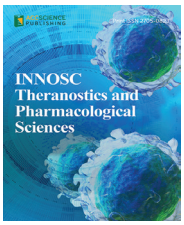
INNOSC Theranostics and Pharmacological Sciences 2022 Vol. 5 (No. 2) pp: 32-44

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INNOSC Theranostics and Pharmacological Sciences

Journal homepage: https://accscience.com/journal/ITPS



RESEARCH ARTICLE

Inhibitory Potential of Chitosan Derivatives against Severe Acute Respiratory Syndrome Coronavirus 2: An *In Silico* Prospective

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(This article belongs to the *Special Issue: Environmental Impact and Health Development in Therapeutic and Pharmacological Research*)

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Received: June 13, 2023; ***Accepted:*** August 3, 2023; ***Published:*** August 17, 2023; ***DOI:*** https://doi.org/10.36922/itps.1077

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Abstract:

The present work was designed to investigate the antiviral potential of novel monomeric and oligomeric chitosan derivatives through *in silico* approaches. The goal was to identify potent broad-spectrum antiviral compounds as promising drug candidates against severe acute respiratory syndrome coronavirus 2 and understand their mode of action. Chitosan biopolymer and its derivatives were virtually screened against the spike glycoprotein and human angiotensin-converting enzyme 2 (ACE2) receptor of novel coronavirus-19. Hydroxypropyl trimethyl ammonium chloride chitosan (HTCC), a polymeric chitosan, has been reported to interact with the corona viral spike (S) protein and blocks its interaction with the ACE2 receptor. The enhancement of antiviral activity relies on better biocompatibility, structural correlations, variation in the degree of deacetylation, and molecular weight of modified chitosan derivatives. The chitosan derivatives constructively interact with viral S protein. Among the chitosan derivatives, N-carboxymethyl chitosan (NCMC) displayed efficient binding affinity. Comparing NCMC to mHTCC, monomeric chitosan, for their interaction with the S protein, receptor binding domain site, and ACE2 receptor, NCMC displayed better binding affinity of −7.9, −6.3, and −7.4 with binding energies of −6.2, −4.8, and −5.5 kcal/mol, respectively. Furthermore, through flexible docking, the interactions of the S protein with ACE2 receptor and ligand mHTCC-S protein complex and NCMC-S protein complex with ACE2 receptor were calculated, showing an efficient reduction of binding energy from −901.2 kJ/mol to −765.06 kJ/mol and −814.72 kJ/mol, respectively. This points to the decrease binding affinity of the viral S protein for the ACE2 receptor in the presence of NCMC/mHTCC. For the first time, the computational study envisages the antiviral efficiency of NCMC, mHTCC, and biocompatible chitosan derivatives as a preventive intervention against COVID-19.

Keywords: Chitosan, mHTCC, Severe acute respiratory syndrome coronavirus 2, S protein, ACE2 receptor, Molecular interaction

1. Introduction

Novel coronavirus-19 (nCoV-19) pandemic has emerged as a global health crisis, resulting in 18,055,630 deaths globally, yet a definitive cure remains elusive. The urgent need of the hour is to search for a safer and more potent drug with a high virucidal effect against nCoV-19 [1,2]. Therefore, the investigation of novel bioactive polymers for identification and cataloging becomes a fundamental necessity [3]. Chitosan, a poly [β-(l-4)-2-amino-2-deoxy-D-glucopyranose], is a profusely available

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natural biopolymer with repeated chains of N-acetyl glucosamine and glucosamine. It possesses physical and biological functionality, is biodegradable, and demonstrates biocompatibility with various organs, tissues, and cells, obviated with cytotoxic and noxious effects [4-6]. The efficiency of chitosan in biomedical applications, such as drug and vaccine delivery, antimicrobial properties (bactericidal, virucidal, fungicidal, and anti-parasitic effects), wound healing, chitosan-guided bone regeneration, and the preparation of artificial skin, hydrogel, and bio lenses for eyes have revolutionized polymer science [5,7-11]. Chitosan's intermolecular and intramolecular hydrogen bonds contribute to its high crystallinity, making it almost insoluble in water and therefore limiting its applications to some extent. However, its adhesion capacity is amplified by the presence of several reactive groups, making it an excellent natural biopolymer. Moreover, chitosan's hydrophobicity and pH sensitivity enable heterogeneous biomedical applications. By reacting with various organic acids and their derivatives, chitosan can have aliphatic or aromatic acyl groups added to its chain [4,5]. Carboxylated chitosan, for instance, possesses higher water solubility and exhibits better thickening, heat preservation, film formation, flocculation, and kneading properties than chitosan alone. The hydrophilic positively charged quaternary ammonium group plays a crucial role in its properties. The higher degree of substitution (DS) in carboxyl chitosan improves water solubility, increases its charge and strength, and weakens hydrogen bonding. Carboxymethyl chitosan, an active compound in the biomedical and pharmaceutical fields, exhibits antibacterial properties that promote wound healing. In addition, it demonstrates lipid-lowering, anti-arteriosclerosis, antiviral, anti-tumor, anti-coagulation, and hypoglycemic effects [12,13].

