International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 8, Issue 4 -2021

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.04.013

Warfarin could play a key role in preventing the thrombotic complications in severe COVID-19 patients by suppressing the activity of Nsp3's macrodomain of sars-cov-2: *in silico* study

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Abstract

Covid-19 (coronavirus disease-2019) is a pandemic disease caused by the newly emerged coronavirus species called SARS-CoV-2. The number of deaths and spread of disease is at a higher rate than previously emerged epidemic diseases namely SARS (2003) and MERS (2012). Despite the higher mortality rate, patients with thrombotic complications were found to be infrequent in the SARS and MERS epidemic. The cytokine expression promoting activity of Nsp3 protein is drawing attention to work more on it. Cytokines play a role in activation of leukocytes, which further leads to thrombosis formation by the help of endothelial cells. The macrodomain domain of Nsp3 protein regulates innate immunity and plays a role in virulence. Here in this paper we targeted 'macrodomain' of Nsp3 protein of SARS-cov-2, which could play a vital role in increasing the risk of thrombotic complications by regulating the innate immunity response in severe covid-19 patients. The active site of the macro-domain was predicted using the Castp server. In this current study, FDA approved anticoagulants have been used for screening studies which are known for the prevention of thrombotic complications. Warfarin showed promising results on the basis of best binding affinity/lowest minimum energy of -8.5 kcal/mol and Hydrogen bond interaction study among the selected lead compounds. To a greater distance we studied protein-ligand interaction to check the intra-molecular interaction of macrodomain-Warfarin complex. This study will further help practitioners to understand the significance of 'Warfarin' as an effective anticoagulant agent to treat severe covid-19 patients.

Keywords: COVID-19, Thrombosis, Nsp3 protein, Warfarin, Bioinformatics

1. Introduction

1.1 COVID-19 and Thrombosis

Covid-19 (coronavirus disease-2019) is a pandemic disease caused by the newly emerged coronavirus species called SARS-CoV-2 [1]. The number of deaths and spread of the disease is at a higher rate than previously emerged epidemic diseases namely SARS (Severe Acute Respiratory Syndrome-2003) and MERS (Middle East Respiratory Syndrome-2012) [2]. Many of the symptoms are similar in these three diseases, but some of the more lethal symptoms are shown by this novel coronavirus. Coagulopathy such as venous thromboembolism (VTE), disseminated intravascular coagulation(DIC), pulmonary embolism, deep vein thrombosis, thrombosis and arterial thrombosis were found in different COVID-19 patient studies [2-4]. Excess inflammatory response followed by coagulopathy (thrombotic conditions) may be leading to sudden death in covid-19 patients [5]. Although mortality rates were higher in SARS (overall=9% in elderly persons= 50%) and MERS (overall= 35%), they were spread over limited areas of earth. The mortality rate of covid-19 is found to be varying in each country. Despite the higher mortality rate, patients with thrombotic complications were found to be very rare in SARS and MERS epidemic [3,5]. Several studies demonstrated an increased level of pro-inflammatory cytokines (IL-6, TNF-α (Tumour necrosis factor α)) found in severe covid-19 patients [5].

1.2 Role of cytokines in thrombosis

Cytokines are the small glycoproteins which play a key role in regulation of host immune response [6,7]. Over 50 types of cytokines produced by lymphoid tissue and epithelial cells interactively form a network to fight against infectious diseases caused by microorganisms [7,8]. Proinflammatory cytokines such as interleukin-6 (IL-6), neutrophils, C-reactive protein give the initial response in the first line of defence against pathogens but, their excessive production could lead to severe systemic inflammatory response syndrome [9,10]. Cytokines play a role in activation of leukocytes and endothelial cells which further leads to thrombosis formation [11].

