Saudi Pharmaceutical Journal 32 (2024) 101986

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Saudi Pharmaceutical Journal

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Utilizing sinapic acid as an inhibitory antiviral agent against MERS-CoV PLpro

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ARTICLE INFO

Keywords:
MERS-CoV
Papain-like protease
Sinapic acid
Antiviral agent
Thermal Shift Assay

ABSTRACT

Concerns about the social and economic collapse, high mortality rates, and stress on the healthcare system are developing due to the coronavirus onslaught in the form of various species and their variants. In the recent past, infections brought on by coronaviruses severe acute respiratory syndrome coronaviruses (SARS-CoV and SARS-CoV-2) as well as middle east respiratory syndrome coronavirus (MERS-CoV) have been reported. There is a severe lack of medications to treat various coronavirus types including MERS-CoV which is hazard to public health due to its ability for pandemic spread by human-to-human transmission. Here, we utilized sinapic acid (SA) against papain-like protease (PLpro), a crucial enzyme involved in MERS-CoV replication, because phyto-medicine derived from nature has less well-known negative effects. The thermal shift assay (TSA) was used in the current study to determine whether the drug interact with the recombinant MERS-CoV PLpro. Also, inhibition assay was conducted as the hydrolysis of fluorogenic peptide from the Z-RLRGG-AMC-peptide bond in the presence of SA to determine the level of inhibition of the MERS-CoV PLpro. To study the structural binding efficiency Autodock Vina was used to dock SA to the MERS-CoV PLpro and results were analyzed using PyMOL and Maestro Schrödinger programs. Our results show a convincing interaction between SA and the MERS protease, as SA reduced MERS-CoV PLpro in a dose-dependent way IC_{50} values of 68.58 μM (of SA). The TSA showed SA raised temperature of melting to 54.61 °C near IC_{50} and at approximately 2X IC_{50} concentration (111.5 μM) the Tm for SA + MERS-CoV PLpro was 59.72 °C. SA was docked to MERS-CoV PLpro to identify the binding site. SA bound to the blocking loop (BL2) region of MERS-CoV PLpro interacts with F268, E272, V275, and P249 residues of MERS-CoV PLpro. The effectiveness of protease inhibitors against MERS-CoV has been established and SA is already known for broad range biological activity including antiviral properties; it can be a suitable candidate for anti-MERS-CoV treatment.

1. Introduction

Coronaviruses, which generally infect humans, comprise six subtypes belonging to four genera that cause respiratory diseases, such as pneumonia, bronchitis, and acute respiratory distress syndrome (ARDS) (Fung and Liu, 2019). Middle East respiratory syndrome corona-virus (MERS-CoV) is one of the coronaviruses that infect humans and was first reported from Saudi Arabia in 2012 (Zaki et al., 2012). Owing to the ability of swift transmission from human-to-human, MERS-CoV rapidly spread to more than 30 countries, causing approximately 800 deaths from 2012 to 2019(Adhikari et al., 2019; Al Mutair and Ambani, 2020).

Complete sequencing of the 30-kb genomic DNA of MERS-CoV revealed that it belongs to group C β-coronaviruses. The open reading frame 1 (ORF1) sequence of MERS-CoV encodes S protein, E protein, N protein, and structural proteins, including ORF1a, which encodes two proteases, papain-like protease (PLpro) and 3C-like protease (3CLpro) (Báez-Santos et al., 2014; Phan et al., 2018; Woo et al., 2012). Proteases are essential for viral RNA synthesis, replication, and propagation in host cells. Owing to their conserved regions, diverse roles in viral pathogenesis, and interactions with the hosts, proteases are prime targets for the inhibition of viruses (Anderson et al., 2009; Lin et al., 2014). In recent work, MERS-CoV PLpro and 3CLpro were expressed in cells so

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https://doi.org/10.1016/j.jsps.2024.101986
Received 5 October 2023; Accepted 5 February 2024
Available online 8 February 2024

that they could be used as biosensors, which has equipped researchers with the tools for rapid development of protease inhibitors. However, further research is required for the development of novel MERS drugs, which are cost-effective and potent with minimal side effects (Kilianski et al., 2013).

Many inhibitors of these proteases, which have promising therapeutic uses, are emerging, including the treatment of neurological diseases such as Alzheimer's disease (Hasanbasic et al., 2016) and cancers (López-Otín and Matrisian, 2007), as well as those caused by parasite-like fungi (Yike, 2011) and viral infections including HIV(Cheenpracha et al., 2006), hepatitis (Abian et al., 2013), and herpes (Waxman and Darke, 2000). These proteases are also necessary for the transmission of various illnesses. Inhibitors of HIV-1 proteases (Nutan et al., 2013) are notable because of the rapidity with which they are made available to humans. Many protease inhibitors for other coronaviruses including SARS-2 main protease (Mpro) and the papain-like protease (PLpro) are been developed and under clinical trials like GRL0617, YM155, cryptotanshinone and peptide-drug conjugates (PDCs) (Brian Chia and Pheng Lim, 2023; Tan et al., 2022). Intriguingly, the viral aspartic protease inhibitors used to treat HIV infections also sup-pressed the growth of Candida. Currently, several protease inhibitors that reduce or stop disease development are being generated. Protease inhibitors should be extremely powerful and highly selective in their binding to specific proteases to be useful as bio-logical tools. To be considered potential medications, protease inhibitors must also display suitable pharmacokinetic and pharmacodynamic features (Anderson et al., 2009).

