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In Silico Studies of Synthetic Sulfatide as a Potential Drug Candidate Against Covid-19

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ABSTRACT

Sulfatides play various roles in many biological processes such as cancer metastasis, viral infections and regulation in nerve cells. The sulfatide molecules are related with hypertension diseases in which ACE2 (Angiotensin converting enzyme) is important for regulating blood pressure. ACE2 is also a key receptor for Covid-19 and highly expressed many different tissue types. Understanding the interaction between the sulfatides and ACE2 might be a key factor to develop potential novel treatments against Covid-19. Here we studied the interaction of main protease enzyme (6LU7) of Covid-19 with native sulfatide(A), chitosan based synthetic sulfatide(B) and inhibitor N3, through in silico studies such as molecular docking, molecular dynamics, ADMET prediction and target selection analysis. The compounds A, B and N3 bind the virus protease enzyme with docking score of -5.420, -6.009, -6.161 kcal/mol respectively indicates synthetic sulfatide binds better than native sulfatide and comparable to N3. Besides, molecular dynamics studies were carried out to reveal the stability of the complexes of interest. ADMET and target prediction studies carried out to reveal pharmacological properties and toxicity of the complexes and synthetic sulfatide found to be a drug-like molecule. We anticipate that computational investigation of virus interaction mechanisms will be an important starting point for experimental research in drug development efforts against Covid-19.

Keywords: Sulfatide, Covid-19, Drug design, Molecular docking, MD simulation.

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Introduction

The recent outbreak emerged in the late 2019, named Covid-19 by the World Health Organization (WHO), have been a global challenge for scientific community to find an immediate cure[1]. Phylogenetical analysis show that the newly emerged virus is similar to the early versions including the SARS-CoV (Severe acute respiratory syndrome–coronavirus) and MERS-CoV (Middle East respiratory syndrome coronavirus) which were emerged in 2002 and 2012 respectively, so that the new virus is named as SARS-CoV-2 because of genetic similarity[2]. It is found that most of the Covid-19 infected patients show symptoms in a broad range such as difficulty in breathing, weakness, fever, vomiting, loss of taste and smell and dry cough[3-6]. In order to enter to host cell, the SARS-CoV-2 uses ACE2 receptors which are type I membrane proteins found on the surface of mammalian cells, especially in lungs, heart, kidneys, and intestine. These receptors are associated with the metabolism of angiotensin (Ang), a peptide hormone that regulates vasoconstriction and blood pressure[2, 7, 8]. The hypertensive patients use the ACE inhibitor drugs to control their blood pressure; however, they express the ACE2 more than normal due to the inhibitor drug consuming[9]. As a result, hypertensive patients having Covid-19 are at more risk than normal individuals[10]. In addition, differences in expression levels of ACE2 between children and adults is suggested recently to explain why Covid-19 is milder in children than adults[11]. Therefore, computational efforts to shed light on sulfatides' interactions will be helpful to understand the underlying mechanisms of Covid-19.

The sulfatides are expressed in liver tissue and found abundantly in neural systems [6,12]. The sulfatide prevents human paravirus influenza type 3 to enter COS-7 cells [13]. It has been reported that the concentration of sulfatide in the blood of children may be higher than in adults [14]. The low sulfatide concentration increases hypertension risk two times than having high amount of sulfatide in blood[15-17]. Changes in the expression of hepatic cerebroside sulfotransferase (CST), the key enzyme involved in sulfatide synthesis, are the primary determinants of serum sulfatide amount [16]. Regarding Covid-19, it could be that high amount of the sulfatide might reduce the infection ability or disease severity and vice-verse[6, 18]. In their cell culture experiments Davies et al. observed that fenofibrate drug decreased Covid-19 infection significantly and fenofibrate is thought to increase the amount of sulfatide in the blood[19]. We synthesized and characterized chitosan based synthetic sulfatide both chemically and biologically and have published elsewhere[12].

