Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

*Review*
**Volume 13, Issue 2, 2024, 72**
https://doi.org/10.33263/LIANBS132.072

# Recent Developments in the Antiviral Activity of Glycyrrhizic Acid and its Derivatives Against SARS-CoV-2: Computational Aspects

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**Received: 15.03.2023; Accepted: 2.07.2023; Published: 30.06.2024**

**Abstract:** Natural product-based drugs have been extensively used to treat several diseases over the past few decades. Evidence over the past decades has demonstrated that glycyrrhizic acid, the major active constituent of licorice root, and its derivatives are potent candidates for the inhibition of SARS-CoV-2. In this review article, we have highlighted the inhibitory action of glycyrrhizic acid and its derivatives toward SARS-CoV-2 by computational studies. A comparison was made by comparing the binding affinities to the viral targets of different glycyrrhizic acid derivatives under consideration, as well as the documented molecular locations of interaction with the SARS-CoV-2 proteins. It was concluded that glycyrrhizic acid and its derivatives displayed promising inhibition against the target proteins of SARS-CoV-2. The current study also offers molecular information that serves as the foundation for future proposals to alter the structural properties of specific chemicals to improve their ability to interact with proteins.

**Keywords:** Licorice root; Glycyrrhizin; Binding energy; Docking; Natural products; Inhibition mechanisms



## 1. Introduction

Medicinal plants have gained much attention during the coronavirus pandemic due to the effectiveness of extracted components against numerous viral diseases. Licorice is also reported to be one of the medicinal plants largely used worldwide for its therapeutic properties. So far, several research groups have reported a wide range of pharmacological activities of licorice root and the isolated components such as antiviral, anti-cancer, antioxidant, anti-inflammatory, anti-diabetic, anti-parasitic, anti-bacterial, anti-angiogenic, anti-obesity, anti-arthritic, neuroprotective, anti-osteogenic, and many others [1-12].

The licorice root has been reported to inhibit several viruses over the past decades. Glycyrrhizin, also known as glycyrrhizinic acid or glycyrrhizic acid, a triterpene glycoside, as shown in Figure 1, is the major active constituent of licorice root (*Glycyrrhiza glabra* Linn.). Evidence over the past decades has demonstrated that glycyrrhizic acid is mainly responsible for the vast range of biological activities of licorice root, including antiviral activity against the severe acute respiratory syndrome (SARS) virus [13-15].

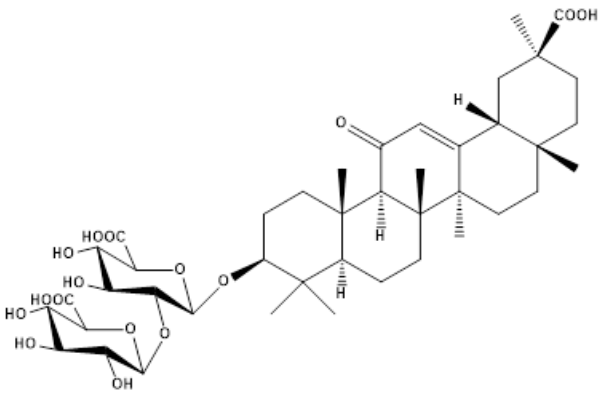



**Figure 1.** Structure of Glycyrrhizic acid (CID_14982).

Several research groups investigated the antiviral activity of compounds formed by modifying the glycyrrhizic acid structure and found that the resulting compounds blocked SARS-CoV replication at even lower concentrations than the parent molecule [16]. Computational chemists focus on antiviral drugs that target specific regions of the virus life cycle to reduce virus replication. Thus, we have reviewed the antiviral activity of various derivatives of glycyrrhizic acid, reported in the recent past, along with their inhibition mechanisms against SARS-CoV-2 infection based on computational techniques.

Glycyrrhizic acid and its derivatives have also exhibited antiviral activity against many other viruses like herpes, hepatitis, influenza, and human immunodeficiency virus (HIV). In addition, antiviral activity against some animal viruses like infectious bronchitis virus (IBV), duck hepatitis virus (DHV), infectious bursal disease virus (IBDV), porcine epidemic diarrhea virus (PEDV), porcine reproductive and respiratory syndrome virus (PRRSV) has also been reported in the past by glycyrrhizic acid and its derivatives [17-18].

Next, glycyrrhetic acid, also known as glycyrrhetinic acid or enoxolone, a pentacyclic triterpene, is formed by the hydrolysis of glycyrrhizic acid. It has been reported to show inhibition against the cytopathic effect of various RNA and DNA viruses, for example, Herpes Simplex virus-1 (HSV-1), vaccinia, vesicular stomatitis virus (VSV), and Newcastle disease [19]. Carino and the group reported that glycyrrhetinic acid reduces the ACE2/RBD binding based on *in silico* and *in vitro* studies. It binds with the RBD pocket of the S protein of SARS-CoV-2 (Binding energy value = −8.6kcal $mol^{-1}$) through hydrophobic and polar interactions [20]. Binding energy indicates how strongly a ligand binds to a target. The greater the negative value of binding energy, the better the binding of the ligand to the receptor.

## 2. Antiviral activity against SARS-CoV-2

Chen and Du performed MD (Molecular Dynamics) simulations to predict the binding affinity of glycyrrhizic acid with the type I integral membrane protein, Angiotensin Receptor Enzyme-2 (ACE2), which has been reported to be a functional receptor of SARS-CoV-2. The ligand interacts with the active site residues Arg559, Gln388, Arg393, and Asp30, as shown in Figure 2. They found that glycyrrhizic acid is a potential candidate to target ACE2 and inhibit the entry of SARS-CoV-2 into the cell [21-22].

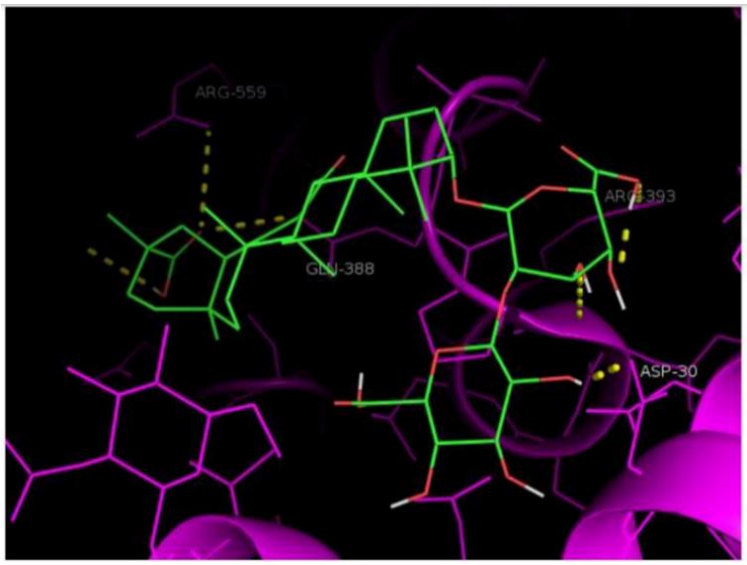



**Figure 2.** Binding sites for glycyrrhizic acid and ACE2 enzyme interactions (Binding energy = −9 kcal $mol^{-1}$). Adapted from [21]. Copyright © 2020 The Authors.

