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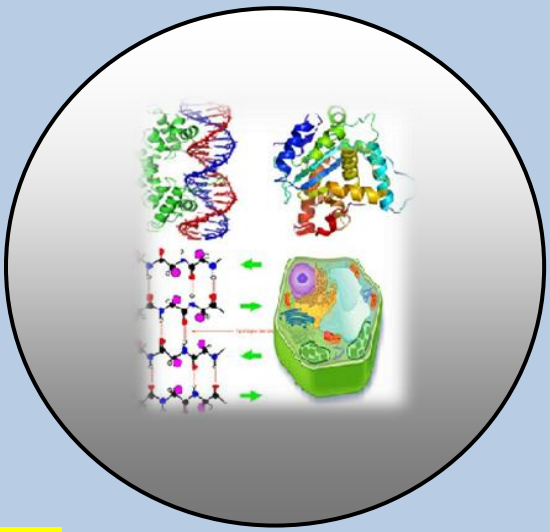

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RESEARCH PAPER

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Molecular Docking and Drug Designing Studies on Swine Flu H1N1 Virus

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ABSTRACT

In the present investigation we have performed computational molecular docking simulation studies against the Neuraminidase enzyme of Swine flu H1N1 virus. As we know that there is an alarming rise in drug resistant varieties and various subtypes and probability of a pandemic flu by genetic re-assortment. Thus, hinders an urgent attention for the scientific community to work together and gather information on fighting novel inhibitors or vaccine against this virus. In this context, our effort aims on targeting the active site of Neuraminidase enzyme against quercetin and its derivatives. The result obtained was interesting as some of the derivatives could inhibit the enzyme thereby hinting that these compounds can be prescribed whenever the next outbreak occurs.
Keywords: H1N1, Neuraminidase, Swine flu and Molecular docking.

INTRODUCTION

Swine influenza generally known as the pig influenza or swine flu is an infection caused by any one among the several types of swine influenza viruses. Swine influenza virus (SIV) or swine-origin influenza virus (S-OIV) is any strain of the influenza family of viruses that is

endemic in pigs **[Kimura et al., 1997, Matsuzaki et al., 2002 and Lynch and Walsh, 2007].** Since 2009, some of the known SIV strains include influenza C and the subtypes of influenza A known as H1N1, H1N2, H2N1, H3N1, H3N2, and H2N3 **[Ma et al., 2007, Shin et al., 2006, Kothalawala et al., 2006 and Yassine et al., 2007].**

Swine influenza virus is very common throughout pig populations in the world **[Olsen, 2002 and Taubenberger and Morens, 2006]**. The transmission of the virus from pigs to humans is possible but not common and does not always lead to human flu, it only leads the production of antibodies in the blood [**Vana and Westover, 2008 and Kimura et al., 1998].** But if transmission does cause human flu, and it is called zoonotic swine flu. People with regular exposure to pigs are at increased risk of swine flu infection **[Wells et al., 1991, Gray et al., 2007 and Patterson and Pyle, 1991].**

The influenza virion is roughly spherical. It is an enveloped virus; the outer layer is a lipid membrane which is taken from the host cell in which the virus multiplies **[Thacker and Janke, 2008**]. Inserted into the lipid membrane are "spikes", which are proteins—actually glycoproteins, because they consist of protein linked to sugars—known as HA (hemagglutinin) and NA (neuraminidase) [**Yu et al., 2008 and Lindstrom et al., 2004].** These are the proteins that determine the subtype of influenza virus (A/H1N1, for example). The HA and NA are important in the immune response against the virus; antibodies (proteins made to combat infection) against these spikes may protect against infection [**Myers et al., 2007, Retailliau et al., 1980 and Schonberger et al., 1979]**. The NA protein is the target of the antiviral drugs Relenza and Tamiflu. Also embedded in the lipid membrane is the M2 protein, which is the target of the antiviral adamantanes amantadine and rimantadine [**Haber et al., 2009 and Kaplan et al., 1982]**. Swine flu spread very rapidly worldwide due to its high human-to-human transmission rate and due to the frequency of air travel [**Vellozzi et al., 2009]**. In 2015 the instances of Swine Flu substantially increased to five year highs with over 10000 cases reported and 660 deaths in India **[Gray et al., 2007].**

In the present investigation, we trageted the NA protein of H1N1 which is required for the viral survival. The active site of the neuraminidase was targeted with quercetin and its analogues. The result was very interesting as it was evident that some of the analogues of quecertin inhibiting the active site of the neuraminidase enzyme. Thus, these findings would gain more attention in developing novel inhibitors against H1N1 Swine flu virus.

