Sulphated polysaccharides from seaweeds as potential entry inhibitors and vaccine adjuvants against SARS-CoV-2 RBD spike protein: a computational approach

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Abstract
The health pandemic (covid-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected, to date, over 233 million people and over 4.77 million deaths worldwide. Therefore, it is important to offer a wide understanding of the ability of potential antiviral biomolecules to combat this virus, which begins to develop new variants, potentially more transmissible and infectious. Sulphated polysaccharides (SPs) from seaweeds have been reported for their antiviral activity and their potential as vaccine adjuvants. However, their effect against SARS-CoV-2 remains very limited. In this study, seventeen ligands of SPs were screened for effective interaction with the domain (RBD) spike protein using a computational approach. The obtained results indicated that two PS ligands (Xylan sulphate and Heparin tetrasaccharide N-sulphated) effectively bind to the RBD protein and could efficiently inhibit viral entry. Overall, data support the potential importance of SPs against SARS-CoV-2 as entry inhibitors and vaccine adjuvants.

Highlights
- Seventeen ligands of sulphated polysaccharides (SPs) were subjected to a molecular docking to assess their interaction with SARS-CoV-2 RBD spike protein;
- Two SP ligands showed an effective binding affinity to the RBD protein and could efficiently inhibit viral entry;
- One effective SP ligand (Xylan Sulphate) was predicted using ADMETTox evaluation as good inhibitor;
- Effective candidates can have an importance against SARS-CoV-2 as entry inhibitors and vaccine adjuvants.

1. Introduction
The novel SARS-CoV-2 is the infectious agent responsible for the global COVID-19 (coronavirus disease 2019) outbreak [1]. To date, the epidemic has spread around the world and has infected over 233 million people and over 4.77 million deaths [2].

SARS-CoV-2 is an enveloped positive-sense RNA virus that belongs to a group of β-coronavirus [3]. From attachment; and through the stages of the viral cycle (penetration, transcription, translation, replication of the genome), the SARS-CoV-2 virus interacts with various targets [4]. During interaction, therapeutic protein targets of SARS-CoV-2, known to be essential at different stages of the viral cycle including 3CLpro (main protease), PLpro (papain-like protease), SGp-RBD (spike glycoprotein-receptor binding domain), RdRp...
(RNA dependent RNA polymerase) and ACE2 (angiotensin-converting enzyme 2) are involved and therefore constitute a good targets of drug research [5]. The mechanism of blocking viral attachment through hindering the binding of the virus to the cell surface receptor is very essential in early stopping the action of the virus [6].

The initial step of viral invasion into host cells start by the binding of viral particles to the host cell surface via electrostatic interaction, and the subsequent process is then achieved by transforming the unstable reversible electrostatic binding into stable irreversible binding [6]. Coronaviruses use the homotrimeric spike glycoprotein (comprising a S1 subunit and S2 subunit in each spike monomer) on the envelope to bind to their cellular receptors [7].

The CoV spike (S) glycoprotein is a key target for vaccines, therapeutic antibodies, and diagnostics [8]. Glycosylated S proteins cover the surface of SARS-CoV-2 which the RBD (receptor binding domain) of the S1 subunit mediates binding by recognizing host cell receptors, angiotensin-converting enzyme 2 (ACE2) leading to cell attachment, and fusion during viral infection [5,9]. SARS-CoV-2 RBD binds to ACE2 with an affinity in the low nanomolar range, indicating that the RBD is a key functional component within the S1 subunit that is responsible for binding of SARS-CoV-2 by ACE2 [5,10]. Thereafter, the RdRp facilitates the viral genome replication [27]. The 3CLpro and PLpro act as proteases in the process of proteolysis of the viral polyprotein into functional units [5,10].

Therefore, the search of natural biomolecules with antiviral efficacy is currently a challenge for the scientific community for the discovery of efficient SARS-CoV-2 antiviral agents [11,12], especially with the emergence and spread of new SARS-CoV-2 variants [13,14]. The choice of an effective antiviral agent depends on its potential compatibility to occupy and block the site of attachment, which renders the virus unable to complete the subsequent infection process [15,16].

