**Eryngium creticum** L.: Chemical Characterization, SARS-CoV-2 Inhibitory Activity, and *In Silico* Study

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**ABSTRACT:** Phytochemical investigation of *Eryngium creticum* L. has resulted in isolation of five compounds, including four compounds that are reported from the plant for the first time. Compound 1 was identified as (E)-rosmarinic acid, meanwhile, compound 2 was isolated as an (E/Z)-rosmarinic acid mixture. Interestingly, the E/Z-isomeric mixture was about 4 times as active as the single E-isomer toward the severe acute respiratory syndrome coronavirus 2 3-chymotrypsin-like protease (3CL\(\text{pro}\)), \(IC_{50} = 6.062\) and 25.75 \(\mu M\), respectively. Utilizing combined molecular docking and molecular dynamics (MD) techniques, the binding affinities and features of the isolated compounds were evaluated against 3CL\(\text{pro}\). Compound 2Z demonstrated a higher binding affinity for 3CL\(\text{pro}\) than 2E, with docking scores of −8.9 and −8.5 kcal/mol and MM-GBSA/150 ns MD binding energies of −26.5 and −22.1 kcal/mol, respectively. This justifies the superior activity of the E/Z-isomeric mixture versus the single E-isomer. Structural and energetic analyses revealed the stability of 2Z and 2E compared to the reference HIV-1 protease inhibitor, lopinavir. Besides, DFT calculations demonstrated the more energetic stability of 2E compared to 2Z, which justifies the difficulty in isolating the Z-isomer in a pure form, where it readily isomerizes to the E-isomer.

1. INTRODUCTION

*Eryngium creticum* L., Apiaceae is commonly known as “snakeroot”. It is an edible salad plant that is widely distributed in the Eastern Mediterranean region. It is widely used in traditional medicine as a bitter tonic and a remedy for snake and scorpion bites. The root is used traditionally for the treatment of kidney stones, liver diseases, skin diseases, infections, edema, sinusitis, inflammations, poisoning, anemia, and infertility.1

Regarding the reported biological activities, *Eryngium creticum* is reported to possess antisnake and antiscorpion venom properties2 and antimicrobial,3 antimalarial,4 cytotoxic,5 and hypoglycemic activities.6

There are some studies addressing the chemical constituents of essential oils.7,8 However, the literature regarding the non-volatile constituents is still very scarce.9 Previous studies reported the isolation of quercetin, \(\beta\)-sitosterol glucoside, a phloroglucinol glycoside, a monoterpenic glucoside, a methyl ketone, a unique sesquiterpene, and two coumarins.10

The scarce literature on the non-volatile constituents of *E. creticum* prompted us to investigate its chemistry. In this work, five compounds were isolated from the roots of *E. creticum*. They were identified as (E)-rosmarinic acid 1, (E/Z)-rosmarinic acid mixture 2, panaxadiol 3, (E)-15-hydroxy-9,16-heptadecadiene-11,13-diyn-8-one 4, and \(\beta\)-sitosterol 5.

The coronavirus disease-19 (COVID-19) pandemic is a devastating worldwide crisis with serious economic, social, and political consequences. The disease is caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Up until now, there has been no approved global antiviral drug for controlling the pandemic. Due to its crucial role in the SARS-CoV-2 life cycle, studies have focused on finding potential inhibitors of the main protease, also known as M\(\text{pro}\) or 3CL\(\text{pro}\). 3CL\(\text{pro}\) dominates the maturation of functional polyproteins that are essential for the viral replication process.10 Being a highly conserved protein across all CoVs with no human homologs and its dominant role in SARS-CoV-2 replication make 3CL\(\text{pro}\) an ideal drug target for COVID-19.11

Natural products remain the wealthiest source of medicinal agents, as 51% of the marketed drugs (1981–2019) were derived or inspired by natural compounds. In addition, more than 60% of the market protease inhibitors are related to natural compounds.12

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Rosmarinic acid is reported as a broad inhibitor against a diverse group of viruses including hepatitis B virus, influenza viruses, enterovirus 71, herpes simplex virus, human immunodeficiency virus (HIV), and Japanese encephalitis virus. According to recent studies, rosmarinic acid showed medium inhibitory activity against SARS-CoV-2 replication and SARS-CoV-2 3CLpro. This prompted us to investigate the inhibitory potential of E-rosmarinic acid versus (E/Z)-rosmarinic acid mixture against the main protease of coronavirus.

