Research Article

Isolation and In Silico Anti-COVID-19 Main Protease (M\text{pro}) Activities of Flavonoids and a Sesquiterpene Lactone from \textit{Artemisia sublessingiana}

Roza I. Jalmakhanbetova,1 Yerlan M. Suleimen,2 Masayoshi Oyama,3 Eslam B. Elkaeed,4 Ibrahim. H. Eissa,5 Raigul N. Suleimen,6 Ahmed M. Metwaly,7 and Margarita Yu. Ishmuratova8

1 Kazakh University of Technology and Business, Nur-Sultan, Kazakhstan
2 The Laboratory of Engineering Profile of NMR Spectroscopy, Sh. Ualikhanov Kokshetau University, Kokshetau, Kazakhstan
3 Laboratory of Pharmacognosy, Gifu Pharmaceutical University, Gifu, Japan
4 Department of Pharmaceutical Sciences, College of Pharmacy, AlMaarefa University, Ad Diriyah 13713, Riyadh, Saudi Arabia
5 Pharmaceutical Medicinal Chemistry & Drug Design Department, Faculty of Pharmacy (Boys), Al-Azhar University, Cairo 11884, Egypt
6 L.N. Gumilyov Eurasian National University, Nur-Sultan, Kazakhstan
7 Pharmacognosy and Medicinal Plants Department, Faculty of Pharmacy (Boys), Al-Azhar University, Cairo, Egypt
8 Department of Botany, E.A. Buketov Karaganda State University, Karaganda, Kazakhstan

Correspondence should be addressed to Yerlan M. Suleimen; syerlan75@yandex.kz and Ahmed M. Metwaly; ametwaly@azhar.edu.eg

Received 5 February 2021; Accepted 8 May 2021; Published 27 May 2021

Academic Editor: Leena Gupta

Copyright © 2021 Roza I. Jalmakhanbetova et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The emergence of the COVID-19 pandemic declared the huge need of humanity for new and effective antiviral drugs. The reported antimicrobial activities of \textit{Artemisia sublessingiana} encouraged us to investigate the ethanol extract of its aerial parts which led to the isolation of six flavonoids and a sesquiterpenoid. The structures of the isolated compounds were elucidated by EI-MS, 1D, and 2D NMR spectroscopic methods to be (1) eupatilin, (2) 3'4'-dimethoxyluteolin, (3) 5,7,3'4',5’-trimethoxyflavone, (4) hispidulin, (5) apigenin, (6) velutin, and (7) sesquiterpene lactone 8α,14-dihydroxy-11,13-dihydromelampolide. The isolated compounds were in silico examined against the COVID-19 main protease (M\text{pro}) enzyme. Compounds 1–6 exhibited promising binding modes showing free energies ranging from −6.39 to −6.81 (kcal/mol). The best binding energy was for compound 2. The obtained results give hope of finding a treatment for the COVID-19 pandemic.

1. Introduction

COVID-19 is the pandemic caused by the new coronavirus strain SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). The pandemic started in Wuhan, China, at the end of 2019 and spread all over the world [1]. By December 2020, COVID-19 infected more than 35 million patients and caused more than a million deaths according to the WHO [2]. Unfortunately, there is no accessible treatment for COVID-19 till now. The available treatment for infected patients is just symptomatic treatment by using anticoagulants, oxygen therapy, analgesics, and some research drugs [3]. Coronavirus have caused serious diseases to humans before, such as Middle East respiratory syndrome (MERS-CoV) which appeared in 2012 and severe acute respiratory syndrome (SARS-CoV) in 2003 [4, 5].

