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by Herin Setianingsih, Erina Yatmasari, M. Fathi Ilmawan, Hilyatuz Zahroh, Eka Diyah Pl

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Research Article

Targeting Multiple SARS-Cov-2 And Human Proteins: In Silico Approach For COVID-19 Drug Repurposing

HERIN SETIANINGSIH[1]*, ERINA YATMASARI[1], M. FATHI ILMAWAN[1], HILYATUZ ZAHROH[2], EKA DIYAH PUTRI LESTARI[3]
[1]Faculty of Medicine, Hang Tuah University
[2]Faculty of Medicine, Yarsi University
[3]Faculty of Sciences, Brawijaya University
*Corresponding Author
Email: herin.setianingsih@hangtuah.ac.id
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ABSTRACT

The ongoing Coronavirus Disease 2019 (COVID-19) pandemic has killed more than one million lives and infected almost 40 million people around the world. In the absence of approved antiviral drugs and vaccines for COVID-19, drug repurposing could be an effective strategy to fast forward COVID-19 drug discovery process. In this study, we screened 160 potential drugs from Therapeutic Target Database against 13 protein targets (12 SARS-CoV-2 proteins and 1 human protein) using an inverse docking approach. Our preliminary result showed that suramin, a poly-sulfonated compound used to treat sleeping sickness, came out with the strongest binding affinity against 3 protein targets (Spike protein, Nucleocapsid protein, ACE2). Suramin formed the strongest complexes with spike protein (prefusion) and nucleocapsid protein (binding affinity: -11.2 Kcal/mol, each) from SARS-CoV-2. The best candidate was also evaluated through molecular dynamics simulation. It is clearly confirmed that this active compound has stable binding during 10 ns simulation. We concluded that drug repurposing based on virtual screening technique revealed that Suramin is the most potential to bind nucleocapsid and Spike protein of SARS-CoV-2. ACE2 is also considered as a new target of Suramin.

Keywords: protein target, spike protein, nucleocapsid, ACE2, virtual screening.

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is currently a global problem including in Indonesia, as it has caused pandemics with high rates of transmission and death. The problem is further complicated because there is still no effective treatment to treat this infectious disease[1]. The development and trial of new antiviral drugs and vaccines take a long time, so efforts to reposition drugs that have been approved by the Food and Drug Administration (FDA) are the best alternatives. Antiviral drugs and symptom reliever due to coronavirus (CoV) circulating and FDA approved have not existed at all that target functional protein CoV namely, nucleocapsid protein (NP). Meanwhile, NP is an important protein associated in the Life cycle of CoV [2].

Coronavirus (CoV) belongs to the family Coronaviridae which can cause zoonotic diseases with common flu symptoms to severe and deadly. Coronavirus types that have spread and are known to cause infectious diseases in severe categories include, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), and most recently COVID-19 disease caused by SARS-CoV 2. COVID-19 or Coronavirus Disease has come to the attention of the global community including Indonesia because it has caused respiratory disorders with high rates of transmission and death in more than 100 countries[1]. The pandemic is known to stem from a pneumonia outbreak in Wuhan City, China at the end of 2019. However, to date there is still no effective treatment to treat this infectious disease. In addition, the development and trial of new antiviral drugs and vaccines takes a considerable time. Thus, the quickest solution is to repurpose the drug. The concept of drug repurposing is an attempt to reposition the target and type of disease of a drug that has been approved or fda approved. This is mainly because some drugs are only approved for one disease, but actually also have the potential for activity for other indications. In addition, several studies have suggested the concept of stabilizing effective interactions (PPI) proteins in the discovery and development of antivirals [3][4][5].

In addition, this solution can significantly reduce the time and cost of drug development, especially the toxicity and pharmacokinetic profile of such drugs is widely known and tested. The number of

FDA approved drugs has also steadily declined over the past three decades, so drug re-use can accelerate the marketing of other new drugs[6]. Whereas in order for a drug or active compound to be targeted against NP SARS-CoV-2 must have a small mass (less than 500 Da) and meet at least 2 requirements Lipinski Rule of Five [7], so that the type of polysaccharides will not be suitable.

