The streptazolin- and obscuriolide-type metabolites from soil-derived *Streptomyces alboniger* YIM20533 and the mechanism of influence of γ-butyrolactone on the growth of *Streptomyces* by their non-enzymatic reaction biosynthesis†

Na Luo,†a Ya-Bin Yang,†b‡ Xue-Qiong Yang,b Cui-Ping Miao,a Yi-Qing Li,a Li-Hua Xu,a Zhong-Tao Ding b and Li-Xing Zhao a,∗a

Eleven new compounds with streptazolin- and obscuriolide-type skeletons were isolated from soil-derived *Streptomyces alboniger* obtained from Tibet, China. Two types of unprecedented skeletons of obscuriolide dimer and an obscuriolide-type compound with an aromatic polyketide of pentanone substituted at the benzene ring were determined by spectral data analysis. Compound 11 was the first evidence of two nitrogens in streptazolin-type structures. Compound 1 indicated an inhibitory effect on nitric oxide production in LPS-activated macrophages with an inhibition ratio of 51.7% at 50 μM, and on anticoagulant activity on platelet activating factor (PAF)-induced platelet aggregation with an inhibition ratio of 26.0 ± 9.1% at 200 μg mL⁻¹. 11 had anti-acetylcholinesterase activity with an inhibition ratio of 27.2% at a concentration of 50 μM. Mechanistic aspects of the non-enzymatic reaction as well as a more detailed picture of the biosynthetic relationships of the streptazolin- and obscuriolide-type metabolites are described. Acidic and basic conditions can inhibit the growth of *Streptomyces*, and γ-butyrolactones were found to be hormones controlling antibiotic production in *Streptomyces*. In the pH fermentation tests, acylation of γ-butyrolactones was successfully used to explain the mechanism of influence on the growth of *Streptomyces*.

Introduction

*Streptomyces* spp. have been thoroughly investigated for more than 70 years as they produce a number of diverse bioactive compounds, especially clinical chemotherapeutics, such as antibacterial, anticancer, and immunosuppressive agents. It has been proposed that there are more genes encoding the potential production of small molecules than have been found, indicating that in-depth research should be developed to activate the silent genes.1,2 New biotechnological techniques, such as gene mining and gene-regulating methods, have been applied to stimulate *Streptomyces* strains to produce new molecules.3,4 At the same time, bioactive compound production also is related to nutritional regulators, morphological development, physiological processes and other hormone or hormone-like production in the *Streptomyces* culture.5 Mining novel bioactive compounds from microorganisms harbored in under-explored niches has proved an efficient method in pharmaceutical research and development.6,7 Streptazolin, mainly produced by members of the genus *Streptomyces*, presents antibiotic and antifungal activities.8,9 Some synthesis research on the compound and its analogues has also been developed.10,11 Recent research has proved that the compound can also increase bacterial killing and elaboration of immunostimulatory cytokines by macrophages.12 The diene and oxazolidinone moieties are considered as the pharmacophore in streptazolin and its analogues.13,14 Obscuriolides, a series of butyrolactone derivatives produced by *Streptomyces viridochromogenes* (strain Tü 2580), present weak inhibitory activity against phosphodiesterases.15,16 γ-Butyrolactones play an autoregulation role in antibiotic production and differentiation for many members of the genus *Streptomyces* by...
modulating the DNA binding activity of cognate receptor proteins.\textsuperscript{17} Novel butyrolactones as signaling molecules should be involved in many functional aspects in microbial life, which would be useful for synthetic biology.\textsuperscript{18} In our effort to search for new biological compounds from \textit{Streptomyces} harbored in different niches, eight new butyrolactones (obscurolie-type), two new streptazolin-type compounds, a new metabolite and two new natural products were isolated from the culture broth of \textit{Streptomyces alboniger} YIM20533, an isolate obtained from a soil sample from Tibet, China. Their structures were determined and interpreted by 1D NMR, 2D NMR, and HR-ESIMS data (Fig. 1). In this research, we report the isolation, structural elucidation, bioactivities and the mechanism of influence on the growth of \textit{Streptomyces} via the biosynthesis of butyrolactone derivatives.

