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Acatulides A-G, neuroprotective macrolides from *Acaulium album* H-JQSF

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ABSTRACT

Fungal symbionts co-evolve with hosts and microbial co-inhabitants to acquire an unpredictable potential for producing novel bioactive metabolites, but the knowledge about the topic remains patchy and superficial. Here we present the chemical characterization of acatulides A–G (**1**–**7**) as architecturally unprecedented macrolides from the solid-state culture of *Acaulium album* H-JQSF, an arthropod-associated fungus. The acatulide structures were elucidated by spectroscopic analysis, modified Mosher's method and single-crystal X-ray diffraction. The plausible biosynthetic pathways for compounds **1**–**4** are proposed. Interestingly, acatulides B–D (**2**–**4**) and G (**7**) were demonstrated to be neuroprotective against the 1-methyl-4-phenylpyridinium (MPP^+)-induced damage to SH-SY5Y cells and nematode *Caenorhabditis elegans* (*C. elegans*).

Arthropods belong to the invertebrate animal, but usually have a distinct exoskeleton, segmented body and jointed appendage. They distribute widely in nature and are evidenced to be the most numerous in the animal kingdom [1]. Some arthropod species such as *Malaphis chinensis* and *Bombyx mori* have long been used as traditional Chinese medicines [2]. On the other hand, arthropods are generous hosts for microbial symbiont communities including fungi that have been a rich source of chemically fascinating and biologically potent secondary metabolites [3,4]. However, only a small fraction of arthropod-derived fungi has been investigated chemically and biologically [5].

In continuation of our efforts to characterize skeletally unprecedented bioactives from fungi [6,7], *Acaulium* sp. H-JQSF (re-identified herein as *Acaulium album* H-JQSF) was isolated from the isopod *Armadillidium vulgare* that has been used since ancient times as an insect-based folk medicine for treating chronic bronchitis, amenorrhea and malaria [8]. The *A. album* strain is so active in secondary metabolism that arrays of architecturally undescribed macrolides were characterized from its culture [9–11]. However, during our campaign of identifying the *A. album* metabolites, some minor metabolites failed to be isolated simply because their abundance was closely around the high performance liquid chromatography (HPLC) detection limit. In order to characterize such low-abundance metabolites, the fungus was regrown on the rice solid-state medium on a larger scale. Here, we present the characterization of another collection of macrolides with intriguing molecular frameworks, named acatulides A–G (**1**–**7**, Fig. 1), of which acatulides B–D (**2**–**4**) and G (**7**) were demonstrated to protect the SH-SY5Y cells and the nematode [*Caenorhabditis elegans* (*C. elegans*)] from the 1-methyl-4-phenylpyridinium (MPP^+) injury.

Acatulide A (**1**) was evidenced to have a molecular formula of $C_{21}H_{26}O_9$ with 9 degrees of unsaturation from the Na^+-liganded molecular ion at *m/z* 445.1458 (calcd for $C_{21}H_{26}O_9Na$, 445.1469) in its high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra of **1** (Table S1 and Figs. S2–S8 in Supporting information) displayed the resonances due to three carbon-carbon double bonds as well as three methyls, a methoxy, three methylenes, four methines (3 oxygenated), and three ester or carboxylic acid groups. Furthermore, **1** was shown to be tricyclic by its molecular formula and two dimensional (2D) NMR experiments [$^1H-^1H$ correlation spectroscopy ($^1H-^1H$ COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple-bond correlation spectroscopy (HMBC) and (nuclear overhauser enhance-

