, Significantly active against SARS-CoV-2 RBD.(13) SN00293542 -9.81, SN00213181 -9.07, SN00296151 -9.07, SN00350811-8.39, SN00162745- -8.27, SN00168969 -8.25, SN00007464 -8.19 Glide score more when compared to currently use Ritonavir- -7.48, Lipinavir- -6.94, Nelfinavir --5.93. The above compounds have good affinity to the receptor due to more lipophilic character and also due to hydrogen bonding. The results are summarized in the Table 1. The best-affinity modes of all the docked

compounds withCOVID-19 Spike RBD (PDB id: PDB 6YZ5) are shown in Figure 2. Almost all the compounds are docked in the same binding pocket. (14)

Table: 1 Glide XP docking values (kcal/mol) for the acquired hits in the dynamic site of SARS –CoV-2 Spike (6YZ5)

S.NO	Compounds	GScore	LipophilicEvdW	HBond
1	SN00293542	-9.81	-1.01	-7.38
2	SN00213181	-9.07	-0.63	-7.1
3	SN00296151	-9.07	-0.88	-6.71
4	SN00350811	-8.39	-1.54	-6.0
5	SN00162745	-8.27	-0.97	-5.2
6	SN00168969	-8.25	-1.73	-5.85
7	SN00007464	-8.19	-0.76	-5.86
8	SN00213037	-7.78	-0.98	-5.28
9	SN00216711	-7.7	-1.55	-5.27
10	32581-42-3	-7.46	-1.56	-4.46
11	SN00216710	-7.44	-1.14	-5.14
12	SN00334894	-7.35	-0.04	-5.68
13	102040-09-5	-7.32	-0.63	-5.41
14	SN00347999	-7.28	-1.58	-4.49
15	SN00330810	-7.15	-1.32	-4.45
16	SN00165563	-7.06	-1.75	-5.97
17	SN00403420	-7.04	-0.94	-4.87
18	SN00382588	-7.02	-1.88	-5.19
19	20633-84-5	-6.96	-2.43	4.93
20	SN00041592	-6.83	-0.55	4.77
21	139933-53-2	-6.71	0.79	4.67
22	SN00114482	-6.64	-1.52	6.43
23	474794-52-0	-6.54	-1.97	3.75
24	SN00249174	-6.51	-0.61	4.96
25	SN00352807	-6.39	-1.41	4.06
26	SN00143458	-6.37	-0.78	4.2
27	SN00215944	-6.26	-0.97	4.35
28	SN00379716	-6.18	-1.24	3.43
29	SN00038781	-5.7	-0.96	4.07
30	SN00332128	-5.69	-1.19	3.38
31	Ritonavir (Std)	-7.48	-6.57	-1.7
32	Lipinavir (Std)	-6.94	-6.01	-1.12
33	Nelfinavir (std)	-5.93	-4.86	-1.18

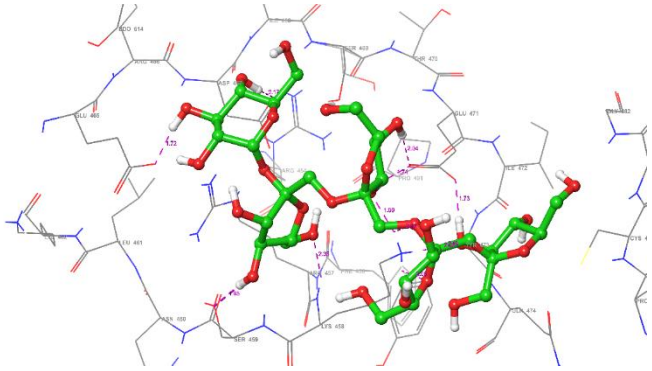

Figure 4. Docked poses of all compounds with SARS –CoV-2 Spike (6YZ5)

