Anti-Inflammatory, Antiallergic and COVID-19 Main Protease (M\textsuperscript{pro}) Inhibitory Activities of Butenolides from a Marine-Derived Fungus \textit{Aspergillus costaricaensis}

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## Supporting Information

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Experimental section

General experimental procedures

Ready-made TLC plates (silica gel 60 F254, Merck®, Darmstadt, Germany) were applied for screening procedures. To visualize TLC plates, a laboratory UV lamp at short (254 nm) and long (365 nm) wavelengths were used or by spraying developed TLC plates with anisaldehyde reagent and then heated. Preparative TLC separations were performed using Flat Bottom TLC Chamber (Camag®, Muttenz, Switzerland). Chromatographic workup procedures were conducted implementing column chromatography implementing silica gel 60 (0.04–0.063, Merck®, Germany) and Sephadex® LH-20 (Sigma-Aldrich®, Burlington, MA, USA) as stationary phases. Perkin-Elmer-241 MC polarimeter was used for measuring optical rotation values. Fine purification steps were performed using preparative HPLC (Agilent®, Santa Clara, CA, USA) with Zorbax Eclipse XDB-C18 preparative column (9.4 mm × 250 mm, L × ID; 5 µm particle size) at a flow rate of 2 mL/min and UV screening detection at 210 to 330 nm. A standard gradient elution was applied using (MeOH in Water) as follows: 0 min, 10% MeOH; 5 min, 10% MeOH; 40 min, 90% MeOH, applying a flow rate of 1 mL/min. An Agilent 600 MHz spectrometer (USA) was used for 1D (¹H and ¹³C NMR) and 2D NMR spectra (chemical shifts in ppm). Deuterated chloroform-­d and methanol-d₄ NMR solvents (Sigma-Aldrich®, Taufkirchen, Germany) were used to dissolve isolated compounds.

Sponge and Fungal Strain Material

Aspergillus costaricaensis was derived from Petrosia ficiformis, a marine sponge collected by one co-author (B.G.) from Çıralı, Antalya, Turkey in July, 2018. For fungal identification, Sabouraud 4% dextrose agar medium (SDA, Merck, Germany) was used to cultivate the fungus at room temperature (Nüve, Turkey). Fungal identification was
determined based on DNA amplification and ITS (Internal Transcribed Spacer) sequencing data analysis as reported before in literature\(^1\) and it was identified as *Aspergillus costaricaensis* (GenBank accession number MT273951). A voucher specimen of fungal strain was kept at Department of Pharmacognosy, Faculty of Pharmacy, Ankara University (B.K.).

**Extraction, isolation and purification**

The isolated and identified *Aspergillus costaricaensis* fungus was cultured in 10 pieces of 2 L Erlenmeyer flasks each containing 100-mL solid rice medium prepared using autoclaved 100 g rice and 100 mL distilled water containing 3.5% artificial sea salt. Cultivation was kept in darkness at room temperature and under static conditions for 30 days.

Fermentation process was discontinued by ethyl acetate (EtOAc) (3 × 350 mL) added to each flask to cease fungal growth. Afterwards, flasks were set on shaker for 12 h then filtered. All EtOAC filtrates were collected and concentrated under vacuum till attaining a solid residue (4.9 g). Then, the crude raw extract was subjected to liquid–liquid partitioning between *n*-hexane and 90% aqueous MeOH where both fractions were separated and concentrated under reduced pressure. The aqueous 90% MeOH phase (2.7 g) was then subjected to vacuum liquid chromatography (VLC) using silica gel 60 as a stationary phase and *n*-hexane:EtOAc (25:75) as a starting mobile phase followed by a gradient elution development starting from DCM (100%) to MeOH (100%) with a 10%-increment interval affording eleven fractions (AC–1~AC–11).

