Molecular modelling studies unveil potential binding sites on human serum albumin for selected experimental and *in silico* COVID-19 drug candidate molecules

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Article info

**Article history:**
Received 10 August 2021
Revised 11 September 2021
Accepted 13 September 2021
Available online 17 September 2021

**Abstract**

Human serum albumin (HSA) is the most prevalent protein in the blood plasma which binds an array of exogenous compounds. Drug binding to HSA is an important consideration when developing new therapeutic molecules, and it also aids in understanding the underlying mechanisms that govern their pharmacological effects. This study aims to investigate the molecular binding of coronavirus disease 2019 (COVID-19) therapeutic candidate molecules to HSA and to identify their putative binding sites. Binding energies and interacting residues were used to evaluate the molecular interaction. Four drug candidate molecules (*β*-D-N4-hydroxycytidine, Chloroquine, Disulfiram, and Carmofur) demonstrate weak binding to HSA, with binding energies ranging from $-5$ to $-6.7$ kcal/mol. Ivermectin, Hydroxychloroquine, Remdesivir, Arbidol, and other twenty drug molecules with binding energies ranging from $-6.9$ to $-9.5$ kcal/mol demonstrated moderate binding to HSA. The strong HSA binding drug candidates consist of fourteen molecules (Saquinavir, Ritonavir, Dihydroergotamine, Daclatasvir, Paritaprevir, etc.) with binding energies ranging from $-9.7$ to $-12.1$ kcal/mol. All these molecules bind to different HSA subdomains (IA, IB, IIA, IIB, IIIA, and IIIB) through molecular forces such as hydrogen bonds and hydrophobic interactions. Various pharmacokinetic properties (gastrointestinal absorption, blood-brain barrier permeation, P-glycoprotein substrate, and cytochrome P450 inhibitor) of each molecule were determined using SwissADME program. Further, the stability of the HSA-ligand complexes was analyzed through 100 ns molecular dynamics simulations considering various geometric properties. The findings of this study might be useful in understanding the mechanism of COVID-19 drug candidates binding to serum albumin protein, as well as their pharmacodynamics and pharmacokinetics.

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1. Introduction

In December 2019, Wuhan, Hubei Province, China, reported numerous cases of a new respiratory disease. By January 2020, it had been proven that these illnesses were caused by a new coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease came to be known as coronavirus disease 2019 (COVID-19) (Ashour et al., 2020). SARS-CoV-2 is a new betacoronavirus that shares 79% of its genome sequence with severe acute respiratory syndrome coronavirus (SARS-CoV) and...
50% similarity with Middle East respiratory syndrome coronavirus (MERS-CoV) (Lu et al., 2020). It has a genomic structure that is similar to that of other betacoronaviruses. The six functional open reading frames (ORFs) are replicase (ORF1a/ORF1b), spike (S), envelope (E), membrane (M), and nucleocapsid (N) in order from 5’ to 3’. Between the structural genes, there are additional seven potential ORFs encoding accessory proteins (Chan et al., 2020). The demand for new antiviral drugs against SARS-CoV-2 continues as the COVID-19 pandemic spreads quickly (Villamagna et al., 2020). There is currently insufficient evidence that any antiviral drugs available today may effectively treat COVID-19 pneumonia. However, numerous clinical trials on possible antiviral treatments are now underway (Zhaori et al., 2020). The antiviral treatments can be classified into two groups—the one that targets the coronavirus, either by inhibiting a key viral enzyme involved in genome replication or by preventing its entry into human cells and the second that is intended to influence the human immune system, either by enhancing the innate response against viruses or by suppressing the inflammatory processes that lead to lung damage (Tu et al., 2020). The majority of these drugs were developed for different infections before being repurposed for COVID-19. The method of repurposing existing antiviral agents authorized or under development for other viral diseases has been embraced in light of the necessity to discover an effective treatment for symptomatic individuals (Singh et al., 2020). The SOLIDARITY study, an international clinical trial, was recently initiated by the World Health Organization (WHO) to address this issue. Lopinavir and ritonavir, with interferon β and chloroquine, and remdesivir are among the drugs tested in this study (Uddin et al., 2020). These drugs like others need to bind to the drug carrier protein known as human serum albumin which influences their activity and half-life.

Human serum albumin (HSA) is a single-chain, non-glycosylated polypeptide with a molecular weight of 66,500 Da and 585 amino acids (Yamasaki et al., 2013). The structure of HSA indicates the existence of three domains, namely domains I (residues 1–195), II (196–383), and III (384–585), which are not only topologically identical but also have comparable three-dimensional structures, as anticipated by amino acid sequence comparison (He and Carter, 1992; Yang et al., 2014). The polypeptide chain has an approximate dimension of 80 \times 80 \times 30 \text{ Å} and forms a heart-shaped structure (Sugio et al., 1999). Except for Cys34 (in domain I), HSA has 35 cysteine residues, all of which are involved in disulfide bond formation, which helps to stabilize the protein. Interdomain and inter-subdomain interactions have a crucial role in the stability of the HSA molecule, according to crystallographic evidence (Yamasaki et al., 2013). It is the most abundant protein in blood plasma that serves a variety of important physiological functions. HSA controls colloidal osmotic pressure and transports a variety of endogenous substances such as fatty acids (FA), hormones, bile acids, amino acids, metals, and toxic metabolites, among others (Yang et al., 2014). Furthermore, through binding with HSA, a wide range of drugs are transported to their target organs/tissues (Yamasaki et al., 2013). As a result, HSA not only sequesters bound pharmaceuticals from oxidation and impacts drug distribution in vivo, but it also changes drug pharmacokinetic and pharmacodynamic characteristics (Tayyab and Feroz, 2020).