2. Materials and Methods

2.1 Nsp3 proteins

Nsp3 is the largest non-structural protein out of eleven NSP (Non-structural proteins) encoded by the 'ORF1a' gene of coronavirus [12]. The nsp3 protein is a multi-functional protein with approximately 16-17 different domains and is 200 KD in molecular weight [12,13]. It is also said that nsp3 protein alters cytokine expression to decrease host immune response and could increase thrombotic complications [12]. Its activity of promoting cytokine expression is drawing attention to work more on it [13]. The macro-domain of Nsp3 protein, which is also called 'ADRP domain' or 'X domain' is said to play a vital role in virulence [13]. The exact catalytic mechanism of the macro-domain domain is still a mystery [14]. Keep *et. al.* [14] hypothesized that macro-domain domain regulates innate immunity and plays a role in virulence. Although the macro-domain domain was present in nsp3 protein of all three viruses, thrombotic complications are found to be more prevalent in covid-19. Furthermore, we did computational screening of FDA approved anticoagulants to check their activity against Nsp3 protein's macro-domain.

2.2 Active site prediction

The active site or motif residues of nsp3's macro-domain have been predicted using the Castp server (Computed Atlas of Surface Topography of proteins – link: http://sts.bioe.uic.edu/castp/.), which provides the motif residues with their name and number [15].

2.3 Virtual screening and Docking

Virtual screening is an extensively used technique for identification of best lead compounds against specific targets or proteins from a compound library of thousands of compounds [16]. We screened the anticoagulants which are already known to prevent the thrombotic complications [17]. We used Autodock-vina, a free, command-line-based software for screening of these compounds [18]. After a successful screening, we analyzed the results based on the lowest minimum energy level of these drugs/compounds.

2.4 Protein-ligand interaction

Nsp3 protein's macro-domain and Warfarin interaction has been checked by using a web-based tool protein–ligand interaction profiler (PLIP URL-https://projects.biotec.tu-dresden.de/plip-web/plip) [19]. Hydrogen bonds play a pivotal role in providing stability to protein-ligand complexes [20]. Hydrogen bonds are validated using bond length which should range from 2.4 to 3.5 Å.

3. Results and Discussion

3.1 Nsp3's macro-domain

We have used the structure of nsp3 protein's macro-domain of SARS-CoV-2 in '.pdb' file format from NCBI's (National Center for Biotechnology Information) structure database resource (https://www.ncbi.nlm.nih.gov/) [Table no 01 and Figure no 01].

Figure no 01

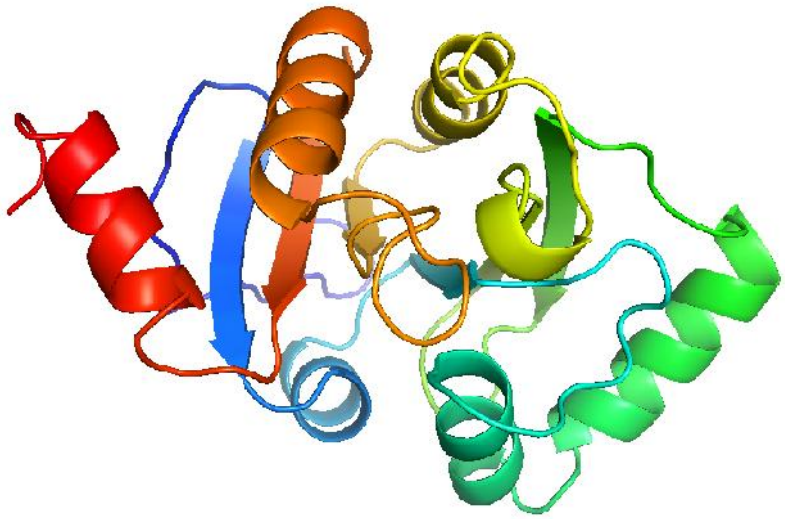


Figure showing macrodomain of Nsp3 protein includes amino acids from 1 to 173 of chain A visualized in Pymol.