Based on our literature studies, it can be known that antiviral drugs that are currently developed and used to treat COVID-19 mostly target two types of corona virus proteins, namely, NSP (Non-structural protein) and SP (Structural protein). NSP consists of RpRd (RNA-directed RNA polymerase), PL-PRO (papain-like protease), and 3CLpro (3C-like protease). While SP consists of S (Spike glycoprotein) and E (Envelope small membrane protein), so another novelty of this study is to target other SP proteins, namely, nucleopsid (N) viruses that are very rarely targeted for antiviral drugs to overcome diseases caused by CoV infection are included in the category of approved drugs. Research that has been, too, is still in the approach stage in silico [8][9]. N-protein is a very important protein because it is associated with the life cycle of CoV. This NP has been said to play a role in the core formation of the virus, as it can package the viral genome into a long, flexible, helical RNP complex. Then dimerized domain N-terminal (NTD) of protein CoV N virus that ultimately interacts with the C-terminal part (N-CTD) that both have RNA-binding activity is closely related to the assembly process of the viral RNA genome. In addition, NP also plays a role in the transcription process of the viral genome [10].

METHODS

Ligand Datasets

Candidates for drug repurposing compounds are collected from the Therapeutic Target Database (http://db.idrblab.net/ttd/). The types of compounds collected are divided into two, namely small molecule drugs (152 compounds) and protein drugs (8 compounds). Furthermore, the 3D structures of the drug compounds are collected in format (.sdf) from the PubChem (https://pubchem.ncbi.nlm.nih.gov) database. Then, the data collection of antiviral function scores from each compound is done using passonline online software (http://www.way2drug.com/PASSOnline/).

SARS-CoV-2 Protein Target

Before determining the target protein, we studied the genome structure of SARS-CoV-2 and the resulting proteins. The genome size of SARS-CoV-2 ranges from 29,903 bp (NC_045512.2). SAR-CoV-2 has 4 structural proteins, 16 non-structural proteins and 9 accessory proteins. Then, we conduct protein screening that will be targeted in the docking process based on the existence and quality of the 3D structure of proteins contained in the database. From the results of the screening, there are 14 protein structures from SARS-CoV-2 that we use as molecular docking targets. In addition, we also use the ACE2 protein structure of humans as a target. The protein used is spike protein (prefusion) (PDB ID: 6VSB); nsp3 or PLpro (PDB ID: 6WX4); Mpro or nsp5 or 3CLpro (PDB ID: 7BQY); nucleocapsid protein (PDB ID: 6M3M); nsp9 (PDB ID: 6W4B); orf9b (PDB ID: 6Z4U); nsp10/16 (PDB ID: 6W4H); nsp12/7/8 with co-factor (PDB ID: 6M71); nsp15 (PDB ID: 6VWW); orf3a (PDB ID: 6XDC); orf7a (PDB ID: 6W37); orf8 (PDB ID: 7JTL); and ACE2 (PDB ID: 6M18) downloaded from the RCSB DATABASE of PDB (https://www.rcsb.org/) in format (.pdb) (**Table.1**)

Preparation of Ligands and Macromolecules

Protein preparation was performed using Discovery Studio 2019 program by BIOVIA. Water molecules and ligands were removed from the 3D structures. The prepared structures were stored back in PDB (.pdb) format.

Furthermore, the preparation process also carried out on ligands used PyRx 0.9.8 software to lower or minimize the free energy of the ligands as well as convert compounds into AutoDock ligands. The pre-prepared ligans are then saved in PDB (.pdb) format.

Molecular Docking and Visualisation of Protein-Ligand Interaction

The docking process is done by using inverse virtual screening of AutoDock Vina 1.1 from PyRx 0.9.8 software. After docking is complete, the results are saved in PDB (.pdb) format, then visualized and interpreted using PyMOL v.2.3.2.1, Discovery Studio 2019 Client and LigPlot+ v.1.4.5.