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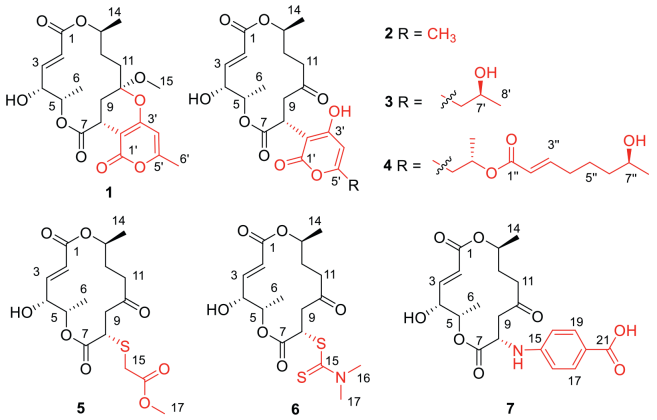


Fig. 1. Structures of acatulides A–G (**1**–**7**) from *A. album* H-JQSF.

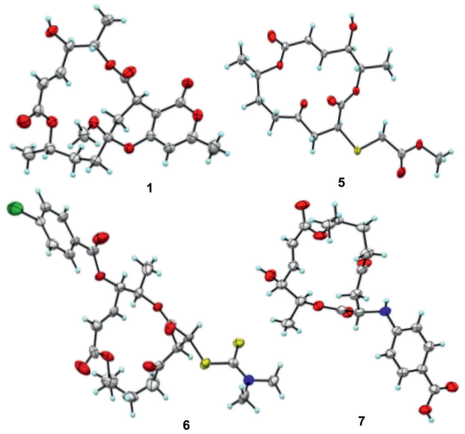


Fig. 3. The X-ray structures of compounds **1**, **5**, **6** and **7**. Displacement ellipsoids are drawn at the 50% probability level.

ment spectroscopy (NOESY)]. The $^{1}H–^{1}H$ COSY spectrum of this substance shows H-2/H-3/H-4/H-5/H_3-6 and H-8/H-9 and H-11/H-12/H-13/H_3-14 correlations. The HMBC spectrum shows H-3/C-1, H-5/C-7, H-9/C-7, H-12/C-10, and H-13/C-1 correlations, allowing us to elucidate the existence of a 14-membered macrolide. The 14-membered macrolide motif in the molecule of **1** was indicated by comparing its ^{1}H and ^{13}C NMR data with those of acaulones A and B, acaudiol A and acaulide [9]. However, as illustrated in Fig. 2, **1** was shown to possess a 3,4-disubstituted 6-methyl-2-pyrone motif as evidenced from the HMBC correlation of H-4′ with C-2′ (δ_C 96.5), C-3′ (δ_C 162.9), C-5′ (δ_C 161.6) and C-6′ (δ_C 20.0). The 6-methyl-2-pyrone and 14-membered macrolide motifs were pieced together through the $C_8–C_{2'}$ bond and the $C_{10}–O–C_{3'}$ linkage according to the degrees of unsaturation and HMBC correlations of H-8 with C-1′ and C-3′ as well as of C-10 (δ_C 102.8) with H-15.

To understand its stereochemistry, the NOESY spectrum of **1** was acquired and scrutinized to identify the correlations of the anticipated proton pairs (H-4 *versus* H-8 and H-5 *versus* H-8; Fig. S1 in Supporting information). The NOESY spectrum revealed that H-4, H-5 and H-8 were co-facial. However, the C-4, C-5, C-8, C-10 and C-13 configuration failed to be determined by the NOESY experiment due to the conformation flexibility of the macrodilactone moiety. To clarify the ambiguity, we tried and succeeded in obtaining its single crystal. Subsequent crystallography of **1** through Cu Kα ($\lambda = 1.54178$ Å) radiation established its (4*R*,5*S*,8*R*,10*R*,13*S*)-configuration (Fig. 3).