Contributing to the search for therapeutic agents for COVID-19, the isolated compounds were tested for potential inhibitory activity against 3CLpro. Utilizing a molecular docking technique, binding affinities and features of the isolated compounds were predicted against 3CLpro. Complexes of the docked compounds 2Z- and 2E-3CLpro were then subjected to molecular dynamics (MD) simulations over 150 ns, followed by molecular mechanics-generalized Born surface area (MM-GBSA) binding energy calculations. Postdynamics calculations were carried out to inspect the stability of complexes of 2Z- and 2E-3CLpro. Ultimately, the energetic stability of 2E and 2Z was scrutinized using DFT calculations.

2. RESULTS AND DISCUSSION

2.1. Chemistry. The scarce literature on the non-volatile constituents of E. creticum prompted us to investigate its chemistry. Five compounds were isolated (Figure 1), and their NMR data are shown in Figures S1−S30. As shown in Table 1, the 1H NMR spectrum of compound 1 showed trans-olefinic protons at δH 7.54 (d, 16.0) and 6.26 (d, 16.0)
Figure 2. Expansion of the $^1$H NMR spectrum of compound 2 showing the gradual increase of the $Z$–$E$ isomer ratio from 1:1 to 4:1 upon storage under dark condition. (a) Light condition, (b) light condition after 12 h, (c) dark condition, and (d) dark condition after 12 h.
That Z-degradation product of the daylight, moisture, and di- solid state, form because the rosmarinic acid is not stable enough to be isolated in a pure conditions or by using a catalyst. As previously reported, under UV irradiation, to a lesser extent under thermal to the literature, an equilibrium mixture or photostationary state. According to the literature because Z/rosmarinic acid was identified as (E)-rosmarinic acid 1, and compound 2 was identified as an (E/Z)-rosmarinic acid mixture 2. The Z/E isomeric ratio was 1:1 and 4:1 under light and dark conditions, respectively, as discussed below.

Even though compounds 1 and 2 were isolated using HPLC, it was difficult to isolate the Z-isomer (2Z) as a single compound. The Z-isomer (2Z) was unstable, and it was immediately converted to an E-isomer (2E). The Z–E conversion of rosmarinic acid is reported under thermal, catalytic, photochemical, or solvent conditions, which leads to an equilibrium mixture or photostationary state. According to the literature, Z-rosmarinic acid is converted into the E-form under UV irradiation, to a lesser extent under thermal conditions or by using a catalyst. As previously reported, Z-rosmarinic acid is not stable enough to be isolated in a pure form because the Z–E conversion needs low energy and can readily occur in visible light and at low temperatures.

On the other side, the E–Z conversion of rosmarinic acid is reported after storage in tetrahydrofuran, ethanol, or methanol, whether in darkness or daylight and at different temperatures. The E–Z conversion occurs at a higher extent in protic solvents rather than in aprotic solvents. Meanwhile, in the solid state, E-rosmarinic acid is stable even after exposure to daylight, moisture, and different temperatures. It is reported that Z-rosmarinic acid is not a natural compound but a degradation product of the E-isomer.

Upon isolation of compounds 1 and 2, they were kept in the solid state under light condition and at room temperature. When the proton NMR spectrum was recorded for compound 2 in CD3OD under light condition, the 2Z/2E isomeric ratio was about 1:1 (Figure 2a). The recording of the proton NMR spectrum was repeated after 12 h under light and the 2Z/2E isomers also existed in equilibrium (Figure 2b).

In an attempt to isolate the 2Z isomer in a pure form, the compounds were reisolated again by HPLC. This time the compounds were kept in the solid state under dark condition. The proton NMR spectrum of compound 2 was recorded in CD3OD under dark condition, and the 2Z/2E isomeric ratio was 4:1 (Figure 2c). Even though the Z/E isomeric ratio increased from 1:1 to 4:1 under dark condition, the 2Z isomer could not be isolated in a pure form. The NMR experiments were repeated after 12 h upon storage in deuterated methanol under dark condition, and the 2Z/2E isomer ratio remained almost stable at 4:1 (Figure 2d). These results are in accordance with the literature because Z-rosmarinic acid is reported to be not stable enough to be isolated in a pure form.

The remaining compounds were identified based on the analysis of their spectral data (Figures S1–S5) and comparison to the literature as follows: panaxadiol 3,21 (E)-15-hydroxy-9,16-heptadecadiene-11,13-diyne-8-one 4,9 and β-sitosterol 5.22 To the best of our knowledge, apart from compound 5, these compounds are reported for the first time from E. creticum in this study.