Seaweeds provide a great variety of bioactive compounds with antimicrobial activity. The main components of them are usually polysaccharides [17]. Among these natural and safe bioactive substances, sulphated polysaccharides (SPs) are recognized to possess various biological activities including anticoagulant, antiviral, and immuno-inflammatory activities [16]. The major SPs are galactans, fucans, ulvans and their derivatives synthesized by the three major groups of marine algae, brown algae (Phaeophyta), red algae (Rhodophyta), and green algae (Chlorophyta), respectively [18]. These bioactive substances don’t have equivalents in terrestrial plants and resemble the chemical and biological properties of mammalian glycosaminoglycans [19].

SPs are considered as efficiency candidates in antiviral activities against several viruses, among them the coronavirus family [20], which inhibit the viral infection in different phases of the viral life cycle and/or by improving the host antiviral immune response [21]. Their efficiencies appear to be based on their abilities to interfere with the entry process by blocking the pathogenic virus receptors [21,22,23]. Furthermore, SPs show other potentialities, which make it possible to promote antiviral efficacy via an immunity action. Several experimental studies demonstrate that SPs from seaweeds have potential vaccine adjuvant properties, with immunostimulatory activity on macrophages, dendritic cells and other types of immune cells [24,25]. In a recent study, Kim et al [26] found that both monomeric and trimeric SARS-CoV-2 spike bind more tightly to immobilized heparin with remarkably high affinity and it prefers long, heavily sulphated structures. Despite their antiviral potential, SPs effect against SARS-CoV-2 remain very limited.

In this study, seventeen SPs from seaweeds were selected on the basis of their potential antiviral activities and screened for their effective interaction with the SARS-CoV2 RBD spike protein using a computational approach.

2. Material and methods

2.1. Ligand preparation

We selected seventeen compounds from literature representing different ranges of SPs to screen their potential inhibitory action against SARS-CoV-2. The repeating units as a basic structure of each SPs were considered as ligands; and were prepared for molecular docking with AutoDock Tools [27]. Discovery studio 2020 was used to visualize the interactions between ligands and their target protein [28]. The 2D and 3D structures of ligands conjugated to protein are presented in supplementary I. All the parameters were set to default.

2.2. Preparation of protein

The structure of SARS-CoV-2 RBD was obtained from the Research Collaboratory for Structural Bioinformatics, the Protein Data Bank RCSB PDB (PDB ID: 6VW1) [29] with 2.1 Å as a resolution. Then, it was prepared for molecular docking using AutoDock Tools [27] by removing defects such as missing hydrogen atoms, water, charge states, incorrect bond order assignments, orientations of various groups, and missing side chains.

2.3. Molecular docking

AutoDock Vina [30] was used to predict binding affinities (kcal/mol) between RBD protein of SARS-CoV-2 and 17 ligands of SPs using a Lamarckian genetic algorithm [27]. The grid box size was set at 120 Å for x, y and z, and the grid centre was set to −24.98, 14.11, and 57.25 for
x, y and z respectively. The standard docking protocol was applied which is based on the population size of 150 randomly placed individuals, a maximum number of 250000 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Twenty-five independent docking runs were carried out for each inhibitor and cluster tolerance was kept at 1.0 Å.

### 2.4. ADMETox profiling

The term ADME/Tox refers to the absorption, distribution, metabolism, excretion, and toxicity of medicines. The in silico ADME/Tox profile is a valuable technique for predicting the pharmacological and toxicological characteristics of drug candidates, particularly during the pre-clinical stage. In silico models have been used to enhance ADME/Tox predictions. The application of these models has notably contributed to medication optimization and preventing late-stage failures, which is essential since such failures result in significant wasteful investment of time and money [31].

ADMETox properties were studies for the best-docked compound for further analysis and evaluation to give more insight about our most activated compound (Xylan sulphate).

#### 3. Results and discussion

##### 3.1. Molecular docking

Molecular docking results of the seventeen candidates are presented in Table 1. Heparin was used as a positive control for the screening of the interaction of all candidates with SARS-CoV-2 RBD protein, which the binding energy is −7.0 kcal/mol. The interactions between SARS-CoV-2 RBD protein and all candidates show various types of molecular contacts (Table 1). The completely docking results were included in the Supplementary Table 2. The results showed the potentialities of candidates, especially those with high binding affinities to SARS-CoV-2 RBD protein using several interactions and types of bonds. Candidates with a docking score between −8.3 and −7.0 kcal/mol are considered as interesting compounds that can be proposed as potential compounds to inhibit the SARS-CoV-2. Among them, two candidates, Xylan sulphate and Heparin tetrasaccharide N-sulphated showed the highest binding affinities, and demonstrated lower binding energies compared to the positive control, with −8.3, −7.3 kcal/mol, respectively (Table 1).