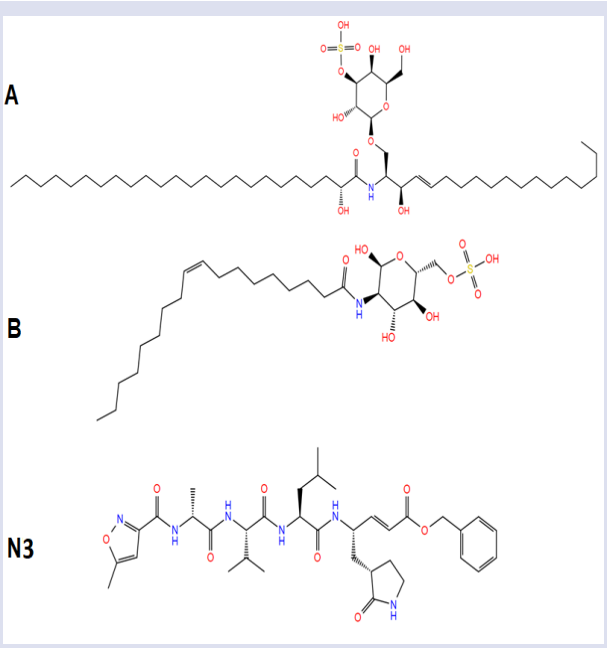

Figure 1. 2D structures of compounds A, B, and N3

Molecular docking studies were performed in order to reveal and compare the binding properties of the natural ligand (A), the synthetic derivative (B) under investigation and the N3, ligand of crystallized form for comparison[20]. In addition, molecular dynamics simulations were employed to verify the stability of protein-ligand complexes. Both studies showed that chitosan based synthetic sulfatide can play an important role for inhibition ACE2 as receptor for Covid-19.

Material and Methods

Docking Studies

Maestro 12.8 of Schrödinger (Schrödinger Release 2021-4: Maestro, Schrödinger, LLC, New York, NY, 2021) software was used in all molecular docking studies. The structures of the ligands were drawn with 2D Sketcher software. The ligands were minimized using LigPrep, a utility of Schrodinger (Schrödinger Release 2021-4: Maestro, Schrödinger, LLC, New York, NY, 2021). The X-ray structure of the target protein (PDB ID: 6LU7) was downloaded from the RCSB Protein Data Bank (www.rcsb.org)[21, 22]. Schrödinger's modules, Protein Preparation Wizard Prime, Impact, Epik, Prime (Schrödinger Release 2021-4: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2021; Impact, Schrödinger, LLC, New York, NY; Prime, Schrödinger, LLC, New York, NY, 2021.)[22] and Propka[23] were used for removing ligands and solvent molecules in protein, adding hydrogens, assigning charges and deleting polar hydrogens for clarity. Grid maps were created with the Maestro (Schrödinger Release 2021-4: MacroModel, Schrödinger, LLC, New York, NY, 2021). grid generation panel, and prepared ligands were docked in this grid map 100 times in standard precision (SP) mode using the Glide software[24, 25]

Molecular Dynamics Simulations

MD simulations were carried out by the Desmond (Schrödinger Release 2021-4: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2021. Maestro-Desmond Interoperability Tools, Schrödinger, New York, NY, 2021.)[26] module through Maestro of Schrödinger suite in order to investigate stability and interaction profiles of protein-ligand complexes for 50ns. Backbone RMSDs, the average distance between the backbone atoms of the protein–ligand structures, were plotted to compare the structural and dynamical properties[27].

