



Article

Curtachalasin Y1–Y13, Anti-Inflammatory Cytochalasins from the Soil Fungus *Xylaria* sp. Y01

Yi-Yun Yuan ^{1,2,†}, Yang Xie ^{1,†}, Xi Zhou ², Liang Tu ², Ying-Meng Leng ², Qi-An Chen ^{1,2}, Qing-Hui Xiao ^{1,2}, Shao Liu ^{1,2} , Wen-Xuan Wang ²  and Jing Li ^{1,2,*} 

¹ Department of Pharmacy, National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha 410008, China

² Xiangya School of Pharmaceutical Sciences, Central South University, Changsha 410083, China

* Correspondence: lijingliyun@csu.edu.cn

[†] These authors contributed equally to this work.

Abstract

Twenty-seven cytochalasins, including thirteen previously undescribed analogs with a 5/6/6/6 tetracyclic skeleton (curtachalasin Y1–Y13), were isolated from the rice-based culture of a soil-derived fungus, *Xylaria* sp. Y01. Their structures were determined through comprehensive analysis of spectroscopic data and quantum chemical calculations. All isolates were evaluated for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide-stimulated RAW264.7 macrophages. Curtachalasin Y1 (**12**), 5,6-dihydro-7-oxo-18-desoxy-19,20-epoxycytochalasin C (**21**), and 7-oxo-19,20-epoxycytochalasin C (**22**) exhibited potent inhibition, with IC₅₀ values of 55.4, 43.8, and 17.8 μM, respectively, compared to 14.9 μM for the positive control dexamethasone. Furthermore, these compounds suppressed lipopolysaccharide (LPS)-induced release of pro-inflammatory cytokines IL-6, MCP-1, and TNF-α, confirming their anti-inflammatory activity. Preliminary mechanistic investigation indicated that compound **22** exerts its anti-inflammatory effects in RAW264.7 cells by downregulating CXC motif chemokine ligand 10 (CXCL10) and upregulating suppressor of cytokine signaling 3 (SOCS3) expression. These results expand the structural diversity of cytochalasins from *Xylaria* species and provide a basis for further exploration of their anti-inflammatory potential.

Keywords: cytochalasins; *Xylaria* sp.; anti-inflammatory activity; structure elucidation

1. Introduction

Fungi of the genus *Xylaria*, members of the family Xylariaceae within the order Xylariales, constitute a diverse and ecologically significant group [1]. With approximately 300 recognized species, this globally distributed genus spans temperate to tropical climates [2]. *Xylaria* species, primarily saprophytic or endophytic, represent a rich source of chemically diverse secondary metabolites. These compounds exhibit a broad spectrum of bioactivities, including anticancer, antimicrobial, cytotoxic, and anti-inflammatory properties [2].

Cytochalasins are a prominent class of fungal metabolites derived from polyketide-nonribosomal peptide synthetase hybrids and possess diverse biological functions [3]. Since the initial discovery of cytochalasins A and B in 1966 from *Phoma* sp. S 298 and *Helminthosporium dematioideum*, respectively, over 500 cytochalasins have been reported [3,4]. These compounds are predominantly isolated from fungi belonging to the genera *Xylaria*, *Chaetomium*, *Aspergillus*, *Penicillium* and *Zygosporium* [3]. Recent decades have seen significant progress



Academic Editor: Guan-Jhong Huang

Received: 7 June 2026

Revised: 6 July 2026

Accepted: 8 July 2026

Published: 15 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

not only in expanding the known chemical diversity of this class but also in elucidating their biological activities, molecular targets, and mechanisms of action [5].

Soil-derived fungi are renowned for their capacity to produce secondary metabolites with unique chemical diversity, including pyridone derivatives [6], cytochalasins [7], steroids [8], and sesquiterpenes [9]. Among these, the curtachalasin—characterized by a distinctive 5/6/6/6 tetracyclic skeleton—were first reported from the fungus *Xylaria curta* E10. To date, a total of 18 curtachalasin analogs have been identified [6,10,11], and their various activities, such as cytotoxicity and antibacterial, antifungal, and immunosuppressive effects, have been investigated.

During a search for novel anti-inflammatory cytochalasins, a fungal strain isolated from a soil sample collected in Kunming, China, and identified as *Xylaria* sp. Y01 was investigated. This study led to the isolation of twenty-seven cytochalasins from its rice-based culture. These include thirteen new curtachalasin-type compounds, designated curtachalasin Y1–Y13 (1–13), alongside fourteen known analogs: curtachalasin J (14) [10], curtachalasin X4 (15) [11], curtachalasin X5 (16) [11], curtachalasin G (17) [10], 13-hydroxy-14-chloro-13,14-dihydro-19,20-epoxycytochalasin D (18) [12], 19-epi-cytochalasin P1 (19) [13], cytochalasin C1 (20) [14], 5,6-dihydro-7-oxo-18-desoxy-19,20-epoxycytochalasin C (21) [12], 7-oxo-19,20-epoxycytochalasin C (22) [12], 5,6-dihydro-7-oxo-19,20-epoxycytochalasin C (23) [13], 19,20-epoxycytochalasin C (24) [15], 19,20-epoxycytochalasin D (25) [15], 6,7-dihydro-7-oxo-cytochalasin C (26) [16], and cytochalasin C (27) (Figure 1) [17]. All isolates were tested for their ability to inhibit nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. Compounds 12, 21, and 22 showed potent inhibition, with IC_{50} values of 55.4, 43.8, and 17.8 μ M, respectively (positive control dexamethasone: 14.9 μ M). These three compounds also reduced LPS-induced secretion of the pro-inflammatory cytokines IL-6, MCP-1, and TNF- α . Preliminary mechanistic studies revealed that compound 22 exerts its anti-inflammatory effect in RAW264.7 cells through downregulation of CXC motif chemokine ligand 10 (CXCL10) and upregulation of suppressor of cytokine signaling 3 (SOCS3) expression. This report describes their isolation, structural elucidation, and evaluation of anti-inflammatory properties.

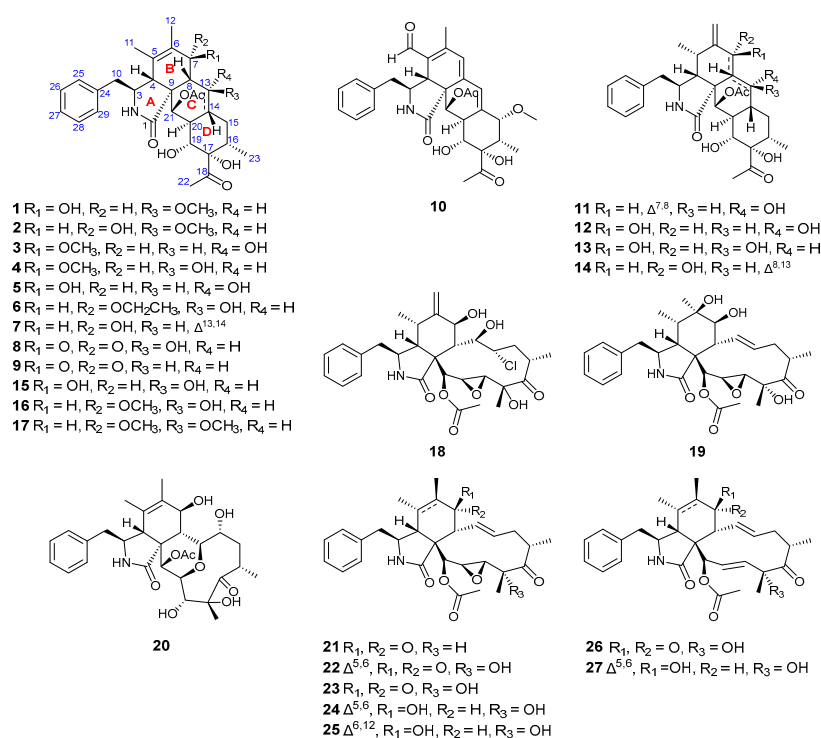


Figure 1. Chemical structures of compounds 1–27.

2. Results and Discussion

Compound **1** was obtained as a white powder. The molecular formula was determined as $C_{31}H_{41}NO_8$ by HRESIMS (m/z 578.2724 $[M + Na]^+$ $\Delta -0.2$ ppm), corresponding to 12 degrees of unsaturation. Its UV spectrum exhibited absorptions at 210 and 260 nm. The IR spectrum displayed characteristic bands for an ester group (1733 cm^{-1}) and hydroxy groups (3346 cm^{-1}). The ^1H and ^{13}C NMR data of **1** (Table 1) displayed signals characteristic of a cytochalasan skeleton [7], including a monosubstituted phenyl group at δ_{H} 7.13 (2H, d, $J = 7.7$ Hz), 7.30 (2H, t, $J = 7.7$ Hz), and 7.23 (1H, t, $J = 7.7$ Hz); one methyl doublet at δ_{H} 0.79 (3H, d, $J = 6.5$ Hz), two methylene groups at δ_{H} 2.86 (1H, dd, $J = 13.4, 6.8$ Hz) and 2.94 (1H, dd, $J = 13.4, 8.4$ Hz), δ_{H} 1.85 (1H, overlapped), and 1.45 (1H, ddd, $J = 12.5, 12.1, 12.1$ Hz), a ketone carbon (δ_{C} 212.4), one amide carbonyl (δ_{C} 175.2), an ester carbonyl (δ_{C} 172.1), one tetrasubstituted double bond (δ_{C} 125.0 and 133.0), five methyl singlets (δ_{C} 14.2, 17.4, 21.2, 25.3, 59.9), two sp^3 -hybridized quaternary carbons (δ_{C} 50.4 and 83.6), an N-methyl group (δ_{C} 59.4), four oxygenated methines (δ_{C} 70.7, 71.5, 72.2, 84.5), and five high-field methines (δ_{C} 36.1, 39.5, 41.6, 43.2, 48.6). Analysis of the ^1H - ^1H COSY spectrum revealed three spin-spin coupling systems, namely $\text{H}_2\text{-10/H-3/H-4}$, $\text{H-25/H-26/H-27/H-28/H-29}$, and $\text{H-7/H-8/H-13/H-14(H}_2\text{-15/H-16/H}_3\text{-23)/H-20/H-21(H-19)}$ (Figure 2). Key HMBC correlations (Figure 2) between NH and C-3/C-4/C-9, H-3 and C-1, H-4 and C-1/C-9, H-8 and C-9, $\text{H}_3\text{-11 and C-4/C-5/C-6}$, as well as $\text{H}_3\text{-12 and C-5/C-6/C-7}$ established the connectivity of rings A and B. Further HMBC correlations between 7-OH and C-6/C-7/C-8, H-21 and C-9, $\text{H}_3\text{-23 and C-15/C-16/C-17}$, $\text{H}_3\text{-22 and C-17/C-18/C-19}$, together with COSY data, completed the assembly of rings B, C, and D. The attachment of the phenyl group at C-10 was indicated by HMBC correlations from $\text{H}_2\text{-10 to C-24}$ (δ_{C} 137.7) and to C-25/C-29 (δ_{C} 129.2). Thus, the planar structure of **1** was determined as shown in Figure 2.

