New antimicrobial compounds produced by *Seltsamia galinsogisoli* sp. nov., isolated from *Galinsoga parviflora* as potential inhibitors of FtsZ

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A total amount of 116 fungal strains, belonging to 30 genera, were acquired from the rhizosphere soil and plant of *Galinsoga parviflora*. A strain SYPF 7336, isolated from the rhizospheric soil, was identified as *Seltsamia galinsogisoli* sp. nov., by morphological and molecular analyses, which displayed high antibacterial activity. In order to study the secondary metabolites of *Seltsamia galinsogisoli* sp. nov., nine compounds were successfully separated from the strain fermentation broth, including two new compounds and seven known compounds. Their structures were elucidated based on spectral analysis including 1D and 2D NMR. All the separated compounds were evaluated for their antimicrobial activities. Compounds 2, 5 and 1 displayed antimicrobial activities against *Staphylococcus aureus* with MIC values of 25, 32 and 75 μg/mL, respectively. Moreover, morphological observation showed the coccoid cells of *S. aureus* to be swollen to a volume of 1.4 to 1.7-fold after treatment with compounds 1, 2 and 5, respectively. Molecular docking was carried out to investigate interactions of filamentous temperature-sensitive protein Z (FtsZ) with compounds 1, 2 and 5.

Secondary metabolites coming from microorganism represent a large number of diverse components which have been treated as potential candidates for drugs\(^1\text{-}^5\). Currently, due to the increase of antibiotic resistance, there is an urgent need for novel classes of lead compounds and novel mechanisms to confront the antibiotic crises\(^6\text{-}^7\).

Microbial filamentous temperature-sensitive protein Z (FtsZ) is a novel target for drug discovery, which plays a key role in cell division\(^8\text{-}^9\). The inhibitors of FtsZ prevent the cellular fission of bacteria, which lead to apoptosis of bacteria\(^10\text{-}^12\). Therefore, morphological observation of microbial fission and molecular docking between the lead compound and FtsZ were accomplished to explore the possible mechanism\(^13\text{-}^14\). Up to now, the discovered FtsZ inhibitors are divided into natural products (sanguinarine, berberine, totarol, curcumin and cinnamaldehyde) and synthetic small molecules (PC190723, UCM53, CCR-11)\(^15\text{-}^19\). At present, PC190723 has been the candidate drugs to enter the clinical trials, which inspires scientists to make more effort to find potential FtsZ inhibitors as antibacterial agents\(^20\text{-}^22\).

The endophytic fungi are known as a source of abundant secondary metabolites for functional bioactive substances. As we know, only a small amount of microbes have been studied so far, and a vast number of new taxa waiting for discovery, especially those separated from medicinal plants. This leads us to isolate and evaluate the potential pharmacological activity of bioactive compounds produced by endophytic fungi.

In the present study, the endophytic fungi diversity of the traditional Chinese herb, *Galinsoga parviflora* was surveyed and 116 fungal strains were isolated from the whole plant (stems, leaves and roots) which attributed to 30 genera. Forty-three percent of the strains revealed antimicrobial abilities against at least one kind of human pathogenic microorganisms. Strain SYPF 7336, presented the strongest antibacterial activity, could not be affiliated to any known taxon, so it was identified as a novel species of genus *Seltsamia* by phylogenetic analyses, given the name *Seltsamia galinsogisoli*.\(^23\text{-}^24\)
Seltsamia galinsogisoli sp. nov. The genus Seltsamia, (Cucurbitariaceae) was first proposed in 2018, and the only strain Seltsamia ulmi CBS 143002 was isolated on corticated Ulmus glabra in Norway23. Seltsamia ulmi produces pyriform, black ascomata and cylindrical asci. Asci contain 8 uni- to partly biseriately arranged ascospores and ascospores are fusoid to subclavate with 3 main septa. Due to none of the secondary metabolites of the genus Seltsamia having been recorded, the compounds and bioactivities of the novel species, strain SYPF 7336, were explored in the present study. Further, the molecular docking analysis was carried out for mechanism investigation.