The attained fractions were screened by TLC plates and analytical HPLC runs. Fractions AC–2, AC–4 and AC–6 were selected for further preparative TLC and/or HPLC purification procedures. Fraction AC–2 (261 mg), eluted with DCM (100%), was rechromatographed on CC using silica gel 60 as a stationary phase and petroleum
ether:EtOAc as a mobile phase using gradient elution of the following ratios (4:1, 3:1, 2:1, 1:1) with each of 300 mL yielding 1 (2.4 mg) and 6 (1.7 mg). Fraction AC-4 (223 mg) eluted with DCM:MeOH (80:20) was applied on CC using Sephadex LH-20 (as a stationary phase) and methanol (as a mobile phase) to afford 2 (1.2 mg) and 3 (2.3 mg). Fraction AC-6 (240 mg) eluted with DCM:MeOH (60:40) was similarly subjected to CC using Sephadex LH-20 and methanol followed by preparative HPLC for final purification to obtain 4 (1.8 mg) and 5 (1.3 mg).

**Anti-Inflammatory Assay**

The human neutrophils were isolated from venous blood of healthy adult volunteers (20–30 years old) following the reported procedure.\(^2\) Elastase release by the fMLF/CB-activated neutrophils was measured using elastase substrate (N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide) according to the previous method.\(^4\)

**Antiallergic Assay**

The mucosal mast-cell derived rat basophilic leukemia cells (RBL-2H3, Bioresource Collection and Research Center, Hsin-Chu, Taiwan), were grown in DMEM containing 10% FBS, antibiotics (100 U/mL penicillin, and 100 μg/mL streptomycin) in incubator at 37 °C. The cytotoxic effects towards RBL-2H3 cells were evaluated using methylthiazole tetrazolium (MTT) method as described previously.\(^2\) The level of degranulation in mast cells was then assessed according to the previous literature.\(^3\) Briefly, the RBL-2H3 cells were seeded for A23187-induced assay in 96-well plate or, separately, for antigen-induced assay in 48-well plate treated with 0.5 μg/mL anti-DNP IgE overnight. The samples (10 and 100 μM) were added to the wells and incubated 30 min. The cells were then activated by the addition of 0.5 μM A23187 for A23187-induced assay, or 100 ng/mL DNP-BSA for antigen-induced assay, in both cases for 30 min. Azelastine served as a positive control. The amount of β-hexosaminidase was
then measured utilizing substrate (p-nitrophenyl-N-acetyl-D-glucosaminide) as described before.\[3\]

**Molecular Modelling Studies**

Docking studies of investigated isolated natural products with target proteins were done with Autodock Vina similar to what we have done before.\[5\] In our case, human neutrophil elastase (1H1B) and the main protease (M\[^{\text{pro}}\]) of SARS-CoV-2 (6LU7) crystal structures were used. Co-crystallized ligands as well as prepared test compounds were docked in a cubic grid box with side length of 25 Å centered on co-crystalized ligand using exhaustiveness of 16. In every case, the top 9 binding modes or poses were arranged according to their docking scores and the obtained interactions with targets were analyzed. The pose with the best score and a binding pose similar to co-crystalized ligands was reported.

**Molecular Dynamic (MD) Simulation**

GROMACS 2020.3 MD package was used to run dynamics simulation.\[6\] CHARMM36 all-atom force field was used to generate parameters for target proteins while those of the ligands were generated from SwissParam.\[7,8\] All simulations were run in a dodecahedron box filled with explicit 3-site water model (TIP3P) and neutralized by Na\[^+\] or Cl\[^-\] ions.\[9\] Minimization was done using steepest descent algorithm for a maximum of 5000 steps. Systems were initially equilibrated using Canonical ensemble then isothermal–isobaric ensemble for 100 ps each. The temperature was kept constant around 300 K using a thermostat (the V-rescale algorithm) and pressure was kept at 1 atm by the Parrinello-Rahman barostat.\[10,11\] The LINear Constraint Solver (LINCS) algorithm\[12\] was used for bond’s length constraints and Particle mesh Ewald (PME) method\[13\] was used for long-range electrostatics calculations. For all simulations, a time step of 2 femtoseconds was used. A cut-off distance of 1.2 nm was
used for van der Waals interactions. Coordinates obtained from crystal structure files or from docking poses were used as initial coordination for the dynamics.