### 2.2. In Silico Drug Discovery

2.2.1. Molecular Docking.

The outstanding performance of the AutoDock4.2 software in disclosing the inhibitor-3CLpro binding mode was previously reported, giving a predicted binding mode for XF7 with a root-mean-square deviation (rmsd) of 0.20 Å, compared to the resolved experimental binding mode.23 Therefore, a molecular docking technique was used to predict the binding modes and affinities of the isolated compounds for 3CLpro, and these were compared to those of lopinavir as a reference. The predicted docking scores and binding features for the isolated compounds are listed in Table 2. The 2D and 3D representations of binding modes of the isolated compounds inside the active site of 3CLpro are displayed in Figure S27. Most of the studied compounds demonstrated almost identical docking poses within the 3CLpro’s active site, forming fundamental hydrogen bonds with GLU166 and THR190 (Figure S27). A wide range of binding affinities were noticed, that is, a range of −5.4 to −8.9 kcal/mol (Figure S27).

The anticipated docking scores, 3D and 2D molecular interaction patterns of the top potent isolated compounds (2Z and 2E), and lopinavir within the binding pocket of 3CLpro are depicted in Figure 3. It can be seen from the data in Figure 3 that compounds 2Z and 2E demonstrated promising binding affinities against 3CLpro with docking scores of −8.9 and −8.5 kcal/mol, respectively. Compound 2Z exhibited 10 hydrogen bonds with LEU141, GLY143, SER144, CY5145, HIS163, GLU166, GLN189, and THR190 amino acids with bond lengths ranging from 1.72 to 3.01 Å (Figure 3).

Compared to compound 2Z, compound 2E demonstrated seven hydrogen bonds with GLY143, SER144, HIS163, GLN189, THR190, and GLN192, with bond lengths ranging from 1.88 to 2.55 Å (Figure 3).

Among the investigated FDA-approved drugs, lopinavir (DrugBank code: DB01601) is a HIV protease inhibitor,24,25 which was utilized as a positive control. Lopinavir manifested a

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**Table 2.** Predicted Docking Scores and Binding Features for the Isolated Compounds and Lopinavir toward SARS-CoV-2 3CLpro

| compounds | docking scores (kcal/mol) | binding features
|------------|--------------------------|-----------------
| lopinavir  | −9.8                     | HIS164 (2.62 Å), SER144 (3.09 Å), LEU141 (1.96 Å), GLY143 (2.01 Å)
| 2Z         | −8.9                     | LEU141 (1.84, 2.44 Å), GLY143 (2.56 Å), SER144 (2.65 Å), CYS145 (3.01 Å), HIS163 (2.03 Å), GLU166 (1.72 Å), GLN189 (2.16 Å), THR190 (1.99, 1.98 Å)
| 2E         | −8.5                     | GLY143 (2.55 Å), SER144 (2.20 Å), HIS163 (2.04 Å), GLN189 (2.03 Å), THR190 (1.98 Å), 199, GLN192 (2.14 Å)
| 5          | −7.4                     | THR190 (2.12 Å)
| 3          | −5.6                     | ASP187 (1.94 Å), THR190 (1.83 Å)
| 4          | −5.4                     | TYR54 (2.56 Å), THR190 (1.70 Å)

*aConventional hydrogen bonds only are presented. For the other interactions, see Figure S27.*

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great binding affinity with a docking score of $-9.8$ kcal/mol, exhibiting four hydrogen bonds with LEU141, HIS163, GLY143, and SER144, with bond lengths ranging from 1.96 to 3.09 Å (Figure 3).

### 2.2.2. Molecular Dynamics and Post-MD Analyses.

Toward more reliable binding affinities, MD simulations were performed for the $2Z$ and $2E$ compounds in complex with 3CL\textsuperscript{pro}. The binding energies ($\Delta G_{\text{binding}}$) were then calculated using the MM-GBSA approach on the basis of the collected snapshots for 3CL\textsuperscript{pro} over the production stage of 150 ns. The average structures for $2Z$, $2E$, and lopinavir inside the 3CL\textsuperscript{pro} active site during the simulation time of 150 ns are depicted in Figure S28. The calculated MM-GBSA binding energies are depicted in Figure 4.