MATERIAL AND METHODS

Protein preparation

The three-dimensional crystal structure of H1N1 enzyme (PDB ID: 3B7E) determined by X-ray crystallography was retrieved from the Protein Databank (http://www.rcsb.org) and it was imported in the Molegro Virtual Docker [MVD] (Molegro APS: MVD 5.0) software. For docking purposes, all the water molecules along with the cofactors and the associated ligands were removed since they were not considered during scoring.

The 3B7E enzyme of consists of 2 chains (A & B). Considering the chains are identical and independent of one another, only chain A from the enzyme was imported in the MVD to perform the molecular docking simulation. This procedure reduced the computation time.

Compound retrieval

The two-dimensional and three-dimensional structure of quercetin was generated using Cambridge Soft Chem Office, 2010 (Cambridge Soft Corporation, 2010).

Additionally, a chemical structure search of Quercetin was performed at the NCBI Pub Chem database to retrieve the related compounds and analogues. The search parameters were set at 90 % similarity subjected to Lipinski rule of five filters. For docking purposes, the 2D structure of the 73 compounds from the Pub Chem compound search were converted to three-dimensional format using Chem Office, 2010. The energy of these compounds was optimized using MM2 force field methods and saved as tripos sybyl mol2 file format using Chem Office, 2010.

Molecular docking

The potential ligand binding site of H1N1 enzyme was predicted using Molegro Virtual Docker [MVD] (Molegro APS: MVD 5.0). The binding cavity was set at the site X: -0.30.23; Y: 10.92; Z: -21.64 within a constraint of radius 15 A° with a volume of 172.032 $A°^3$ and a surface area of 504 $A°^2$. The method adopted to determine the potential binding site is a grid-based cavity prediction algorithm. As described by Thomsen and Christensen initially a discrete grid with a resolution of 0.8 A° covering the protein is created and it is placed in a sphere with a radius of 1.4 A °. It is then checked whether the sphere will overlap as determined by the Van der Waals radii of the protein atoms. Then, each accessible grid point is checked for whether it is part of a cavity or not. The final step is to find out the connected regions. Two grid points are connected if they are neighbors. The cavities found are then ranked according to their volume. Bond flexibility of all the ligands imported in the MVD and the side chain flexibility of the active site amino acid residues of the protein within the cavity is set with a tolerance of 1.10 and strength of 0.90 for docking simulations. RMSD threshold for multiple cluster poses is set at 2.00 A°. The docking algorithm is set at a maximum iteration of 1500 with a simplex evolution size of 50 and a minimum of 10 runs. Molecular docking is carried out using Molegro Virtual Docker (MVD). MVD is based on a differential evolution algorithm; the solution of the algorithm considers the sum of the intermolecular interaction energy between the ligand and the protein and the intra-molecular interaction energy of the ligand. The docking energy scoring function is based on the modified piecewise linear potential (PLP) with new hydrogen bonding and electrostatic terms included.

RESULTS AND DISCUSSION

The optimized potentials of the compound retrieved from the NCBI Pub Chem database and their optimized energies are shown in Table 1. The optimization method used in the present investigation is based on the Allinger's potential function which includes one of two possible electrostatic terms: one based on bond dipoles, or one based on partial atomic charges. The addition of a charge-dipole interaction term allows for a combined approach, where partial charges are represented as bond dipoles, and charged groups, such as ammonium or phosphate, are treated as point charges. Also the MM2 includes the addition of a quartic bond stretching term, troublesome negative bond stretching energies which appear when long bonds are treated by Allinger's force field. The quartic bond stretching term is required primarily for molecular dynamics; it has little or no effect on low energy conformations. While the cut offs for electrostatic and van der Waals terms implemented in optimizing the 73 compounds greatly improve the computation speed for large molecules by eliminating long range interactions from the computation. From the table 1, it is revealed that all most all the 73 compounds used in the present investigation are stable and geometrically optimized.

Table 1. Optimized energies of the compounds used in the present investigation.