\section*{Results and discussion}

The molecular formula of streptalbonin A (1) was determined as C\textsubscript{19}H\textsubscript{23}NO\textsubscript{5} from HR-ESIMS analysis. The $^1$H and $^{13}$C NMR spectroscopic analysis, including DEPT, clearly showed two methyls, one methylene, twelve methines, two olefinic quaternary carbons, and two carbonyl carbons, which indicated the skeleton of obscurolie.\textsuperscript{15} An additional pentane should be substituted at the benzene ring, which was confirmed by the COSY correlations between H-7 and H-8, H-10 and H-11, and the HMBC correlations from H-11 to C-9 and C-10; H-7 and H-8 to C-9; and H-7 to C-3 and C-5. Compound 1 has an unprecedented skeleton with a pentane substituted at the benzene ring in obscurolie-type compounds. The fraction structure of this compound, an aromatic polyketide, is rarely found in natural products. The other COSY correlations of H-2/H-3; H-5/H-6; and H-2/H-3/H-4/H-5/H-6/H-7/H-8, and the HMBC correlations from H-2 to C-1, C-3 and C-4; H-4 to C-1, C-5, and C-6; and H-8 to C-6 and C-7 also confirmed the skeleton of obscurolie (Fig. 2). The \textit{trans} orientation at C-7 and C-8 was determined by the coupling constant at 15.6 Hz. The relative configuration between H-3 and H-4 of 1 was determined as \textit{trans} by the ROESY correlation of H-3/H-5. The configurations (3S, 4R) of streptalbonin A at C-3 and C-4 and \textit{trans} orientation at C-5 and C-6 were determined by comparing the NMR spectrum with those for other obscuroliodes and pseudonocardides also found in actinomycetes and their biogenesis.\textsuperscript{15,19} Streptalbonin A exists in two diastereomers at C-7 just as the obscurolie A\textsubscript{2} reported in previous work.\textsuperscript{15} The configuration of C-10 of compound 1 was determined as R by comparing the NMR and optical

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Structures of compounds 1–9, 11, and 13–15 isolated from \textit{Streptomyces alboniger} YIM20533.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{The COSY and HMBC correlations in compounds 1–6, and 8, 9, 11, and 13–15.}
\end{figure}
rotation with those for (R)-2-hydroxy-5-phenylpent-4-en-3-one formed by microorganism enzymes. The fraction structure (R)-2-hydroxy-5-phenylpent-4-en-3-one existed in compound 1.

The molecular formula of streptalonin B (2) was determined as C₁₄H₁₈NO₄ from HR-ESIMS analysis. The spectroscopic analysis also showed the skeleton of obscurolide. The COSY correlations of H-2'/H-3'; H-5'/H-6'; and H-2/H-3/H-4/H-5/H-6/H-7/H-8, and the HMBC correlations from H-2, H-3 and H-4 to C-1; H-4, H-7 and H-8 to C-6; H-3, H-4 and H-7 to C-5; H-3 to C-1'; and H-3' and H-5' to C-1' and C-7' confirmed this speculation. The structure difference compared with other known obscurolides was the acetyl at C-7, which was determined by the weak HMBC correlation from H-2' to H-7 (Fig. 2). The relative configurations between H-3 and H-4 were determined as trans by the NOEY correlations of H-2'/H-4; H-3/H-5. The configurations of streptalonin B and the trans orientation at C-5 and C-6 were determined by comparing the NMR results with those for compound 1.

The molecular formula of streptalonin C (3) was determined as C₁₄H₁₈NO₄ from HR-ESIMS analysis. The spectroscopic analysis clearly showed the skeleton of obscurolide. The COSY correlations of H-2'/H-3'; H-5'/H-6'; and H-2/H-3/H-4/H-5/H-6/H-7/H-8, and the HMBC correlations from H-2, H-3, and OCH₃ to C-1; H-4, H-7, H-8 to C-6; H-4, H-7 to C-5; H-3', H-5' to C-1' and C-7' confirmed this skeleton (Fig. 2). The acetyl at C-7 was confirmed by the chemical shift of H-7 compared with those of compound 2. The configurations of streptalonin C at C-3, C-4 were determined by comparing the NMR spectrum with that of compound 2.

The molecular formula of streptalonin D (4) was determined as C₁₄H₁₄NO₃ from HR-ESIMS analysis. The ¹H and ¹³C NMR spectroscopic analysis clearly showed the skeleton of obscurolide as in compounds 1–3. The COSY correlations of H-2'/H-3'; H-5'/H-6'; and H-2/H-3/H-4/H-5/H-6, and the HMBC correlations from H-2, H-3 and H-4 to C-1; H-5, H-6 and H-8 to C-7; H-3 and H-4 to C-5; H-3' and H-5' to C-1' and C-7'; and H-2' and H-6' to C-4' also confirmed this structure (Fig. 2). The relative configurations between H-3 and H-4 of 4 were determined as trans by the NOEY correlations of H-3/H-5. The configurations of streptalonin D at C-3 and C-4 were determined as 3S, 4R by comparing the NMR spectrum with that for compound 3 and biogenesis.

The molecular formula of streptalonin E (5) was determined as C₁₆H₂₃NO₄ from HR-ESIMS analysis. The ¹H and ¹³C NMR spectroscopic analysis clearly showed the skeleton of obscurolide. The COSY correlations of H-2'/H-3'; H-5'/H-6'; H-2/H-3; and H-4/H-5/H-6, and the HMBC correlations from H-2, H-3 and H-4 to C-1; H-5, H-6 and H-8 to C-7; H-5 to C-3 and C-4; H-3' and H-5' to C-1' and C-7'; H-8' to C-7'; and H-2' and H-6' to C-4' also confirmed this structure (Fig. 2). The relative configurations between H-3 and H-4 were determined as trans by the NOEY correlations of H-3/H-5. The configurations of streptalonin E were determined to be the same as those found for the other compounds.