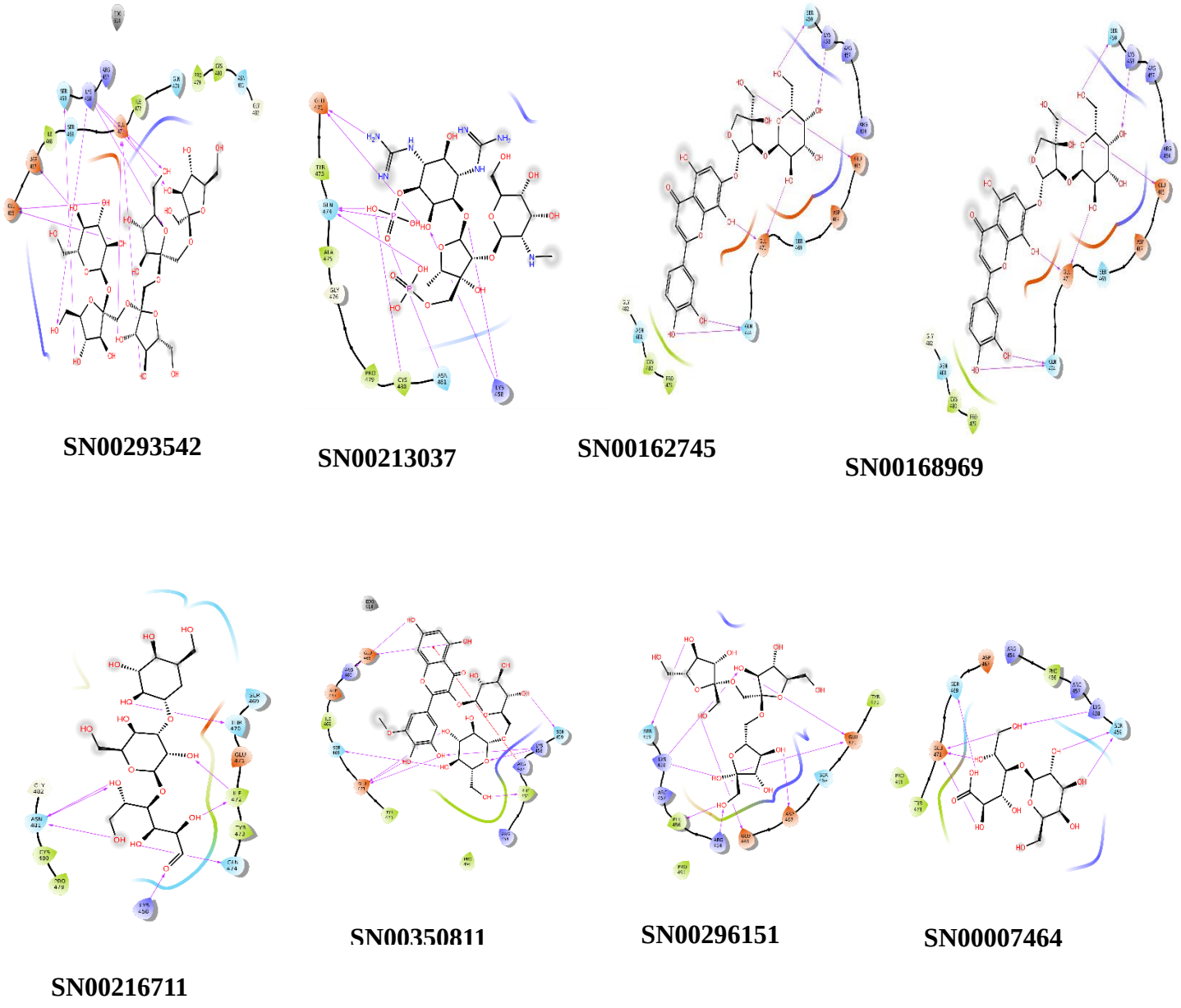

Figure 5. 2D Interaction of top 8 compounds SARS –CoV-2 Spike (6YZ5)

Table 2. Number of hydrogen bonds and interacting residues for the obtained hits in the active site SARS –CoV-2 Spike (6YZ5)

S.NO	COMPOUNDS	AMINO ACID INTERACTION
1	SN00293542	SER-459, LYS-458, ARG-457, ASP-467, ILE- 468, SER-469, GLU-471, GLN- 472, PRO- 479, CYS-480, ASN – 481, GLY-482.
2	SN00213037	GLU- 471, TYR- 473, GLN – 474, ALA- 475, GLY- 476, LYS-458, PRO-479, CYS- 480, ASN -481.
3	SN00162745	SER-459, LYS- 458, ARG- 457, ARG- 454, GLU-465, ASP-467, SER-469, GLN -474, GLY-482, ASN-481, CYS-480, PRO-479.
4	SN00168969	LYS- 458, GLY- 482, ASN- 481, CYS-480, PRO- 479, SER- 477, GLY-476, GLN – 474, TYR- 473, ILE- 470, GLU-471.
5	SN00216711	SER-469, THR-470, GLU-471, ILE-472, THY-473, GLN-474, LYS-458, PRO-479, CYS-480, ASN- 481, GLY- 482.
6	SN00350811	TYR- 473, GLU-471, SER-469, ILE-468, ASP- 467, ARG-466, GLU-465, PRO-491, ARG-454, PHE- 456, ARG-457,LYS- 458, SER- 459
7	SN00296151	THY-473, GLU-471, SER-469, ASP-467, GLU- 465, SER-459, LYS – 458, ARG- 457, PHE- 456, ARG- 454, PRO-491.
8	SN00007464	PHY- 473, GLU-471, SER- 469. ASP-467, ARG-454, PRO-491, ARG-457, LYS- 458, SER-459.