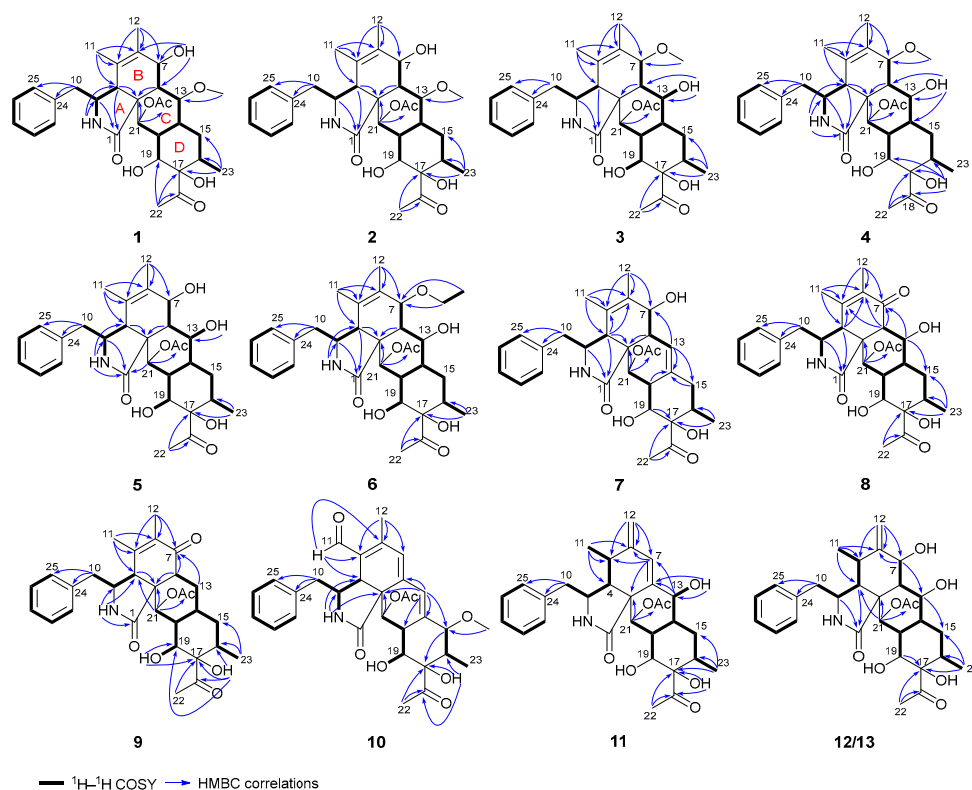


Figure 2. Key ^1H - ^1H COSY and HMBC correlations of compounds **1**–**13**.

Stereochemical analysis relied on the coupling constants of the cyclohexane rings (B, C, and D). The observed large vicinal couplings for H-7 (9.7 Hz), H-8 (10.0, 9.7 Hz), H-13 (10.4, 10.0 Hz), H-19 (10.5 Hz), and H-20 (12.3, 10.5, 2.1 Hz) indicated axial orientations for these protons, whereas the small coupling of H-21 (2.1 Hz) suggested an equatorial orientation. Key NOESY correlations defined the relative configuration. Correlations between H-3/H₃-11, H-4/H-21, and H-21/H₂-10 indicated that H-3 and the ring B are on one face of ring A, whereas H-4, H₂-10, and H-21 lie on the opposite face. Additional NOESY cross peaks between H-8/H-14, H-14/H-16, H-16/H-19, and H-19/H₃-22 defined one spatial cluster, while correlations between H-7/H-13 and H-13/H_{ax}-15/H-20 defined another. Because H-7 and H-8 are axially oriented but point in opposite directions, these two clusters reside on opposite faces of the molecule. The resulting relative configuration was depicted in Figure 3. The absolute configuration of **1** was determined by ECD calculation (Figure 4). The calculated ECD curve for the isomer 3*S*,4*R*,7*S*,8*S*,9*R*,13*S*,14*R*,16*S*,17*S*,19*R*,20*S*,21*R*-**1** at the ω B97X-D/TZVP level (IEFPCM, acetonitrile) showed excellent agreement with the measured data. Therefore, the structure of **1** was fully assigned and named curtachalasin Y1.

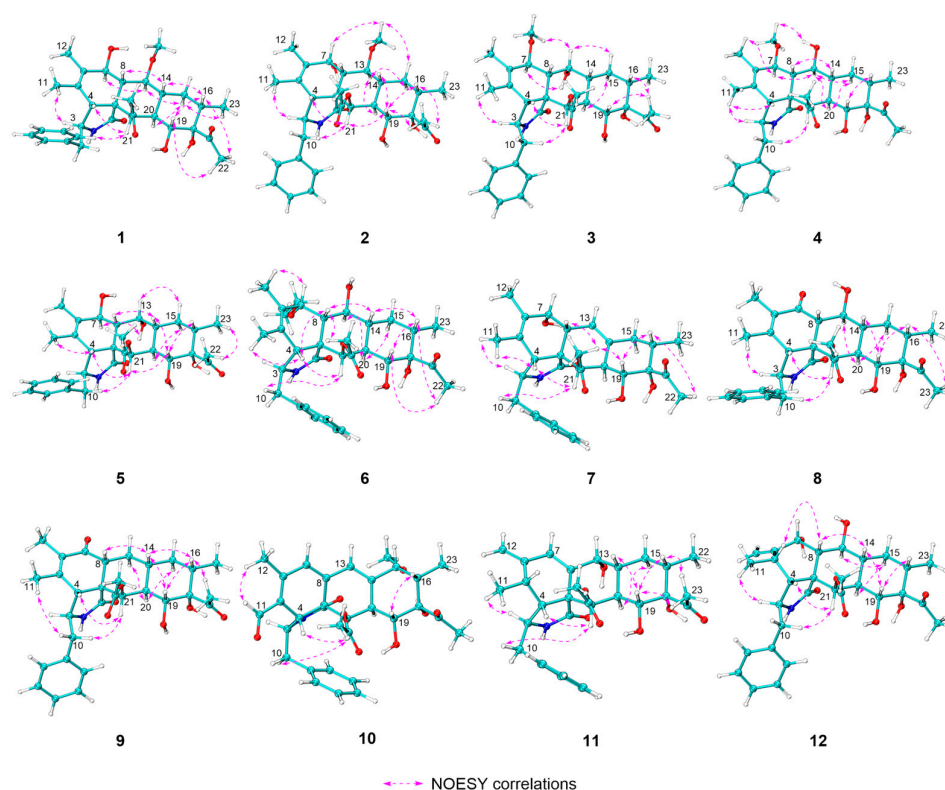


Figure 3. Key NOESY correlations of compounds **1–12**, depicted with their lowest-energy conformations (coordinates are provided in Tables S20–S32).

Table 1. ¹H (600 MHz) NMR data of **1–4** in CDCl₃ (δ in ppm, *J* in Hz).

No.	1	2	3	4
3	3.38 m	3.44 dd (7.8, 7.5)	3.46 br dd (9.2, 6.4)	3.34 br dd (8.4, 6.8)
4	2.37 br s	2.37 br s	2.40 br s	2.37 br s
7	4.25 d (9.7)	4.11 d (2.7)	3.75 d (10.5)	3.94 br d (9.8)
8	1.98 dd (10.0, 9.7)	1.94 m	1.93 overlapped	1.92 dd (9.8, 9.5)
10	2.86 dd (13.4, 6.8)	2.94 dd (13.4, 7.8)	2.97 dd (13.6, 9.2)	2.95 dd (13.6, 8.4)
11	2.94 dd (13.4, 8.4)	2.88 dd (13.4, 7.5)	2.88 dd (13.6, 6.4)	2.88 dd (13.6, 6.8)
12	1.42 s	1.40 s	1.46 s	1.42 s
13	1.68 s	1.78 s	1.68 s	1.65 s
13	4.65 dd (10.4, 10.0)	4.17 (dd 10.3, 10.3)	4.00 ddd (11.5, 3.5, 3.5)	4.51 dd (9.5, 9.5)

Table 1. Cont.

No.	1	2	3	4
14	1.73 m	1.58 overlapped	1.68 m	1.57 m
15	1.85 overlapped	1.92 m	1.96 ddd (12.6, 12.4, 12.1)	2.04 ddd (12.5, 3.7, 3.7)
16	1.45 ddd (12.5, 12.1, 12.1)	1.46 ddd (12.3, 12.3, 12.4)	1.44 overlapped	1.36 ddd (12.5, 12.5, 12.5)
19	1.84 overlapped	1.84 m	1.93 overlapped	1.85 m
20	3.39 d (10.5)	3.40 dd (10.5, 6.8)	3.39 dd (10.5, 7.5)	3.41 dd (10.4, 7.4)
21	2.41 ddd (12.3, 10.5, 2.1)	2.56 ddd (11.0, 10.5, 1.7)	2.38 ddd (12.4, 10.5, 2.0)	2.45 ddd (12.4, 10.4, 1.9)
22	5.43 d (2.1)	5.38 d (1.7)	5.53 d (2.0)	5.47 d (1.9)
23	2.26 s	2.22 s	2.26 s	2.26 s
25/29	0.79 d (6.5)	0.79 d (6.7)	0.78 d (6.3)	0.77 d (6.9)
26/28	7.13 d (7.7)	7.15 d (7.4)	7.14 d (7.4)	7.14 d (7.2)
27	7.30 t (7.7)	7.30 t (7.4)	7.32 t (7.4)	7.31 t (7.2)
27	7.23 t (7.7)	7.23 t (7.4)	7.25 t (7.4)	7.24 t (7.2)
21-OAc	2.26 s	2.26 s	2.24 s	2.26 s
7-OCH ₃ /13-OCH ₃	3.57 s	3.60 s	3.62 s	3.49 s
NH	5.87 s		5.70 s	5.54 br s
7-OH	5.16 s			
13-OH			5.93 d (11.5)	4.88 s
17-OH				3.67 s
19-OH			2.83 d (7.5)	

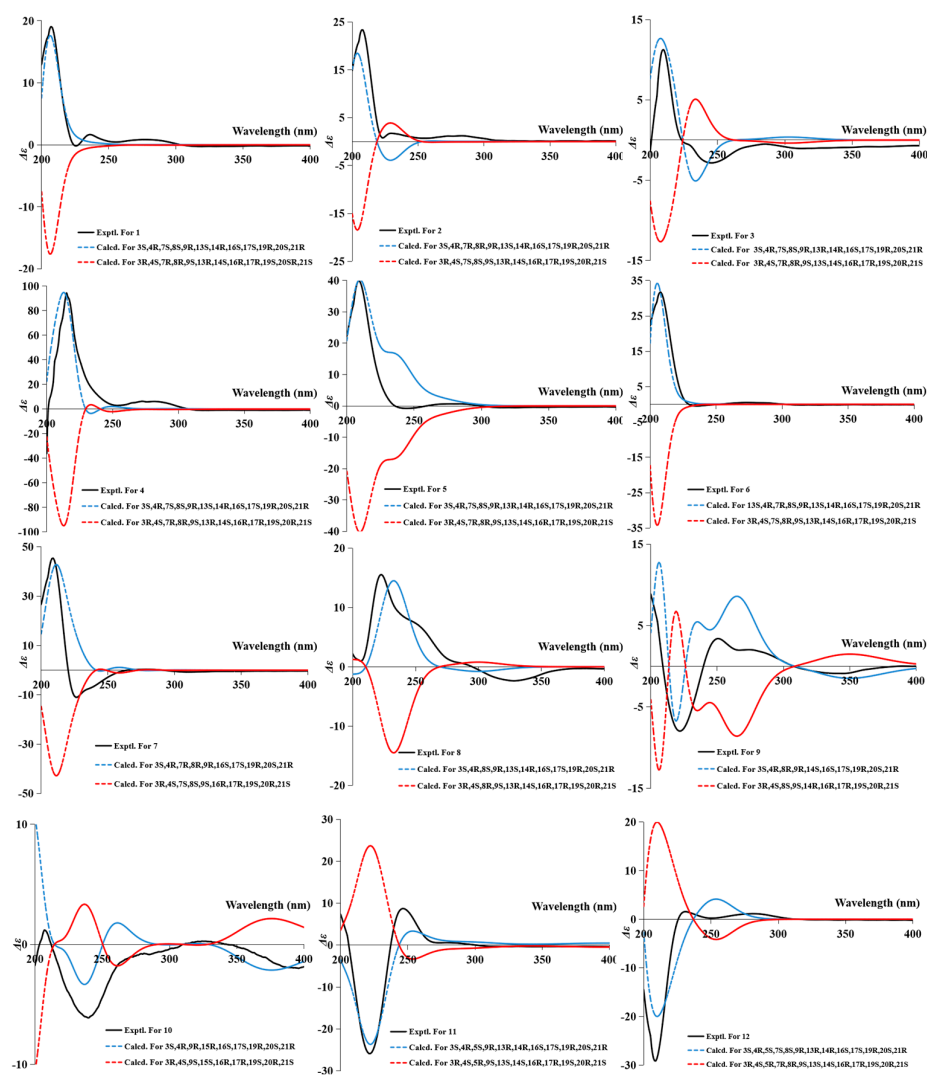


Figure 4. Calculated and experimental ECD spectra of compounds 1–9.