SN	CID	Stretch-Bend	Torsion	Non-1,4 VDW	Dipole/Dipole	Total Energy
1	CID26034	0.1057	-12.1968	-3.2152	3.6309	25.9415 kcal/mol
2	CID147651	0.1715	-9.8835	0.1371	2.5872	36.1007 kcal/mol
3	CID627207	0.0103	-14.4428	-5.9758	-0.7804	10.7567 kcal/mol
4	CID628780	0.0103	-14.4428	-5.9758	-0.7804	10.7567 kcal/mol
5	CID632128	0.2295	-10.1307	1.2120	2.7127	43.4030 kcal/mol
6	CID797370	0.0694	-12.1460	-3.7246	2.4747	24.0380 kcal/mol
7	CID5280417	0.0644	-10.0471	-1.9081	2.9344	28.1148 kcal/mol
8	CID5280666	0.1633	-13.8982	-5.7425	-0.3632	9.7386 kcal/mol
9	CID5280681	-0.0135	-10.0628	-2.8349	3.0252	21.0619 kcal/mol
10	CID5280682	0.1183	-10.1327	-0.7737	3.1030	35.4491 kcal/mol
11	CID5281604	0.0241	-12.4180	-4.1253	3.7014	18.8780 kcal/mol
12	CID5281612	-0.0385	-15.6050	-2.9489	1.8187	12.0864 kcal/mol
13	CID5281654	-0.0184	-12.2279	-1.5163	4.6192	23.6830 kcal/mol
14	CID5281691	-0.0027	-12.2631	-5.1808	4.0276	18.0104 kcal/mol
15	CID 5281699	-0.0291	-12.2835	-5.0222	4.2145	18.4087 kcal/mol
16	CID5281953	0.0264	-13.9013	-3.3761	3.7335	25.3608 kcal/mol
17	CID5282154	-0.0477	-14.2565	-5.3179	3.6838	16.9217 kcal/mol
18	CID5315126	-0.0402	-12.9030	-7.2931	4.6389	14.4858 kcal/mol
19	CID5316900	0.0449	-9.7697	1.6473	3.5776	33.8183 kcal/mol
20	CID5318214	-0.0114	-15.5087	-3.1975	1.6275	11.7190 kcal/mol
21	CID5319731	0.0449	-9.7697	1.6473	1.6275	11.7190 kcal/mol
22	CID5320496	0.0397	-15.5150	-2.1278	1.7670	19.1553

						kcal/mol
23	CID5320287	0.0550	-12.4164	-4.0710	4.1359	25.4326 kcal/mol
24	CID5320945	0.0659	-12.5229	-0.5858	4.5267	30.7564 kcal/mol
25	CID5378244	-0.0123	-12.3124	-4.5953	2.5434	16.9685 kcal/mol
26	CID5380905	0.0396	-10.0586	-1.7391	3.1805	28.4499 kcal/mol
27	CID5400219	-0.1106	-15.1643	-9.9469	0.9532	-1.3047 kcal/mol
28	CID5464381	0.0495	-15.3649	1.2819	2.1670	24.4280 kcal/mol
29	CID5481966	-0.0366	-15.0212	-5.9991	4.6524	14.8879 kcal/mol
30	CID5482937	-0.6279	-12.7576	-1.4334	6.8336	151.1925 kcal/mol
31	CID5487855	0.0640	-12.2103	-0.0696	4.6102	31.7825 kcal/mol
32	CID5489501	0.0905	-9.8039	-0.8476	2.6620	29.0687 kcal/mol
33	CID6105183	-0.0982	-13.4868	-9.7486	2.0271	0.3776 kcal/mol
34	CID6452329	0.0444	-12.3723	-5.7220	3.9411	19.3798 kcal/mol
35	CID6477684	-0.0427	-10.7253	-4.5083	0.2814	12.1240 kcal/mol
36	CID6481478	0.0571	-11.9980	-5.0142	2.2176	22.4853 kcal/mol
37	CID9799499	-0.0346	-14.1233	-8.6739	4.3026	12.5940 kcal/mol
38	CID9839293	-0.0005	-9.8411	-2.5569	1.5367	19.7844 kcal/mol
39	CID9949390	-0.0284	-12.2577	-5.0988	4.1963	18.3390 kcal/mol
40	CID10473561	-0.8931	-9.3350	-4.4781	10.6653	156.7942 kcal/mol
41	CID10493280	0.0043	-15.5863	-2.7708	0.1830	10.5220 kcal/mol
42	CID10807373	0.1217	-13.7821	-2.0617	2.0323	31.0577 kcal/mol
43	CID11347622	-1.1517	-2.3047	-3.4343	1.6832	100.6037 kcal/mol
44	CID11474580	0.1381	-10.6513	-3.5325	1.2718	22.7630 kcal/mol
45	CID13942543	0.2319	-9.5978	4.5902	3.1241	48.7731

