Aspulvins A–H, Aspulvinone Analogues with SARS-CoV-2 M<sub>pro</sub> Inhibitory and Anti-inflammatory Activities from an Endophytic Cladosporium sp.

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ABSTRACT: Eight new aspulvinone analogues, aspulvins A–H (1–8) and aspulvinones D, M, O, and R (9–12), were isolated from cultures of the endophytic fungus Cladosporium sp. 7951. Detailed spectroscopic analyses were conducted to determine the structures of the new compounds. All isolates displayed different degrees of inhibitory activity against the severe acute respiratory syndrome coronavirus 2 main protease (SARS-CoV-2 M<sub>pro</sub>) at 10 μM. Notably, compounds 9, 10, and 12 showed potential SARS-CoV-2 M<sub>pro</sub> inhibition with IC₅₀ values of 10.3 ± 0.6, 9.4 ± 0.6, and 7.7 ± 0.6 μM, respectively. For all compounds except 3 and 4, the anti-inflammatory activity occurred by inhibiting the release of lactate dehydrogenase (LDH) with IC₅₀ values ranging from 0.7 to 7.4 μM. Compound 10 showed the most potent anti-inflammatory activity by inhibiting Casp-1 cleavage, IL-1β maturation, NLRP3 inflammasome activation, and pyroptosis. The findings reveal that the aspulvinone analogues 9, 10, and 12 could be promising candidates for coronavirus disease 2019 (COVID-19) treatment as they inhibit SARS-CoV-2 infection and reduce inflammatory reactions caused by SARS-CoV-2.

The novel coronavirus SARS-CoV-2, the causative agent of COVID-19, can cause symptoms of severe acute respiratory syndrome and has caused significant mortality and morbidity worldwide.1,2 However, there are currently few effective drugs against SARS-CoV-2, and the virus is still spreading across the globe. The SARS-CoV-2 gene encodes important polyprotein precursors, which are then digested into several nonstructural proteins, predominantly by the main protease (M<sub>pro</sub>). The ability of M<sub>pro</sub> to process polyproteins into functional proteins during viral replication makes it an ideal antiviral target. Thus, formulating antiviral drugs that target SARS-CoV-2 M<sub>pro</sub> could offer novel strategies for treating SARS-CoV-2.3−5 Small molecules such as ebselen, carmofur, tideglusib, and the natural product shikonin are promising candidates against SARS-CoV-2 because of their strong inhibitory effects on M<sub>pro</sub>.6 The structural diversity of the natural products and their important role against SARS-CoV-2 prompted us to investigate additional candidates with improved activities.7,8

The SARS-CoV-2 infection is characterized by a strong inflammatory response involving the production of cytokines and chemokines. These interacting biological signals are major causes of fatal pneumonia.2 Designing drugs with both antiviral and anti-inflammatory properties could be effective in the treatment of COVID-19.9 Studies have shown that traditional Chinese medicines inhibit viral replication and ease excessive inflammatory responses caused by viruses,9 such as Lianhuaqingwen10 and Liu Shen capsule.11 Traditional medicines have a limitation as most of their active components are not known. Therefore, identifying the target inhibitor with antiviral and anti-inflammatory activities from natural products could be a promising strategy for obtaining lead compounds against COVID-19. To do this work, we focused on isolating the natural products based on the target SARS-CoV-2 M<sub>pro</sub> from endophytic fungi.

The Cladosporium sp. 7951 is an endophytic plant fungus isolated from a medicinal plant Paris polyphylla var. yunnanensis (Franch.) Hand.-Mazz. Cladosporium is one of the largest genera of dematiaceous hyphomycetes. The Cladosporium genus has been of interest in recent years because of its ability...
RESULTS AND DISCUSSION

Compound 1 was obtained as a yellow amorphous solid. Its molecular formula C_{27}H_{28}O_{7} was established by high-resolution electrospray ionization mass spectrometry (HRESIMS), indicating 14 degrees of unsaturation. The 13C and DEPT NMR spectra of 1 showed 27 carbon signals, including four methyls (δC 26.0, 25.9, 21.2, and 17.9), two methines (δC 133.2, 131.2, 130.6, 123.7, 116.1, 108.5, 104.9, and 70.7), and 13 nonprotonated carbons (δC 171.9, 165.8, 157.3, 154.8, 154.1, 142.1, 133.4, 129.8, 126.0, 113.3, 111.6, 99.0, and 78.4). The NMR spectra of compound 1 were like those of 11,22 except for the absence of a double bond at C-8′ and C-9′, as well as the presence of methylene (δC 70.7) at C-8′ and a nonprotonated carbon (δC 78.4) at C-9′. The HRESIMS analysis confirmed an increment of 16 mass units as compared to that of 11. These data indicate that compound 1 has a dihydropyran fused to the benzene.