Table no 01

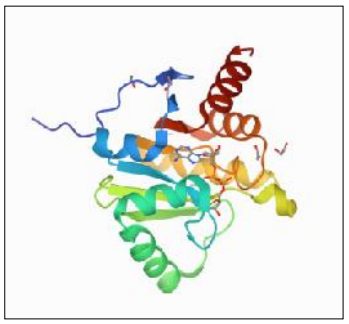
Sr no	Name	PDB ID	Structure
1	Crystal structure of SARS-CoV-2 (Covid-19) NSP3 macrodomain in complex with ADP-ribose	6YWL	

Table showing nsp3 protein's structure taken from NCBI's structure database.

3.2 Active site prediction results

The active site of nsp3's macro-domain has been predicted using Castp server [Table no 02] for grid-box assessment and virtual screening studies [Figure no 02].

Table no 02

Sr no	Residue No	Residue Name	Sr no	Residue No	Residue Name
1	21	ALA	16	50	ALA
2	22	ASP	17	97	GLY
3	23	ILE	18	125	PRO
4	38	ALA	19	126	LEU
5	39	ALA	20	127	LEU
6	40	ASN	21	128	SER
7	43	LEU	22	129	ALA
8	44	LYS	23	130	GLY
9	45	HIS	24	131	ILE
10	46	GLY	25	132	PHE
11	47	GLY	26	154	ALA
12	48	GLY	27	155	VAL
13	49	VAL	28	156	PHE
14	50	ALA	29	157	ASP
15	160	LEU	-	-	-

Table showing predicted active site residues/ motifs using CastP server.

Figure no 02

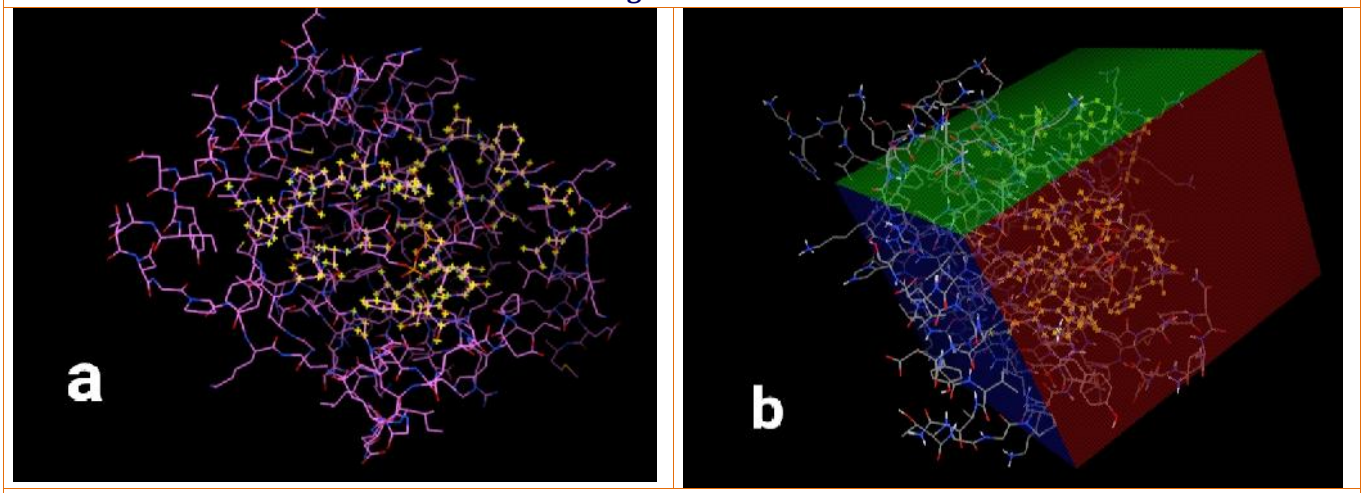


Figure showing a) predicted motif residues of active site for nsp3's macrodomain and b) Gridbox assessment for the predicted active site using AutoDock Vina

3.3 Virtual screening and Docking analysis

We selected 'Warfarin', as the best drug with binding affinity or the lowest minimum energy level of -8.5 kcal/mol which is a sign of strong intramolecular interaction between protein and ligand [see Table no 03 and Figure no 03]. Docked complex of the protein's macro-domain and 'Warfarin' with binding affinity of -8.5 kcal/mol is visualized under UCSF Chimera [Figure no 04].