						kcal/mol
46	CID13964547	0.0634	-15.6018	-1.0557	1.4177	19.8948 kcal/mol
47	CID13964548	0.0892	-15.5021	-1.2705	1.2294	19.5812 kcal/mol
48	CID13964549	0.0808	-15.4875	2.2636	1.8061	25.1383 kcal/mol
49	CID14079475	-0.0097	-14.0139	-3.7146	2.4541	19.4122 kcal/mol
50	CID14162696	0.0883	-12.2276	0.2961	4.1907	31.4855 kcal/mol
51	CID14162697	0.1559	-10.0757	3.7756	3.1238	41.6407 kcal/mol
52	CID15227607	0.0772	-12.3664	-3.0944	3.7975	26.2694 kcal/mol
53	CID15895793	0.0489	-13.9844	-3.1916	2.0759	23.9009 kcal/mol
54	CID18372853	-0.0411	-16.5276	-8.3852	1.8074	10.3254 kcal/mol
55	CID18372862	0.0361	-12.1984	-3.6013	3.6542	20.0358 kcal/mol
56	CID20159736	0.0787	-9.6251	-3.4105	2.5241	22.9346 kcal/mol
57	CID23320229	0.0609	-8.9823	-3.0236	1.7299	21.3765 kcal/mol
58	CID25202456	0.0306	-15.7408	-0.0739	-3.2930	12.5654 kcal/mol
59	CID44258706	-0.0479	-16.4324	-8.3921	1.8432	10.1369 kcal/mol
60	CID44259511	0.0293	-14.1523	-0.1148	4.2797	31.4787 kcal/mol
61	CID44259518	-0.0188	-11.1232	-0.9960	3.7642	32.2069 kcal/mol
62	CID44259636	-0.0244	-14.1472	-9.0629	3.1012	11.0701 kcal/mol
63	CID44259638	0.0101	-14.1650	-4.4614	3.7164	24.1241 kcal/mol
64	CID44259678	-0.0405	-13.2807	-2.2368	2.8286	19.7828 kcal/mol
65	CID44259679	-0.0320	-13.2823	-2.7150	2.7769	19.4232 kcal/mol
66	CID44610311	0.0575	-15.6041	-2.8980	1.2665	13.6235 kcal/mol
67	CID44610313	0.1311	-13.3698	-3.4425	1.2737	18.6224 kcal/mol
68	CID44610474	0.0961	-13.3738	-3.0212	1.2786	17.3428

						kcal/mol
69	CID46228135	0.1001	-14.6393	-2.1495	0.4586	17.5474 kcal/mol
70	CID46781386	-0.0417	-15.6060	-2.9538	1.8250	12.0684 kcal/mol
71	CID49835129	-0.0537	-16.5348	-11.8617	1.4471	4.8728 kcal/mol
72	CID49849634	-0.0417	-15.6060	-2.9538	1.8250	12.0684 kcal/mol
73	CID53811601	-0.0284	-12.2577	-5.0988	4.1963	18.3390 kcal/mol

Table 2. Molecular docking result of the compounds docked at the active site of H1N1 Neuraminidase.

