Elucidation of the inhibitory activity of plant-derived SARS-CoV inhibitors and their potential as SARS-CoV-2 inhibitors

Martiniano Bello and Md. Kamrul Hasan

Laboratorio de Modelado Molecular, Bioinformática y Diseño de Fármacos de la Escuela Superior de Medicina, Instituto Politécnico Nacional, Mexico City, Mexico; Department of Biochemistry and Molecular Biology, Tejgaon College, National University, Gazipur, Bangladesh

Communicated by Ramaswamy H. Sarma

CONTACT Martiniano Bello bellomartini@gmail.com Laboratorio de Modelado Molecular, Bioinformática y Diseño de Fármacos de la Escuela Superior de Medicina, Instituto Politécnico Nacional, Mexico City, Mexico.

ARTICLE HISTORY
Received 25 January 2021
Accepted 28 May 2021

KEYWORDS
COVID-19; SARS-CoV-2 3CLprO; binding free energy; molecular docking; molecular dynamics simulation

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the 7th coronavirus species discovered that infects humans, is the causative agent of the ongoing COVID-19 viral pandemic (Huang et al., 2020; Guan et al., 2020; Liu et al., 2020). The virus was first discovered in Wuhan in late 2019, causing a pneumonia-like outbreak that quickly spread worldwide (Ludwig & Zarbock, 2020). In March 2020, the World Health Organization (WHO) declared COVID-19 a pandemic due to its extreme outbreak (Brinks & Ibert, 2020) and notified global authorities to take emergency measures. While many countries have successfully combated the pandemic and have been declared COVID-19 free, the pandemic situation has relapsed in several others. To date, approximately 2.68 million lives have been lost globally to COVID-19, with 121.58 million people still infected [https://www.worldometers.info/coronavirus/]; the global COVID-19 situation seems hardly optimistic overall. The virus was initially reported to cause fever, coughing, sneezing, breathing difficulties in noncritical cases, pneumonia, and multiple organ failure, leading to death in severe cases (Huang et al., 2020). Recent studies indicate the possibility of viral infection also causing kidney dysfunction and myocardial injury (Kwong et al., 2018; Li et al., 2020; Nguyen et al., 2016). While SARS-CoV-2 is the third coronavirus that has reached epidemic status after 2002 SARS and 2012 MERS (Liu et al., 2020), it is by far the deadliest of the three and the only one to spread on a global scale in such a short period (Petrosillo et al., 2020; Xie & Chen, 2020).

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus of the genus Betacoronavirus in the family Coronaviridae, which belongs to the order Nidovirales. It has been suggested that SARS-CoV-2 followed an evolutionary transmission cascade similar to that of SARS-CoV and MERS-CoV, both of which have zoonotic origins, with their natural originators being bats (Benvenuto et al., 2020; Li et al., 2020; Zhou et al., 2020). It has also been confirmed that SARS-CoV-2 shares ~80% sequence identity with SARS-CoV (Ceraolo & Giorgi, 2020). While most coronaviruses generally contain six open reading frames (ORFs), SARS-CoV-2 contains 14, among which ORF 1a/b plays the most substantial role in viral proliferation (Chen et al., 2020; Gordon et al., 2020; Masters, 2006). ORF 1a/b translates into two overlapping polypeptides, pp1a and pp1ab, which are cleaved by the main protease 3CLprO and papain-like protease PLprO enzymes, into 16 nonstructural proteins, including a major protein for viral
reproduction such as RdRp (Chen et al., 2020; Khailany et al., 2020; Masters, 2006; Ziebuhr et al., 1997, 2000). It has been discovered that the PLpro enzyme also recognizes the C-terminal sequence of ubiquitin (Baez-Santos et al., 2015), but the 3CLpro enzyme exclusively cleaves polypeptide sequences after a glutamine residue (Zhang, Lin, Sun, et al., 2020). The rest of the genome sequence translates into structural proteins, which include the spike glycoprotein (S), an envelope protein (E), the membrane protein (M), and the nucleocapsid phosphoprotein (N). The spike glycoprotein (S) recognizes the human angiotensin-converting enzyme-2 (ACE-2) receptor, which makes it indispensable for viral propagation (Chen et al., 2020; Woo et al., 2010).

Since the discovery of SARS-CoV-2 in late 2019, scientists have developed various methods to alleviate the severity of the resulting disease and minimize the spread of the infection; after the declaration of a pandemic by the WHO, a global effort emerged for the rapid development of vaccines and specific antiviral treatments. Scientists have vested much effort in developing new antiviral drugs, with an influential group of researchers focusing on drug repurposing, as this method is faster than developing novel medicines. Among the antiviral drug targets that have been studied against coronaviruses, 3CLpro, PLpro, RdRp, and spike glycoprotein S have been treated as significant drug targets for the antiviral treatment of diseases, as they play crucial roles in viral proliferation and infection (Hilgenfeld, 2014; Ibrahim, Abdelrahman, Hussien et al., 2020; Ibrahim, Abdeljawaad, Abdelrahman, et al., 2020; Zhang, Lin, Sun, et al., 2020; Zhang, Lin, Kusov, et al., 2020). The deubiquitinase nature of PLpro makes substrate-derived inhibitors of PLpro also inhibit host-cell deubiquitinases, making drug development targeting PLpro arduous (Baez-Santos et al., 2015). Several FDA-approved RdRp inhibitor drugs, including remdesivir, favipiravir, sofosbuvir, ribavirin, lopinavir, ritonavir, tenofovir, and galidesivir, are effective against a broad range of RNA viruses, including past coronaviruses, and have been tested against SARS-CoV-2 for potential antiviral treatment. So far, only remdesivir has shown a reduction in the recovery period; however, it has shown zero impact on mortality (Beigel et al., 2020; Li & De Clercq, 2020; Mitjá & Clotet, 2020; Sinha & Balayla, 2020; Yavuz & Ünal, 2020). A recent computational study opted for an alternative pathway using structural analogs of FDA-approved RdRp inhibitor drugs. While the result of this analysis was shown to be optimistic, the computational nature of the study makes the possibility of developing effective antiviral drugs uncertain (Hasan et al., 2021). 3CLpro of SARS-CoV-2 proteolytically cleaves the pp1a and pp1ab polyproteins from ORF a/b into functional proteins, a critical step during viral replication, representing an essential target for decreasing the impact of COVID-19 (Zhang, Lin, Sun, et al., 2020). The alignment of 3CLpro of SARS-CoV-2 and 3CLpro of SARS-CoV showed that these proteins share up to 95% sequence identity, indicating that SARS-CoV 3CLpro inhibitors may function similarly against SARS-CoV-2. Different theoretical studies repurposed from 3CLpro of SARS-CoV have been published to identify new inhibitors of monomeric (Andrianov et al., 2020; Jimenez-Alberto et al., 2020; Li & De Clercq, 2020) and dimeric 3CLpro of SARS-CoV-2 (Bello, 2020; Bello et al., 2020), with the latter being the conformation of the active enzyme (Graziano et al., 2006). Taking advantage of this information, the present study assessed three naturally available plant-derived SARS-CoV 3CLpro inhibitors, namely, tannic acid, 3-isothiocyanatin-3-gallate, and theaflavin-3,3-digallate, which have been previously confirmed to have potent SARS-CoV 3CLpro inhibitor activity (Chen et al., 2005); these inhibitors were virtually docked against both SARS-CoV and SARS-CoV-2 using molecular docking analysis, protein-ligand interactions, molecular dynamic simulations, and free energy calculations to predict their potential for antiviral treatment of SARS-CoV-2 using nutraceuticals.