HRESIMS analysis of compound **2** gave an identical molecular formula of $C_{31}H_{41}NO_8$ (m/z 1133.5575 $[2M + Na]^+ \Delta$ 0.0 ppm) to that of **1**. The 1H and ^{13}C NMR data of **2** closely resembled those of **1**. Analysis of 2D NMR data confirmed that **1** and **2** shared the same planar structure (Figure 2). Notably, the ^{13}C NMR chemical shifts of **2** differed by more than 2 ppm from those of **1** at C-1, C-5, C-7, C-12, and C-13, implying a stereochemical difference in rings B and C. The large coupling constants of H-13 (10.3, 10.3 Hz) indicated its axial orientation. Key NOESY cross peaks between H-7/13-OCH₃/H_{eq}-15 confirmed that these protons are on the same face of the molecule. To verify the relative configuration assignment, GFN2NMR calculations were performed [18]. Comparison of the experimental NMR data for **1** and **2** with their respective GFN2NMR-calculated values supported the stereochemical deductions (Tables S1 and S2). The calculated ECD curve for 3*S*,4*R*,7*R*,8*R*,9*R*,13*S*,14*R*,16*S*,17*S*,19*R*,20*S*,21*R*-**2** at the ω B97X-D/TZVP level (IEFPCM, MeOH) showed excellent agreement with the measured data. Therefore, the structure was fully assigned and named curtachalasin Y2.

Compound **3** was obtained as a white powder with the molecular formula $C_{31}H_{41}NO_8$ determined by HRESIMS (m/z 556.2914 $[M + H]^+ \Delta$ +1.6 ppm), identical to those of **1** and **2**. The 1H and ^{13}C NMR spectra also showed similar signals, except that the ^{13}C NMR chemical shifts of **3** at C-7 and C-13 were observed at δ_C 76.5 and 66.7, respectively (δ_C 70.7 and 84.5 for **1**, δ_C 67.8 and 78.8 for **2**). These significant discrepancies imply a structural difference at these positions. The HMBC correlation (Figure 2) between 7-OCH₃ and C-7 indicated that the methoxy group was attached to C-7. 1H - 1H COSY correlations and coupling constants indicated that the hydroxyl group at δ_H 5.93 (1H, d, J = 11.5 Hz) is attached to C-13. The remaining 2D NMR connections were consistent with those of **1**. Key NOESY correlations between H-8/H-14/H-16/H-19/H₃-22 and 7-OCH₃/H-13/H_{eq}-15 established the relative configuration as shown in Figure 2. The calculated ECD curve at the M062X/6-311+G* (IEFPCM, acetonitrile) level for 3*S*,4*R*,7*S*,8*S*,9*R*,13*R*,14*R*,16*S*,17*S*,19*R*,20*S*,21*R*-**3** matched the experimental ECD well. Therefore, the structure of compound **3** was fully assigned and named curtachalasin Y3.

Compound **4** was obtained as a white powder with the molecular formula $C_{31}H_{41}NO_8$ determined by HRESIMS (m/z 1133.5592 $[2M + Na]^+ \Delta$ +1.5 ppm), identical to that of compounds **1**–**3**. The 1H and ^{13}C NMR, HSQC, 1H - 1H COSY, and HMBC spectra confirmed that **3** and **4** shared the same planar structures (Figure 2). However, significant differences in chemical shifts (>5 ppm) were observed at C-7 and C-13 (Table 2). The coupling constants of H-7 (9.8 Hz) and H-13 (9.5, 9.5 Hz) indicated that H-7 and H-13 were axially oriented. In contrast, the H-13 proton in **3** occupied an equatorial position. The altered orientation of the 13-OH group likely affected the chemical shifts in C-7 and C-13 (Figure 3). This speculation was supported by GFN2NMR ^{13}C NMR calculations for **3** and **4** (Table S6). Moreover, key NOESY cross peaks between H-7/H-13/H_{ax}-15 and 7-OCH₃/13-OH confirmed that the difference between **3** and **4** was the configuration at position C-13 (Figure 3). To determine the absolute configuration of **4**, ECD predictions were performed. The calculated ECD curve at the M062X/6-311+G* (IEFPCM, acetonitrile) level for 3*S*,4*R*,7*S*,8*S*,9*R*,13*S*,14*R*,16*S*,17*S*,19*R*,20*S*,21*R*-**4** matched the experimental ECD well. Therefore, the structure of **4** was fully assigned and named curtachalasin Y4.

Compound **5** was obtained as a white powder with the molecular formula $C_{30}H_{39}NO_8$ established by HRESIMS (m/z 542.2752 $[M + H]^+ \Delta$ +0.6 ppm). Analysis of the 1H and ^{13}C NMR data (Table 3) revealed that the structure of **5** closely resembled that of **3**, with the key difference being the replacement of the methoxy group at C-7 in **3** with a hydroxy group. The coupling constants of H-7 (J = 10.7 Hz), H-8 (J = 10.7, 3.3 Hz) supported axial configurations for both protons. Moreover, NOESY correlations of H-7/13-OH indicated that they were on the same side of the ring system (Figure 3). Compared to the known compound **14**,

5 is presumed to be its isomerized product at C-13. The ^{13}C NMR chemical shifts of **14** and **5** were compared in the same solvent (CDCl_3), and clear differences were observed near the isomerization position (Table S33). GFN2NMR was also used to calculate their ^{13}C NMR chemical shifts, confirming the structural assignments. The absolute configuration of **5** was further determined to be 3*S*,4*R*,7*S*,8*S*,9*R*,13*R*,14*R*,16*S*,17*S*,19*R*,20*S*,21*R* by ECD calculation at the B3LYP/TZVP (IEFPCM, MeOH) level of theory (Figure 4).

Table 2. ^{13}C (150 MHz) NMR data of 1–4 in CDCl_3 (δ in ppm).

No.	1	2	3	4
1	175.2, C	177.5, C	176.8, C	174.9, C
3	59.4, CH	59.1, CH	60.2, CH	60.0, CH
4	48.6, CH	49.0, CH	49.4, CH	49.0, CH
5	125.0, C	127.8, C	125.3, C	127.0, C
6	133.0, C	131.8, C	134.0, C	133.0, C
7	70.7, CH	67.8, CH	76.5, CH	83.2, CH
8	43.2, CH	43.7, CH	43.2, CH	43.2, CH
9	50.4, C	49.5, C	51.6, C	50.8, C
10	43.8, CH_2	44.2, CH_2	43.5, CH_2	43.9, CH_2
11	17.4, CH_3	17.4, CH_3	17.4, CH_3	17.4, CH_3
12	14.2, CH_3	18.4, CH_3	14.7, CH_3	13.4, CH_3
13	84.5, CH	78.8, CH	66.7, CH	72.0, CH
14	39.5, CH	39.8, CH	37.5, CH	39.1, CH
15	31.5, CH_2	31.9, CH_2	31.2, CH_2	31.6, CH_2
16	36.1, CH	36.3, CH	36.0, CH	36.1, CH
17	83.6, C	83.9, C	83.8, C	83.7, C
18	212.4, C	213.3, C	212.4, C	212.1, C
19	72.2, CH	72.5, CH	72.6, CH	72.4, CH
20	41.6, CH	41.6, CH	38.1, CH	40.5, CH
21	71.5, CH	71.9, CH	72.0, CH	71.3, CH
22	25.3, CH_3	25.4, CH_3	25.0, CH_3	24.9, CH_3
23	15.0, CH_3	15.0, CH_3	14.3, CH_3	14.6, CH_3
24	137.7, C	137.5, C	137.2, C	137.4, C
25/29	129.2, CH	129.2, CH	129.0, CH	129.1, CH
26/28	129.0, CH	129.0, CH	128.9, CH	128.9, CH
27	127.1, CH	127.2, CH	127.1, CH	127.0, CH
	172.1, C	171.9, C	171.6, C	171.9, C
21-OAc	21.2, CH_3	21.2, CH_3	21.0, CH_3	21.1, CH_3
7-OCH ₃ /13-OCH ₃	59.9, CH_3	60.9, CH_3	60.9, CH_3	59.1, CH_3

Compound **6** was obtained as a white powder with the molecular formula $\text{C}_{32}\text{H}_{43}\text{NO}_8$ determined by HRESIMS (m/z 592.2907 $[\text{M} + \text{Na}]^+ \Delta +4.4$ ppm). Compared to **4**, the ^1H NMR data of **6** revealed the presence of an additional ethyl group, characterized by a methyl group at δ_{H} 1.09 (3H, dd, $J = 7.0, 7.0$ Hz) and a methylene group at δ_{H} 3.53 (1H, dq, $J = 9.2, 7.0$ Hz) and 3.64 (1H, dq, $J = 9.2, 7.0$ Hz). Additionally, the chemical shift in C-7 was significantly different from that of **4**, with a downfield shift of 8.9 ppm. HMBC correlations between the ethyl group and C-7 indicated that the ethoxy group is attached to C-7 (Figure 2). The observed NOE correlations between H-4 and H-8 confirmed their co-facial orientation on ring B, with both protons adopting axial positions (Figure 3). The coupling constant between H-7 and H-8 (2.1 Hz) indicated that H-7 is equatorially oriented. The remainder of the planar structure and the relative configuration of **6** were determined to be identical to those of **4** (Figure 2). Finally, the absolute configuration of **6** was determined to be 3*S*,4*R*,7*R*,8*S*,9*R*,13*S*,14*R*,16*S*,17*S*,19*R*,20*S*,21*R* by ECD calculation at the $\omega\text{B97X-D/TZVP}$ (IEFPCM, MeOH) level of theory (Figure 4).

Table 3. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data of **5–7** (δ in ppm, J in Hz).

No.	5 ^a		6 ^b		7 ^c	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		178.5, C		176.1, C		176.8, C
3	3.81 dd (7.4, 7.4)	61.5, CH	3.40 m	59.3, CH	3.29 overlapped	61.4, CH
4	2.90 s	50.3, CH	2.29 br s	49.1, CH	2.53 br s	49.0, CH
5		125.9, C		129.2, C		128.4, C
6		135.8, C		130.2, C		135.3, C
7	4.83 br d (10.7)	66.2, CH	4.00 d (2.1)	74.3, CH	3.78 br d (10.8)	70.0, CH
8	2.49 dd (10.7, 3.3)	45.5, CH	1.85 overlapped	46.2, CH	2.40 m	41.0, CH
9		53.3, C		48.8, C		50.9, C
10	3.30 overlapped 3.37 dd (13.3, 7.4)	44.2, CH ₂	1.84 overlapped 2.86 br d (7.4)	45.3, CH ₂	3.15 dd (13.2, 9.2) 3.00 dd (13.2, 5.5)	43.8, CH ₂
11	1.46 s	17.5, CH ₃	1.35 s	19.3, CH ₃	1.15 s	17.5, CH ₃
12	1.97 s	15.4, CH ₃	1.76 s	17.4, CH ₃	1.63 s	14.8, CH ₃
13	4.80 ddd (11.4 3.6, 3.3)	67.8, CH	4.62 dd (10.9, 9.0)	69.1, CH	5.81 dd (4.4, 2.1)	120.6, CH
14	2.10 dddd (12.3, 12.3, 3.9, 3.6)	39.0, CH	1.42 m	41.3, CH		134.5, C
15	2.62 ddd (12.7, 12.4, 12.3)	32.4, CH ₂	1.42 m	31.7, CH ₂	2.28 m	38.2, CH ₂
	1.53 ddd (12.7, 3.6, 3.6)		1.86 m		2.05 dd (14.1, 4.4)	
16	2.15 overlapped	36.5, CH	1.82 m	36.2, CH	1.91 m	37.2, CH
17		85.6, C		83.8, C		85.9, C
18		215.8, C		213.1, C		214.6, C
19	4.04 d (10.4)	73.9, CH	3.41 overlapped	72.8, CH	3.69 d (10.7)	73.9, CH
20	3.29 overlapped	38.8, CH	2.79 ddd (10.8, 10.8, 2.3)	41.1, CH	2.85 m	43.6, CH
21	6.41 s	72.9, CH	5.31 d (2.3)	72.6, CH	5.80 d (4.1)	72.3, CH
22	2.44 s	26.5, CH ₃	2.27 s	25.7, CH ₃	2.26 s	25.9, CH ₃
23	0.95 d (6.6)	15.7, CH ₃	0.79 d (6.7)	14.8, CH ₃	0.75 d (6.7)	15.1, CH ₃
24		139.3, C		137.6, CH		139.9, C
25/29	7.23 overlapped	129.9, CH	7.15 d (7.3)	129.2, CH	7.30 overlapped	130.7, CH
26/28	7.26 overlapped	129.0, CH	7.29 t (7.3)	128.8, CH	7.32 overlapped	129.7, CH
27	7.27 overlapped	126.9, CH	7.22 t (7.3)	126.9, CH	7.21 t (7.7)	127.7, CH
		170.8, C		171.9, C		172.6, C
21-OAc	2.29 s	20.9, CH ₃	2.20 s	21.0, CH ₃	2.19 s	21.0, CH ₃
7-OCH ₂ CH ₃			3.53 dq (9.2, 7.0)	66.7, CH ₂		
7-OCH ₂ CH ₃			3.64 dq (9.2, 7.0)			
NH	9.88 s		1.09 dd (7.0, 7.0)	15.6, CH ₃		
13-OH	7.09 d (11.4)		5.91 s			
17-OH			3.69 br s ^d			
19-OH	6.59 br s		3.01 s			

^a Measured in pyridine-*d*₅ ^b Measured in CD₃Cl ^c Measured in CD₃OD ^d Assigned by the chemical shift value.