Drugs that target the virus or host and its immune system are potential therapeutics for diseases caused by coronaviruses. Resveratrol, a well-known phytoalexin, and emodin, an anthraquinone, have demonstrated strong inhibitory effects against MERS-CoV and severe acute respiratory syndrome (SARS)-CoV in *in vitro* experiments (Duca et al., 2019). Curcumin, hesperetin, hesperidin, tangeretin, naringenin, tet-ra-O-galloyl-beta-D-glucose (TGG), and luteolin have been shown to be effective against SARS-CoV *in vitro* (Ubani et al., 2020; Yamamoto et al., 2016; Yi et al., 2004). The SARS-CoV surface protein binds with TGG and luteolin, preventing the virus from entering the host cell (Yi et al., 2004). Studies using molecular docking and protease inhibition assays have revealed that polyphenols such as epigallocatechin gal-late (EGCG a phytoconstituent of tea), quercetin, kaempferol, and isoliquiritigenin are powerful inhibitors of the SARS-CoV-2 protease (Nguyen et al., 2012; Park et al., 2017).

Sinapic acid (SA; C11H12O5), one of the most prevalent hydroxycinnamic acids, ex-ists in both free and ester forms and contains bioactive carboxylic acids (Nićiforović, 2014). SA is a ubiquitous phytoconstituent found in a variety of plant sources such as spices, citrus and berry fruits, vegetables, cereals, and oilseed crops (Chen, 2016). Several studies have been conducted to examine the efficacy of SA. According to previous reports, SA is useful in treating a wide range of clinical conditions, including oxidative stress, inflammation, cancer, hypoglycemia, neurodegeneration and anxiety (Alaofi, 2020; Huang et al., 2022; Raish et al., 2022; Shahid et al., 2022). SA was reported to show inhibitory activity against SARS-CoV-2 by targeting its envelope protein (Bahun et al., 2022; Orfali et al., 2021). Moreover, the IC_{50} of sinapic acid against envelope protein was 2.5 μg/ml.

It is worth mentioning we have previously investigated small molecules such as Doxorubicin and acyclovir against MERS-CoV PLpro (Alaofi et al., 2022). Only Doxorubicin showed inhibitory activity against MERS-CoV PLpro while acyclovir did not. Here, as polyphenols are naturally present in food and have a long history of use by humans, there is little chance of harm from them. In this study, we examined the inhibitory effect shown by SA against MERS-CoV PLpro by investigating the effects of SA on recombinant MERS-CoV PLpro. We also analyzed whether the drugs interacted with MERS-CoV PLpro and studied the structural changes that occurred in MERS-CoV PLpro in the presence of SA.

2. Materials and methods

2.1. Chemicals

Z-RLRGG-AMC acetate was purchased from Bachem (CA, USA) and SA from Carbosynth Ltd. (United Kingdom). MagicMedia and SYPRO™ dye were purchased from Thermo Fisher Scientific (Rockford, IL, USA). Pierce™ BCA Protein Assay Kit, poly-His primary and HRP-conjugated secondary antibodies were obtained from Thermo Fisher Scientific (Santa Cruz, CA, USA). Laemmli buffer and Precision Plus Protein™ marker were acquired from Bio-Rad (CA, USA), Annexin V-FITC apoptosis kit (Biovision, United Kingdom).

2.2. Protein expression and purification

The MERS-CoV PLpro sequence with a His-tag at its C-terminus (GenBank: AFS88944) was cloned into pET28b + between NcoI and XhoI sites to generate pMERS-CoV PLpro.BL21(DE3) (Alaofi et al., 2022). The transformed cells were allowed to grow in MagicMedia containing 50 μg/ml of kanamycin for 18 h at 30 °C. The cells were harvested by centrifuging at 4,000 g at 4 °C, stored at −80 °C, and then used as required. The cell pellet was resuspended in lysis buffer composed of phenylmethylsulfonyl fluoride (PMSF), 5 % (v/v) glycerol, 50 mM Tris-HCl, 250 mM NaCl, 10 mM imidazole, and 2 mM beta-mercaptoethanol (BME), pH 8.0. Following three rounds of sonication for five seconds each at 40 % amplitude to lyse the cell pellet, His-GraviTrap was used to isolate polyhistidine-tagged proteins using affinity chromatography (GE Healthcare, Uppsala, Sweden). Lysis buffer (25 ml) was used to equilibrate the column before adding the cell lysate. This buffer did not contain PMSF but contained 30 mM imidazole. The rMERS-Plpro were eluted using an elution buffer containing (50 mM Tris-HCl, 250 mM NaCl, 200 mM imidazole, 2 mM BME, pH8.0). The eluates were then dialyzed once more over the course of the entire night using 14 kDa molecular weight cut-off (MWCO) dialysis tubing. Fresh protein was eluted, and the protein concentration was calculated for each inhibition and binding assay using bicinchoninic acid assay kit.

2.3. MERS-CoV PLpro inhibition assay

Protease inhibition was performed as previously described by Alaofi et al. (Alaofi et al., 2022). Briefly, the hydrolysis of the Z-RLRGG-AMC-peptide bond significantly increased the fluorescence of AMC, allowing the conversion to be monitored. A 384-well plate assay was performed using a plate with a black flat bottom to calculate the IC50 values for SA. The reactants included the following components: 20 mM Tris buffer (pH 8.0), 30 μM Z-RLRGG-AMC, and different concentration of SA. The reaction volume was 50 μL. The experiments began with the addition of PLpro, resulting in an enzyme concentration of 60 nM (excitation wavelength [Ex] = 360 nm; emission wavelength [Em] = 460 nm; gain = 40). The plates were shaken for 30 s, and after 30 min of incubation, the fluorescence generated upon the release of AMC from the peptide was analyzed using a Biotek HT Microplate Reader (USA). The IC50 values were calculated using GraphPad Prism.

