Antibacterial Potential of *Streptomyces showdoensis* BYC17 from the Nest of *Odontotermes formosanus*

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Research article

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Abstract

Background: With the continuous exploration and application of antibiotics, many common diseases have been treated while the evolution of drug-resistant bacteria has increased. The immediate raised of antibiotic-resistance makes it necessary to research special microorganisms for finding novel bioactive substances against drug-resistant bacteria. Insect-associated microbes have special metabolic pathways and are valuable resource base for the research and development of new antibiotics. The *Odontotermes formosanus* has formed a unique self-defense mechanismin the long-term evolution. Hence, nest of *O. formosanus* is a potential material for screening actinomycetes and compounds with bacteriostatic activity.

Methods: The strain BYC17 was identified by morphological observation and 16S rDNA sequencing analysis, and the bacteriostasis test of BYC17 was carried on. The active component was separated and purified after screening, and the structure of the active monomer compound was determined by spectral analysis. Finally, the bacteriostatic effect of the active monomer compound was tested.

Results: BYC17 was identified as *Streptomyces showdoensis* with antimicrobial activity against all three test bacteria. The monomer compound BYC17-01 was isolated from BYC17 and identified as izumiphenazine A. Under the concentration of 90 μg/6 mm filter paper, the inhibition zones of the monomer compound BYC17-01 against *Staphylococcus aureus*, *Escherichia coli* and *Micrococcus tetragenus* were 13.0, 9.0 and 11.1 mm respectively.

Conclusions: This study demonstrates that izumiphenazine A produced by strain BYC17 hold the potential to be used against various human pathogenic microorganisms, particularly *S. aureus* and *M. tetragenus* .

Introduction

Antibiotics are often used to treat infections caused by tiny pathogens and make great contributions to human health. With the extensive research and utilization of antibiotics, the infection of many common diseases has been reduced and the evolution of drug-resistant bacteria has also increased [1]. However, the resistance among pathogenic bacteria to antibiotics has become more and more serious in recent years. Some pathogenic bacteria even have multi-drug resistance and cross resistance. The number of drug-resistant bacteria also shows a rapid growth trend [2]. Drug-resistance of microbial pathogens to antibiotic microbial drugs has become a global concern [3]. The emergence and spread of drug-resistant bacteria are caused by the mixing of various related conditions. Among them, many problems of drug-resistant bacteria are related to misuse or abuse of antibiotic microbial drugs and acquired drug resistance genes [4, 5]. If this problem cannot be solved as soon as possible, then we will probably enter the “life of post antibiotics” in which no medicine is available [6]. In order to avoid damages to human health, it is an important way to find drug-derived compounds with new structural characteristics or new functional characteristics to deal with rapid changes of pathogenic microorganisms. During the long-term

evolution with the host, insect-associated microbes have special metabolic pathways and are valuable resource base for the research and development of new antibiotics. Therefore, lots of antibacterial active substances from insect-associated actinomycetes is one of the current methods to resist drug resistance of pathogenic microorganisms [7].

The *Odontotermes formosanus* lives in a dark, humid and sealed environment, but it is safe and sound. Termite-termitarium association is essential and momentous. The *O. formosanus* has formed a unique self-defense mechanismin the long-term evolution. Studies have shown that the MV32^T was isolated from intestinal tract of south African termite *Amitermes hastatus* actinomycetes, its had good antibacterial activity against *Mycobacterium aurum* A+ [8]. The strain FSPNRU 102 that isolated from termite gut is belonging to *Streptomyces niveoruber* and it produced bioactive compound with broad spectrum activities against Gram-positive (such as *Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (such as *Pseudomonas aeruginosa* and *Escherichia coli*) bacteria [9]. Microtermolides A and the known antibiotic vinylamycin have similarity in their structures, which was isolated from a *Streptomyces* sp. strain associated with fungus-growing termites [10]. Natalamycin A, a compound isolated from termitarium actinomycete M56 in Natal, South Africa, has good inhibitory activity on termite toadstools [11]. In this experiment, we identified strain BYC17 from termitarium of *O. formosanus*, determined antibacterial activities against various human pathogenic microorganisms. Further tracing and isolating the active components of the active strains and identifying their structures are aimed at finding novel antibacterial lead compounds and laying a certain foundation for the creation of new antibiotics.

