Computational Docking Study of Calanolides as Potential Inhibitors of SARS-CoV-2 Main Protease

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Despite the nationwide effort provided to combat the COVID-19 pandemic, we have yet to approve a specific antiviral treatment against the SARS-CoV-2. We have studied the molecular interactions between two anti-HIV-1 natural drugs, +(-) calanolide A and -(-) calanolide B, and the active site of $3CL^{pro}$ through a computational docking method. Our promising results show that the two compounds of this study are potential inhibitors of the SARS-CoV-2 $3CL^{pro}$ through strong binding to its catalytic dyad. Considering its progress in clinical trials as an anti-HIV-1 treatment, we suggest that +(-) calanolide A is a good candidate for the treatment of COVID-19.

Introduction

The current emergence of the recent Coronavirus outbreak COVID-19 in Wuhan, Hubei Province, China has quickly become the first coronavirus pandemic according to the World Health Organization (WHO) [1]. The Previous Coronavirus outbreaks such as Severe Acute Respiratory Syndrome (SARS-CoV) in China and the Middle East Respiratory Syndrome (MERS-CoV) in Saudi Arabia were less contagious and showed less viral spread levels compared to the new SARS-CoV2. Although the reproductive number (R0) of COVID-19 is high, its fatality rate is lower than SARS (9.5%) and MERS (34.4%) [2].

As of 18 January 2022, data from the WHO indicate that the worldwide total laboratory-confirmed COVID-19 cases are around 300 million with almost 5.6 million deaths. In the same report, new cases seem to be reduced in Western Pacific Region and Africa compared to Europe and Americas where the most important numbers of the newly confirmed cases were reported [3]. The virus transmission may occur through exposure to droplets produced by coughing or direct contact with

infected persons. Symptoms appear after a medium incubation period of 5.2 days [4]. At the onset, patients may express cough, fever, headache, and fatigue. In severe cases, dyspnea and pneumonia with ground-glass opacities were observed. However, some patients remain asymptomatic and do not express any symptoms all over the infection period [4, 5]. More recently, it has been noted that patients infected with COVID-19 may also report anosmia and dysgeusia [6]. Those symptoms were associated with thrombocytopenia, lymphopenia, and high levels of proinflammatory cytokines e.g. IL7, TNFα [7]. Moreover, new symptoms such as neuropsychiatric symptoms can appear with the new variants of the virus e.g 501.V2 and B.1.1.7 [8].

SARS-CoV-2 belongs to the ß-coronavirus genus, the Coronavirinae subfamily and the Coronaviridae family. Those are enveloped positive-sense (+) single-stranded RNA viruses. SARS-CoV-2 main targets are human lung epithelial cells that express angiotensin-converting enzyme 2 (ACE2) on their surface [9].

The receptor-binding domain of the virus spike glycoprotein binds to ACE2 on the epithelial cell surface inducing receptor-mediated endocytosis [10]. The virus then releases its nucleocapsid in the cytoplasm. Once there, the viral replication starts involving both viral and cellular factors. Two open reading frames ORFa1 and ORFb1 on the viral RNA are translated into replicase polyproteins pp1a and pp1ab containing nonstructural but functional polypeptides that are involved in viral replication and transcription [11]. The SARS-CoV-2 main protease (M^{pro}), also known as 3-chymotrypsin-like cysteine protease ($3CL^{pro}$) accomplishes the proteolysis of ppa1 and pp1ab into functional polypeptides [12]. $3CL^{pro}$ is a cysteine protease containing a Cysteine (Cys)-histidine (His) dyad at the active site. HIS residue enhances the nucleophilicity of the CYS through the deprotonation of the CYS thiol. Once deprotonated, the nucleophilic CYS attacks the carbon of the reactive peptide bond [13]. The $3CL^{pro}$ action on replicase polyproteins is a fundamental step in the viral cycle, which makes this enzyme a main target of antiviral therapy against SARS-CoV-2.