Sinha and co-workers [23-24] performed molecular docking studies on SARS-CoV-2 main protease (PDB ID: 6LU7), spike glycoprotein (PDB ID: 6VSB) and Nsp15 endoribonuclease (PDB ID: 6W01), with the help of Autodock vina software, and the findings of the work demonstrated that glycyrrhizic acid exhibited the highest binding energy (-9.2 kcal $mol^{-1}$) with the active site of the spike glycoprotein compared to the other two proteins, as given in Table 1. Binding-interaction analysis showed different types of non-covalent interactions, such as hydrogen bonding, hydrophobic, and π-cation, with the enzyme's active site of the SARS-CoV-2, as tabulated in Table 1. In addition to these interactions, the active site of $M^{pro}$ was also stabilized through alkyl and π-alkyl bonds with Leu27, Cys145, and His41 [25]. The orientation adopted by the ligand and the interactions shown by the ligand inside the active site of main protease ($M^{pro}$), spike glycoprotein, and Nsp15 endoribonuclease of the SARS-CoV-2 are shown in Figure 3.

**Table 1.** The binding energy and interaction parameters of the ligand in the enzyme's active site of the SARS-CoV-2.

| S.No. | Enzyme | Binding energy (kcal $mol^{-1}$) | H-bonding interactions | Hydrophobic interactions | π-cation interactions | Charge center | Reference |
|---|---|---|---|---|---|---|---|
| **1.** | **Main protease** | -8.6 | Ser144, His41, Cys145, Glu166, Gly143 | Leu50, Glu166 | - | - | [22] |
| **2.** | **Spike glycoprotein** | -9.2 | His625, Arg319, Glu298, Val320, Gln321 | Gln321, Tyr343, Val320, Ile624, The323 | His625 | His625 | [23] |
| **3.** | **Nsp15 endoribonuclease** | -8.3 | His235, Cys291, Ser294, Thr341 | Tyr343 | His235, Lys290, His250 | His235, Lys290, His250, | [23] |

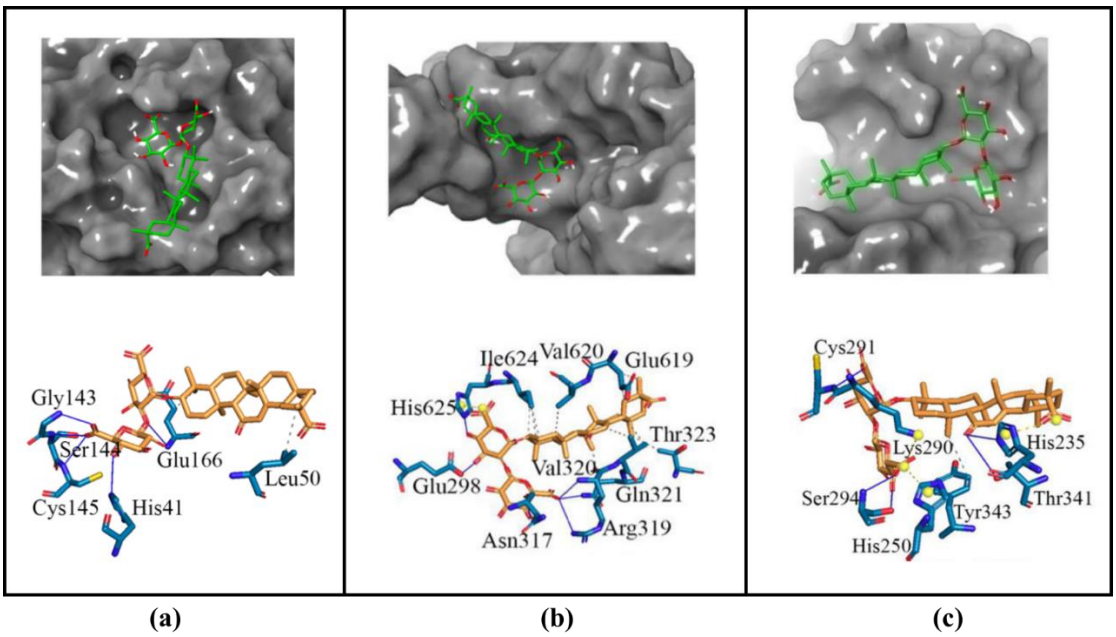



**Figure 3.** The binding mode (above) and the binding interactions (below) of glycyrrhizic acid in the active site of **(a)** main protease ($M^{pro}$) (PDB ID: 6LU7); **(b)** spike glycoprotein (PDB ID: 6VSB), **(c)** Nsp15 endoribonuclease (PDB ID: 6W01), of the SARS-CoV-2. Adapted from [24]. Copyright © 2021 The Authors.

The nanomaterials have also been reported to be effective in preventing and rapidly diagnosing COVID-19 [26-27]. Recently, Zhao and co-workers synthesized glycyrrhizic acid nanoparticles hydrothermally and investigated their antiviral activity against SARS-CoV-2 *in vitro* and *in vivo*, as demonstrated in Figure 4. They evaluated the *in vivo* therapeutic effects of glycyrrhizic acid nanoparticles in lungs liver, and on LPS-induced hyperinflammation with no noticeable toxicity [28].

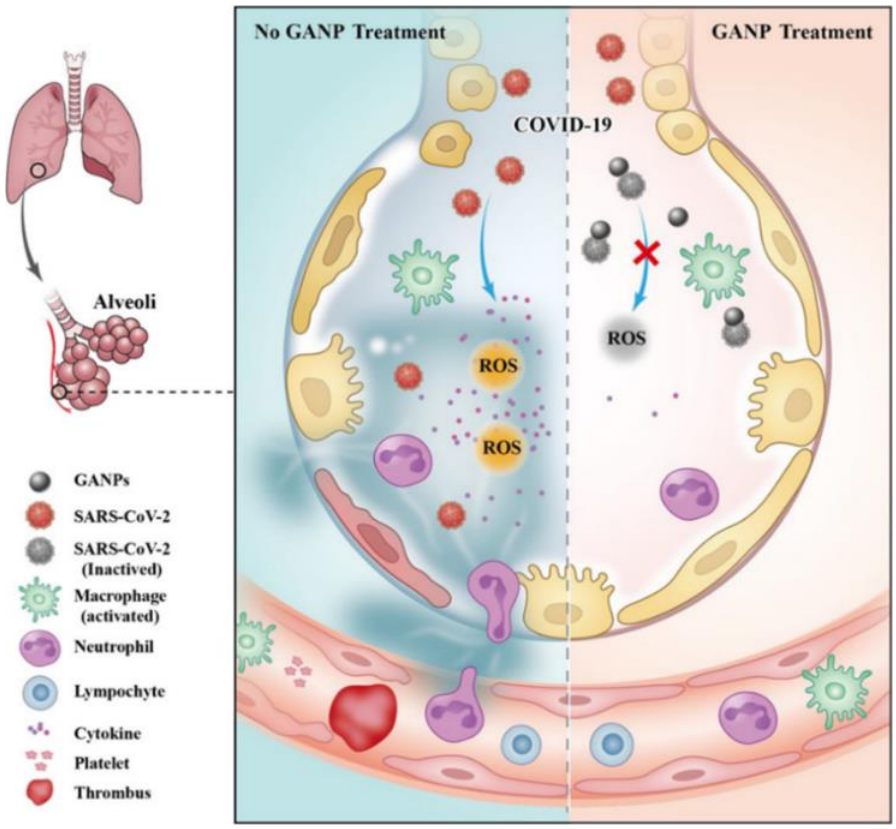
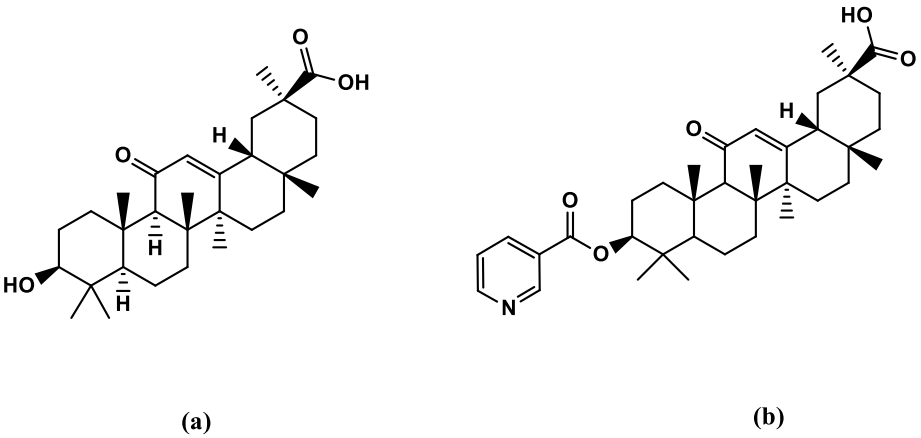



**Figure 4.** Schematic representation of glycyrrhizic acid nanoparticles effective against SARS-CoV-2 Adapted from [28]. Copyright © 2021 The Authors.