In recent years, the most commonly used quaternary ammonium salts are 2,3-epoxypropyl trimethyl ammonium chloride and 3-chloro-2-hydroxypropyl trimethyl ammonium chloride and N, N, N- trimethyl ammonium chloride [6,14-16]. Quaternary ammonium chitosan salt has demonstrated superior antibacterial, antiviral, biocompatible, biodegradable, non-toxic, mucoadhesive, and anti-inflammatory properties, rendering them ideal filler fibers in dressing materials. As a mucoadhesive biopolymer, chitosan nanoparticles effectively encapsulate both hydrophilic and lipophilic chemotherapeutic drugs, significantly improving the pharmacokinetic profile of the drug by increasing its solubility and stability in the biological system. Research has shown that hydroxypropyl trimethyl ammonium chloride chitosan (HTCC), a chitosan polymer, interacts with the coronaviral spike (S) protein, effectively blocking its interaction with cellular receptors, thereby preventing the entry of the virus into susceptible cells. This interaction highlights the potential of HTCC as a potent broad-spectrum antiviral drug, effectively hampering the replication of severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) and MERS CoV in VeroE6/human aerial epithelium (HAE)/Huh-7 cell cultures, suggesting HTCC's inhibitory activity against the viral spike protein and its entry receptor interaction [17-19]. However, the exact mechanism of action for these quaternary ammonium chitosan remains unclear and their antiviral mode of action against other viruses has yet to be elucidated [18].

The S protein of the virus interacts with the host cell membrane receptor called angiotensin-converting enzyme 2 (ACE2), a monomeric membrane-associated receptor on human cells, enabling SARS-CoV-2 to enter host cells. According to Jena *et al.*, 2021 [2], the receptor binding domain (RBD) of the S-glycoprotein of SARS-CoV-2 binds ACE2. This RBD region is identical to that of SARS-CoV. Targeting the RBD region of the spike protein could potentially impede viral attachment to the ACE2 receptor and prevent viral entry into host cells, making it a promising target for viral infection prevention [17]. While recent research has proposed various natural products for developing or repurposing anti-SARS-CoV2 drugs that target the spike glycoprotein, to the best of our knowledge, none have explored the potential of chitosan and its derivatives for this purpose [18,19].

In a recently published research [16-18], several chitosan derivatives, namely, N-octadecanoyl-N-3-carboxy propionyl chitosan, palmitoyl-trimethyl-chitosan, N-carboxymethyl chitosan (NCMC), N,O-carboxymethyl chitosan, and carboxy ethyl chitosan, demonstrated effective actions against viral S protein and its

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interaction with the cell receptor. Molecular docking and binding affinity studies revealed the potential of these chitosan derivatives as antiviral agents, particularly in their interaction with the S protein of SARS-CoV-2 **(Figure 1)**. Notably, hydroxypropyl trimethyl ammonium chloride chitosan (mHTCC), N,O-carboxymethyl chitosan, glycerol chitosan, methyl methacrylate chitosan, monomeric chitosan, NCMC, carboxy ethyl chitosan, N-octadecanoyl-N-3-carboxy propionyl chitosan, and palmitoyl-trimethyl-chitosan displayed binding affinities (ΔGb) of −6.2, −5.9, −4.9, −5.3, −7.9, −7.2, −6.4, and −6.9 kcal/mol, respectively. Among these, NCMC and mHTCC exhibited the highest binding affinities toward S protein as a polymer and monomer, respectively. Therefore, mHTCC and NCMC were selected for further analysis of their protein-protein interactions with the S protein-ACE2 complex.

Utilizing computational methods for this study not only reduced biological waste but also saved research time and costs. Therefore, the use of computational approaches allowed us to explore natural compound-based therapeutic interventions for COVID-19. The results also demonstrated the strong binding affinity of NCMC and mHTCC for both the S protein and the RBD site of the S protein.

2. Materials and methods

2.1. Selection of monomeric and oligomeric subunits of chitosan derivatives

The monomeric and oligomeric subunits of chitosan derivatives, which exhibit improved water solubility, pH sensitivity, stability, biocompatibility, and biodegradability resulting from alkylation, carboxylation, acylation, and quaternization, were selected for interaction studies with the S protein of SARS-CoV-2. The decision to choose these subunits was based on their reported antiviral spectrum and inhibitory activity against highly pathogenic human coronaviruses, as cited in **Table 1**. The monomeric and oligomeric subunits include mHTCC, N,O-carboxymethyl chitosan, glycerol chitosan, methyl methacrylate chitosan, monomeric chitosan, NCMC, carboxy ethyl chitosan, N-octadecanoyl-N-3-carboxy propionyl chitosan, and palmitoyl-trimethyl-chitosan.

2.2. Construction of 3D structures of chitosan derivatives

The 3D structures of chitosan and its modified derivatives were established using their canonical smiles strings retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Conversion

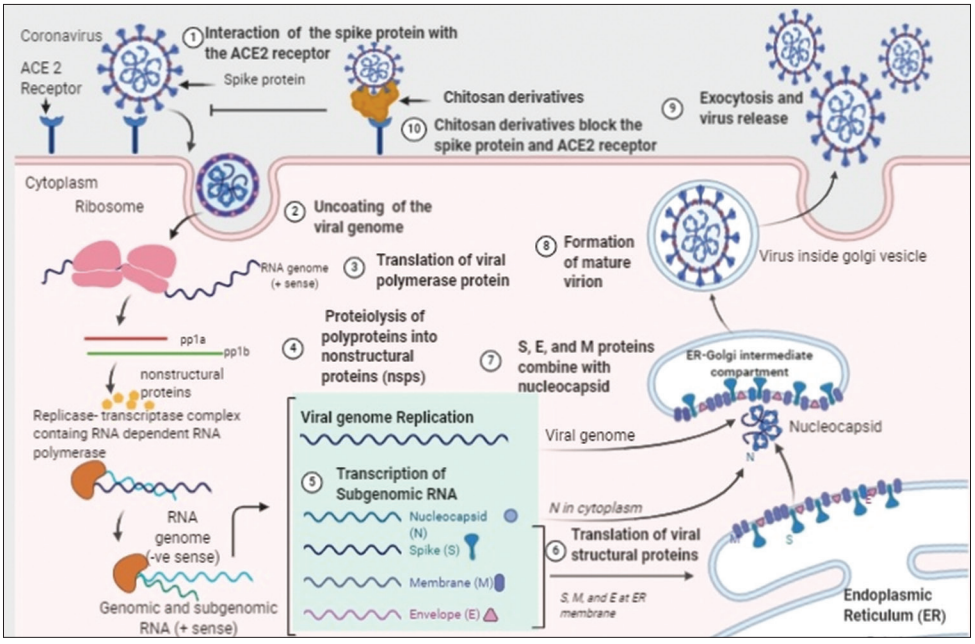


Figure 1. The attachment, entry, and multiplication of the severe acute respiratory syndrome coronavirus 2 in the host cell and its transport to the outer surface of the cell. Figure is generated using BioRender software.