It is apparent from Figure 4 that compounds $2Z$ and $2E$ showed satisfactory binding affinities ($\Delta G_{\text{binding}}$) with values of
−26.5 and −22.1 kcal/mol, respectively. Compared with lopinavir (ΔG<sub>binding</sub> = −33.1 kcal/mol), the calculated MM-GBSA binding energies were in line with the predicted docking scores, demonstrating the high potency of lopinavir over the other investigated compounds.

MM-GBSA binding energy of the investigated compounds with 3CL<sub>pro</sub> was decomposed to explore the predominant interactions between the compound and target. According to the data, it was found that the binding energy of 2Z and lopinavir was dominated by E<sub>vdw</sub> interactions with an average value of −40.2 and −46.1 kcal/mol, respectively (Figure 4). Besides, the E<sub>ele</sub> interactions of 2Z and lopinavir were favorable, with an average value of −28.0 and −25.1 kcal/mol, respectively (Figure 4). For compound 2E, E<sub>vdw</sub> and E<sub>ele</sub> contributions were −24.4 and −47.3 kcal/mol, respectively (Figure 4). Together these results demonstrated the promising binding affinity of compounds 2Z and 2E with 3CL<sub>pro</sub>.

The interaction nature and stability of 2Z and 2E inside the active site were estimated using structural and energetic analyses. Structural and energetic analyses, including energy per frame, center-of-mass distance (CoM), and RMSD were performed over 150 ns MD simulations.

The stability of 2Z and 2E inside the 3CL<sub>pro</sub> active site was estimated and compared to lopinavir using the correlation between the binding energy per frame and time. MM-GBSA binding energy was subsequently evaluated per frame for three promising compounds against 3CL<sub>pro</sub> and displayed in Figure 5. The most interesting aspect of this graph is the overall stability of three identified compounds over 150 ns MD simulations with average values of −26.9, −22.1, and −33.1 kcal/mol for 2Z, 2E, and lopinavir, respectively.

Interestingly, investigating the CoM distance between 2Z, 2E, and lopinavir and the key amino acid residue GLU166 through the 150 ns MD simulations would reflect a strong indication of the high stability of the identified compounds inside the 3CL<sub>pro</sub> active site. The CoM distances were inspected over the 150 ns MD simulations and shown in Figure 6. What stands out in Figure 6 is that the average CoM distance between the identified compounds and the key amino acid residue GLU166 was approximately constant, with average CoM distances of 9.7, 9.8, and 10.8 Å, respectively. Figure 6 reveals the high stability of the identified compounds in complex with SARS-CoV-2 3CL<sub>pro</sub>.

The structural changes of 2Z-3CL<sub>pro</sub>, 2E-3CL<sub>pro</sub>, and lopinavir-3CL<sub>pro</sub> complexes were evaluated using RMSD. The conformational change of backbone atoms of the most promising compounds in complex with 3CL<sub>pro</sub> has been compared with initial conformations over 150 ns MD simulations and shown in Figure 7. As shown in Figure 7,
the RMSDs were noticed to be below 0.22 nm, demonstrating the overall stability of these compounds inside the 3CL\textsuperscript{pro} active site. These results confirmed that three compounds are tightly bonded in the active site and do not affect the overall topology of 3CL\textsuperscript{pro}.

To pinpoint backbone steadiness and conformational changes of the 2Z-3CL\textsuperscript{pro}, 2E-3CL\textsuperscript{pro}, and lopinavir-3CL\textsuperscript{pro} complexes, the root-mean-square fluctuation (RMSF) of C\textalpha was estimated and depicted in Figure 8. RMSF is used to analyze the parts of the structure that are oscillating from their mean structure the most (or least). As shown in Figure 8, the average number of hydrogen bonds was detected, implying that the obtained structures represent the true minima on the corresponding conformational energy surfaces. The DFT energetic results showed that the stability of trans-isomer \textit{2E} was higher than the cis-isomer \textit{2Z} conformer by 1.51 kcal/mol.