HSA’s remarkable ability to bind a wide range of drugs is one of its most distinguishing features (Yang et al., 2014). Given the large quantity of HSA in plasma, drug binding affinity to HSA is an essential consideration when developing novel therapies. Furthermore, the interaction of drugs that bind to HSA at the same time might alter HSA binding behaviour and potentially affect the drugs’ therapeutic efficacy (Tessersonatis and Alevizou, 2008). The structures of HSA-ligand complexes have shown not only where distinct drug binding sites on HSA are located, but also reveal how several drugs interact with HSA (Yang et al., 2013, 2012). When studying the processes determining the pharmacological effects of these molecules, understanding drug binding characteristics to HSA is critical (Yang et al., 2014). In this study, the molecular mechanism underlying the interaction between drug candidate molecules for COVID-19 and human serum albumin was investigated. The interaction between the drug candidate molecules and HSA was evaluated in terms of binding energies and binding site identification through molecular docking studies. The pharmacokinetic properties of the COVID-19 drug candidate molecules were also computationally investigated. The dynamic behaviour of the free HSA and HSA-ligand complexes were further explored through molecular dynamics simulations in an aqueous environment.

2. Materials and methods

2.1. Retrieval of drug candidate molecules

A total of 55 chemical structures were downloaded from PubChem database (Kim et al., 2016) which comprises 16 experimental drug candidate molecules for COVID-19 (Set A) (Caly et al., 2020; Choy et al., 2020; Dai et al., 2020; Devaux et al., 2020; Jin et al., 2020; K.Y. Wang et al., 2020, M. Wang et al., 2020, X. Wang et al., 2020), 26 in silico drug candidate molecules for COVID-19 i.e. 15 molecules in Set-B from others’ publications (Beck et al., 2020; Hall Jr. and Ji, 2020; Ho, 2020; Ke et al., 2020; Pant et al., 2020), 11 molecules in Set C from our previous publications (Gurung et al., 2020a, 2020b, 2020c), and 13 control drug molecules that bind serum albumin proteins (Set D) (Varshney et al., 2010). The compounds that lacked a 3D structure were transformed using OpenBabel version 2.4.1 (O’Boyle et al., 2011) and optimized with the MMFF94 force field (Halgren, 1996).

2.2. Retrieval of protein structures

The three-dimensional X-ray crystal structure of human serum albumin was obtained from Protein Data Bank (http://www.rcsb.org/) using PDB ID: 6HSC. The structure consists of human serum albumin in complex with Aristolochic acid at a resolution of 1.9 Å.

2.3. Molecular docking studies

The binding of COVID-19 drug candidate molecules to HSA was studied using AutoDock Vina program which is based on a sophisticated gradient optimization method (Trott and Olson, 2010). The serum protein was prepared by removing the heteroatoms such as ions, water molecules and cocrystal ligands and the addition of polar hydrogen atoms and Kollman charges. The ligands were prepared by adding hydrogen atoms, Gasteiger charges and optimally defining the torsions. Blind docking was performed using a grid box centred at XYZ coordinates of \(-71.2957, 1.2889\) and \(14.0226\) with dimensions of \(X: 98.2245\ \text{Å}, Y: 51.0570\ \text{Å}\) and \(Z: 90.0794\ \text{Å}\) and exhaustiveness were set to 8. The binding conformations were clustered and ranked based on their binding affinities. The molecular interactions such as hydrogen bonds and hydrophobic interactions between HSA and compounds were evaluated using LigPlot + program version 1.4.5 (Laskowski and Swindells, 2011).

2.4. Determination of pharmacokinetic properties

Various pharmacokinetic properties (gastrointestinal absorption, blood-brain barrier permeation, F-glycoprotein substrate, and cytochrome P450 inhibitor) of each molecule were determined using SwissADME tool (Daina et al., 2017).
Fig. 1. The chemical structures of selected experimental and in silico COVID-19 drug candidate molecules used in the study.
2.5. Molecular dynamics simulation

The trajectories of the free HSA and HSA-ligand complexes were studied through 100-ns of MD simulations using GROMMACHine for Chemical Simulations (GROMACS) 2019.2 software (Hess et al., 2008) with GROMOS96 43a1 force field. The free HSA and the HSA-ligand complexes were prepared for MD simulation by considering a cubic box of 1 Å spacing and solvated with simple point charge (SPC216) waters. A leap-frog time integration algorithm was used for integrating Newton’s equations of motion. The systems were neutralized by adding an appropriate number of counterions and subsequently energy minimized. The systems were subjected to production MD run for 100 ns in NPT ensemble. PRODRG web server (Schüttelkopf and Van Aalten, 2004) was used to generate topology of the ligand. MD analysis was performed by choosing parameters such as RMSD (root mean square deviation), RMSF (root mean square fluctuation), Rg (radius of gyration), total SASA (solvent accessible surface area) and the number of hydrogen bonds (NHBs). Graphs were plotted using Xmgrace tool.