Acatulide B (**2**) was determined to have a molecular formula of $C_{20}H_{24}O_9$, 14 Da less than that of **1** (*vide supra*), according to its protonated molecular ion at *m/z* 409.1497 in its HR-ESI-MS ($C_{20}H_{25}O_9$ requires 409.1493). The structure of **2** was suggested to be similar to that of **1** by comparing both sets of ^{1}H and ^{13}C NMR spectra (Table S1). However, the methoxy resonances (δ_H 2.91 and δ_C 48.9) in the spectra of **1** disappeared in those of **2**, and the ketal carbon signal of **1** ($\delta_{C\text{-}10}$ 102.8) was replaced by a ketone resonance line at δ_C 207.0 in **2**. All ^{1}H and ^{13}C NMR spectral data of **2** were assigned by the 2D NMR experiments (Figs. S9–S15 in Supporting information), suggesting that a pyrone motif was present in the molecule of **2**. The proposal was confirmed by the HMBC correlation of H-4′ with C-2′ (δ_C 98.5), C-3′ (δ_C 166.3) and C-5′ (δ_C 161.5), and of H-8 with C-1′ (δ_C 163.5) and C-3′ (δ_C 166.3). Both **1** and **2** were produced in the same culture of the fungus, and thus their high similarity in the NMR spectral data suggested that **2** shared most likely the same configuration with **1**.

The HR-ESI-MS spectrum of acatulide C (**3**) displayed a Na^+-liganded molecular ion at *m/z* 475.1586, indicative of its molecular formula $C_{22}H_{28}O_{10}$ (calcd. for $C_{22}H_{28}O_{10}Na$, 475.1575), which possessed an extra 44 Da in comparison to that of **2**. The ^{1}H and ^{13}C NMR spectra of **3** suggested that it was an analog of **2**. This was confirmed by its 2D NMR experiments ($^{1}H–^{1}H$ COSY, HMBC and NOESY), which allowed the exact assignment of all NMR signals (Fig. 2 and Figs. S16–S22 in Supporting information). In particular, the coupling sequence from H-6′ through H_3-8′ disclosed through the $^{1}H–^{1}H$ COSY spectrum indicated that the methyl group on C-5′ of 2 was replaced by a 2-hydroxypropanyl residue in that of **3** (Fig. 2). The configuration of **3** was deduced to be identical to that of **2** from their close similarity in the NMR spectra (Table S1),

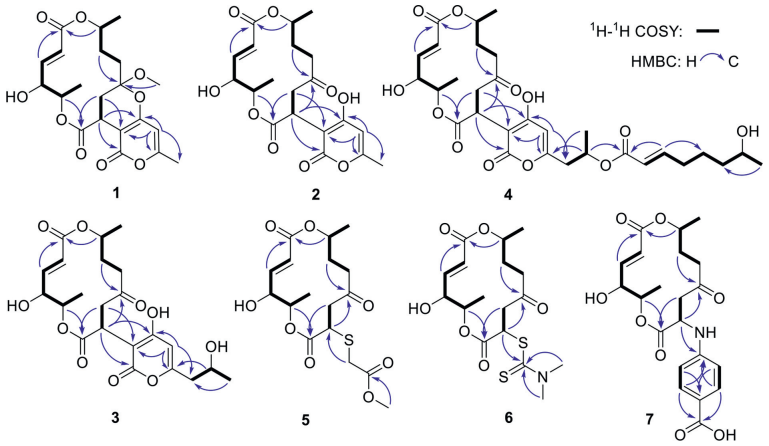


Fig. 2. The key 2D NMR ($^{1}H–^{1}H$ COSY and HMBC) correlation of acatulides A–G (**1**–**7**).