Materials And Methods

Identification of strain BYC17

The strain BYC17 was inoculated in Gao's No. 1 solid medium and cultured at 28 ℃ [12]. The external morphological characteristics of the colony were observed on time. The strain BYC17 was identified by 16S rDNA gene sequencing followed by a sequence similarity search [13]. 16S rDNA gene was amplified using the universal eubacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [14]. Each 50 μL reaction volume contained 10 pmol genomic DNA, 10 × PCR Buffer reaction buffer 5 μL, dNTP 1 μL, 1μL each primer, and 0.25 μL Taq DNA polymerase. The PCR reactions were performed in Proflex PCR System using the following reaction conditions: pre-denaturation at 95 ℃ for 5 min, followed by 30 cycles at 94 ℃ for 1 min, 54 ℃ for 1 min, 72 ℃ for 1 min 30 s, and final extension at 72 °C for 10 min [15]. The amplified fragments were analyzed by agarose gel electrophoresis and further purified. The purified amplicons were sent to Shanghai Shenggong Company for sequencing. The pairwise sequence alignment of 16S rDNA gene sequence was done and compared with the other related *Streptomyces* spp. Retrieved from EzBioClud server. Neighbor joining method was used to construct phylogenetic tree based on bootstrap values (1000 replications with MEGA6 software) [16, 17].

Antibacterial activity of the crude extract of BYC17

The fresh mycelium of the purified strain BYC17 cultivated on Gao's No.1 medium at 28 ± 0.5 ℃ was inoculated into 250 mL Erlenmeyer flasks each containing 100 mL of Gao's No. 1 liquid medium followed by a 7-day's incubation at 28 ± 0.5 ℃ on a rotary shaker with an agitation for 180 rpm, the bacteria were filtered with gauze and the fermentation broth was retained. According to the literature [18], the filter paper method of agar diffusion method was used to determine the inhibitory activity of the crude extract against three pathogenic bacteria.

Extraction of active compound

The seed solution was done by carrying out fermentation in Erlenmeyer flasks (250 ml), containing 100 ml of Gao's No. 1 liquid medium inoculated at 28 ℃ for 7 days under agitation at 180 rpm. Then it was fed into a 1000 mL Erlenmeyer flasks containing 200 mL of Gao's No. 1 liquid medium, and fermented in a rotary haker at 28 ℃ for 8 days at 180 rpm, with a total fermentation of 16 L. The fermentation broth was filtered with 3 layers of gauze at room temperature. For the extraction of antibacterial metabolites, the supernatant was extracted three times with ethyl acetate using solvent-solvent extraction technique. The separated organic phase was concentrated using the rotary evaporator and redissolved in respective solvent.

Purification and structure elucidation of active compound

For the analysis of antibacterial metabolites, the ethyl acetate extract was separated by thin layer chromatography (TLC) using dichloromethane: Methanol=10: 1 as solvent system and the developed chromatogram was observed under UV light. To purify the antibacterial compound, ethyl acetate extract was subjected to silica gel chromatography and gel column chromatography. The column was packed with silica gel using dichloromethane as solvent and eluted step-wise with 100% dichloromethane, 100: 1, 100: 2, 100: 4, 100: 8, 100: 16, 100: 32, 100: 64 of dichloromethane: Methanol, 100% Methanol. Fraction D (CH_2Cl_2/MeOH, 100: 4) was further chromatographed over silica gel to give two subfractions (D-1 and D-2). ^{1}H NMR and ^{13}C NMR spectra were recorded with TMS used as an internal standard and the mass spectrometry (MS) spectra were obtained to identify the structures of the secondary metabolites.

Antibacterial activity of purified compound

The antibacterial activity of the purified compound was assayed using the agar diffusion method [18]. The zones of inhibition were measuredin millimetres and gentamicin sulfate as positive control.