The molecular formula of streptalonin F (6) was determined as C₁₅H₂₁NO₃ from HR-ESIMS analysis. The ¹H and ¹³C NMR spectroscopic analysis clearly showed the skeleton of obscurolide. The COSY correlations of H-2'/H-3'; H-5'/H-6'; and H-2/H-3/H-4/H-5/H-6/H-7/H-8, and the HMBC correlations from H-2, H-3 and H-4 to C-1; H-8 to C-6 and C-7; H-3' and H-5' to C-1' and C-7'; and H-2' and H-6' to C-4' also confirmed this structure (Fig. 2). The trans orientation at C-6 and C-7 was determined by the NOEY correlations between H-5 and H-7, and H-6 and H-8. The relative configurations between H-3 and H-4 of 6 were determined as trans by the NOEY correlations of H-3/H-5. The configurations of streptalonin F at C-3 and C-4 were determined as 3S and 4R by comparing the NMR spectrum with that for compound 5. The configuration of C-5 was elucidated as S by comparing the NMR data with those of musacins also isolated from Streptomyces. Streptalonin G (7) was determined to be the diastereoisomer of streptalonin F (6) by NMR, 2D-NMR and CD analysis. The difference between compounds 6 and 7 was the configuration of C-5, which was found to be S for 6 and R for 7 by NMR analysis.

The molecular formula of streptalonin H (8) was determined as C₂₉H₃₄N₂O₆ from HR-ESIMS analysis. The ¹H and ¹³C NMR spectroscopic analysis clearly showed the skeleton of obscurolide. The COSY correlations of H-2'/H-3'; H-5'/H-6'; and H-2/H-3/H-4/H-5/H-6/H-7/H-8, and the HMBC correlations from H-2, H-3 and H-4 to C-1; H-8 to C-6 and C-7; H-3' and H-5' to C-1'; and H-2' and H-6' to C-4' also confirmed this structure. The key correlations from H-7 to C-3', 4', and 5' indicated that the C-7' is connected to C-4' (Fig. 2), which showed an unprecedented streptalonin dimer. The trans orientation at C-5 and C-6 was determined by comparing the NMR results with those for other compounds isolated in this strain. The relative configurations between H-3 and H-4 of 8 were determined as trans by the NOEY correlations of H-3/H-5. The configurations of streptalonin H at C-3 and C-4 were determined as 3S, 4R by comparing the NMR results with those for compound 3.

The molecular formula of streptalonin I (9) was determined as C₁₅H₂₁NO₃ from HR-ESIMS analysis. The ¹H and ¹³C NMR spectroscopic analysis clearly showed two methyls, five methines, two olefinic quaternary carbons, and two carbonyl carbons. The COSY correlations of H-2'/H-3'; H-5'/H-6'; and H-3/H-4, and the HMBC correlations from H-1 and H-4 to C-3; H-3 to C-1'; H-3' and H-5' to C-1' and C-7'; and H-2' and H-6' to C-4' also confirmed this structure (Fig. 2). The configuration of this compound was determined as R by comparing the optical rotation for this compound with that for (R)-2-hydroxybutan-2-one, which is also found in Streptomyces. Compound 10 was determined as 8α-hydroxystreptazolone by spectroscopic analysis. The molecular formula of streptalin A (11) was determined as C₁₄H₁₈NO₄ from HR-ESIMS analysis. Comparing the NMR data of compounds 10 and 11, these compounds have similar structures, except for streptalin A (11) with a p-aminobenzoic acid substituted at C-5, which was confirmed by the HMBC correlation from H-5 to C-1'. The COSY correlations of H-11/H-4/H-5 and H-8/H-9/H-10, and the HMBC correlations from H-4, H-10, and H-11 to C-2; H-5, H-8, and H-11 to C-7; H-4, H-5, and H-13 to C-6; H-8 to C-9; H-3' and H-5' to C-1' and C-7'; and H-2' and H-6' to C-4' also confirmed this structure (Fig. 2). The configurations of this compound were determined to be the same as those for...
8α-hydroxystreptazolone by comparing the NMR spectra, and the NOESY correlations of H-4/H-11; H-4/H-2', and H-5/H-8 also confirmed this elucidation.

4α,5-Dihydrostreptazolin (12) was determined by spectroscopic analysis and it was the first time that it was isolated from a natural source. Strepalbin B (13) was determined as C_{11}H_{12}NO_{3} from HR-ESIMS analysis. Comparing with the NMR data of 8α-hydroxy-streptazolone, strepalbin B (13) had a hydroxyl at C-12 instead of the carbonyl in 8α-hydroxy-streptazolone, and this structure was determined by the COSY correlations of H-9/H-10 and H-11/H-4/H-5, and the HMBC correlations of H-4, H-10, H-11/C-2; H-4, H-5, H-8, H-11/C-6, C-7; H-9, H-10/C-8; and H-13/C-6, C-12 (Fig. 2). The configurations of this compound were determined to be the same as for 8α-hydroxy-streptazolone by comparing the NMR spectra, and the NOESY correlations also confirmed it. The configuration of C-12 in compound 13 was not determined owing to the low amount.