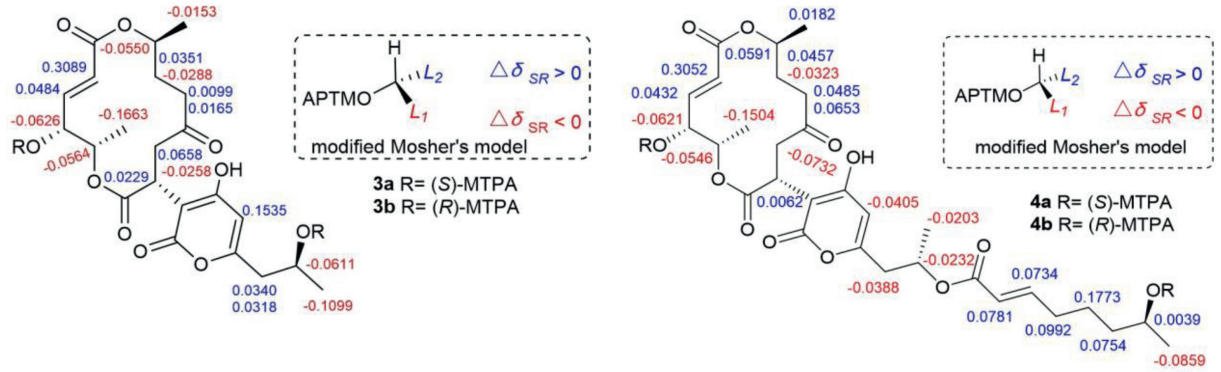


Fig. 4. Absolute configuration of compounds **3** and **4** *via* Mosher's method. Values of $\Delta\delta_{SR}$ [$\Delta(\delta_S - \delta_R)$] are given in ppm. Positive and negative regions are colored in blue and red, respectively.

except for C-7′ which was determined to have an *S*-configuration by the modified Mosher's method (Fig. 4 and Figs. S23–S26 in Supporting information) [12].

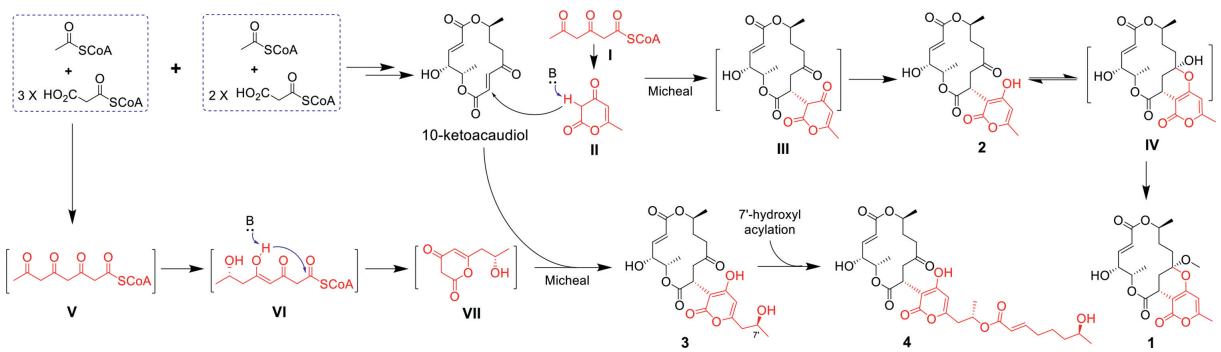
Acatulide D (**4**) was found to have a molecular formula $C_{30}H_{40}O_{12}$ with 11 degrees of unsaturation according to its HR-ESI-MS (*m/z* 615.2984 $[M+Na]^+$, calcd. for 615.2993). Comparison of the ^1H and ^{13}C NMR spectral data of **4** and **3** (Table S1) revealed that **4** might form from the 7-hydroxyoct-2-enoylation of 7′–hydroxy group of **3**. This assumption was confirmed by its 2D NMR spectra ($^1H-^1H$ COSY, HMBC and NOESY), which facilitated unequivocal assignments of all ^1H and ^{13}C NMR signals (Table S1, Fig. 2, Figs. S1 and S27–S33 in Supporting information). The configuration of **4** was deduced to be identical to that of **3** from their close similarity in the NMR spectra (Table S1), except for C-7′′ whose *S* configuration was established by the modified Mosher's method (Fig. 4 and Figs. S34–S37 in Supporting information) [12].