Table no 03

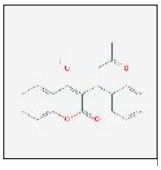
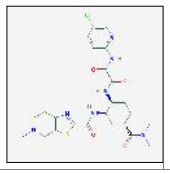
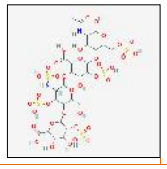
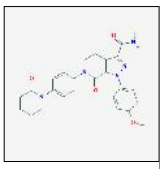
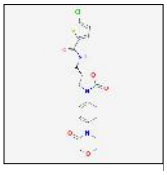
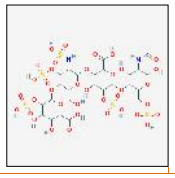
Sr no	Name of drug	Type of drug	Pubchem ID	Structure	Affinity
1	Warfarin	VKAs	CID: 54678486		-8.5
2	Dabigatran	NOACs	CID: 216210		-8.4
3	R-enantiomer acenocoumarol	VKAs	CID: 54676537		-8.0
4	Edoxaban	NOACs	CID:10280735		-8.0
5	Heparin	Anticoagulant	CID: 772		-8.0
6	Apixaban	NOACs	CID: 10182969		-7.7
7	Rivaroxaban	NOACs	CID: 9875401		-7.6
8	Clexane (Low mol. wt. heparin)	Anticoagulant	SID: 349990081		-6.6

Table showing virtual screening results of anticoagulants against macro-domain of Nsp3 with Name of drug, Type of drug, Pubchem Compound ID, Structure, lowest minimum energy level (kcal/mol).