Afterward, Puttaswamy and the group performed *in silico* studies and reported that glycyrrhizic acid has seven rotatable bonds, sixteen hydrogen bond acceptors, and eight hydrogen bond donors. The glycyrrhizic acid formed a hydrogen bond with His296 of the target protein, i.e., human transmembrane serine protease 2 (TMPRSS2), with a binding energy value of -9.5 kcal $mol^{-1}$, whereas glycyrrhetic acid formed a hydrogen bond with Ser441 of TMPRSS2 with a binding energy value of -7.7kcal $mol^{-1}$. Based on scoring functions, molecular docking was carried out using PyRx'"" 's PyRx's AutoDock Vina feature. Further, Discovery Studio 3.5 and PyMOL103 were used to examine the interactions between the ligand and protein [29].




**(a)** **(b)**

**Figure 5.** The structure of **(a)** glycyrrhetic acid or glycyrrhetinic acid (CID_10114); **(b)** nicotinamide of glycyrrhetic acid [30-31].

Recently, Fomenko and the group synthesized glycyrrhizic acid nicotinates and investigated their antiviral activity against SARS-CoV-2 and HIV-1. The acylation of glycyrrhizic acid with nicotinic acid results in a mixture containing mono-, di-, tri-, and tetra nicotinates. Therefore, nicotinamide of glycyrrhetic acid, as shown in Figure 5 (b), was prepared to analyze the biological effects of the glycosidic fragment. The formed mixture was very effective against SARS-CoV-2 with minimal toxic effects [30].

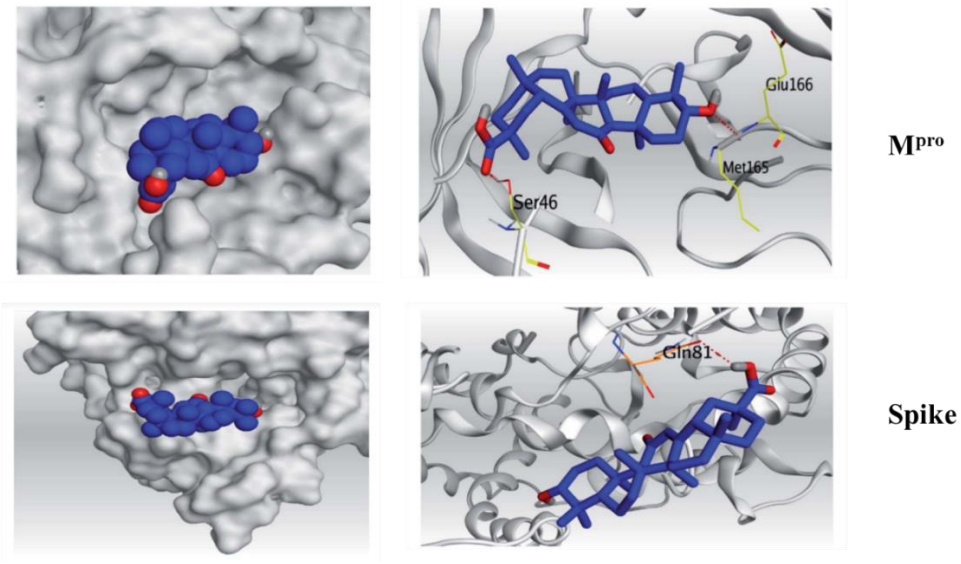



**Figure 6.** Positioning (left) and binding interactions (right) of glycyrrhetinic acid inside $M^{pro}$ and S pockets of SARS-CoV-2. Adapted from [32]. Copyright © 2021 The Authors.

Elebeedy and group [32] performed molecular docking studies to investigate the anti-SARS-CoV-2 activity of glycyrrhetinic acid against the main protease (PDB ID: 6LU7) and

the spike (PDB ID: 6VW1) receptors using MOE 2019 suite. Positioning and binding interactions of the molecule inside $M^{pro}$ and S pockets of SARS-CoV-2 are depicted in Figure 6.

Binding scores and RMSD (Root Mean Square Deviation) values are important measures used in molecular docking and molecular dynamics simulations to assess the quality of protein-ligand interactions. They provide valuable insights into the strength and stability of the protein and ligand interaction. A more negative binding score generally indicates a more favorable and stronger interaction between the protein and ligand, and a lower RMSD value indicates a closer similarity and better agreement between the initial and final structures, suggesting a stable and well-maintained interaction.

The glycyrrhetinic acid showed binding interactions with Gln81 in the spike pocket with a binding score of -6.90 and RMSD value of 2.14 and with Ser46 and Glu166 in $M^{pro}$ pocket of SARS-CoV-2 with a binding score of -6.77 and RMSD value of 1.59 [32].

After that, Zígolo and group [33] assessed the interaction of several plant-derived compounds with $M^{pro}$ (PDB ID: 6LU7) and S protein (PDB ID: 6VSB) through molecular docking studies using the Autodock 4.2 program. Visual Molecular Dynamics 1.9.1 (VMD) was used to visualize protein-ligand complexes. They reported the binding energy value of -19.22 kcal $mol^{-1}$ for glycyrrhizic acid with Spike protein and -8.03 kcal $mol^{-1}$ for glycyrrhetinic acid with $M^{pro}$ protein. The better binding affinity of glycyrrhizic acid is attributed to more interactions with S protein residues. It formed six hydrogen bonds, *viz.* four with A chain residues, i.e., Phe855 (two H-bonds), Asp979 and Val976, and two with C chain residues, i.e., Asp571 and Asp574. Further, it showed hydrophobic interactions with fourteen residues, i.e., Leu546, Thr547, Gly548, Thr549, Arg567, Asp568, Thr572, Thr573, Ile587, Thr588, Pro589, Thr49, Asn856, and Asn978. Next, glycyrrhetinic acid formed hydrogen bonds with four catalytic residues. i.e., Gln189, Asn142 (two H-bonds) and Thr26, and one hydrophobic interaction with Leu4 residue, as shown in Figure 7.