Molecular Dynamic Simulation

Molecular dynamics simulation is done with YASARA Dynamic software developed by GmbH Biosciences. The first step, each file included a single spike that has been prepared as control, the docking result of spike-suramin and spike-apilimod complex inputted into the program by choosing Options then selected Macro & Movie menu and lastly selected Set Target. Furthermore, macro inputs are carried out to simulate molecular dynamics that have previously been pre-prepared in the variable part, namely the

temperature of 310K and the default pH of 7.4. The next step, in *macro md_run* also sets the running time 7500 ps (7.5 ns). This running time is a quorum of time that represents a simulation of physiological conditions. This simulation uses forcefield AMBER03 and snapshot storage every 25 ps. Analysis of RMSD and potential energy obtained by running *macro md_analyze*. Furthermore, RMSF analysis is run with *macro md_ analyzeres*, while re-run running results are done by running *macros md_play*. The last step, the results are recorded with the IceCream Screen Recorder application with the output result in the form of a file with the extension (.wmv).

Table 1. List of protein target for virtual screening

No.	Macromolecules	PDB ID	Method	Resolution	Seq Length	Positions	Chains	Ref
1	Spike Protein (prefusion)	6VSB	EM	3.46 Å	1288	1-1208	A/B/C	[11]
2	nsp3/PLpro	6wx4	XRD	1.66 Å	326	1562-1879	D	[14]
3	Mpro/nsp5/3CLpro	7bqy	XRD	1.70 Å	306	3264-3569	A	[14]
4	Nucleocapsid Protein	6m3m	XRD	2.70 Å	136	41-174	A/B/C/D	[11]
5	nsp9	6w4b	XRD	2.95 Å	117	4141-4253	A/B	[12]
6	orf9b	6z4u	XRD	1.95 Å	97	1-97	A/B	[11]
7	nsp10/16	6w4h	XRD	1.80 Å	301 (A), 142 (B)	6799-7096 (nsp 16), 4254-4392 (nsp 10)	A (nsp 16), B (nsp 10)	[12]
8	nsp12/7/8-cofactor	6m71	EM	2.90 Å	942 (A), 83 (C), 198 (B,D)	4393-5324 (RdRp/nsp 12), 3860-3942 (nsp 7), 3943-4140 (nsp 8)	A (nsp 12), C (nsp 7), B, D (NSP 8)	[14]
9	nsp15	6vww	XRD	2.20 Å	370	6453-6798	A/B	[13]
10	orf3a	6xdc	EM	2.90 Å	284	1-275	A/B	[12]
11	orf7a	6w37	XRD	2.90 Å	67	16-82	A	[13]
12	orf8	7jtl	XRD	2.04 Å	107	18-121	A/B	[13]
13	ACE2	6M18	EM	2.90 Å	814		B, D	[12]

RESULTS AND DISCUSSIONS

Virtual Screening for Drug Repurposing

Docking result was evaluated and indicated that Suramin is the most potential candidate (**Table 2**, **Table 4**, **Table 6**). The first potential target, Spike protein, is one of structural protein of SARS-CoV-2 which plays pivotal role during viral entry. Coronavirus entry into host cells is mediated by the transmembrane spike (S) glycoprotein that forms homotrimers protruding from the viral surface [15]. Visualizes of the interaction result between spike protein and the 10 best ligand indicates that in general the active site of the S protein are Ser19, Gln24, Phe28, Asp30, Lys31, His34, Asp38, Tyr41, Gln42, Met82, Tyr83, Lys353, Gly354, Asp355, Lys417, Gly446, Tyr449, Tyr453, Leu455, Phe486, Asn487, Tyr489, Gln493, Gly496, Thr500, Asn501, Gly502, and Tyr505 (**Figure 1**). But then based on binding affinity it can be known that Suramin came on top of docking result with spike protein (**Table 2**). Suramin binds to both hydrophobic (shown in brown) and hydrophilic (shown in blue) amino acids of the spike (**Figure 2a**). In general there are 3 types of bonds namely hydrogen bonds, hydrophobic bonds and electrostatic bonds (**Figure 2b, Tabel 5**). Amino acids involved in hydrogen bonds include A:Ser967; A:Asn969; C:Arg355; C:Arg466; C:Arg567; A:Ile231; C:Phe464; A:Ser975; A:His49; A:Gly199; and A:Gly232. Hydrogen bond is very important in looking at the affinity of a drug on the binding site, the more hydrogen bond type bond will be stronger intercation between the drug and the target. The strength of a hydrogen bond can be seen from the distance, where the closer 2 Å then the stronger, so that the bond that dominates and strengthens the interaction between suramin and S protein are interaction of H atom from ligand with O atom from A:Ile231 (2.84071 Å), C:Phe464:O (2.04653 Å) and A:Ser975 (2.69769 Å). Furthermore the amino acids

