

ORIGINAL ARTICLE

Gilvocarcin HE: a new polyketide glycoside from *Streptomyces* sp

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A new antimicrobial polyketide glycoside (1) and three known compounds, gilvocarcins H (2), V (3) and M (4) were isolated from the ethyl acetate extract of *Streptomyces* sp. QD01-2. The structure of (1) was established by spectroscopic analysis, X-ray single crystal diffraction and CD spectra. The new compound (1) showed potent antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*, with MIC values of 0.5–5.0 μM . Compound (1) also showed weak cytotoxicity against the MCF-7, K562 and P388 cell lines, with IC₅₀ values of 36, 39 and 45 μM , respectively. The results showed that the vinyl side chain helps to increase the cytotoxicity and antimicrobial activities of gilvocarcin-type glycosides. In addition, the NMR data of gilvocarcin H (2) were also reported for the first time.

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Keywords: antimicrobial activity; cytotoxicity; gilvocarcin HE; polyketide glycoside; *Streptomyces*

INTRODUCTION

Producing 13 000 bioactive metabolites described, cultured bacteria have been a prolific resource for drug discovery.¹ About 70% of these bioactive compounds have been isolated from cultured actinomycetes.² Among them, polyketide metabolites represent a major source for novel chemotherapeutics.³ A particularly promising lead structure is gilvocarcin V (3), the prototype of a family of benzo[*d*]naphtha[1,2-*b*]pyran-6-one glycosides from *Streptomyces* spp.^{4–8} Members of this family generally exhibit high antimicrobial and antitumor activities.⁹ Structurally these gilvocarcin-type polyketide glycosides differ in the nature of the sugar moiety and the side chain at C-8.^{10–14} As part of our ongoing search for bioactive secondary metabolites from actinomycetes,^{15–17} strain *Streptomyces* sp. QD01-2 was isolated from the 3-m-deep soil collected at Huiquan Square near Qingdao First Beach, China. Its secondary metabolites showed antibacterial activity against *Staphylococcus aureus* at a concentration of 100 $\mu\text{g ml}^{-1}$. And a series of peaks with the similar UV absorptions to those of gilvocarcin-type polyketide glycosides^{4–8} were observed at λ_{max} 242, 274, 314 and 386 nm in the HPLC-UV profile (Supplementary Figure S7). Chemical investigation resulted in the isolation of a new gilvocarcin, gilvocarcin HE (1), along with the known gilvocarcins H (2),¹⁸ V (3),⁵ and M (4) (Figure 1).⁵ New compound (1) displayed significant antimicrobial activities against *S. aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*, with MIC values of 0.5, 2.8, 5.0 and 3.6 μM , respectively. It also showed cytotoxicity against the MCF-7, K562 and P388 cell lines, with IC₅₀ values of 36, 39 and 45 μM , respectively.

RESULTS

Physico-chemical properties

Compound (1): colorless needles (MeOH); $[\alpha]_{\text{D}}^{23}$ –122 (*c* 0.15, MeOH); HR-ESI-MS *m/z* 513.1757 [M+H]⁺ (calculated for C₂₇H₂₉O₁₀, 513.1761); UV (MeOH) λ_{max} (log ϵ) 242 (2.51), 244 (2.46), 271 (1.80), 274 (1.94), 314 (0.73), 386 (0.71) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 220 (+0.3), 243 (–2.3), 300 (+0.3) nm; IR ν_{max} (KBr) 3398, 2926, 2856, 1685, 1651, 1619, 1457, 1433, 1371, 1306, 1247, 1166, 1083, 1053 cm^{–1}; ¹H and ¹³C NMR (see Table 1).

Compound (2): yellow amorphous powder; $[\alpha]_{\text{D}}^{23}$ –208 (*c* 0.15, MeOH); ESI-MS 499[M+H]⁺; ¹H and ¹³C NMR (see Table 1, Supplementary Figures S1 and S2).

Compound (3): yellow amorphous powder; $[\alpha]_{\text{D}}^{23}$ –205 (*c* 0.15, MeOH); ESI-MS 495[M+H]⁺; CD (MeOH) λ_{max} ($\Delta\epsilon$) nm 220 (+4.6), 243 (–1.5), 303 (+0.3); ¹H and ¹³C NMR (see Table 1, Supplementary Figure S8).

Biological activity

The antimicrobial activities of these four compounds were evaluated by an agar dilution method.²⁰ The results showed that compounds 1–4 displayed antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* (Table 2), while compounds 1–4 were inactive against *Aerobacter aerogenes* and *Pseudomonas aeruginosa* (MIC > 100 μM). Compounds (1) and (3) are more active than compounds (2) and (4), and (2) is more active than (4), indicating that both unsaturation and oxidation at the side chain result in an increase of antimicrobial activities of gilvocarcin-type glycosides.