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Table 1. Type of modification, solubility parameters, molecular mass, antiviral spectrum, and mechanism of action of the selected chitosan derivatives

No.	Chitosan derivatives	Type of chitosan modification (molecular mass [g/mol])	Advantages of the polymer	Antiviral spectrum	Mode of action	References
1	Hydroxypropyl trimethyl ammonium chloride chitosan (mHTCC)	Quaternary ammonium addition (342.8)	Increases solubility, enhances mucoadhesion, prolongs the resistance, and increases pH sensitivity.	Hepatitis virus C, human coronaviruses (HCoVs), human immune deficiency virus-1 (HIV-1)	Inhibits the viral spike protein and host cell ACE2 receptor interaction and blocks viral propagation.	[17,18,37]
2	N,O-carboxymethyl chitosan	Methylation (291.2)	Enhances solubility in polar solvents with increasing biocompatibility.	HIV-1, Friend murine leukemia helper virus (F-MuLV), HSV	Inhibits the virus coat protein and host cell receptor interaction.	[20,30]
3	Glycerol chitosan	Glycerolyation (279.3)	Enhances penetrability and increases mucoadhesion.	Rotavirus, norovirus, adenovirus	Blocks viral replication.	[38]
4	Methyl methacrylate chitosan	Ammonium addition and alkylation (291.3)	Increases drug sustainability, slows down drug release, and increases penetrability.	Adenovirus and chicken pox and smallpox viruses	Inhibits viral protein synthesis.	[30]
5	Monomeric chitosan	No modification (180.18)	Enhances biocompatibility and biodegradability but is less soluble	Newcastle virus, influenza virus	Causes viral lysis.	[5]
7	NCMC	Methylation (559.5)	Enhances solubility in polar solvents with increasing biocompatibility	HIV-1, F-MuLV, HSV	Inhibits the virus coat protein and host cell receptor interaction.	[12]
8	Carboxy ethyl chitosan	Alkylation (573.5)	Increases solubility and stability	HIV-1, HSV, influenza virus	Inhibits the virus coat protein and host cell receptor interaction.	[20,30]
9	N-octadecanoyl-N-3-carboxy propionyl chitosan	Carboxylation and alkylation (868)	Increases solubility and enhances immunostimulatory activity and mucoadhesion.	Human noroviruses, murine viruses, feline caliciviruses	Interrupts the virus replication as well as interaction with the host.	[30]
10	Palmitoyl-trimethyl-chitosan	Methylation and palmitoylation (782)	Enhances access to the cell membrane and stabilizes the drug sustainability.	HSV, influenza virus, HIV-1	Inhibits viral polymerases and multiplication inside the host cell.	[31]

Abbreviations: HSV: Herpes simplex virus; ACE2: Angiotensin-converting enzyme 2.

into 3D structures was done using CHIMERA 1.11.2. Subsequently, these structures were used to evaluate their interactions with the spike protein of SARS-CoV-2 and the ACE-2 receptor [20].

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2.3. Structure and sequence analysis of S protein and ACE- 2 receptor

The cryogenic electron microscopic structure of the S protein of SARS-CoV-2 (Protein Data Bank [PDB] ID: 6vsb) with a resolution of 3.46 Å and the X-ray diffraction structure of the ACE-2 receptor (PDB ID: 1r42) with a resolution of 2.2 Å were retrieved from the PDB. The FASTA sequences of the other reported human coronaviruses (HCoV-229E, MERS-CoV, HCoV-NL63, and SARS-CoV) were obtained from the PDB database. These sequences were then used for multiple sequence alignment to identify the similarity of the amino acid sequences of the three chains of the spike protein.

2.4. Phylogenetic analysis

The FASTA sequence of the S-protein was retrieved from the PDB database and evolutionary analysis of genetic distance and diversity was conducted using MEGA-X. Phylogenetic analysis was performed using the Substitution Model Jones-Taylor-Thornton (JTT) [21], and standard error estimates were obtained through a bootstrap procedure (1000 replicates). The phylogenetic tree was then constructed using the maximum likelihood statistical method.

2.5. Molecular docking

The evaluation of the free binding energy of energy-minimized chitosan subunits and their monomeric and oligomeric derivatives with the S protein and ACE2 receptor was conducted using the molecular docking program AutoDock Tools 1.5.6. Molecular docking studies were also performed to assess the binding affinity of chitosan derivatives, namely, mHTCC and NCMC, with the RBD of the S protein. The protein binding affinity of S protein, RBD, and ACE2 receptor with chitosan monomeric and oligomeric derivatives was examined using AutoDock Vina1.1.2. Various parameters, such as binding affinity, receptors interacting atom, receptor pocket atom, receptor-ligand interaction site, atomic contact energy (ACE), and side amino acid residues, were studied to identify the binding sites of the S protein and ACE2 receptor [22]. The results of the docking studies were visualized and analyzed using Discovery Studio 2017 R2 Client [23-25].