Compound 14 was determined as C_{10}H_{12}O_{3} from HR-ESIMS analysis. The ^1H and ^13C NMR spectroscopic analysis clearly showed three methylenes, four methines, two olefinic quaternary carbons, and one carbonyl carbon. There were also a disubstituted benzene and a butyric acid in the structure of 14, named as 4-[2-hydroxyphenyl]butyric acid, which was determined by the COSY correlations of H-3/H-4/H-5/H-6 and H-7/H-8/H-9, and HMBC correlations of H-8, H-9/C-10; H-7, H-8/C-11; H-7/C-2, C-6; H-6/C-1, C-2; and H-4/C-2, C-6 (Fig. 2). This structure was first isolated in natural products.

Compound 15 was determined as C_{9}H_{12}N_{2}O_{2}S from HR-ESIMS analysis. The ^1H and ^13C NMR spectroscopic analysis clearly showed two methylenes, three methines, one olefinic quaternary carbon, and two carbonyl carbons. The structure of 15 was determined by the COSY correlations of H-2/H-3 and H-7/H-8/H-9, and HMBC correlations of H-2, H-2'/C-1'; H-3, H-7/C-5; and H-7, H-8, H-9/C-6 (Fig. 2). This structure was first isolated in natural products.

Some new compounds were evaluated for their nitric oxide inhibition [1, 2, 3, 4, 5, 6, 7, 8, 9, 11, obscurolide A_1, obscurolide A_2 (ref. 15), anticoagulant activity (1, 8, 9, and 11), and anti-acetylcholinesterase activity (1, 8, 9, and 11). Compound 1 indicated an inhibitory effect on nitric oxide production in LPS-activated macrophages with an inhibition ratio of 51.7% at 50 μM, and anticoagulant activity on platelet activating factor (PAF)-induced platelet aggregation with an inhibition ratio of 26.0 ± 9.1% at 200 μg mL⁻¹. 11 had anti-acetylcholinesterase activity with an inhibition ratio of 27.2% at a concentration of 50 μM. The other tested compounds had no obvious activities in the corresponding assays.

The obscurolides were isolated from Streptomyces viridochromogenes and turned out to be inhibitors of cyclic AMP phosphodiesterase. Previous work has focused on their structure and bioactivity. Only one paper described the manipulation of fermentation pattern to initiate obscurolide biosynthesis, and no detailed process was discussed. Unexpectedly, a non-enzymatic reaction was found to be a key step in the production of these obscurolide- and streptazolin-type compounds. The common precursor 4-aminobenzoic acid was also isolated from this strain, and 4,4'-methyleneedianiline is a common industrial chemical, so they might be the bioprecursors in compounds 1, 8, and 11.

Conclusions

Eleven new compounds were isolated from Streptomyces albo-niger, which was derived from soil in Tibet, China. Two types of unprecedented skeletons of obscurolide dimer and an obscurolide-type compound with a pentanone substituted at the benzene ring core were found in this work. Compound 1 indicated an inhibitory effect on nitric oxide production in LPS-activated macrophages and anticoagulant activity on platelet activating factors (PAF)-induced platelet aggregation. 11 exhibited anti-acetylcholinesterase activity. Mechanistic aspects of
the non-enzymatic reactions of the biosynthetic relationships of the streptazolin and obscurolide-type metabolites were exhibited. The acylation of γ-butyrolactones was successfully used to explain the mechanism of influence of acid–base properties on the growth of *Streptomyces*.

**Experimental**

**General experimental procedure**

Silica gel (200–300 mesh; Qingdao Marine Chemical Group Co.), Lichroprep RP-18 (Beijing Greenherbs and Technology and Development Co.) and Sephadex LH-20 (GE Healthcare Co.) were used for column chromatography. 1D and 2D NMR spectra were obtained on a Bruker AVANCE 500, 600 MHz NMR instrument (Bruker). MS spectra were recorded with an Agilent G3250AA (Agilent) and an AutoSpec Premier P776 spectrometer (Waters). ORs were obtained on a Jasco P-1020 polarimeter. Circular dichroism spectra were obtained using an Applied Photophysics Chirascan spectrometer (Applied Photophysics Ltd.).

**Biological material and cultivation of actinomycetic strain**

The actinomycetic strain YIM20533 was isolated from the soil in Tibet, China. The species was identified as *Streptomyces albogínger* based on morphological and genetic (16S rRNA gene sequence) analyses. A voucher specimen was deposited at the Yunnan Institute of Microbiology, Kunming, P.R. China. This bacterium was cultivated on 80 L scale using 8 L seed medium (yeast extract 0.4%, glucose 0.4%, malt extract 0.3%, deca-