The heteronuclear multiple bond correlation (HMBC) from H-2′ to C-2 and C-7′, H-7′ to C-3′, C-4′, C-8′, and C-9′, and H-8′ to C-3′ confirmed the presence of a dihydropyran fused to the benzene at C-3′ and C-4′. Furthermore, the 1H−1H COSY supported the correlations between H-7′ and H-8′. The Z-configuration of the 4-ene was deduced from the chemical shifts of C-2 and C-5 (δC 99.0 and 108.5, respectively), which differ markedly from those of the E-configuration (δC 105 and 115, approximately).21 The structure of compound 1 was established as shown and named aspulvin A.

Compound 2, a yellow amorphous solid, had the same HRESIMS data as compound 1, indicating they were isomers. The 1H and 13C NMR data confirmed that compounds 1 and 2 had similar structures. However, high-performance liquid chromatography (HPLC) analysis suggested that compound 2 was less polar than compound 1. An in-depth 2D NMR analysis confirmed that compounds 1 and 2 were different. From the HMBC data, the correlations from H-5 to C-3, C-4, C-6″, H-2′ to C-2 and C-7′; H-7′ to C-3′, C-4′, C-8′, and C-9′; and H-2″ to C-2″, C-3″, C-4″, C-8″, and C-9″ were found, together with the Z−1H COSY correlations of H-7′ and H-8′, H-6″, and H-7″ and H-8″. The key 1H−1H COSY and HMBC correlations are shown in Figure 1.

The evidence indicated a switch of the substituents attached at C-3′/C-4′ and C-3″/C-4″, as compared to compound 1. Thus, the chemical structure of compound 2 is as depicted and named aspulvin B.

Compound 3, a yellow solid with an [M + H]+ ion at m/z 481.1862, is consistent with the molecular formula C_{27}H_{30}O_{8}. The NMR spectra showed that compounds 2 and 3 were similar. The differences were the shifts of C-8′ and C-9′ (124.3
and 132.7), which were replaced by 70.7 and 78.3. These data indicated that compound 3 bears two dihydropyrans fused to the two benzenes, respectively. The $^1$H−$^1$H COSY correlations between H$_2$-7$'$ and H-8$'$, and H$_2$-7$''$ and H-8$''$, supported the presence of two dihydropyran systems. Furthermore, HMBC correlations from H-2$'$ to C-7$'$, H$_2$-7$'$ to C-3$'$, and H-8$'$ to C-3$''$, as well as H-2$''$ to C-7$''$, H$_2$-7$''$ to C-3$''$, and H-8$''$ to C-3$''$, confirmed the presence of a dihydropyran fused to each benzene. The structure of 3 was finally determined and named aspulvin C.

Compound 4, a yellow solid, had a molecular formula of C$_{27}$H$_{30}$O$_9$, as determined by HRESIMS, indicating 13 degrees of unsaturation. The $^{13}$C NMR data for 4 were like those of 1 except for C-8$''$ and C-9$''$. Instead of a double bond ($\delta_C$ 123.7 and 133.4) present in 1, the shifts of C-8$''$ and C-9$''$ in 4 were 80.0 and 73.9, indicating the presence of two hydroxy groups. The HMBC spectrum showed correlations from H$_2$-7$''$ to C-4$''$ and C-8$''$, and from H-8$''$ to C-3$''$ and C-9$''$, suggesting the side-chain at C-3$''$ in 4 was a 2,3-dihydroxy-3-methylbutyl group. In comparison with 1, HRESIMS analysis confirmed an additional 34 mass units (two hydroxy groups). The structure of 4 was determined as shown and named aspulvin D.