Results
Species delineation and classification. A total of 116 fungal strains were acquired from the soil and plant of Galinsoga parviflora, which were divided into 30 groups according to morphological characteristics and phylogenetic analyses. Twelve genera, Acremonium, Beauveria, Trichoderma, Periconia, Gibberella, Discos, Scolcosbasidium, Sporoblomexes, Pyrenochaeta, Peyronellaea, Chaetopyrena and Seltsamia, were found only in the soil. Twelve genera, Sarocladium, Talaromyces, Botryotinia, Cadophora, Phomopsis, Volutella, Epicoccum, Mucor, Plectosphaerella, Cylindrocarpon, Clonostachys, and Phialophora just survived in the plant. Six genera, Cladosporium, Paraphoma, Penicillium, Alternaria, Fusarium, and Phoma could simultaneously survive in the soil and plant of G. parviflora (Fig. 1).

A strain SYPF 7336, isolated from the rhizosphere soil of G. parviflora, was most close to Seltsamia ulmi when using ITS sequence BLAST, but they did not match well in 13 different positions. So, the phylogenetic analyses were carried out based on two loci (ITS and LSU) to clarify the taxon status of strain SYPF 7336. In the MP analysis, 1938 characters were constant, 87 were parsimony-uninformative, and 152 were parsimony-informative. After phylogenetic analysis, the best MP tree was shown in Fig. 2 (TL = 576, CI = 0.575, RI = 0.630, RC = 0.362, HI = 0.425). In this tree, strain SYPF 7336 was placed in genus Seltsamia and formed a sister clade together with S. ulmi. The ITS and LSU sequences were deposited in GenBank with accession numbers KU759584 and KU759581, respectively (Table 1).

Description of Seltsamia galinsogisoli Tianyuan Zhang & Yixuan Zhang, sp. nov. MB 820393. Seltsamia galinsogisoli (Ga.lin.so.gi'so'li. N.L. gen. n. galinsogisoli of soil of a G. parviflora, got from Huludao city, Liaoning Province, northeast of China).

Vegetative hyphae hyaline, smooth walled. Pycnidia subglobose to globose, brown to dark brown, 70–125 × 45–96 μm. Surface roughened by colourless hyphal appendages (Fig. 3a,b). Conidiogenous cells phallicid, hyaline, smooth walled, 9–19.2 × 1.4–4.2 μm (Fig. 3c–e). Conidia 1-celled, hyaline, smooth, cylindrical, slightly curved, 2.5–4.3 × 0.8–1.2 μm (Fig. 3f).

Colonies on PDA attaining 36.6 mm diameter after 11 d at 26 °C, surface floccose, grey to dark grey, reverse grey to dark grey (Fig. 4a,b). Colonies on PNA attaining 25.9 mm diameter after 11 d at 26 °C, surface floccose, grey, reverse grey (Fig. 4c,d). Colonies on CMA attaining 30.2 mm diameter after 11 d at 26 °C, surface floccose, dark grey to dark green, reverse dark green to black (Fig. 4e,f). Colonies on MEA attaining 33.5 mm diameter after 11 d at 26 °C, surface velvety, grey with 5–8 radial and 1 annular groove, reverse grey with 5–8 radial and 1 annular cracks (Fig. 4g,h). Colonies on OA attaining 29.8 mm diameter after 11 d at 26 °C, surface floccose, grey to dark olive green, reverse dark olive green (Fig. 4i,j).

Type specimen. China, Liaoning province, Huludao city, 40°82′26.5″N, 119°78′52.0″E, Sep 2014, from the rhizosphere of G. parviflora. Ex-type culture CBS 140956 = CGMCC 3.17981 = SYPF 7336.

Identification of the compounds. Seltsamiayu (1) (Fig. 5) was isolated as white flakes. The absorption bands at 3448 (strong wide wave), 1636.6 cm⁻¹ in the IR spectrum suggested the presence of hydroxyl carboxylic.
Figure 2. Maximum Parsimony (MP) tree based on analysis of a combined dataset of ITS and LSU sequence data. MP bootstrap support values (MPB above 50%) and Bayesian posterior probabilities (BPP; above 70%) are given at the nodes (MPB/BPP). *Drechmeria panacis* sp. nov. is denoted by bold letters.