2.4. Thermal shift assay (TSA)

Differential scanning fluorimetry (DSF) was performed to quantify the amount of SA interacting with the MERS-CoV PLpro protein using Applied Biosystems 7500 Real-Time PCR Systems. Test chemicals, whose concentrations ranged from 13.94 to 2230 μM, were added shortly after dilution in the reaction buffer containing MERS-CoV PLpro final concentration of 2 mol/L. The buffer served as a standard and the mixture was incubated at 25 °C for 30 min. SYPRO Orange dye was used to record the fluorescence signal at gradient temperature of 1 °C/min from 25 to 95 °C. The melting temperature (Tm) in the presence of test compounds and the Tm in the presence of buffer and protein was used to

calculate the melting temperature (ΔTm). TSA-CRAFT, a webserver (https://tbtlab.org/tsacraft.html) that analyzes RT-PCR data by Boltzmann equation fitting, was used to process and generate data (Alaofi et al., 2022; Huynh and Partch, 2015; Lee et al., 2019).

2.5. Docking of sinapic acid to MERS-CoV PLpro

Autodock Vina was used to dock SA (Pubmed ID 637775) to the MERS-CoV PLpro dynamic (simulated in a box of water) structure obtained from our previous work. Briefly, water molecules were deleted and polar hydrogen charges were added. The MERS-CoV PLpro grid box (X 83.893, Y 85.280, and Z 38.837 Å) encompassed most of the PLpro structure, with a spacing of 0.375 Å. Nine poses were obtained from Auto-dock Vina, and four of them were docked in the same region (groove). The docking results were visualized and analyzed using PyMOL and Maestro Schrödinger programs (W. (2020). Schrödinger, 2020).

2.6. Cytotoxicity assay

The A549 human lung carcinoma cell line was cultivated at 37 °C in a 5 % CO2 atmosphere using Dulbecco's Modified Eagle's Medium (DMEM) supplied by Hyclone, USA, enriched with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin, both from Hyclone and Invitrogen, USA, respectively. Cells at a density of 1×10^6 cells per well were seeded in a 6-well culture plate and incubated for 24 h. Following this, the cells were treated with sinapic acid at concentrations of IC_{50} and $2XIC_{50}$ and incubated for an additional 24 h. Cytotoxic effects were evaluated using a previously established method (Ahmad Ansari et al., 2023; Alangari et al., 2023).

For the analysis of cell apoptosis via flow cytometry, A549 cells, again at 1×10^6 cells per well, were cultured in a 6-well plate for 24 h. Post incubation, they were treated with sinapic acid at IC_{50} concentrations for another 24 h. Subsequently, the cells were collected, washed, and stained with Annexin V-fluorescein isothiocyanate (FITC) for 20 min, followed by propidium iodide (PI) staining at a concentration of 50 μg/mL for 10 min. These staining procedures were conducted in cold conditions and protected from light. Finally, the stained cells were subjected to flow cytometry analysis.

3. Results

3.1. SDS-PAGE and Western blot analysis

PL protease was initially produced in E. coli and subsequently validated by SDS-PAGE analysis, which is displayed in (Fig. 1A, lane 4) as a 37 kDa rMER-CoV PLpro prified protein. We successfully expressed the MERS-PLpro gene at high levels in BL21 DE3 E. coli self-induced culture. By utilizing His-tag primary antibody in a Western blot examination of the MERS-PLpro, the expression was further verified (Fig. 1B, lane 1 and 2) (Ahmed L. Alaofi et al., 2022).

3.2. MERS-CoV PLpro inhibition assay

To determine the effect of SA on MERS-CoV PLpro, the fluorescence from the AMC moiety was measured as part of a dose-dependent protease inhibition assay. Enzymatic activity was assessed using the accepted method for the continuous enzymatic inhibition test (Alaofi et al., 2022), and inhibition curves were constructed by keeping enzyme and substrate concentrations constant while varying the amount of medication (Fig. 2). Various concentrations of SA were used, and the inhibition of the enzymatic activity was assessed by examining the first slope of each curve (Fig. 2B). Notably, SA affected enzyme activity in a dose-dependent manner (Fig. 2A). Nonlinear regression analysis (employing a simple inhibition model) was conducted to examine the inhibition of enzymatic activity at different doses and time points. IC_{50} values of 68.58 μM (of SA) after 30 min of incubation indicated that SA may bind to the BL-2 loop of the enzyme and function as a competitive inhibitor (Table 1).

By conducting assays to assess PLpro cleavage activity across a broad spectrum of substrate concentrations, we observed that the reaction velocities did not reach a point of saturation even at the highest concentration tested. Consequently, we estimated the apparent Km to be 74 μM based on a dataset that displayed incomplete saturation (Fig. 1b). Additionally, we observed a steady increase in cleavage activity over the course of an hour, across various enzyme concentrations (Fig. 1a). For our screening purposes, we opted to use 2 μM of enzyme and 30 μM of substrate.