2.6. Protein-protein interaction

Protein-protein interaction studies were carried out using flexible docking to investigate the interactions of specific protein complexes [1,2]. First, the interactions between the S protein with mHTCC and NCMC were examined using molecular docking. Subsequently, the resulting S protein-mHTCC and S protein-NCMC complexes were studied in their interaction with the ACE2 receptor. To assess the binding affinity of the S protein for the ACE2 receptor in the presence of mHTCC and NCMC similarly, the interaction between the S protein and ACE2 receptor without mHTCC and NCMC was also analyzed [26,27]. For this analysis, the clusPro 2.0 software (https://cluspro.bu.edu/publications.php) was employed, utilizing the fast Fourier transform (FFT) docking methods. The selections of the filtered conformations were based on the assessment of empirical free energy. Both the lowest de-solvation and electrostatic energies were taken into account for the evaluation of free energy. Piper being a FFT-based rigid docking tool, serves the clusPro clustering program for measure the native sites by providing 1000 low energy outcomes [28]. The native site is assumed to possess a wide range of free energies to yield a great number of results. Initially, a sample of approximately 10^9 positions of the ligand with respect to the receptor was taken. From this set, only the top 10^3 positions were selected for further analysis, considering all relative ligand positions corresponding to the receptor.

3. Results

3.1. Phylogenetic analysis

The S protein of SARS-CoV-2 showed the highest sequence identity (73.9090%) with SARS-CoV, while its lowest amino acid sequence similarity was observed with HCoV-229E (10.6077%). In addition, the S protein shares 22.9037% sequence identity with MERS-CoV and 18.5559% with HcoV-NL63. Phylogenetic tree analysis of SARS-CoV-2 and SARS-CoV reveals their close relationship, as they share the same operational taxonomic unit (out) **(Figure S1)**.

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3.2. Molecular docking

3.2.1. Interaction of different monomeric subunits of chitosan with the S protein

The binding modes of monomeric units of chitosan and its derivative with the S protein were investigated using AutodockVina1.1.2. The free binding energies (ΔGb) of the S protein with energy-minimized chitosan monomeric and polymeric units, as well as its derivative, namely, mHTCC, N,O-carboxymethyl chitosan, glycerol chitosan, methyl methacrylate chitosan, monomeric chitosan, NCMC, carboxy ethyl chitosan, N-octadecanoyl-N-3-carboxy propionyl chitosan, and palmitoyl-trimethyl-chitosan, were calculated as −6.2, −5.9, −4.9, −5.3, −7.9, −7.2, −6.4, and −6.9 kcal/mol, respectively. Further information on the type of modification, solubility parameters, molecular mass, antiviral spectrum, and mechanism of action of the selected chitosan derivatives can be found in **Figures 2**, **3**, and **S2**–**S7**. **Table 2** provides a brief elaboration on the binding energy, interacting amino acids of the S protein, and the type of interaction with the selected chitosan derivatives. The 2D interaction between the S protein and the selected chitosan derivatives is provided in **Figures 2**, **3**, and **S2**–**S7**.

3.2.2. Interaction studies of mHTCC with the S protein

The mHTCC unit exhibits specific interaction with the S protein, particularly at the RBD site of the S protein and ACE2 receptor, with binding affinities recorded as −6.2, −4.8 and −5.5 kcal/mol, respectively. The present investigation elucidates that HTCC directly binds with certain amino acids of the S protein, including Glu1017, Ala1016, Ile1013, Thr961, Gln762, Ala958, Gln954, Arg1019, Arg1014, Gln954, Gln1010, Gln954, Arg765, and Leu1012, and those presented in the RBD, such as Ser438, Asp442, Phe342, Ala344, Asn440, Asn343, Asn437, Thr345, Trp436, Leu441, and Leu441. In addition, interactions with the ACE2 receptor involve Lys562, Tyr196, Gln102, and Ala99 (data on the interactions with the ACE2 receptor are not provided). These interactions primarily involve conventional hydrogen bonds, carbon-hydrogen bonds, unfavorable positive-positive interactions, alkyl bonds, pi-sigma bonds, pi-pi T-shaped interactions, van der Waals interactions, and unfavorable donor-donor interactions. **Figure 2** illustrates the 2D interaction of mHTCC with the S protein.

3.2.3. Interaction studies of NCMC with the S protein

The binding affinity exhibited by NCMC toward the S protein was found to be the highest, followed by carboxy ethyl chitosan, with binding energies of −7.9 and −7.2 kcal/mol, respectively. Nevertheless, the binding affinity of NCMC with the S protein was even higher than that exhibited by mHTCC, involving the participating amino acids, namely, Ala1016, Ala1016, Glu1017, Glu1017, Glu1017, Arg1019, Arg1019, Ala1020, Ala1020, Ser1021, Asn1023, Asn1023, Leu1024, Leu1024, Thr1027, Thr1027, Glu1031, Phe1042, Phe1042, Ala1016, Ala1020, Asn1023, Leu1024, Thr1027, Arg1039, Arg1039, Arg1039, and Arg1039. Further investigation into the binding energy of NCMC involved its interaction with the RBD of the S protein and ACE2 receptor. The binding affinity of NCMC for the RBD of the S protein was found to be -6.3 kcal/mol, involving the participating amino acids, such as Asn343, Arg509, Thr345, Ala344, Leu441, Trp436, Asp442, Ser438, Ser373, Ala372, Ser375, Asn437, Asn440, Ser375, and Phe374. Similarly, NCMC binding to ACE2 receptor exhibited binding energy of −7.4 kcal/mol, with primary participating amino acids including Asn397, Ala396, Arg514, Glu398, Asp206, Gln102, Ser511, Tyr510, Trp203, Tyr199, Gly205, Tyr202, Tyr196, Glu208, Gln98, Leu95, Lys562, and Ala396. These interactions involved conventional hydrogen bonds, carbon-hydrogen bonds, unfavorable positive-positive interactions, alkyl bonds, pi-alkyl bonds, pi-sigma bonds, alternative charges, salt bridges, van der Waals interactions, and unfavorable donor-donor interactions (data not provided). **Figure 3** illustrates the 2D interaction of NCMC with the S protein, the RBD of the S protein, and the ACE2 receptor, as well as participating amino acids at the RBD and ACE2 receptor, and the binding of NCMC with the S protein.