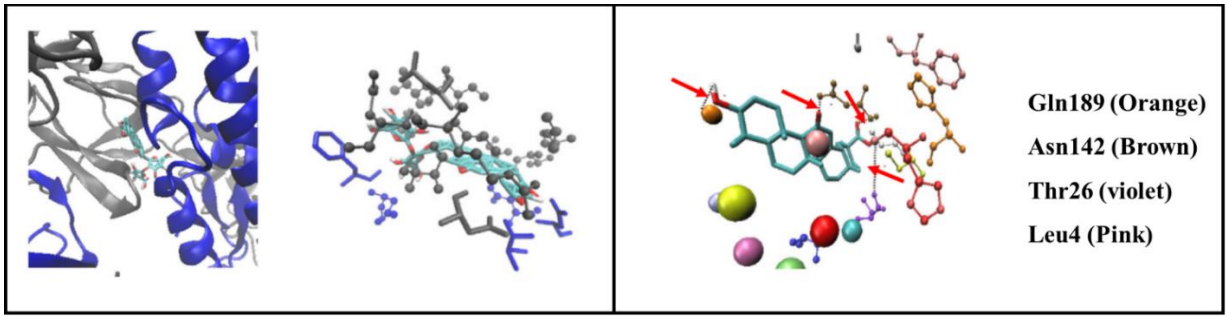



**Figure 7.** The binding interactions of **(a)** glycyrrhizic acid with S protein; **(b)** glycyrrhetinic acid with $M^{pro}$ protein. Adapted from [33]. Copyright © 2021 The Authors under CC BY NC ND 4.0 License.

Yi and the group [34] reported that triterpenoids isolated from licorice could be potential candidates for developing antiviral drugs against SARS-CoV-2. They screened 125 licorice components employing ligand-based virtual screening by targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. They performed density functional theory (DFT) calculations to optimize the molecules under study at B3LYP/6-311G(d) level in the gas phase using Gaussian 09W software, and MD simulations were performed with the help of AutoDock Vina and AutoDockTools v1.5.6 software. The triterpenoids exhibited higher binding affinity in the target pocket to inhibit the infection. Among all the considered

triterpenoids, four compounds, *viz.* glycyrrhizic acid, glycyrrhetinic acid, 3-O-β-D-glucuronosyl-glycyrrhetinic acid, and licorice-saponin A3, showed higher affinity towards RBD. The structures of 3-O-β-D-glucuronosyl-glycyrrhetinic acid and licorice-saponin A3 are given in Figure 8. The binding interactions of these four compounds with RBD are illustrated in Figure 9. Moreover, the binding modes of all four compounds with surrounding residues of RBD and ACE2 are displayed in Figure 10.

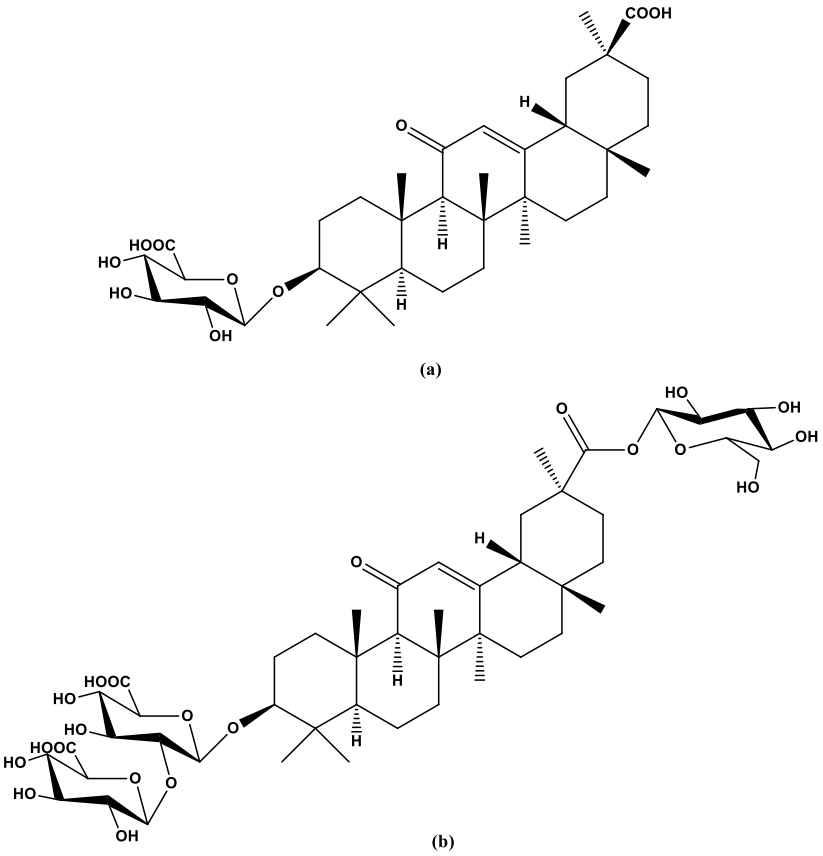



**Figure 8.** Structure of **(a)** 3-O-β-D-glucuronosyl-glycyrrhetinic acid; **(b)** licorice-saponin A3

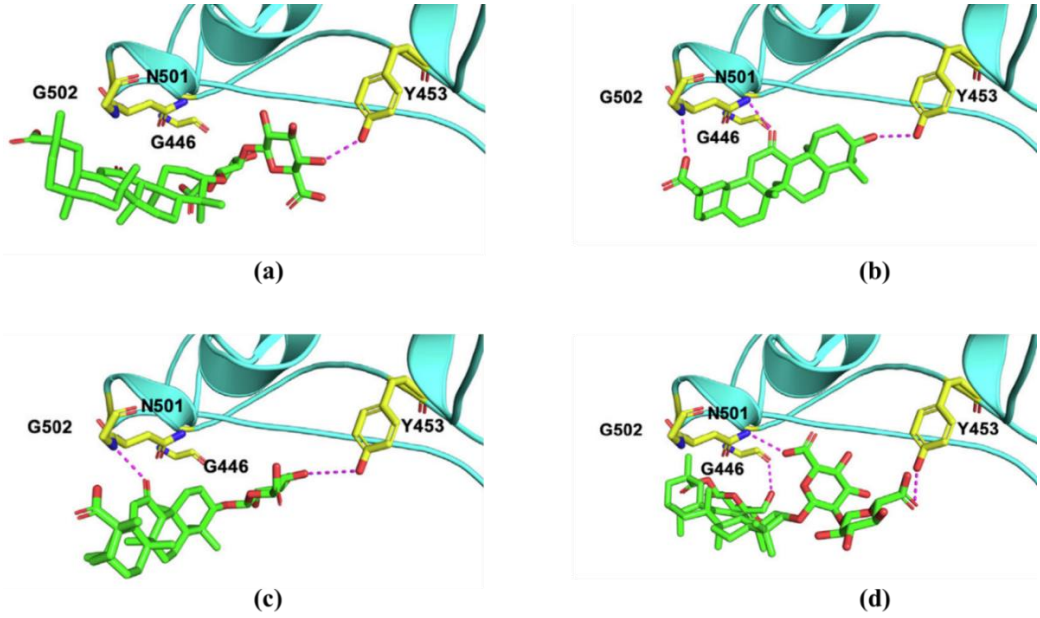



**Figure 9.** The binding interactions of **(a)** glycyrrhizic acid; **(b)** glycyrrhetinic acid; **(c)** 3-O-β-D-glucuronosyl-glycyrrhetinic acid; **(d)** licorice-saponin A3, with RBD (PDB ID: 6M0J). Magenta dashes represent hydrogen bonds. Adapted from [34]. 