involved in this type of hydrophobic bonding are A:Asp198; Gly199; C:Leu518; C:Phe464; C:Arg466; and C:Leu518. Hydrophobic bonding have a crucial function as a marker of the hydrophobic niche area of a drug binding site that will help maintain the viability of the drug.

Table 2. Docking result for drug repurposing of Spike protein

Binding Affinity	rmsd/ub	rmsd/lb	Drug Name
-11.2	8.994	4.894	Suramin
-10.4	12.577	2.655	Vazegepant
-10	14.581	4.009	Elbasvir
-9.8	41.343	35.431	Telmisartan
-9.7	30.791	23.937	Anidulafungin
-9.5	4.886	3.094	Montelukast
-9.5	28.336	26.512	Ivermectin
-9.5	16.085	12.066	Sirolimus
-9.4	4.315	3.169	Apilimod
-9.4	3.554	2.894	Irbesartan

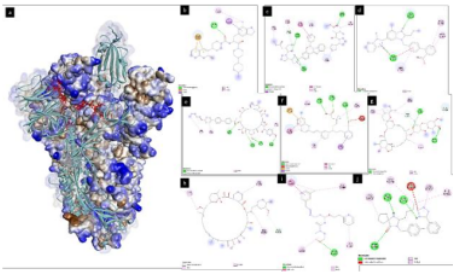

Fig.1: Binding site (a) and molecular interaction of Spike protein with Vazegepant (b), Elbasvir (c), Telmisartan (d), Anidulafungin (e), Montelukast (f), Ivermectin (g), Sirolimus (h), Apilimod (i), and Irbesartan (j).

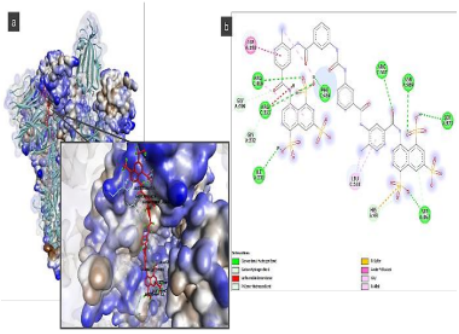

Fig.2: Binding site and molecular interaction of Suramin and Spike protein, 3D (a) and 2D (b).