After almost two years of the pandemic, data indicate that it remains a global health threat. Basing on the available data about the virus existing variants and vaccines with multiples characteristics, a mathematical model was developed to simulate the virus spread. The study results showed that a scenario of uncontrolled new wave of a new variant would happen even with vaccination and spread control measures, indicating that the health situation is still critical [14]. To this day, we have yet to approve an available treatment against COVID-19. First-line treatments include acetaminophen for fever and guaifenesin for nonproductive cough. Considering their possible adverse effects

based on previous epidemic [15], corticoids are applied only in short-term small dosage in severe cases to restore hemodynamic stability [16]. Melatonin was proposed as a safer anti-inflammatory agent for managing excessive inflammation features of COVID-19 [17]. Antimalarials, chloroquine, and hydroxychloroquine were among the most discussed treatment options against COVID-19 effects. *In vitro* studies have proved Chloroquine efficacy against multiple micro-organisms including coronaviruses via the inhibition of binding to cell receptors, endosome-mediated viral entry, post-translational modification of viral proteins, or blocking the viral replication cycle. That may depend on the pathogen under study. It was therefore suggested that hydroxychloroquine is more effective against SARS-CoV-2, but both of them showed promising results, and their clinical trials were highly recommended [18, 19, 20].

Anti-HIV and Broad-spectrum antiviral agents are also suggested for treating the SARS-CoV-2 infection. National Health Commission of the People's Republic of China recommends IFN- α and Lopinavir/Ritonavir combination as antiviral treatments. Therefore, Remdesivir, an eventual treatment of the Ebola virus infection, seems to provide more efficiency against the COVID-19 [21]. More recently, anticoagulant therapy was recommended to control the thromboembolic complications associated with the COVID-19 infection although an anticoagulant related hemorrhage may occur [22].

Furthermore, Traditional Chinese Medicine (TCM) had a part in coronaviruses related diseases treatment since 2003. Nowadays, patient data obtained from clinical trials in China of more than 60,000 COVID-19 cases showed high effectiveness of TCM against the disease's complications when associated with antiviral treatments [23]. In fact, natural products may play a crucial role in COVID-19 outbreak management. Despite their confirmed active antiviral effects, many bioactive compounds haven't been tested yet. Those include pyranocoumarins, effective antiviral agents with diverse mechanisms of action and molecular targets [24].

Calanolides are a family of HIV reverse transcriptase inhibitor pyranocoumarins isolated from tropical plants of the genus Calophyllum. In the last two decades, several *in vivo* and *in vitro* studies have noted that calanolides are potent agents against *Mycobacterium tuberculosis*, human cytomegalovirus and HIV-1 drug-resistant mutant strains [25, 26, 27]. Regarding their promising antiviral activities compared to other existing antivirals, they may be good candidates as drugs in COVID-19 treatment. Hence, we report molecular docking results of calanolides eventual inhibiting effect of the SARS-CoV-2 main protease.

Materials and methods

SARS-CoV-2 3CL^{pro} three-dimensional structure

SARS-CoV-2 main protease is a dimer that targets many cleavage sites on the replicase polyproteins. Each protomer A or B of the dimer contains ß-six-stranded antiparallel barrels constituting domains I and II where the active sites are located. A five helices globular cluster (Domain III) from each protomer is involved in the dimerization [28]. The dimerization of the enzyme is important for its activity but only protomer A is active in the dimer [29]. A 3D model of the protomer A in the active state based on a crystal structure (resolution: 1.75 Å) was downloaded from the Protein Data Bank (http://www.rcsb.org) as a PDB file (PDB ID:6Y2E) [28].