**Extraction and isolation of compounds**

After 7 days of growth, the mycelia were removed from the cultures (80 L) by filtration. The filtrate was extracted with isomeric ethyl acetate (EtOAc) 3 times, and the solvent was removed under vacuum to obtain the EtOAc extract (20.0 g). The EtOAc extract was separated into five fractions (Fr. 1–Fr. 5) by column chromatography on silica gel (200–300 mesh), eluting with a stepwise CHCl₃/MeOH gradient (CHCl₃: CHCl₃/MeOH 50 : 1 v/v; CHCl₃/MeOH 25 : 1 v/v; CHCl₃/MeOH 12.5 : 1 v/v; MeOH). Fr. 3 was divided into three parts (Fr. 3–1 to Fr. 3–3) using a Sephadex LH-20 column with MeOH. Fr. 3–1 was isolated using a Lichroprep RP-18 column with H₂O/MeOH (60 : 30 v/v) and further purified using a silica gel column with petroleum ether/EtOAc gradient (4 : 1 to 2 : 1) to afford compounds 9 (10.5 mg) and 14 (4.6 mg). Fr. 3–2 was isolated using a Lichroprep RP-18 column with H₂O/MeOH gradient (v/v 90 : 10 to 50 : 50) to give four subfractions, Compounds 10 (2.7 mg), 11 (2.4 mg), 12 (2.1 mg), and 15 (3.6 mg) were obtained from Fr. 3.2.2 using a silica gel column with petroleum ether/EtOAc gradient (4 : 1 to 1 : 1). Fr. 4 was separated into four fractions (Fr. 4–1 to Fr. 4–4) using a Sephadex LH-20 column with MeOH, then Fr 4–1 was isolated using a Lichroprep RP-18 column with H₂O/MeOH gradient (v/v 90 : 10 to 50 : 50) and further purified by a silica gel column with a petroleum ether/EtOAc gradient (8 : 1 to 3 : 1) to afford compounds 4 (3.2 mg), 5 (3.6 mg), 6 (3.0 mg) and 7 (2.5 mg). Fr. 4–3 was isolated using a Lichroprep RP-18 column with H₂O/MeOH gradient (v/v 90 : 10 to 50 : 50) and further purified using a silica gel column with a petroleum ether/EtOAc gradient (5 : 1 to 2 : 1) to afford compounds 1 (10.2 mg), 2 (4.3 mg) and 3 (2.2 mg). Fr. 4–4 was isolated using a Lichroprep RP-18 column with H₂O/MeOH (v/v 80 : 20) and further purified using a silica gel column with a petroleum ether/EtOAc gradient (5 : 1 to 2 : 1) to afford compound 13 (3.2 mg). Fr. 5 was divided into three parts (Fr. 5–1 to Fr. 5–3) using a Sephadex LH-20 column with MeOH and compound 8 (11.8 mg) was obtained from Fr 5.3 using a silica gel column with a petroleum ether/EtOAc gradient elution (3 : 1 to 0 : 1).

**Compound 1.** [α]D° 5.3 (c 0.1, MeOH). HR-ESIMS m/z: 346.1627 [M + H]⁺, calcd for C₁₉H₂₃NO₅: 346.1654. H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 1.

**Compound 2.** [α]D° 12.5 (c 0.2, MeOH). HR-ESIMS m/z: 356.1114 [M + Na]⁺, calcd for C₁₇H₂₃NO₆Na: 356.1110. H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 1.

**Compound 3.** [α]D° −6.6 (c 0.1, MeOH). HR-ESIMS m/z: 366.1556 [M + H]⁺, calcd for C₁₅H₁₇NO₅: 366.1553. ¹H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 1.

**Compound 4.** [α]D° 23.2 (c 0.2, MeOH). HR-ESIMS m/z: 292.1178 [M + H]⁺, calcd for C₁₃H₁₈NO₄: 292.1185. H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 2.

**Compound 5.** [α]D° 15.5 (c 0.09, MeOH). HR-ESIMS m/z: 292.1550 [M + H]⁺, calcd for C₁₃H₁₈NO₄: 292.1549. H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 2.

**Compound 6.** [α]D° −5.0 (c 0.4, MeOH). HR-ESIMS m/z: 314.0975 [M + Na]⁺, calcd for C₁₅H₁₇NO₅Na: 314.1004. H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 2.

**Compound 7.** [α]D° −2.2 (c 0.2, MeOH). HR-ESIMS m/z: 314.0977 [M + Na]⁺, calcd for C₁₅H₁₇NO₅Na: 314.1004. H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 3.

**Compound 8.** [α]D° 0.6 (c 0.2, MeOH) HR-ESIMS m/z: 529.2310 [M + Na]⁺, calcd for C₂₉H₃₄N₂O₆Na: 529.2315. H-NMR (MeOD, 400 MHz) and ¹³C-NMR (MeOD, 100 MHz) in Table 3.

**Compound 9.** [α]D° −11.5 (c 0.1, MeOH). HR-ESIMS m/z: 208.0973 [M + H]⁺, calcd for C₁₄H₁₄NO₄: 208.0974. H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 3.

**Compound 10.** [α]D° 5.3 (c 0.15, MeOH). HR-ESIMS m/z: 357.1086 [M − H]⁺, calcd for C₁₄H₁₂N₂O₄: 357.1087. H-NMR (MeOD, 600 MHz) and ¹³C-NMR (MeOD, 150 MHz) in Table 4.