Results And Discussion

Identification of strain BYC17

The colony of strain BYC17 was rapid growing, dry, powdery substance, brown-white; villous hyphae on the edge, mycelium yellowish brown; tight texture, close to medium growth. In comparison of cultural

characteristics to those of actinomycetic species described in previous literature [19], it suggested that strain BYC17 belonged to the genus *Streptomyces*. A 1404 base pairs (bp) strain of the16S rDNA gene was amplified from the strain BYC17. The BLAST matching analysis from EzBioCloud showed the 16S rDNA gene sequence of the strain had a high similarity (99.64%) to that of *Streptomyces showdoensis* (accession number: AB184389). This is also reflected in the phylogenetic tree where the BYC17 strain showed at the same branch as *S. showdoensis* (**Fig. 1**). Therefore, the BYC17 strain was identified as *S. showdoensis.*

It was reported that the actinobacteria could be introduced to termite comb from surrounding soil [20, 21]. Strain BYC17 was mainly relevant with actinobacteria from soil. We speculated that strain BYC17 isolated from termites nest may come from the surrounding soil, and then compare the difference between termites nest actinomycetes and actinomycetes in the surrounding environment is an important breakthrough point to understand the specificity of termites nest [22].

Antibacterial activity of the crude extract of BYC17

BYC17 strain displayed antibacterial activity with varying degree of inhibition against all three test bacteria. Under concentration of 90 µg/6 mm filter paper, the diameter of inhibition zones (ZOI) of BYC17 strain on *S. aureus*, *E. coli* and *M. tetragenus* were 13.2, 8.0, 9.4 mm respectively (**Fig. 2**). Moreover, thin layer chromatography (TLC) results showed that BYC17 strain had various secondary metabolites and might be easy to isolate and purify. Accordingly, strain BYC17 was suitable for further studies.

Isolation and structural identification of active compound from strain BYC17

The structure of the bioactive compound was elucidated using the silica gel column chromatography and the gel column chromatography. We used gel chromatography to carry out fine separation. The eluent was eluted with dichloromethane: methanol (100: 4) polarity and recrystallized to obtain the compound BYC17-01. The monomer compound BYC17-01 was red amorphous powder, dissolved in dimethyl sulfoxide. We tested its ^{1}H NMR (**Fig. 3**) and ^{13}C NMR (**Fig. 4**). The spectral data of purified compound confirmed the presence of various functional groups such as hydroxyls, alkenes, carbonyl groups which are the characteristics of compound. The compound was characterized by spectral data and comparisons with reference [23]. We found that its spectral data were basically consistent with izumiphenazine A. Therefore, BYC17-01 was identified as izumiphenazine A based on the following evidences and its structure was shown in **Fig. 5**.

^{1}H NMR (DMSO-*d*6) *δ*: 3.69 (1H, dd, 7.2Hz14.8 Hz), 4.19 (1H, dd, 6.5 Hz14.8 Hz), 4.80 (1H, m), 5.34 (1H, dd, 3.3 Hz 6.1 Hz), 5.59 (1H, dd, 6.1 Hz10.4 Hz), 6.33 (1H, d, 3.3 Hz), 7.11 (1H, d, 7.9 Hz), 7.32 (1H, d, 7.7 Hz), 7.38 (1H, d, 7.9 Hz), 7.59 (1H, t, 7.9 Hz), 7.71 (1H, d, 7.7 Hz), 7.81 (1H, t, 7.7 Hz), 8.12 (1H, s), 10.30 (1H, s), 10.77 (1H, s), 14.34 (1H, brs); ^{13}C NMR (DMSO-*d*6) *δ*: 33.3, 39.1, 39.2, 39.4, 39.5, 39.6, 39.8, 39.9, 71.5, 88.6, 111.6, 111.7, 118.0, 118.3, 130.5, 131.2, 137.3, 138.7, 142.2, 149.7, 153.1, 153.4, 153.5, 158.7, 165.7.

Antibacterial activity results of monomer compound BYC17-01

Under the concentration of 90 μg/6 mm filter paper, monomer compound BYC17-01 had certain inhibitory effect on the tested pathogenic bacteria. The diameter of inhibition zone (ZOI) for *S. aureus* was 13.0 mm, which was slightly weaker than the positive control gentamicin sulfate, with ZOI of 19.2 mm. BYC17-01 had weak activity against *E. coli* and *M. tetragenus* and the ZOI was less than 12.0 mm (**Table 1**).