The proteases, especially main protease (M\text{pro}), play a vital role in the life cycle of coronaviruses [6]. M\text{pro} is a cysteine protease that is enrolled in the maturation cleavage events within the polyprotein’s precursors [7, 8].
2. Materials and Methods

2.1. General Experimental Procedures. Column chromatography separations (CC) were performed on glass columns packed with silica gel (ASTM, 230–400 mesh, Merck, LTD, Japan). Thin-layer chromatography (analytical and preparative thin-layer chromatography (TLC) was performed on silica gel 60 F 254 glass plates (Merck, LTD, Japan). Solvents were visualized under UV light (254 and 366 nm) and by spraying with 10% H$_2$SO$_4$ reagent followed by heating. Isolated compounds were identified by 1D and 2D NMR analysis ($^1$H NMR (DMSO, 500 MHz) δ (ppm): 13.05 (s, 5-OH), 10.73 (s, 7-OH), 6.98 (s, H-3), 6.65 (s, H-8), 7.57 (d, $J = 2.5$ Hz, H-2′), 7.12 (d, $J = 8.5$ Hz, H-5′), 7.68 (dd, $J = 2.0, 8.5$ Hz, H-6′), 7.36 (s, 6-OCH$_3$), 3.88 (s, 3′-OCH$_3$), 3.86 (s, 4′-OCH$_3$); $^1$H NMR (CDCl$_3$, 500 MHz) δ (ppm): 13.08 (s, 5-OH), 6.52 (s, 7-OH), 6.61 (s, H-3), 6.59 (s, H-8), 7.34 (brs, H-2′), 6.98 (d, $J = 8.5$ Hz, H-5′), 7.52 (brd, $J = 8.5$ Hz, H-6′), 4.05 (s, 6-OCH$_3$), 3.99 (s, 3′-OCH$_3$), 3.97 (s, 4′-OCH$_3$); $^{13}$C NMR (DMSO, 500 MHz) δ (ppm): 163.38 (C-2), 103.37 (C-3), 182.21 (C-4), 152.74 (C-5), 131.35 (C-6), 157.32 (C-7), 94.38 (C-8), 152.43 (C-9), 104.13 (C-10), 122.92 (C-1′), 109.40 (C-2′), 149.00 (C-3′), 152.11 (C-4′), 111.67 (C-5′), 120.02 (C-6′), 59.97 (6-OCH$_3$), 55.73 (3′-OCH$_3$), 55.85 (4′-OCH$_3$).) were expressed in δ ppm. Mass spectra (EIMS) were recorded on an IT-TOF-MS spectrometer.

2.2. Plant Material. The aerial parts of A. sublessingiana were collected 90 km from Kyzylorda city, Kazakhstan (Kyzylkum sand desert). The material was authenticated by Professor M. Ishmuratova, Department of Botany, E.A. Buketov Karaganda University, Republic of Kazakhstan. A sample was deposited in the herbarium of the Faculty of Biology and Geography.

2.3. Extraction and Isolation. Air-dried powered above-ground parts of A. sublessingiana (1.0 kg) were ground and extracted with EtOH for 1 day. The extract was filtered, and the extraction process was repeated twice. The combined extracts were evaporated under reduced pressure to yield a crude extract of 93 g. The total crude extract was subjected to column chromatography over silica gel eluting with hexane and gradually increasing the polarity with acetone (up to 100%) and then MeOH. The fractions were studied on TLC and combined into twenty-three fractions (1F–23F). Compound (1) (216 mg) was separated from fraction 17F. Further chromatography of fraction 18F (2.5 g) on a column of silica gel with chloroform-acetone (in a manner of increasing polarity) gave compound 2 (5 mg). Fraction 18F4 (0.04 g) was dissolved in a solvent and repeatedly washed to give 3 (28 mg). Fraction 19F (2.91 g) was further fractionated on a silica gel column (60 g) eluting with chloroform-methanol (in a manner of increasing polarity) to give (7) (68.8 mg).