2.6. Binding free energy analysis

The binding free energy ($\Delta G_{\text{bind}}$) of the compounds was calculated using LARMD program (Yang et al., 2020) which uses the following equation (Equation 1).

$$\Delta G_{\text{bind}} = \Delta E_{\text{bind}} - T \Delta S_{\text{col}} - T \Delta S_{\text{conf}}$$  (1)

### Table 1

| Molecules | Name                  | PubChem ID | Binding Energy (kcal/mol) | Binding strength | Binding region |
|-----------|-----------------------|------------|---------------------------|------------------|----------------|
| A1        | Ivermectin            | 6321424    | −9.4                      | Moderate         | IB-IIA-IIIB    |
| A2        | Hydroxychloroquine    | 3652       | −7.1                      | Moderate         | IIA            |
| A3        | Arbidol               | 131411     | −7.3                      | Moderate         | IB-IIA         |
| A4        | Ebselen               | 3194       | −8.5                      | Moderate         | IIA-IB-III     |
| A5        | Remdesivir            | 121304016  | −8.9                      | Moderate         | IIA-III        |
| A6        | Lopinavir             | 92727      | −9.1                      | Moderate         | IB-IIA-IIIA    |
| A7        | Emetine               | 10219      | −8.1                      | Moderate         | IB-IIA-IIIA    |
| A8        | Homoharringtoninine   | 285033     | −6.9                      | Moderate         | IIA-III        |
| A9        | Chloroquine           | 2719       | −6.5                      | Weak             | IIA-IB         |
| A10       | β-D-N4-hydroxycytidine (NHC) | 197020 | −6.5     | Weak             | IA-IA          |
| A11       | Disulfiram            | 3117       | −9.3                      | Weak             | IIA            |
| A12       | Tidoglusib            | 11313622   | −10                      | Strong           | IB-IIA-IIIB-III|
| A13       | Carmofur              | 2577       | −6.7                      | Weak             | IIA-III        |
| A14       | Shikonin              | 479503     | −8.9                      | Moderate         | IIA-IB         |
| A15       | Compound 11a          |            | −9.3                      | Moderate         | IB-IIA         |
| A16       | Compound 11b          |            | −10.4                     | Strong           | IIA-IB-III     |
| B17       | Zanamivir             | 60855      | −7.8                      | Moderate         | IIA            |
| B18       | Indinavir             | 5362440    | −9.7                      | Strong           | IB-IIA-IIIA    |
| B19       | Saquinavir            | 441243     | −10.2                     | Strong           | IB-IIA         |
| B20       | Atazanavir            | 146192     | −8.7                      | Moderate         | IB-IIA-IIIA    |
| B21       | Bedaquiline           | 5388906    | −8.7                      | Moderate         | IB-IIA-IIIA    |
| B22       | Brequininar           | 57030      | −9                       | Moderate         | IB-IIA         |
| B23       | Celecoxib             | 2662       | −9.8                      | Strong           | IIA-III        |
| B24       | Clofazimine           | 2794       | −9.5                      | Moderate         | IIA-III        |
| B25       | Clovirapten           | 15171      | −11.2                     | Strong           | IIA-III        |
| B26       | Gemcitabine           | 60750      | −7.3                      | Moderate         | IIA            |
| B27       | Tolcapone             | 4659569    | −9.3                      | Moderate         | IB             |
| B28       | Vismodegib            | 24776445   | −8.5                      | Moderate         | IB-IIA         |
| B29       | Cobicistat            | 25151504   | −9.3                      | Moderate         | IIA-III        |
| B30       | Ritonavir             | 392622     | −10.1                     | Strong           | IIA-III        |
| B31       | Carunavir             | 213039     | −10.9                     | Moderate         | IB-IIA-III     |
| C32       | Ergotamine            | 8223       | −11.5                     | Strong           | IIA-IB-III     |
| C33       | Dihydroergotamine     | 10531      | −11.9                     | Strong           | IB-IIA         |
| C34       | Bonducelpin D         | 10835061   | −7.9                      | Moderate         | IIA-IB         |
| C35       | Glicapreirr           | 6082839    | −9.7                      | Moderate         | IB-IIA-III     |
| C36       | Daclatasvir           | 25154714   | −12.1                     | Strong           | IB-IIA         |
| C37       | Paritapreirr          | 45110509   | −10.5                     | Strong           | IIA-IB-III     |
| C38       | Vincapsusine          | 11646359   | −7.6                      | Moderate         | IB-IIA         |
| C39       | Alloylumbine          | 120716     | −9.7                      | Strong           | IIA-III        |
| C40       | Gummadiol             | 21722930   | −9.9                      | Strong           | IA-IB          |
| C41       | ZINC000254565785      |          | −9.2                      | Moderate         | IIA            |
| C42       | ZINC000726422572      |          | −9                      | Moderate         | IA-IA          |
| C43       | Indomethacin          | 3715       | −8.6                      | –               | IIA            |
| C44       | Furosemide            | 3440       | −8                       | –               | IIA            |
| C45       | Warfarin              | 54678486   | −8.7                      | –               | IIA-IB         |
| C46       | Bexiprolecine         | 5904       | −8.4                      | –               | IIA-IB         |
| C47       | Chloropromamide       | 2727       | −7.5                      | –               | IIA-IB-III     |
| C48       | Phenytoin             | 1775       | −9.3                      | –               | IA             |
| C49       | Diazepam              | 3016       | −7.7                      | –               | IIA            |
| C50       | Naprofen              | 3672       | −7.4                      | –               | IIA-IB         |
| D51       | Naproxen              | 156391     | −8.3                      | –               | IIA-IB         |
| D52       | Clonofibrate          | 2796       | −7                       | –               | IIA-III        |
| D53       | Chlorpromazone        | 2726       | −7.1                      | –               | IIA            |
| D54       | Imipramine            | 3696       | −7.9                      | –               | IA             |
| D55       | Quinidine             | 441074     | −7.8                      | –               | IB-IIIA        |