Compound 5 was a yellow solid with the sodium-adduct mass ion peak at m/z 505.1835 [M + Na]$^+$ and the molecular formula C$_{27}$H$_{30}$O$_9$. Signals in the NMR spectra of compound 5 were similar to those of compound 4. The shifts of C-8$''$ and C-9$''$ of 5 were the same as those of 4 (δ$_C$ 80.0 and 73.9). These data indicated that there is a 2,3-dihydroxy-3-methylbutyl group at C-3$''$. The most significant differences as compared to 4 were the shifts of C-8$'$ and C-9$'$ (δ$_C$ 124.4 and 132.6), suggesting that there is an isopentyl unit at C-3$'$ in compound 5 instead of a dihydropyran. The HMBC and $^1$H−$^1$H COSY correlations of 5 as shown in Figure 1 indicated that there is an isopentyl and 2,3-dihydroxy-3-methylbutyl group at C-3$'$ and C-3$''$, respectively. Finally, the structure of 5 was determined and named aspulvin E.

Compound 6, a yellow solid, had a molecular formula of C$_{27}$H$_{30}$O$_7$ as deduced by HRESIMS, indicating 13 degrees of unsaturation. The NMR spectra of 6 were roughly analogous to those of 5, except for the absence of methylene (δ$_C$ 80.0) and the presence of methine (δ$_C$ 44.9) in the $^{13}$C NMR spectra of 6. The $^1$H−$^1$H COSY correlations between H$_2$-7$''$ and H-8$''$ and the HMBC correlations from H$_2$-7$''$ to C-2$''$, C-4$''$, and C-8$''$, as well as from H$_2$-8$''$ to C-7$''$ and C-9$''$, were consistent with a 3-hydroxy-3-methylbutyl group at C-3$''$. Thus, the structure of 6 was elucidated and named aspulvin F.

Compound 7, a yellow solid, had a molecular formula of C$_{27}$H$_{28}$O$_7$ from HRESIMS data and an unsaturation of 14. Its molecular formula was similar to that of compounds 1 and 2, indicating that they were possible isomers. HPLC analysis suggested that compound 7 was less polar than compound 1 and more polar than compound 2. As compared to 2, compound 7 showed shifts in the signal H$_2$-8$''$ from δ$_H$ 3.76 to 4.63. Also, the $^{13}$C NMR data signals of C-8$''$ and C-9$''$ shifted from δ$_C$ 70.3 to 91.0 and from δ$_C$ 78.7 to 72.5, respectively. These differences in the shifts of the $^1$H and $^{13}$C NMR signals indicated the presence of a dihydrofuran in 7 instead of the

Figure 1. Key $^1$H−$^1$H COSY, HMBC, and correlations of compounds 1–8.
Finally, the structure of unsaturation. The $^{13}$C and DEPT NMR spectra of based on the result of HRESIMS, which indicated 13 degrees molecule candidates protecting cells from COVID-19 infection.

SARS-CoV-2 Mpro inhibition activity was measured in the presence of increasing concentrations of potent compounds. Dose–response curves for IC$_{50}$ values were determined by nonlinear regression. $n = 3$ biological replicates.

Figure 2. (A) SARS-CoV-2 M$^{pro}$ inhibition of compounds 1–12 at 10 μM. Ebselen (1 μM) was used as the positive control. **P < 0.01. (B–D) SARS-CoV-2 M$^{pro}$ inhibition activity was measured in the presence of increasing concentrations of potent compounds. Dose–response curves for IC$_{50}$ values were determined by nonlinear regression. $n = 3$ biological replicates.

dihydropyran present in 2. The $^1$H–$^1$H COSY and HMBC spectra further supported this. The HMBC correlations from H-2" to C-4" and C-7", and from H$_2$-7" to C-4", confirmed the presence of a dihydropyran fused to the benzene at C-3" and C-4". Compound 7 and aspulvinone F had similar structures, especially the only one chiral carbon at C-8". The rotation value of 7 ($[\alpha]$_{D}^{27} = -57.2$) is similar to that of aspulvinone F ($[\alpha]$_{D}^{27} = -59$), indicating that the chiral carbon C-8" in 7 is consistent with the configuration of aspulvinone F. Finally, the structure of 7 was determined, and aspulvin G was given as its trivial name.