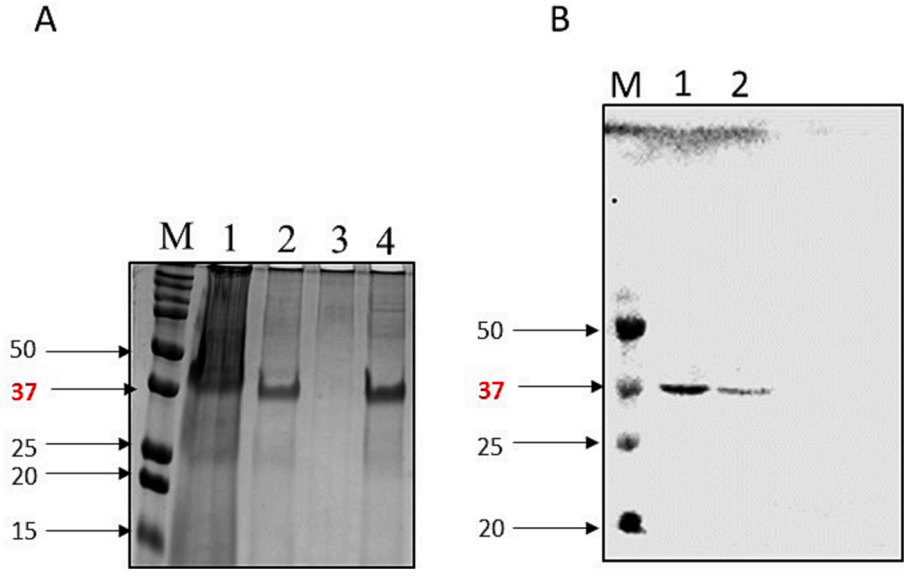

Fig. 1. (A) SDS-PAGE 10 % analysis of MERS-PLpro expression: 37. kDa. M = protein marker, lane 1 = crude extract, lane 2 = first purification elute, lane 3 = wash, lane 4 = Final elute. (B). Western blot M = Protein marker lane 1 = crude extract, lane 2 = Final elute.

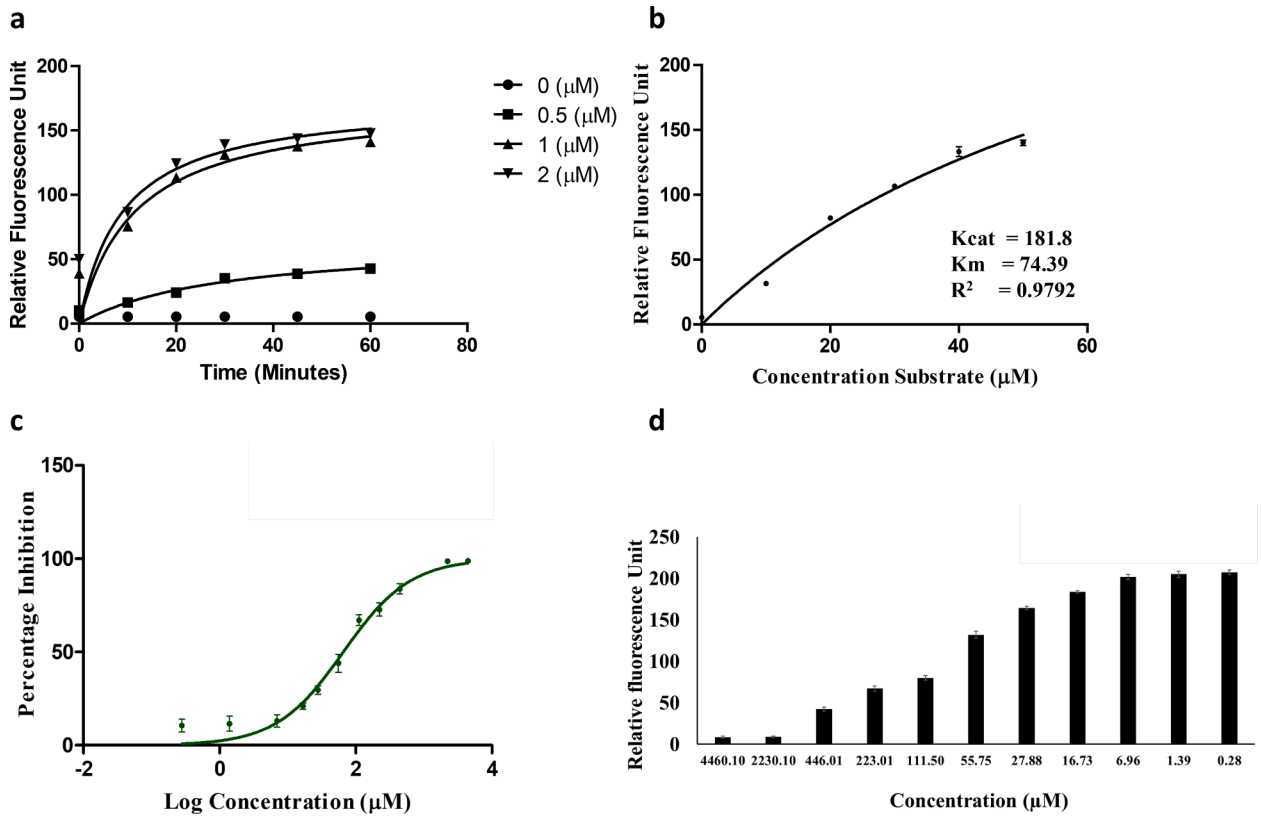

Fig. 2. (a) Titration of MERS-Plpro against was plotted against time at constant substrate concentration (b) Kinetic constants of MERS-PLpro. The reaction initial rates of PLpro were plotted against different substrate concentrations to yield the kcat and Km values (c) Dose-response curve. (d) Percent inhibition of MERS-CoV PLpro obtained from the results of the protease inhibition assay, in which dissociation of the Z-RLRGG-7-Amino-4-methylcoumarin-3-acetic substrate was observed. The percent inhibition values were plotted on the dose–response curve to estimate the IC_{50} value. Values represent the mean ± SD of three replicates.