Ligand	MolDock Score[a]	Rerank Score[b]	Interaction[c]	Internal[d]	HBond[e]
CID5315126	**-115.43**	**-96.89**	**-148.68**	**33.25**	**-8.39**
CID9799499	**-112.84**	**-96.24**	**-152.77**	**39.93**	**-10.42**
CID20159736	**-109.96**	**-95.20**	**-145.77**	**35.81**	**-4.82**
CID11474580	**-117.85**	**-94.57**	**-151.13**	**33.29**	**-7.59**
CID10807373	**-108.98**	**-93.40**	**-142.67**	**33.69**	**-1.75**
CID23320229	**-114.82**	**-93.36**	**-136.92**	**22.09**	**-4.82**
CID5319731	**-106.17**	**-92.53**	**-140.65**	**34.48**	**-10.80**
CID44610313	**-112.83**	**-92.03**	**-135.17**	**22.34**	**-5.57**
CID44610311	**-111.78**	**-91.96**	**-137.27**	**25.49**	**-4.83**
CID5481966	**-106.22**	**-91.22**	**-138.22**	**32.00**	**-5.20**
CID147651	**-108.81**	**-90.96**	**-141.47**	**32.66**	**-4.54**
CID797370	**-103.71**	**-90.81**	**-128.39**	**24.68**	**-9.90**
CID5282154	**-103.03**	**-90.59**	**-140.37**	**37.34**	**-8.68**
CID44259678	**-105.84**	**-90.34**	**-141.03**	**35.19**	**-6.89**
CID9949390	**-107.52**	**-89.95**	**-135.51**	**27.99**	**-8.08**
CID44259679	**-99.93**	**-88.74**	**-133.53**	**33.60**	**-6.06**
CID5280417	**-104.07**	**-87.93**	**-136.37**	**32.30**	**-4.33**
CID44258706	**-101.01**	**-87.83**	**-133.81**	**32.80**	**-6.28**
CID5280681	**-104.53**	**-87.55**	**-135.14**	**30.61**	**-5.79**
CID18372853	**-102.78**	**-87.49**	**-133.57**	**30.79**	**-2.41**
CID9839293	**-104.98**	**-87.43**	**-133.87**	**28.89**	**-3.71**
CID44259636	**-97.02**	**-86.74**	**-129.57**	**32.55**	**-3.92**

a - Moldock score is derived from the PLP scoring functions with a new hydrogen bonding term and new charge schemes

b - The rerank score is a linear combination of E-inter (steric, Van der Waals, hydrogen bonding, electrostatic) between the ligand and the protein, and E-intra (torsion, sp2–sp2, hydrogen bonding, Van der Waals electrostatic) of the ligand weighted by pre-defined coefficients

c - The total interaction energy between the pose and the protein

d - The internal energy of the pose

e - Hydrogen bonding energy

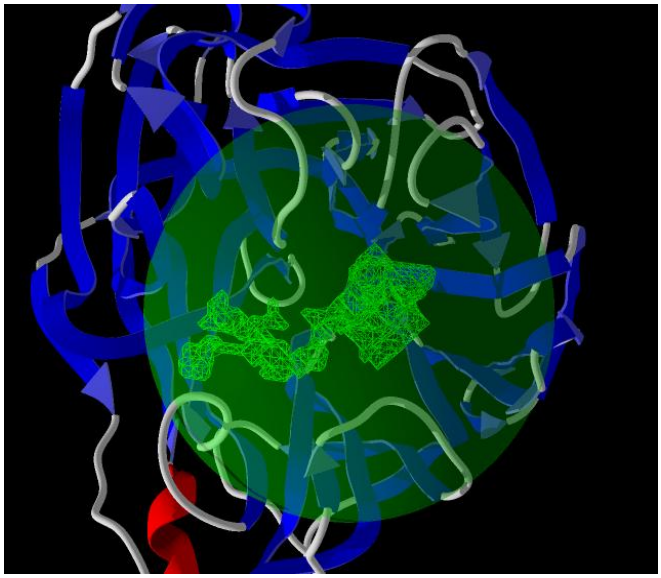
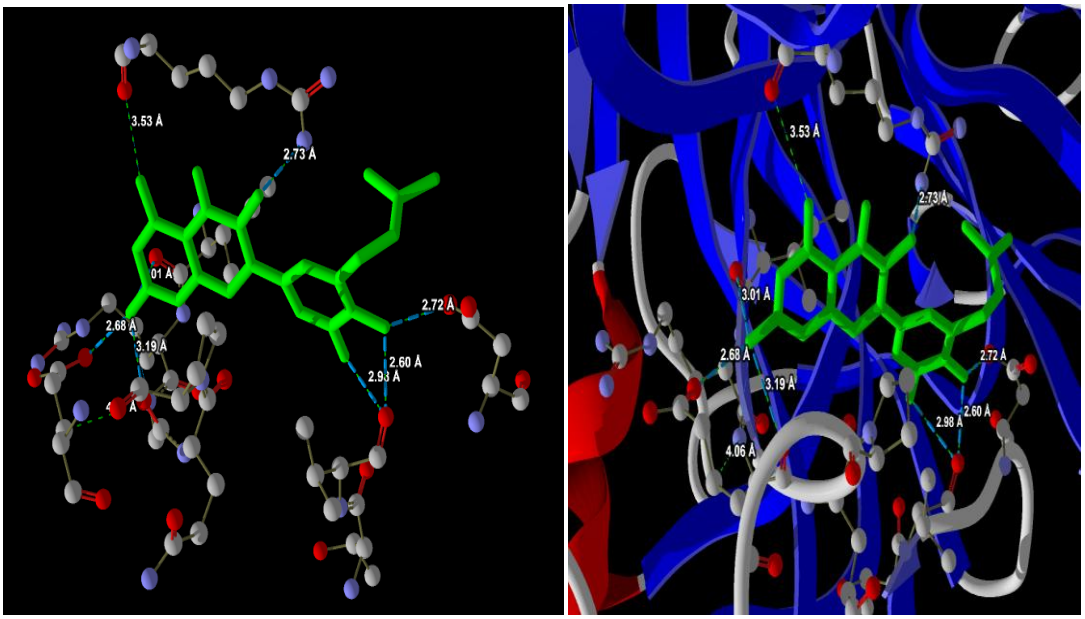