2. Methods

2.1. Preparation of systems

Tannic acid, theaflavin-3,3-digallate, and 3-isothiocyanatin-3-gallate were obtained from ChemSpider (http://www.chemspider.com/) and optimized at the AM1 level with Gaussian 09W software (Frisch et al., 2009). The X-ray crystallography structures of SARS-CoV-2 3CLpro (PDB ID: 6LU7, 2.16 Å) and SARS-CoV 3CLpro (PDB ID: 2GX4, 1.93 Å) were employed to construct the protein-ligand complexes.

2.2. Molecular docking

The three compounds were docked on dimeric SARS-CoV-2 3CLpro and SARS-CoV 3CLpro using the software AutoDock Tools 1.5.6 and AutoDock 4.2 (Morris et al., 2009). Hydrogen atoms were added to the ligands and protein atoms, and Kollman and Gasteiger partial charges were given for the receptor and ligand, respectively. The grid box was placed on the binding site of each monomeric subunit of dimeric SARS-CoV-2 3CLpro and SARS-CoV 3CLpro with grid xyz points of 70 × 70 × 70 Å and a grid space of 0.375 Å. Ligand location was assessed using a Lamarckian genetic algorithm as employed elsewhere (Yadava et al., 2013; Yadava, 2018). The docking runs were set to 100, and the population in the Lamarckian genetic algorithm was 150. The 100 docked poses were clustered into groups with RMSD values lower than 1.0 Å. The protein-ligand complex with the lowest binding free energy was designated as the initial conformer to start MD simulations. The docking was validated by replicating the binding mode of inhibitor N3 and TG-0205221 on SARS-CoV-2 3CLpro (PDB ID: 6LU7) and SARS-CoV 3CLpro (PDB ID: 2GX4) with RMSD values lower than 1.0 Å (Figure 1S, supplementary material).

2.3. MD Simulations

MD simulations were performed with AMBER16 software (Case et al., 2005) using the tail atom ff14SB force field (Duan et al., 2003). The three compounds’ force fields were determined using AM1-BCC atomic charges and the general Amber force field (GAFF) (Wang et al., 2004). Each system
obtained through docking was neutralized with 0.10 M NaCl
and then solvated in a dodecadic box of 12.0 Å using the
TIP3P water model (Jorgensen et al., 1983). The minimization
and equilibration of solvated and neutralized systems
consisted of the following steps: minimization through 1000
steps using the steepest descent method and 3000 steps
using the conjugate gradient method. Afterward, the com-
plexes were heated through 200 ps, and then, the density

Figure 1. Interactions of the complex between tannic acid and 3CL\textsuperscript{pro} of SARS-CoV-2. Map of tannic acid interactions at subunit 1 (A) and subunit 2 (B). The figure was constructed with Maestro Schrödinger version 10.5.
was equilibrated through 200 ps; finally, the systems were equilibrated with 600 ps of constant pressure equilibration at 310 K. MD simulations were run for 100 ns with triplicate experiments under an NPT ensemble at 310 K. The electrostatic forces were described by the PME method (Darden et al., 1993). A 10 Å cutoff was selected for the van der Waals interactions. The SHAKE algorithm (Van Gunsteren Berendsen, 1977) was employed to constrain bond lengths at their equilibrium values. Temperature and pressure were preserved using the weak-coupling algorithm (Berendsen et al., 1984). The MD results were analyzed using AmberTools16, whereas the images were built using Maestro Schrödinger version 10.5 (Schrödinger, 2016). Hydrogen bonds (H-bonds) were identified based on the following criteria: a maximum distance of 3.5 Å, a minimum donor angle of 120°, and a minimum acceptor angle of 90.0°. Representative protein-ligand complexes were obtained using a cluster analysis employing the kclust algorithm present in the MMTSB toolset (http://www.mmtsb.org/software/mmtsbtoolset.html).

2.4. Binding free energy and per-residue decomposition calculations

The MMGBSA (Miller et al., 2012, Gohlke & Case, 2004) approach was used to determine the binding free energy (ΔGbind) values for the complexes and determine the per-residue decomposition energy. Five hundred snapshots at time intervals of 100 ps were taken over the equilibrated time (last 50 ns), representing the simulation time where the average energies converged (Figure 25, supplementary material). Before the analysis, all counterions and water molecules were removed, and a salt concentration of 0.10 M was considered with the implicit solvation model (Onufriev et al., 2004). The ΔGbind calculation and per-residue decomposition analysis were performed as described elsewhere (Bello & García-Hernandez, 2014), and the ΔGbind values signify the average values of three experiments.

3. Results and discussion

3.1. Docking results

3.1.1. Docking interactions between TA and 3CLpro of SARS-CoV-2 and SARS-CoV

The tannic acid (TA)-docked complex with SARS-CoV-2 3CLpro (SARS-CoV-2 3CLpro/TA) and SARS-CoV 3CLpro (SARS-CoV 3CLpro/TA) showed docking scores of −9.48 and −9.60 kcal, respectively (Table 1S, supplementary material). The SARS-CoV-2 3CLpro/TA complex showed a total of 11 H-bonds in 8 residues of subunit 1 (Figure 3SA, supplementary material). Among these residues, four were similar to those in the complex between TA and SARS-CoV 3CLpro (SARS-CoV 3CLpro/TA) (Figure 4SA, supplementary material). Via H-bonding interactions of Glu166, residues Phe140, Gly143, Glu166, and Gln189 of subunit 1 differed between the SARS-CoV-2 3CLpro/TA complex and SARS-CoV-2 3CLpro/TA complex, as Glu166 had a total of 5 H-bonding interactions (Figure 5S, supplementary material) with TA in the SARS-CoV 3CLpro/TA complex but 3 H-bonds in the SARS-CoV-2 3CLpro/TA complex. Additionally, both complexes had a common H-bonding interaction with residue 46, which was a Ser residue in the SARS-CoV-2 3CLpro/TA complex but an Ala residue in the SARS-CoV 3CLpro/TA complex.