Table 1. Program parameters of MD stimulation studies

Force Field	OPLS3E[28]
Solvation	Crystallographic Water (TIP3P)
Counter Ions	Na + Cl-
Ensemble	(Npt) Of Nose–Hoover Thermostat 300k Barostat 1bar
Boundary Conditions	Orthorhombic Periodic Boundary Conditions
Buffer Region	10 Å
Any Deleted Molecules	Water, Etc.
Minimization Algorithm	1000 Steps Of Steepest Descent Followed By Conjugate Gradient
Adjusting The Concentration Of The System	0,15M NaCl

ADME Prediction

ADME (Adsorption, Distribution, Metabolism and Excretion) evaluation is a key step to analyze the pharmacodynamics properties of the molecules to be used as a drug. The 2D structures of the compounds were drawn using the 2D Sketcher module of the Maestro program. Smiles data of the compounds were transferred to the SWISS-ADME[29] online program and various physicochemical parameters, Lipophilicity, Water Solubility, Lipinski rules, and drug likeness scores of the given compounds were calculated[30].

Target Prediction

Molecular target studies are used to predict the effects of small molecules in the body. These may cause cross-reactivity with other proteins or cause side effects[31]. Using the Swiss Target Prediction website[29] (https://www.swisstargetprediction.ch), the smile formula of the molecules was examined by applying it to the search bar.

Toxicity Prediction

The toxicology prediction of small drug candidates must be known before applying them to the animal or human model. In this case, pkCSM [24] database (is used for details of toxicological effects such as AMES Toxicity, human maximum tolerance dose, hERG-I inhibitor, hERG-II inhibitor, LD50, LOAEL, Hepatotoxicity, Skin Toxicity, T. pyriformis toxicity, and Minnow toxicity). The website was accessed and SMILES of the sulfatides were entered into the website search bar and the toxicity mode was selected[32].

Results and Discussion

Molecular Docking and MD Simulations

Molecular docking studies were carried out to examine the interactions of ligands with residues in the active site of the target protein (6LU7). For the validation of the docking studies, the N3 in the crystal structure of the protein was removed, minimized, redocked and the RMSD value was calculated 0.526 Å. The compounds A, B and N3 bind the virus protease enzyme with docking score of -5.420, -6.009, -6.161 kcal/mol respectively (Table 2).

Table 2. Docking scores of compounds, A, B, and N3 with 6LU7 PDB encoded protein

Compounds	Docking Scores (kcal/mol)
A	-5.420
B	-6.009
N3	-6.161

The molecular docking score of the compound B was higher than the scores of compound A and also very close to the docking score of N3. Similar to N3, compound B interacted hydrogen bonded in the active site of the enzyme with GLU166, GLN189 and hydrophobicly with LEU27, MET49, LEU141, PHE140, CYS145, MET165, LEU167, PRO168, ALA191. It made polar interactions with THR190, and charged (positive) interaction with ARG188. It also made a hydrogen bond with THR190 (Figure 2).