| Species                          | Culture accession No. | GenBank accession No. |
|---------------------------------|-----------------------|-----------------------|
| *Allocucurbitaria botulispora*   | CBS 142452^T          | LT592932 LN907416    |
| *Astragalicola amorpha*          | CBS 142999^T          | MF795753 MF795753    |
| *Cucitella opalis*               | CBS 142405^T          | MF795754 MF795754    |
| *Cucurbitaria berberidis*        | CBS 130806^T          | LT717673 KC506793    |
| *Cucurbitaria oromediterranea*   | CBS 142399^T          | MF795761 MF795761    |
| *Neocucurbitaria acerina*        | CBS 142403            | MF795768 MF795768    |
| *Neocucurbitaria aquatica*       | CBS 297.74^T          | LT623221 EU754177    |
| *Neocucurbitaria cava*           | CBS 257.68^T          | JF740260 EU754199    |
| *Neocucurbitaria cisticola*      | CBS 142402^T          | MF795772 MF795772    |
| *Neocucurbitaria hakeae*         | CPC 28920^T           | KY173436 KY173526    |
| *Neocucurbitaria irregularis*    | CBS 142791^T          | LT592916 LN907372    |
| *Neocucurbitaria juglandicola*   | CBS 142390^T          | MF795773 MF795773    |
| *Neocucurbitaria keratinophila*  | CBS 121759^T          | EU885415 LT623215    |
| *Neocucurbitaria populi*         | CBS 142393^T          | MF795774 MF795774    |
| *Neocucurbitaria unguis-hominis* | CBS 111112            | LT623222 GQ387623    |
| *Neocucurbitaria vachelliae*     | CBS 142397^T          | MF795787 MF795787    |
| *Paracucurbitaria corni*         | CBS 248.79            | LT963672 GQ387608    |
| *Paracucurbitaria italicca*      | CBS 234.92^T          | LT623219 EU754176    |
| *Parafenestella mackenziei*      | MFLUCC 16-145^T       | KY563071 KY563074    |
| *Parafenestella ostyaee*         | MFLUCC 17-0097^T      | KY563072 KY563075    |
| *Parafenestella pseudoplatsani*  | CBS 142392^T          | MF795788 MF795788    |
| *Protofenestella ulmi*           | CBS 143000^T          | MF795791 MF795791    |
| *Pseudopyrenochaeta lycopersici* | CBS 306.65^T          | NR_103581 EU754205   |
| *Pseudopyrenochaeta terrestris*  | CBS 282.72^T          | LT623228 LT6232216   |
| *Pyrenochaeta nobilis*           | CBS 407.76^T          | EU930011 EU754206    |
| *Seltsamia galinsogisi*          | CBS 140956^T          | KU759584 KU759581    |
| *Seltsamia ulmi*                 | CBS 143060^T          | MF795794 MF795794    |

Table 1. Strains used in the phylogenetic analyses and their corresponding GenBank accession numbers. ^\text{a}\text{ New accession numbers produced in this study are bold.}
acid carbonyl, and carbonyl functionalities (Figs S1–S6). The molecular formula of compound 1 was determined as C_{16}H_{14}O_{6} on the basis of its ion [M + Na]^+ at m/z 325.2738 obtained by HRESI/MS. The 1D NMR spectra of compound 1 (Table 2) indicated the presence of two benzene rings [δC 162.2, 159.9, 146.6, 145.9, 140.1, 129.8, 126.7, 115.6, 115.5, 109.5, 105.0, 100.8; δH 7.04 s, 6.90 s, 6.51 d (J = 2.2 Hz), 6.50 d (J = 2.2 Hz)] and one methoxy [δC 55.4, δH 3.8 s]. The spectra of 1 were in part very similar to those of alter lactone except for the absence of a methylene group. The HMBC spectra indicated the presence of long-range correlations (Fig. 6) from H-1′ with C-2, C-3, and C-4, from H-5 with C-4a, C-7 and C-11b, from H-9 with C-7a and C-11, combined with the HSQC and HRESIMS data, the structure of compound 1 was verified as shown in Fig. 5 and it was given the common name Pyrenochaeta yu.