We found *S. showdoensis* has been reported in the literature that maleimycin is a bicyclic maleimide antibiotic, it inhibits *E. coli*, *S. aureirs*, *Microbacterium phlei*, and leukemia L-1210 cells [24]. At present, the research on the activity of izumiphenazine A is still less and needs further research.

Conclusions

In conclusion, strain BYC17 was identified as *S. showdoensis* by 16 s rDNA sequence analysis, and had antibacterial activities against all three tested bacteria. The ethyl acetate extract of BYC17 had good bacteriostatic effect on *S. aureus* with ZOI of more than 13.0 mm. The ethyl acetate extract also had some inhibitory activity on *E. coli* and *M. tetragenus* with ZOI of more than 8 mm. An active compound BYC17-01 was obtained from the ethyl acetate extract of fermentation broth by silica gel adsorption chromatography and thin layer chromatography, and identified as izumiphenazine A. The compound BYC17-01 has good bacteriostatic effect on *S. aureus* and *M. tetragenus* with ZOI of more than 11 mm, which was slightly weaker than the positive control. There has not been a report on bacteriostatic activity of izumiphenazine. Therefore, the strain BYC17 has the potential to develop into microbial antibiotic, which is worthy of further study. As for the safety of the strain BYC, the mechanism of active action and the identification of other active compounds, further research is needed.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

YL-Z designed the research. YL-Z supervised the study. W-C, J-X, JL-L, YW Z, ZD H and CP-Y performed the experiments and analyzed the data. W-C and J-X wrote the manuscript. All authors revised the manuscript and approved the final version for submission.

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Table 1

Table 1 Inhibition zones diameter of inhibiting effect of BYC17-01 on three pathogenic bacteria (mm).

Pathogens	BYC17-01	Gentamycin sulfate
S. aureus	13.0 ± 1.2	19.2 ± 2.8
E. coli	9.0 ± 0.8	24.5 ± 1.6
M. tetragenus	11.1 ± 1.0	42.0 ± 3.2