Compound 8 had an elemental composition of C$_{22}$H$_{20}$O$_6$ based on the result of HRESIMS, which indicated 13 degrees of unsaturation. The $^{13}$C and DEPT NMR spectra of 8 showed 22 carbon signals, including two methylenes ($\delta$C 25.9 and 17.9), one methine ($\delta$C 132.9, 130.1, 129.4, 123.9, 116.0, 107.8, 105.7, and 105.3), and 11 nonprotonated carbons ($\delta$C 176.2, 175.1, 157.7, 157.4, 156.5, 145.6, 133.0, 129.5, 126.8, 113.7, and 95.7). The $^{13}$C and DEPT NMR spectra of 8 were like those of 11, except for the absence of one isoprene unit. The HMBC correlations from H-2" to C-4" and C-7", from H$_2$-7" to C-4" and C-9", and from H-10" to C-8" and C-9" indicated that the isoprene unit is connected to C-3" of compound 8. On the basis of the evidence described above, the structure of 8 was determined and named aspulvin H.

The structures of the known compounds (9–12) were determined by comparing their spectroscopic data with the literature values and identified as aspulvinones D, M, O, and R, respectively. It is reported that SARS-CoV-2 M$^{pro}$ plays a central role in viral replication, which makes it a key drug target. Small molecule candidates protecting cells from COVID-19 infection with high potency were discovered by high-throughput filtering, target designing, and other means. Previous research showed that isoaspulvinone E, an aspulvinone derivative, has anti-influenza A activity, and a docking experiment demonstrated its effect on SARS-CoV-2 M$^{pro}$ but lacked additional evidence. These research results hinted at the inhibitory effect on SARS-CoV-2 M$^{pro}$ of all isolates presented have. Because SARS-CoV-2 M$^{pro}$ is a vital antiviral target, all isolates were tested for their SARS-CoV-2 M$^{pro}$ inhibitory activity. The results show that they all displayed different degrees of inhibitory activity against SARS-CoV-2 M$^{pro}$ at 10 μM, as shown in Figure 2. Among them, compounds 9, 10, and 12 are the most potent antiviral candidates with IC$_{50}$ values of 10.3 ± 0.6, 9.4 ± 0.6, and 7.7 ± 0.7 μM, respectively.

Considering that SARS-CoV-2 can lead to excessive inflammatory reaction, it would be important if the drug had antiviral and anti-inflammatory activities. Hence, all isolates were evaluated for their anti-inflammatory activities. Except for compounds 3 and 4, all compounds in a macrophage pyroptosis model inhibited the release of LDH with IC$_{50}$ values varying from 0.7 to 7.4 μM (Figure 3A and B). In addition to showing the most potent LHD inhibitory activity, compound 10 inhibited Casp-1 cleavage (Figure 3C) and IL-1β maturation (Figure 3D) and reduced the number of pyroptic cells (Figure 3E) significantly. Compound 10 also blocked propidium iodide (PI) uptake in nigericin stimulated J774A.1 cells in a dose-responsive manner (Figure 4). These results suggested that compound 10 displayed significant anti-inflammatory activity by inhibiting NLRP3 inflammasome activation.
Uppsala, Sweden). Semipreparative HPLC was performed on an instrument (Germany), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Tokyo, Japan), LiChroprep RP-18 gel (40 μm), and AVIII-600 instruments using tetramethylsilane (TMS) as the internal standard. Delta (δ) values gave chemical shifts. Meanwhile, HRESIMS was performed on a Thermo Fisher LTQ Orbitrap XL hybrid ion trap mass spectrometer. Column chromatography was monitored by UV detector at 254 nm. Silica gel plates (GF254, Qingdao Marine Chemical Inc., Qingdao, China) were used for analytical thin layer chromatography (TLC). TLC monitored the fractions, visualizing the spots by UV light (254 nm), spraying 10% H2SO4 in ethanol, followed by heating.

**EXPERIMENTAL SECTION**

**General Experimental Procedure.** A Rudolph Autopol VI polarimeter measured optical rotations, while IR spectra were obtained on a Thermo Scientific Nicolet 10X spectrophotometer with KBr pellets. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer. NMR spectra were recorded on Bruker AM-400 MHz spectrometer with KBr pellets. UV spectra were recorded on a Shimadzu UV-2401A spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer. NMR spectra were recorded on Bruker AM-400 spectrophotometer.