Preparation of Ligands

Regarding their important action against HIV-1 strains, enantiomers +(-) calanolide A and -(-) calanolide B with the molecular formula ($C_{22}H_{26}O_5$) were chosen for the study. +(-) calanolide A and -(-) calanolide B are the main pyranocoumarins isolated from various species of *Calophyllum* (Fig. 1) [30]. 3D conformers of the two ligands were obtained from the Pubchem open chemistry database as SDF files (https://pubchem.ncbi.nlm.nih.gov/) [31] and then converted into PDB files using Open Babel 2.3.2 software [32]. The chemical structures of +(-) calanolide A and -(-) calanolide B were modeled using the Gauss View 5.0 software. Molecular energy optimization of the ligand structures was performed using Gaussian 09 software [33] with the density functional theory (DFT) method using Becke's three-parameter hybrid functional (B3LYP) through the 6-311G (d,p) basis set. We used Nelfinavir as a drug control. Nelfinavir is an approved anti-HIV-1 considered as a potent SARS-CoV-2 3CL^{pro} inhibitor [11].

Molecular docking

We used Auto dock 1.5.6 applying the Vina scoring function to perform docking calculations [34]. After deleting water molecules and other non-protein atoms, the PDB format of the protein was modified by adding polar hydrogens, Kolman and Gasteiger charges. PDB ligand files were also converted to a PDBQT format. A grid box of (84 x 88 x 58 Å) (x,y,z sizes), and spacing of 0.375 Å was centered at (x, y, z) (-16.609, -33.619, 17.843) around the active site of the enzyme. Conformations were then visualized through Discovery Studio 2020 [35] and PyMOL software [36].

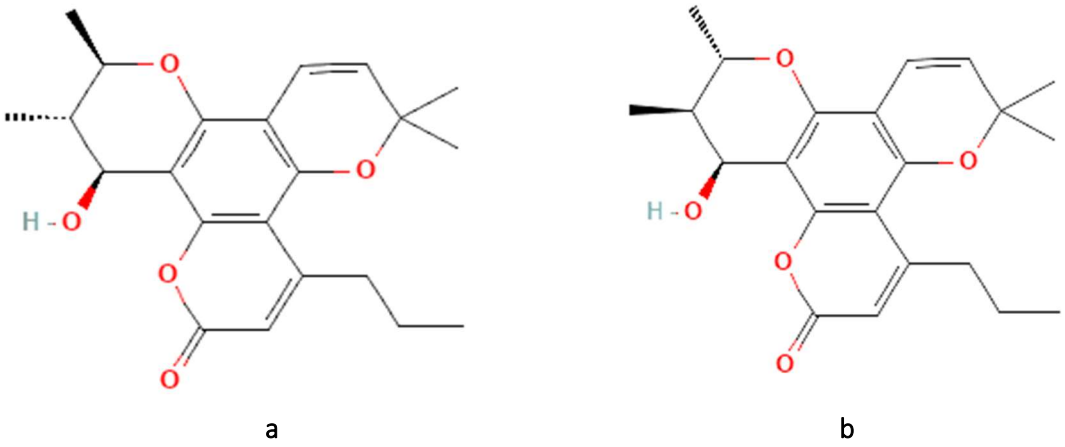

Figure 1. 2D structure of +(-) calanolide A (a) and -(-) calanolide B (b). (https://pubchem.ncbi.nlm.nih.gov/) [31]

Results and discussion

Molecular docking provides an essential piece of information about interactions between ligands and proteins and predicts the best conformations according to their interaction profile. It can improve the knowledge about new and approved drugs and their ability to modify proteins functions. In this study, we evaluated the ability of +(-) calanolide A and -(-) calanolide B, two patented anti-HIV-1 compounds [37], to inhibit the SARS-CoV-2 M^{pro} activity by binding to its active site using computational docking based on the enzyme crystal structure. We also used Nelfinavir as a drug control in our study. Nelfinavir is a recommended drug for the COVID-19 treatment [38]. It has provided the best inhibition activity against the SARS-CoV-2 replication *in vitro* among a series of HIV-1 protease inhibitors [39]. The best binding conformations are those in which ligands bind exactly to the conserved dyad or amino acids of the active site pocket i.e. Gly143, Ser144 forming with Cys145, the canonical oxyanion hole of the enzyme [28]. Docking results show that the two ligands successfully bind to the active site with a binding energy of -7.16 and -6.73 kcal/mol respectively for +(-) calanolide A and -(-) calanolide B. Calanolide A binds to the active site of the enzyme as strong as Nelfinavir that provides the lowest free binding energy of -7.2. Also, the calculated inhibition constants (K_i) values of +(-) calanolide A, -(-) calanolide B and nelfinavir were significantly lower (Table1) as compared to other coumarin derivatives that we have studied (the results are not presented in this paper). It is well established that the smaller the K_i the better the affinity of the ligand. In a comparative study of experimental and calculated values of monoamine oxidase-B inhibitors, there was an acceptable correlation between the docking estimated and the