3.3. Protein-protein interaction

The best ten docking models with different free energies were obtained from the ClusPro web server, using the total root mean square deviation value as the grouping criteria [29]. From these models, we

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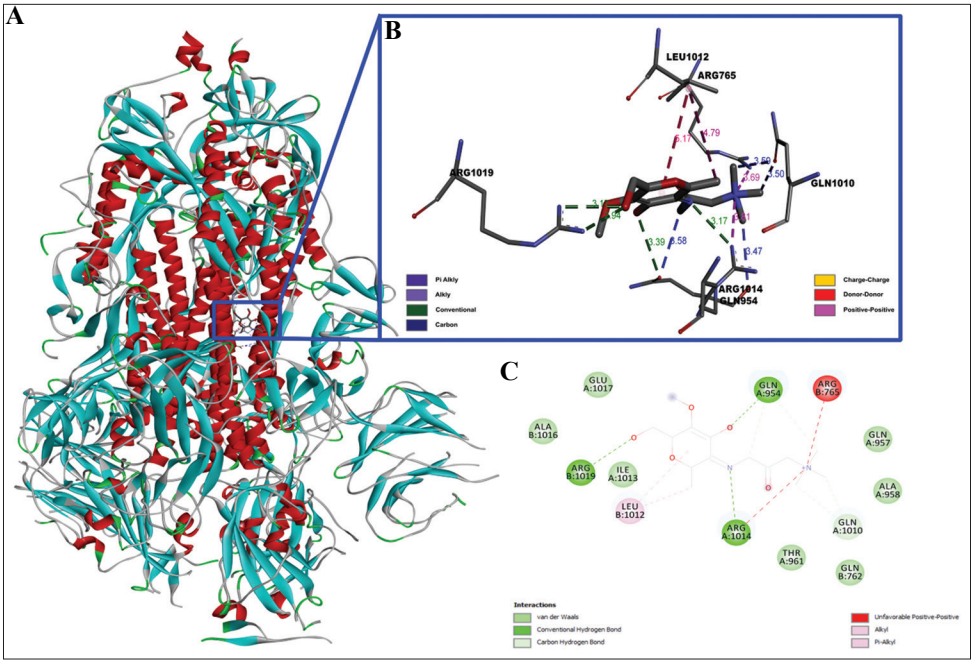


Figure 2. (A) Molecular docking of mHTCC with the S protein. (B) Brief 3D representation of mHTCC and S protein interaction including bond types and length. (C) 2D representation of mHTCC and S protein with participating amino acids and types of bonds.

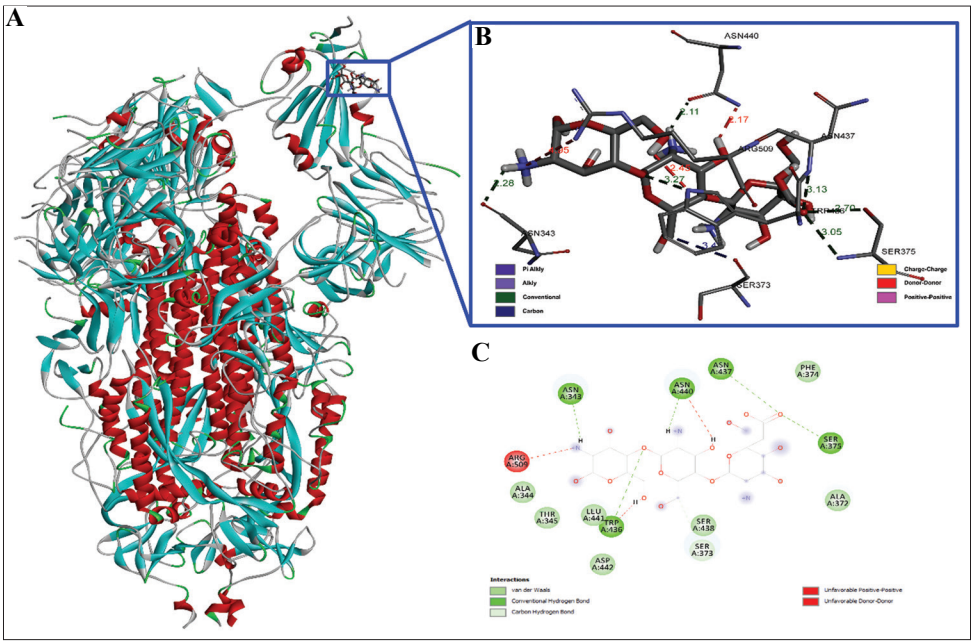


Figure 3. (A) Molecular docking of N-carboxymethyl chitosan (NCMC) with the S protein. (B) Brief 3D representation of NCMC and S protein interaction, including bond types and length. (C) 2D representation of NCMC and S protein with participating amino acids and types of bonds.

selected and analyzed five ClusPro docking models based on the probability of interaction between the S protein and derivatives of chitosan and the predicted binding sites of the ACE2 receptor, as well as the lowest binding energy observed during these interactions. The average binding energy for all five binding positions involved in S protein-ACE2 interaction is −901.2 kJ/mol. Nevertheless, in the presence of mHTCC and NCMC, the average binding energy for S protein-ACE2 interaction decreased to −765.06 kJ/mol and −814.72 kJ/mol, respectively (**Table 3**). These findings indicate that the free energy

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Table 2. 2D representation of the molecular docking of the chitosan and its derivatives explaining the type of interaction and amino acids involved with detailed insight into the type of chitosan modification, advantages, its virucidal spectrum, and mechanism of action