Nucleocapsid is one of structural protein of SARS-CoV-2, which plays pivotal role during viral replication. N-protein is a very important protein because it is associated with the life cycle of CoV. This NP has been said to play a role in the core formation of the virus, as it can package the viral genome into a long, flexible, helical RNP complex. Then dimerized domain N-terminal (NTD) of protein CoV N virus that ultimately interacts with the C-terminal part (N-CTD) that both have RNA-binding activity is closely related to the assembly process of the viral RNA genome. In addition, NP also plays a role in the transcription process of the viral genome [10]. Visualizes of the interaction result between spike protein and the 10 best ligand indicates that in general the active site of the N protein are Thr55, Ala56, Arg89, Ala91, Thr92, Arg93, Arg94, Ser106, Tyr110, and Tyr112 (**Figure 3**). But then based on binding affinity, same as result from S protein, Suramin also came as best of docking result with nucleocapsid protein (**Table 3**). Suramin binds to both hydrophobic (shown in brown) and hydrophilic (shown in blue) amino acids of the N protein (**Figure 4a**). In general there are 4 types of bonds namely hydrogen bonds, hydrophobic bonds, electrostatic bonds, and unfovarable bond (**Figure 4b, Tabel 5**). Amino acids involved in hydrogen bonds include A:Thr92; A:Tyr110; A:Tyr112; B:Lys66; B:Arg69; B:Glu63 and A:Arg89. The interactions bond that dominates and strengthens the interaction between suramin and N protein are interaction of O atom from ligand with OH atom from A:Tyr112 (2.87729 Å), N atom from B:Lys66 (2.85069 Å) and B:Arg69 (2.83705 Å), also H atom from A:Thr92 (2.26128 Å). Furthermore the amino acids involved in this type of hydrophobic bonding are A:Thr50; B:Lys66; A:Pro118; A:Pro152; B:Arg69; and A:Ala51. As explained

before hydrophobic bonding have a crucial function as a marker of the hydrophobic niche area of a drug binding site that will help maintain the viability of the drug; but from this complex there were also one unfavorable bond (D:Asn155) that which will precisely cause the binding of the drug to the target to be weaken.

Table 3. Docking result for drug repurposing of Nucleocapsid protein

Binding Affinity	rmsd/ub	rmsd/lb	Drug Name
-11.2	2.161	1.522	Suramin
-10.6	10.876	5.37	Vazegepant
-10.6	13.567	9.214	Ivermectin
-10.4	3.339	2.107	Elbasvir
-9.3	15.646	8.123	Brilacidin
-9.2	2.032	1.213	Anidulafungin
-9.1	2.81	0.082	Tradipitant
-9	2.851	1.806	Sanglifehrin A
-9	9.809	4.762	Telmisartan
-8.9	8.633	5.029	Zanubrutinib

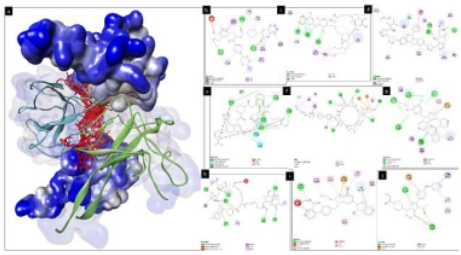

Fig.3: Binding site (a) and molecular interaction of Nucleocapsid protein with Vazegepant (b); Ivermectin (c); Elbasvir (d); Brilacidin (e); Anidulafungin (f); Tradipitant (g); Sanglifehrin A (h); Telmisartan (i) and Zanubrutinib (j).

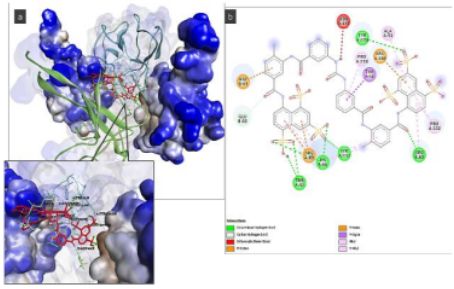

Fig.4: Binding site and molecular interaction of Suramin and Nucleocapsid protein; 3D (a) and 2D (b).

Previous studies showed that ACE2 mediates SARS-CoV-2 entry into human cells. ACE2 could mediate SARS-CoV-2 S-mediated entry into cells; establishing it as a functional receptor for this newly emerged coronavirus. The SARS-CoV-2 SB engages human ACE2 (hACE2) [15]. Therefore; it serves as an attractive target for COVID-19 antiviral drug. Visualizes of the interaction result between spike protein and the 10 best ligand indicates that in general the active site of the ACE2 are Asp30; His34; Tyr41; Met82; Tyr83; Gln325; and Glu329 (**Figure 5**). But then based on binding affinity; same as two result before; Suramin also showed the most negative binding affinity of all the ligands (**Table 4**). Suramin favorably binds to hydrophilic amino acid in ACE2 than hydrophobic ones. (**Figure 6a**). In general there are 3 types of bonds namely hydrogen bonds; hydrophobic bonds; and electrostatic bonds (**Figure 6b; Tabel 5**). Amino acids involved in hydrogen bonds include D:Gln98; D:Gln102; D:Tyr196; D:Asp206; D:Asn394 and D:Lys562. The interactions bond that dominates and strengthens the interaction between suramin and ACE2 are interaction of O atom from ligand with OH atom from D:Tyr196 (2.9924 Å). Furthermore the amino acids involved in this type of hydrophobic bonding are D:Leu73; D:Trp203; and D:Lys74. In addition to hydrophobic bonds other types of bonds in the form of van der wals also stabilize drug inetraksi with target proteins only the influence is weaker because the bond distance is above 4 Å.