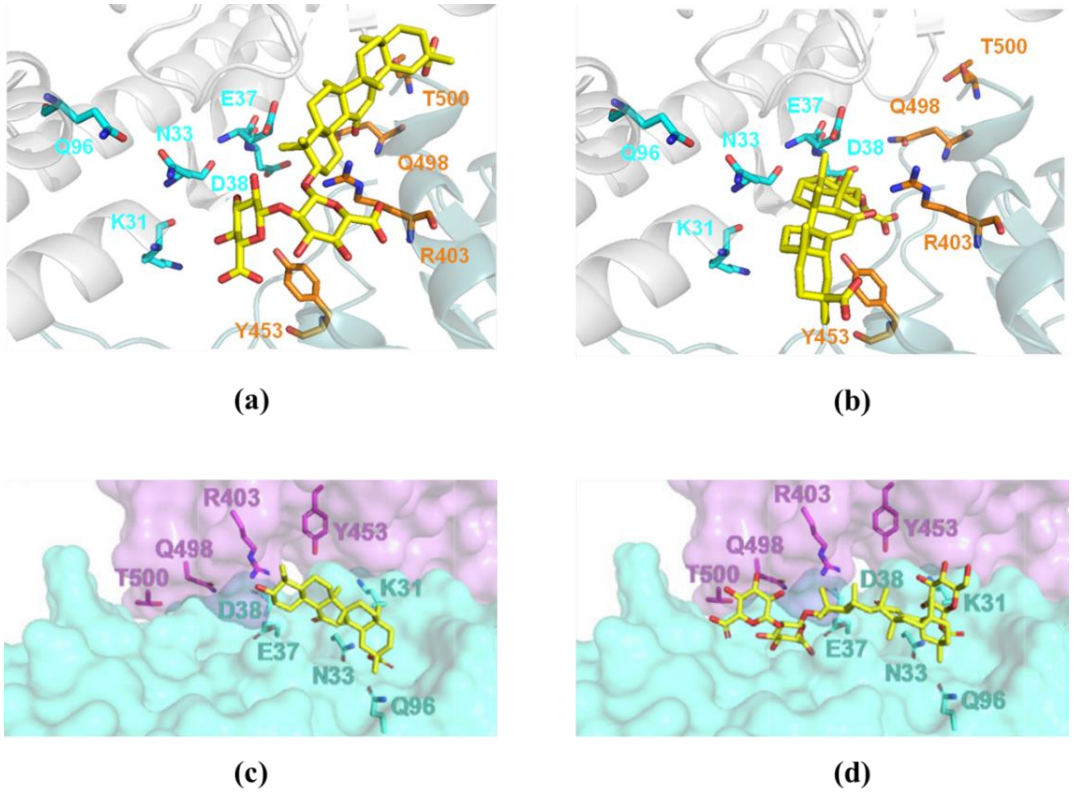



**Figure 10.** The binding modes of **(a)** glycyrrhizic acid; **(b)** 3-O-β-D-glucuronosyl-glycyrrhetinic acid; **(c)** glycyrrhetinic acid; **(d)** licorice-saponin A3, showing surrounding residues of RBD and ACE2 Adapted from [34]. 

The findings of the work showed that glycyrrhetinic acid targets spike protein RBD of SARS-CoV-2, whereas licorice-saponin A3 binds to the Nsp7 protein of SARS-CoV-2. Although both molecules belong to the same class of triterpenoids, they target different proteins for inhibition [34].

Mahdian and co-workers [35] performed virtual screening for some selected drugs with ACE2, $3CL^{pro}$, $PL^{pro}$, HR1, and transmembrane protease serine 2 (TMPRSS2) protein targets of SARS-CoV-2, generated using homology modeling in the SWISS-MODEL workspace. The authors reported that glycyrrhizic acid is one of the drugs having a better binding affinity with the ACE2, RBD, $PL^{pro,}$ and $3CL^{pro}$ protein targets, as tabulated in Table 2, and thus could be considered as a therapeutic option for the SARS-CoV-2 infection. A more negative docking score (indicating a more favorable interaction) would correlate with higher binding affinity. Further, the glycyrrhizic acid shows better binding affinity toward $PL^{pro}$, as reflected by its more negative docking score compared to other target proteins.

**Table 2.** The docking score and hydrogen bonding interactions of the ligand in the enzyme's active site of the SARS-CoV-2.

| S.No. | Enzyme | Docking score | H-bonding interactions |
|---|---|---|---|
| **1.** | **RBD** | -8.6 | Glu411, Arg454, Arg457, Asp467, Ser469, Cys480, Gly482, Ile488 |
| **2.** | **$PL^{pro}$** | -10.7 | Glu487, Asp924, Ser925, Thr1456, Arg1546, Ser1560 |
| **3.** | **$3CL^{pro}$** | -8.8 | Asn119, Asn142, Ser144, His163, His164, Glu166, Gln189 |
| **4.** | **ACE2** | -8.8*<br>-7.8# | Ser43, Ser47, Glu87, Gln101, Asn194, Thr196, Glu208, Ala348, Asp382, Gly395, Asn397, His401, Glu402 |

*Complex of glycyrrhizic acid with ACE2 (residues near Lys31 and Tyr41)
# Complex of glycyrrhizic acid with ACE2 (residues near Tyr82-84)

The hydrogen bonding interactions of the compound in the enzyme's active site of the SARS-CoV-2 are also given in Table 2. The glycyrrhizic acid was observed to form more hydrogen bonds with ACE2.

Yu and the group [36] considered five hundred ingredients extracted from eighteen herbs and performed molecular docking studies using MOE software. They identified glycyrrhizic acid as one of the potential candidates to disrupt the interaction between the ACE2 protein and RBD of the SARS-CoV-2. The carboxylic group present in the molecule formed hydrogen bonds with Asp405 and Arg408. Further, the carbonyl group showed a hydrogen bonding interaction with Arg403. In addition, the glycosyl also formed a hydrogen bond with Tyr453, as depicted in Figure 11.

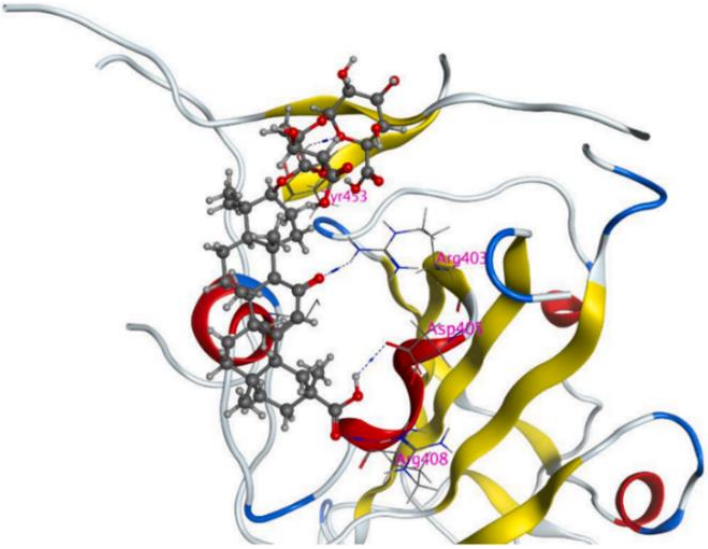



**Figure 11.** The binding interactions of glycyrrhizic acid in the active site of RBD of SARS-CoV-2. Adapted from [36]. Copyright © 2022 The Authors.

Maddah and group [37] proposed some compounds isolated from licorice root which showed high binding affinity towards SARS-CoV-2 targets *viz*. main protease ($M^{pro}$), RNA-dependent RNA polymerase (RdRp), spike receptor-binding domain (RBD), human angiotensin-converting enzyme 2 (hACE2), papain-like protease ($PL^{pro}$), endoribonuclease non-structural protein (Nsp15), using computational tools. Among the considered compounds, the glycyrrhizic acid and licorice saponin C2, the structure shown in Figure 12, exhibited high affinity to all the targets and seems to have a dominant inhibitory effect against SARS-CoV-2. The different interactions of both the compounds, i.e., glycyrrhizic acid and licorice saponin C2, with the residues of each target binding pocket, are illustrated in Figure 13 and Figure 14, respectively.