No.	Chitosan derivatives	Binding affinity (kcal/mol)	Types of interaction	Interacting AA name; chain name; AA number
1	Hydroxypropyl trimethyl ammonium chloride chitosan	−6.2	van der Waals	Glu A:1017, Ala B:1016, Ile A:1013, Thr A:961, Gln B:762, Ala A:958, Gln A:954
			Conventional hydrogen bond	Arg B:1019, Arg A:1014, Gln A:954
			Carbon-hydrogen bond	Gln A:1010, Gln A:954
			Unfavorable positive-positive	Arg B:765
			Alkyl	Leu B:1012
2	N,O-carboxymethyl chitosan	−5.9	van der Waals	Thr C:549, Thr C:572, Ile C:587, Asp A:745, Met A:740, Tyr A:741, Cys A:743, Asn A:856, Phe C:541, Leu A:977, Leu A:966, Asn A:978, Thr C:572, Val A:976
			Conventional hydrogen bond	Arg A:1000, Ile A:742, Thr C:573
			Unfavorable donor-donor	Arg A:1000
			Carbon-hydrogen bond	Asp C:571
3	Glycerol chitosan	−4.9	van der Waals	Leu A:977, Arg A:100, Ile A:742, Val A:976, Leu A:966, Thr C:572, Asp C:571, Pro C:589, Thr C:549, Asn A:978
			Conventional hydrogen bond	Cys A:743, Thr C:573
			Carbon-hydrogen bond	Tyr A:741, Met A:740
			Salt bridge	Asp A:745
4	Monomeric chitosan	−5.3	van der Waals	Lys C:1028, Leu C:1024, Thr A:1027
			Conventional hydrogen bond	Glu A:780, Gln A:784, Ala A:1026, Ser A:1030, Phe C:1042
			Alternative charge	Asp C:1041, Glu C:725
5	NCMC	−7.9	van der Waals	Ala A:1016, Ala B:1016, Glu A:1017, Glu B:1017, Glu C:1017, Arg B:1019, Arg C:1019, Ala A:1020, Ala B:1020, Ser C:1021, Asn A:1023, Asn C:1023, Leu B:1024, Leu C:1024, Thr B:1027, Thr C:1027, Glu B:1031, Phe A:1042, Phe B:1042
			Conventional hydrogen bond	Ala C:1016, Ala C:1020, Asn B:1023, Leu A:1024, Thr A:1027, Arg B:1039, Arg C:1039, Arg A:1039
			Unfavorable donor-donor	Arg A:1039
6	Carboxy ethyl chitosan	−7.2	van der Waals	Gly A:757, Ser A:758, Cys A:760, Leu A:754, Cys A:738, Leu A:754, Thr A:739, Ser A:750, Asn A:764, Thr A:768, Leu C:303
			Alternative charge	Asp A:737
			Conventional hydrogen bond	Gln C:314, Thr A:761, Thr C:315, Thr C:302
			Unfavorable positive-positive	Arg A:765, Lys C:304
			Unfavorable donor-donor	Arg A:765, Lys C:304

(Cont'd...)

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Table 2. *(Continued)*

No.	Chitosan derivatives	Binding affinity (kcal/mol)	Types of interaction	Interacting AA name; chain name; AA number
7	N-octadecanoyl-N-3-carboxy propionyl chitosan	−6.4	van der Waals	Pro A:665, Val B:772, Thr B:768, Asn B:764, Tyr A:913, Gln A:914, Ala B:766, Gln A:1010, Gln A:954, Arg A:1014, Gln A:957, Gly B:769, Leu B:1012, Gln B:762, Ala A:958, Thr B:761, Leu A:908
			Conventional hydrogen bond	Thr A:902, Ile :912
			Unfavorable positive-positive	Arg B:765
8	Palmitoyl-trimethyl-chitosan	−6.9	van der Waals	Gly A:667, Ile A:666, Gln A:613, Lys B:733, Ile A:312, Val B:772, Thr B:768, Tyr A:313, Leu A:303, Asn A:953, Ile B:770, Leu B:1012, Gly B:769, Gln A:954, Gln A:957
			Conventional hydrogen bond	Asp A:950, Arg B:765, Ala B:766
			Carbon-hydrogen bond	Asp A:950
			Alternative charge	Glu B:773
			Unfavorable positive-positive	Arg A:1014, Arg B:1019

Abbreviation: NCMC: N-carboxymethyl chitosan.

Table 3. Protein-protein interactions of S protein with ACE2 receptor, S protein-mHTCC complex with ACE2 receptor, and S protein-NCMC complex with ACE-2 receptor

Macromolecule	Binding positions	Lowest energy (kJ/mol)	Average lowest energy (kJ/mol)
S protein-ACE2	Red	−928.9	−901.2
S protein-ACE2	Green	−923	
S protein-ACE2	Blue	−902.4	
S protein-ACE2	Violet	−853.3	
S protein-ACE2	Light blue	−898.4	
S protein with mHTCC-ACE2	Red	−785.4	−765.06
S protein with mHTCC-ACE2	Green	−783.7	
S protein with mHTCC-ACE2	Blue	−750.4	
S protein with mHTCC-ACE2	Violet	−756.3	
S protein with mHTCC-ACE2	Light Blue	−749.5	
S protein with NCMC-ACE2	Red	−863.5	−814.72
S protein with NCMC-ACE2	Green	−850.2	
S protein with NCMC-ACE2	Blue	−741.1	
S protein with NCMC-ACE2	Violet	−797.3	
S protein with NCMC-ACE2	Light Blue	−821.5	

Abbreviations: NCMC: N-carboxymethyl chitosan; ACE2: Angiotensin-converting enzyme 2; HTCC: Hydroxypropyl trimethyl ammonium chloride chitosan.

ΔGb is reduced due to the binding of mHTCC to the S protein, as depicted in **(Figure 4** and **Figure S8)**. This finding complies with the study by Milewska *et al.* [17], indicating that both polymeric and monomeric HTCC reduce the affinity of the spike protein for the host cell ACE2 receptor. As a result, the virus replication and multiplication inside the host tissue are significantly reduced.