**Determination of elastase enzymatic activity**

Elastase enzymatic activity assay was performed using cell-free model as described before. The neutrophil suspension (6 × 10⁵ cells·mL⁻¹) was activated by CB (1.5 μg·mL⁻¹) and fMLF (0.1 μM) and the supernatant containing elastase was collected and incubated with the test compounds. Then, after the addition of the substrate for elastase (methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide), the absorbance at 405 nm was measured to quantify the effect of the compounds on elastase enzymatic activity.

**Coronavirus 229E assay**

The cytopathic effects on human coronavirus 229E (HCoV-229) were measured as previously described. The host cells (Huh7 cells, human liver carcinoma cell line) were infected with the coronavirus 229E (load of 9TCID50 which represents Median Tissue Culture Infectious Dose). The sample or vehicle was added and cells were incubated for 6 days at 33 °C. Then the surviving cells were then treated with MTT (3-[4.5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) and absorbance was measured to evaluate the percentage of surviving cells.

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Figure S1. Chemical structures of 1-6.

Figure S2. Human coronavirus 229E (HCoV-229E) protective activity. The cells infected by HCoV-229E were treated with the compounds (1, 3, 5) (orange bar) or vehicle (gray bar). The different values between the orange and gray bars would indicate protective effects against HCoV-229E infection. The uninfected cells were treated with the compounds (1, 3, 5) (dark blue bar) or vehicle only (cyan bar), which served as a control to indicate the samples non-toxicity towards uninfected cells. Veh; vehicle. Data for compounds 1 and 3 were shown in Molecules 2021, 26, 3354, 10.3390/molecules26113354.
Table S1. Comparison of $^1$H and $^{13}$C NMR Data for compound (1) and butyrolactone I.

| pos. | 1 | Butyrolactone I [12,13] |
|------|---|------------------------|
|      | $\delta_H$ (J [Hz])$^a$ | $\delta_C$, type$^b$ | $\delta_H$ (J [Hz])$^c$ | $\delta_C$, type$^d$ |
| 1    | 170.4, C                  |                        | 167.9, C                  |                        |
| 2    | 146.7, C                  |                        | 138.1, C                  |                        |
| 3    | 139.8, C                  |                        | 127.5, C                  |                        |
| 4    | 86.8, C                   |                        | 84.8, C                   |                        |
| 5    | 3.46, d (14.6)            | 39.6, CH$_2$           | 3.47, d (14.0, 2H)        | 38.1, CH$_2$           |
|      | 3.41, d (14.6)            |                        |                          |                        |
| 6    | 171.7, C                  |                        | 169.8, C                  |                        |
| 7    | 3.78, s (3H)              | 53.8, CH$_3$           | 3.82, s (3H)              | 53.4, CH$_3$           |
| 1$'$ | 123.2, C                  |                        | 121.1, C                  |                        |
| 2$'$ | 7.59, d (8.8)             | 130.4, CH              | 7.50, d (8.0)             | 128.8, CH              |
| 3$'$ | 6.87, d (8.8)             | 116.6, CH              | 6.78, d (8.0)             | 115.8, CH              |
| 4$'$ | 159.3, C                  |                        | 157.9, C                  |                        |
| 5$'$ | 6.87, d (8.8)             | 116.6, CH              | 6.78, d (8.8)             | 115.8, CH              |
| 6$'$ | 7.59, d (8.8)             | 130.4, CH              | 7.50, d (8.0)             | 128.8, CH              |
| 1$''$| 125.1, C                  |                        | 123.1, C                  |                        |
| 2$''$| 6.41, d (2.1)             | 132.4, CH              | 6.50, m                   | 128.4, CH              |
| 3$''$| 129.1, C                  |                        | 114.1, C                  |                        |
| 4$''$| 155.1, C                  |                        | 153.8, C                  |                        |
| 5$''$| 6.49, d (8.1)             | 115.0, CH              | 6.50, m                   | 114.1, CH              |
| 6$''$| 6.53, dd (8.1, 2.1)       | 129.8, CH              | 6.50, m                   | 127.5, CH              |
| 7$''$| 3.07, m (2H)              | 28.7, CH$_2$           | 3.13, d (7, 2H)           | 27.6, CH$_2$           |
| 8$''$| 5.06, tp (7.4, 1.5)       | 123.6, CH              | 5.06, t (7.0)             | 122.4, CH              |
| 9$''$| 133.0, C                  |                        | 131.4, C                  |                        |
| 10$''$| 1.67, d (1.3, 3H)        | 26.0, CH$_3$         | 1.67, s (3H)             | 25.5, CH$_3$         |
| 11$''$| 1.57, d (1.3, 3H)        | 17.8, CH$_3$         | 1.62, s (3H)             | 17.5, CH$_3$         |