experimental Ki values. Although, Ki experimental values may be lower and experiments remain the best solution to confirm the inhibitors efficiency [40]. We suggest that the two compounds may provide an important antiviral activity against the new SARS-CoV-2 as well.

Table 1. Interactions characteristic of calanolides A, B, nelfinavir and $3CL^{pro}$

Ligands	+(-) calanolide A	-(-) calanolide B	Nelfinavir
Binding energy	-7.16	-6.73	-7.20
(K_i) M	-6.73	11.73	5.28
Number of H bonds	-7.20	4	2

Calanoide A forms three hydrogen bonds (H bonds) with the active Cys145. Calanolide A accepts two H bonds of (H…O: 3.03 Å) and (H…O: 2.08 Å) from the sulfhydryl, and the amino groups of Cys 145 respectively and donates one H bond from the hydroxyl group of the pyranol moiety accepted by the sulfur of Cys 145(H…S: 2.55 Å) (Fig.2, a, c). Also, it forms six hydrophobic bonds with Cys145, His41, Met49, Met165 (2 bonds), and Leu27 (Fig. 3, a). Calanolide B binds tightly to the active dyad of the enzyme. It accepts two H bonds of (H…O: 3.01 Å) and (H…O: 1.93 Å) from the sulfhydryl and the amino groups of Cys145 respectively. Cys145 sulfhydryl and the double-bonded nitrogen of the imidazole ring nitrogen accepts two H bonds from the hydroxyl group of the pyranol moiety of (S…H: 2.34 Å) and (N…H: 2.58 Å) respectively (Fig. 2, b, d).

Additionally, four hydrophobic bonds are formed between calanolide B and His41 (2 bounds), Cys145 and Met49 (Fig. 3, b). Although H bonds are the strongest bonds formed in the complex, hydrophobic bonds contribute to the complex stability by projecting the ligand thoroughly in the binding site [42]. Moreover, 2D diagrams of calanolides A and B complexes with the active site of the $3CL^{pro}$ show Pi-sulfur bonds formed between the Cys145, and the two rings of the chromen-2-one moiety of ligands, which may strengthen the formed complexes (Fig. 4).