After heating and equilibration, the systems were subjected to production MD run for 100 ns in NPT ensemble.
Fig. 2. Binding poses and molecular interactions of the drug molecules with HSA- (A) HSA_A13 (B) HSA_C34 (C) HSA_A16 and (D) HSA_D43. The domain I of HSA consists of subdomains IA coloured green (residues 5–107) and IB coloured orange (residues 108–196); domain II comprises subdomains IIA coloured blue (residues 197–297) and IIB coloured yellow (residues 298–383); domain III has subdomains IIIA coloured magenta (residues 384–497) and IIIB coloured cyan (residues 498–582). Hydrophobic interactions are shown as semi-arcs with red eyelashes, whereas hydrogen bonds are shown as green dashed lines.
Table 2
Pharmacokinetic properties of the selected molecules.

| Molecule | Lipinski violations | Veber violations | ESOL Class | GI absorption | BBB permeant | Pgp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor |
|----------|---------------------|------------------|------------|---------------|--------------|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| A1       | 2                   | 1                | Poorly soluble | Low           | No           | Yes           | No              | No              | No              | No              | No              |
| A2       | 0                   | 0                | Soluble     | High          | Yes          | No            | Yes             | No              | No              | No              | Yes             |
| A3       | 0                   | 0                | Moderately soluble | High       | No           | No            | No              | Yes             | Yes             | Yes             | Yes             |
| A4       | 0                   | 0                | Soluble     | High          | Yes          | No            | No              | No              | No              | No              | No              |
| A5       | 2                   | 2                | Moderately soluble | Low        | No           | Yes           | No              | No              | No              | Yes             | No              |
| A6       | 1                   | 1                | Poorly soluble | High         | No           | Yes           | No              | No              | No              | No              | Yes             |
| A7       | 0                   | 0                | Moderately soluble | High       | Yes          | Yes           | No              | No              | No              | No              | No              |
| A8       | 1                   | 1                | Soluble     | High          | No           | No            | No              | No              | No              | Yes             | Yes             |
| A9       | 0                   | 0                | Moderately soluble | High       | Yes          | No            | No              | No              | No              | No              | Yes             |
| A10      | 0                   | 0                | Very soluble | Low           | No           | No            | No              | No              | No              | No              | No              |
| A11      | 0                   | 0                | Soluble     | High          | No           | No            | Yes             | Yes             | No              | No              | Yes             |
| A12      | 0                   | 0                | Moderately soluble | High       | Yes          | No            | Yes             | Yes             | Yes             | No              | No              |
| A13      | 0                   | 0                | Soluble     | High          | No           | No            | Yes             | No              | No              | No              | No              |
| A14      | 0                   | 0                | Soluble     | High          | No           | No            | Yes             | No              | Yes             | No              | No              |
| A15      | 0                   | 1                | Moderately soluble | High      | No           | Yes           | No              | No              | Yes             | No              | No              |
| A16      | 0                   | 0                | Soluble     | High          | No           | No            | No              | Yes             | Yes             | Yes             | Yes             |
| B17      | 2                   | 1                | Highly soluble | Low          | No           | Yes           | No              | No              | No              | No              | No              |
| B18      | 1                   | 1                | Moderately soluble | High       | No           | Yes           | No              | No              | No              | No              | No              |
| B19      | 2                   | 2                | Moderately soluble | Low        | No           | Yes           | No              | No              | No              | No              | Yes             |
| B20      | 2                   | 2                | Poorly soluble | Low           | No           | Yes           | No              | No              | No              | No              | Yes             |
| B21      | 2                   | 0                | Poorly soluble | Low           | No           | Yes           | Yes             | No              | No              | Yes             | Yes             |
| B22      | 0                   | 0                | Poorly soluble | High          | No           | Yes           | Yes             | No              | No              | No              | No              |