In the case of subunit 2 of the SARS-CoV-2 3CLpro/TA complex (Figure 3B, supplementary material), a total of 11 H-bonds were formed by nine residues; among these, two residues were similar to the SARS-CoV 3CLpro/TA complex (Figure 4B, supplementary material). Ser139 and Gln189, through H-bonding interactions with Gln189, differed between the SARS-CoV 3CLpro/TA complex and SARS-CoV-2 3CLpro/TA complex, as Gln189 formed a total of 2 H-bonding interactions with TA in the SARS-CoV-2 3CLpro/TA complex but only one H-bond in the SARS-CoV 3CLpro/TA complex. Subunit 1 of the SARS-CoV-2 3CLpro/TA complex had a total of 16 polar contacts with TA, and 11 of these contacts held similar positions in the SARS-CoV 3CLpro/TA complex, including Phe140, Leu141, Cys145, Met165, Met166, Leu167, Pro168, and Ala191 of chain A and ile213 and Cys300 of chain B. Subunit 2 of the SARS-CoV-2 3CLpro/TA complex also had 16 polar contacts with TA; eight residues, namely, Asn214 in subunit 1 and Thr25, His41, Ser139, Asn142, Ser144, Gln189, and Gln192 in subunit 2, were found to be shared. Both complexes also had a common polar residue, no. 169 of chain B, although in the SARS-CoV-2 3CLpro/TA complex, it was a His residue rather than Thr169 in the SARS-CoV-3CLpro/TA complex. Additionally, subunit 1 of the SARS-CoV-2 3CLpro/TA complex was observed to have a total of 14 hydrophobic contacts with TA; among them, ten contacts were shown to be similar to the SARS-CoV 3CLpro/TA complex, including Met49, Phe140, Leu141, Cys145, Met165, Leu167, Pro168 and Ala191 of chain A and ile213 and Cys300 of chain B. Subunit 2 of the SARS-CoV-2 3CLpro/TA complex formed a total of 22 hydrophobic contacts with TA; among them, 10 contacts were shown to be similar in the SARS-CoV 3CLpro/TA complex, including Phe3 and ile213 of subunit 1 and Leu27, Met49, Phe140, Leu141, Cys145, Met165, Leu67, and Ala191 of subunit 2. From a comparative observation, TA as a ligand showed a considerably higher binding affinity for the SARS-CoV-2 3CLpro enzyme than for the SARS-CoV 3CLpro enzyme.

3.1.2. Docking interactions between TF3 and 3CLpro of SARS-CoV-2 and SARS-CoV

Theaflavin-3,3-digallate (TF3) was docked with SARS-CoV-2 3CLpro (SARS-CoV-2 3CLpro/TF3) and SARS-CoV 3CLpro (SARS-CoV 3CLpro/TF3), showing docking scores of −7.20 and −7.44 kcal, respectively (Table 1S, supplementary material). Subunit 1 of the SARS-CoV-2 3CLpro/TF3 complex displayed 4 H-bonds by three residues of subunit 1 (Figure 6SA, supplementary material); among these, only one residue was similar to that in the SARS-CoV 3CLpro/TF3 complex (Figure 6SC, supplementary material), namely, Glu166 of subunit 1. The H-bonding interactions of Glu166 differed between the SARS-CoV 3CLpro/TF3 complex and SARS-CoV-2 3CLpro/TF3 complex: Glu166 formed a total of 3 H-bonding interactions...
with TF3 in the SARS-CoV 3CL\textsuperscript{pro}/TF3 complex but only 2 H-bonds in the SARS-CoV-2 3CL\textsuperscript{pro}/TF3 complex. In subunit 2 of the SARS-CoV-2 3CL\textsuperscript{pro}/TF3 complex, 4 H-bonding interactions were observed (Figure 6SB, supplementary material), of which only one residue, Glu166, was similar to that in the SARS-CoV 3CL\textsuperscript{pro}/TF3 (Figure 6SD, supplementary material) complex, which had a total of 8 H-bonds. However, it must be noted that the Glu166 residue of the SARS-CoV 3CL\textsuperscript{pro}/
TF3 complex formed 2 H-bonds, unlike that of the SARS-CoV-2 3CLpro/TF3 complex, which only formed 1 H-bond. SARS-CoV-2 3CLpro/TF3 complex subunit 1 included nine polar contacts with TF3, of which seven were common with the SARS-CoV-2 3CLpro/TF2B and SARS-CoV 3CLpro (Yadava et al., 2015, 2017). Theaflavin-3-gallate (TF2B) docked with SARS-CoV-2 3CLpro showed a docking score of $-9.44$ kcal in each subunit. Notably, 3CLpro of SARS-CoV-2 was shown to have a total of 17 H-bonds in the subunit (Figure 1), three of which were from side chains, with the others from residue backbones; among these, 5 H-bonds were in positions similar to those in the SARS-CoV 3CLpro/TA complex (Table 2, supplementary material). Therefore, the first 30 ns were removed from the 100 ns simulation for further analysis.

### Table 1. Binding free energy components of complexes between ligands and SARS-CoV-2 3CLpro (in units of kcal/mol).

| System                  | $\Delta F_{ele}$ | $\Delta F_{pol}$ | $\Delta G_{ele,ind}$ | $\Delta G_{pol,ind}$ | $\Delta G_{solv}$ |
|-------------------------|------------------|------------------|-----------------------|-----------------------|------------------|
| SARS-CoV-2 3CLpro/TAsub1| $-127.72$ (7.5)  | $-92.62$ (16.98) | $160.15$ (14.34)      | $-14.90$ (0.80)       | $-75.11$ (7.5)   |
| SARS-CoV-2 3CLpro/TAsub2| $-134.35$ (6.5)  | $-110.38$ (22.48)| $189.13$ (18.24)      | $-17.53$ (0.60)       | $-73.13$ (7.1)   |
| SARS-CoV-2 3CLpro/TF3sub1| $-58.22$ (4.7)   | $-37.47$ (7.4)   | $75.07$ (5.4)          | $-7.65$ (0.40)        | $-28.27$ (4.3)   |
| SARS-CoV-2 3CLpro/TF3sub2| $-64.26$ (5.0)   | $-55.98$ (9.4)   | $92.38$ (7.8)          | $-8.01$ (0.34)        | $-35.88$ (4.5)   |
| SARS-CoV-2 3CLpro/TF2Bsub1| $-61.08$ (5.0)   | $-86.30$ (12.9)  | $109.28$ (10.0)       | $-7.87$ (0.30)        | $-45.97$ (4.0)   |
| SARS-CoV-2 3CLpro/TF2Bsub2| $-50.46$ (5.8)   | $-57.06$ (17.7)  | $81.96$ (13.0)         | $-6.68$ (0.73)        | $-32.24$ (6.6)   |
| SARS-CoV-3CLpro/TAsub1  | $-95.40$ (7.8)   | $-134.79$ (21.7) | $183.47$ (18.0)       | $-13.53$ (0.73)       | $-60.26$ (8.8)   |
| SARS-CoV-3CLpro/TAsub2  | $-62.45$ (7.4)   | $-148.31$ (19.55)| $188.58$ (16.38)      | $-10.44$ (0.60)       | $-32.63$ (4.8)   |
| SARS-CoV-3CLpro/TF3sub1  | $-63.13$ (6.0)   | $-66.67$ (16.8)  | $104.84$ (13.0)       | $-8.53$ (0.50)        | $-35.48$ (3.3)   |
| SARS-CoV-3CLpro/TF3sub2  | $-62.83$ (4.1)   | $-52.83$ (11.4)  | $86.65$ (10.2)         | $-6.52$ (0.46)        | $-25.29$ (3.4)   |
| SARS-CoV-3CLpro/TF2Bsub1| $-59.94$ (5.1)   | $-33.25$ (9.3)   | $63.95$ (9.6)          | $-5.38$ (0.60)        | $-26.63$ (4.0)   |
| SARS-CoV-3CLpro/TF2Bsub2| $-46.94$ (5.7)   | $-82.32$ (13.5)  | $110.97$ (10.0)       | $-7.09$ (0.38)        | $-25.38$ (4.3)   |