Table 1
Melting point (Tm) of MERS-CoV PLpro in the presence of SA calculated on the basis of the dissociation of SYPRO dye.

	SA (111.5 μM)	Indomethacin (279.5 μM)	MERS-CoV-2 PLpro
Tm (°C)	59.72	42.80	41.36
ΔTm (°C)	18.36	1.44	–
R^2	0.9986	0.9803	0.9482

3.3. Thermal shift assay

To validate the fact that SA inhibited MERS-PLpro in a dose-dependent manner, we performed a thermal shift investigation of the protein stability and ligand interactions, where we found that the results were consistent with those obtained in the inhibition assay. The basic procedure of a thermal shift experiment also known by thermofluor assay-involves the incubation of natively folded proteins in aqueous solution in presence of SYPRO Orange and ligand. In presence of thermal gradient and simultaneously measuring the SYPRO Orange fluorescence emission spectrum, it is possible to monitor the denaturation of the protein thereby establishing the binding of ligand to the protein. The variation in melting temperature (ΔTm; mean = 12.54 °C) for various concentrations of SA ranged from 1.49 to 19.09 °C. The results obtained from the enzymatic protease inhibition experiment were compatible with those of the thermal shift assay (Fig. 3). It is interesting that in both our experiments, SA suppressed MERS-CoV-2 PLpro in a dose-dependent manner. Notably, indomethacin (an antiviral medication) did not alter the Tm of MERS-CoV PLpro significantly at any concentration, suggesting that there may not have been any interaction between indomethacin (279.5 μM) and PLpro (Fig. 3). The increasing of SA concentrations resulted in increased Tm of MERS-CoV PLpro; indicated a proportional relationship between SA concentrations and the protease (Fig. 4).

3.4. Sinapic acid (SA) binding site

The results showed that SA was bound to a groove region close to the BL2 loop of MERS-CoV PLpro. The BL2 residues F268, E272, V275, and P249 interacted with SA (Fig. 5). Notably, SA showed hydrophobic interactions with MERS-PLpro residues P249, F268, A274, V275, and Y278, charged interactions with R167, E272, and D164, and polar interactions with S166 and T307. The BL2 loop plays a crucial role in protease activity. The BL2 loop can block the catalytic. It is probable that the SA molecule binds to the PLpro palm domain and impedes the catalytic activity of the protease. Furthermore, binding to this region might have increased the stability of the tertiary structure. Our thermal stability assay results suggested that binding to this area might have also stabilized the tertiary structure (i.e., increased the Tm) of MERS-CoV PLpro.Fig. 5.

3.5. Cytotoxicity of sinapic acid (SA) in A549 cells

The flow cytometry data suggests that the treatment with sinapic acid induces a no increase in early and late apoptosis in A549 cells when compare to control. The MTT assay results support this finding, showing no reduction cell viability after sinapic acid treatment, indicative of no cytotoxic effects at IC_{50} and twice the IC_{50} concentration which is in agreement with the previous reports (Fig. 6)(Ahmad Ansari et al., 2023; Nićiforović, 2014; Orfali et al., 2021).