| B23      | 0                   | 0                | Moderately soluble | High       | No           | No            | Yes             | No              | No              | Yes             | No              |
| B24      | 1                   | 0                | Poorly soluble | Low           | No           | No            | No              | No              | No              | No              | No              |
| B25      | 0                   | 0                | Poorly soluble | High          | No           | No            | No              | Yes             | No              | No              | No              |
| B26      | 0                   | 0                | Very soluble | High          | No           | No            | No              | No              | No              | No              | No              |
| B27      | 0                   | 0                | Soluble     | High          | No           | No            | No              | Yes             | No              | No              | Yes             |
| B28      | 0                   | 0                | Moderately soluble | High      | No           | No            | No              | Yes             | No              | No              | Yes             |
| B29      | 2                   | 2                | Poorly soluble | Low           | No           | Yes           | No              | No              | No              | No              | Yes             |
| B30      | 2                   | 2                | Poorly soluble | Low           | No           | Yes           | No              | No              | No              | No              | Yes             |
| B31      | 1                   | 2                | Moderately soluble | Low        | No           | Yes           | No              | No              | No              | No              | Yes             |
| C32      | 1                   | 0                | Moderately soluble | High       | No           | Yes           | No              | Yes             | Yes             | Yes             | Yes             |
| C33      | 1                   | 0                | Moderately soluble | High       | No           | Yes           | No              | No              | No              | Yes             | Yes             |
| C34      | 0                   | 0                | Soluble     | High          | No           | Yes           | No              | No              | Yes             | No              | No              |
| C35      | 0                   | 0                | Poorly soluble | Low           | No           | Yes           | No              | No              | No              | No              | No              |
| C36      | 2                   | 2                | Poorly soluble | Low           | No           | Yes           | No              | No              | Yes             | No              | Yes             |
| C37      | 2                   | 1                | Poorly soluble | Low           | No           | Yes           | No              | No              | No              | Yes             | Yes             |
| C38      | 0                   | 0                | Soluble     | High          | Yes          | No            | No              | No              | No              | Yes             | No              |
| C39      | 0                   | 0                | Moderately soluble | High       | Yes          | No            | No              | No              | No              | Yes             | No              |
| C40      | 0                   | 0                | Soluble     | High          | No           | No            | No              | No              | Yes             | No              | Yes             |
| C41      | 0                   | 0                | Moderately soluble | High       | Yes          | No            | Yes             | Yes             | Yes             | No              | Yes             |
| C42      | 0                   | 0                | Moderately soluble | High       | No           | No            | Yes             | Yes             | Yes             | Yes             | Yes             |
where $D_{E\text{bind}}$ is the binding energy, $T_{D_{SS}}\text{sol}$ is the solvation entropy and $T_{D_{SC}}\text{conf}$ is the conformational entropy. While entropy was computed using an empirical method (Hao et al., 2009; Pan et al., 2008), the enthalpy was derived using the Molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) or molecular mechanics generalized Born surface area (MM/GBSA) method (Hou et al., 2011).

### 3. Results

A total of 55 molecules comprising of experimental ($N = 16$, Set A1-A16) and in silico COVID-19 drug candidate molecules ($N = 26$) which include set B17-B31 ($N = 15$) and set C32-C42 ($N = 11$) along with the control drugs (known binders of HSA) ($N = 13$, set D43-D55) were docked into the human serum albumin protein using the blind docking method (Fig. 1). The molecular docking results of the compounds are represented in Table 1. On comparing the binding energy scores with the control data set ($\leq 9.3$ kcal/mol), the selected COVID-19 drug candidate molecules were classified into three different categories- weak, moderate and strong binding molecules. Four drug candidate molecules viz., Chloroquine (A9), b-D-N4-hydroxycytidine (A10), Disulfiram (A11), and Carmofur (A13) demonstrate weak binding to HSA, with binding energies ranging from $5$ to $6.7$ kcal/mol, according to molecular docking studies. Ivermectin (A1), Hydroxychloroquine (A2), Arbidol (A3), Ebselen (A4), Remdesivir (A5), Lopinavir (A6),