Compound **7** was obtained as a white powder with the molecular formula C₃₀H₄₃NO₈ determined by HRESIMS (m/z 1069.5055 [2M + Na]⁺ Δ +2.1 ppm). Compared to **2**, the ^1H and ^{13}C NMR data of **7** indicated that **7** lacks a methoxy group and two methine groups, and instead possesses an additional double bond signal at δ_{C} 120.6 and 134.5. 2D NMR analysis confirmed **7** as a 13,14-dehydrogenated product derived from **2**. The absolute configuration of **7** was further determined by ECD calculation at the B3LYP/TZVP (IEFPCM, acetonitrile) level of theory (Figure 4). Therefore, the structure of **7** was determined.

Compound **8** was obtained as a white powder with the molecular formula C₃₀H₃₇NO₈ determined by HRESIMS (m/z 1101.4925 [2M + Na]⁺, Δ −0.5 ppm). Compared to **4**, the ^1H and ^{13}C NMR data of **8** indicated that it lacks a methoxy group and contains an additional carbonyl group, observed at δ_{C} 199.3. HMBC correlations between H₃-12/H-8/H-13 and C-7 indicated the position of the carbonyl group. The 2D NMR data proved that **8** is a 7-

oxidized product derived from **4** (Figure 3). The absolute configuration of **8** was confirmed by ECD calculation at the B3LYP/TZVP (IEFPCM, MeOH) level of theory (Figure 4).

Compound **9** was obtained as a white powder with the molecular formula $C_{30}H_{37}NO_7$ determined by HRESIMS (m/z 1069.5045 $[2M + Na]^+$, Δ +1.1 ppm). Compared to **8**, it contains one fewer oxygen atom. Comparison of the 1D and 2D NMR data of **9** with those of **8** indicated that **9** possesses an additional methylene at C-13 (δ_C 26.6) (Figure 3), indicating that **9** is a deoxygenized product at C-13 from **8**. The absolute configuration of **9** was determined to be 3*S*,4*R*,9*R*,15*R*,16*S*,17*S*,19*R*,20*S*,21*R* by ECD calculation at the M062X/6-311+G* (IEFPCM, acetonitrile) level of theory (Figure 4). Therefore, the structure of **9** was determined.

Compound **10** was obtained as a white powder. Its molecular formula was determined to be $C_{31}H_{35}NO_8$ by HRESIMS (m/z 572.2257 $[M + Na]^+$, Δ +0.3 ppm), indicating an index of hydrogen deficiency of 15. The UV spectrum of **10** in MeOH exhibited absorption maxima at 230 and 365 nm, suggesting the presence of multiple conjugated structures. The 1H , ^{13}C NMR, DEPT, and HSQC spectra revealed characteristic features (Tables 4 and 5), including a monosubstituted benzene ring with signals at δ_H 7.22 (2H, overlapped), 7.30 (2H, t, J = 7.6 Hz), and 7.23 (1H, overlapped), an aldehyde group at δ_H 10.15 (1H, s), a methoxy group at δ_H 3.40 (3H, s), one methyl doublet at δ_H 1.06 (3H, d, J = 7.0 Hz), three methyl groups attached to double bonds at δ_H 2.10 (3H, s), 2.30 (3H, d, J = 1.7 Hz), and 2.31 (3H, s), an amide group at δ_C 173.2, one ester group at δ_C 171.6, three double bonds at δ_C 128.1, 128.7, 131.2, 137.4, 137.4, and 147.5, and a methylene group at δ_C 44.2. The 1H - 1H COSY and HMBC signals indicated a 5/6/6/6 fused ring system scaffold, as observed for **1–9** (Figure 2). The HMBC correlations of H-7 to C-5/C-6/C-9, H-13 to C-8/C-9/C-14/C-15/C-20, H-21 to C-9, and H₃-12 to C-5/C-6/C-7 validated the planar structure of rings B and C. The aldehyde proton signal was shifted upfield, indicating its conjugation with double bonds. The HMBC correlation between the aldehyde proton and C-4/C-5/C-6 established the position of aldehyde group. The chemical shift in C-15 and the HMBC correlations of the methoxy group to C-15 suggested the position of the methoxy group (Figure 2). The remainder of the planar structure was consistent with that of **1–9** (Figure 2). Additionally, NOESY correlations between H-16 and H-19 indicated that they are located on the same side of ring D and both occupy axial positions (Figure 3). The coupling constants between H-15 and H-16 (J = 2.8 Hz) suggested that H-15 is in an equatorial position. The NOESY correlations between H-4 and H₂-10/H-21 indicated that the benzyl group and H-4 are both located on the same side of ring A. Based on the relative configuration of **10** as shown in Figure 3, the absolute configuration was determined to be 3*S*,4*R*,9*R*,15*R*,16*S*,17*S*,19*R*,20*S*,21*R* by ECD calculation on the M062X/6-311+G* (IEFPCM, MeOH) level of theory (Figure 4).

Compound **11** was obtained as a white powder with the molecular formula of $C_{30}H_{37}NO_7$, determined by HRESIMS (m/z 546.2460 $[M + Na]^+$, Δ −0.6 ppm). Its 1H and ^{13}C NMR spectra indicated the same basic scaffold as **1–10** (Figure 2). Compound **11** possesses a sp^2 methylene group, with signals at δ_H 5.10 (1H, br s), 5.15 (1H, br s), and δ_C 113.4. The HMBC correlations between H₂-12 and C-5/C-6/C-7, and between H-7 and C-5/C-6/C-9, as well as the 1H - 1H COSY correlations of H₂-10/H-3/H-4/H-5/H₃-11 revealed the structure of rings A and B (Figure 2). The relative configuration of rings C and D, as supported by 2D NMR data, was consistent with that of **3** (Figure 2). NOESY correlations between H-3 and H₃-11 indicated that they are located on the same side of ring B. The observed NOESY correlations between H-14 and H-19, together with the coupling constants of H-19, indicated that both protons adopt an axial orientation on the same side of ring D (Figure 3). Moreover, the coupling constants between H-13 and H-14 suggested that H-13 is in an equatorial position. The relative configuration of

11 was fully assigned (Figure 3). The absolute configuration of **11** was determined to be 3*S*,4*R*,5*S*,9*R*,13*R*,14*R*,16*S*,17*S*,19*R*,20*S*,21*R* by ECD calculation at the M062X/6-311+G* (IEFPCM, acetonitrile) level of theory (Figure 4).

Table 4. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data of **8** and **9** in CDCl_3 (δ in ppm, J in Hz).

No.	8		9	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		173.8, C		174.3, C
3	3.48 br dd (7.6, 7.4)	59.0, CH	3.78 ddd (8.8, 8.8, 3.3)	57.9, CH
4	2.75 br s	48.9, CH	2.89 d (8.8)	52.9, CH
5		148.8, C		147.2, C
6		133.1, C		132.3, C
7		199.3, C		195.8, C
8	2.72 d (9.6)	48.9, CH	2.64 (overlapped)	42.7, CH
9		50.4, C		53.7, C
10	3.08 dd (13.5, 7.6) 2.96 dd (13.5, 7.4)	43.5, CH_2	3.37 dd (13.7, 3.1) 2.63 dd (13.7, 10.8)	42.8, CH_2
11	1.58 s	19.2, CH_3	2.05 s	22.0, C
12	1.72 s	11.8, CH_3	1.89 s	11.9, C
13	4.80 dd (9.6, 9.6)	68.2, CH	1.52 overlapped 2.35 ddd (12.8, 2.6, 2.6)	26.6, CH
14	1.59 overlapped	37.9, CH	1.54 overlapped	29.4, CH
15	2.06 overlapped	31.3, CH_2	1.50 overlapped	35.8, CH_2
16	1.36 ddd (12.7, 12.7, 12.5) 1.82 br dq (12.5, 6.7)	36.0, CH	1.26 br dd (12.3, 12.3) 1.85 ddq (12.3, 4.1, 6.7)	36.0 C
17		83.7, C		84.3, C
18		212.4, C		211.4, C
19	3.43 d (10.4)	72.3, CH	3.42 dd (10.1, 10.1)	72.0, CH
20	2.40 ddd (12.4, 10.3, 2.0)	41.4, CH	2.83 ddd (10.1, 10.1, 2.6)	42.0, CH
21	5.61 br s	70.8, CH	5.54 d (2.6)	72.6, CH
22	2.27 s	24.9, CH_3	2.21 s	24.0, CH_3
23	0.76 d (6.7)	14.6, CH_3	0.69 d (6.7)	14.7, CH_3
24		137.0, C		136.9, C
25/29	7.18 d (7.7)	129.1, CH	7.18 d (7.4)	128.9, CH
26/28	7.33 t (7.7)	129.0, CH	7.35 t (7.4)	129.4, CH
27	7.27 t (7.7)	127.3, CH	7.28 t (7.4)	127.6, CH
21-OAc		171.4, C		170.6, C
	2.30 s	21.1, CH_3	1.91 s	20.6, CH_3
NH	6.03 s		5.47 s	
17-OH			3.89 s	
19-OH			2.09 s	

Compound **12** was obtained as a white powder with the molecular formula $\text{C}_{30}\text{H}_{39}\text{NO}_8$, determined by HRESIMS (m/z 542.2748 $[\text{M} + \text{H}]^+$, Δ -0.2 ppm), containing one more oxygen atom than **11**. Compounds **12** and **11** share very similar structures, except for the absence of one double bond and the presence of two additional sp^3 methine groups (δ_{C} 43.1, 69.4) (Table 6), as evidenced by a comparison of their ^1H and ^{13}C NMR data. 2D NMR confirmed that **12** was substituted with a hydroxyl group at C-7 (Figure 2). The coupling constants of H-7 (10.5 Hz) suggested that H-7 and H-8 are both in axial positions. The remaining relative configurations were consistent with those of **11**, as supported by coupling constants and NOESY correlations (Figure 3). The absolute configuration of **12** was confirmed by ECD calculation at the B3LYP/TZVP (IEFPCM, acetonitrile) level of theory (Figure 4).

Table 5. ^1H (600 MHz) NMR data of **10–13** (δ in ppm, J in Hz).