$^a$ Measured in methanol-$d_4$ at 600 MHz.  
$^b$ Measured in methanol-$d_4$ at 150 MHz.  
$^c$ Measured in chloroform-$d_6$ at 200 MHz.  
$^d$ Measured in DMSO-$d_6$ at 50 MHz.
Figure S3. HPLC chromatogram and HRESIMS of 1
Figure S4. $^1$H-NMR spectrum of 1 in methanol-$d_4$ at 600 MHz.
Figure S5. $^{13}$C-NMR spectrum of 1 in methanol-$d_4$ at 150 MHz.
Figure S6. $^1$H–$^1$H COSY spectrum of 1 in methanol-$d_4$ at 600 MHz.
Figure S7. gHMBC spectrum of 1 in methanol-$d_4$ at 600 MHz.
Figure S8. gHMOC spectrum of 1 in methanol-$d_4$ at 600 MHz.
Figure S9. NOESY spectrum of 1 in methanol-$d_4$ at 600 MHz.
Table S2. Comparison of $^1$H and $^{13}$C NMR Data for compound (2) and butyrolactone VI.

| pos. | $\delta_H$ (Hz)$^a$ | $\delta_C$, type$^b$ | $\delta_H$ (Hz)$^c$ | $\delta_C$, type$^d$ |
|------|---------------------|---------------------|---------------------|---------------------|
| 1    | 170.4, C            | 168.0, C            |
| 2    | 139.9, C            | 138.4, C            |
| 3    | 129.2, C            | 127.4, C            |
| 4    | 86.8, C             | 85.2, C             |
| 5    | 3.45, s (2H)        | 3.46, d (14.4)      |
|      |                     | 3.45, d (14.4)      |
| 6    | 171.6, C            | 170.0, C            |
| 7    | 3.79, s (3H)        | 3.79, s (3H)        |
|      | 53.9, CH$_3$        | 52.8, CH$_3$        |
| 1’   | 7.58, d (8.8)       | 7.63, d (8.8)       |
|      | 123.2, C            | 122.0, C            |
| 2’   | 6.87, d (8.8)       | 6.98, d (8.8)       |
|      | 116.6, CH$_3$       | 115.7, CH$_3$       |
| 3’   | 159.4, C            | 158.0, C            |
| 4’   | 6.87, d (8.8)       | 6.98, d (8.8)       |
|      | 116.6, CH$_3$       | 115.7, CH$_3$       |
| 5’   | 13.5, d (8.8)       | 13.5, d (8.8)       |
|      | 125.6, C            | 124.3, C            |
| 6’   | 6.62, d (2.1)       | 6.61, d (2.1)       |
|      | 134.4, CH$_3$       | 133.2, CH$_3$       |
| 1''  | 127.3, C            | 126.3, C            |
| 2''  | 155.9, C            | 155.1, C            |
| 3''  | 6.51, d (8.1)       | 6.56, m             |
|      | 115.9, CH$_3$       | 115.5, CH$_3$       |
| 6''  | 6.49, dd (8.1, 2.1) | 130.5, CH$_3$       |
|      | 6.56, m             | 129.5, CH$_3$       |
| 7''  | 2.69, dd (14.1, 2.3)| 34.0, CH$_2$        |
|      | 2.54, dd (14.1, 10.0)| 2.65, dd (14.2, 2.0)|
|      |                     | 2.58, dd (14.2, 9.6)| 33.6, CH$_2$ |
| 8''  | 3.44, dd (10.0, 2.3)| 80.6, CH$_3$        |
|      | 3.48, dd (9.6, 2.0) | 80.1, CH$_3$        |
| 9''  | 73.8, C             | 71.8, C             |
| 10'' | 1.18, s (3H)        | 25.5, CH$_3$        |
|      | 1.19, s (3H)        | 24.8, CH$_3$        |
| 11'' | 1.17, s (3H)        | 25.1, CH$_3$        |
|      | 1.18, s (3H)        | 24.2, CH$_3$        |