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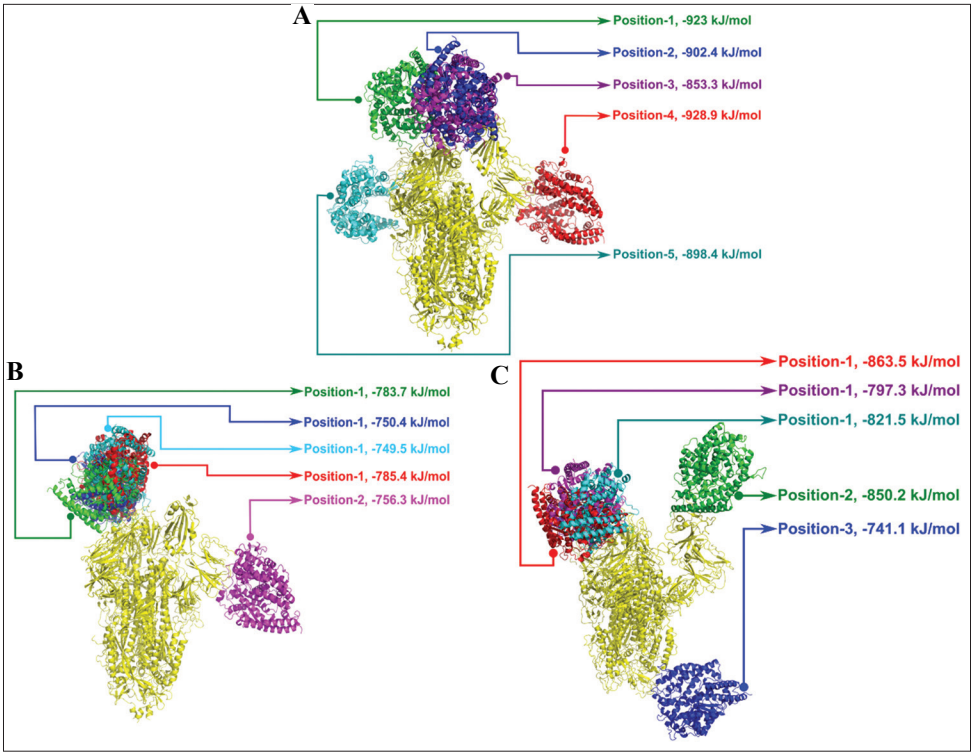


Figure 4. Protein-protein interaction of (A) angiotensin-converting enzyme 2 (ACE2) receptor with the S protein, (B) S protein-mHTCC complex with ACE2 receptor, and (C) S protein-N-carboxymethyl chitosan complex with ACE2 receptor.

4. Discussion

Chitosan derivatives have been reported to influence inhibitors of various human pathogenic viruses, including influenza virus, HIV, Newcastle virus, human norovirus, murine virus, feline calicivirus, hepatitis C virus, Friend murine leukemia helper virus (F-MuLV), herpes simplex virus (HSV), pox virus, and human coronavirus [20,30]. In the present study, we focused on the usage of biodegradable and biocompatible chitosan and its derivatives, which exhibit potent inhibition against spike proteins of coronaviruses. We thoroughly evaluated the effect of molecular weight, configuration, and DS on different monomeric and oligomeric units of chitosan derivatives and their interaction with the spike protein. Milewska *et al.* [17] reported a decline in viral infection rate and multiplication when employing HTCC, a polymeric derivative of chitosan, in the culture medium of human airway epithelium cell lines. HTCC was also identified as a broad-range inhibitor of the other human coronaviruses in its polymeric form, with the DS varying from 57% to 77% [18]. In our study, we evaluated the interaction affinity of the monomeric unit of mHTCC with the spike protein, RBD of the S protein and the ACE2 receptor. The protein-protein interaction study revealed the binding efficiency of mHTCC and the S protein complex with ACE2 receptor, indicating that mHTCC inhibits the binding affinity of the S protein with ACE 2 receptor. These findings are consistent with prior research.

In a recent study by Malik *et al.* [31], trimethyl chitosan (TMC) was identified as a potent chitosan derivative with properties such as high aqueous solubility, stability over a wide range of ionic conditions, and mucoadhesive properties, making it an effective adjuvant molecule. TMC was found to induce strong antibody responses and robust cell-mediated immunity. Alongside TMC, HTCC, a cationic chitosan derivative, has been proven to be effective as an adjuvant when co-administered with the hepatitis E virus (HEV) recombinant polypeptide vaccine through the intramuscular route. Vaccination using HTCC as an adjuvant was associated with an increase in serum HEV-specific IgG antibodies, splenocyte proliferation, and the growth of CD4+ and CD8+ T lymphocytes, and IFN-γ-secreting T lymphocytes in peripheral blood.

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These findings suggest that HTCC has a strong immuno-enhancing effect [32].

The current alarming situation necessitates urgent research to proactively address the pathogenesis of SARS-CoV-2 and prevent future outbreaks. The viral entry site is composed of the RBD domain of the S protein (ranges from Arg 319 to Phe591) and the N-terminal peptidase domain of ACE2 receptor (ranges from Ser19 to Asp615). When ligands mHTCC bind with RBD, they interact with Ser438, Asp442, Phe342, Ala344, Asn440, Asn343, Asn437, Thr345, Trp436, Leu441, and Leu441, which fall within the range of amino acids 319–591 of the RBD domain. This region is well-known for its role in binding to host cells. Similarly, mHTCC binds with ACE2 receptor involving Lys562, Tyr196, Gln102, and Ala99, which are part of the virus entry site (**Figure 2**). Moreover, NMNC binds at the RBD site, interacting with Asn343, Arg509, Thr345, Ala344, Leu441, Trp436, Asp442, Ser438, Ser373, Ala372, Ser375, Asn437, Asn440, Ser375, and Phe374 (**Figure 3**). In addition, NMNC binds with ACE2 receptor at amino acids Asn397, Ala396, Arg514, Glu398, Asp206, Gln102, Ser511, Tyr510, Trp203, Tyr199, Gly205, Tyr202, Tyr196, Glu208, Gln98, Leu95, Lys562, and Ala396.