No.	10 ^a	11 ^a	12 ^b	13 ^a
3	3.47 br d (11.4)	3.49 ddd (10.5, 7.0, 3.1)	3.34 m	3.25 br dd (7.9, 6.5)
4	3.29 br s	2.35 dd (7.0, 5.3)	2.17 br dd (6.3, 2.2)	2.05 dd (5.6, 2.1)
5		2.61 m	2.54 dq (6.8, 6.3)	2.64 m
7	5.99 s	6.52 br s	4.26 d (10.5)	4.25 d (9.4)
8			2.19 dd (10.5, 3.5)	2.20 dd (10.0, 9.4)
10	3.62 dd (11.7, 3.5)	3.16 dd (13.7, 3.1)	2.73 dd (13.1, 9.3)	2.76 dd (13.4, 7.9)
	2.84 dd (13.4, 11.7)	2.53 dd (13.7, 10.5)	2.93 dd (13.1, 5.3)	2.67 dd (13.4, 6.5)
11	10.15 s	1.29 d (7.3)	0.42 d (6.8)	0.72 d 6.5
12	2.30 d (1.7)	5.15 br s	5.15 s	5.20 br s
		5.10 br s	4.95 s	4.97 br s
13	6.31 br d (2.6)	4.00 dd (11.8, 3.2)	3.96 dd (3.2, 3.2)	4.56 dd (10.0, 10.0)
14		1.76 m	1.81 dddd (12.6, 12.3, 3.2, 3.2)	1.58 ddd (12.1, 10.0, 3.4)
15	3.63 br d (2.8)	1.38 dd (10.0, 4.3)	1.85 ddd (12.6, 12.5, 12.5)	1.90 ddd (13.0, 3.8, 3.4)
		1.91 m	1.33 ddd (12.6, 3.6, 3.3)	1.30 br dd (13.0, 12.1)
16	2.13 dq (7.1, 2.8)	1.90 m	1.99 m	1.83 m
19	3.53 dd (10.6, 8.6)	3.45 dd (10.2, 8.9)	3.49 d (10.5)	3.42 d (10.4)
20	3.19 ddd (10.6, 3.8, 3.0)	2.94 ddd (12.9, 10.2, 2.5)	2.45 ddd (12.3, 10.5, 2.2)	2.49 ddd (12.1, 10.4, 2.2)
21	5.68 d (3.8)	5.54 d (2.6)	5.51 d (2.2)	5.28 d (2.2)
22	2.31 s	2.26 s	2.26 s	2.25 s
23	1.06 d (7.0)	0.77 d (5.8)	0.76 d (6.7)	0.76 d (6.6)
25/29	7.22 overlapped	7.13 d (7.4)	7.21 overlapped	7.11 d (7.3)
26/28	7.30 t (7.6)	7.32 t (7.4)	7.30 t (7.0)	7.28 t (7.3)
27	7.23 overlapped	7.25 t (7.4)	7.21 overlapped	7.20 t (7.3)
21-OAc	2.10 s	2.17 s	2.26 s	2.25 s
15-OCH ₃	3.40 s			
NH	5.46 s	5.62 br s		6.04 s
13-OH		4.85 d (11.8)		
17-OH	4.59 s	3.72 s ^c		
19-OH	2.75 d (8.6)			

^a Measured in CDCl₃ ^b Measured in CD₃OD ^c Assigned by the chemical shift value.**Table 6.** ^{13}C (150 MHz) NMR data of **10–13** (δ in ppm).

No.	10 ^a	11 ^a	12 ^b	13 ^a
1	173.2, C	176.1, C	178.7, C	175.3, C
3	62.0, CH	54.4, CH	55.8, CH	53.4, CH
4	42.9, CH	50.2, CH	48.3, CH	47.6, CH
5	131.2, C	34.3, CH	32.8, CH	32.1, CH
6	147.5, C	143.3, C	151.1, C	148.4, C
7	128.7, CH	132.8, CH	69.4, CH	73.9, CH
8	137.4, C	134.1, CH	43.1, CH	42.9, CH
9	52.0, C	53.5, C	54.0, C	51.3, C
10	44.2, CH ₂	44.4, CH ₂	44.2, CH ₂	44.4, CH ₂
11	190.0, CH	15.6, CH ₃	12.9, CH ₃	12.9, CH ₃
12	17.8, CH ₂	113.4, CH ₂	114.0, CH ₂	114.1, CH ₂
13	128.1, CH	75.2, CH	68.8, CH	73.8, CH
14	137.4, C	38.1, CH	39.3, CH	40.3, CH
15	85.6, CH ₂	30.9, CH ₂	32.4, CH ₂	31.3, CH ₂
16	39.1, CH	35.8, CH	36.9, CH	36.0, CH
17	84.7, C	83.8, C	85.7, C	84.0, C
18	215.2, C	212.0, C	215.2, C	212.3, C

Table 6. Cont.

No.	10 ^a	11 ^a	12 ^b	13 ^a
19	73.9, CH	72.5, CH	74.0, CH	72.6, CH
20	40.4, CH	38.6, CH	38.9, CH	41.1, CH
21	71.8, CH	74.7, CH	73.6, CH	72.5, CH
22	29.3, CH ₃	24.9, CH ₃	26.0, CH ₃	25.1, CH ₃
23	11.5, CH ₃	14.8, CH ₃	15.4, CH ₃	14.9, CH ₃
24	138.6, C	137.2, C	138.8, C	137.3, C
25/29	129.2, CH	129.2, CH	130.7, CH	129.3, CH
26/28	129.0, CH	128.7, CH	129.6, CH	128.9, CH
27	126.9, CH	127.3, CH	127.8, CH	127.1, CH
	171.6, C	171.3, C	172.4, C	171.7, C
21-OAc	21.0, CH ₃	20.8, CH ₃	20.9, CH ₃	21.1, CH ₃
15-OCH ₃	56.6, CH ₃			

^a Measured in CDCl₃ ^b Measured in CD₃OD.

Compound **13** was obtained as a white powder with the molecular formula C₃₀H₃₉NO₈, determined by HRESIMS (m/z 542.2739 [M + H]⁺, Δ −1.8 ppm). Compounds **13** and **12** possess the same planar structures as revealed by NMR data. However, they are two different compounds with different retention times in the HPLC chromatogram (Figure S152). Their main difference lies in the methyl carbon at C-13 (δ_C 73.8 for **13** and 68.8 for **12**), suggesting epimerization at this position. The coupling constants of H-13 (J = 10.0, 10.0 Hz) suggested that H-8, H-13, and H-14 are all in axial positions (Table 5), which was confirmed by the NOESY correlations observed between H-7 and H-13. The remaining relative configurations were consistent with those of **12**, as supported by NOESY correlations (Figure 2). The absolute configuration of **13** was confirmed by ECD calculation at the B3LYP/TZVP (IEFPCM, MeOH) level of theory (Figure 5). Moreover, the ¹³C NMR chemical shift calculations of compounds **1–13** were performed using GFN2NMR (Figures S2–S19). As a result, all of the calculated data fitted well with the corresponding experimental data, indicating the high confidence of their structure assignment.

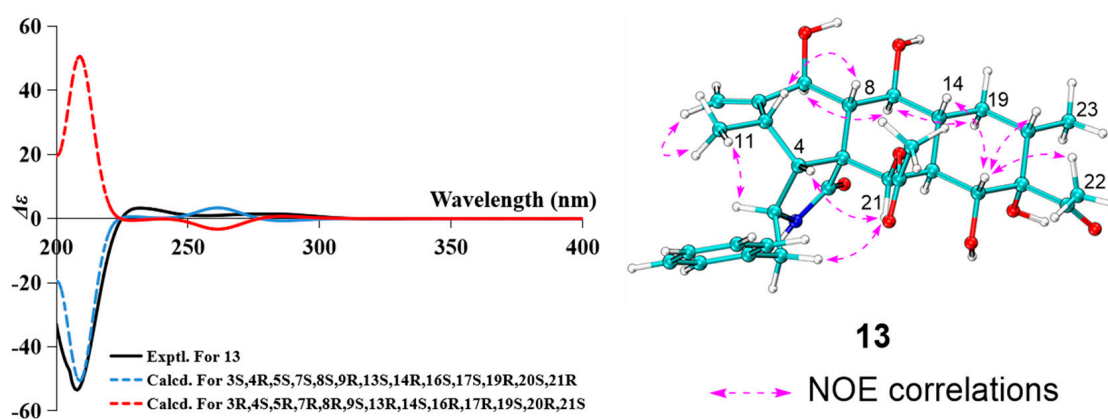


Figure 5. Key NOE correlations, the experimental and calculated ECD curves of **13** and its enantiomers.

To determine the anti-inflammatory activity of the cytochalasins, compounds available in sufficient quantities (**1**, **5**, **7**, **8**, **12**, **13–16**, and **18–27**) were evaluated for their inhibitory effects on NO production in the LPS-induced RAW264.7 cell model, using dexamethasone as a positive control. First, CCK-8 analysis was used to assess cytotoxicity against RAW264.7 cells. Sixteen cytochalasins (**1**, **5**, **7**, **8**, **12**, **13–16**, **19–23**, **26**, and **27**) exhibited >90% cell viability after treatment at 30 μ M and were selected for further evaluation. The inhibitory activity on NO production was assessed by measuring nitrite/nitrate levels in

the supernatant of LPS-induced RAW264.7 cells. Compared with the vehicle-treated LPS group, treatment with compounds **12**, **21** and **22** significantly reduced NO levels, with IC₅₀ values of 55.4, 43.8, and 17.8 µM, respectively. Notably, the effect of compound **22** approached that of dexamethasone (IC₅₀: 14.9 µM). The remaining compounds showed IC₅₀ values exceeding 60 µM (Table 7).

Table 7. The IC₅₀ values of tested compounds for inhibitory effects on LPS-induced NO production of RAW264.7 macrophages.

Compounds	IC ₅₀ (µM)
1, 5, 7, 8, 13–16, 19, 20, 23, 26, and 27	>60
12	55.4 ± 0.3
21	43.8 ± 5.5
22	17.8 ± 0.7
Dexamethasone	14.9 ± 1.3

Previous studies have shown that RAW264.7 cells release large amounts of pro-inflammatory cytokines, including IL-6, MCP-1, and TNF-α, in response to LPS-mediated inflammatory responses. Therefore, whether these compounds exert an inhibitory effect on the release of pro-inflammatory cytokines was investigated. The ELISA results indicated that IL-6, MCP-1, and TNF-α levels were significantly decreased by treatment with compounds **12**, **21**, and **22** (Figure 6), confirming their anti-inflammatory effects.

RNA-sequencing-based transcriptome analysis was performed on RAW264.7 cells treated with compound **22** after LPS induction to preliminarily explore the potential mechanism. This high-throughput method enabled the identification of genes and pathways altered in response to **22** treatments. A total of 217 DEGs were identified between **22**-treated and untreated RAW264.7 cells, as shown in the volcano plot (Figure 7A), with 126 genes upregulated and 91 downregulated. KEGG pathway enrichment analysis indicated that the DEGs identified in RAW264.7 cells treated with **22** (compared to untreated cells) were related to the TNF signaling pathway, which is closely involved in inflammatory responses. DEGs such as CXCL10 and C-C motif ligand 2 (CCL2) were significantly downregulated, whereas SOCS3 was significantly upregulated by treatment with **22** (Figure 7B,C). Further mRNA expression analysis showed that treatment with **22** increased SOCS3 mRNA expression in RAW264.7 cells compared to controls (Figure 7D, $p < 0.01$). Moreover, CXCL10 and TNF-α levels in RAW264.7 cells were reduced in a dose-dependent manner following **22** treatment (Figure 7E,F, $p < 0.001$). To further validate these results, Western blotting was performed, revealing that treatment with **22** increased SOCS3 protein expression in a dose-dependent manner compared to LPS-induced controls (Figure 7G). Previous research has indicated that CXCL10 plays a key role as an inflammatory mediator [19,20], whereas SOCS3 serves as an anti-inflammatory factor and negatively regulates TNF-α activation [21]. Therefore, compound **22** likely inhibits the TNF signaling pathway and reduces inflammatory responses in RAW264.7 cells through a mechanism that involves CXCL10 and SOCS3.

Due to the limited initial amount of compound **22** (approximately 2 mg), the remaining material is no longer sufficient to support further experiments after several rounds of bioactivity screening and mechanistic studies. An additional sample will be obtained through semi-synthesis from other compounds (e.g., **24**) to meet the requirements of subsequent in-depth research.