$^a$ Measured in methanol-$d_4$ at 600 MHz. $^b$ Measured in methanol-$d_4$ at 150 MHz. $^c$ Measured in acetone-$d_6$ at 500 MHz. $^d$ Measured in acetone-$d_6$ at 125 MHz.
Figure S10. HPLC chromatogram and HRESIMS spectrum of 2
Figure S11. $^1$H-NMR spectrum of 2 in methanol-$d_4$ at 600 MHz.
Figure S12. $^{13}$C-NMR spectrum of 2 in methanol-$d_4$ at 150 MHz.

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Figure S13. $^1$H–$^1$H COSY spectrum of 2 in methanol-$d_4$ at 600 MHz.
Figure S14. gHMBC spectrum of 2 in methanol-$d_4$ at 600 MHz.
Figure S15. gHMOC spectrum of 2 in methanol-$d_4$ at 600 MHz.
Figure S16. NOESY spectrum of 2 in methanol-$d_4$ at 600 MHz.
Table S3. Comparison of $^1$H and $^{13}$C NMR Data for compound (3) and butyrolactone V.

| pos. | $^1$H ($^J$/Hz)$^a$ | $^13$C, type$^b$ | $^1$H ($^J$/Hz)$^c$ | $^13$C, type$^d$ |
|------|---------------------|------------------|---------------------|-------------------|
| 1    | 169.6, C            |                  | 167.8, C            |                   |
| 2    | 137.6, C            |                  | 138.3, C            |                   |
| 3    | 129.7, C            |                  | 127.5, C            |                   |
| 4    | 86.1, C             |                  | 85.1, C             |                   |
| 5    | 3.51, d (14.7)      | 38.8, CH$_2$     | 3.48, d (14.4)      | 38.4, CH$_2$      |
|      | 3.41, d (14.7)      |                  | 3.46, d (14.4)      |                   |
| 6    | 169.9, C            |                  | 169.9, C            |                   |
| 7    | 3.73, s (3H)        | 53.7, CH$_3$     | 3.79, s (3H)        | 52.8, CH$_3$      |
| 1'   | 122.0, C            |                  | 121.9, C            |                   |
| 2'   | 7.56, d (9.0)       | 129.6, CH        | 7.62, d (8.8)       | 129.3, CH         |
| 3'   | 6.90, d (9.0)       | 116.2, CH        | 6.98, d (8.8)       | 115.7, CH         |
| 4'   | 157.3, C            |                  | 158.0, C            |                   |
| 5'   | 6.90, d (9.0)       | 116.2, CH        | 6.98, d (8.8)       | 115.7, CH         |
| 6'   | 7.56, d (9.0)       | 129.6, CH        | 7.62, d (8.8)       | 129.3, CH         |
| 1''  | 118.4, C            |                  | 124.7, C            |                   |
| 2''  | 6.51, d (2.1)       | 132.1, CH        | 6.48, d (2.0)       | 131.7, CH         |
| 3''  | 124.8, C            |                  | 119.7, C            |                   |
| 4''  | 152.0, C            |                  | 152.3, C            |                   |
| 5''  | 6.53, d (8.1)       | 116.7, CH        | 6.49, d (8.3)       | 116.0, CH         |
| 6''  | 6.51, dd (8.1, 2.1) | 128.9, CH        | 6.60, dd (8.3, 2.0) | 129.2, CH         |
| 7''  | 2.82, dd (17.0, 5.0)| 30.9, CH$_2$     | 2.74, dd (16.5, 5.5)| 31.2, CH$_2$      |
|      | 2.58, dd (17.0, 6.0)|                  | 2.52, dd (16.5, 8.4)|                   |
| 8''  | 3.74, m             | 69.7, CH         | 3.69, dd (8.4, 5.5) | 69.0, CH          |
| 9''  | 76.8, C             |                  | 76.9, C             |                   |
| 10'' | 1.24, s (3H)        | 22.0, CH$_3$     | 1.29, s (3H)        | 19.3, CH$_3$      |
| 11'' | 1.21, s (3H)        | 24.8, CH$_3$     | 1.14, s (3H)        | 25.3, CH$_3$      |