3.1.3. Docking interactions between TF2B and 3CLpro of SARS-CoV-2 and SARS-CoV

Theaflavin-3-gallate (TF2B) docked with SARS-CoV-2 3CLpro (SARS-CoV-2 3CLpro/TF2B) and SARS-CoV 3CLpro (SARS-CoV 3CLpro/TF2B) showed docking scores of $-8.12$ and $-6.40$ kcal, respectively (Table 1, supplementary material). The 3CLpro/theaflavin-3-gallate (TF2B) complex of SARS-CoV-2 subunit 1 formed 7 H-bonding interactions (Figure 7SA, supplementary material) between 5 residues of the receptor-ligand; among them, only one residue was similar to that in the SARS-CoV 3CLpro/TF2B complex (Figure 7SC, supplementary material): Gln189 of subunit 1 and Ser1 of subunit 2. The H-bonding interactions of Glu166 and Thr190 differed between the SARS-CoV-2 3CLpro/TF2B complex subunit 1 and Ser1 of subunit 2, were also present in the SARS-CoV 3CLpro/TA complex. On the other hand, subunit 2 of SARS-CoV-2 3CLpro/TF2B also made a total of 11 polar contacts with TF2B: 9 residues, namely, Thr25, Thr26, His41, Asn142, Ser144, His163, His172, and Asn189 of subunit 1 and Ser1 of subunit 2, were also present in the SARS-CoV 3CLpro/TF2A complex. Notably, 3CLpro of SARS-CoV-2 was shown to have a total of 17 H-bonds in the subunit (Figure 1), three of which were from side chains, with the others from residue backbones; among these, 5 H-bonds were in positions similar to those in the SARS-CoV 3CLpro/TA complex (Table 2, supplementary material). Therefore, the first 30 ns were removed from the 100 ns simulation for further analysis.

3.2. MD Simulations

3.2.1. Convergence of MD simulations

Root mean squared deviation (RMSD) and radius of gyration (Rg) analysis showed that bound SARS-CoV-2 3CLpro and SARS-CoV 3CLpro reached equilibrium between 10 and 30 ns (Figures 8S and 9S, supplementary material). Therefore, the first 30 ns were removed from the 100 ns simulation for further analysis.

3.2.2. Interactions between TA and 3CLpro of SARS-CoV-2 and SARS-CoV

The complex of TA docked with 3CLpro of SARS-CoV-2 showed a docking score of $-9.44$ kcal in each subunit. Notably, 3CLpro of SARS-CoV-2 was shown to have a total of 17 H-bonds in the subunit (Figure 1), three of which were from side chains, with the others from residue backbones; among these, 5 H-bonds were in positions similar to those in the SARS-CoV 3CLpro/TA complex (Figure 2): Cys145, Glu166, Arg188 and Thr190 of subunit 1 and Ser1 of subunit 2. The H-bonding interactions of Glu166 and Thr190 differed between the SARS-CoV 3CLpro/TA complex and the SARS-CoV-2 3CLpro/TA complex, as Glu166 had a total of 3 H-bonding interactions with TF2B in the SARS-CoV-2 3CLpro/TF2B complex but only 1 H-bond in the SARS-CoV 3CLpro/TF2B complex. SARS-CoV-2 3CLpro/TF2B complex subunit 1 made a total of 11 polar contacts with TF2B: 9 residues, namely, Thr25, Thr26, His41, Asn142, Ser144, His163, His172, and Asn189 of subunit 1 and Ser1 of subunit 2, were also present in the SARS-CoV 3CLpro/TF2A complex. On the other hand, subunit 2 of SARS-CoV-2 3CLpro/TF2B also made a total of 11 polar contacts with TF2B: 5 residues, Thr25, Thr26, His41, Asn142, and Asn189, of subunit 2 were also present in the SARS-CoV 3CLpro/TF2B complex. Regarding hydrophobic interactions, it was found that the eight hydrophobic contacts of SARS-CoV-2 3CLpro/TF2B complex subunits 1 and 5 were similar to those of SARS-CoV 3CLpro/TF2B complex subunit 1; these residues were Leu27, Met49, Phe140, Leu141, and Cys145. SARS-CoV-2 3CLpro/TF2B complex subunit 2 also had 8 hydrophobic contacts, with 5 hydrophobic contacts similar to those of SARS-CoV 3CLpro/TF2A complex subunit 2: Leu27, Met49, Leu141, Cys145, and Met165 from subunit 2. These complexes were further submitted to optimization through MD simulations to observe the prevalence of the interactions identified through docking studies as performed elsewhere (Yadava et al., 2015, 2017).
bonding interactions (Figure 10SA-B, supplementary material) with TA in the SARS-CoV 3CLpro/TA complex. In contrast, only one H-bond (Figure 11SA-B, supplementary material) was formed in the SARS-CoV-2 3CLpro/TA complex, and Thr190 had a total of 2 H-bonding interactions with TA in the SARS-CoV-2 3CLpro/TA complex, while only one H-bond formed in the SARS-CoV-2 3CLpro/TA complex.