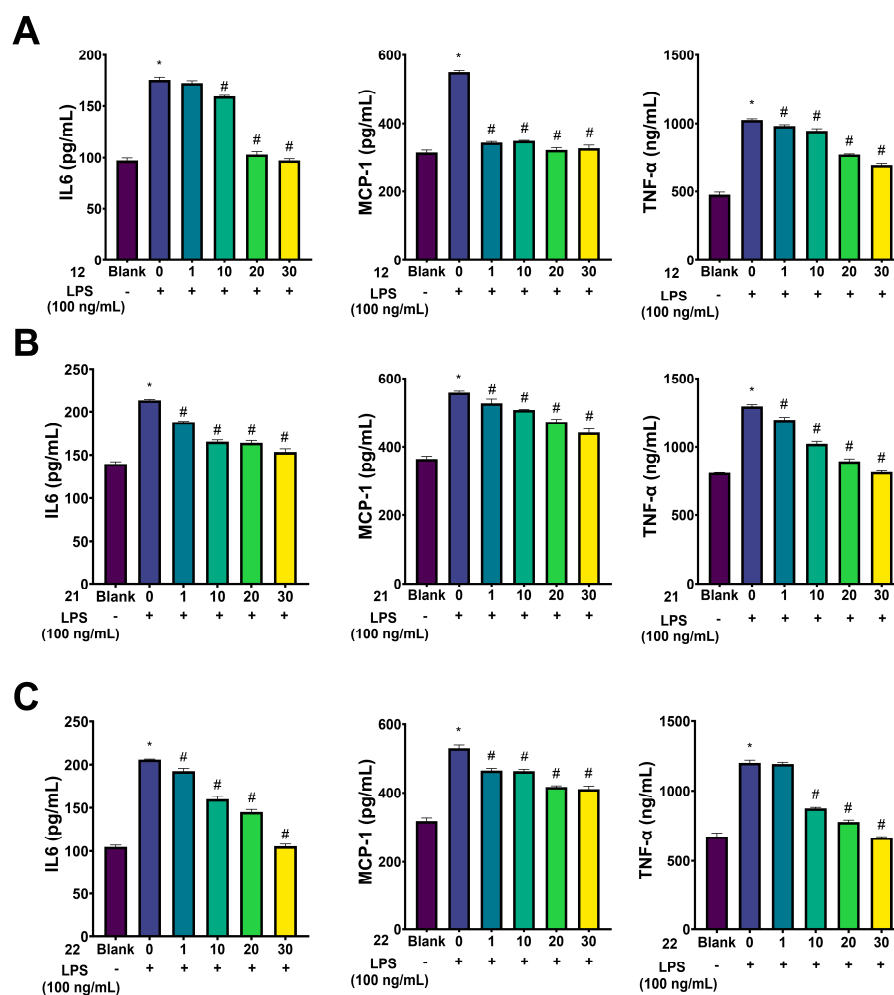


Figure 6. Levels of TNF- α , MCP-1, and IL-6 were measured by ELISA in cells treated with compounds 12 (A), 21 (B), and 22 (C), using vehicle-treated cells as the control group. * $p < 0.05$ vs. blank group, # $p < 0.05$ vs. control group.

In summary, twenty-seven cytochalasins (1–27) from a soil fungus *Xylaria* sp. Y01 are reported, including thirteen previously undescribed ones (1–13). Compounds 1–17 possess a 5/6/6/6 tetracyclic skeleton. The structures of the new compounds were elucidated by extensive spectroscopic methods and quantum chemical calculations. The inhibitory activity of all isolates on LPS-stimulated NO production in mouse RAW264.7 macrophages was evaluated. The results indicated that compound 22 exhibited anti-inflammatory effects in RAW264.7 cells by upregulating SOCS3 expression. Therefore, compound 22 represents a potential candidate for the research and development of treatments for inflammatory diseases. Structure–activity relationship analysis correlates the superior potency of compound 22 with the C-5/C-6 double bond and C-7 ketone carbonyl group, as these moieties are either absent or modified in less active analogs. This finding reinforces the importance of these structural elements for bioactivity and guides future optimization of cytochalasan derivatives. Although the present work emphasizes anti-inflammatory activity, additional biological profiling, including cytotoxicity and immunosuppressive assays, is necessary. Further studies addressing these aspects, particularly for compound 22, are warranted.

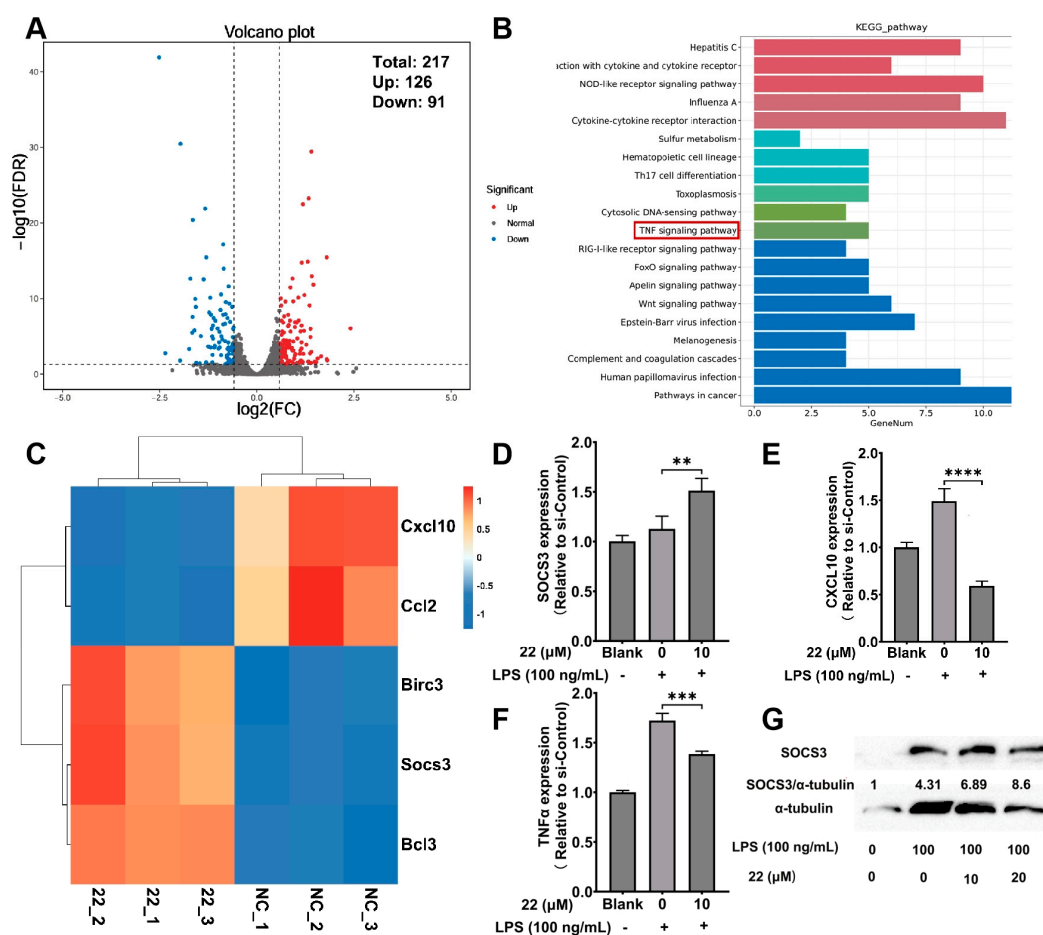


Figure 7. Compound 22 exhibited anti-inflammatory effects in RAW264.7 cells by upregulating SOCS3 expression. (A) Volcano plot of DEGs in RAW264.7 cells treated with compound 22 versus control cells. The x-axis represents $\log_2$ (fold change), and the y-axis represents $-\log_{10}$ (false discovery rate, FDR). Blue dots indicate downregulated genes; red dots indicate upregulated genes. (B) KEGG pathway enrichment analysis of DEGs. Colors indicate the regulation status of the pathways: red represents up-regulation, blue represents down-regulation, and cyan represents normal/non-significant changes. The red box highlights the "TNF signaling pathway". (C) Heatmap of DEGs in RAW264.7 cells treated with compound 22 versus control cells. (D–F) RT-qPCR analysis of SOCS3, CXCL10, and TNF- α mRNA levels in RAW264.7 cells following treatment with compound 22. (G) Western blot analysis of SOCS3 protein levels in RAW264.7 cells after treatment with compound 22. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ vs. control group.

3. Materials and Methods

3.1. General Experiment Procedures

UV spectra were recorded on a Cary 300 spectrometer (Agilent Technologies, Santa Clara, CA, USA). Experimental ECD data were collected using a ChirascanTM-plus CD instrument (Applied Photophysics, London, UK). Optical rotations were measured on a Rudolph Autopol IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA), and IR spectra were acquired on a Shimadzu FT-IR spectrometer (Shimadzu Corporation, Kyoto, Japan) with KBr pellets. HRESIMS spectra were recorded on an Agilent 6500 series Q-TOF mass spectrometer (Agilent Technologies, Singapore). NMR experiments (^1H at 600 MHz and ^{13}C at 150 MHz) were performed on Bruker spectrometers (Bruker BioSpin, Rheinstetten, Germany). For chromatographic separation, D101 macroporous adsorption resin (Tianjin Haoju, Tianjin, China) and silica gel (200–300 and 300–400 mesh; Qingdao Marine, Qingdao, China) were used for column chromatography. Preparative

HPLC was performed on an E-Classical P3500 system (Dalian Elite Analytical Instruments Co., Ltd., Dalian, China) equipped with a DAD detector, using SinoChrom ODS-AP or Supersil C8 columns (5 μ m, 10 $\times$ 250 mm, Dalian Elite Analytical Instruments Co., Ltd., Dalian, China). TLC was run on precoated silica gel GF254 plates (Qingdao Marine Chemical, Qingdao, China), and spots were visualized under UV light (254 or 356 nm) or by spraying with 10% H₂SO₄ in EtOH followed by heating. The purity of each compound tested in the bioassays was verified to be higher than 96% by HPLC analysis.

3.2. Fungal Material

The fungal strain *Xylaria* sp. Y01 was isolated from a soil sample collected from Kunming, Yunnan Province, China, in 2022. Within 24 h of collection, a nutrient-limited medium (1 g/L dextrose, 15 g/L agar) containing 50 mg/L streptomycin was inoculated with 100 μ L of a suspension prepared from 1 g of soil in 250 mL of sterile water containing 50 mg/L streptomycin. The mixture was incubated at 25 °C for 7 days. Pure fungal colonies were selected and cultured on potato dextrose agar (PDA). The methods for sample processing and fungal species identification followed the procedures outlined in a previous publication [18]. The fungal specimen is deposited at the Xiangya School of Pharmaceutical Sciences, Central South University, Changsha, China.

3.3. Fermentation and Isolation

The hyphae of *Xylaria* sp. Y01 were cultured in SDA liquid medium at 25 °C for 10 days. The resulting cultures were then inoculated onto rice medium in 500 mL Erlenmeyer flasks and incubated at 25 °C for 40 days. The rice cultures of *Xylaria* sp. Y01 (10 kg) were harvested and extracted with 20 L of ethyl acetate at room temperature. The crude extract was obtained by evaporation under vacuum. The extract was partitioned five times between water and ethyl acetate, and the combined organic layers were concentrated under reduced pressure to yield a brown residue (150 g). The crude extract was applied to a macroporous resin D101 column (20 cm $\times$ 30 cm) and eluted successively with 0% (discarded), 90%, and 100% aqueous methanol to obtain three fractions (Fr. A–C). Fr. A was further subjected to silica gel column chromatography and eluted with a petroleum ether (PE)–ethyl acetate (EA)–methanol gradient (*v/v/v*, 1:0:0 to 0:0:1) to yield five fractions (Fr. A1–A5).