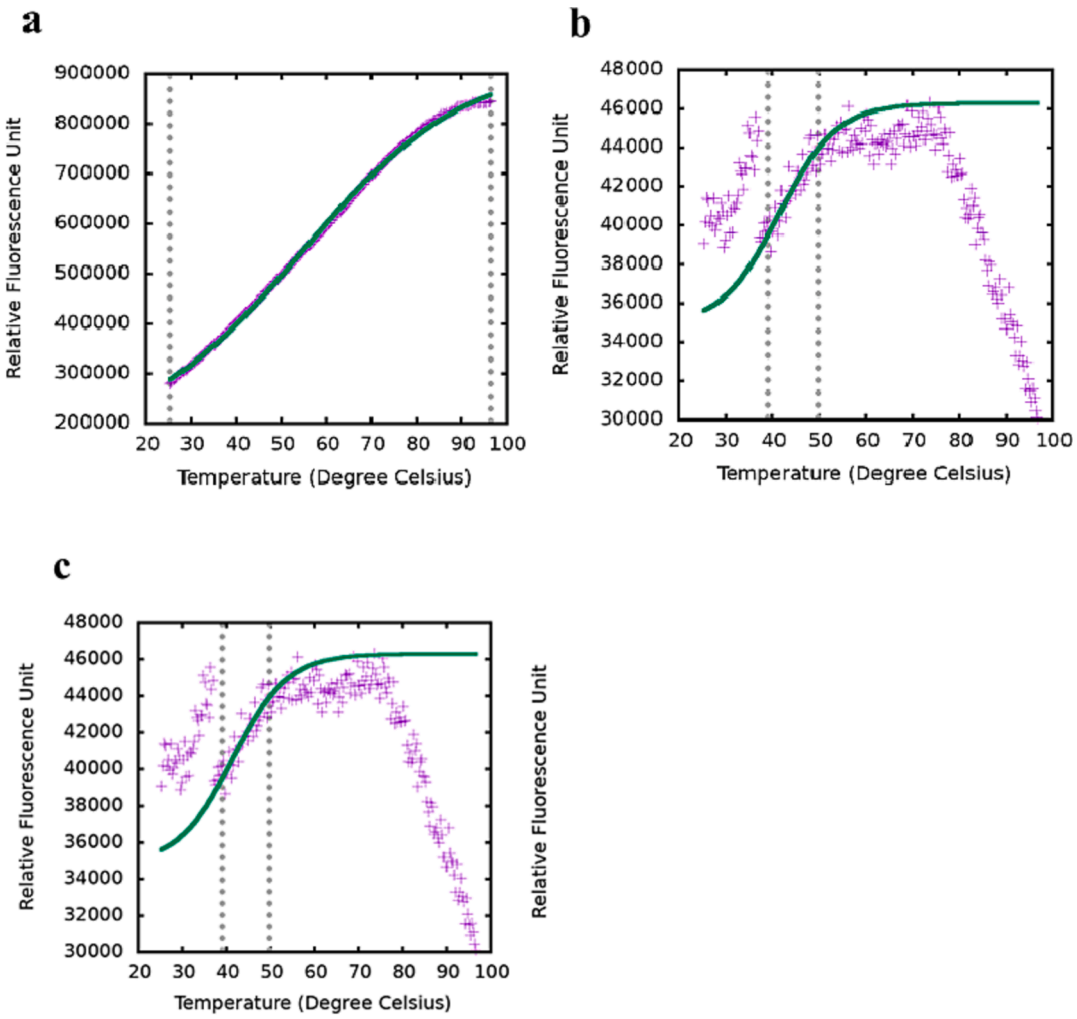

Fig. 3. The melting temperature (Tm) of MERS-CoV-2 PLpro obtained from the results of the thermal shift assay (TSA). The figure was plotted using TSA-CRAFT software (34). (A) representative image of Tm profile in the presence of 55.8 μM SA, (B) representative image of Tm profile in the presence of 280 μM indomethacin, and (C) Tm of MERS-CoV-2 PLpro.

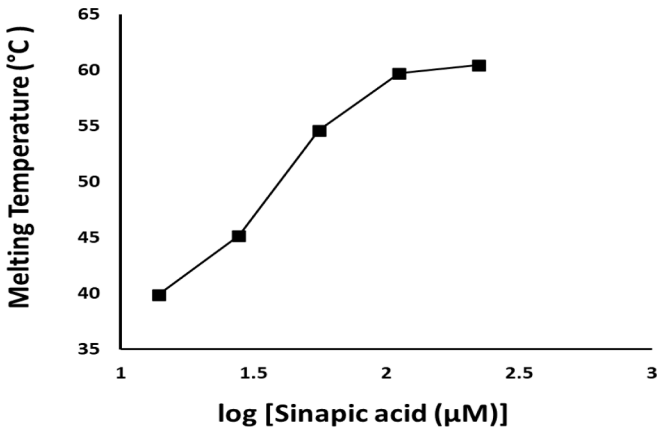

Fig. 4. The melting temperature (Tm) of MERS-CoV PLpro as a function of sinapic acid (uM). PLpro Tm was elevated as sinapic acid concentration increased.

4. Discussion

The COVID-19 epidemic has increased the demand for pharmacologically effective medication against MERS-CoV. The reservoir of active chemicals must function through a variety of mechanisms with high efficacy, low sensitivity to viral alterations, and resistance to treatment. Since nonstructural protein 3 (nsp3) are highly conserved across different coronavirus strains, medications that target CoV proteases can help with some of the issues of resistance to treatment. Small or large-scale screening of CoV protease inhibitors using experimental and/or computational methods can aid in drug development or repurposing. In addition to the investigation of synthetic small molecules, screening of products obtained from plant sources is also crucial as it can lead to the development of promising, safe, effective, and economical antiviral medications. Rapid and continuous characterization of MERS-CoV proteins is essential for the development of novel therapeutics.

The bulk of viral components and protein–protein interactions has been well defined, allowing for the execution of structure-based virtual screening trials. One of the most prevalent secondary metabolites in the plant world, phenolic compounds, have demonstrated therapeutic promise against a variety of clinical diseases, including viral infections (Annunziata et al., 2020; Bahun et al., 2022; Park et al., 2017). Dose-dependent efficacy of SA in the inhibition of MERS-CoV PLpro was successfully evaluated by a continuous enzyme assay using Z-RLRGG-AMC, a peptidic fluorescence resonance energy transfer (FRET) substrate, to determine the level of inhibition of the enzyme's functionality by SA. SA showed a high degree of antiviral activity (IC_{50} 68.58 μM). In *in vitro* experiments, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication was found to be specifically suppressed by SA,