Drug from all repurposing result that came as top result; Suramin is an anti-parasitic drug used in sleeping sickness therapy caused by trypanosoma. Suramin also has a broad spectrum antiviral effect so it is used for the treatment of several diseases caused by viruses; including HIV; hepatitis C virus; herpes simplex type 1; Zika virus; dengue virus; Chikungunya and others. The suramin mechanism when used

as an anti-virus SARS-COV-2 will work at the stage of inhibiting viral replication; as research has been conducted using Vero E6 cells which can show inhibition of virus replication [16]. Suramin also inhibits SARS-COV-2 replication in the primary human airway epithelial cell cultures (HAE) model.

Table 4. Docking result for drug repurposing of ACE2

Binding Affinity	rmsd/ub	rmsd/lb	Drug Name
-11	74.637	68.463	Suramin
-9.7	69.470	66.361	Vazegepant
-9.6	69.143	64.529	ABBV-744
-9.6	64.358	55.744	Anidulafungin
-9.5	89.923	85.923	Telmisartan
-9.3	82.964	75.727	Zanubrutinib
-9.2	16.30	4.754	Elbasvir
-9.2	74.174	70.248	Relacatib
-9.1	86.823	82.76	Ibrutinib
-9.1	11.934	3.997	Sanglifehrin A

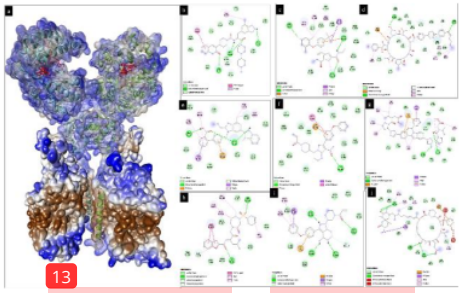

Fig.5: Binding site (a) and molecular interaction of ACE2 with Vazegepant (b); ABBV-744 (c); Anidulafungin (d); Telmisartan (e); Zanubrutinib (f); Elbasvir (g); Relacatib (h); Ibrutinib (i) and Sanglifehrin A (j).

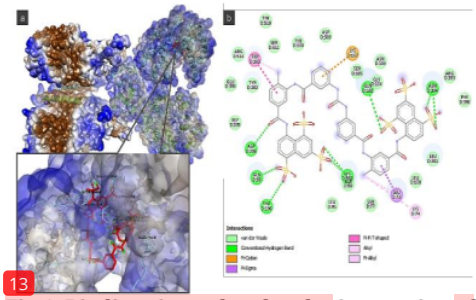

Fig.6: Binding site and molecular interaction of Suramin and ACE2; 3D (a) and 2D (b).