The bindings of mHTCC and NCMC with the RBD involve interactions such as conventional hydrogen bonds, carbon-hydrogen bonds, unfavorable positive-positive interactions, alkyl bonds, pi-sigma bonds, pi-pi T-shaped interactions, van der Waals interactions, and unfavorable donor-donor interactions. Furthermore, the binding of mHTCC and NCMC with ACE2 protein also revealed the presence of conventional hydrogen bonds, carbon-hydrogen bonds, unfavorable positive-positive interactions, alkyl bonds, pi-alkyl bonds, pi-sigma bonds, alternative charges, salt bridges, van der Waals interactions, and unfavorable donor-donor interactions. Similarly, the ligands mHTCC and NCMC, when bound to ACE2 sites, function as the region or medium responsible for the entry of the viral S protein. Our findings also demonstrated strong binding of the ligands mHTCC and NCMC to the RBD site of the S protein. Thus, the chitosan derivatives mHTCC and NCMC demonstrated a dual role, strongly binding to the S protein and impeding viral entry into the host cell ACE2 receptor. These findings align with the research conducted by Milewska *et al.* in 2016 and 2021 [17,18].

In addition to mHTCC, carboxylated chitosan derivatives have demonstrated efficient inhibitory activity against the S protein, yielding binding energies of −7.9, −7.2, and −5.9 kcal/mol for NCMC, N-carboxy ethyl chitosan, and N,O-carboxymethyl chitosan, respectively. These values were ascertained through interactions with the S protein. NCMC exhibited a promising docking score with both the RBD of the S protein and the ACE2 receptor. This promising result paves the way for further investigations, positioning it as a prospective candidate for robust biopolymer development against SARS-CoV-2. Our study's observations reveal the enhanced binding affinity of the carboxylated derivatives of chitosan subunits toward the S protein. Molecular docking studies reveal the efficacy of HTCC, NCMC, N,O-carboxymethyl chitosan, and N-carboxy ethyl chitosan in establishing efficient binding affinity against the S protein of SARS-CoV-2. In this context, the results of this computational investigation indicate that the compounds listed above can be considered for prospective antiviral drugs against SARS-CoV2.

A recent research demonstrated that silymarin-chitosan nanoparticles (Sil-CNPs) can act as a potent antiviral agent for adenovirus 5 (ADV5) and SARS-CoV-2. The researchers evaluated the drug's cytotoxic activity on Vero and Vero E6 cell lines and found it non-cytotoxic while improving its bioavailability and physicochemical qualities. The enhanced antiviral activity of Sil-CNPs may be attributed to their ability to inhibit the viral host ACE2 receptor, thus preventing the virus from attaching to cells [33]. A separate study underscores the reinforcement of bronchoalveolar lavage and local mucosal immunity within the lungs, facilitated by the chitosan-mediated nanovaccine. This innovative approach exhibits the capacity to strengthen the host's defense mechanisms against infection without causing systemic harm [34]. In addition, chitosan/α-Ag_2WO_4 composites emerge as compelling agents with high efficacy in eliminating pathogenic microorganisms, such as *Escherichia coli*, methicillin-susceptible *Staphylococcus aureus*, and the yeast strain *Candida albicans* [35]. These composites also exhibit the ability to inactivate

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SARS-CoV-2 on direct contact, achieved through the production of reactive oxygen species. Another study about chitosan aimed to develop mucoadhesive, chemically cross-linked chitosan hydrogels using malic and glutaric acid, without supplementary excipients, solubilizing agents, or catalysts, and examine their interaction with certain natural ACE2 receptor inhibitors [36]. These hydrogels were intended for use as a nasal formulation to reduce the risk of COVID-19 infection. Furthermore, protein-protein interaction studies revealed notable reductions in the binding energy of the S protein with ACE2 in the presence of chitosan and its derivatives. As a result, both compounds are likely to prevent the attachment of the RBD region of S Protein to the ACE2 receptor [37,38]. It is important to note that, however, these findings necessitate further experimental validation. The carboxylic group emerges as an important structural feature that contributes to the heightened affinity of chitosan for this target. These groups also exhibit the capacity to enhance water solubility and flocculating capacity, which may enhance the *in vivo* efficacy [5].

5. Conclusion

The novel COVID-19, responsible for the recent pandemic, stands as a global threat with no established curative drug. Instead, the predominant approach involves managing the illness through supportive therapies employing repurposed drugs. Notably, NCMC, mHTCC, and various biocompatible chitosan derivatives have exhibited significant antiviral activity *in-silico*. These compounds hold potential as inhibitors of SARS-CoV-2 infections. Within this context, the results of this computational investigation propose that NCMC and mHTCC warrant further investigation as potential contenders for future antiviral medications targeting SARS-CoV2. Nonetheless, it is important to underscore that additional experimental validation is imperative to corroborate these findings.

Acknowledgments

The authors are thankful to the World Bank-OHEPEE for supporting the Centre of Excellence in Integrated Omics and Computational Biology Utkal University for the manpower support. The manpower support from DBT Government of India, New Delhi to the Department of Biotechnology, Utkal University, and The National Centre for Cell Science (NCCS), Pune, is gratefully acknowledged.

Funding

World Bank-OHEPEE and DBT Government of India.

Conflict of interest

The authors declare no conflicts of interest.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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