**Fungal Material and Fermentation.** The endophytic fungus Cladosporium sp. 7951 was isolated from a medicinal plant collected from Kunming of Yunnan Province, China. The endophytic fungus Cladosporium sp. 7951 was identified on the basis of sequence (GenBank accession no. MZ994501) analysis of its internal transcribed spacer (ITS) region amplified using ITS4 (5'-3': TCCTCCGCTTATTGATATGC) forward and ITS5 (5'-3': GGAAGGTAAAAGTCAAGG) reverse primers. The Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission, and Ministry of Education, Yunnan Minzu University, Kunming, China, maintained the fungus. The fungal strain was cultured on potato dextrose agar (PDA) slants at 30 °C for 3 days. Agar plugs were aseptically cut into small pieces of about 0.5 × 0.5 × 0.5 cm3, and 20 pieces of agar plugs were inoculated in 10 sterile Erlenmeyer flasks (500 mL), each containing 200 mL of potato dextrose broth, pH 6.5. Ten flasks of the inoculated media were incubated at 28 °C on a rotary shaker at 200 rpm for 4 days to prepare the seed culture. Rice fermentation was carried out in 100 Fernbach flasks (500 mL), each containing 80 g of rice and 120 mL of H2O sterilized by autoclaving. Each flask was inoculated with 10 mL of the seed inoculum and incubated at 28 °C for 30 days.

**Extraction and Isolation.** The fermented rice was extracted using methanol (MeOH) to obtain crude extract after the organic solvent was recovered. The total extract (97 g) was chromatographed on MCI gel CC and eluted stepwise in a MeOH–H2O gradient (5:5–1:0, v/v) to afford major fractions A–E by TLC. Fraction A (9 g) was chromatographed on a Sephadex LH-20 CC resulting in seven subfractions, A1–A7. Subfraction A4 was subjected to RP-C18 elution using MeOH–H2O mixtures (5:5–1:0, v/v) and 0.5% formic acid, to yield 10 fractions. Subsequently, the 60% MeOH sample (71 mg) was eluted by semipreparative HPLC using isocratic MeCN–H2O gradient (5:5–1:0, v/v) to obtain 4 (20 mg, tR = 5.9 min) and 5 (21 mg, tR = 15.7 min). The 65% MeOH sample (90 mg) was isolated by semipreparative HPLC using isocratic MeCN–H2O–HCOOH (8:12:0.1, v/v/v) to obtain 6 (9 mg, tR = 8.7 min) and 7 (9 mg, tR = 9.3 min), and the 70% MeOH sample (33 mg) was purified by HPLC (MeCN–H2O–HCOOH 9:1:0.1, v/v/v) to obtain 8 (9 mg, tR = 9.1 min) and 12 (8 mg, tR = 9.6 min). In addition, a 75% MeOH sample (43 mg) was separated by HPLC (MeCN–H2O–HCOOH, 11:9:0.1, v/v/v) to give 1 (9 mg, tR = 12.6 min), 7 (3 mg, tR = 15.1 min), and 2 (10 mg, tR = 12.5 min).

Figure 3. (A) LDH release rate was detected in all compounds in J774A.1 mouse macrophages. MCC950 (100 nM) was used as a positive control. Compounds with an LDH release level lower than 50% were marked by arrows. (B) IC50 values of potent compounds in an LDH release assay (n = 2). (C, D) Western blot assays and grayscale analysis of treatment with 10 followed by LPS + nigericin costimulation. (E) Pyroptotic cells were counted in six different fields for each well in inverted fluorescence microscopy. ***P < 0.001, **P < 0.01, P < 0.05.
$t_R = 16.2$ min). Last, an 80% MeOH sample (130 mg) was purified by HPLC (MeCN–H$_2$O–HCOOH, 14:6:0.1, v/v/v) and 0.5% formic acid to yield 11 (68 mg, $t_R = 9.0$ min) and 9 (18 mg, $t_R = 12.2$ min). Sephadex LH-20 CC and eluent of MeOH were used to obtain compound 8 (37 mg) from subfraction A6.

Aspulvin A (1). Yellow amorphous solid; [α]$^D_{28}$ +9.6 (c 0.09, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 197 (3.79), 336 (3.36), 375 (3.35) nm; IR (KBr) $\nu$$_{max}$ 3411, 2977, 2926, 2726, 1718, 1599, 1503, 1432, 1280, 1099 cm$^{-1}$; for $^1$H and $^{13}$C NMR data, see Tables 1 and 2; HRESIMS $m/z$ 465.1909 [M + H]$^+$ (calcd for C$_{27}$H$_{29}$O$_7$, 465.1908).