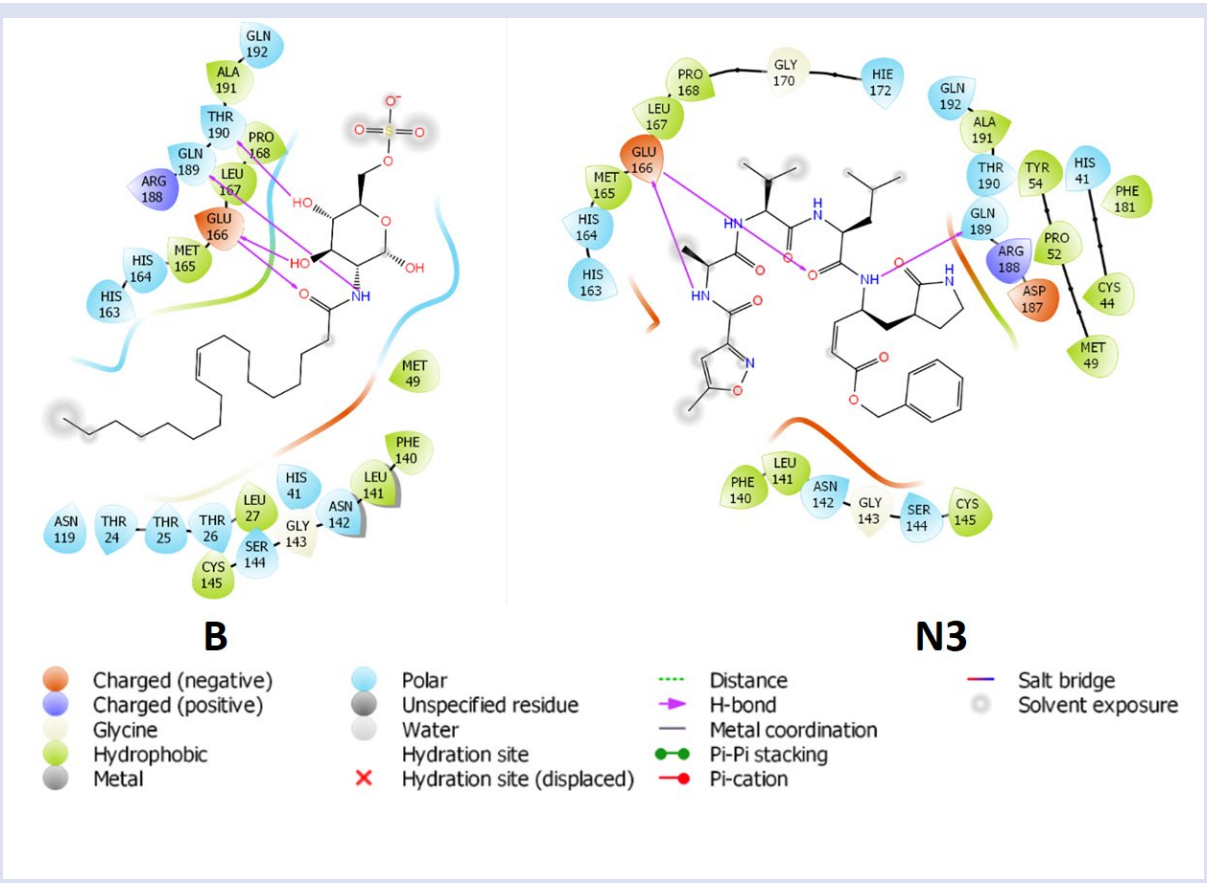

Figure 2. 2D interaction of compounds B and N3 with the active site of 6LU7 PDB-encoded protein

In addition to molecular docking studies, and MD Simulation studies were carried out with compounds A, B, and N3. RMSD values of compounds A, B, and N3 to analyze structural deviations and stability are shown in Figure 3. Simulations performed for 50 ns and RMSD values of the alpha carbons (Cα) of the enzyme (6LU7) in all three analyzes were seen to vary up to 3.2 Å (Figure 3). Despite the slight shifts observed, synthetic sulfatide complex B reached stability after 20 ns, with less deviations compared to compound A. Compound B and

native ligand-protein compound N3 show similar RMSD values.

The interaction of residues in the active site of the enzyme with compounds A and B were also investigated. In Figure 4, the residues interact with the ligands are shown and they are observed to be constant throughout the simulation. In particular, it was determined that GLU166 and GLU189 distinctly took place in binding with both compounds.