Table.3 Prime MMGB-SA binding free energy values (kcal/mol) for the acquired hits in the dynamic site SARS –CoV-2 Spike (6YZ5)

S.NO	Compounds	MMGBSA dG_Bind	MMGBSA_dG_ Bind_Coulomb	_MMGBSA_dG_Bind_Covalent	MMGBSA_dG_Bind_Hbond	MMGBSA_dG_Bind_Lipo
1	SN00293542	-54.44	-35.28	3.85	-11.12	-6.57
2	SN00213181	-40.96	-39.46	13.31	-7.39	-4.15
3	SN00296151	-40.38	-19.99	3.11	-5.59	-10.5
4	SN00350811	-51.78	-44.93	-2.7	-8.38	-2.16
5	SN00162745	-53.65	-32.14	4.11	-8.27	-12.63
6	SN00168969	-52.48	-41.9	8.2	-7.52	-9.37
7	SN00007464	-20.08	10.81	-17.31	-4.2	-2.28
8	SN00213037	-51.76	-64.21	10.58	-4.96	-3.85
9	SN00216711	-51.12	-44.86	-5.73	-5.58	-8.29

10	32581-42-3	-25.74	-29.88	-4.12	-5.99	-4.09
11	SN00216710	-15.03	-12.15	1.78	-5.94	-1.19
12	SN00334894	-49.05	-42.88	10.8	-9.33	-5.38
13	102040-09-5	-32.74	-25.6	-5.74	-6.91	-5.16
14	SN00347999	-28.28	-19.63	3.54	-4.19	-9.85
15	SN00330810	-30.53	-31.63	13.17	-9.07	-3.82
16	SN00165563	-67.69	-52.82	19.82	-11.3	-13.5
17	SN00403420	-30.41	-6.71	-5.94	-3.84	-1.41
18	SN00382588	-45.31	-23.16	-6.02	-3.42	-7.34
19	20633-84-5	-46.49	-32.3	1.36	-3.86	-7.89
20	SN00041592	-48.64	-42.06	4.7	-6.15	-7.94
21	139933-53-2	-30.22	-12.49	-0.04	-6.37	-12.08
22	SN00114482	-31.43	-12.19	-0.37	-4.13	-1.59
23	474794-52-0	-24.8	-22.3	-6.35	-6.36	-0.74
24	SN00249174	-40.88	-65.09	8.24	-8.69	-2.14
25	SN00352807	-23.77	-27.5	7.52	-6.84	-7.34
26	SN00143458	-23.78	-8.27	-8.43	-4.78	-6.65
27	SN00215944	-20.82	-22.88	4.18	-5.17	3.57
28	SN00379716	-28.37	-20.15	1.13	-2.57	-2.45
29	SN00038781	-47.71	-66.18	0.68	-6.23	-4.44
30	SN00332128	-42.9	-34.04	4.54	-4.51	-8.61

Molecular docking was additionally assessed with MM-GBSA free-restricting vitality which is identified with the postscoring approach for COVID19 Spike protein RBD (6YZ5.pdb) target and the values are shown in the Table 3. From the results of MM-GB/SA studies the dG bind valueswere observed in the range of –67.69- Kcal/mol to -15.03 and also dGvdw values, dG lipophilic values and the energies are positively contributing toward total binding energy. The accuracy of docking is confirmed by examining the lowest energy poses predicted by the scoring function. The Glide score and MM-GBSA free energy are obtainedby the docking of ligands into the coupling pocket are more stable.

CONCLUSION

From the results of docking study that supernatural compounds demonstrated better arrangement at dynamic site. The *in-silico* structuring strategy embraced in the present investigation helped for recognizing some lead molecules and furthermore may somewhat clarify their useful impact for further determinations like and *in vivo* assessments. The results from the *in-silico* study exhibited that many of the super natural compound may be useful against COVID-19. The supernatural compound SN00293542 –9.81, SN0021318 -9.07, SN00296151 –9.07 are significantly active against SARS-CoV-2. From the results of MM-GB/SA studies the dG bind values were observed in the range of –67.69- Kcal/mol to -15.03 and also dGvdw values, dG lipophilic values and the energies are positively contributing toward total binding energy. Spike RBD with remedial possibilities and are probably going to be helpful after further refinement. In conclusion of this study SNP Compounds may be useful remedy in the prevention of Spike protein SARS-CoV-2.

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Tamilnadu, India.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests.

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