**Compound 11.** [α]D° 16.0 (c 0.2, MeOH). HR-ESIMS m/z: 240.0869 [M − H]⁺, calcd for C₁₄H₁₂N₂O₄: 240.0872. H-NMR (MeOD, 500 MHz) and ¹³C-NMR (MeOD, 125 MHz) in Table 4.

**Compound 12.** HR-ESIMS m/z: 179.0813 [M − H]⁻, calcd for C₁₀H₁₂O₃: 179.0708. ¹H-NMR (CDCl₃, 600 MHz) δ: 7.04 (1H, d, J = 7.8 Hz, H-6), 6.98 (1H, t, J = 7.8 Hz, H-4), 6.72 (1H, t, J = 7.8 Hz, H-5), 6.72 (1H, d, J = 7.8 Hz, H-3), 2.85 (2H, t, J = 10.4 Hz, H-7), 2.62 (2H, t, J = 7.2 Hz, H-9), 1.87 (2H, t, J = 7.2 Hz, H-8). ¹³C-NMR (CDCl₃, 150 MHz) δ: 178.1 (CH-10), 155.0 (C-2), 129.8 (C-6), 127.8 (C-1), 126.7 (C-4), 119.1 (C-5), 114.6 (C-3), 33.6 (C-9), 29.2 (C-7), 25.1 (C-8).
The AChE inhibitory activities of the compounds were measured using Ellman et al. 26 with slight modification. Tacrine (Sigma, purity > 99%) was used as a positive control with a final concentration of 0.333 μM with an inhibition ratio of 57.4%. The NO inhibitory activity of these compounds was determined using the Griess reagent assay for NO production. L-NMMA was used as the positive control with an inhibition ratio of 55.04 ± 2.80% at a concentration of 50 μM. The in vitro anticoagulant activities were investigated using the prothrombin time (PT) method for compounds 1, 9, and 11, the activated partial thromboplastin time (APTT) for compounds 1, 8, 9, and 11, thrombin time (TT) for compounds 1, 9, and 11, adenosine diphosphate (ADP) induced platelet aggregation for compound 1 and PAF-

### Table 1 13C NMR and 1H NMR data for compounds 1–3 in MeOD

| Compound 15. HR-ESIMS m/z: 235.0512 [M + Na]+, calcd for C9H12N2O2SNa: 235.0517. 1H-NMR (MeOD, 600 MHz) δ: 7.03 (1H, q, J = 1.3 Hz, H-9), 6.16 (1H, q, J = 1.3 Hz, H-7), 6.20 (1H, q, J = 1.2 Hz, H-8), 3.38 (2H, t, J = 6.6 Hz, H-2), 3.12 (2H, t, J = 6.6Hz, H-3), 1.92 (3H, s). 13C-NMR (MeOD, 150 MHz) δ: 180.5 (C-5), 172.1 (C-1′), 129.8 (C-6), 124.3 (C-9), 115.0 (C-7), 109.6 (C-8), 39.3 (C-2′), 26.8 (C-3′), 21.1 (C-2′). Bioactivity assay The AChE inhibitory activities of the compounds were assayed by the spectrophotometric method developed by Ellman et al. 26 with slight modification. Tacrine (Sigma, purity > 99%) was used as a positive control with a final concentration of 0.333 μM with an inhibition ratio of 57.4%. The NO inhibitory activity of these compounds was determined using the Griess reagent assay for NO production. L-NMMA was used as the positive control with an inhibition ratio of 55.04 ± 2.80% at a concentration of 50 μM. The in vitro anticoagulant activities were investigated using the prothrombin time (PT) method for compounds 1, 9, and 11, the activated partial thromboplastin time (APTT) for compounds 1, 8, 9, and 11, thrombin time (TT) for compounds 1, 9, and 11, adenosine diphosphate (ADP) induced platelet aggregation for compound 1 and PAF-