Fr. A1 was purified by pre-HPLC (flow rate 2.5 or 3 mL/min) to obtain **12** (26.6 mg, 40% acetonitrile, *t_R* = 22.0 min, SinoChrom ODS-AP column), **13** (68.2 mg, 50% methanol, *t_R* = 35.0 min, Supersil C8: 10.0 mm $\times$ 250 mm, 5 μ m), and **16** (4.1 mg, 67% acetonitrile, *t_R* = 20.0 min, Supersil C8 column). Fr. A2 was fractionated over a Microporous resin (MCI) and eluted with methanol–H₂O (*v/v* 20:80, 40:60, 60:40, 80:20, 100:0, 20 mL/min) to yield five subfractions (A2a–A2e). The remaining Fr. A2a was purified by pre-HPLC (flow rate 2.5 or 3 mL/min) to yield **5** (50.3 mg, 59% methanol, *t_R* = 30 min, SinoChrom ODS-AP column), **6** (3.6 mg, 63% methanol, *t_R* = 31.5 min, SinoChrom ODS-AP column), **7** (9.4 mg, 31% acetonitrile, *t_R* = 47 min, Supersil C8 column), **8** (5.3 mg, 59% methanol, *t_R* = 41.0 min, SinoChrom ODS-AP column), **14** (8.3 mg, 33% acetonitrile, *t_R* = 38 min, Supersil C8 column), **15** (5.0 mg, 33% acetonitrile, *t_R* = 36.5 min, Supersil C8 column), and **17** (1.2 mg, 59% methanol, *t_R* = 33 min, SinoChrom ODS-AP column). The remained Fr. A2b was purified by pre-HPLC (flow rate 2.5 or 3 mL/min) successively to yield **2** (1.1 mg, 39% acetonitrile, *t_R* = 22.0 min, Supersil C8 column), **3** (1.2 mg, 68% methanol, *t_R* = 33.0 min, SinoChrom ODS-AP column), **4** (0.9 mg, 72% methanol, *t_R* = 25.0 min, SinoChrom ODS-AP column), **9** (1.0 mg, 39% acetonitrile, *t_R* = 22.0 min, SinoChrom ODS-AP column), **10** (0.8 mg, 54% methanol, *t_R* = 28.0 min, SinoChrom ODS-AP column), and **11** (1.3 mg, 68% methanol, *t_R* = 29.0 min, SinoChrom ODS-AP column). The remaining Fr. A2c was purified

by pre-HPLC (flow rate 2.5 mL/min) to yield **1** (10.7 mg, 34% methanol, t_R = 23.0 min, SinoChrom ODS-AP column). The isolation procedure for compounds **18–27** was carried out according to the previously reported method [12].

Curtachalasin Y1 (**1**): Colorless powder. $[\alpha]_D^{25}$ +49.4 (c 1.34, MeOH). IR (KBr): $\nu_{\max}$ 3428.1, 2929.8, 1744.2, 1694.1, 1451.2, 1380.7, 1245.0, 1101.3, 1036.2, 705.2 cm^{-1} . UV (acetonitrile) $\lambda_{\max}$ 210, 260 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Tables 1 and 2. HRESIMS m/z 578.2724 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{31}\text{H}_{41}\text{NO}_8\text{Na}^+$, 578.2725 Δ −0.2 ppm).

Curtachalasin Y2 (**2**): Colorless powder. $[\alpha]_D^{25}$ +303.0 (c 0.14, MeOH). IR (KBr): $\nu_{\max}$ 3380.1, 2924.3, 2862.0, 1749.7, 1706.3, 1671.0, 1451.2, 1375.3, 1234.2, 1030.7, 941.2, 737.8 cm^{-1} . UV (MeOH) $\lambda_{\max}$ 205 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Tables 1 and 2. HRESIMS m/z 1133.5575 $[2\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{62}\text{H}_{82}\text{N}_2\text{O}_{16}\text{Na}^+$, 1133.5575, Δ 0.0 ppm), m/z 1075.5551 $[2\text{M} + \text{H} - 2\text{H}_2\text{O}]^+$ (calcd. for $\text{C}_{62}\text{H}_{79}\text{N}_2\text{O}_{14}^+$, 1075.5526, Δ +2.7 ppm).

Curtachalasin Y3 (**3**): Colorless powder. $[\alpha]_D^{25}$ +54.0 (c 0.10, MeOH). IR (KBr): $\nu_{\max}$ 3467.0, 2401.8, 2959.6, 2924.3, 1733.4, 1700.8, 1679.1, 1646.6, 1386.1, 1356.3, 1256.0, 1106.7, 1041.6 cm^{-1} . UV (acetonitrile) $\lambda_{\max}$ 205 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Tables 1 and 2. HRESIMS m/z 556.2914 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{31}\text{H}_{42}\text{NO}_8^+$, 556.2905, Δ +1.6 ppm).

Curtachalasin Y4 (**4**): Colorless powder. $[\alpha]_D^{25}$ +9.6 (c 0.08, MeOH). IR (KBr): $\nu_{\max}$ 3439.9, 2921.6, 2346.5, 1747.0, 1696.6, 1443.1, 1378.0, 1245.0, 1038.9 cm^{-1} . UV (acetonitrile) $\lambda_{\max}$ 205 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Tables 1 and 2. HRESIMS m/z 1133.5592 $[2\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{62}\text{H}_{82}\text{N}_2\text{O}_{16}\text{Na}^+$, 1133.5575, Δ +1.5 ppm), m/z 578.2746 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{31}\text{H}_{41}\text{NO}_8\text{Na}^+$, 578.2725, Δ +3.6 ppm).

Curtachalasin Y5 (**5**): Colorless powder. $[\alpha]_D^{25}$ +31.9 (c 2.52, MeOH). IR (KBr): $\nu_{\max}$ 3429.0, 2924.3, 2311.2, 1749.7, 1714.4, 1638.4, 1459.4, 1375.3, 1256.0, 1228.8, 1025.3 cm^{-1} . UV (in MeOH) $\lambda_{\max}$ 205 and 280 nm. ^1H NMR (600 MHz in pyridine- d_5) and ^{13}C NMR (150 MHz in pyridine- d_5) data, see Table 3. HRESIMS m/z 542.2752 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{30}\text{H}_{40}\text{NO}_8^+$, 542.2749, Δ +0.6 ppm).

Curtachalasin Y6 (**6**): Colorless powder. $[\alpha]_D^{25}$ +165.4 (c 0.44, MeOH). IR (KBr): $\nu_{\max}$ 3467.0, 2919.0, 1749.7, 1706.3, 1673.7, 1451.2, 1378.0, 1231.5, 1022.6, 702.5 cm^{-1} . UV (MeOH) $\lambda_{\max}$ 205 and 285 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Table 3. HRESIMS m/z 592.2907 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{32}\text{H}_{43}\text{NO}_8\text{Na}^+$, 592.2881, Δ +4.4 ppm).

Curtachalasin Y7 (**7**): Colorless powder. $[\alpha]_D^{25}$ +9.6 (c 1.00, MeOH). IR (KBr): $\nu_{\max}$ 3514.5, 3449.6, 3355.0, 2965.4, 2930.3, 2854.5, 1748.3, 1699.6, 1599.5, 1437.3, 1372.4, 1231.7, 1083.0, 96.13, 707.0, 471.7 cm^{-1} . UV (acetonitrile) $\lambda_{\max}$ 205 nm. ^1H NMR (600 MHz in CD_3OD) and ^{13}C NMR (150 MHz in CD_3OD) data, see Table 4. HRESIMS m/z 1069.5055 $[2\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{60}\text{H}_{74}\text{N}_2\text{O}_{14}\text{Na}^+$, 1069.5033, Δ +2.1 ppm).

Curtachalasin Y8 (**8**): Colorless powder. $[\alpha]_D^{25}$ +115.3 (c 0.66, MeOH). IR (KBr): $\nu_{\max}$ 3412.7, 2956.9, 2859.2, 2859.2, 1676.4, 1581.5, 1416.0, 1264.0, 1101.3, 1036.2, 808.3 cm^{-1} . UV (MeOH) $\lambda_{\max}$ 205 and 243 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Table 4. HRESIMS m/z 1101.4925 $[2\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{60}\text{H}_{74}\text{N}_2\text{O}_{16}\text{Na}^+$, 1101.4931, Δ −0.5 ppm).

Curtachalasin Y9 (**9**): Colorless powder. $[\alpha]_D^{25}$ +350.7 (c 0.12, MeOH). UV (acetonitrile) $\lambda_{\max}$ 205 and 250 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Table 4. HRESIMS m/z 1069.5045 $[2\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{60}\text{H}_{74}\text{N}_2\text{O}_{14}\text{Na}^+$, 1069.5033, Δ +1.1 ppm), m/z 1047.5227 $[2\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{60}\text{H}_{75}\text{N}_2\text{O}_{14}^+$, 1047.5213, Δ +0.9 ppm). The IR spectrum was not measured because of its low sample amount.

Curtachalasin Y10 (**10**): Colorless powder. $[\alpha]_D^{25} -416.0$ (c 0.03, MeOH). UV (MeOH) $\lambda_{\max}$ 230 and 365 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Tables 5 and 6. HRESIMS m/z 572.2257 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{31}\text{H}_{35}\text{NO}_8\text{Na}^+$, 572.2255, $\Delta +0.3$ ppm). The IR spectrum was not measured because of its low sample amount.

Curtachalasin Y11 (**11**): Colorless powder. $[\alpha]_D^{25} +199.7$ (c 0.17, MeOH). IR (KBr): $\nu_{\max}$ 3420.8, 2967.7, 2921.6, 2875.5, 1736.0, 1709.0, 1681.8, 1432.3, 1375.3, 1228.8, 1036.2 cm^{-1} . UV (acetonitrile) $\lambda_{\max}$ 210 and 263 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Tables 5 and 6. HRESIMS m/z 546.2460 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{30}\text{H}_{37}\text{NO}_7\text{Na}^+$, 546.2463, $\Delta -0.6$ ppm).

Curtachalasin Y12 (**12**): Colorless powder. $[\alpha]_D^{25} +87.7$ (c 2.66, MeOH). IR (KBr): $\nu_{\max}$ 3428.1, 3158.8, 2970.0, 2911.7, 2875.6, 1745.6, 1712.3, 1673.4, 1456.9, 1376.3, 1354.1, 1226.4, 1018.2, 943.2, 890.5, 732.2, 704.5 cm^{-1} . UV (acetonitrile) $\lambda_{\max}$ 205 nm. ^1H NMR (600 MHz in CD_3OD) and ^{13}C NMR (150 MHz in CD_3OD) data, see Tables 5 and 6. HRESIMS m/z 542.2748 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{30}\text{H}_{40}\text{NO}_8^+$, 542.2749, $\Delta -0.2$ ppm).

Curtachalasin Y13 (**13**): Colorless powder. $[\alpha]_D^{25} +4.2$ (c 8.52, MeOH). IR (KBr): $\nu_{\max}$ 3118.5, 2966.9, 2878.7, 2346.6, 1735.0, 1696.4, 1222.4, 1114.9, 1062.5, 985.4, 1010.2, 1037.7 cm^{-1} . UV (MeOH) $\lambda_{\max}$ 205 nm. ^1H NMR (600 MHz in CDCl_3) and ^{13}C NMR (150 MHz in CDCl_3) data, see Tables 5 and 6. HRESIMS m/z 542.2739 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{30}\text{H}_{40}\text{NO}_8^+$, 542.2749, $\Delta -1.8$ ppm).

3.4. Quantum Chemical Calculations

Candidate conformers were initially explored with Crest (version 2.12) at the GFN0 theoretical level [22,23], followed by optimization at the GFN2-xTB [24] level, where an energy cutoff of 4 kcal/mol was applied to discard higher-energy species. The retained conformers were then subjected to geometry optimization and vibrational frequency analysis at the B3LYP-D3(BJ)/TZVP (IEFPCM) level. The calculated ECD spectra were then simulated using SpecDis version 1.71 [25], and the contributions of conformers were Boltzmann-averaged based on Gibbs free energy. All DFT calculations were performed using the Gaussian 16 software package [26]. The optimized geometries, relative energies, and conformational populations of all calculated structures are provided in the Supplementary Material.

3.5. Cell Culture and Cell Viability Assay

RAW264.7 cells (Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd., Shanghai, China) were cultured in DMEM (NEST Biotechnology Co., Ltd., Wuxin, China) containing 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, and 2 mg/mL gentamicin, and maintained at 37 °C in a humidified atmosphere with 5% CO_2 . For the assay, cells were seeded into 96-well plates at a density of 5×10^4 cells per well and exposed to compounds **1**, **5**, **7**, **8**, **12**, **13–16**, and **18–27** at a concentration of 30 μM for 24 h under the same conditions. Afterwards, 10 μL of CCK-8 reagent was added to each well, and the plates were further incubated at 37 °C for 4 min. Absorbance was read at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA), with values obtained from untreated cells set as 100% viability. No significant cytotoxicity was observed; therefore, concentrations below 30 μM of these nineteen cytochalasins were used in subsequent experiments.

3.6. NO Production Inhibitory Activity

The nitrite content in culture supernatants, as an indicator of NO release, was quantified employing the Griess reagent. RAW264.7 cells were treated with lipopolysaccharide (LPS, 100 ng/mL) in the presence or absence of the nineteen cytochalasins (30 μM) for 24 h. A 100 μL aliquot of the cell culture supernatant was mixed with 100 μL of Griess reagent

and incubated for 10 min at room temperature. The absorbance was then measured at 540 nm.