Figures

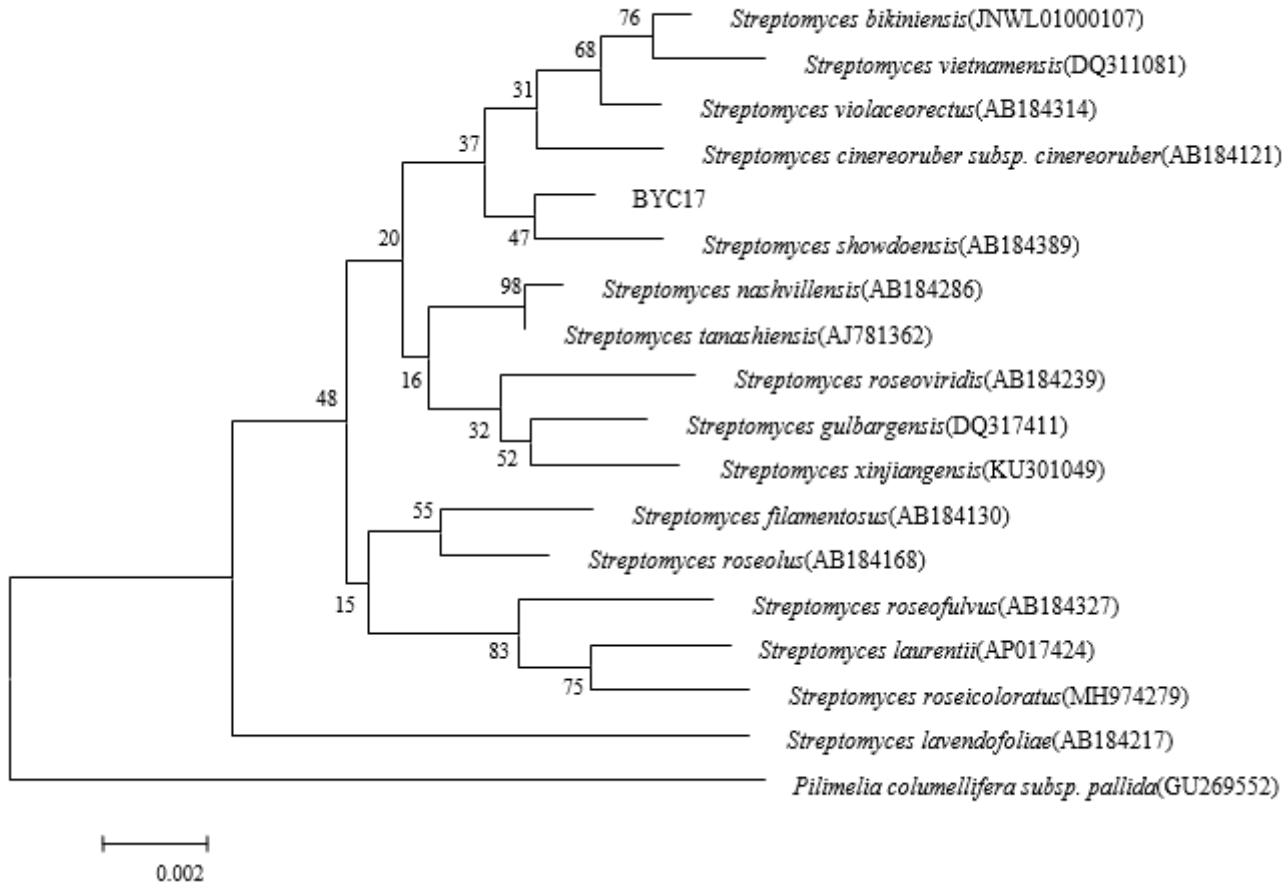

Figure 1

Phylogenetic tree based on the16S rDNA gene sequences of BYC 17 and Streptomyces related strains.

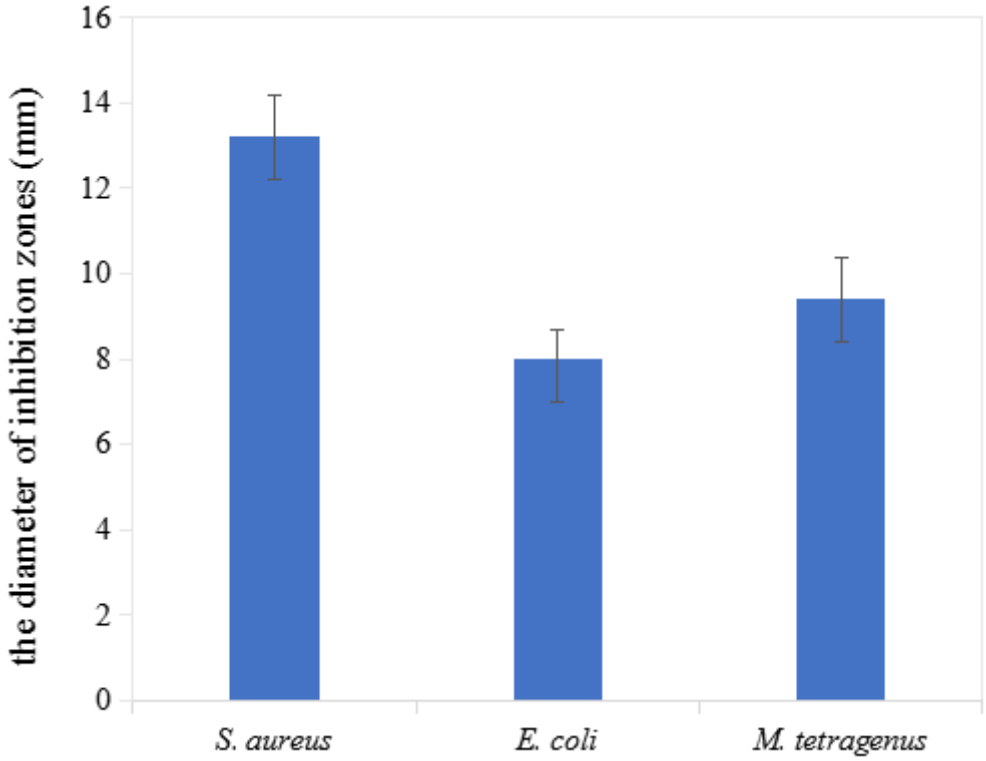

Figure 2

Inhibition zones diameter of inhibiting effect of BYC17 on three pathogenic bacteria (mm).