In this study, the main bioactive contents of the aerial parts of A. sublessingiana (Krasch. ex Poljak) Poljak. (Synonium Seriphidium sublessingianum (Krasch ex Poljakov)) have been investigated. This paper reports the isolation, structural determination, and in silico anti-COVID-19 main protease (Mpro) activities of six flavonoids and one sesquiterpene lactone from A. sublessingiana. Their structures were determined by spectrum analysis of 1D, 2D NMR, and ESI-MS data.

2.4. Compound Identification. 5,7-Dihydroxy-6,3′,4′-trimethoxyflavone (eupatilin) (1): yellow crystals, C$_{13}$H$_{18}$O$_7$, $^1$H NMR (DMSO$_d_6$, 500 MHz) δ (ppm): 13.05 (s, 5-OH), 10.73 (s, 7-OH), 6.98 (s, H-3), 6.65 (s, H-8), 7.57 (d, $J = 2.5$ Hz, H-2′), 7.12 (d, $J = 8.5$ Hz, H-5′), 7.68 (dd, $J = 2.0, 8.5$ Hz, H-6′), 7.36 (s, 6-OCH$_3$), 3.88 (s, 3′-OCH$_3$), 3.86 (s, 4′-OCH$_3$); $^1$H NMR (CDCl$_3$, 500 MHz) δ (ppm): 13.08 (s, 5-OH), 6.52 (s, 7-OH), 6.61 (s, H-3), 6.59 (s, H-8), 7.34 (brs, H-2′), 6.98 (d, $J = 8.5$ Hz, H-5′), 7.52 (brd, $J = 8.5$ Hz, H-6′), 4.05 (s, 6-OCH$_3$), 3.99 (s, 3′-OCH$_3$), 3.97 (s, 4′-OCH$_3$); $^{13}$C NMR (DMSO$_d_6$, 500 MHz) δ (ppm): 163.38 (C-2), 103.37 (C-3), 182.21 (C-4), 152.74 (C-5), 131.35 (C-6), 157.32 (C-7), 94.38 (C-8), 152.43 (C-9), 104.13 (C-10), 122.92 (C-1′), 109.40 (C-2′), 149.00 (C-3′), 152.11 (C-4′), 111.67 (C-5′), 120.02 (C-6′), 59.97 (6-OCH$_3$), 55.73 (3′-OCH$_3$), 55.85 (4′-OCH$_3$).
5.7-Dihydroxy-3',4'-dimethoxyflavone (3): yellow powder, C_{19}H_{14}O_{5}, 1H NMR (DMSO, 500 MHz) δ (ppm): 12.99 (s, 5-0H), 10.70 (s, 7-0H), 9.60 (s, 3'-0H), 6.92 (s, H-3), 6.60 (s, H-8), 7.17 (dd, J = 1.5, 4.5 Hz, H-2'), 7.17 (dd, J = 1.5, 4.5 Hz, H-6'), 3.70 (s, 6-OCH$_3$), 3.70 (s, 4'-OCH$_3$), 3.80 (s, 5'-OCH$_3$). 13C NMR (DMSO, 500 MHz) δ (ppm): 163.25 (C-2), 104.22 (C-3), 182.23 (C-4), 152.46 (C-5), 131.41 (C-6), 153.57 (C-7), 94.28 (C-8), 152.78 (C-9), 104.22 (C-10), 125.88 (C-1'), 102.12 (C-2'), 157.46 (C-3'), 139.63 (C-4'), 150.91 (C-5'), 107.68 (C-6'), 59.95 (6-OCH$_3$), 60.08 (4'-OCH$_3$), 56.15 (5'-OCH$_3$). HRESIMS: m/z 337.0786 [M + Na]$^+$. C_{19}H_{22}O$_7$Na, calc. 337.0788.