3.7. RNA Extraction and Sequencing

Total RNA was extracted using mirVana™ miRNA Isolation Kit (Ambion, Thermo Fisher Scientific, Waltham, MA, USA) according to the supplier's recommendations. RNA quality and concentration were assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA); only samples with an RNA integrity number (RIN) ≥ 7 proceeded to library construction. cDNA libraries were constructed using the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, Inc., San Diego, CA, USA; Paired-End, PE150) as per the manufacturer's instructions, and subsequently sequenced on an Illumina HiSeq™ 2500 platform, producing 125/150 bp paired-end reads.

3.8. Differential Expression and KEGG Pathway Analyses

Gene expression levels were calculated as FPKM values with Cufflinks (v2.2.1, University of Washington, Seattle, WA, USA), and read counts per gene were derived using HTSeq-count (v0.11.2, European Molecular Biology Laboratory, Heidelberg, Germany). Differentially expressed genes (DEGs) were identified with the DESeq R package (v4.2.1), applying the functions estimateSizeFactors and nbinomTest. Significance was defined as a p -value < 0.05 and an absolute fold change > 2 or < 0.5 . Hierarchical clustering was performed to reveal expression patterns among DEGs, while KEGG pathway enrichment was carried out in R based on the hypergeometric distribution.

3.9. Enzyme-Linked Immunosorbent (ELISA) Assay

Levels of TNF- α , IL-6, and MCP-1 in cell culture supernatants were determined using commercial ELISA kits from Aifang (Changsha, China), following the provided protocols.

3.10. RT-PCR Analysis

RAW264.7 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS (NEST Biotechnology Co., Ltd., Nanjing, China) and 1% penicillin/streptomycin, and kept at 37 °C with 5% CO₂. RT-PCR was used to detect gene expression. Total RNA was purified with an RNA isolation kit (Vazyme Biotechnology, No. RC112-01, Nanjing, China), and cDNA was synthesized using a reverse transcription kit. RT-PCR was performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotechnology, No. Q711-02, Nanjing, China). The following primers were used:

TNF- α -F: 5'-GCCTCTTCTCATTCTGCTTGTTGG-3'

TNF- α -R: 5'-GTGGTTTGTGAGTGTGAGGGTCTG-3'

SOCS3-F: 5'-ATGGTCACCCACAGCAAGTTT-3'

SOCS3-R: 5'-TCCAGTAGAATCCGCTCTCCT-3'

Cxcl10-F: 5'-CCAAGTGCTGCCGTCATTTTC-3'

Cxcl10-R: 5'-GGCTCGCAGGGATGATTTC-3'

3.11. Western Blotting

The harvested cells were washed with PBS and lysed with protein lysis buffer. Protein concentrations were determined using a BCA Protein Assay Kit (Multisciences, Hangzhou, China). Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to a PVDF membrane (Merck Millipore, Billerica, MA, USA), and incubated with the designated SOCS3 antibody and α -tubulin antibody overnight. After washing three times with TBST and incubating with a secondary antibody at room temperature for 2 h, protein expression was detected with an ECL Prime Western Blotting Kit (NCM Biotech, P10100, Suzhou, China).

4. Statistical Analysis

Data from this experiment are presented as the mean $\pm$ standard error of the mean (mean $\pm$ SEM). Analysis was conducted using one-way or two-way ANOVA with Graph-Pad Prism 9.0.0 software. All statistical tests were two-tailed, with $p < 0.05$ considered statistically significant. Significance levels of $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$ are denoted by *, **, ***, and **** (or #, ##, ###, ####), respectively. Each experiment was performed and analyzed with a minimum of three independent replicates.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27146313/s1>.

Author Contributions: Conceptualization, J.L. and W.-X.W.; Methodology, Y.-Y.Y. and X.Z.; Software, Y.-Y.Y. and Y.-M.L.; Formal analysis, Y.X. and L.T.; Investigation, Q.-A.C. and Q.-H.X.; Resources, W.-X.W. and J.L.; Data curation, X.Z.; Writing—original draft preparation, Y.-Y.Y. and Y.X.; Writing—review and editing, W.-X.W. and J.L.; Visualization, Y.-Y.Y. and Y.X.; Supervision, W.-X.W. and J.L.; Project administration, S.L. and J.L.; Funding acquisition, W.-X.W., Y.X. and J.L. All authors have read and agreed to the published version of the manuscript.

Funding: The work was financially supported by National Natural Science Foundation of China (Nos. 82173713, 82304794), Natural Science Foundation of Hunan Province (No. 2024JJ8127), Project of Hunan Administration of Traditional Chinese Medicine (No. B2023059), and Postgraduate Scientific Research Innovation Project of Hunan Province (Nos. 2025ZZTS0913, 2026ZZTS0160, 2025ZZTS0170).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank the Institute for Advanced Study, Central South University, for NMR measurement.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Becker, K.; Stadler, M. Recent progress in biodiversity research on the Xylariales and their secondary metabolism. *J. Antibiot.* **2021**, *74*, 1–23. [CrossRef] [PubMed]
2. Helaly, S.E.; Thongbai, B.; Stadler, M. Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. *Nat. Prod. Rep.* **2018**, *35*, 992–1014. [CrossRef] [PubMed]
3. Zhao, Y.L.; Long, X.W.; Wu, H.; Deng, J. Recent advances in the total synthesis of cytochalasan natural products using bioinspired strategies. *Org. Chem. Front.* **2022**, *9*, 6979–6998. [CrossRef]
4. Aldridge, D.C.; Armstrong, J.J.; Speake, R.N.; Turner, W.B. The structures of cytochalasins A and B. *J. Chem. Soc.* **1967**, *2*, 1667–1676. [CrossRef]
5. Scherlach, K.; Boettger, D.; Remme, N.; Hertweck, C. The chemistry and biology of cytochalasans. *Nat. Prod. Rep.* **2010**, *27*, 869–886. [CrossRef] [PubMed]
6. Spiteller, P. Chemical ecology of fungi. *Nat. Prod. Rep.* **2015**, *32*, 971–993. [CrossRef] [PubMed]
7. Zhang, D.; Li, S.; Fan, M.; Zhao, C. The novel compounds with biological activity derived from soil fungi in the past decade. *Drug Des. Devel. Ther.* **2022**, *16*, 3493–3555. [CrossRef] [PubMed]
8. Weng, J.; Li, S.; Ma, R.; Shi, Q.; Meng, X.; Zhou, G.; Qin, L.; Li, H. New secondary metabolites from the soil-derived *Aspergillus versicolor* QC812. *Steroids* **2025**, *220*, 109629. [CrossRef] [PubMed]
9. Yaosanit, W.; Rukachaisirikul, V.; Phongpaichit, S.; Preedanon, S.; Sakayaroj, J. Sesquiterpenes from the soil-derived fungus *Trichoderma citrinoviride* PSU-SPSF346. *Beilstein J. Org. Chem.* **2022**, *18*, 479–485. [CrossRef] [PubMed]
10. Wang, W.X.; Cheng, G.G.; Li, Z.H.; Ai, H.L.; He, J.; Li, J.; Feng, T.; Liu, J.K. Curtachalasin, immunosuppressive agents from the endophytic fungus *Xylaria* cf. *curta*. *Org. Biomol. Chem.* **2019**, *17*, 7985–7994. [CrossRef] [PubMed]
11. Chen, W.; Yu, M.; Chen, S.; Gong, T.; Xie, L.; Liu, J.; Bian, C.; Huang, G.; Zheng, C. Structures and biological activities of secondary metabolites from *Xylaria* spp. *J. Fungi* **2024**, *10*, 190. [CrossRef] [PubMed]

12. Yuan, Y.Y.; Li, Y.; Li, J.; Lu, W.Y.; Liang, A.L.; Xu, P.J.; Wang, W.X. Chemical constituents from a soil fungus *Xylaria* sp. Y01 and their anticancer potential. *J. Mol. Struct.* **2025**, *1321*, 140274. [[CrossRef](#)]
13. Chen, Z.; Chen, Y.; Huang, H.; Yang, H.; Zhang, W.; Sun, Y.; Wen, J. Cytochalasin P1, a new cytochalasin from the marine-derived fungus *Xylaria* sp. SOF11. *Z. Naturforsch. C* **2017**, *72*, 129–132. [[CrossRef](#)] [[PubMed](#)]
14. Wang, W.X.; Feng, T.; Li, Z.H.; Li, J.; Ai, H.L.; Liu, J.K. Cytochalasins D1 and C1, unique cytochalasins from endophytic fungus *Xylaria* cf. *curta*. *Tetrahedron Lett.* **2019**, *60*, 150952. [[CrossRef](#)]
15. Espada, A.; Rivera-Sagredo, A.; de la Fuente, J.M.; Hueso-Rodríguez, J.A.; Elson, S.W. New cytochalasins from the fungus *Xylaria hypoxylon*. *Tetrahedron* **1997**, *53*, 6485–6492. [[CrossRef](#)]
16. Minato, H.; Katayama, T.; Matsumoto, M.; Katagiri, K.; Matsuura, S.; Sunagawa, N.; Hori, K.; Harada, M.; Takeuchi, M. Structure-activity relationships among zygosporin derivatives. *Chem. Pharm. Bull.* **1973**, *21*, 2268–2277. [[CrossRef](#)] [[PubMed](#)]
17. Aldridge, D.C.; Turner, W.B. Structures of cytochalasins C and D. *Chem. Soc.* **1967**, *4*, 923–928. [[CrossRef](#)]
18. Ai, W.J.; Li, J.; Cao, D.; Liu, S.; Yuan, Y.Y.; Li, Y.; Tan, G.S.; Xu, K.P.; Yu, X.; Kang, F.; et al. A Very deep graph convolutional network for ¹³C NMR chemical shift calculations with density functional theory level performance for structure assignment. *J. Nat. Prod.* **2024**, *87*, 743–752. [[CrossRef](#)] [[PubMed](#)]
19. Rokni, M.; Khomeijani-Farahani, M.; Soltani, T.; Jamshidi, A.; Mahmoudi, M.; Farhadi, E. Understanding the pleiotropic effects of CXCL10/IP-10 in the immunopathogenesis of inflammatory rheumatic diseases: Implications for better understanding disease mechanisms. *Int. Immunopharmacol.* **2025**, *153*, 114456. [[CrossRef](#)] [[PubMed](#)]
20. Miura, M.; Kitaura, H.; Ohori, F.; Narita, K.; Ren, J.; Noguchi, T.; Marahleh, A.; Ma, J.; Lin, A.; Fan, Z.; et al. Role of CXCL10 released from osteocytes in response to TNF- α stimulation on osteoclasts. *Sci. Rep.* **2025**, *15*, 3040. [[CrossRef](#)] [[PubMed](#)]
21. Sobah, M.L.; Liongue, C.; Ward, A.C. SOCS proteins in immunity, inflammatory diseases, and immune-related cancer. *Front. Med.* **2021**, *8*, 727987. [[CrossRef](#)] [[PubMed](#)]
22. Pracht, P.; Bohle, F.; Grimme, S. Automated exploration of the low-energy chemical space with fast quantum chemical methods. *Phys. Chem. Chem. Phys.* **2020**, *22*, 7169–7192. [[CrossRef](#)] [[PubMed](#)]
23. Grimme, S.; Bannwarth, C.; Shushkov, P. A robust and accurate tight-binding quantum chemical method for structures, vibrational frequencies, and noncovalent interactions of large molecular systems parametrized for all spd-block elements (Z = 1–86). *J. Chem. Theory Comput.* **2017**, *13*, 1989–2009. [[CrossRef](#)] [[PubMed](#)]
24. Bannwarth, C.; Ehlert, S.; Grimme, S. GFN2-xTB—an accurate and broadly parametrized self-consistent tight-binding quantum chemical method with multipole electrostatics and density-dependent dispersion contributions. *J. Chem. Theory. Comput.* **2019**, *15*, 1652–1671. [[CrossRef](#)] [[PubMed](#)]
25. Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G. SpecDis: Quantifying the comparison of calculated and experimental electronic circular dichroism spectra. *Chirality* **2013**, *25*, 243–249. [[CrossRef](#)] [[PubMed](#)]
26. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Petersson, G.A.; Nakatsuji, H.; et al. *Gaussian*, version 16, revision B.01; Gaussian, Inc.: Wallingford, CT, USA, 2016.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.