Table 5. Bond interaction of Suramin with Spike protein; Nucleocapsid protein and ACE2 as target for drug repurposing

Complex ID	Hydrogen Bond Position	Hydrogen Bond Length (Å)	Hydrophobic Interaction	Other Bond Position
Spike-Suramin	A:SER967:OG - N:LIG1:O	3.01549	A:ASP198:C;O; GLY199:N - N:LIG1	N:LIG1:S - A:HIS49
	A:ASN969:ND2 - N:LIG1:O	3.02149	N:LIG1:C - C:LEU518	
	C:ARG355:NH1 - N:LIG1:O	3.01257	C:PHE464 - N:LIG1:C	
	C:ARG466:N - N:LIG1:O	3.00694	N:LIG1 - C:ARG466	
	C:ARG567:NH1 - N:LIG1:O	3.06627	N:LIG1 - C:LEU518	
	N:LIG1:H - A:ILE231:O	2.84071		
	N:LIG1:H - C:PHE464:O	2.04653		

	N:LIG1:H - A:SER975:OG	2.69769		
	A:HIS49:CE1 - N:LIG1:O	3.64127		
	A:GLY199:CA - N:LIG1:O	3.20038		
	A:GLY232:CA - N:LIG1:O	3.75057		
Nucleocapsid-Suramin	A:THR92:N - N:LIG1:O	3.18392	A:THR50:CB - N:LIG1	A:ARG150:NH2 - N:LIG1
	A:TYR110:OH - N:LIG1:O	3.37319	N:LIG1:C - B:LYS66	B:ASP64:OD2 - N:LIG1
	A:TYR112:OH - N:LIG1:O	2.87729	N:LIG1 - B:LYS66	N:LIG1:S - N:LIG1
	B:LYS66:NZ - N:LIG1:O	2.85069	N:LIG1 - B:LYS66	
	B:LYS66:NZ - N:LIG1:O	3.16571	N:LIG1 - A:PRO118	
	B:ARG69:N - N:LIG1:O	2.83705	N:LIG1 - B:LYS66	
	N:LIG1:H - N:LIG1:O	1.89691	N:LIG1 - A:PRO152	
	N:LIG1:H - A:THR92:O	2.26128	N:LIG1 - B:ARG69	
	N:LIG1:H - N:LIG1:O	1.99258	N:LIG1 - A:ALA51	
	B:GLU63:CA - N:LIG1:O	3.65367	N:LIG1 - A:PRO152	
	A:ARG89:NH1 - N:LIG1	3.34453		
	A:ARG89:NH2 - N:LIG1	3.98165		
ACE2-Suramin	D:GLN98:NE2 - N:LIG1:O	3.19477	D:LEU73:CB - N:LIG1	D:LYS187:NZ - N:LIG1
	D:GLN102:NE2 - N:LIG1:O	3.00254	D:TRP203 - N:LIG1	N:LIG1:S - N:LIG1
	D:TYR196:OH - N:LIG1:O	2.9924	N:LIG1:C - D:LEU73	
	D:ASP206:N - N:LIG1:O	3.23083	N:LIG1:C - D:LYS74	
	D:ASN394:ND2 - N:LIG1:O	3.0619	D:TRP203 - N:LIG1:C	
	D:ASN394:ND2 - N:LIG1:O	3.15003		
	D:LYS562:NZ - N:LIG1:O	3.19007		
	D:LYS562:NZ - N:LIG1:O	3.25467		

Interaction Stability Evaluation

Analysis of the total potential energy of single spike and spike complex with suramin was obtained after running macro md_analyze. The results of the analysis of both generally show that the potential energy will experience a very significant increase from 0 ns to 0.35 ns of running time. This shows the occurrence of an energy initiation process to achieve an energy stability. However; fluctuations began to appear at 0.4 ns of running time; indicating that both types of samples experienced changes in molecular bond energies. This fluctuation continues even though it has reached a stable potential energy range (equilibrium phase) at -9445247.4 kJ / mol (**Figure 7a**). Up fluctuation

means that there is a strengthening of the bonds in the molecule; whereas the decrease in potential energy can be interpreted as a result of the relaxation of molecular bonds [17]. In addition; the fluctuation tendency between single spike and suramin spike complex is not much different; so it can be concluded that the suramin ligand in the complex does not cause significant conformational changes (**Figure 9**).