### 2.3. 3CL\textsuperscript{pro} Inhibitory Activity

Recently, E-rosmarinic acid has been reported to inhibit SARS-CoV-2 replication and SARS-CoV-2 3CL\textsuperscript{pro}\textsubscript{\textit{13}}. However, the activity of the \textit{Z}-isomer has not been reported yet. This prompted us to investigate and compare the 3CL\textsuperscript{pro} inhibitory potential of compounds 1 and 2 (2Z and 2E) (Table 3). E-Rosmarinic acid 1 showed an IC\textsubscript{50} value of 25.75 \mu M, meanwhile (E/Z)-rosmarinic acid mixture 2 was more active with an IC\textsubscript{50} value of 6.062 \mu M, and lopinavir showed an IC\textsubscript{50} value of 0.148 \mu M. Interestingly, the E/Z-isomeric mixture (6.062 \mu M) was about 4 times as active as the single E-isomer (25.75 \mu M). In order to justify the superior activity of the E/Z-isomeric mixture, the binding affinities and features of both isomers against 3CL\textsuperscript{pro} were further investigated by molecular docking and MD techniques. It is worth noting that rosmarinic acid is reported to exhibit an \textit{in silico} high binding affinity toward 3CL\textsuperscript{pro}\textsubscript{\textit{26–29}}. The compound is also reported to exhibit broad-spectrum antiviral activity.

| Table 3. Inhibitory Activity of the Isolated Compounds against 3CL\textsuperscript{pro} |
|-----------------------------------------------|
| compound                                      | IC\textsubscript{50} (\mu M) |
| 1                                            | 25.75 ± 2.1                 |
| 2                                            | 6.062 ± 0.54                |
| lopinavir\textsuperscript{\textsuperscript{a}} | 0.148 ± 0.01                |

\textsuperscript{a}Standard.

### 3. MATERIALS AND METHODS

#### 3.1. General Experimental Procedures

NMR spectral analysis was performed using a Bruker DRX 600 NMR spectrometer from Bruker Daltonics (500 MHz for \textit{1}\text{H} and 125 MHz for \textit{13}\text{C}) and Varian INOVA-600 (600 MHz for \textit{1}\text{H} and 150 MHz for \textit{13}\text{C}). ESI-TOF-MS spectra were measured with a Bruker microTOF mass spectrometer. The high-resolution mass (HR-FAB-MS) spectrum was measured with a JEOL JMS 700 spectrometer. Chromatographic separation was carried out using Merck Silica gel G 63-200, preparative thin-layer chromatography (RP-C18 F254 glass plates, 20 × 20 cm × 0.25 mm thick) and RP HPLC using Cosmosil AR-II, 250 × 10 mm i.d with a JASCO PU2089 gradient pump and a PU2075 UV/VIS detector. Thin-layer chromatography was carried out using Merck precoated silica gel F254 plates.

#### 3.2. Plant Material

The plant material consists of the root part of \textit{Eryngium criticum} L. It was collected from Borg El Arab, Alexandria, Egypt, and authenticated by Dr. Ibrahim Mashaly, Professor of Ecology, Faculty of Science, Mansoura University. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University (02-16-ECMansoura).

#### 3.3. Extraction and Isolation

About 912 g dry powdered root parts were extracted with MeOH (5 × 4 L methanol) to afford 250 g extract. The extract was dissolved in MeOH/H\textsubscript{2}O (50:50) and portioned with \textit{n}-hexane, CH\textsubscript{2}Cl\textsubscript{2}, EtOAc, and \textit{n}-BuOH to afford 21, 7, 14, and 55 g, respectively.

The \textit{n}-hexane fraction was fractionated over a silica gel column (36 × 3.25, 400 g) using \textit{n}-hexane/EtOAc as a solvent system to afford seven major groups (1–7). Group 2, eluted with 10% EtOAc in \textit{n}-hexane, was purified over a preparative
silica TLC using n-hexane/EtOAc (8/2) to afford compound 3. Group 3, eluted with 15% EtOAc in n-hexane, afforded compound 5 by crystallization. Group 5, eluted with 30% EtOAc in n-hexane, was purified over a preparative silica TLC using n-hexane/EtOAc (8/2) to afford compound 4. Compounds 1 and 2 were purified from the n-BuOH fraction as a single spot. They were isolated by HPLC (20% CH$_3$CN, containing 0.1% HCOOH, Cholester) at a flow rate of 1 mL/min. UV monitoring was done at 254 nm.

3.4. DFT Calculations. To thoroughly elucidate the energetic stability of the Z- and E-conformations of compound 2, DFT calculations were carried out using Gaussian 09 software. The investigated conformers were first optimized using Becke’s three-parameter and Lee–Yang–Parr correlation functionals with the Pople split-valence double-zeta basis set with polarization function (6-31G*). Upon the optimized conformers, the vibrational frequency calculations were then performed to verify the true minima of the obtained structures. The single-point energies were calculated at the optimization level of theory.