Ligands interact with sites on the protein surface, which several amino acids were involved in the binding

| No | Ligand name | Binding energies ($\Delta$G) Kcal/mol | Types of Interactions |
|----|-------------|--------------------------------------|----------------------|
| 1  | Sulphated Galactan (G) | −5.3 | 5 H-bonding with LYS 357, ARG 466, ARG 355 |
|    |             |         | 4 $\pi$ – Sulphur with, TYR 396, ARG 466, ARG 355 |
| 2  | Pyranosic arabinan (G) | −5.7 | 9 H-bonding with GLN 498, ASN 501, GLY 496, GLN 493, TYR 453 |
|    |             |         | 1 $\pi$ – Sulphur with, TYR 449 |
| 3  | Rhamnan sulphate (G) | −5.2 | 3 H-bonding with MET 430, PHE 515, |
|    |             |         | 1 Carbon Hydrogen bond with ASP 428 |
| 4  | Ulvanobiose (G) | −5.3 | 5 H-bonding with SER 349, TYR 351, ASN 450, LYS 452 |
|    |             |         | 1 Carbon Hydrogen bond with PRO 348 |
| 5  | Sulphated fucoidan (B) | −6.9 | 8 H-bonding with PHE 347, SER 349, TYR 351, ASN 450, TYR 451, LYS 452, ARG 509 |
|    |             |         | 2 $\pi$ – Donor Hydrogen-bonds with LYS 346, PRO 348, TYR 451 |
| 6  | Sulphated homogeneous Galactan (B) | −5.9 | 4 H-bonding with PHE 347, ASN 450, ASN 448, SER 349 |
| 7  | Sulphated fucose (B) | −5.1 | 6 H-bonding with PHE 347, SER 349, ASN 448, ASN 450 |
|    |             |         | 1 $\pi$ – Sulphur with, TYR 351 |
| 8  | Sulphated Galactofucan (B) | −6.3 | 5 H-bonding with PHE 347, SER 349, LYS 452, ARG 509 |
|    |             |         | 1 $\pi$ – Sulphur with PHE 464 |
| 9  | Sulphated fucan (B) | −6.0 | 2 H-bonding with TYR 396, GLU 516 |
|    |             |         | 3 $\pi$ – Sulphur with TYR 351, LYS 452, ARG 509 |
|    |             |         | 1 Carbon Hydrogen bond with LYS 346 |
| 10 | Porphyrin (R) | −5.0 | 6H-bonding with ASN 437, ARG 439, ASN 440 |
|    |             |         | 1 Carbon Hydrogen bond with THR 372 |
| 11 | $\kappa$-carrageenan (R) | −6.5 | 2 H-bonding with ASN 450 |
|    |             |         | 2 Carbon Hydrogen bonds with PRO 348, ILE 441 |
| 12 | $\lambda$-carrageenan (R) | −5.8 | 8 H-bonding with PHE 373, ASN 437, ARG 439, GLN 506 |
|    |             |         | 1 Carbon Hydrogen bond with ASN 437 |
| 13 | $\eta$-carrageenan (R) | −6.3 | 3 H-bonding with TYR 449, ASN 450 |
|    |             |         | 1 Carbon Hydrogen bond with ILE 441 |
| 14 | Poligeenan (R) | −6.2 | 3 H-bonding with LYS 403, GLN 493 |
| 15 | Xylan sulphate (R) | −8.2 | 3 H-bonding with ASP 428, MET 340 |
|    |             |         | 1 $\pi$ – Sulphur with ARG 355, ARG 439, ASP 428, PHE 464, GLU 516 |
|    |             |         | 1 $\pi$ – $\pi$ shaped with PHE 464 |
| 16 | Heparin tetrasaccharide N-sulphated (R) | −7.3 | 5 H-bonding with ARG 355, LYS 356, LYS 462 |
|    |             |         | 1 $\pi$ – Alkyl bond with LYS 356 |
| 17 | Heparin(R) | −7.0 | 10 H-bonding with PHE 342, ASN 343, ALA 344, TYR 345, PHE 374, TRP 436, ARG 439, ASN 440, GLN 506, ARG 509 |
|    |             |         | 2 $\pi$ – Sulphur with PHE 373, TRP 436 |
|    |             |         | 2 Carbon Hydrogen bonds with, ASN 343, ASN 437 |