In the case of subunit 2 of the SARS-CoV-2 3CLpro/TA complex (Figure 1), a total of 15 H-bonds (3 of which were H-bonded side chains) were formed, while the SARS-CoV 3CLpro/TA complex (Figure 2) formed only eight H-bonds (5 of which were H-bonded side chains). Similarities were noticed in only 2 locations: Cys145 of subunit 2 (H-bonded backbone) and Asn214 of subunit 1 (H-bonded side chain). Another notable difference between the SARS-CoV-2 3CLpro/TA and SARS-CoV 3CLpro/TA complexes was that in the SARS-CoV 3CLpro/TA complex, there were 2 double H-bonding interactions (H-bonded side chain) with TA by Glu47 and Glu166 (Figure 10SC-D, supplementary material) of subunit 2, which were absent in the SARS-CoV-2 3CLpro/TA complex, whereas a triple H-bonding interaction (H-bonded backbone) was observed with His164 of subunit 2. Subunit 1 of the SARS-CoV-2 3CLpro/TA complex had a total of 12 polar contacts with TA, and 7 of these contacts held similar positions in the SARS-CoV 3CLpro/TA complex: residues His41, Asn142, Ser144, His163, Gln189, and Thr190 of chain A and Ser1 of subunit 2. Subunit 2 of the SARS-CoV-2 3CLpro/TA complex had a total of 14 polar contacts with TA, while the SARS-CoV 3CLpro/TA complex had only 7 polar contacts, with 5 residues, namely, Asn214 of chain A and His41, Asn142, Ser144, and Gln189 of subunit 2, found in common. Analysis of the hydrophobic interactions showed that subunit 1 of the SARS-CoV-2 3CLpro/TA complex had a total of 16 hydrophobic contacts with TA. Among these contacts, seven were shown to be similar to those in the SARS-CoV 3CLpro/TA complex, namely, Leu167, Pro168, Met49, Met165, Cys145, and Phe140 of chain A and Cys300 of subunit 2. Subunit 2 of the SARS-CoV-2 3CLpro/TA complex formed a total of 17 hydrophobic contacts with TA; six contacts were shown to be similar to those in the SARS-CoV 3CLpro/TA complex, namely, Val303 of subunit 1 and Cys44, Met49, Leu50, Phe140 and Met165 of subunit 2. From a comparative observation, TA as a ligand played only 2 protein-ligand H-bonds (backbones) through residues His164 and Arg188 (Figure 3A), bearing no similarities to the 6 residues from subunit 1 of the SARS-CoV 3CLpro/TF3 complex (Figure 3C), which formed 7 H-bonds (4 backbones, 3 side chains) since Glu166 of subunit 1 formed 2 H-bonds with TF3 (Figure 10SE-F, supplementary material). In subunit 2 of the SARS-CoV-2 3CLpro/TF3 complex (Figure 3B), 4 H-bonding interactions (backbones) were observed, of which two residues, His164 and Glu166 (Figure 11SC-D, supplementary material), were similar to those in the SARS-CoV 3CLpro/TF3 complex (Figure 3D), which only formed 2 H-bonds in total. However, it must be noted that Glu166 of the SARS-CoV 3CLpro/TF3 complex formed a side-chain H-bond (Figure 10SG-H, supplementary material), unlike the H-bonded backbone of the residue from the SARS-CoV-2 3CLpro/TF3 complex. SARS-CoV-2 3CLpro/TF3 complex subunit 1 formed eight polar contacts with TF3, of which four were common to the SARS-CoV 3CLpro/TF3 complex, namely, Glu189, His41, Asn142, and His163, and subunit 2 formed 5 polar contacts with TF3 and compared to 7 polar contacts in SARS-CoV 3CLpro/TF3 complex subunit 2, where two residues were found to be similar: His41 and His164. Subunit 1 of the SARS-CoV-2 3CLpro/TF3 complex formed 4 hydrophobic contacts with TF3, of which three residues were common to those in SARS-CoV 3CLpro/TF3 complex subunit 1: Leu27, Cys145, and Met165. On the other hand, subunit 2 of the SARS-CoV-2 3CLpro/TF3 complex formed seven hydrophobic contacts; when compared with the SARS-CoV 3CLpro/TF3 complex, three residues identical to those in subunit 1 were found to be in common: Leu27, Cys145, and Met165.

3.2.3. Interactions between TF2B and 3CLpro of SARS-CoV-2 and SARS-CoV

The 3CLpro/TF2B complex of SARS-CoV-2 subunit 1 formed 5 H-bonding interactions between the receptor ligands (Figure 4A). Residues Cys145, Glu166, and Gln189 formed these interactions, with Glu166 forming 3 H-bonds (Figure 11SE-F, supplementary material); on the other hand, the SARS-CoV 3CLpro/TF2B complex formed three side-chain H-bonds with residues His163 and His164 (Figure 4C) and one H-bond with Glu166 (Figure 10SI-J, supplementary material). For subunit 2 of the SARS-CoV-2 3CLpro/TF2B complex (Figure 48), a total of 7 H-bonds formed (3 backbones, four side chains) through residues Thr26, Asn142, His164, and Glu166 (Figure 10SK-L, supplementary material); when compared to the 5 H-bonding interactions of the SARS-CoV 3CLpro/TF2B complex (Figure 4D), the only similarity was the interaction with Glu166 (Figure 10SK-L and 115G-H, supplementary material). SARS-CoV-2 3CLpro/TF2B complex subunit 1 made six polar contacts with TF2B, among which five residues, His41, Asn142, Ser144, His163, and Asn189, were similar to the five polar contacts of the SARS-CoV 3CLpro/TF2B complex. On the other hand, for subunit 2, both the SARS-CoV-2 3CLpro/TF2B and SARS-CoV 3CLpro/TF2B complexes made seven similar polar contact ligands: Thr25, Thr26, His41, Thr45, Asn119, Asn142, and His172 from subunit 2. Moreover, it was found that the five hydrophobic contacts of SARS-CoV-2 3CLpro/TF2B complex subunit 1 were similar to 5 out of 6 hydrophobic contacts of SARS-CoV-2 3CLpro/TF2B complex subunit 1; the common residues were Met49, Phe140, Leu141, Cys145, and Met165. The six hydrophobic contacts of SARS-CoV-2 3CLpro/TF2B complex subunit 2 were similar to 6 out of ten hydrophobic contacts of SARS-CoV 3CLpro/TF2B complex subunit 2: Leu27, Met49, Phe140, Leu141, Cys145, and Met165 from subunit 2.
3.4. Binding free energy calculations