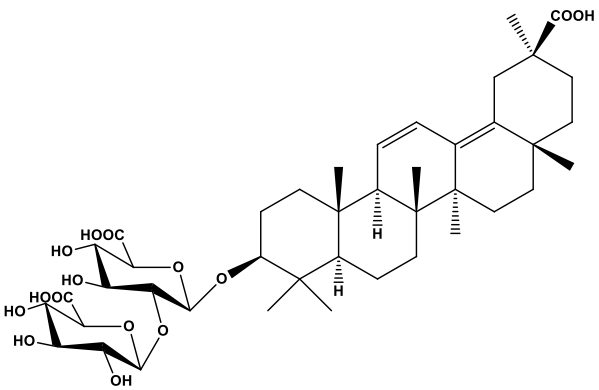



**Figure 12.** Structure of licorice saponin C2.

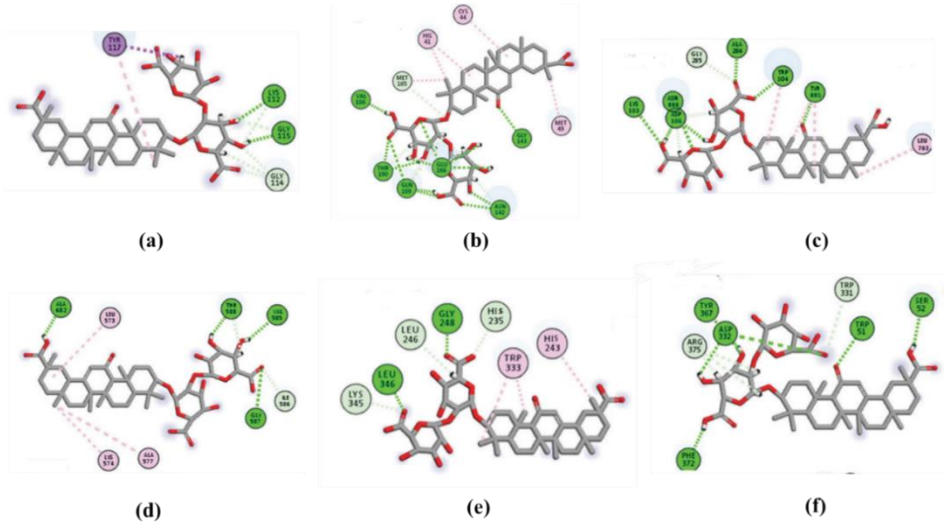



**Figure 13.** The binding interactions of glycyrrhizic acid in the active site of **(a)** RBD (PDB ID: 6LZG); **(b)** $M^{pro}$ (PDB ID: 6Y2E); **(c)** $PL^{pro}$ (PDB ID: 6W9C); **(d)** RdRp (PDB ID: 7BTF); **(e)** Nsp15 (PDB ID: 6VWW); **(f)** hACE2 (PDB ID: 6LZG), of the SARS-CoV-2 (Inspired from [37]).

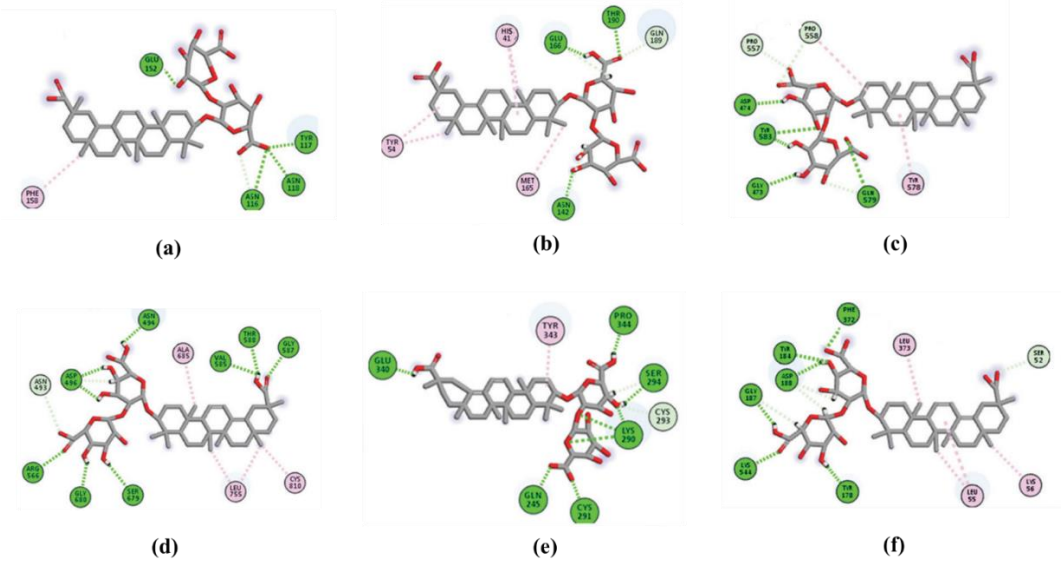



**Figure 14.** The binding interactions of licorice saponin C2 (CID_452864) in the active site of **(a)** RBD; **(b)** $M^{pro}$; **(c)** $PL^{pro}$; **(d)** RdRp; **(e)** Nsp15; **(f)** hACE2, of the SARS-CoV-2 (Inspired from [37]).

The detailed binding interactions of both the ligands in the enzyme's active site and docking energy values calculated through the MM/GBSA approach for all the protein targets are tabulated in Table 3. The molecules considered represent the most negative binding energy value for the Mpro target; thus, they are considered to be the significant target binding pocket for SARS-CoV-2 inhibition.

**Table 3.** The binding energy and different interactions of the ligand in the enzyme's active site of the SARS-CoV-2.

| S.No. | Enzyme | Docking energy (kcal $mol^{-1}$) | | H-bonding interactions | | Hydrophobic interactions | |
|---|---|---|---|---|---|---|---|
| | | Glycyrrhizic acid | Licorice saponin C2 | Glycyrrhizic acid | Licorice saponin C2 | Glycyrrhizic acid | Licorice saponin C2 |
| 1. | RBD | -10.19 | -9.75 | Tyr117, Asn169, Gly170 | Lys112, Gly115, Asn116, Tyr117, Phe158 | Lys85, Tyr121, Tyr163 | Tyr117, Leu120, Tyr157, Phe158 |

| S.No. | Enzyme | Docking energy (kcal mol$^{-1}$) | | H-bonding interactions | | Hydrophobic interactions | |
|---|---|---|---|---|---|---|---|
| | | Glycyrrhizic acid | Licorice saponin C2 | Glycyrrhizic acid | Licorice saponin C2 | Glycyrrhizic acid | Licorice saponin C2 |
| **2.** | **M$^{pro}$** | -13.12 | -12.00 | Glu166, Thr190, Gln192, Thr24, | Asn142, Glu166, Thr190, Gln192 | Thr24, Thr25, Met49, Gln189 | Thr24, Thr25, Met49, Gln189 |
| **3.** | **PL$^{pro}$** | -11.81 | -11.41 | His235, Cys291, Ser294, Thr341 | Gly478, Asp479, Arg481, Tyr583, Thr611 | Leu783, Asp785, Tyr885, Asn888, Trp104 | Pro557, Pro558, Tyr574, Asn577, Pro609 |
| **4.** | **RdRp** | -11.58 | -11.07 | Cys810, Ser811, Lys497, Arg566, Gly587, Thr588 | Asn493, Lys497, Arg563, Ser679, Ala685, Gln570 | Ile586, Ala682, Tyr686 | Lys590, Leu755 |
| **5.** | **Nsp15** | -9.87 | -9.24 | Thr341 | Cys291, Val292, Ser294, Pro344, Leu346 | Asp240, Cys291, Val292, Ser294 | Glu340, Thr341, Tyr343 |
| **6.** | **hACE2** | -11.59 | -11.43 | Leu373, Phe22, Trp51, Leu55 | Asp12, Asp20, Glu19 | Asp332, Phe372, Asn376 | – |

Next, the MM/GBSA (Molecular mechanics with generalized Born and surface area solvation) was utilized to estimate the energy corresponding to different interactions in the binding pocket, like van der Waals ($\Delta E_{vdW}$) and electrostatic ($\Delta E_{ele}$) interactions. Further, solvation energies ($\Delta G_{sol}$) were also reported as the total of polar and non-polar solvation energies. In addition, $\Delta G_{bind}$ represents the sum of all three energies. The summary of energy contributions for the glycyrrhizic acid and licorice saponin C2 (in parentheses) with each target is given in Table 4. The high negative free energy values for the interaction of both the molecules with enzyme M$^{pro}$ indicate favorable binding with this target.