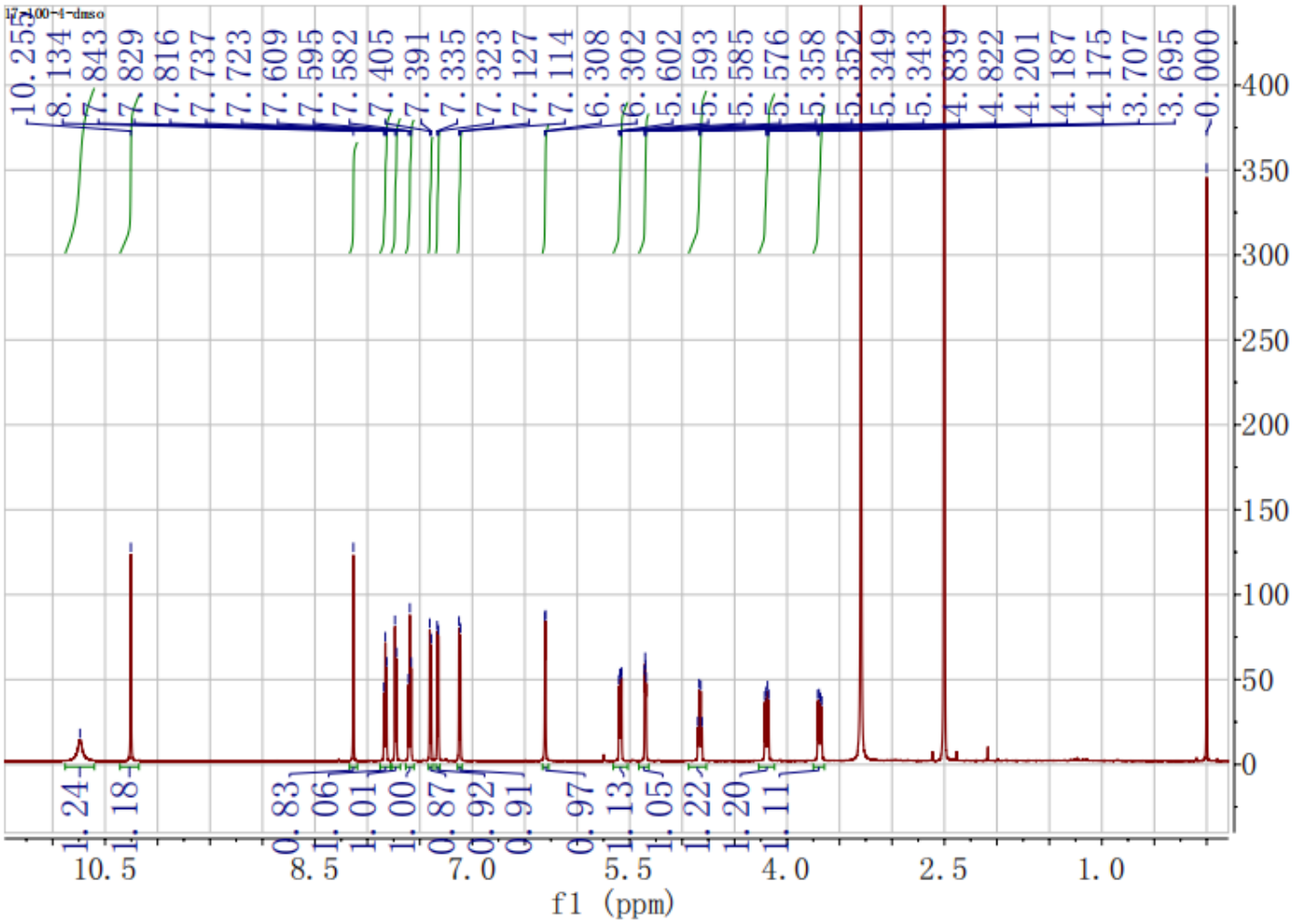

Figure 3

The 1H NMR of BYC17-01 in DMSO-d6.