**Table 2** (continued)

| Molecule | Lipinski #violations | Veber #violations | ESOL Class | GI absorption | BBB permeant | Pgp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor |
|----------|----------------------|------------------|------------|---------------|--------------|--------------|-----------------|-----------------|----------------|----------------|----------------|
| D43      | 0                    | 0                | Moderately soluble | High         | Yes          | No            | Yes             | Yes             | No             | No             | No             |
| D44      | 0                    | 0                | Soluble     | High          | No           | No            | Yes             | No              | No             | No             | No             |
| D45      | 0                    | 0                | Soluble     | High          | No           | No            | Yes             | Yes             | Yes            | No             | No             |
| D46      | 0                    | 0                | Soluble     | High          | No           | No            | No              | No              | No             | No             | No             |
| D47      | 0                    | 0                | Soluble     | High          | No           | No            | No              | No              | No             | No             | No             |
| D48      | 0                    | 0                | Soluble     | High          | No           | No            | No              | No              | No             | No             | No             |
| D49      | 0                    | 0                | Soluble     | High          | Yes          | No            | Yes             | Yes             | Yes            | No             | No             |
| D50      | 0                    | 0                | Soluble     | High          | No           | No            | No              | No              | No             | No             | No             |
| D51      | 0                    | 0                | Soluble     | High          | No           | No            | No              | No              | No             | No             | No             |
| D52      | 0                    | 0                | Soluble     | High          | Yes          | No            | Yes             | No              | No             | No             | No             |
| D53      | 1                    | 0                | Moderately soluble | High         | Yes          | No            | Yes             | Yes             | Yes            | No             | No             |
| D54      | 0                    | 0                | Moderately soluble | High         | No           | No            | Yes             | No              | No             | No             | No             |
| D55      | 0                    | 0                | Soluble     | High          | Yes          | No            | No              | No              | No             | No             | No             |

**Table 3**

Average geometric properties of the systems calculated during 100 ns MD simulation studies.

| Systems | RMSD (nm) | Rg (nm) | Total SASA (nm²) | Number of Hydrogen bonds |
|---------|-----------|--------|-----------------|------------------------|
| HAS     | 0.448642 ± 0.04833 | 2.61873 ± 0.02697 | 264.0154 ± 6.698827 | 482.4416 ± 11.10697 |
| HSA_A13 | 0.62448 ± 0.06459  | 1.45710 ± 0.50422 | 267.8342 ± 5.879287 | 481.6254 ± 11.67786 |
| HSA_C34 | 0.49058 ± 0.05781  | 0.609009 ± 0.092277 | 265.9578 ± 8.20947 | 475.4775 ± 11.42339 |
| HSA_A16 | 0.509058 ± 0.078123 | 0.322053 ± 0.045001 | 269.5354 ± 8.744237 | 475.3816 ± 11.46369 |
| HSA_D43 | 0.478107 ± 0.0692  | 0.612155 ± 0.058157 | 268.3333 ± 0.03179 | 475.3816 ± 11.46369 |

**Table 4**

Statistics of binding free energy calculation of HSA-ligand complexes (kcal/mol).

| Protein-ligand complexes | ELE | VDW | GAS | PBSOL | PBTO | GBSOL | GBTO | -TS | $\Delta G_{\text{pe}}$ | $\Delta G_{\text{fb}}$ |
|--------------------------|-----|-----|-----|-------|------|-------|------|-----|-----------------------|-----------------------|
| HSA_A13                  | -2.87 ± 1.23 | -40.49 ± 1.95 | -43.36 ± 2.46 | 12.10 ± 2.24 | -31.26 ± 2.43 | 9.61 ± 1.45 | -33.75 ± 1.99 | 14.46 ± 1.65 | -16.80 ± 19.29 |
| HSA_C34                  | 6.76 ± 3.16   | -50.79 ± 2.92 | -57.54 ± 3.52 | 24.72 ± 3.08 | -32.83 ± 2.69 | 18.29 ± 2.43 | -39.25 ± 2.61 | 22.73 ± 2.74 | -10.10 ± 16.52 |
| HSA_A16                  | -6.45 ± 2.00  | -65.66 ± 2.67 | -72.12 ± 3.37 | 25.51 ± 2.48 | -46.60 ± 3.44 | 16.39 ± 1.86 | -55.72 ± 2.94 | 22.38 ± 2.19 | -24.22 ± 33.34 |
| HSA_D43                  | 15.79 ± 5.50  | -37.93 ± 2.87 | -22.13 ± 6.28 | -9.15 ± 5.10 | -31.30 ± 5.07 | -5.57 ± 5.30 | -27.72 ± 7.74 | 15.76 ± 2.06 | -15.54 ± 11.96 |