### Table 2 13C NMR and 1H NMR data for compounds 4–6 in MeOD

| Pos. | δ_H |  δ_c | Pos. | δ_H |  δ_c | Pos. | δ_H |  δ_c |
|------|-----|-----|------|-----|-----|------|-----|-----|
| 1    | 176.0 | 8  | 1    | 176.3 | 8  | 1    | 176.7 | 8  |
| 2    | 2.37, 2.99 (m) | 34.3 | 2    | 2.39, 2.97 (m) | 34.2 | 2    | 2.39, 2.64 (m) | 35.4 |
| 3    | 4.09 (m) | 54.5 | 3    | 4.12 (m) | 54.3 | 3    | 3.88 (m) | 54.2 |
| 4    | 4.73 (m) | 85.2 | 4    | 4.70 (m) | 84.8 | 4    | 4.05 (m) | 72.9 |
| 5    | 5.74, 5.83 (m) | 124.5 | 5    | 5.80 (m) | 127.2 | 5    | 5.65 (m) | 131.0 |
| 6    | 5.74, 5.83 (m) | 139.0 | 6    | 5.80 (m) | 133.9 | 6    | 5.65 (m) | 131.7 |
| 7    | 4.34 (m) | 71.7 | 7    | 5.26 (m) | 69.8 | 7    | 5.16 (m) | 70.5 |
| 8    | 1.12 (d, J = 6.6 Hz) | 21.9 | 8    | 1.18 (d, J = 6.6 Hz) | 18.8 | 8    | 1.07 (d, J = 6.6 Hz) | 18.9 |
| 1'   | 149.9 | 1'  | 1'   | 151.3 | 1'  | 1'   | 152.1 | 1'  |
| 1'   | 149.9 | 8   | 1'   | 151.3 | 1'  | 1'   | 152.1 | 1'  |
| 1'   | 149.9 | 8   | 1'   | 151.3 | 1'  | 1'   | 152.1 | 1'  |
| 1'   | 149.9 | 8   | 1'   | 151.3 | 1'  | 1'   | 152.1 | 1'  |
| 1'   | 149.9 | 8   | 1'   | 151.3 | 1'  | 1'   | 152.1 | 1'  |
| 1'   | 149.9 | 8   | 1'   | 151.3 | 1'  | 1'   | 152.1 | 1'  |
| 1'   | 149.9 | 8   | 1'   | 151.3 | 1'  | 1'   | 152.1 | 1'  |

| Pos. | δ_H |  δ_c | Pos. | δ_H |  δ_c | Pos. | δ_H |  δ_c |
|------|-----|-----|------|-----|-----|------|-----|-----|
| 1    | 176.0 | 8  | 1    | 176.3 | 8  | 1    | 176.7 | 8  |
| 2    | 2.45(dd, J = 5.4 Hz, 18.0 Hz) | 34.8 | 2    | 2.39 (dd, J = 5.4 Hz, 18.0 Hz) | 35.0 | 2    | 2.43 (dd, J = 1.8 Hz, 18.0 Hz) | 35.6 |
| 3    | 4.13 (m) | 53.7 | 3    | 4.04 (m) | 54.4 | 3    | 4.34 (m) | 50.6 |
| 4    | 4.40 (m) | 84.6 | 4    | 4.37 (m) | 84.9 | 4    | 4.34 (m) | 88.3 |
| 5    | 1.93, 2.09 (m) | 27.2 | 5    | 1.91, 2.07 (m) | 27.2 | 5    | 4.24 (m) | 72.4 |
| 6    | 2.70 (m) | 38.3 | 6    | 2.68 (m) | 38.4 | 6    | 5.57 (m) | 129.2 |
| 7    | 208.7 | 7    | 208.7 | 58.2 (m) | 128.9 |
| 8    | 2.14 (s) | 28.4 | 8    | 2.13 (s) | 28.4 | 8    | 1.69 (d, J = 6.0 Hz) | 16.3 |
| 1'   | 151.3 | 1'  | 151.3 | 1'  | 151.3 | 1'  | 151.3 | 1'  |
| 2'   | 6.64 (d, J = 8.0 Hz) | 111.6 | 2'   | 6.63 (d, J = 8.4 Hz) | 112.9 | 2'   | 6.66 (d, J = 9.0 Hz) | 111.6 |
| 3'   | 7.82 (d, J = 8.0 Hz) | 131.4 | 3'   | 7.13 (d, J = 8.4 Hz) | 129.4 | 3'   | 7.81 (d, J = 9.0 Hz) | 131.4 |
| 4'   | 118.6 | 4'  | 118.6 | 4'  | 118.6 | 4'  | 118.6 | 4'  |
| 5'   | 7.82 (d, J = 8.0 Hz) | 131.4 | 5'   | 7.13 (d, J = 8.4 Hz) | 129.4 | 5'   | 7.81 (d, J = 9.0 Hz) | 131.4 |
| 6'   | 6.64 (d, J = 8.0 Hz) | 111.6 | 6'   | 6.63 (d, J = 8.4 Hz) | 112.9 | 6'   | 6.66 (d, J = 9.0 Hz) | 111.6 |
| 7'   | 169.1 | 7'  | 169.1 | 7'  | 169.1 | 7'  | 169.1 | 7'  |
| 8'   | 3.31 (s) | 56.3 | 8'   | 3.31 (s) | 56.3 | 8'   | 3.31 (s) | 56.3 |
induced platelet aggregation for compound 1. Heparin was used as the positive control with PT at 37.3 ± 1.08 s at the tested concentration of 160 μg mL⁻¹. Low molecular weight heparin (LMWH) was used as the positive control for APTT at 79.7 ± 0.36 s, and TT at 18.5 ± 2.06 s at the tested concentration of 35.6 μM. Ticagrelor and GB were used as positive controls in ADP and PAF-induced platelet aggregation for the inhibition ratios at 69.8 ± 7.8% (final concentration: 5 μg mL⁻¹) and 93.4 ± 5.1 (%) (final concentration: 37 μg mL⁻¹), respectively. The cytotoxicity of the compounds against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines was determined in vitro by the 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium (MTS) method. Taxol was used as a positive control with IC₅₀ < 0.008 μM.

pH-static fermentation

The fermentation process was analyzed for a total of 7 days. 48 hours after inoculation, the pH-value was adjusted to pH 2.5, 3.5, 4.5, 6.0, 7.0, 8.0 with citric acid and NaOH. The growth of Streptomyces was observed at different times.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This research work was financially supported by the National Natural Science Foundation of China (81560571, 21562045, 81660582, 31660532), and a project of Yunling Scholars of Yunnan Province.