RMSD (Root Mean Square Deviation) is an analysis result score that provides information on conformational changes in a macromolecule that acts as a receptor after the interaction process with a particular ligand. The analysis of single spike RMSD values and the spike-suramin complex was obtained after running the md_analyze macro. RMSD can also be used as a standard deviation of conformational changes; with standards <2 nm and> 2 nm generally applied to docking results [18]. RMSD is sufficient data to represent the stability of the two samples under simulation conditions (temperature 310k and pH 7.4). The dynamic stability referred to is the absence of significant conformational changes; which is better known as the unfolding process. The RMSD standard for a protein receptor simulation results is 3 nm; where if the RMSD value of a protein ≥ 3 nm is a sign that the protein has undergone a conformational change that is far different from its native condition [17]. In general; the RMSD value of the two samples has a tendency to increase with the presence of several fluctuations. However; the average RMSD yield of the complex is lower than the folding single spike at 0.25 ns; while the unfolding process is required by the spike domain as the initiation of the fusion process (**Figure 8b**). So it can be concluded that the ligand in the form of drug suramin can slow down the unfolding of the A domain and therefore the fusion between the spike and ACE2 as a natural ligand for the SARS-COV-2 spike can be slowed and potentially inhibited (**Figure 7b, Figure 9b**). In addition to the RMSD of the whole molecule; the YASARA program can also be used to see the RMSD of the ligand configuration; which in this study is a Suramin. The RMSD value of the Suramin ligand of the spike-suramin complex shows high fluctuations with a tendency to increase at the 0 ns to 3 ns simulation time and even to 4.5 nm; which means that it undergoes a conformational change that is much different from the initial suramin in the complex or it can also be possible to break ties [17]. In addition; even though it decreased again at 3.2 ns; the RMSD value did not decrease to below 3 so that it could be said that suramin had a conformational change and there was a possibility of weakening the bond between the spike and suramin (**Figure 4c**).

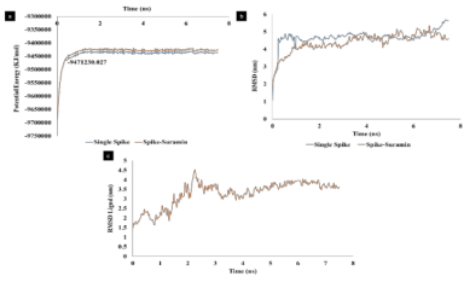

Fig.7: Evaluation of binding stability based on energy (a), RMSD (b), and Ligand RMSD (c).

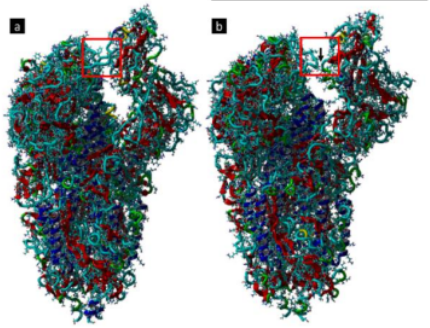

Fig.8: Conformation of single Spike protein before (a) and after (b) molecular dynamic simulation. Arrow indicates there is a weakening of the bond that becomes the crew of unfolding domain A in preparation for the fusion/entry process of S protein of SARS-CoV-2 to bind with ACE2 receptor

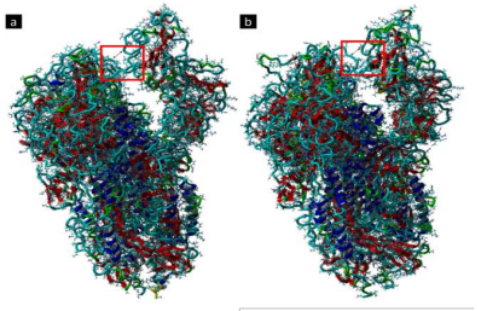

Fig.9: Conformation of complex Spike-Suramin before (a) and after (b) molecular dynamic simulation. Two-way arrow indicates no weakening of ties and precisely the distance between the two subdomains is getting smaller.

CONCLUSION

We concluded that drug repurposing based on virtual screening technique revealed that Suramin is the most potential to bind nucleocapsid and

Spike protein of SARS-CoV-2. ACE2 is also considered as a new target of Suramin.

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