Aspulvin B (2). Yellow amorphous solid; [α]$^D_{28}$ −3.5 (c 0.09, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 198 (3.77), 335 (3.41) nm; IR (KBr) $\nu$$_{max}$ 3419, 2977, 2929, 2722, 1603, 1496, 1431, 1265, 1088 cm$^{-1}$; for $^1$H and $^{13}$C NMR data, see Tables 1 and 2; HRESIMS $m/z$ 465.1913 [M + H]$^+$ (calcd for C$_{27}$H$_{30}$O$_7$, 465.1908).

Aspulvin C (3). Yellow amorphous solid; [α]$^D_{28}$ −8.1 (c 0.09, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 201 (3.71), 325 (3.30), 374 (3.18) nm; IR (KBr) $\nu$$_{max}$ 3425, 2975, 2932, 1723, 1605, 1497, 1267, 1142, 1100 cm$^{-1}$; for $^1$H and $^{13}$C NMR data, see Tables 1 and 2; HRESIMS $m/z$ 481.1862 [M + H]$^+$ (calcd for C$_{27}$H$_{30}$O$_9$, 481.1857).

Aspulvin D (4). Yellow amorphous solid; [α]$^D_{28}$ +10.6 (c 0.10, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 199 (3.74), 325 (3.37), 374 (3.25) nm; IR (KBr) $\nu$$_{max}$ 3422, 2977, 2932, 1721, 1602, 1503, 1434, 1280, 1120 cm$^{-1}$; for $^1$H and $^{13}$C NMR data, see Tables 1 and 2; HRESIMS $m/z$ 499.1965 [M + H]$^+$ (calcd for C$_{27}$H$_{30}$O$_9$, 499.1963).

Aspulvin E (5). Yellow amorphous solid; [α]$^D_{28}$ +14.9 (c 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 240 (3.33), 335 (3.36), 374 (3.30) nm; IR (KBr) $\nu$$_{max}$ 3400, 2974, 2930, 1721, 1601, 1504, 1431, 1261, 1087 cm$^{-1}$; for $^1$H and $^{13}$C NMR data, see Tables 1 and 2; HRESIMS $m/z$ 505.1835 [M + Na]$^+$ (calcd for C$_{27}$H$_{30}$O$_9$Na, 505.1833).

Aspulvin F (6). Yellow amorphous solid; [α]$^D_{28}$ +5.2 (c 0.07, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 339 (3.47) nm; IR (KBr) $\nu$$_{max}$ 3390, 2971, 2927, 1720, 1599, 1506, 1429, 1271, 1089 cm$^{-1}$; for $^1$H and $^{13}$C NMR data, see Tables 1 and 2; HRESIMS $m/z$ 489.1884 [M + Na]$^+$ (calcd for C$_{27}$H$_{29}$O$_8$Na, 489.1884).

Aspulvin G (7). Yellow amorphous solid; [α]$^D_{28}$ −57.2 (c 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 331 (3.27) nm; IR (KBr) $\nu$$_{max}$ 3400, 2974, 2926, 2854, 1713, 1606, 1491, 1384, 1246, 1088, 966 cm$^{-1}$; for $^1$H and $^{13}$C NMR data, see Tables 1 and 2; HRESIMS $m/z$ 465.1909 [M + H]$^+$ (calcd for C$_{27}$H$_{29}$O$_7$, 465.1908).

SARS-CoV-2 M$^{pro}$ Inhibition Assay. On the basis of fluorescence resonance energy transfer, SARS-CoV-2 M$^{pro}$ inhibition was performed via an Enhanced 2019-nCoV M$^{pro}$/3CL$^{pro}$ Inhibitor Screening Kit (Beyotime Biotechnology, Shanghai, China). Compounds were dissolved in dimethyl sulfoxide. The reaction mixture contained 92 μL of assay buffer, 5 μL of test compound solution, 1 μL of SARS-CoV-2 M$^{pro}$, and 2 μL of substrate in a black 96-well plate. After 5 min of incubation, the relative fluorescence unit (RFU) was read on a Spectra Max i3x microplate reader platform (Molecular Devices, U.S.) at the excitation of 325 nm and the emission of 393 nm.
Table 1. $^1$H NMR Data of Compounds 1−8 (δ in ppm, J in Hz)