Acatulide E (**5**) was obtained as colorless prisms. Its molecular formula $C_{17}H_{24}O_8S$ was deduced by its HR-ESI-MS spectrum ($[M+Na]^+$ at *m/z* 411.1075, calcd. for $C_{17}H_{24}O_8SNa$, 411.1084). The 1D and 2D NMR spectra of **5** indicated the coexistence of 14-membered macrodiolide and methyl-2-mercaptoacetate substructures (Figs. S38–S44 in Supporting information). The methyl-2-mercaptoacetate and 14-membered macrolide motifs were pieced together through the C_8–S–C_{15} linkage according to the HMBC correlations of H-15 (δ_H 3.94) with C-8 (δ_C 40.2) (Fig. 2). The (4*R*,5*S*,8*S*,13*S*)-configuration of **5** was clarified by its single-crystal X-ray diffraction (Fig. 3) with a Flack parameter of 0.06 (2).

Acatulide F (**6**) was found to have a molecular formula $C_{18}H_{26}NO_6S_2$ with 5 degrees of unsaturation according to its HR-ESI-MS (*m/z* 426.1022 $[M+Na]^+$, calcd for 426.1016). Comparison of the ^1H and ^{13}C NMR spectral data of **5** and **6** (Table S2 and Figs. S45–S51 in Supporting information) revealed the existence of 14-membered macrodiolide substructure. Compound **6** exhibits a dimethylcarbamodithioic acid motif as evidenced from the HMBC correlation of H_3-16 with C-15 (δ_C 194.9) and H_3-17 with C-15 (δ_C 194.9). The dimethylcarbamodithioic acid and 14-membered macrolide motifs were pieced together through the C_8–S–C_{15} linkage according to the HMBC correlations of H-8 (δ_H 5.23) with C-15 (Fig. 2). To clarify its stereochemistry, we established anomalous dispersion effects in X-ray diffraction measurements on the crystal of *p*-bromobenzoyl ester of 4–hydroxy group, and succeeded in obtaining its single crystal. Subsequent crystallography of **6** through single-crystal X-ray diffraction established its (4*R*,5*S*,8*S*,13*S*)-configuration (Fig. 3).

Acatulide G (**7**) was obtained as colorless orthorhombic crystals with a molecular formula of $C_{21}H_{25}NO_8$ as inferred from HR-ESI-MS ion at *m/z* 442.1477 $[M+Na]^+$ (calcd. for 442.1472), suggesting 9 degrees of unsaturation. The 1D and 2D NMR spectra of **7** indicated the coexistence of 14-membered macrodiolide and 4-aminobenzoic acid substructures (Figs. S52–S58). The $^1H-^1H$ COSY spectrum of **7** established correlations of H-16/H-17 and H-19/H-20. The HMBC spectra show correlations between H-17/C-21, H-19/C-21, H-16/C-18, and H-20/C-18, and give the existence of a 4-aminobenzoic acid. The 14-membered macrolide and 4-aminobenzoic acid motifs were pieced together through the C_8–N–C_{15} linkage according to the HMBC correlations of H-8 (δ_H 4.53) with C-15 (δ_C 152.7) (Fig. 2). The single crystal X-ray diffraction (Cu Kα) of **7** confirmed the proposed structure and revealed its (4*R*,5*S*,8*S*,13*S*)-configuration (Fig. 3).

The conserved structural feature of acatulides A–D (**1**–**4**) encouraged us to propose their biosynthetic pathway (Scheme 1). Extending from our previous chemical attention to the fungal strain [9–11], 10-ketoacaudiol is likely the common precursor of **1**–**4**. Acatulide B (**2**) might be formed from a Michael addition of 10-ketoacaudiol to the triketide 6-methyl-pyran-2,4–dione (**II**). Intramolecular hemiketalization of **2** might give intermedi-



Scheme 1. Putative biosynthetic pathway of **1**–**4**.