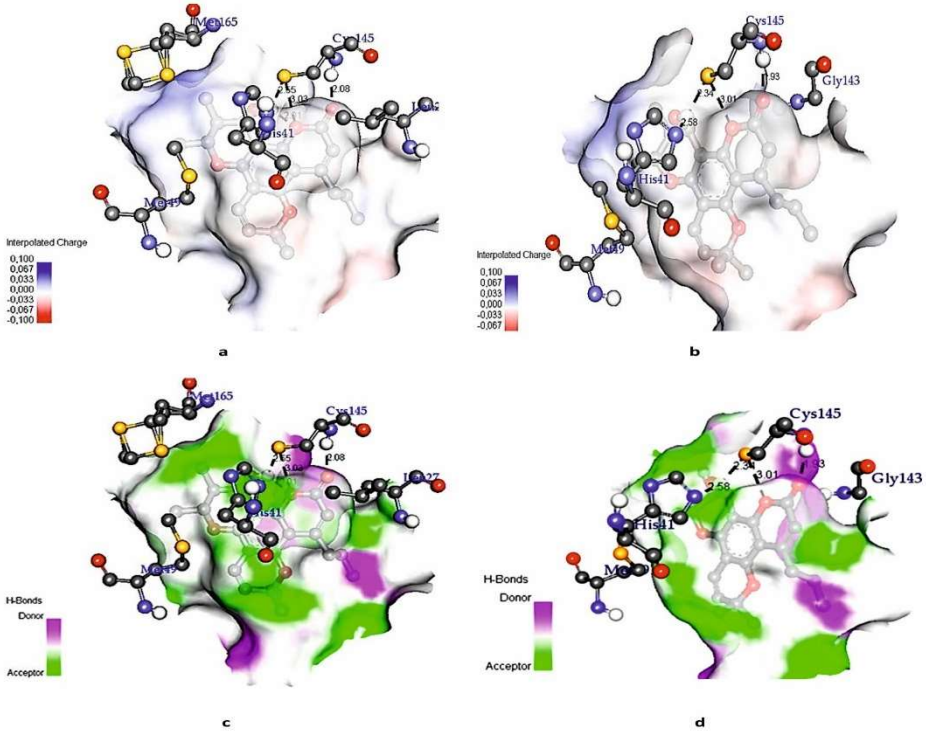

Figure 2. Docking results of calanolide A (a, c) and calanolide B (b, d) bound to the active site of SARS-CoV-2 main protease 3CLpro *(H bonds are presented in black dashed lines, Carbon atoms in grey, Oxygen in red, Nitrogen in blue, Sulfur in yellow and Hydrogen in white)*.

Based on these results, we suggest that +(-) calanolide A and -(-) calanolide B are good candidates as antiviral drugs against the COVID-19. With three H bonds, six hydrophobic bonds and two Pi-sulfur bonds, calanolide A binds more tightly to the active site of the SARS-CoV-2 main protease and may inhibit its activity. These interaction characteristics are promising compared to those of Nelfinavir used as control. Moreover, according the Lipinski's rule, physicochemical properties of a compound are critical determinants of its oral bioavibility. A compound with a good oral bioavibility

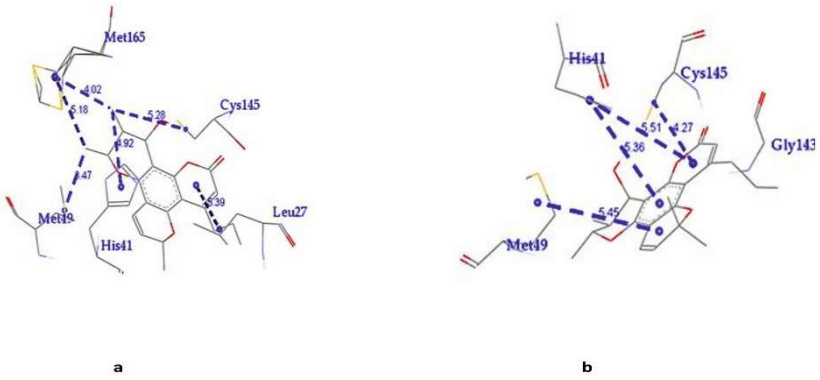

Figure 3. Hydrophobic interactions of calanolide A (a) and Calanolide B (b) with the active site amino acids (*hydrophobic bonds are presented in blue dashed line).*

must have a molecular weight (MW) ≤ 500 g/mol, no more than 5 hydrogen bond donors (HBD), 10 hydrogen bond acceptors (HBA) and 2 rotatable bonds (RB); a n-octanol-water partition coefficient logP ≤ 5 and a polar surface area (PSA) < 140 $Å^2$ [43]. Calanolides A and B characterized by a MW of 370.4 g/mol, one HBD, five HBA, two RB, logP = 3.8 and a PSA= 65 $Å^2$ have no violation of the lipinski's rule indicating their good theoretical oral bioavailability [31]. However, others parameters may limit the oral bioavailability of drug-like molecules particularly the intestinal efflux transporters [44]. Also, 14,6% of available drugs approved by the Food and Drug Administration (FDA) don't obey the Lipinski's rule perfectly [45]. Those points implicate further *in vitro* and *in vivo* pharmacological studies to confirm these drug-like molecules bioavailability. In fact, +(-) calanolide A favorable pharmacokinetic profile was already confirmed *in vivo* [46], and it had made significant progress in preclinical and clinical trials. According to the online clinical studies database (clinicaltrials.gov), providing detailed information about drugs under clinical study, calanolide A completed the phase I as an anti-HIV treatment. Most of the adverse effects of Calanolide A especially dizziness, taste perversion, headache, eructation, and dyspepsia were considered to be mild (grade 1) [47]. Taken together, these points support the possibility of calanolide A to be used as an antiviral treatment against the highly spreading COVID-19 pandemic.