1 Electrostatic energy as calculated by the MM force field; 2Van der Waals contribution from MM; 3Total gas-phase energy; 4Non-polar and polar contributions to solvation based on PB/GB model; 5Final estimated binding free energy calculated from GAS and PBSOL/GBSOL; 6Entropy; 7Binding free energy with entropy.
Emetine (A7), Homoharringtonine (A8), Shikonin (A14), Compound 11a (A15), Zanamivir (B17), Atazanavir (B20), Bedaquiline (B21), Brequinar (B22), Clofazimine (B24), Gemcitabine (B26), Tolaconine (B27), Vismodegib (B28), Cobicistat (B29), Darunavir (B31), Bonducellpin D (C34), Vincapsusine (C38), ZINC000254565785 (C41) and ZINC000726422572 (C42) with binding energies ranging from 6.9 to 9.5 kcal/mol demonstrated moderate binding to HSA. The strong HSA binding drug candidates consist of fourteen molecules Tideglusib (A12), Compound 11b (A16), Indinavir (B18), Saquinavir (B19), Celecoxib (B23), Conivaptan (B25), Ritonavir (B30), Ergotamine (C32), Dihydroergotamine (C33), Glexacrevir (C35), Daclatasvir (C36), Paritaprevir (C37), Alloyoimbine (C39) and Gummadil (C40) with binding energies ranging from −9.7 to −12.1 kcal/mol. All these molecules bind to different HSA sub-domains (IA, IB, IIA, IIB, IIIA, and IIIB) (Table 1) through molecular forces such as hydrogen bonds and hydrophobic interactions. The Carmofur (A13) binds to HSA with a binding energy of −6.7 kcal/mol and the HSA_A13 complex is stabilized by a hydrogen bond with Ser480 and hydrophobic interactions through Gly328, Lys212, Ala213, Leu327, Ala210, Val482, Leu347, Lys351, Leu331, Ala350, Val216 (Fig. 2A). The Bonducellpin D (C34) binds to HSA with a binding energy of −7.9 kcal/mol and the HSA_C34 complex interaction occurs through four hydrogen bonds with Glu153, Ser192, Gln196 and His288 and hydrophobic interactions through Lys199, Lys195, Ala291, Phe157, Glu292, Glu188, Asp451 (Fig. 2B). The compound 11b (A16) binds to HSA with a binding energy of −10.4 kcal/mol and the HSA_A16 complex is stabilized through two hydrogen bonds with Trp214 and Asp451 and hydrophobic...
interactions through Lys195, Lys199, Val482, Ala210, Ser480, Ser202, Leu481, Ser454, Leu457, Leu453, Glu450, Arg485, Val344, Leu198 (Fig. 2C). The control Indomethacin (D43) binds to HSA with a binding energy of \(-8.6\) kcal/mol and the HSA_D43 interaction is facilitated through one hydrogen bond with Ser287 and hydrophobic interactions through Leu260, Ile290, Ala291, Lys199, Arg222, Val241, Trp214, Arg257, His242, Leu238 and Ala261 (Fig. 2D). The pharmacokinetic properties such as gastrointestinal absorption, blood-brain barrier permeation, P-glycoprotein substrate, and cytochrome P450 inhibitor which play a significant role in absorption, distribution, metabolism, excretion, and toxicity (ADMET) were calculated for each molecule (Table 2).

The free HSA and HSA-ligand-bound complexes (HSA_A13, HSA_C34, HSA_A16 and HSA_D43) were subjected to 100 ns of MD simulation to determine the stability of their trajectories. The geometric properties analysed in the study include RMSD, RMSF, Rg, SASA and NHB (Table 3). Root mean square deviation (RMSD) computes the average distance between the backbone atoms of starting structure (reference structure) with superimposed trajectory. The RMSD plot was generated (Fig. 4) which provide further insights into the structural regions of the protein contributing to greater fluctuations. Root mean square fluctuation (RMSF) computes fluctuations (standard deviation) of atomic positions of each amino acid residue in the trajectory. The binding of A13 to HSA causes a significant increase in the amplitude of fluctuations around Ser58-Glu60, Arg114-Arg117 and Asp301 (Fig. 4A). The radius of gyration (Rg) computes the structural compactness of a molecule and the radii of gyration about the x-, y- and z-axes, as a function of time. From the Rg plot (Fig. 5) it is evident that the residues that contribute significantly towards the binding interaction between A13 and HSA include Glu450, Trp214, Ser287 and Ser327. The number of hydrogen bonds for free HSA, HSA_A13, HSA_C34, HSA_A16 and HSA_D43 were 482.4416 ± 11.10697, 470.2488 ± 11.8971, 481.6254 ± 11.67786, 475.4775 ± 11.42339 and 475.3816 ± 11.46369 respectively (Table 3). A decrease in the number of intramolecular hydrogen bond formations may be attributed to the less folded state of the protein upon binding ligand. The number of hydrogen bonds between HSA and ligand in HSA_A13, HSA_C34, HSA_A16 and HSA_D43 were 1.082917 ± 0.867247, 0.588412 ± 0.717235, 2.310689 ± 1.061308 and 1.641359 ± 1.008091 respectively (Table 3).

Molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) and molecular mechanics generalized Born surface area (MM/GBSA) binding free energies of A13 (\(\Delta G = -16.80\) kcal/mol, \(\Delta G_B = -19.29\) kcal/mol), C34 (\(\Delta AP = -10.10\) kcal/mol, \(\Delta G_B = -6.52\) kcal/mol), A16 (\(\Delta AP = -24.22\) kcal/mol, \(\Delta G_B = -33.34\) kcal/mol) and the control (D43) (\(\Delta AP = -15.54\) kcal/mol, \(\Delta G_B = -11.96\) kcal/mol) were tabulated in Table 4. In each of the HSA-ligand complexes, van der Waals energy is the dominant force of molecular interactions. The top ten residues which contribute significantly towards the binding interaction between A13 and HSA include Arg205, Leu347, Ala213, Leu327, Ala210, Lys351, Gly328, Leu331, Ala350 and Val482 (Fig. 8A). In case of HSA_C34 complex, residues such as Gln196, Lys195, Trp214, Ser192, Arg257, Tyr150, Leu238, Glu292, Lys199 and Phe149 has higher contributions (Fig. 8B). The major residues contributing towards the binding interaction between A16 and HSA include Glu450, Trp214,
Ser454, Leu481, Lys199, Val344, Leu198, Lys195, Arg485 and Leu457 (Fig. 8C). In case of HSA_D43 complex, residues such as Arg257, Leu238, Leu260, Ser287, Ile290, Leu219, Ala291, Arg222, Ala261 and Ile264 has higher contribution to the total binding energy (Fig. 8D).