Differences in the binding free energy ($\Delta G_{\text{bind}}$) for the complexes between ligands and the SARS-CoV-2 3CL$^{\text{pro}}$ and SARS-CoV 3CL$^{\text{pro}}$ systems were estimated using the MMGBSA approach, indicating that all of the complexes are energetically favorable and guided mainly through van der Waals energy ($\Delta E_{\text{vdp}}$) and the nonpolar free energy of desolvation ($\Delta G_{\text{npol,sol}}$). Table 1 shows that the ligand reaches a higher affinity in most cases for one of the subunits of the dimeric SARS-CoV-2 3CL$^{\text{pro}}$ and SARS-CoV 3CL$^{\text{pro}}$ systems, which is consistent with the differences found in the map of interactions observed through structural analyses (Figures 1–4) and with previous reports where this energetic behavior has been observed (Bello, 2020; Bello et al., 2020). The average $\Delta G_{\text{bind}}$ values for the ligands coupled at the first and second subunits of SARS-CoV-2 3CL$^{\text{pro}}$ show that TA reaches the highest affinity, followed by TF2B and TF3. Similarly, average $\Delta G_{\text{bind}}$ values for the ligands bound at the two subunits showed that TA binds with the highest affinity to SARS-CoV-2 3CL$^{\text{pro}}$, followed by TF2B and TF3. Interestingly, this tendency is similar to that experimentally reported between these ligands and SARS-CoV-2 3CL$^{\text{pro}}$, showing $IC_{50}$ values of 3, 7, and 9.5 $\mu$M for TA, TF2B, and TF3, respectively (Chen et al., 2005). Comparative analysis of the $\Delta G_{\text{bind}}$ values of TF2B and TF3 with SARS-CoV-2 3CL$^{\text{pro}}$ indicated an affinity similar to that observed for darunavir and indinavir (Bello et al., 2020). Additionally, TA showed a higher association than saquinavir, a potent inhibitor of SARS-CoV-2 3CL$^{\text{pro}}$ previously identified through MMGBSA methods (Bello et al., 2020). Comparisons of the affinity of the three compounds for the SARS-CoV-2 3CL$^{\text{pro}}$ and SARS-CoV 3CL$^{\text{pro}}$ systems showed that they exhibit a higher affinity for SARS-CoV-2 3CL$^{\text{pro}}$ than SARS-CoV 3CL$^{\text{pro}}$. Based on this result, it is evident that TA can be proposed as an anti-COVID-19 clinical drug. TA is an FDA-approved drug used in the treatment of cold sores, diaper rash, and poison ivy. It is also taken by mouth and used directly for bleeding, chronic diarrhea, bloody urine, dysentery, and cancer (https://go.drugbank.com/drugs/DB09372).

3.5. Per-residue decomposition analysis

3.5.1. Per-residue decomposition of TA with 3CL$^{\text{pro}}$ of SARS-CoV-2 and SARS-CoV

Analysis of the residues that contribute the most to the $\Delta G_{\text{bind}}$ value for the complex between TA and subunit 1 of SARS-CoV2 3CL$^{\text{pro}}$ (Table 2S, supplementary material) showed...
that only considering those residues whose $\Delta G_{\text{bind}}$ value was $\geq 2.0 \text{kcal}$, namely, His41, Leu141, Asn142, Cys145, His163, His164, Met165, Glu166, Pro168, Gln189, and Thr190 of subunit 1 and Ser1, Ile213 and Gln299 of subunit 2, contributed significantly to the $\Delta G_{\text{bind}}$ value of the SARS-CoV-2 3CLpro/TA complex (Table 2). Asn142, Cys145, His163, His164, Glu166, Thr190, and Ser1 of subunit 1 participate in H-bond formation (Figure 1A). In subunit 2 of the SARS-CoV2 3CLpro/TA complex (Table 2S, supplementary material), His41, Met49, Ser139, Leu141, Asn142, Cys145, His163, His164, Met165, Pro168, Gln189 and Thr190 of subunit 2 and Phe3, Arg4, Asn214, Val303 and Phe305 of subunit 1 (Figure 1B) contributed significantly to the $\Delta G_{\text{bind}}$ value (Table 2); of these residues, Cys145, Glu166, and Gln189 formed hydrogen bonds (Figure 2B). A comparison of complexation at subunits 1 and 2 indicated that a different residue contributed the most to stabilizing the complexes with both subunits, where only the participation of Cys145 and Glu166 was observed in both subunits of SARS-CoV 3CLpro.

3.5.2. Per-residue decomposition of TF3 with 3CLpro of SARS-CoV-2 and SARS-CoV

Analysis of residues between TF3 and subunit 1 of SARS-CoV-2 3CLpro (Table 3S, supplementary material) showed that Thr25, Thr26, Leu27, His41, Met49, Asn142, Gly143, Ser144, Cys145, His163, His164, Met165, Gln189, Asp187, Arg188, and Gln189 contributed the most to stabilizing the complexes with both subunits, where only the participation of Cys145 and Glu166 was observed in both subunits of SARS-CoV 3CLpro (Figure 3A).
Table 2. Per-residue free energy for complexes of TA, TF3 and TF2B with SARS-CoV-2 3CL\textsuperscript{pro} and SARS-CoV 3CL\textsuperscript{pro} (values kcal/mol).

| Residue | SARS-CoV-2 3CL\textsuperscript{pro}/TA\textsubscript{sub1} | SARS-CoV-2 3CL\textsuperscript{pro}/TF3\textsubscript{sub1} | SARS-CoV-2 3CL\textsuperscript{pro}/TF2B\textsubscript{sub1} | SARS-CoV 3CL\textsuperscript{pro}/TA\textsubscript{sub1} | SARS-CoV 3CL\textsuperscript{pro}/TF3\textsubscript{sub1} | SARS-CoV 3CL\textsuperscript{pro}/TF2B\textsubscript{sub1} |
|---------|----------------|----------------|----------------|----------------|----------------|----------------|
| T25(A)  | -2.016         |                 |                 |                 |                 |                 |
| T26(A)  | -1.132         |                 |                 |                 |                 |                 |
| L27(A)  | -0.92          | -0.765         |                 |                 |                 | -0.694         |
| H41(A)  | -2.256         | -1.611         | -1.841         |                 |                 |                 |
| T45(A)  |                 |                 | -0.599         |                 |                 |                 |
| S/A46(A)| -1.516         |                 |                 |                 |                 |                 |
| E47(A)  |                 |                 | -0.247         |                 |                 |                 |
| D48(A)  | -2.016         |                 |                 |                 |                 |                 |
| M49(A)  | -2.607         | -2.32          | -3.74          |                 |                 |                 |
| L50(A)  |                 | -2.404         |                 |                 |                 |                 |
| F140(A) | -2.081         |                 |                 |                 |                 |                 |
| L141(A) | -1.091         | -2.434         | -0.952         | -1.679         |                 |                 |
| N142(A) | -4.095         | -1.989         | -1.746         | -2.207         | -3.658         |                 |
| G143(A) | -1.018         | -1.423         | -1.59          |                 |                 |                 |
| S144(A) | -0.542         | -1.318         | -0.783         |                 |                 |                 |
| C145(A) | -2.28          | -2.279         | -2.262         | -2.048         | -0.973         | -1.006         |
| H163(A) | -2.764         | -0.971         | -1.104         | -1.723         | -2.154         |                 |
| H166(A) | -2.704         | -1.779         |                 |                 |                 |                 |
| M166(A) | -4.279         | -3.098         | -2.557         | -3.743         | -3.731         | -1.342         |
| E166(A) | -2.067         | -0.71          | -5.495         | -8.312         | -3.029         |                 |
| L167(A) |                 |                 | -2.472         |                 |                 |                 |
| P168(A) | -3.036         |                 |                 |                 |                 |                 |
| D187(A) | -1.202         | -1.034         |                 |                 |                 |                 |
| R188(A) | -0.88          | -1.005         |                 |                 |                 |                 |
| Q189(A) | -2.6           | -2.025         | -3.219         | -3.45          | -2.536         | -0.808         |
| T190(A) | -2.775         |                 |                 |                 |                 |                 |
| S1(B)   | 3.782          |                 |                 |                 |                 |                 |
| I213(B) | -2.123         |                 |                 |                 |                 |                 |
| Q299(B) | -2.415         |                 |                 |                 |                 |                 |