Figure 1. Potential ligand binding site of H1N1 Neuraminidase.

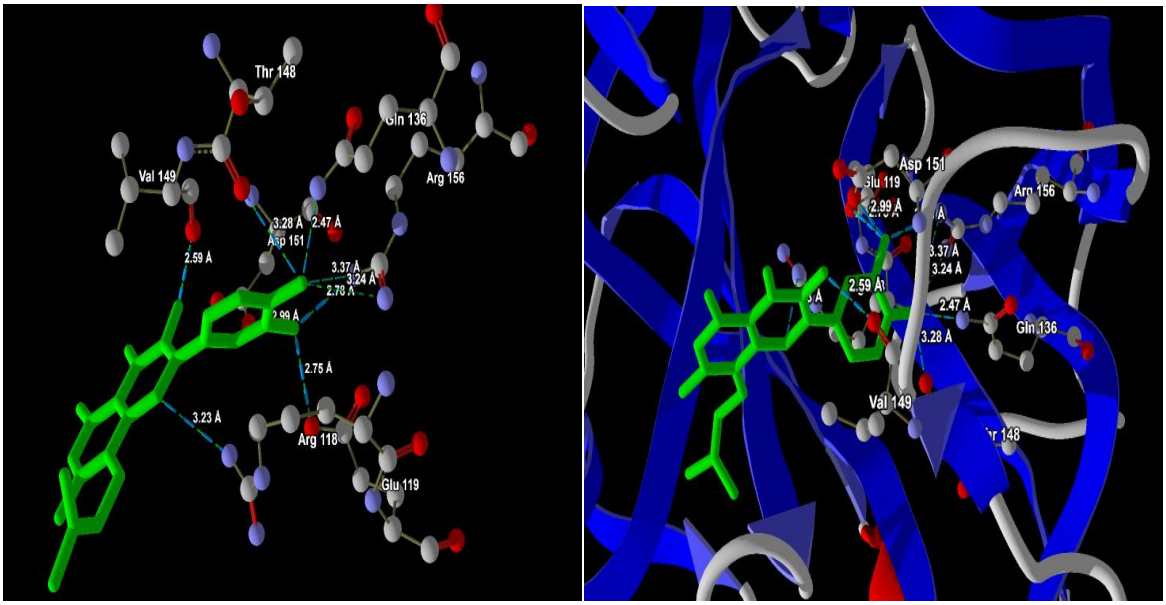


A **B**

Figure 2. Molecular interaction of CID5315126 depicting (A) binding mode and (B) secondary structure molecular interaction with the H1N1 Neuraminidase.