$^a$ Measured in CDCl$_3$-d at 400 MHz. $^b$ Measured in CDCl$_3$-d at 100 MHz. $^c$ Measured in acetone-d$_6$ at 500 MHz. $^d$ Measured in acetone-d$_6$ at 125 MHz.
Figure S17. HPLC chromatogram and HRESIMS spectrum of 3
Figure S18. $^1$H-NMR spectrum of 3 in CDCl$_3$-d at 400 MHz.
Figure S19. $^{13}$C-NMR spectrum of 3 in CDCl$_3$-$d$ at 100 MHz.
Figure S20. $^1$H–$^1$H COSY spectrum of 3 in CDCl$_3$-$d$ at 400 MHz.
Figure S21. gHMBC spectrum of 3 in CDCl$_3$-d at 400 MHz.
Figure S22. gHMOC spectrum of 3 in CDCl₃-d at 400 MHz.
Figure S23. NOESY spectrum of 3 in CDCl$_3$-$d$ at 400 MHz.
Table S4. Comparison of $^1$H and $^{13}$C NMR Data for compound (4) and TMC-256A1.

| pos. | 4         | TMC-256A1 [20] |
|------|-----------|----------------|
|      | $\delta$ (multi, $J$ [Hz])<sup>a</sup> | $\delta$ (multi, $J$ [Hz])<sup>c</sup> |
| 2    | 171.2, C  | 168.3, C       |
| 3    | 6.04, s (1H) | 6.13, s (1H)  |
| 4    | 183.5, C  | 183.5, C       |
| 4a   | 105.7, C  | 102.8, C       |
| 5    | 162.2, C  | 162.2, C       |
| 5a   | 109.3, C  | 106.5, C       |
| 6    | 163.5, C  | 160.5, C       |
| 7    | 6.43, d (1H, 2.2) | 6.41, d (1H, 2.2) |
| 8    | 162.7, C  | 159.9, C       |
| 9    | 6.60, d (1H, 2.2) | 6.63, d (1H, 2.2) |
| 9a   | 140.8, C  | 140.8, C       |
| 10   | 6.94, s (1H) | 7.01, s (1H)  |
| 10a  | 152.4, C  | 152.4, C       |
| 2-Me | 2.38, s (3H) | 2.36, s (3H)  |
| 6-OMe| 3.93, s (3H) | 3.86, s (3H)  |
| 5-OH |         | 14.84, s (1H) |
| 8-OH |         | 10.27, br s (1H) |