Note: sub1 and sub2 denote interactions between the ligand and subunit 1 or 2, respectively, of dimeric SARS-CoV-2 3CL\textsuperscript{pro} and SARS-CoV 3CL\textsuperscript{pro}. A and B denote subunit 1 and 2, respectively.
At the second subunit (Table 25, supplementary material), Leu27, Pro39, His41, Thr45, Met49, Leu50, Cys145, His164, Met165, Glu166, Leu167, Asp187, Arg188, Gln189, and Gln192 (Table 2) were the main contributors to the $\Delta G_{\text{bind}}$ values, of which only His164, Glu166, Asp187, and Gln192 were observed to form hydrogen bonds (Figure 3B). Similar to that observed for the complexes between TA and SARS-CoV-2 3CLpro, complexes between TF3 and SARS-CoV-2 3CLpro shared a high number of similar residues: Leu27, His41, Met49, Cys145, His164, Met165, Glu166, Asp187, Arg188, and Gln189. Between TF3 and subunit 1 of SARS-CoV 3CLpro (Table 35, supplementary material) residues Leu27, His41, Thr45, Ala46, Glu47, Asp48, Phe140, Leu141, Asn142, Cys145, His163, Met165, Glu166, and Gln189 (Table 2) were shown to contribute the most to $\Delta G_{\text{bind}}$ of these residues, Ala46, Asp48, Glu47, Asn142, and Glu166 formed hydrogen bonds with polar moieties of TF3 (Figure 3C). In the second subunit (Table 35, supplementary material), Thr25, Thr26, Leu27, His41, Val42, Phe140, Asn142, Gly43, Ser144, Cys145, His163, His164, Met165, and Glu166 were the main contributors to the affinity (Table 2). Of these residues, only His164 and Glu166 formed hydrogen bonds, with the rest making polar and nonpolar contacts. Comparative analysis of the complexes between TF3 and SARS-CoV-2 3CLpro and SARS-CoV 3CLpro shows that the complexes shared eight residues in common: Leu27, His41, Phe140, Asn142, Cys145, His163, M165, and Glu166.

3.5.3. Per-residue decomposition of TF2B with 3CLpro of SARS-CoV-2 and SARS-CoV

TF2B bound at subunit 1 of SARS-CoV-2 3CLpro (Table 25, supplementary material) indicates that His41, Ser46, Met49, Phe140, Leu141, Asn142, Gly143, Ser144, Cys145, His163, Met165, Glu166, Asp187, Arg188, and Gln189 (Table 2) are the primary stabilizers of the complex, with Cys145, Glu189, and Glu166 forming hydrogen bonds with polar atoms of TF2B and the rest creating polar and nonpolar interactions (Figure 4A). The complex between TF2B and subunit 2 of SARS-CoV-2 3CLpro (Table 25, supplementary material) was mainly stabilized by Thr25, Thr26, Leu27, Ser46, Met49, Phe140, Leu141, Asn142, Gly143, Cys145, His163, His164, Met165, Glu166, and Gln189 (Table 2); of these residues, Thr26, Asn142, His164 and Glu166 formed hydrogen bonds with the polar groups of TF2B (Figure 4B). Comparison of the residues involved in the stabilization of TF2B by both subunits shows a high number of similar residues: Ser46, Met49, Phe140, Leu141, Asn142, Gly143, Cys145, His163, His164, Met165, Glu166, and Gln189.

In subunit 1 of SARS-CoV 3CLpro (Table 35, supplementary material), TF2B was mainly stabilized by His41, Ala46, Met49, Phe140, Leu141, Asn142, Gly43, Ser144, Cys145, His163, Met165, and Gln189 (Table 2), of which only His163 formed a hydrogen bond, with the rest making polar and nonpolar interactions (Figure 4C). In contrast, in subunit 2 of SARS-CoV 3CLpro (Table 35, supplementary material), TF2B was mainly stabilized by Thr25, Thr26, Leu27, His41, Val42, Cys44, Thr45, Ala46, Met49, Tyr118, Asn119, Leu141, Ans142, Gly143, Cys145, Met165, and His172 (Table 2), with His41, Gly143 and His172 forming hydrogen bonds (Figure 4D). A comparison of the residues in both subunits of SARS-CoV 3CLpro indicates that eight residues are present in both subunits, namely, His41, Ala46, Met49, Leu141, Asn142, Gly43, Cys145, and Met165, a lower number than that observed for the complexes between TF2B and SARS-CoV-2 3CLpro.

In general, this analysis takes into consideration the participation of His41 and Cys145, two conserved residues (Nukoolkarn et al., 2008), in molecular recognition and highlights the relevance of other residues (Met49, Asn142, His163, Met165, Glu166, Asp187, and Gln189) in the stabilization of ligands; these residues are similar to those previously observed for ligand stabilization in SARS-CoV-2 3CLpro and SARS-CoV 3CLpro systems (Bello, 2020; Bello et al., 2020).

4. Conclusion

In this contribution, we first performed docking studies of three plant-derived compounds previously reported to be SARS-CoV 3CLpro inhibitors on dimeric SARS-CoV-2 3CLpro and SARS-CoV 3CLpro, after which 100-ns MD simulations combined with the MMGBSA approach were employed to compare the results. Our results showed that the binding affinity of the three natural compounds in complex with SARS-CoV 3CLpro reproduced the experimental affinity tendency, in which tannic acid showed the highest association. Comparing the binding affinity of the three compounds between and SARS-CoV-2 3CLpro and SARS-CoV 3CLpro revealed that the compounds exhibited a higher affinity for SARS-CoV-2 3CLpro than SARS-CoV 3CLpro, suggesting that these three compounds may have potential as inhibitors of SARS-CoV-2 3CLpro. In addition, per-residue free energy decomposition allowed identification of hot-spot residues (His41, Met49, Cys145, Asn142, His163, Met165, Glu166, Asp187, and Gln189), which contributed significantly to the total binding affinity. Of these residues, His41 and Cys145 are conserved residues that are considered necessary for ligand binding.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The work was supported by grants from CONACYT (CB-A1-S-21278) and SIP/IPN (20210516).