Galinsogisoliyu (2) was separated as a brown powder, [α]_{D}^{20} + 3.7 (c 0.25, MeOH). The absorption bands at 3443 (strong wide wave), 1703 and 1642 cm$^{-1}$ in IR spectrum suggested the presence of hydroxyl carboxylic acid carbonyl, and carbonyl functionalities (Figs S7–S12). The HRESIMS indicated the presence of an ion peak at m/z 347.4509 [M + Na]^+ (calcd. for C_{19}H_{32}O_{4}Na, 347.4504), indicating the molecular formula of C_{19}H_{32}O_{4}. The 1H NMR spectrum of 2 (Table 2) indicated the presence of five methyl groups at δH 0.92 (t, 3H), 0.97 (t, 3H), 0.849 (m, 3H), 0.877 (m, 3H), 1.59 (m, 3H), one methoxyl groups at δH 3.68 (3H, m) [Henrick et al. 1975, Watanabe et al. 2000]. It also showed four methylene protons at δH 4.05 (2H, m, H-8), 2.66 (2H, m, H-9), 1.95 (m, 1H, H-15a) and 1.75 (m, 1H, H-15b), five methine protons at 5.85 (1H, s, H-2), 4.83 (1H, m, H-5), 3.6 (1H, m, H-7), 3.6 (1H, m, H-7), 2.92 (1H, brs, H-16); The 13C NMR spectrum (Table 2) indicated the presence of two carbonyl carbons (δC 174.3 and 193.0), a pair of olefinic carbon (δC 174.3 and 193.0), five methyl carbons (δC 22.2*2, 20.7, 13.4 and 8.2), one oxygenated methylene proton carbon (δC 58.9), three methylene carbons (δC 46.4, 32.4 and 23.5). The 1H and 13C NMR spectra of 2 were similar to those of 4-methyl-5,6-dihydro-2H-pyran-2-one except for the absence of two side chains. The planar structure of 2 (Fig. 6) was established by the 2D NMR data. The 2D NMR spectra of compound 2 (Fig. 6) indicated the presence of long-range correlations from H-2 with C-1, C-3 and C-4, H-5...
with C-1, C-14 and C-15, H-9 with C-11 and C-13 and H-15 with C-5 and C-14. According to the above evidence, the structure of 2 was verified as shown in Fig. 5 and it was given the common name Galinsogisoliyu.

Additionally, the discovery metabolites of Seltsamia galinsogisoli sp.nov. resulted the isolation of seven known compounds (3–9), including 1,3-Benzenediol,5-(2-hydroxypropyl) (3)\(^{27}\), 3,4-Dihydroxy-2-methyl-7-[prop-1-eynyl]-3,4-dihydro-2H-pyra[4,3-b]pyran-5-one (4)\(^{18-30}\), 1H-2-Benzopyrylan-1-one,6,8-dihydroxy-3-(2-hydroxypropyl)
The antimicrobial results of compounds 1–9. All the separated metabolites were tested for antimicrobial effects against five common pathogenic bacteria, \textit{S. aureus}, \textit{B. subtilis}, \textit{P. aeruginosa}, \textit{K. pneumonia} and \textit{Bacillus cereus}. The results were shown in Table 3.

Compounds 2, 5 and 1 showed antimicrobial activities against \textit{S. aureus} with MIC values of 25, 32 and 75 μg/mL, respectively. Compounds 3–4, 6–7 and 8–9 showed weak antimicrobial effects.

Morphological observation and molecular docking. The cells of \textit{S. aureus} treated with compounds were observed carefully (Fig. 7). Interestingly, the coccoid cells of \textit{S. aureus} were swelled to larger volume after treatment with compound 1 (1.4 fold), 2 (1.7 fold) and 5 (1.6 fold), respectively. In order to explain the possible mechanism, FtsZ, key protein of cell division\cite{35}, was explored for molecular docking study.

The docking simulation of active compounds 1, 2 and 5 to \textit{S. aureus} (PDB:ID 3VOB) (Fig. 8) resulted in the binding energies of −109, −125, and −113 kcal/mol, respectively (Table 4). Thus, compound 2 had the best binding energies with FtsZ. Furthermore, the binding patterns were also different. Compound 2 displayed four hydrogen bonds and one more hydrophobic bond to relevant residues comparing with compound 5 (Table 4). Compound 1 showed two hydrogen bonds and five hydrophobic bonds to relevant residues. However, an unfavorable bump LEU261 was observed for compound 1 (Table 4). Five hydrophobic bonds for compound 1 were observed and considered to make major contribution to the combinations. The interactions between FtsZ with compounds 1–2 and 5 are displayed in Table 4.