<sup>a</sup> Measured in methanol-$d_4$ at 600 MHz. <sup>b</sup> Measured in methanol-$d_4$ at 150 MHz. <sup>c</sup> Measured in DMSO-$d_6$ at 400 MHz. <sup>d</sup> Measured in DMSO-$d_6$ at 100 MHz.
Figure S24. $^1$H-NMR spectrum of 4 in methanol-$d_4$ at 600 MHz.
Figure S25. $^{13}$C-NMR spectrum of 4 in methanol-$d_4$ at 150 MHz.
Figure S26. $^1$H–$^1$H COSY spectrum of 4 in methanol-$d_4$ at 600 MHz.
Figure S27. gHMBC spectrum of 4 in methanol-$d_4$ at 600 MHz.
Figure S28. gHMOC spectrum of 4 in methanol-$d_4$ at 600 MHz.
Table S5. Comparison of $^1$H and $^{13}$C NMR data for compound (5) and rubrofusarin B.

| pos. | 5                          | Rubrofusarin B [21]                     |
|------|---------------------------|----------------------------------------|
|      | $\delta_\text{H}$ (multi, $J$ [Hz])$^a$ | $\delta_\text{H}$ (multi, $J$ [Hz])$^c$ | $\delta_\text{C}$, type$^b$ | $\delta_\text{C}$, type$^d$ |
| 2    | 170.2, C                  | 167.4, C                               |
| 3    | 6.07 (d, 1.0)             | 5.98 (s)                               | 107.8, CH                  | 107.3, CH                  |
| 4    | 185.9, C                  | 184.2, C                               |
| 4a   | 105.1, C                  | 104.3, C                               |
| 5    | 163.2, C                  | 162.6, C                               |
| 5a   | 109.3, C                  | 108.4, C                               |
| 6    | 161.8, C                  | 160.6, C                               |
| 7    | 6.46 (d, 2.2)             | 6.38 (d, 2.2)                          | 98.5, CH                   | 97.2, CH                   |
| 8    | 163.4, C                  | 161.5, C                               |
| 9    | 6.76 (d, 2.2)             | 6.56 (d, 2.2)                          | 99.1, CH                   | 97.8, CH                   |
| 9a   | 142.9, C                  | 141.0, C                               |
| 10   | 7.10 (s)                  | 6.94 (s)                               | 102.6, CH                  | 101.0, CH                  |
| 10a  | 154.6, C                  | 153.3, C                               |
| 2-CH$_3$ | 2.40 (3H, d, 1.0)           | 20.5, CH$_3$                           | 2.35 (3H, s)               | 20.6, CH$_3$               |
| 6-OCH$_3$ | 3.93 (3H, s)                   | 56.4, CH$_3$                           | 3.99 (3H, s)               | 56.0, CH$_3$               |
| 8-OCH$_3$ | 3.91 (3H, s)                   | 56.0, CH$_3$                           | 3.91 (3H, s)               | 55.4, CH$_3$               |

$^a$ Measured in methanol-$d_4$ at 600 MHz. $^b$ Measured in methanol-$d_4$ at 150 MHz. $^c$ Measured in chloroform-$d$ at 300 MHz. $^d$ Measured in chloroform-$d$ at 75 MHz.
Figure S29. $^1$H-NMR spectrum of 5 in methanol-$d_4$ at 600 MHz.
Figure S30. $^{13}\text{C}$-NMR spectrum of 5 in methanol-$d_4$ at 150 MHz.
Figure S31. $^1$H–$^1$H COSY spectrum of 5 in methanol-$d_4$ at 600 MHz.
Figure S32. gHMBC spectrum of 5 in methanol-$d_4$ at 600 MHz.
Figure S33. gHMOC spectrum of 5 in methanol-\textit{d}_4 at 600 MHz.
Figure S34. NOESY spectrum of 5 in methanol-$d_4$ at 600 MHz.
Table S6. Comparison of $^1$H and $^{13}$C NMR data for compound (6) and methyl $p$-hydroxyphenyl acetate.