5,7-Dihydroxy-3',4'-dimethoxymethoxyflavone (3): yellow amorphous powder, C_{19}H_{14}O_{5}. 1H NMR (DMSO, 500 MHz) δ (ppm): 12.93 (s, 5-0H), 10.85 (s, 7-0H), 6.98 (s, H-3), 6.21 (d, J = 2.5 Hz, H-6), 6.54 (d, J = 1.8 Hz, H-8), 7.57 (d, J = 1.8 Hz, H-2'), 7.13 (d, J = 8.6 Hz, H-5'), 7.69 (dd, J = 1.8, 8.7 Hz, H-6'), 3.88 (s, 3'-OCH$_3$), 3.91 (s, 4'-OCH$_3$). HRESIMS: m/z 337.0686 [M + Na]$^+$. C_{19}H_{22}O$_7$Na, calc. 337.0683.

2.5. Docking Studies Experiment. The crystal structure of the target enzymes COVID-19 main protease (Mprotein) (PDB ID: 6lu7, resolution: 2.16 Å) was downloaded from Protein Data Bank (http://www.pdb.org). Molecular operating environment (MOE) was used for the docking analysis [38]. In these studies, the free energies and binding modes of the examined molecules against Mprotein were determined. At first, the water molecules were removed from the crystal structure of Mprotein, retaining only one chain which is essential for binding. The cocrystallized ligand (PRD-002214) was used as a reference ligand. Then, the protein structure was protonated, and the hydrogen atoms were added. Next, the energy was minimized; and the binding pocket of the protein was defined [39].

The structures of the examined compounds and the cocrystallized ligand were drawn using ChemBioDraw Ultra 14.0 and saved in SDF format. Then, the saved file was opened using MOE software, and 3D structures were protonated. Next, the energy of the molecules was minimized. The validation process was performed for the target receptor by running the docking process for only the cocrystallized ligand. Low RMSD values between docked and crystal conformations indicate valid performance [40, 41]. The docking procedures were carried out utilizing a default protocol. In each case, 30 docked structures were generated using genetic algorithm searches. The output from MOE software was further analyzed and visualized using Discovery Studio 4.0 software [42, 43].

3. Results and Discussion

3.1. Compounds Isolation. Using different chromatographic techniques, seven compounds have been isolated from the ethanol extracts of the aerial parts of A. sublesingiana. The obtained compounds were identified using different 1D and 2D NMR spectroscopic methods to be (1) eupatilin [44, 45], (2) 3',4'-dimethoxymethoxyflavone [46–48], (3) 5, 7, 3'-trihydroxy-6,4',5'-trimethoxyflavone [49], (4) hispidulin [50, 51], (5) apigenin [52, 53], (6) velutin [54], and (7) sesquiterpene lactone 8a,14-dihydroxy-11,13-dihydromelampolide [55–57]. The chemical structures of compounds (1–7) were confirmed by comparison of the reported spectral data in the literature (Figure 1).