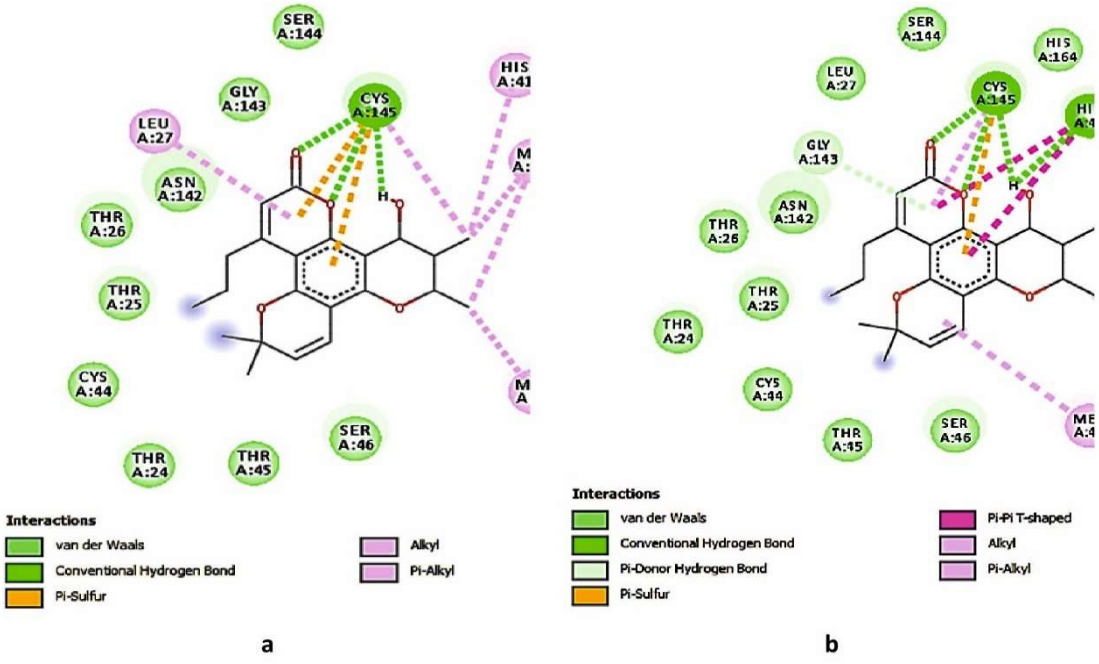

Figure 4. 2D diagram of docking results of calanolide A (a) and calanolide B (b) in the active of SARS-CoV-2 main protease $3CL^{pro}$.

Conclusion

We provide new insight into the COVID-19 treatment through molecular docking based on proven anti-HIV natural drugs, +(-) calanolide A and -(-) calanolide B, Nelfinavir as a drug control and SARS-CoV-2 main protease $3CL^{pro}$ as a drug target. We have studied the molecular interactions between these compounds and amino acids of the $3CL^{pro}$ catalytic site. As +(-) calanolide A and -(-) calanolide B bind strongly to the catalytic dyad of the enzyme, we suggest that they are potent inhibitors of $3CL^{pro}$ and SARS-CoV-2 viral cycle consequently. Among these two compounds, we noted that +(-) calanolide A provides the best binding affinity to the active site of the virus enzyme. Further experimental and clinical studies may confirm the calanolide A efficiency in the COVID-19 treatment and provide a better contribution to the ongoing effort to combat the COVID-19 pandemic.

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