Discussion

All the endophytic fungi isolated from the rhizosphere of \textit{G. parviflora} were fermented and the crude extracts of each strain were tested for the microbial activities (Table S1). Forty-three percent of the strains showed antimicrobial activities against at least one kind of human pathogenic microorganisms. These results provide references for further study of the strains.

Among the stains, SYPF 7336, showed the best antibacterial activity. The strain SYPF 7336 was carefully studied and identified as \textit{Seltsamia galinsogisoli} sp. nov. by morphology and molecular analyses, and only pycnidia was observed whereas no perfect stage available. This is the first-found of pycnidia in the genus \textit{Seltsamia} for only perfect stage was recorded whereas no asexual information in the publication in \textit{S. ulmi}, which was isolated from \textit{Hapalocyctis bicaudata} on corticated \textit{Ulmus glabra} in Norway in 2018\cite{23}. Though differences of the reproductive body between the two species could not be compared, they are remarkably different species based on the
Figure 7. Electron micrographs of *S. aureus* in the absence (A) or presence of compounds 1 (B), 2 (C) and 5 (D). (A) Untreated control cells of *S. aureus* with average diameter length of 691 nm. (B) Cells of *S. aureus* with average diameter length of 768 nm. (C) Cells of *S. aureus* with average diameter length of 823 nm. (D) Cells of *S. aureus* with average diameter length of 815 nm.

Figure 8. In silico docking simulation of compounds 1, 2, 5 and ligand to FtsZ of *S. aureus*. (A1–D1) H-bond interactions between 1, 2, 5 and ligand to FtsZ of *S. aureus*. (A2–D2) Hydrophobic interaction between 1, 2, 5 and ligand to FtsZ of *S. aureus* in a 3D docking model.
phylogenetic analyses (Fig. 2). Moreover, the difference between the two species are that Seltsamia galinsogisoli sp. nov. produces dark grey colonies with annular and radical grooves on MEA

Another aim of this study is to isolate antimicrobial secondary metabolites secreted by the novel strain, Seltsamia galinsogisoli sp. nov. expands the host range of this genus. Cucurbitaricaceae. Two new compounds (1–2) and seven known compounds (3–9) (Fig. 5) were purified, identified and tested for their antimicrobial abilities against S. aureus, B. subtilis, P. aeruginosa, K. pneumonia, and E. coli. As results, compounds 2, 5 and 1 displayed well antibacterial activities toward S. aureus with MIC values of 25, 32 and 75 μg/mL, respectively. These results from the present work provide further information about the diversity and activities of compounds in the genus Seltsamia.

FtsZ is a pop target for drug discovery in recent years. The gene of FtsZ has the ability of high conservation and presented almost in all bacteria.

Table 4. Binding residues involved in the formation of hydrophobic bonds and hydrogen bonds with compounds 1, 2, 5 and ligand. “—” No bond connected “*” Multiple bond connected.

| Comd. | Docking score (Kcal/mol) | Unfavorable Bump | Conventional hydrogen bond | Hydrophobic bond |
|-------|-------------------------|-----------------|--------------------------|-----------------|
| 1     | −109                   | LEU261          | LEU209, LEU208           | ASN208, THR309, ASN263, GLY196, THR265 |
| 2     | −125                   | —               | ILE228*2, THR309, VAL297 | ASN263*2, THR309, THR265 |
| 5     | −113                   | —               | —                        | THR309, ASN263, LEU209 |
| ligand| −167                   | —               | LEU209, ASN263, THR296, VAL207, GLY196, THR309, VAL203 | ILE228, ILE311, ILE197, LEU200, ASP199, VAL297, MET226 |

Methods

General experimental procedures. Optical rotations were recorded using a P-2000 Digital Polarimeter (JASCO, United Kingdom). IR spectra were measured on an Equinox55 spectrophotometer in KBr discs (Bruker Optik BmbH, Ettlingen, Germany). The 1D- and 2D-NMR spectra were recorded at 600 for 1H and 150 MHz for 13C (Bruker, Rheinstetten, Germany). HR-ESI-MS data were acquired on a Bruker Customer micrOTOF-Q 125 mass spectrometer (MA, Germany). Solvents were purchased from Tianjin Kemiou Chemical Reagent Company (Tianjin, China), MeOH and CH3CN for HPLC analysis were chromatographic grades (Merck, Darmstadt, Germany). Silica gel (200–300 mesh, Qingdao Marine Chemistry Ltd, Qingdao, China) were used for column chromatography.