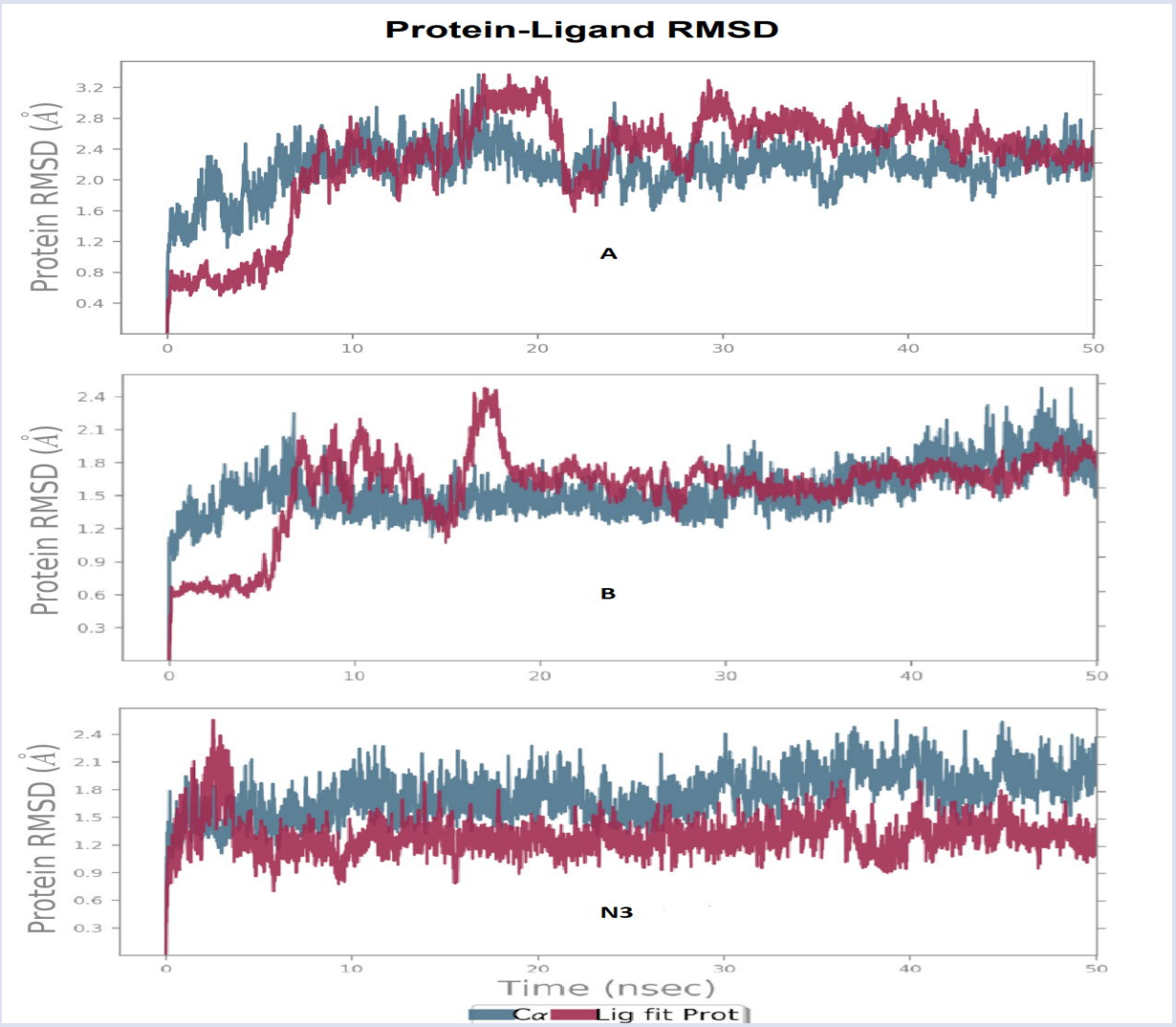

Figure 3. RMSDs for compounds A, B, and N3

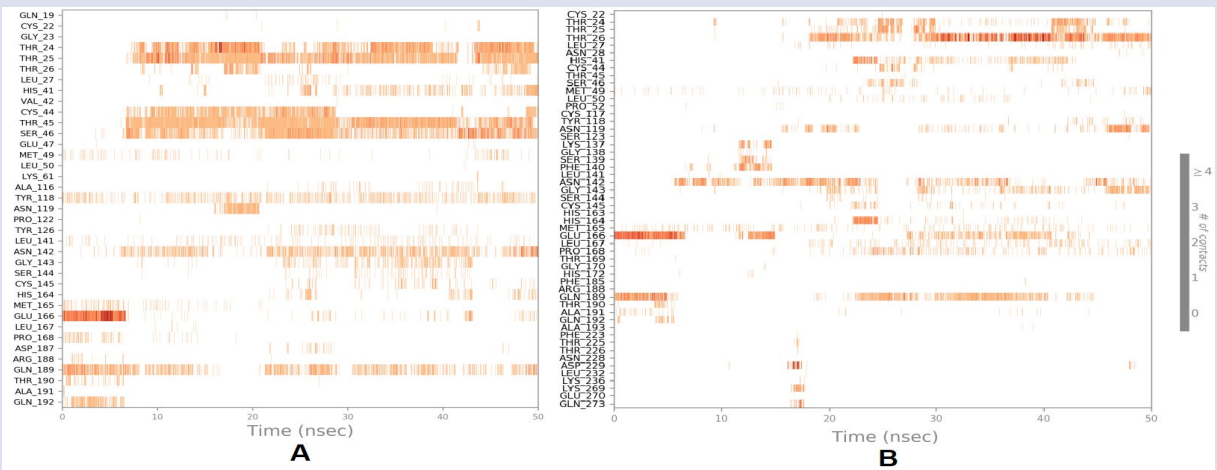

Figure 4. The residues interact with compounds A and B

ADME Prediction

After submission of the ligand molecules to SWISSADME database, the results were obtained in Table 3.

Table 3: Estimated Physicochemical, Lipophilicity, Water Solubility, Pharmacokinetics, Drug likeness, ADMET properties of A, B and N3

Physicochemical Properties	***A***	***B***	***N3***
Molecular weight	908.32 g/mol	523.68 g/mol	680.79 g/mol
Num. H-bond acceptors	12	9	9
Num. H-bond donors	7	5	5
TPSA	220.69 Å²	171.00 Å²	197.83 Å²
Lipophilicity	***A***	***B***	***N3***
Log $P_{o/w}$ (iLOGP)	7.74	3.39	3.94
Consensus Log $P_{o/w}$	9.25	2.90	2.73
Water Solubility	***A***	***B***	***N3***
Log *S* (ESOL) ; Class	-11.69; Insoluble	-4.26; Moderately soluble	-4.89; Moderately soluble
Log *S* (Ali) ; Class	-19.21; Insoluble	-7.24; Poorly soluble	-7.18; Poorly soluble
Pharmacokinetics	***A***	***B***	***N3***
GI absorption	Low	Low	Low
BBB permeant	No	No	No
P-gp substrate	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	Yes
Drug likeness	**A**	**B**	**N3**
Lipinski	No; 3 violations: MW>500, NorO>10, NHorOH>5	Yes; 1 violation: MW>500	No; 2 violations: MW>500, NorO>10
ADMET	***A***	***B***	***N3***
AMES toxicity Categorical (Yes/No)	No	No	No
Max. tolerated dose (human) Numeric (log mg/kg/day)	0.435	0.299	-0.424
hERG I inhibitor Categorical (Yes/No)	No	No	No
hERG II inhibitor Categorical (Yes/No)	No	No	Yes