| no. | 1$^\text{a}$ | 2$^\text{a}$ | 3$^\text{a}$ | 4$^\text{a}$ | 5$^\text{b}$ | 6$^\text{b}$ | 7$^\text{b}$ | 8$^\text{b}$ |
|-----|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 5   | 6.24 (s)     | 6.25 (s)     | 6.27 (s)     | 6.25 (s)     | 6.22 (s)     | 6.27 (s)     | 6.21 (s)     | 6.14 (s)     |
| 2$'$| 7.50 (s)     | 7.52 (s)     | 7.54 (s)     | 7.53 (s)     | 7.50 (s)     | 7.55 (s)     | 7.57 (s)     | 7.67 (d, 8.5) |
| 3$'$| 6.30 (s)     | 6.40 (s)     | 6.31 (s)     | 6.29 (s)     | 6.35 (s)     | 6.42 (s)     | 6.32 (s)     | 6.24 (s)     |
| 7$'$| 2.94 (dd, 16.2, 5.2) | 3.20 (dd, 16.2, 5.2) | 2.97 (dd, 16.2, 5.2) | 2.93 (dd, 15.8, 4.3) | 3.17 (dd, 16.1, 7.2) | 3.23 (dd, 16.1, 7.2) | 3.20 (dd, 16.1, 7.2) | 3.17 (dd, 16.1, 7.2) |
| 8$'$| 3.72 (dd, 16.2, 7.3) | 5.20 (d, 7.3) | 3.75 (dd, 16.2, 7.3) | 3.72 (t, 7.3) | 5.21 (t, 7.3) | 5.32 (t, 7.3) | 5.31 (t, 7.3) | 5.30 (t, 7.3) |
| 10$'$| 1.23 (s)     | 1.70 (s)     | 1.26 (s)     | 1.23 (s)     | 1.67 (s)     | 1.74 (s)     | 1.72 (s)     | 1.72 (s)     |
| 11$'$| 1.30 (s)     | 1.71 (s)     | 1.33 (s)     | 1.30 (s)     | 1.67 (s)     | 1.74 (s)     | 1.72 (s)     | 1.72 (s)     |
| 2$''$| 7.47 (s)     | 7.52 (s)     | 7.55 (s)     | 7.57 (s)     | 7.53 (s)     | 7.55 (s)     | 7.69 (s)     | 7.39 (s)     |
| 5$''$| 6.75 (d, 8.3) | 6.74 (d, 8.4) | 6.76 (d, 8.5) | 6.79 (d, 8.2) | 6.76 (d, 8.3) | 6.78 (d, 8.4) | 6.74 (d, 8.3) | 6.72 (d, 8.4) |
| 6$''$| 7.45 (d, 8.3) | 7.48 (d, 8.4) | 7.51 (d, 8.5) | 7.51 (d, 8.2) | 7.48 (d, 8.3) | 7.49 (d, 8.4) | 7.47 (d, 8.3) | 7.47 (d, 8.4) |
| 7$''$| 3.25 (d, 7.4) | 3.03 (dd, 16.6, 5.0) | 3.06 (dd, 16.7, 5.1) | 2.98 (d, 14.3) | 2.95 (d, 13.8) | 2.69 (m) | 3.20 (d, 8.7) | 3.25 (d, 7.3) |
| 8$''$ | 5.31 (t, 7.4) | 3.76 (t, 5.8) | 3.78 (dd, 7.2, 5.1) | 3.62 (d, 9.5) | 3.59 (dd, 10.1, 1.4) | 1.77 (m) | 4.63 (t, 8.7) | 5.28 (t, 7.3) |
| 10$''$ | 1.72 (s) | 1.26 (s) | 1.28 (s) | 1.23 (s) | 1.21 (s) | 1.28 (s) | 1.23 (s) | 1.70 (s) |
| 11$''$ | 1.73 (s) | 1.32 (s) | 1.35 (s) | 1.23 (s) | 1.21 (s) | 1.28 (s) | 1.26 (s) | 1.70 (s) |