Figure no 03

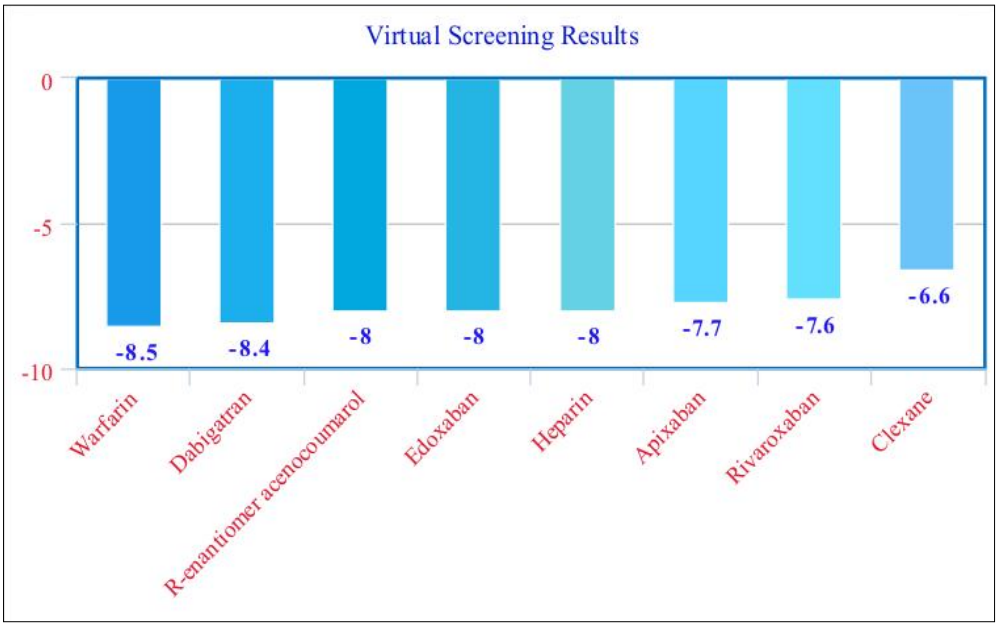


Figure showing diagrammatic representation of virtual screening results

Figure 04

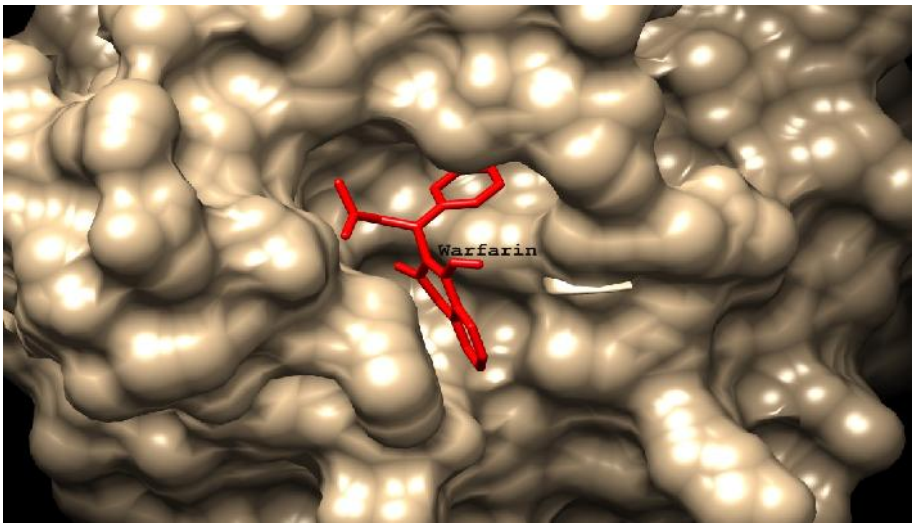


Figure showing anticoagulant drug 'Warfarin' binding to macrodomain of sars-2's Nsp3 protein with binding affinity of -8.5 kcal/mol, visualized under UCSF Chimera.

3.4 Interaction analysis

Amino acids of Nsp3's macro-domain protein namely PHE- Phenylalanine (157A), ASP- Aspartic acid (158A) are involved in formation of two hydrogen bonds with ligand Warfarin's atoms namely 1336 [O2], 1336 [O2] respectively [Figure no 05 and Table no 04].

Figure 05

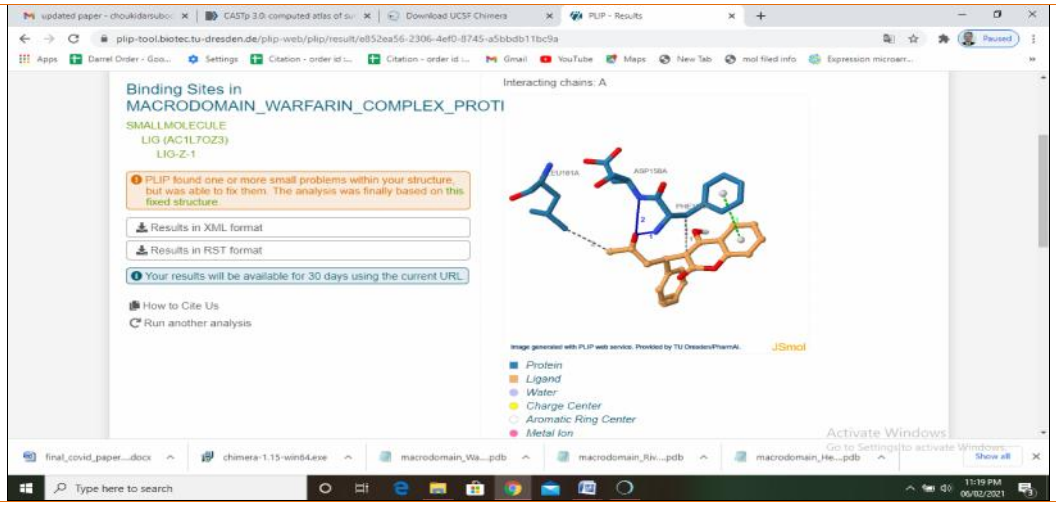


Figure showing Nsp3's macro-domain and Warfarin's interaction result using PLIP a Protein-Ligand Interaction Profiler server (Hydrogen bonds shown in blue color).