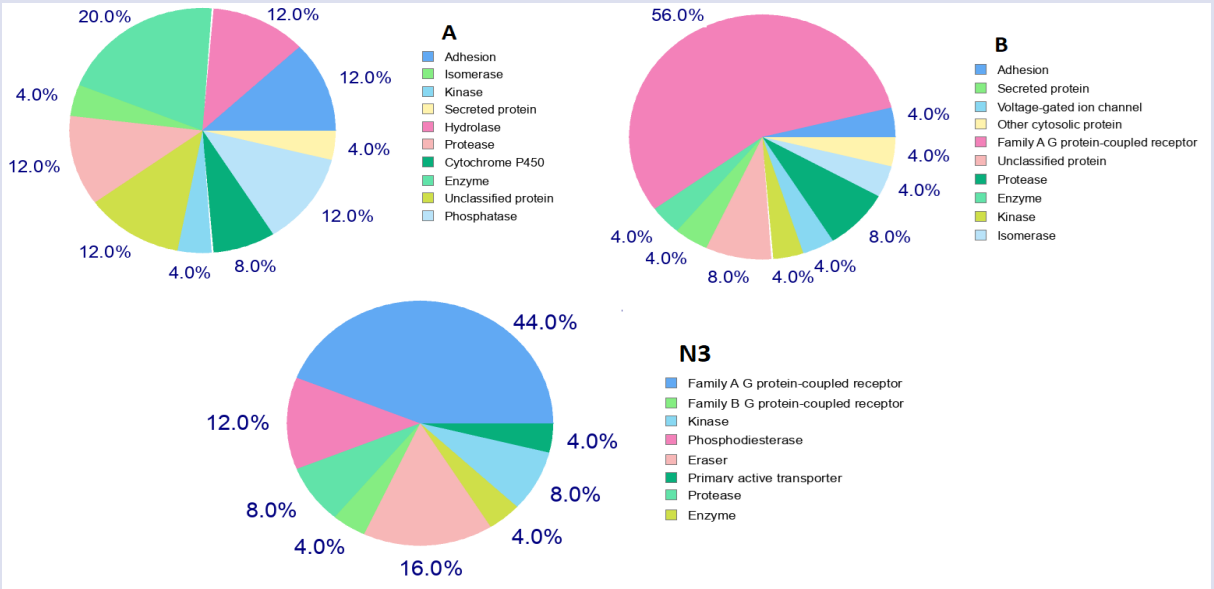

Figure 5. Top-25 targets predicted for A, B, and N3

The pie-chart graphs of the target prediction analysis are given in Figure5 for the top-25 targets as displayed in the website. The pie chart predicts the following observations for native sulfatide (Figure 5): 12% of adhesion, 4% of isomerases, 4% of Kinase, 4% of Secreated protein, 12% of hydrolases, 12% of protease, 8% of cytochrome p450, 20% of Enzymes, 12% of unclassified protein and 12% of phosphatases. The pie chart also predicts for chitosan based synthetic sulfatide (Figure 5): 4% of Adhesion, 4% of Secreted protein, 4% Voltage-gated ion channel, 4% Other cytosolic protein, 56% of Family A G protein-coupled receptor, 8% Unclassified protein, 8% of Protease, 4% of Enzyme, 4% of Kinase, 4% of Isomerase. Target prediction analysis for N3 is as follows (Figure 5): 44% of Family A G protein-coupled receptor, 4% of Family B G protein-coupled receptor,8% Kinase, 12% Phosphodiesterase, 16% Eraser, 4% Primary active transporter, 8% of protease, 4% of Enzymes. Native and chitosan based sulfatides are specific to only P-selectin.

Preliminary estimates of different properties of drugs (such as physicochemical properties, toxicity, absorption, distribution, metabolism, and excretion from the body, solubility in water and fat, and interaction with some enzymes in the body) can be obtained through various computer programs. These properties are evaluated by considering whether the obtained numerical results fall within the reference ranges. Bioavailability is an important criterion for evaluating any synthetic drug for clinical trials. Transportation properties can be determined by the TPSA value [33, 34]. TPSA < 140 $A^{\circ 2}$ is the standard value for gastrointestinal absorption[35] and TPSA>90 $A^{\circ 2}$ means low blood brain barrier (BBB) penetration[36]. Absorption percent (%ABS) was calculated by using %ABS = 109 − (0.345 × TPSA) is another factor for bioavailability (%Abs>50 high, %Abs< 30 low)is low or high[37, 38]. logBB term is used to predict brain penetration for compounds of interest. For the compounds of interest values greater than 0.3 means a penetration, while values less than -1.0 indicate a poor diffusion[39]. Toxicity for T. Pyriformis, a protozoa bacteria, is predicted by the pIGC50 (> −0.5 µg/L toxic) and Minnow toxicity is predicted by the LC50 (log LC50 < -0.3 toxic) respectively. In addition, lipophilicity determined by Log Po/w - Consensus Log Po/w values and water solubility determined by LogS (ESOL) and LogS (Ali) values are important predictors for drug-delivery properties[40-42]. Some of the cytochrome P450 enzymes (CYP1A2, CYP3A4, CYP2C19, CYP2D6 and CYP2C9) are essential to metabolize many drugs[43], so pharmacokinetic interactions with these enzymes are also predicted.