**Table 4.** The summary of energy contributions for the glycyrrhizic acid and licorice saponin C2 (in parentheses) with each target. The standard deviation is reported as ±sd with ΔGbind values.

| S.No. | Enzyme | $\Delta E_{vdW}$ | $\Delta E_{ele}$ | $\Delta G_{sol}$ | $\Delta G_{bind}$ |
|---|---|---|---|---|---|
| **1.** | **RBD** | -30.11 (-28.16) | -16.97 (-11.50) | 26.27 (21.097) | -20.81±1.23 (-18.56±0.95) |
| **2.** | **M$^{pro}$** | -72.41 (-56.20) | -58.87 (-55.25) | 63.30 (63.12) | -67.98±4.10 (-48.33±3.05) |
| **3.** | **PL$^{pro}$** | -62.34 (-55.18) | -40.22 (-40.73) | 69.99 (72.15) | -32.57±2.08 (-23.75±1.65) |
| **4.** | **RdRp** | -48.16 (-35.56) | -40.93 (-38.78) | 53.98 (40.78) | -35.11±2.44 (-33.56±2.53) |
| **5.** | **Nsp15** | -40.51 (-40.67) | -66.95 (-65.46) | 67.66 (69.88) | -39.79±3.11 (-36.47±4.05) |
| **6.** | **hACE2** | -60.32 (-54.58) | -69.50 (-68.58) | 70.43 (85.91) | -59.39±5.03 (-37.25±3.38) |

Rehman and group [38] investigated some natural compounds against protein targets of SARS-CoV-2 using molecular docking and MD simulations with YASARA structure ver. 20.7.4. Based on ligand-protein interactions, the authors reported that glycyrrhizic acid and its metabolite glycyrrhetinic acid fall in the list of phytochemical ligands which exhibited a higher binding affinity for the different targets, as given in Table 5. In addition, they studied the interactions of selected ligands with many non-specific human blood proteins. It was found that glycyrrhizic acid exhibited the highest binding energy of -11.36 kcal mol$^{-1}$ against DdB1

(damage-specific DNA binding protein), and the residues involved in the interactions are given in Table 5.

**Table 5.** The binding energy (in kcal $mol^{-1}$) and residues present in the binding sites of glycyrrhizic acid and glycyrrhetinic acid with different targets of SARS-CoV-2.

| S.No. | Enzyme | Binding energy (kcal $mol^{-1}$) | | Active site residues | |
|---|---|---|---|---|---|
| | | Glycyrrhizic acid | Glycyrrhetinic acid | Glycyrrhizic acid | Glycyrrhetinic acid |
| **1.** | Spike | −9.49<br><br>(−9.29)* | −8.08<br><br>(−7.99)* | **Arg1000**, Asn978, Leu977, Val976, Ser975, Ser967, Leu966, Lys964, Val963, Asn856, Phe855, **Asp745**, **Gly744**, Cys743, Ile742, **Tyr741**<br><br>(**Arg1000,** Asn978, Leu977, Val976, Ser975, Ser967, Leu966, Lys964, Val963, Asn856, Phe855, **Asp745**, **Gly744**, Cys743, Ile742, **Tyr741**, Met740, Lys304, His49, Val47)* | Gly283, Tyr279, Pro225, Glu224, Arg44, Phe43, **Val42**, **Lys41**, Asp40, Tyr38<br><br>(Pro1143, Gln1142, Asp1139, Tyr1138, Val1137, Thr1136, Asn1135, Ile1115, **Ile1114**, Phe1103, Trp1102, His1101)* |
| **2.** | $M^{pro}$ | -9.57<br><br>(-9.46)# | -9.19 | Gln189, Glu166, Met165, His164, His163, Cys145, **Gly143**, Asn142, **Asn119**, Tyr118, Met49, Ser46, Cys44, His41, Gly29, Leu27, **Thr26**, Thr25, Thr24<br><br>(**Asp289**, Leu287, Leu286, Leu272, Tyr239, Asn238, **Tyr237**, **Thr199**, Thr198, Asp197, Thr196, **Gly195**, **Ala194**, Val171, Thr169, Val137, Thr135, Asn133, Arg131)# | Glu290, **Asp289**, **Glu288**, **Leu287**, Leu286, Leu272, Tyr239, **Tyr237**, Thr199, Asp197, Lys137, Arg131, Lys5 |
| **3.** | RdRp | −10.52<br><br>(−9.96)# | – | **Ser814, Cys813**, Phe812, **Glu811**, Trp800, Cys799, **Lys798**, Ala762, Asp761, **Asp760**, **Ser759**, Arg624, Asp623, Cys622, Lys 621, Pro620, Tyr619, Asp618, **Trp617**, Thr556, Arg555, **Ala554**, Arg553, Lys551, Tyr455, **Asp452**,<br><br>(**Ser814**, Cys813, **Glu811**, Trp800, **Lys798**, Asp761, **Asp760**, **Ser759**, Arg624, Asp623, Cys622, Lys621, Asp618, **Trp617**, Arg555, **Ala554**, Arg553, Tyr455, **Asp452**)# | – |
| **4.** | Helicase (Nsp13) | -11.57 | -9.91 | **Arg560**, Asn559, Asn557, His554, **Asp534**, Val533, Thr532, Asn519, | Ser236, Leu235, Pro234, Glu136, Phe133, Leu132, Arg129, Phe24, Pro23, |

| S.No. | Enzyme | Binding energy (kcal mol$^{-1}$) | | Active site residues | |
|---|---|---|---|---|---|
| | | Glycyrrhizic acid | Glycyrrhetinic acid | Glycyrrhizic acid | Glycyrrhetinic acid |
| | | | | **Asn516**, Tyr515, Pro514, Ser486, **Ser485**, Phe422, Leu417, Thr416, Gly415, Thr413, Leu412, Arg409, Pro408, Ala407, Pro406, Leu405, **Lys202**, **Asn177**, Leu176, Pro175 | Arg22, Arg21, **Arg15**, Val6, Ala4 |
| **5.** | E-channel protein | −10.07 | -9.72 | Asn64, Arg61, Tyr57, Pro54, Leu51, Ser50, Val49, Val47, **Ile46**, Cys43, Tyr42, Leu39, Arg38, Leu37, Ala36,Leu34, Ile33, Ala32, Leu31, Thr30, Val29, **Leu28**, Leu27 | **Arg61**, Tyr57, Pro54, Leu51, Val47, Ile46, Thr35, Ala32, Leu31, Thr30, Val29, Leu28, Leu27 |
| **6.** | Ddb1 | -11.36 | – | Leu314, Cys313, Glu312, Arg263, Asn262, His261, Cys260, Val259, Ala221, Met218, Ser217, Tyr171, Phe169, Lys168, Asp166, Pro126, Asp125, Ile124, Ile123, Ile121, Thr118, Leu66, Glu65, Thr20, Val19, Cys18, Gly17, Asn16 | – |

*Docking to the closed state of the protein.
#The binding energy and active site residues at another binding site for the given target.
Active site residues shown in bold showed hydrogen bonding interactions.