8a,14-Dihydroxygermacra-1(10)E,4-Eien-6β,7α,11β-H-12,6-olide (8a,14-dihydroxy-11,13-dihydromelampolide) (7): colorless crystals, C_{21}H_{22}O_{5}. 1H NMR (CDCl$_3$, 500 MHz) δ (ppm): 5.49 (m, 2H, δ = 7.0, 9.0, 16.0 Hz, H-1), 2.14 (m, H-2a), 1.89 (m, H-2b), 2.16 (m, H-3a), 1.91 (m, H-3b), 5.01 (brd, δ = 10.0 Hz, H-5), 4.53 (t, J = 10.0 Hz, H-6), 2.12 (m, H-7), 3.90 (brs, H-8), 2.32 (brd, δ = 15.0 Hz, H-9a), 2.25 (dd, δ = 3.0, 15.0 Hz, H-9b), 2.57 (m, H-11), 1.43 (d, δ = 6.0 Hz, H-13), 4.33 (brd, δ = 12.0 Hz, H-14a), 4.13 (brd, δ = 12.0 Hz, H-14b), 1.83 (brs, H-15), 2.84, 3.90 (brs, each, OH-3, 4). 13C NMR (CDCl$_3$, 500 MHz) δ (ppm): 128.73 (C-1), 25.22 (C-2), 38.05 (C-3), 138.58 (C-4), 124.60 (C-5), 77.26 (C-6), 55.17 (C-7), 73.75 (C-8), 35.15 (C-9), 139.02 (C-10), 41.73 (C-11), 179.45 (C-12), 16.41 (C-13), 69.22 (C-14), 17.24 (C-15).
The crystallized ligand (PRD-002214) showed binding energy of −7.83 kcal/mol. The detailed binding mode of the crystallized ligand was as follows: making three hydrogen bonds with Phe140, His163, and Glu166, the 2-oxopyrrolidin-3-yl moiety occupied the first pocket of the enzyme. Additionally, tert-butyl carbamate moiety occupied the second pocket of \( \text{M}^{\text{pro}} \). Furthermore, the phenyl ring of phenylalanine moiety occupied the third pocket of the receptor, forming hydrophobic interaction with His41. Finally, ethyl propionate moiety was incorporated in the fourth pocket (Figures 2–4).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Binding free energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−6.53</td>
</tr>
<tr>
<td>2</td>
<td>−6.81</td>
</tr>
<tr>
<td>3</td>
<td>−6.51</td>
</tr>
<tr>
<td>4</td>
<td>−6.44</td>
</tr>
<tr>
<td>5</td>
<td>−6.39</td>
</tr>
<tr>
<td>6</td>
<td>−6.55</td>
</tr>
<tr>
<td>7</td>
<td>−4.94</td>
</tr>
<tr>
<td>Cocrystallized ligand (PRD-002214)</td>
<td>−7.83</td>
</tr>
</tbody>
</table>

Compound (2) showed the best binding mode and highest binding energy of −6.81 kcal/mol. The 7-hydroxy-6-methoxy-4H-chromen-4-one moiety occupied the first pocket of \( \text{M}^{\text{pro}} \), forming three hydrogen bonds with Phe140.
Figure 3: Mapping surface showing the cocrystallized ligand (PRD-002214) occupying the active pocket of the COVID-19 main protease.

Figure 4: 2D interaction of the cocrystallized ligand (PRD-002214) in the active site of the COVID-19 main protease.

Figure 5: Compound (2) docked into the active site of the COVID-19 main protease. The hydrogen bonds are represented in green dashed lines, and the hydrophobic interactions are represented in orange dashed lines.

Figure 6: Mapping surface showing compound (2) occupying the active pocket of the COVID-19 main protease.

Figure 7: 2D interaction of compound (2) in the active site of the COVID-19 main protease.
and His163. Also, it formed one hydrophobic interaction with His163. Additionally, 1,2-dimethoxybenzene moity occupied the second pocket of M^{Pro} forming two hydrophobic interactions with Met165 and His41 (Figures 5–7).

4. Conclusions

This study focused on the phytochemical and the in silico biological investigation against the COVID-19 main protease (M^{Pro}) of six flavonoids and one sesquiterpene lactone obtained from A. sublessingiana. Eupatilloin, 3', 4'-dimethoxytoluene, 5, 7, 3'-trihydroxy-6, 4',5'-trimethoxyllavone, velutin, and 8α,14-dihydroxy-11,13-dihydromelampolide were isolated from Artemisia species for the first time. Compound (2) exhibited the best binding mode with a binding energy of −6.81 kcal/mol against COVID-19 main protease (M^{Pro}). The obtained results open a window of hope to find an effective cure to the pandemic of COVID-19. Further in vitro and clinical studies should be conducted on compound 2 to confirm its potential against the contagious virus SARS-CoV-2.

Data Availability

NMR data of the isolated compounds are available.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the Matsumae International Foundation grant (Japan) and has been funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant no. AP 05130941).

Supplementary Materials

Supplementary materials contain the ¹H, ¹³C, 2D NMR, and ESI-MS data for compounds 1–7. (Supplementary Materials)

References