| pos. | $^1$H (multi, $J$ [Hz])$^a$ | $^1$C, type$^b$ | $^{13}$H (multi, $J$ [Hz])$^c$ |
|------|-----------------|-------------|-----------------|
| 1    | 174.6, CO       | 40.9, CH$_2$ | 3.52 (2H, s)    |
| 2    | 3.53 (2H, s)    | 3.52 (2H, s) |
| 3    | 126.3, C        | 116.3, CH   | 6.65 (2H, d, 8.0)|
| 4, 4' | 6.72 (2H, d, 8.4)| 6.72 (2H, d, 8.4)|
| 5, 5' | 7.07 (2H, d, 8.4)| 7.07 (2H, d, 8.0)|
| 6    | 157.6, CO       | 157.6, CO   | 52.4, CH$_3$    |
| 7    | 3.66 (3H, s)    | 3.73 (3H, s) |

$^a$ Measured in methanol-$d_4$ at 600 MHz. $^b$ Measured in methanol-$d_4$ at 150 MHz. $^c$ Measured in chloroform-$d$ at 200 MHz.
Figure S35. $^1$H-NMR spectrum of 6 in methanol-$d_4$ at 600 MHz.
Figure S36. $^{13}$C-NMR spectrum of 6 in methanol-$d_4$ at 150 MHz.
Figure S37. gHMBC spectrum of 6 in methanol-$d_4$ at 600 MHz.
Figure S38. gHMOC spectrum of 6 in methanol-$d_4$ at 600 MHz.
Table S7. Inhibitory activity of compounds (1–6) on A23187- and antigen-induced degranulation.

| Compound                        | % Viability, RBL-2H3 [a] | % Inhibition of A23187-Induced Degranulation [b] | % Inhibition of Antigen-Induced Degranulation [c] |
|---------------------------------|--------------------------|-----------------------------------------------|-----------------------------------------------|
| Butyrolactone VI (2)            | >90%                     | IC₅₀ (μM) [d] >100                             | IC₅₀ (μM) [d] >100                             |
| Butyrolactone V (3)             | >90%                     | IC₅₀ (μM) [d] >100                             | IC₅₀ (μM) [d] >100                             |
| TMC-256A1 (4)                   | >90%                     | IC₅₀ (μM) [d] >100                             | IC₅₀ (μM) [d] >100                             |
| Rubrofusarin B (5)              | >90%                     | IC₅₀ (μM) [d] >100                             | IC₅₀ (μM) [d] >100                             |
| Methyl p-hydroxyphenyl acetate (6)| >90%                     | IC₅₀ (μM) [d] >100                             | IC₅₀ (μM) [d] >100                             |

[a] The cytotoxicity of samples to RBL-2H3 was evaluated using MTT viability assay. Results are presented as mean (n = 3) compared to untreated control (DMSO). All samples were nontoxic towards RBL-2H3 cells. [b] Azelastine (10 μM) was used as a positive control and inhibited 34.5 ± 2.5% of A23187-induced degranulation. The results are presented as mean ± S.E.M. (n = 3). [c] Azelastine (10 μM) was used as a positive control and inhibited 35.5 ± 8.1% of antigen-induced degranulation. The results are presented as mean ± SEM (n = 3). [d] Concentration necessary for 50% inhibition (IC₅₀). Note. Butyrolactone I (1) previously showed dose-dependent inhibition of A23187-induced degranulation (IC₅₀ = 39.7 μM) and antigen-induced degranulation (IC₅₀ = 41.6 μM) while butyrolactone V (3) was inactive. [25] Values marked as “>100” represent inactive samples (insignificant inhibition of degranulation at 100 μM, below 20%).

Table S8. Average parameters during the MD production run for tested complexes with SARS-CoV-2 Main Protease (Mₚₚ).