References

1 J. Begani, J. Lakhani and D. Harwani, *Ann. Microbiol.*, 2018, 68, 419–432.
2 Y. P. Chen, Q. Liu, H. Gao, H. P. Lin, H. Y. Tian, K. Hong, J. Li, R. W. Jiang, X. S. Yao and J. S. Tang, *RSC Adv.*, 2014, 4, 63324–63327.
3 D. Nichols, N. Cahoon, E. M. Trakhtenberg, L. Pham, A. Mehta, A. Belanger, T. Kanigan, K. Lewis and S. S. Epstein, *Appl. Environ. Microbiol.*, 2010, 76, 2445–2450.
4 B. B. Hou, L. Y. Tao, X. Y. Zhu, W. Wu, M. J. Guo, J. Ye, H. Z. Wu and H. Z. Zhang, *Appl. Microbiol. Biotechnol.*, 2018, 102, 4101–4115.
5 S. Kitani, K. T. Miyamoto, S. Takamatsu, E. Herawati, H. Iguchi, K. Nishitomi, M. Uchida, T. Nagamitsu,
S. Omura, H. Ikeda and T. P. Nihira, Proc. Natl. Acad. Sci. U. S. A., 2011, 108, 16410–16415.

6 K. Tiwari and R. K. Gupta, Crit. Rev. Biotechnol., 2012, 32, 108–132.

7 Y. Takahashi and T. Nakashima, Antibiotics, 2018, 7, 45.

8 I. Djinni, W. Djoudi, S. Souagui, F. Rabia, S. Rahmouni, I. Mancini and M. Kecha, J. Microbiol. Methods, 2018, 148, 161–168.

9 C. L. Yang, Y. S. Wang, C. L. Liu, Y. J. Zeng, P. Cheng, R. H. Jiao, S. X. Bao, H. Q. Huang, R. X. Tan and H. M. Ge, Mar. Drugs, 2017, 15, 244.

10 F. Z. Li, N. C. Warshakoon and M. J. Miller, J. Org. Chem., 2004, 69, 8836–8841.

11 I. Nomura and C. Mukai, Org. Lett., 2002, 4, 4301–4304.

12 B. M. Trost, C. K. Chung and A. B. Pinkerton, Angew. Chem., Int. Ed., 2004, 43, 4327–4329.

13 J. A. Perry, K. Koteva, C. P. Verschoor, W. Wang, A. M. E. Bowdish and G. D. Wright, J. Antibiot., 2015, 68, 40–46.

14 G. Zappia, P. Menendez, G. D. Monache, D. Misiti, L. Nevola and B. Botta, Mini-Rev. Med. Chem., 2007, 7, 389–409.

15 H. Hoff, H. Drautz, H. P. Fiedler, H. Zahner, J. E. Schultz, W. Keller-Schierlein, S. Philipps, M. Ritzau and A. Zeeck, J. Antibiot., 1992, 45, 1096–1107.

16 M. Ritzau, S. Philipps, A. Zeeck, H. Hoff and H. Zahner, J. Antibiot., 1993, 46, 1625–1628.

17 E. Takano, Curr. Opin. Microbiol., 2006, 9, 287–294.

18 M. Biarnes-Carrera, R. Breitling and E. Takano, Curr. Opin. Chem. Biol., 2015, 28, 91–98.

19 X. M. Zhang, D. F. Zhang, W. J. Li and C. H. Lu, Helv. Chim. Acta, 2016, 99, 191–196.

20 A. Cosp, C. Dresen, M. Pohl, L. Walter, C. Röhr and M. Müller, Adv. Synth. Catal., 2008, 350, 759–771.

21 A. Schneider, J. Spath, S. Breiding-Mack, A. Zeeck, S. Grabley and R. Thiericke, J. Antibiot., 1996, 49, 438–446.

22 B. M. Nestl, A. Bodlenner, R. Stuermer, B. Hauer, W. Kroutil and K. Faber, Tetrahedron: Asymmetry, 2007, 18, 1465–1474.

23 F. C. Pollak and R. G. Berger, Appl. Environ. Microbiol., 1996, 62, 1295–1299.

24 H. Yamada, S. Aoyagi and C. Kibayashi, Tetrahedron Lett., 1996, 37, 8787–8790.

25 R. Thiericke and M. Zerlin, Nat. Prod. Lett., 1996, 8, 163–16726.

26 G. L. Ellman, K. D. Courtney, V. J. Andres and R. M. Featherstone, Biochem. Pharmacol., 1961, 7, 88–95.