3.5. In Silico Drug Discovery. 3.5.1. Target Preparation. The crystal structure of 3-chymotrypsin like protease (3CL$^{pro}$; PDB code: 6LU7) was selected as a template for all in silico calculations. Crystallographic water molecules, ions, as well as heteroatoms were stripped out. The H++ server was utilized to investigate the protonation state of 3CL$^{pro}$, and all missing hydrogen atoms were added.

3.5.2. Inhibitor Preparation. The chemical structures of the isolated compounds were manually constructed, and their 3D structures were generated using Omega2 software. All compounds were then energetically minimized using the MMFF94S force field with the help of SYBYL software.

3.5.3. Molecular Docking. Molecular docking calculations were carried out using AutoDock4.2.6 software. For molecular docking calculations, the pdbqt file for the 3CL$^{pro}$ target was prepared according to the AutoDock protocol. All docking parameters were conserved to their default values, except the number of genetic algorithm (GA) run and the eval were set to 250 and 25,000,000, respectively. The docking grid was set to 60 Å × 60 Å × 60 Å with polarization function (6-31G*). Upon the optimized conformers, the vibrational frequency calculations were then performed to verify the true minima of the obtained structures. The single-point energies were calculated at the optimization level of theory.

3.6. 3CL$^{pro}$ Inhibitory Activity. Inhibition of 3-chymotrypsin-like protease (3CL$^{pro}$) enzyme activity was measured using the Fluorogenic 3CL$^{pro}$ Assay Kit (BPS Bioscience #79955, San Diego CA, USA) according to the manufacturer’s instructions. It is a fluorescence resonance energy transfer-based assay. The principle of the assay depends on measuring the proximity of two fluorophores in the substrate. The substrate is an internally quenched fluorescent peptide (DABCYL-KTSAVLQSGFRKME-EDANS). Upon proteolysis by 3CL$^{pro}$, the substrate is cleaved to generate a highly fluorescent peptide fragment (SGFRKME-EDANS). The increase in fluorescence intensity is directly proportional to the activity of the enzyme.

Just prior to use, dithiothreitol (DTT) was dissolved in the assay buffer at a concentration of 1 mM. The enzyme was diluted in the assay buffer containing DTT to a final concentration of 3–5 ng/μL. The substrate (5 mM) was diluted 1:20 in assay buffer containing DTT, to make a 250 μM solution. The final concentration of the substrate in a 50 μL reaction was 50 μM. The inhibitor and the test samples were dissolved in dimethyl sulfoxide (DMSO) and serially diluted with the assay buffer, so that the final concentration of DMSO in the assay does not exceed 1%. The samples were prepared at a concentration 5-fold higher than the final concentration in the reaction mixture. The positive control consisted of 30 μL diluted enzyme, 10 μL sample buffer, and 10 μL substrate. The inhibitor control consisted of 30 μL diluted enzyme, 10 μL inhibitor (lpinavir), and 10 μL substrate. The test sample consisted of 30 μL diluted enzyme, 10 μL sample, and 10 μL substrate. The blank consisted of 30 μL assay buffer, 10 μL sample buffer, and 10 μL substrate. The enzyme was preincubated with the inhibitor/test sample for 30 min at room temperature with slow shaking. The reaction was started by the addition of the substrate solution to each well. The reaction was incubated at room temperature overnight and sealed with a plate sealer. The fluorescence intensity was measured at an excitation/emission wavelength of 360/460 nm. The “Blank” value was subtracted from all other values.

4. Conclusions

Five compounds were isolated from E. creticum, including four compounds that are reported from the plant for the first time. Compounds 1 and 2 (2E and 2Z) were investigated for their 3CL$^{pro}$ inhibitory activity. Interestingly, the E/Z-isomeric
mixture 2 was about 4 times as active as the single E-isomer 1. Molecular docking and MD simulations demonstrated the promising binding affinity of compounds 2Z and 2E with SARS-CoV-2 main protease (3CLpro), with docking scores of $-8.9$ and $-8.5$ kcal/mol and MM-GBSA binding energies of $-26.5$ and $-22.1$ kcal/mol, respectively. Furthermore, DFT calculations demonstrated the more energetic stability of 2E compared to 2Z, justifying the difficulty of isolating the Z-isomer in a pure form.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c02237.

Spectroscopic data; isolation scheme; and molecular docking of the isolated compounds (1–5) from E. creticum (PDF)

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Notes
The authors declare no competing financial interest.

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