Table no 04

Sr no	Amino acid residue, (chain), atom of protein	Atom of ligand	Hydrogen bond length in (Å)
1	PHE-Phenylalanine, (157A), 1167 [Nam]	1336 [O2]	2.89
2	ASP-Aspartic acid, (158A), 1178 [Nam]	1336 [O2]	3.34

Table showing Hydrogen bonds formed between Nsp3 protein's macro-domain and Warfarin with Nsp3's Amino acid involved in interaction.

4. Conclusion

The current paper reveals the correlation between the 'macro-domain of Nsp3 protein' and 'Warfarin' which may possibly play to reduce the thrombotic complications. Here in this paper we tried to make the correlation that the macro-domain could play a vital role in increasing thrombotic complications. To comply with the stated issue, we utilized artificial intelligence like virtual screening of macro-domain with anticoagulant drugs to highlight the best possible drug for the treatment of current covid-19 patients. BE/LME (binding affinity /lowest minimum energy) was the targeted parameter to analyze virtual screening and their results. 'Warfarin' which is anticoagulant drug showed promising results on the basis of best BE/LME among the selected lead compounds (Warfarin = -8.5 kcal/mol, Dabigatran =-8.4, R-enantiomer acenocoumarol= -8.0,Edoxaban= -8.0, Heparin= -8.0, Apixaban= -7.7, Rivaroxaban= -7.6, Clexane= -6.6 kcal/mol). The best drug candidate 'Warfarin' proved to be based on BE/LME of -8.5 kcal/mol, the greater distance was calculated using a PLIP server to visualize the protein ligand interactions. Two hydrogen bonds observed between Nsp3's residues and Warfarin's atoms indicate the stability of the interaction. Based on the above observations, we conclude 'Warfarin' can be useful for treating severe covid-19 patients with thrombotic complications. Furthermore, clinical studies with different biochemical, physiological parameters confirmation is needed for Warfarin's activity against the macro-domain of nsp3 protein. This study will help practitioners to understand the significance of 'Warfarin' as an effective anticoagulant agent to treat severe covid-19 patients.

Acknowledgments

We thank MGM's Medical College and Hospital, for the ideas and valuable guidance for this work. We thank MGM IBT, for providing the required technical facilities for this work.

Conflicts of interest:

The authors of this paper declare No conflict of interest.

Abbreviations:

1. Covid-19 = coronavirus disease-2019.
2. SARS-CoV-2 = Severe Acute Respiratory Syndrome-Coronavirus-2
3. SARS = Severe Acute Respiratory Syndrome
4. MERS = Middle East Respiratory Syndrome
5. NSP3 = Non structural protein-3
6. ADRP = ADP-ribose-1"-phosphate
7. FDA = Food and Drug Administration
8. VKA's = vitamin K antagonist
9. NOACs = non-vitamin K antagonist oral anticoagulants
10. PLIP = protein–ligand interaction profiler
11. IL-6 = Interleukin-6
12. TNF-α = Tumour necrosis factor α
13. ADRP domain = ADP-ribose-1″-phosphatase domain

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DOI:10.22192/ijarbs.2021.08.04.013

How to cite this article:
Subodh G. C., Dr. Talib S.H. , Dr. Talib Yusuf, Ms. Archana Panche, Sanjay N. Harke, Abdoulaye Diawara. (2021). Warfarin could play a key role in preventing the thrombotic complications in severe COVID-19 patients by suppressing the activity of Nsp3's macrodomain of sars-cov-2: *in silico* study. Int. J. Adv. Res. Biol. Sci. 8(4): 100-108.
DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.04.013