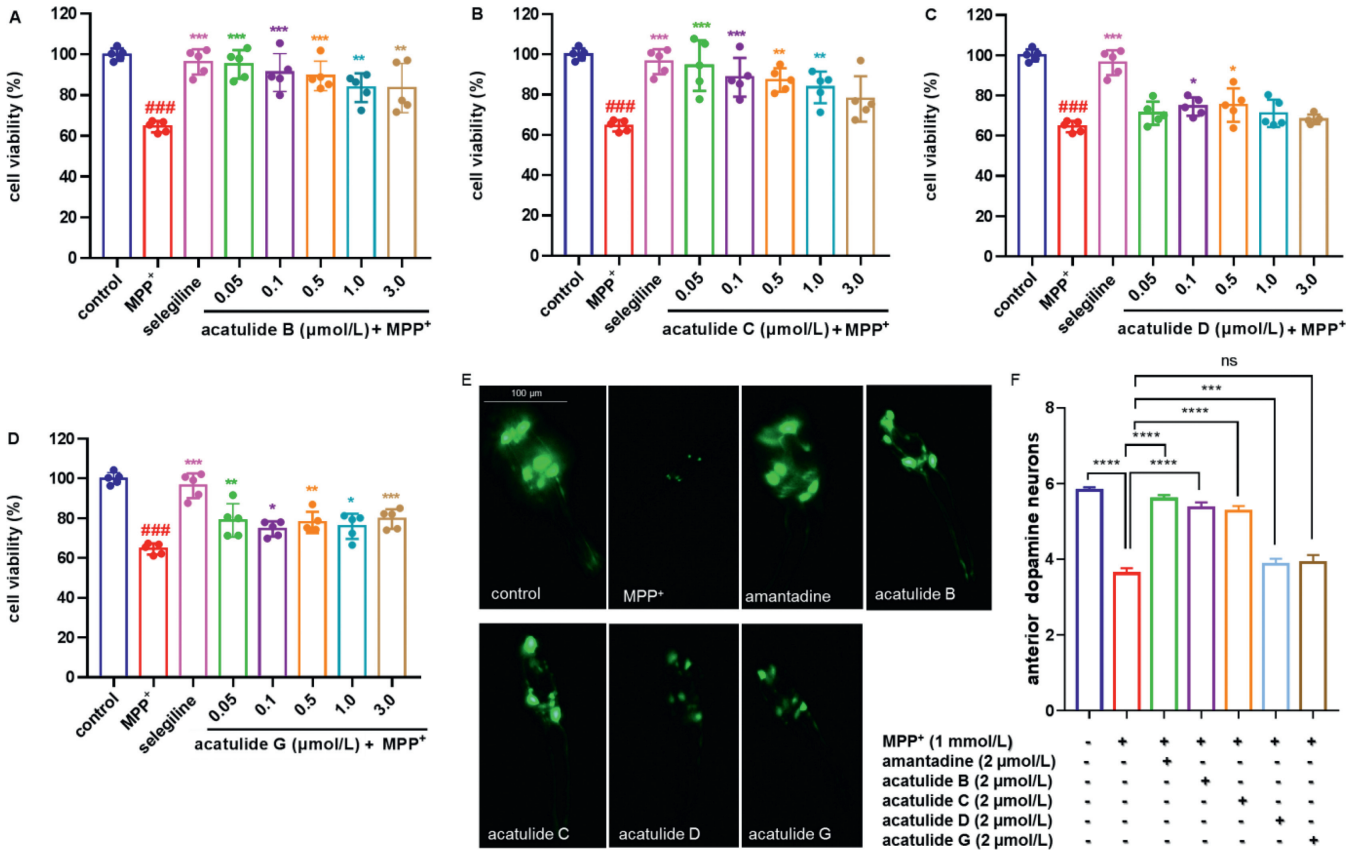


Fig. 5. Neuroprotective effects of **2**–**4** and **7** in the SH-SY5Y cells and *C. elegans* model. (A–D) Protection of SH-SY5Y cells from the MPP^+ injury by acatulides B–D (**2**–**4**; A–C) and acatulide G (**7**; D)/selegiline (50 nmol/L, positive control). (E) Representative immunofluorescence images showing the anterior dopamine neurons of the transgenic strain BZ555 of *C. elegans*. The efficacy was expressed in terms of the brightness of green-fluorescent protein (GFP) tagged to the dopamine neuronal soma, and indicated by the difference of MPP^+- and acautalide/amantadine (positive control)-co-exposed worms from the intact (blank reference) and MPP^+-treated (negative control) animals ($n = 30$–60). Scale bar: 100 μm. (F) Counts of anterior dopamine neurons of bz555 *C. elegans*. Data are the mean ± standard error of the mean (SEM) from three or more independent experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ *vs.* the MPP^+ group, $^{\#\#\#}P < 0.001$ *vs.* the control group are analyzed with one-way-analysis of variance (ANOVA).

ate (**IV**) which tends to give acatulide A (**1**) *via* its ketalization reaction with methanol. Likewise, acatulide C (**3**) could be generated from a Michal addition of 10-ketoacaudiol to the tetraketide 6-(2-hydroxypropyl)-pyran-2,4–dione (**VII**). The 7-hydroxyoct-2-enoylation of 7′–hydroxy group of **3** should yield acatulide D (**4**).

Some oxygenated macrolides have been demonstrated to exhibit neuroprotective activity [11,13,14]. Therefore, **1**–**7** were individually evaluated in the SH-SY5Y cells for the protective action against the neuroinjury by MPP^+, a mitochondrial complex I inhibitor used reliably to induce neuronal cell death in the acquired Parkinson's disease (PD) model [15]. At 50 nmol/L, compounds **2**–**4** and **7** improved the cell viability to 95.14%, 94.39%, 71.23% and 78.94%, respectively, in comparison to the MPP^+ (500 μmol/L) caused cell viability reduction to 64.58% of the untreated counterpart (Figs. 5A–D). In addition, the activity of **2**–**4** decreased with higher concentrations, which may be related to their cytotoxic activity. However, compounds **1**, **5** and **6** showed no neuroprotective activity, which may also be related to their strong cytotoxic activity. Furthermore, **2**–**4** and **7** were also evaluated in the *C. elegans* model for the protective action against the neuroinjury by MPP^+. At 2 μmol/L, **2**–**4** and **7** were found to counteract the injury to the worm neurons by MPP^+ at 1 mmol/L. In particular, **2** and **3** have the potential to more significantly restore the MPP^+-damaged dopamine neurons (Figs. 5E and F), indicating their potential as precursor molecules or sources of inspiration for the antiparkinsonian drug discovery.

Fungal symbionts have been regarded as an important source of bioactive secondary metabolites with potential application in biomedicine and agriculture [16,17]. Arthropod species distribute widely in nature [18] and thus provide a large variety of special and complex niches for fungi to reside and evolve [19]. Therefore, many metabolites produced by the arthropod-associated fungi are structurally intriguing and biologically promising [11,20]. This observation gains an additional confirmation from the present characterization of acatulides A–G (**1**–**7**). The construction of complex molecular architecture in a live cell requires the catalysis of a suite of enzymes, but some biosynthetic steps are spontaneous or non-enzymatic. The structural feature of **1**–**7** as well as other polyketide frameworks generated by the fungus [9–11], suggested that 10-ketoacaudiol is prone to undergo, as rationalized herein, the Michael addition reaction with diverse nucleophiles such as 6-methyl-pyran-2,4–dione, 6-(2-hydroxypropyl)-pyran-2,4–dione, methyl-2-mercaptoacetate, dimethylcarbamodithioic acid and 4-aminobenzoic acid. The finding may be of value for the diversity-oriented synthesis of new chemical entities from the starting materials with the 4-oxopent-2-enoate motif as possessed by 10-ketoacaudiol. Inspired by the Michael addition-based detoxication mechanism in mammals [21], the fungal polyketides with one or more 4-oxopent-2-enoate motifs (this work and Refs. [9–11]) might play roles in chemical defense, which, however, could only be investigated in a suitably mimicked multivariate ecological system.