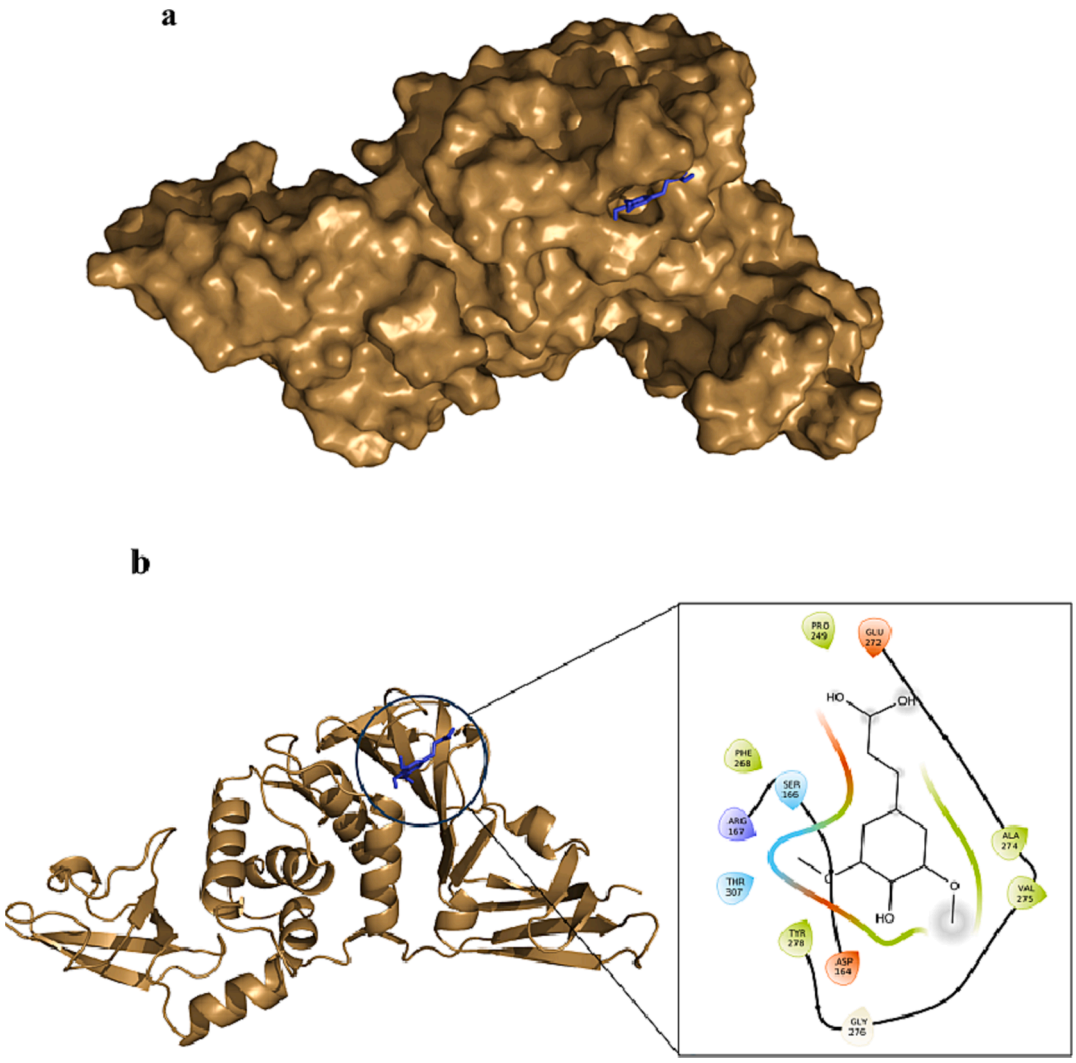

Fig. 5. Binding site of SA (blue) on MERS-CoV PLpro surface representation (brown) and SA in stick representation (blue) (a). In (b), cartoon of MERS-CoV PLpro showed SA interacts with P249, F268, A274, V275, Y278, D164, R167, S166, and T307 residues of MERS-CoV PLpro; indicate by a box of the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

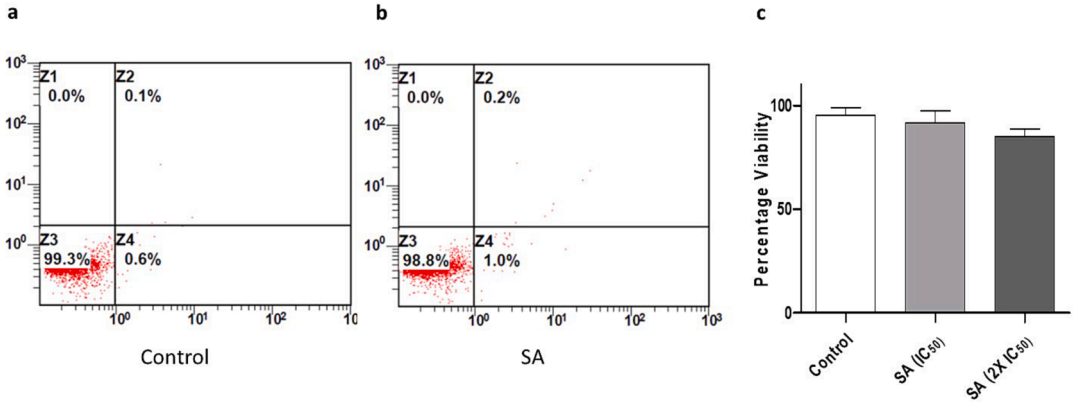

Fig. 6. Evaluation of apoptotic profile of A549 cells post treatment with sinapic acid using flow cytometry. (a) Control cell, (b) Sinapic acid treated cells (c) MTT assay for A549 cells after treatment with sinapic acid.

with an IC_{50} value of 2.69 μg/mL and noticeably low cytotoxicity (Orfali et al., 2021). Moreover, molecular docking studies have shown a similar binding affinity of SA with SARS-CoV-2 (Acar, 2021).