ORCID

Martiniano Bello https://orcid.org/0000-0002-9686-0755
Md. Kamrul Hasan https://orcid.org/0000-0002-3032-7640

References

Andrianov, A. M., Kornoushenko, Y. V., Karpenko, A. D., Bosko, I. P., & Tuzikov, A. V. (2020). Computational discovery of small drug-like
pathogenic coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV. Journal of Medical Virology, 92(5), 491–494. https://doi.org/10.1002/jmv.25709

Ludwig, S., & Zarbock, A. (2020). Coronaviruses and SARS-CoV-2: A brief overview. Anesthesia & Analgesia, 131(1), 93–96. https://doi.org/10.1213/ANE.0000000000004845

Masters, P. S. (2006). The molecular biology of coronaviruses. Advances in Virus Research, 66, 193–292.

Miller, B. R., McGee, T. D., Swails, J. M., Homeyer, N., Gohlke, H., & Nguyen, J. L., Yang, W., Ito, K., Matte, T. D., Shaman, J., & Kinney, P. L. (2015). Theoretical Biology and Medical Chemistry, 30(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Nguyen, J. L., Yang, W., Ito, K., Matte, T. D., Shama, J., & Kinney, P. L. (2016). Seasonal influenza infections and cardiovascular disease mortality. JAMA Cardiol, 1(3), 274–281. Jun. 2016. https://doi.org/10.1001/jamacardio.2016.0433

Nukoolkarn, V., Lee, V. S., Malaisree, M., Arursakulwong, O., & Hanningbua, S. (2008). Molecular dynamic simulations analysis of ritonavir and lopinavir as SARS-CoV 3CLpro inhibitors. Journal of Theoretical Biology, 254(4), 861–867. https://doi.org/10.1016/j.jtbi.2008.07.030

Onufriev, A., Bashford, D., & Case, D. A. (2004). Exploring protein native states and large-scale conformational changes with a modified generalized born model. Proteins: Structure, Function, and Bioinformatics, 55(2), 383–394. https://doi.org/10.1002/prot.20033

Petrosillo, N., Vicente, G., Ergonoul, O., Ippolito, G., & Petersen, E. (2020). COVID-19, SARS and MERS: Are they closely related? Clinical Microbiology and Infection, 26(6), 729–734. Jun. 2020. https://doi.org/10.1016/j.cmi.2020.03.026

Schrödinger, LLC. Maestro, Version 10.S. New York, NY, USA: 2016–1.

Yavuz, S., & Ünal, S. (2020). Antiviral treatment of Covid-19. Turkish Journal of Medical Sciences, 50(S1-1), 611–619. https://doi.org/10.3906/sag-2004-145

Sinha, N., & Balayla, G. (2020). Hydroxychloroquine and covid-19. Postgraduate Medical Journal, 96(1139), 550–555. 2020. https://doi.org/10.1136/postgradmedj-2020-137785

Van Gunsteren, W. F., & Berendsen, H. J. C. (1977). Algorithms for macro-molecular dynamics and constraint dynamics. Molecular Physics, 34(5), 1311–1327. https://doi.org/10.1080/00268977700102571

Wang, J., Wolf, R. M., Caldwell, J. W., Kollman, P. A., & Case, D. A. (2004). Development and testing of a general amber force field. Journal of Computational Chemistry, 25(9), 1157–1174. https://doi.org/10.1002/jcc.20035

Woo, P. C. Y., Huang, Y., Lau, S. K. P., & Yuen, K. Y. (2010, September). Coronavirus genomics and bioinformatics analysis. Viruses, 2(8), 1804–1820. https://doi.org/10.3390/v2081803

Xu, Y., & Chen, Q. (2020, May). Insight into 2019 novel coronavirus — An updated interim review and lessons from SARS-CoV and MERS-CoV. International Journal of Infectious Diseases, 94, 119–124. https://doi.org/10.1016/j.ijid.2020.03.071

Yadava, U. (2018). Search algorithms and scoring methods in protein-ligand docking. International Journal of Endocrinology, 6(6), 359–367.

Yadava, U., Shukla, B. K., Roychoudhury, M., & Kumar, D. (2015). Pyrazolo [3, 4-d] pyrimidines as novel inhibitors of O-acetyl-l-serine sulphydrylase of Entamoeba histolytica: An in silico study. Journal of Molecular Modeling, 21(4), 1–13. https://doi.org/10.1007/s00894-015-2631-3

Yadava, U., Singh, M., & Roychoudhury, M. (2013). Pyrazolo [3, 4-d] pyrimidines as inhibitor of anti-coagulation and inflammation activities of phospholipase A 2: Insight from molecular docking studies. Journal of Biological Physics, 39(3), 419–438. https://doi.org/10.1007/s10867-013-9299-7

Yadava, U., Yadav, V. K., & Yadav, R. K. (2017). Novel anti-tubulin agents from plant and marine origins: Insight from a molecular modeling and dynamics study. RSC Advances, 7(26), 15917–15925. https://doi.org/10.1039/C7RA00370F

Zhang, L., Lin, D., Kusov, Y., Nian, Y., Ma, Q., Wang, J., von Brunn, A., Leslyen, F., Po Lanko, K., Neys, J., de Wilde, A., Snijder, E. J., Liu, H., & Hilgenfeld, R. (2020). z-Ketoamides as broad-spectrum inhibitors of coronavirus and enterovirus replication: Structure-based design, synthesis, and activity assessment. Journal of Medicinal Chemistry, 63(9), 4562–4578. https://doi.org/10.1021/acs.jmedchem.9b01828

Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., & Hilgenfeld, R. (2020). Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved z-ketoamide inhibitors. Science, 368(6489), 409–412. https://doi.org/10.1126/science.abb3405

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., ... Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

Ziebuhr, J., Heusipp, G., & Siddell, S. G. (1997). Biosynthesis, purification, and characterization of the human coronavirus 229E 3C-like protease. Journal of Virology, 71(5), 3992–3997. 1997.https://doi.org/10.1128/jvi.71.5.3992-3997.1997

Ziebuhr, J., Snijder, E. J., & Gorbalenya, A. E. (2000). Virus-encoded proteases and proteolytic processing in the Nidovirales. Journal of General Virology, 81(4), 853–879. https://doi.org/10.1099/0022-1317-81-4-853