Sampling, fungal isolation, morphological study. Samples of soil and plant were collected from the field of a traditional Chinese medical herb G. parviflora in Huludao city, Liaoning province, northeast of China (40°82′26.5″N, 119°78′52.0″E). The samples were conducted as described previously.

The colony morphology of the isolate was studied on PDA, pine-needle agar (PNA), corn meal agar (CMA), malt extract agar (MEA) and oatmeal agar (OA) plates and incubated at 26°C. Mycelium structure was observed under an optical microscope Olympus BX33 (Olympus, Tokyo, Japan).
DNA isolation, PCR and sequencing. The mycelia grown in PDB at 26 °C for 7 days were prepared for DNA isolation. Total genomic DNA was extracted as described previously. The internal transcribed spacer (ITS) region was amplified with primers ITS4 and ITS5. The partial 28S ribosomal RNA (LSU) gene region was amplified with primers LORF and LR. The thermocycling conditions for amplifications had an initial denaturing step of 94 °C for 5 min, 32 cycles of 94 °C for 60 s, 55 °C for 30 s, 72 °C for 90 s, followed by a final elongation step at 72 °C for 7 min. A G1000 Thermal Cycler (BOER, Hangzhou, China) was used for PCR amplification. Amplicons were verified with 1% agarose electrophoresis gel, and the expected bands were excised and purified with an AxyprepTM gel purification kit (Axygen, Hangzhou, China). These fragments were cloned into the pEASY-T5 zero cloning kit (Transgen Biotech, Beijing, China) and followed by sequencing (Sangon Biotech, Shanghai, China). The nucleotide sequences of the genes have been deposited in GenBank (Table 1).

Phylogenetic analyses. All the sequences (Table 1) were aligned by Clustal X (Larkin, Blackshields et al. 2007) and Mega 7.0. Datasets were analyzed using both maximum parsimony (MP) and Bayesian tree inference (BI). MP analyses were performed using PAUP* 4.0b10, a heuristic search option was chosen with random addition of sequences as 1,000 replications; gaps were treated as missing data. BI analyses were run with MrBayes 3.2.4 using the GTR substitution model with gamma-distributed rate variation across sites and a proportion of invariable sites. Two sets of four chains were executed until the standard deviation of split frequencies reached 0.01. Sample frequency was set at 100 and 25% of trees removed as burn-in.

Fermentation and extraction. This assay was performed according to our previous method. Briefly, sterile water (53 ml) and rice (40 g) were mixed to an Erlenmeyer flask (250 ml), which were autoclaved at 121 °C for 30 min. The strain of SYEP 7336 (1 ml) was inoculated in each Erlenmeyer flask (250 ml × 120), which were cultivated at 28 °C for 30 days. The fermented material was extracted using ethyl acetate (12L × 3) to give the crude extract (127 g). Then it was dissolved in 90% MeOH–H₂O (1 L), and extracted by hexane (1 L × 3) to obtain the residue (62 g).

Isolation of secondary metabolites. Silica gel chromatography was used to separate the extract (41.44 g) eluting with CH₂Cl₂/CH₃OH (v/v 80:1–3:1), and yielding four fractions (A-D). Fraction B (10.2 g) was separated by ODS eluting with MeOH-H₂O (v/v 10:90 to 90:10) to give another eight fractions (B1-B7). Fraction B1 (1.6 g) was further subjected to semipreparative HPLC (Agilent 1100 instrument; YMC 5 μm C18 column (250 mm × 10 mm)), eluted with CH₃CN-H₂O (v/v 18:82, 3.5 ml/min) to yield compounds 1 (10 mg), 3 (12 mg) and 8 (10 mg). Subfraction B 3–4 (0.8 g) was further purified by semipreparative HPLC (CH₃CN-H₂O, v/v 20:80, 3.5 ml/min) to produce compounds 2 (10 mg), 4 (13 mg) and 9 (11 mg). Similarly, subfraction B7 (1.2 g) was subjected to semipreparative HPLC, eluted with CH₃CN-H₂O (v/v 22:78, 3.5 ml/min) to afford compounds 5 (13.6 mg), 6 (11.2 mg) and 7 (17 mg).