In conclusion, we have characterized acatulides A–G (**1**–**7**) as skeletally unprecedented macrolides from the solid-state culture of *A. album* H-JQSF. The biosynthetic landscape was rationalized to suggest 10-ketoacaudiol as the common precursor of **1**–**7**,

which tends to undergo its Michael addition reaction with tri- and tetraketides, with or without further acylation at the side chain. The acatulides B–D (**2**–**4**) and G (**7**) protects the SH-SY5Y cells and nematode *C. elegans* from the MPP^+-induced neural injuries. This work deepens the chemical understanding of the arthropod-associated fungi and suggests the potential application of the novel secondary metabolites produced by the fungal symbionts.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.108488.

References

[1] S. Pfeffer, H. Wolf, Anim. Cogn. 23 (2020) 1041–1049.
[2] G.M. Cragg, D.J. Newman, Biochim. Biophys. Acta 1830 (2013) 3670–3695.
[3] Y.H. Shin, J.Y. Beom, B. Chung, et al., Org. Lett. 21 (2019) 1804–1808.
[4] S. Lin, H. Yu, B. Yang, et al., J. Nat. Prod. 83 (2020) 169–173.
[5] S.T. Henriques, Y.H. Huang, K.J. Rosengren, et al., J. Biol. Chem. 286 (2011) 24231–24241.
[6] A.H. Zhang, N. Jiang, X.Q. Wang, et al., Sci. Rep. 9 (2019) 14316.
[7] G.Z. Dai, W.B. Han, Y.N. Mei, et al., Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 1174–1180.
[8] R. Lemmens-Gruber, M.R. Kamyar, R. Dornetshuber, Curr. Med. Chem. 16 (2009) 1122–1137.
[9] T.T. Wang, Y.J. Wei, H.M. Ge, et al., Org. Lett. 20 (2018) 1007–1010.
[10] T.T. Wang, Y.J. Wei, H.M. Ge, et al., Org. Lett. 20 (2018) 2490–2493.
[11] Z.W. Tong, X.H. Xie, T.T. Wang, et al., Org. Lett. 23 (2021) 5587–5591.
[12] H.R. Zhang, L.T. Xu, X.Q. Liu, et al., Chin. Chem. Lett. 28 (2017) 1460–1464.
[13] B. Zhang, T.J. Kopper, X. Liu, et al., CNS. Neurosci. Ther. 25 (2019) 591–600.
[14] R. Alvariño, E. Alonso, R. Lacret, et al., Mol. Pharm. 16 (2019) 1456–1466.
[15] E.M. Prasad, S.Y. Hung, Antioxidants 9 (2020) 1007.
[16] D.S. Li, M.J. Yang, R.F. Mu, et al., Chin. Chem. Lett. 34 (2023) 107469.
[17] Y.C. Xu, L.P. Wang, G.L. Zhu, et al., Chin. Chem. Lett. 30 (2019) 431–434.
[18] W. Jesse, J. Molleman, O. Franken, et al., Glob. Chang. Biol. 26 (2020) 3294–3306.
[19] M.G. Chevrette, C.M. Carlson, H.E. Ortega, et al., Nat. Commun. 10 (2019) 516–527.
[20] W.B. Han, G.Y. Wang, J.J. Tang, et al., Org. Lett. 22 (2020) 405–409.
[21] K. Berhane, M. Widersten, A. Engström, et al., Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 1480–1484.