The thermal stability of MERS-CoV PLpro was determined by performing gradient thermal denaturation using fluorescence TSA. TSA is a quick and reliable method for studying the binding affinities and inhibitory effects of different ligands against proteins. TSA is favored over alternative methods used for the study of stability in high-

throughput screening assays owing to its high rate of reproducibility and rapidity in assessment of protein stabilization. The thermal shift experiment revealed that the Tm ranged from 1.49 to 19.09 °C; SA inhibited MERS-CoV PLpro at various doses. The degree of SA's binding affinity to MERS-CoV PLpro demonstrated that SA is a strong candidate for further optimization and preclinical investigative studies; the apparent changes in the unfolding temperature of MERS-CoV PLpro may have been brought on by the particular interaction of SA with the protease. Polyphenols and flavonoids such as quercetin have been reported to increase the thermal stability of SARS-CoV-2 3CLpro (Abian et al., 2020; Bahun et al., 2022). In addition, the monitoring of melting temperature (Tm) at different concentrations can be a helpful parameter for clinical applications.

The precise set of conformational states of the protein that interact with the ligand help in determining the total stabilizing effect of the ligand. If the ligand has interatomic affinities with most of the conformational states, its effect on protein stability will be minimal. If the preference for nonnative states is high and the ligand shows interatomic interactions with a large proportion of unfolded forms (in addition to the native state), a destabilizing effect may be expected. It is probable that the SA molecule attached to the PLpro thumb domain and impeded the catalytic activity of the protease.

Our analysis of the flexibility of MERS-CoV PLpro indicated that it is dynamic, and as a result, SA interacted with the BL2 loop through BL2 residues F268, E272, V275, and P249. The BL2 loop is crucial for MERS-CoV PLpro activity, and by binding to BL2, it is possible to stop MERS-CoV PLpro from performing catalysis activity. For example, Lee et al. demonstrated that SARS-CoV PLpro inhibitors bind to BL2 rather than the catalytic triad (Arya et al., 2023; Lee et al., 2015). It is important to note that BL2 is a flexible domain and that altering its conformation could result in the inhibition of the binding to sinapic acid.

Our findings support the idea that SA, an antioxidant molecule with well-known pharmacokinetic and pharmacodynamic effects, can be introduced to the diet to aid in the treatment of MERS-CoV infection. It is significant to highlight that the outcomes of our binding experiments, which demonstrate that SA suppresses coronavirus by acting against the SARS-CoV-2 envelope protein, are supported by the previously reported experimental evidence. Our findings show that SA can selectively target MERS-CoV PLpro and prevent it from catalyzing, according to experimental evidence.

Polyphenols including Sinapic acid work as NF-kB down-regulators or Reactive oxygen species (ROS) scavengers to modulate anti-inflammatory responses. IkB kinases are inhibited by polyphenols, which thereby prevent the production of pro-inflammatory genes. Additionally, polyphenols can stimulate HO-1 expression by encouraging the dissociation of the Nrf2-Keap1 complex and block NF-kB via altering the MAPK pathway (Alaofi, 2020; Raish et al., 2022; Shahid et al., 2022). Coronavirus including MERS-CoV has been shown to have a role in interferon antagonism and inhibit NF-κB reporter activity and expression of proinflammatory cytokines. Sinapic acid has the ability to curb inflammation and scavenge ROS which may enhance the cellular mechanism to combat MERS-CoV infectivity (Clementz et al., 2010; Frieman et al., 2009). Our findings support the idea that SA, an antioxidant molecule with well-known pharmacokinetic and pharmacodynamic effects, can be introduced to the diet to aid in the treatment of MERS-CoV infection. It is significant to highlight that the outcomes of our binding experiments, which demonstrate that SA suppresses coronavirus by acting against the SARS-CoV-2 envelope protein, are supported by the previously reported experimental evidence. Our findings show that SA can selectively target MERS-CoV PLpro and prevent it from catalyzing, according to experimental evidence.

5. Conclusions

In the case of MERS-CoV infection, the most common pharmaceutical target is PLpro. Diverse drug discovery methods have been used to identify protease inhibitors. At present, there is a haste among researchers worldwide to present their results to the scientific community to accelerate the drug development process. However, the quality of research should not be compromised because of this haste. Biochemical, binding, and cellular tests should be conducted to elucidate the mechanisms of action of the candidate drugs. Consideration should be given to both target specificity and cellular target interaction in pharmacological characterisation investigations. Coronavirus protease inhibitors can be built as a chemical library with the accumulation of knowledge on protease inhibitors to aid in future drug design investigations. Our evidence-based findings clearly demonstrate the binding of SA with protease, thus inhibiting the protease. A cell-based assay is suggested to establish and reaffirm the antiviral activity of SA.

Funding

The author(s) disclosed the receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by the Research Supporting Project number (RSPD-2024RR560), King Saud University, Riyadh, Saudi Arabia.

CRediT authorship contribution statement

Mudassar Shahid: Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization, Investigation, Validation, Formal analysis, Methodology, Project administration, Software. **Ahmed L. Alaofi:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing, Investigation, Validation, Formal analysis, Methodology, Supervision, Resources, Project administration, Software. **Mushtaq Ahmad Ansari:** Writing – review & editing, Methodology. **Sheikh Fayaz Ahmad:** Data curation, Visualization, Methodology. **Saleh Alsuwayeh:** Supervision, Resources. **Ehab Taha:** Writing – original draft, Investigation, Methodology. **Mohammad Raish:** Conceptualization, Formal analysis, Methodology.

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 of this study extend their appreciation to the Researcher Supporting Project, (RSPD-2024R560), King Saud University, Riyadh, Saudi Arabia, for supporting this study and for funding this work.

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