Compound 1: white flakes; 1H (600 MHz, DMSO-d₆) and ¹³C NMR (150 MHz, DMSO-d₆) data, see Table 2; HR ESI MS/MS m/z 325.2738 [M + Na]⁺ (calcd for C₁₅H₁ₐO₅Na, 325.2733).

Compound 2: yellowish oil; [α] D₂₀ +3.0 (c 0.25, MeOH). 1H (600 MHz, DMSO-d₆) and ¹³C NMR (150 MHz, DMSO-d₆) data, see Table 2; HR ESI MS/MS m/z 307.2551 [M + Na]⁺ (calcd for C₁₅H₁₈O₄Na, 307.2556).

Antimicrobial assay. Bacterial strains used in our anti-bacterial studies are from National Center for Medical Culture Collections (CMCC), and the CMCC numbers are listed as below: B. subtilis (CMCC63501), S. aureus (CMCC26003), P. aeruginosa (CMCC10104), K. pneumonia (CMCC46117), and E. coli (CMCC44102). The antimicrobial assay was performed according to the procedure of Zhu et al. Briefly, DMSO was used to dissolved the positive control and compounds 1–9 at an initial concentration of 10 mg/ml, respectively. Luria-Bertani (LB) media was used for the five pathogenic bacteria for 24 h at 37 °C until the absorbance OD₆₀₀ reached 0.01. Sample frequency was set at 100 and 25% of trees removed as burn-in.

Minimal inhibitory concentration (MIC) of compounds 1, 2 and 5 were assessed against bacteria S. aureus (CMCC26003). The MIC values of the isolated compounds against human pathogenic bacteria were determined by the modified CLSI M38-A method. Briefly, compounds 1, 2 and 5 were dissolved using DMSO with the final concentrations of 100, 50, 25, 12.5, and 6.25 μg/mL, respectively for the MIC determination. The dosage-response curve was drawn according to different concentrations of compound to S. aureus cells growth inhibition rates. The MIC values were calculated from the dosage-response curves. DMSO and Ampicillin were used as the negative control and positive control, respectively.

Molecular docking. This assay was performed according to our previous method. Briefly, the crystal structure of protein obtained from RCSB Protein Data Bank (PDB Code 3Y0V) were used for docking. The 3D structures of the compounds 1–2 and 5 were prepared and Gasteiger–Hückel charges were added using Sybyl software (Tripos, America). The ligand, guanosine-5′-diphosphate, was subjected to energy minimization with Tripos force filed parameters. Blind docking was carried out using Molegro Virtual Docker 4.0 (Molegro ApS, Aarhus, Denmark) program. The 3D docking grid was sufficiently large to cover the protein.

Morphological observation of bacterial fission. To identify whether there are changes in morphology of S. aureus after treated with compounds 1–2 and 5, observations under a transmission electron microscope (TEM, HT7700, Japan) were performed. S. aureus cells were grown at 37 °C on an agar plate, then diluted by LB broth to an OD₆₀₀ of 0.2, and 25 μg/ml compounds 1–2 and 5 were added to the suspensions. Samples of treated cells and controls were further cultivated at 37 °C for 3 h. Then, the bacterial suspensions were dyed with 2% phosphotungstic acid (v/v = 1:1 pH 6.5) for 3–5 min, and transmission scan was performed as previously described.
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**Author Contributions**
T.-Y.Z., Y.-Y.W. and Y.-X.Z. designed and coordinated the research. Y.-Y.W. and H.-J.Y. performed the isolation, purification and structure elucidation of the compounds. T.-Y.Z., M.-Y.Z., J.C. and H.-J.Y. supplied the strain of *Seltsamia galinsogisoli* sp. nov. and finished the morphometric identification and fermentation. M.-Y.Z. and J.C. carried out the antimicrobial activity. Y.-Y.W. and M.-Y.Z. completed the molecular docking and morphological observation of bacterial fission. T.-Y.Z. and Y.-Y.W. wrote the manuscript. Blessings Dube edited the manuscript. All authors read, revised and approved the final manuscript.

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