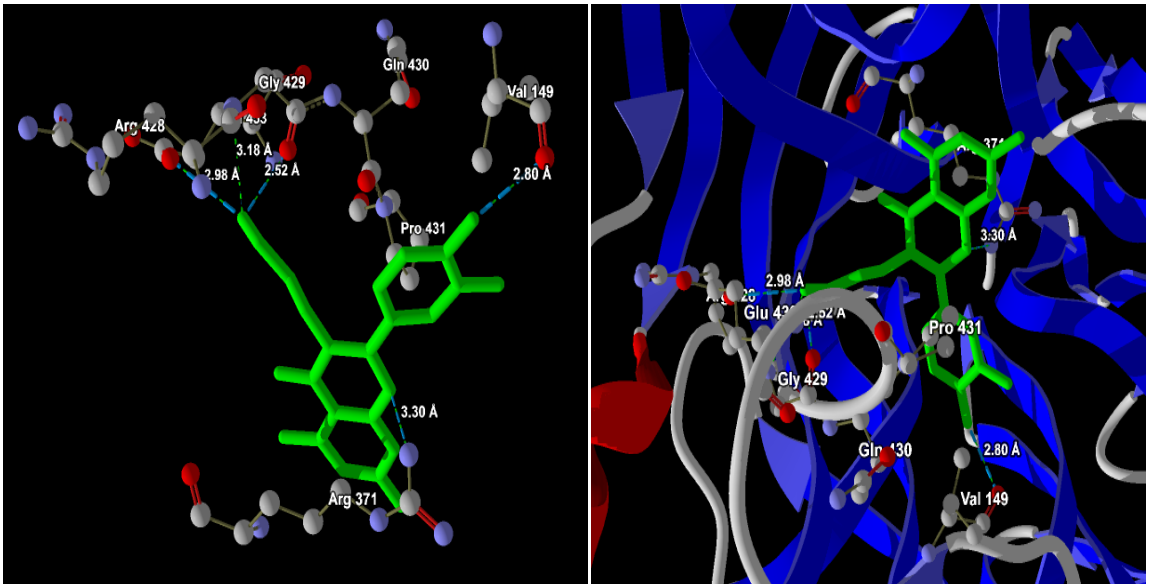
The potential ligand binding site predicted using Molegro Virtual Docker [MVD] for the H1N1 (PDB ID: 3B7E) which have a volume of 172.032 $A°^3$ and a surface area of 504 $A^{°2}$ is shown in Fig. 1. Molecular docking was carried out and the top poses docked at the active site region of the H1N1 neuraminidase enzyme were ranked based on the re-rank score (shown in Table 2). From the docking result, it is observed that the CCID5315126, CID9799499, CID20159736 and CID11474580 have favorable Re-rank score and MolDock score. The re-rank score is a linear combination of E-inter (steric, Van der Waals, hydrogen bonding, electrostatic) between the ligand and the protein, and E-intra (torsion, sp2–sp2, hydrogen bonding, Van der Waals, electrostatic) of the ligand weighted by pre-defined coefficients Thomsen and Christensen.

Besides, these compounds (CID5315126, CID9799499, CID20159736 and CID11474580) have favorable ligand–protein interaction energy with the active site of H1N1 Neuraminidase. Also, a detailed analysis of the top poses of the analogues in terms of ligand–protein interaction energy was carried out and the snaps are shown in Fig 2, Fig 3, Fig 4 and Fig 5 respectively. The ligand–protein interaction energy analysis (both electrostatic and H-bond) were calculated to get a better understanding of the variations between the binding mode of each compound and the molecular factors responsible for the activity.