Li and group [39] studied the antiviral effect of glycyrrhizic acid against SARS-CoV-2, *in vitro* and *in silico*, using an S protein-pseudotyped lentivirus (Lenti-S). Based on AutoDock Vina analysis, they reported that the molecule inhibits infection by blocking S protein binding to host cells. The autodocking analysis of the interaction of glycyrrhizic acid with the S and ACE2 proteins of the SARS-CoV-2 is displayed in Figure 15.

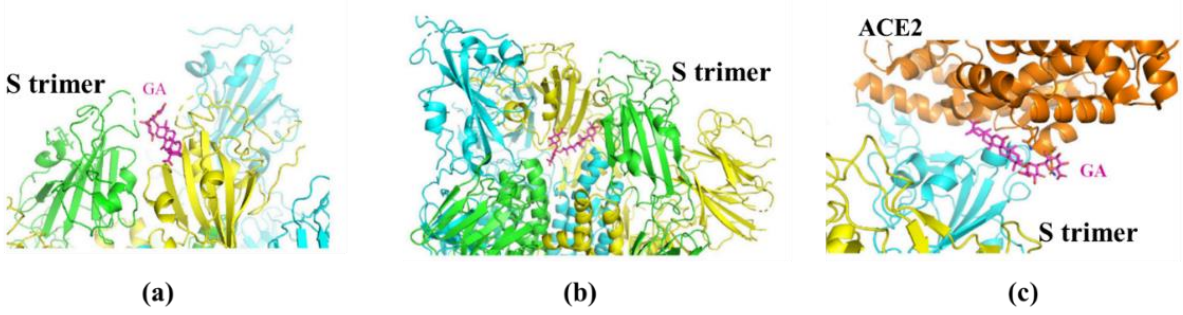



**Figure 15.** The S trimer represents three promoters of the S protein of SARS-CoV-2. (a) anticipated binding of glycyrrhizic acid on the S protein at the S–ACE2 interface (-8.0 kcal mol$^{-1}$) (b) anticipated binding of glycyrrhizic acid on a binding pocket present at the inner side of the RBD (-7.0 kcal mol$^{-1}$) (c) anticipated binding of glycyrrhizic acid on the ACE2 protein at the ACE2–S interface (-4.1 kcal mol$^{-1}$). The respective binding energies are shown in parentheses. Adapted from [39]. Copyright © 2021 The Authors.

Further, several research groups conducted mechanistic studies on the interaction of various antiviral compounds with SARS-CoV-2 mutant variants [40-41]. Recently, Vardhan & Sahoo (2022) studied the interactions of glycyrrhizic acid molecules with S protein RBD of different variants of COVID-19 *viz*. Omicron (B.1.1.529), Delta (B.1.617.2), and wild-type

(WT) (B.1.1.7) are at the binding interface of ACE2, as shown in Figure 16, and the binding energy and interacting residues are summarized in Table 6.

**Table 6.** The binding energy and interactions of glycyrrhizic acid with S protein RBD of Omicron, Delta, and WT variants at the binding interface of ACE2.

| S.No. | Variant | Binding energy (kcal $mol^{-1}$) | Interactions |
|---|---|---|---|
| **1.** | **Omicron** | -8.4 | Conventional H-bond: Ser496<br>Van der Waals: His505, Tyr495, Arg493, Tyr489, Cys488, Gly485, Ala484, Phe456, Leu455, Tyr453 |
| **2.** | **Delta** | -8.7 | Conventional H-bond: Arg452, Ser494, Gly496, Asn501<br>Van de Waals: Tyr505, Gln498, Phe497, Tyr495, Gln493, Phe490, Tyr449, Arg403 |
| **3.** | **WT** | -8.5 | Conventional H-bond: Leu492, Gln493, Ser494<br>Van der Waal: Tyr489, Glu484, Gly482, Asn481, Ile472, Thr470, Phe456, Leu455, Tyr449 |

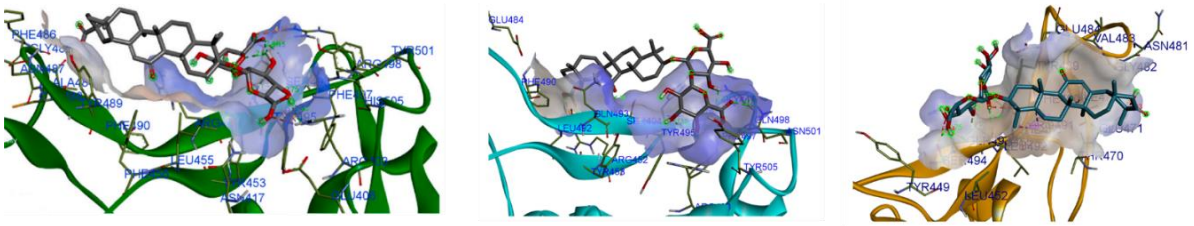



**Figure 16.** Glycyrrhizic acid pose with S protein RBD of (a) Omicron (b) Delta (c) WT variant. Adapted from [42]. Copyright © 2022 The Authors.

The authors reported that the title molecule binds effectively with the S protein RBD of all three variants of COVID-19 with almost equal binding affinity, as tabulated in Table 6. Conventional hydrogen bonds are being formed between the glycyrrhizic acid and the residues Ser494, Gln493, and Leu492 in the case of the WT variant of COVID-19. With a bond distance of 2.24 and 2.75Å, the oxygen ligand formed a close interaction with the Ser494 receptor. The van der Waals interactions with the residues Tyr489, Glu484, Gly482, Asn481, Ile472, Thr470, Phe456, Leu455, and Tyr449 were also observed on the ’” ’receptor’s surface.

Like WT, glycyrrhizic acid also interacts with the Delta RBD residues Asn501, Gly496, Ser494, and Arg452 through traditional H-bonds with bond distances of 2.87, 2.72, 2.83, 2.87, and 2.51Å, respectively, and charge attraction with Arg452. Additionally, the residues Gln498, Phe497, Gln493, Tyr495, Phe490, Tyr505, Arg403, and Tyr449 were creating a Van der Waals affinity with the glycyrrhizic acid.

Further, glycyrrhizic acid successfully binds to the receptor-binding site in Omicron SGp RBD, making four conventional H-bonds with Ser496 with bond distances of 2.44 and 2.15Å. Additionally, glycyrrhizic acid interacts with His505, Tyr495, Arg493, Tyr489, Cys488, Gly485, Ala484, Phe456, Leu455, and Tyr453 through Van der Waal interactions [42].

## 3. Conclusions and perspectives

Here, we have summarized the *in silico* computational docking and simulations performed with glycyrrhizic acid and its derivatives with different protein targets of SARS-

CoV-2 recently. Licorice 'root's glycyrrhizic acid was anticipated to be a key lead chemical in the fight against COVID-19. This review article helps to understand how glycyrrhizic acid and its derivatives inhibit SARS-CoV-2 infection based on different computational techniques, *viz*. all-atom molecular dynamics (MD) simulations, docking energy parameters, and different types of interactions of the ligand with the enzyme's active site. This will also assist in designing natural antiviral drugs effective against different variants of the SARS-CoV-2 in the future.

## Funding

No funding was received.

## Acknowledgments

The authors are thankful to Shyam Lal College, University of Delhi, Delhi; University of Lucknow, Lucknow and GLA University, Mathura, for the infrastructural support.

## Conflicts of Interest

The authors declare no conflict of interest.

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