(G): green; (B): Brown; (R): Red seaweeds.
process. Globally, five kinds of bonds belonging to the
broad category of non-covalent interactions were domi-
nated in interactions (Hydrogen, Carbon–Hydrogen,
\(\pi\)-Sulphure, \(\pi \rightarrow \pi\)-shaped, \(\pi \rightarrow\) Alkyl bonds). It should be
noted that the sulphate groups of ligands were involved
in the majority of bonds, not only via the \(\pi\)-Sulphure
bonds, but also in the other bonds, in particular by
involving the oxygen atoms to binding with amino
acid residues. Lodish et al. [32] reported that although
certain bonds are weak, the coexistence of multiple
noncovalent bonds produces highly stable and specific
associations between different macromolecules.

3.2. Entry inhibitor action

The number and diversity of bonds, involving the sul-
phate group of ligand and the amino acid residues
may be one of the binding efficiency and stability of
the ligand–protein complex. Indeed, the interaction
results of Xylan sulphate and SARS-CoV-2 RBD pro-
tein (Supplementary Table 2) show Hydrogen Bond
with ASP 428, and MET 430, \(\pi \rightarrow \pi\) T-shaped with
PHE 464, \(\pi \rightarrow\) Sulphure with ARG 355, ASP 428, PHE
464, GLU 516 residues. Meanwhile, Heparin tetrasac-
charide, N-sulphated interacts with ARG 355, LYS 356,
LYS 462 residues, forming hydrogen bonds, and \(\pi \rightarrow\)
Alkyl bond with LYS 356 residue (Supplementary Table
2). Notably, it is observed that unlike the bonds recog-
nized in all candidates, Xylan and Heparin tetrasaccha-
ride N-sulphated, in their interactions with SARS-CoV-
2 RBD protein, specifically develop two new types of
bonds (\(\pi \rightarrow \pi\) T-shaped, \(\pi \rightarrow\) Alkyl). These last, although
also qualified as non-covalent, they involve the aro-
matic cycle of amino acids; hence probably their high
affinities in binding to the protein; in addition to the
hydrophilic interaction of the two ligands with the
hydrophilic sites of the active region of the RBD pro-
tein that favoured by the presence of sulphonic groups
(Figure 1).

Apart from these two candidates, none of the
screened candidates were more active than heparin,
an anticoagulant used as the positive control [33].
Indeed, in vitro studies, heparin was recently reported
to exhibit antiviral activity, and was used as antiviral
drugs [26,33]. Other studies report that soluble unfrac-
tionated heparin and its derivatives inhibit host cell
entry of SARS-CoV2 in vitro [34,35].

Nebulized heparin is now being studied [36], and a
separate clinical investigation shows a relation between
heparin and decreased mortality in COVID-19 patients
[37]. Heparin could be a promising option for repur-
posing as a prophylacticCOVID-19 therapy with more
randomized controlled studies in various therapeutic
regimes [37]. However, Xylan sulphate who demon-
strated the more affinity than Heparins to bind SARS-
CoV-2 RBD protein, has not been involved in any study
to demonstrate anti SARS-CoV-2 potential. This result
appears very important in the search for a new drug
combating SARS-CoV-2.

Recently, in vitro studies using the SP fucoidans
from brown algae and iota-carrageenan from red
algae were demonstrated significant antiviral activities
against SARS-CoV-2 [23]. At the cellular mechanism, the
efficiencies of SPs may be due to their interference with
the entry process at the initial attachment by blocking the positive charge of the pathogen surface receptors, to prevent them from binding to the surface of the host cell [24,25]. Despite their antiviral activities, the implication of SPs against SARS-CoV-2 remains very limited [23].

From the obtained results, and in terms of seaweeds origin, we can deduce that the SPs from red algae are more effective, than brown and green ones, with a range of energy, (−8.2 to −5), (−6.9 to −5.1), (−5.7 to −5.3) kcal/mol, respectively.

### 3.3. Potential use of SPs as vaccine adjuvants

Based on our current study, we can hypothesize that small molecules may be formulated as derivatives from SPs. They can be designed and tested to be efficient as entry inhibitors and vaccine adjuvants. Individual sites of the sulphated chains of SPs can act as mimetic natural ligands and interact with the protein [38].