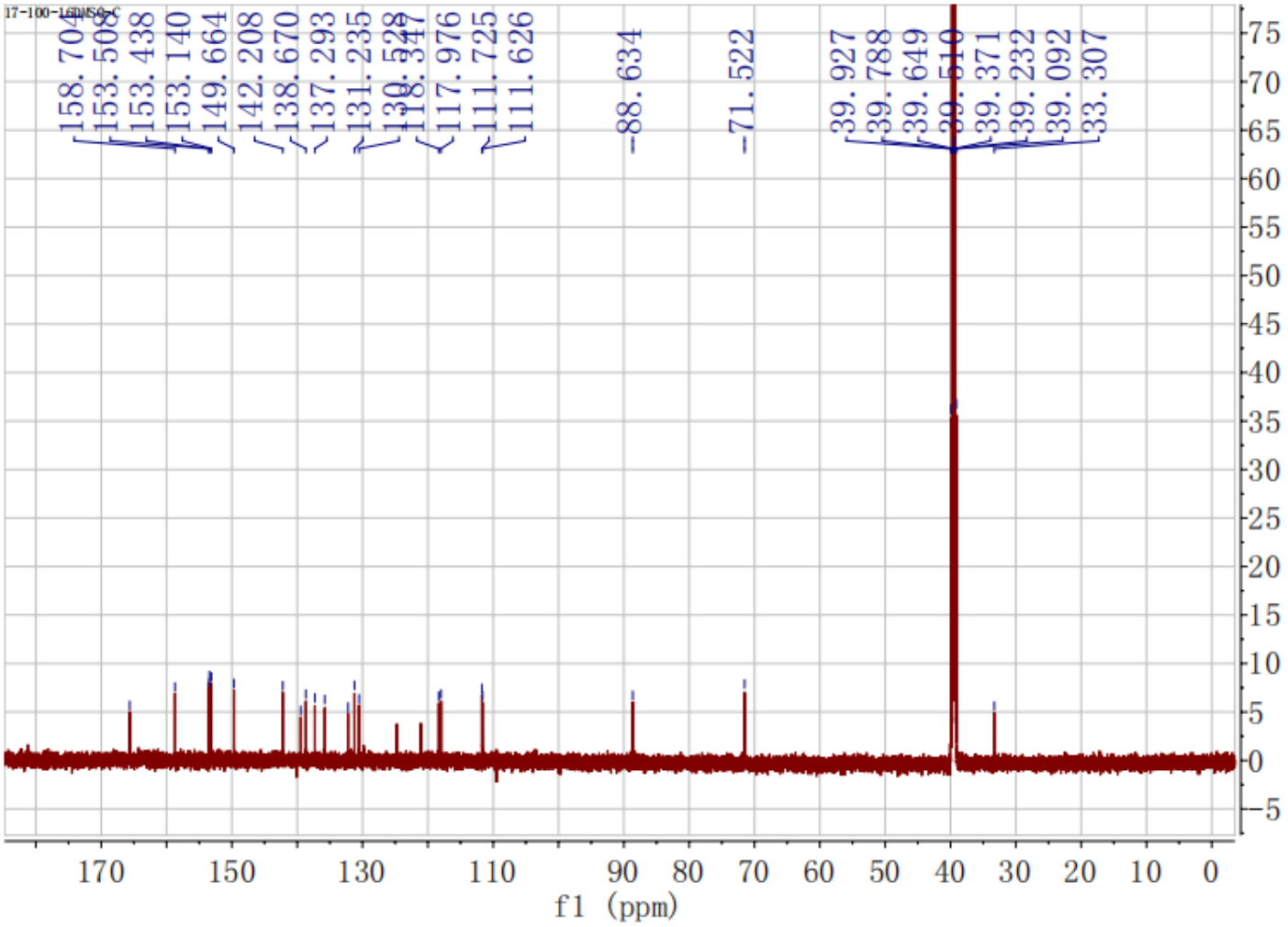

Figure 4

The 13C NMR of BYC17-01 in DMSO-d6.

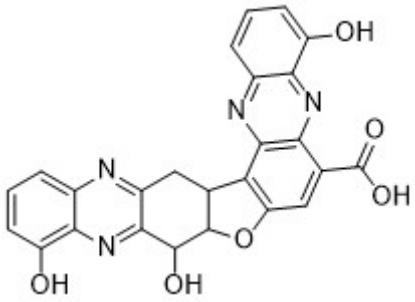

Figure 5

The molecular structure of BYC17-01.