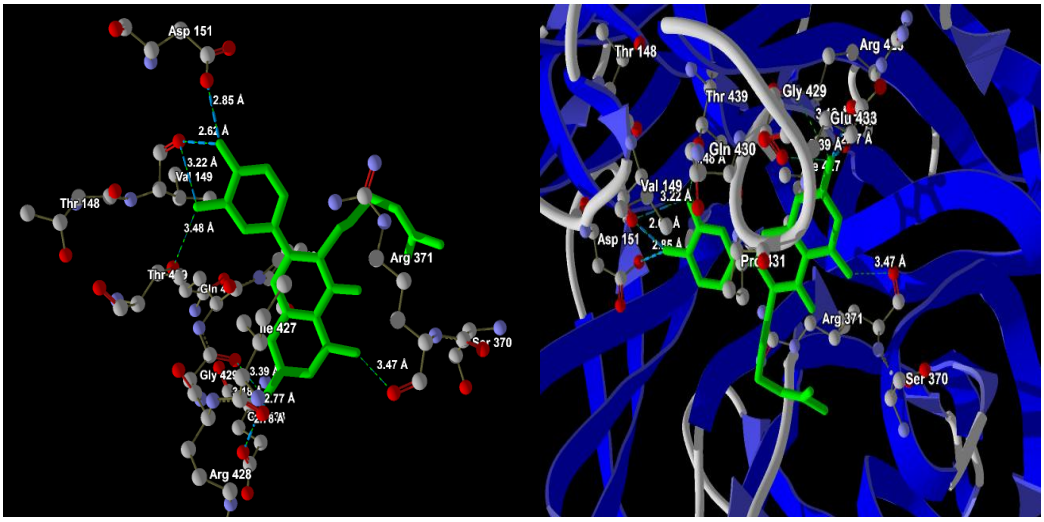
A **B**

Figure 3. Molecular interaction of CID9799499 depicting (A) binding mode and (B) secondary structure molecular interaction with the H1N1 Neuraminidase.



A **B**

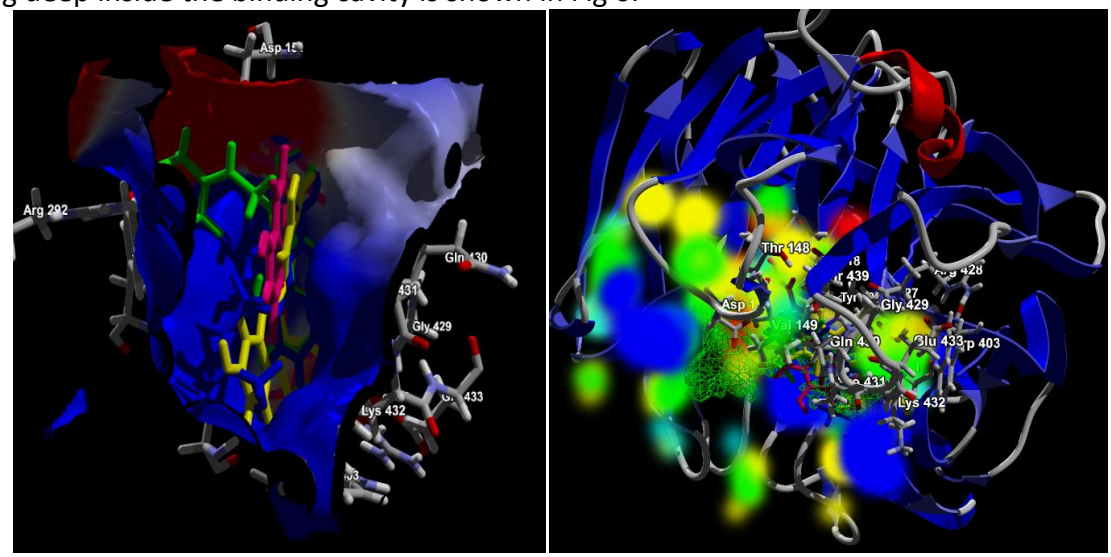
Figure 4. Molecular interaction of CID20159736 depicting (A) binding mode and (B) secondary structure molecular interaction with the H1N1 Neuraminidase.



A **B**

Fig 5. Molecular interaction of CID11474580 depicting (A) binding mode and (B) secondary structure molecular interaction with the H1N1 Neuraminidase.

Additionally, the electrostatic interaction and energy map depicting the top docking hits lying deep inside the binding cavity is shown in Fig 6.



A **B**

Figure 6 (A) Electrostatic interaction and (B) energy map depicting the top docking hits lying deep inside the binding cavity.

CONCLUSION

The Neuraminidase enzyme of Swine flu virus H1N1 was retrieved and molecular docking was carried out against quercetin and its derivatives. The molecular docking revealed CID5315126, CID9799499, CID20159736, and CID11474580 have favorable molecular docking score as evidenced by Mol Dock score, Rerank score and interaction energy. The energy map and electrostatic interaction also reveal these compounds dock at the potential ligand binding site. Hence comes to a conclusion that these compounds can be proposed as a drug for treating swine flu virus.

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