SPs alone or after their attachment to the antigen substrate induce an immune reaction [39]. Several experimental studies demonstrate that SPs from seaweeds have potential vaccine adjuvant properties, with low toxicity, high biocompatibility, safety, as well as their mechanisms of immunomodulatory activity [40,41]. Various observations were reported for a variety of SPs. Fucoidan from brown algae was shown to have stimulatory effects on macrophages, dendritic cells, and other types of immune cells [24,25]. The sulphated galactans from green algae increase the production of inflammatory mediators by macrophages, which enhance both adaptive and innate immunity [40,42]. Carrageenans from red seaweeds stimulate macrophages, lymphocytes, phagocytosis, natural killer cells, antibody-dependent cell cytotoxicity; and the production of inflammatory cytokines [41,43,44].

### 3.4. ADMET and drug-likeness prediction

Water solubility, lipophilicity, and the percentage of intestinal human absorption (HIA) characteristics were used to predict absorption. SwissADME’s Silicos IT LogSw descriptor was used to forecast water solubility. Our compound’s LogSw values were anticipated to be 7.58. Compounds having scores less than (more negative than) 6 on the SwissADME LogSw scale are deemed weakly soluble.

Lipophilicity was determined by calculating the logarithm of the n-octanol/water partition coefficient, which was predicted using SwissADME’s Consensus LogPo/w descriptor. LogPo/w is intimately associated with transport mechanisms such as membrane permeability and distribution to various tissues and organs [45]. A moderate logP (0 log P 3) is a typical guideline for excellent oral bioavailability (good permeability and solubility) [46]. The expected value of logPo/w for our compound 15 (Xylan sulphate) is 2.14.

The LogSw and logPo/w predictions revealed a link between solubility and lipophilicity. The proportion of compound 15 that would be absorbed via the human intestine (% HIA) was estimated to be 19.66 percent using pkCSM-pharmacokinetics.

The glycoprotein P (P-gp) substrate, blood–brain barrier (BBB) permeability, and percentage-unbound descriptors were used to predict distribution. pkCSM-pharmacokinetics was used to predict descriptors. Pgp is an ATP-dependent drug-extracting pump identified in a variety of human tissues. The chemical 15 was anticipated to be P-gp substrates [47]. Our compound’s BBB permeability was anticipated to be −5.193.

SwissADME was used to estimate metabolism by inhibiting the major cytochromes (CYP) of the P450 superfamily, notably CYP2C19, CYP1A2, CYP2C9, CYP3A4, and CYP2D6. Inhibiting CYP enzymes, a key mechanism for metabolism-based drug–drug interactions, often entails competing with another drug for the same enzyme binding site [48]. Compound 15 was expected to inhibit CYP1A2 but not CYP2C19, CYP2C9, CYP2D6, or CYP3A4.

Excretion occurs largely as a result of a mix of hepatic and renal clearance, is related to bioavailability, and is critical in calculating dosage rates to reach steady-state concentrations [49]. Using the total clearance (CLtot) descriptor of pkCSM-pharmacokinetics, excretion values were expected to be −0.109 ml/min/kg.

The compound 15’s toxicity levels were anticipated utilizing pkCSM-pharmacokinetics to predict hepatotoxicity and oral rat acute toxicity LD50 values [46]. 2.489 mol/kg is the predicted LD50 value.

### 4. Conclusion

This study underlines the great potential of SP ligands as entry inhibitors and vaccine adjuvants that may offer promising use as potential agents in treating COVID-19. The molecular docking results validated the two SP ligands (Xylan sulphate and Heparin tetrasaccharide N-sulphated) that have a higher capability to interact with the target SARS-CoV-2 RBD protein. However, Xylene sulphate was predicted to be a potential spike protein of SARS-COV-2 inhibitor using ADMETox evaluation. The Admetox of our inhibitor is good, and it deserves to be studied in the laboratory. Hence, they will be effective as promising entry inhibitors that neutralize the attachment site; and therefore inhibit binding at a very early stage of the virus cycle.

In addition to this capacity, their use as vaccine adjuvants can be a real alternative in the research of the effectiveness of vaccines. From the obtained prediction results, SP ligands can be formulated, as derivatives, and tested to obtain good and safe drugs and/or vaccine adjuvants. Future researches, in vitro, will be
necessary to validate the antiviral potentialities of SP ligands against SARS-CoV-2.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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