The toxicity of drugs is a key factor and should be of great concern[44]. For toxicity, the maximum tolerated dose is important that it is used to estimate the starting dose in phase I clinical trials of drugs. For the maximum tolerated dose, values less than or equal to 0.477 are considered to be low and vice versa[45]. The toxicity of drugs is highly related to the interaction with some important proteins in the body, the chemical nature and dose of the any given drug, and the stage of infection. For example, drugs sold in markets such as cisapride, sertindole, terfanadine inhibit human (hERG) K^+ channels, causing cardiac arrhythmias and ultimately death and for this reason their sale has been stopped[44]. By measuring the toxicity tests using the ProTox-II - Prediction of Toxicity of Chemicals program, more detailed information about the drug can be obtained with computer data[44].

In our study, iLOG $P_{o/w}$ and consensus Log $P_{o/w}$ values for compound A are 7.74 and 9.25 respectively, so it is weak in terms of lipophilicity. On the contrary these values are 2.90-3.39 and 2.73-3.94 for B and N3 indicating both are lipophilic and exhibit good GI properties. Both the ESOL Log S and Ali log S values, suggest that compound A is insoluble, while the others are moderately soluble (see Table 3). The GI is low for all three compounds and all three cannot exceed the BBB. The three drugs do not inhibit any of the p450 inhibitors, except N3 inhibits CYP3A4. This eliminates an important concern in terms of the toxicity of the synthetic drug. The drug likeness property is positive only for B. It only violates the molecular weight rule. However there are many drugs of high molecular weight in phase III[46, 47]. Three drugs can be excreted from the cell as they are substrates of P-gp (see Table 3). Ames toxicity is negative for all three components, which suggest that the molecules are non-carcinogenic in nature. Maximum tolerated doses (log mg/kg/day) for all show eligibility for human use (Table 3). Except N3 gives positive result for hERG II, none exhibit hERG I and hERG II inhibitory property. All these results promise that the synthetic molecule (B) can be safely used as drug.

Conclusion

It has been observed that sulfatide molecules may be of high importance in viral infections. The synthetic sulfatide showed great binding than native sulfatide due to their binding affinity scores. Structural mimic of the compound B was investigated by molecular docking and MD simulation studies. In addition, it was observed that compound B interacts with residues in the active site of the target enzyme similar to N3. ADMET studies showed that compound B did not have a significant predicted toxic effect and was in accordance with Lipinski's five-point rule.

Development of drugs or vaccines against Covid-19 in a short time is a great challenge for scientific community. The novel vaccines are currently been used in many countries. However, viruses can change their genetic material via mutations rapidly, so the vaccines don't keep us protected for a long time against mutated viruses. Efforts for developing anti-viral drugs studies for Covid-19 are slower and less promising than vaccine development. Although there are some drugs used in Coivd-19 treatment protocols, there is currently no drug whose

effectiveness has been proven and approved by authorized institutions. Therefore, rapid development of alternative drug molecules is of vital importance and computational efforts can guide experimental and clinical studies in this field.

While obtaining huge amounts of biological sulfatides can be difficult, synthetic sulfatide is relatively easy to be produced in laboratory. These molecules are candidates to be the starting point for successful drug development against COVID-19.

Conflicts of Interest

The author declares that there is no conflict of interest.

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