$^a$Recorded at 400 MHz in methanol-$d_4$. $^b$Recorded at 600 MHz in methanol-$d_4$. 
Table 2. $^{13}$C NMR Data of Compounds 1–8 (δ in ppm)

| compound | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|----------|----|----|----|----|----|----|----|----|
| no.      |    |    |    |    |    |    |    |    |
| 1        | 171.9 | C | 171.7 | C | 172.1 | C | 172.2 | C |
| 2        | 171.9 | C | 171.7 | C | 172.1 | C | 172.2 | C |
| 3        | 165.8 | C | 164.5 | C | 167.0 | C | 167.0 | C |
| 4        | 142.1 | C | 142.5 | C | 143.0 | C | 142.6 | C |
| 5        | 108.5 | CH | 107.8 | CH | 107.5 | CH | 108.0 | CH |
| 6        | 111.6 | C | 109.6 | C | 111.9 | C | 112.0 | C |
| 7        | 131.2 | C | 131.1 | C | 131.0 | C | 131.2 | C |
| 8        | 113.3 | C | 121.9 | C | 113.2 | C | 113.2 | C |
| 9        | 154.8 | C | 156.9 | C | 154.7 | C | 154.6 | C |
| 10       | 104.9 | CH | 103.7 | CH | 105.0 | CH | 105.0 | CH |
| 11       | 154.1 | C | 153.3 | C | 154.4 | C | 154.3 | C |
| 12       | 31.6 | CH | 28.7 | CH | 31.6 | CH | 28.7 | CH |
| 13       | 70.7 | CH | 124.3 | CH | 70.7 | CH | 70.7 | CH |
| 14       | 78.4 | C | 132.7 | C | 78.3 | C | 78.3 | C |
| 15       | 21.2 | CH | 17.9 | CH | 21.2 | CH | 21.2 | CH |
| 16       | 25.9 | CH | 26.0 | CH | 25.9 | CH | 25.9 | CH |
| 17       | 126.0 | C | 126.9 | C | 127.0 | C | 126.4 | C |
| 18       | 133.2 | CH | 153.2 | CH | 133.2 | CH | 134.8 | CH |
| 19       | 129.8 | C | 121.7 | C | 121.7 | C | 128.5 | C |
| 20       | 157.3 | C | 155.2 | C | 155.1 | C | 157.8 | C |
| 21       | 116.1 | CH | 118.4 | CH | 118.3 | CH | 116.8 | CH |
| 22       | 130.6 | CH | 131.0 | CH | 131.0 | CH | 131.0 | CH |
| 23       | 29.2 | CH | 32.1 | CH | 32.1 | CH | 34.1 | CH |
| 24       | 123.7 | CH | 70.3 | CH | 70.3 | CH | 80.0 | CH |
| 25       | 133.4 | C | 78.1 | C | 78.7 | C | 73.9 | C |
| 26       | 17.9 | CH | 21.4 | CH | 21.3 | CH | 25.1 | CH |
| 27       | 26.0 | CH | 25.9 | CH | 25.9 | CH | 25.6 | CH |

*Recorded at 100 MHz in methanol-d$_4$. *Recorded at 150 MHz in methanol-d$_4$.

NLRP3 Inflammasome Activation. The J774A.1 macrophages were primed with 100 ng/mL lipopolysaccharide (LPS) for 3 h, followed by incubation with compounds or MCC950 and stimulated with the NLRP3 stimulus nigericin (10 μM) for 1 h. The supernatant was collected for the LDH release assay. The LDH release level was determined according to the manufacturer’s manual (Promega, U.S.).

Cell Permeability Assay. J774A.1 cells were primed by LPS (100 ng/mL) for 3 h. Subsequently, the compound (10 μM) was added for 0.5 h followed by stimulation with nigericin (10 μM) for 1 h. The cells were stained using PI (0.5 μg/mL) and Hoechst33342 (3 μg/mL) for 15 min. An inverted fluorescent microscope (Zeiss, Axio Observer 3) was used to observe the fluorescent image immediately.

Western Blot Assays. After NLRP3 inflammasome activation of J774A.1, proteins from the supernatant were obtained. Protein extracts were loaded onto a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 12% gel for electrophoresis and transferred to a nitrocellulose (NC) membrane. After being blocked with 5% nonfat dry milk, membranes were incubated overnight at 4 °C with primary antibodies, including Anti-Goat-IL-1β and Anti-Mouse-Caspase-1 (p20), respectively. Secondary antibodies included donkey antigoat IgG HRP and goat anti-mouse IgG HRP. The target proteins were visualized by chemiluminescence.

ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.1c01003.

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The 1D and 2D NMR, UV, IR, and HRESIMS spectra for compounds 1–8 (PDF)

| inhibition rate (%) = (RFU$^{100\%}$ enzymatic activity − RFU$^{sample}$) / (RFU$^{100\%}$ enzymatic activity − RFU$^{blank}$) × 100% |
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