4. Discussion

There is a high paucity of new antiviral drugs against the COVID-19 pandemic which is caused by a novel betacoronavirus known as SARS-CoV-2. Numerous clinical trials on possible antiviral treatments are still underway and the majority of these drug candidate molecules are the repurposed drugs. The drug molecules in the body are bound to the drug carrier protein know as human serum albumin which influences their activity and half-life. In the process of developing novel therapies, the binding affinity of the drugs towards HSA is an important parameter of study given the large quantity of HSA in plasma. In addition, the therapeutic efficacy of drugs is altered upon binding to HSA (Tesseromatis and Alevizou, 2008). Human serum albumin (HSA) is a non-glycosylated polypeptide with a molecular weight of 66,500 Da (Yamasaki et al., 2013). The polypeptide chain forms a heart-shaped structure with a dimension of approximately 80 × 80 × 30 Å and (Sugio et al., 1999). The presence of three domains, namely domains I (residues 1–195), II (196–383), and III (384–585) are structural characteristics of HSA (He and Carter, 1992; Yang et al., 2014). In our studies, we have characterized the binding energy and putative binding sites of selected anti-COVID-19 drug candidate molecules to HSA. Comparing the binding energies of the control drugs to HSA, we have classified the strength of their binding into weak, moderate, and strong. The

![Fig. 8. Heatmap showing the top ten residues contributing significantly to the total binding free energy of HSA-ligand complexes](image-url)
weak binding molecules include four molecules such as Chloroquine (A9), β-D-N4-hydroxycytidine (A10), Disulfiram (A11), and Carmofur (A13). Molecules such as Ivermectin (A1), Hydroxychloroquine (A2), Arbidol (A3), Ebselen (A4), Remdesivir (A5), Lopinavir (A6), Emetine (A7), Homoharringtonine (A8), Shikonin (A14), Compound 11a (A15), Zanamivir (B17), Atazanavir (B20), Bedaquiline (B21), Brequinar (B22), Clofazimine (B24), Gemcitabine (B26), Tolcapone (B27), Vismodegib (B28), Cobicistat (B29), Darunavir (B31), Bonducellin D (C34), Vincaapuscins (C38), ZINC000254565785 (C41) and ZINC000726422572 (C42) exhibited moderate binding to HSA. The molecules strongly binding to HSA comprises Tideglusib (A12), Compound 11b (A16), Indinavir (B18), Saquinavir (B19), Celecoxib (B23), Conivaptan (B25), Ritonavir (B30), Ergotamine (C32), Dihydroergotamine (C33), Glecaprevir (C35), Daclatasvir (C36), Paritaprevir (C37), Allelohydroimbine (C40) and Gammucolidio (C40). These molecules bind to different domains of HSA through both hydrogen bonds as well as hydrophobic interactions. We have selected one drug molecule from each category-A13 (weak binding), C34 (moderate binding), A16 (strong binding), and a control (D43) for molecular dynamics simulation studies. The dynamic structural changes in the HSA upon binding of these molecules resulted in variations in the geometric properties such as RMSD, RMSF, Rg, total SASA, and the number of hydrogen bonds. Molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) and molecular mechanics general-Born surface area (MM/PBSA) binding energies of the molecules were assessed. We have selected one drug molecule for each category-A13 (weak binding), C34 (moderate binding), A16 (strong binding), and a control (D43) for computational limits in our present studies, the molecular dynamics simulations of only a few selected molecules were performed. It is worthwhile to investigate the number of binding sites, thermodynamic parameters, and dissociation constants using experimental techniques such as fluorescence quenching, isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR) which will provide further insights into the binding of COVID-19 drug candidate molecules to HSA.

5. Conclusion

Human serum albumin (HSA) is an important drug carrier protein that modulates the activity and half-life of the majority of drug molecules. Using molecular docking and dynamics simulation techniques, we were able to characterize the binding sites of the selected experimental and in silico COVID-19 drug candidates. Our study unravels that these drug molecules bind to different domains of HSA using intermolecular forces such as hydrogen bonds and hydrophobic interactions. The drug molecules also show different degrees of binding strength to HSA and the HSA-drug complexes were also stable throughout the molecular dynamics simulation time. Various experimental techniques such as fluorescence quenching, isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR) will shed further light on the thermodynamic binding properties of COVID-19 drugs to HSA.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgments

The authors would like to extend their sincere appreciation to the Researchers Supporting Project number (RSP-2021/306), King Saud University, Riyadh, Saudi Arabia.

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