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Table 1 ^1H -NMR and ^{13}C -NMR data for 1–3 (600, 150 MHz, $\text{DMSO-}d_6$, TMS, δ p.p.m.)

Position	1		2		3	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	152.4 s		152.4 s		152.3 s	
2	112.4 d	6.93 (1H, d, 8.2)	112.2 d	6.92 (1H, d, 8.2)	112.5 d	6.91 (1H, d, 8.2)
3	129.5 d	8.05 (1H, d, 8.2)	129.6 d	8.04 (1H, d, 8.2)	129.5 d	8.04 (1H, d, 8.2)
4	126.7 s		126.5 s		126.7 s	
4a	124.3 s		124.3 s		124.2 s	
4b	142.7 s		142.6 s		142.8 s	
6	160.4 s		160.2 s		160.2 s	
6 ^a	122.4 s		122.3 s		117.6 s	
7	118.1 d	7.96 (1H, s)	118.9 d	7.90 (1H, s)	122.7 d	7.89 (1H, s)
8	150.5 s		142.6 s		139.1 s	
9	115.7 d	7.59 (1H, s)	115.3 d	7.53 (1H, s)	115.3 d	7.63 (1H, s)
10	157.5 s		157.5 s		157.9 s	
10-OCH ₃	57.2 q	4.13 (3H, s)	57.1 q	4.08 (3H, s)	56.7 q	4.05 (3H, s)
10a	122.5 s		122.3 s		123.4 s	
10b	113.6 s		113.6 s		113.4 s	
11	102.1 d	8.47 (1H, s)	102.1 d	8.42 (1H, s)	101.8 d	8.33 (1H, s)
12	153.2 s		153.2 s		153.1 s	
12-OCH ₃	56.9 q	4.11 (3H, s)	56.9 q	4.08 (3H, s)	57.2 q	4.10 (3H, s)
12a	115.3 s		115.3 s		114.9 s	
13	68.1 d	4.93 (1H, m)	62.7 t	4.67 (2H, s)	135.8 d	6.88 (1H, dd, 11.3, 17.5)
14	26.4 q	1.42 (3H, d, 6.5)			119.6 t	5.46 (1H, d, 11.0), 6.10 (1H, d, 17.5)
1'	81.4 d	6.19 (1H, d, 5.5)	81.4 d	6.16 (1H, d, 5.5)	81.4 d	6.15 (1H, d, 5.0)
2'	79.2 d	4.67 (1H, m)	79.2 d	4.66 (1H, m)	79.1 d	4.64 (1H, m)
3'	79.5 d	3.84 (1H, m)	79.4 d	3.86 (1H, m)	79.3 d	3.87 (1H, m)
4'	86.2 d	3.50 (1H, dd, 4.2, 5.8)	86.4 d	3.51 (1H, dd, 4.5, 5.0)	86.2 d	3.51 (1H, dd, 4.3, 5.0)
5'	67.0 d	3.87 (1H, m)	67.0 d	3.84 (1H, m)	66.9 d	3.84 (1H, m)
6'	20.8 q	1.24 (3H, d, 6.4)	20.7 q	1.24 (3H, d, 6.6)	20.7 q	1.25 (3H, d, 6.0)
1-OH		9.70 (1H, s)		9.69 (1H, s)		9.65 (1H, s)
13-OH		5.53 (1H, d, 5.0)		5.57 (1H, brs)		
2'-OH		4.52 (1H, d, 6.6)		4.51 (1H, d, 6.6)		4.50 (1H, d, 6.6)
3'-OH		5.11 (1H, d, 4.9)		5.12 (1H, d, 5.0)		5.11 (1H, d, 5.0)
5'-OH		4.84 (1H, d, 4.9)		4.87 (1H, d, 5.0)		4.87 (1H, d, 5.0)

Abbreviations: brs, broad singlet; d, doublet; dd, doublet of doublets; m, multiplet; s, singlet.

Table 2 The MIC values of compounds 1–4 against pathogens (μM)

Compound	1	2	3	4
<i>Staphylococcus aureus</i>	0.5	0.5	0.1	1.5
<i>Escherichia coli</i>	2.8	5	3	5
<i>Bacillus subtilis</i>	5.0	20	1.5	> 50
<i>Candida albicans</i>	3.6	25	4	25

Table 3 The IC₅₀ values of compounds 1–4 against three tumor cell lines (μM)

Compound	1	2	3	4
MCF-7	36	38	1.8	17
K562	39	31	0.8	15
P388	45	35	1.1	21

The cytotoxicities of compounds 1–4 were also evaluated against MCF-7, K562 and P388 cell lines by the MTT method.²¹ The results showed that compound (3) was the most active against the tested tumor cell lines, indicating that the vinyl side chain might have an important role in cytotoxicity of gilvocarcin-type glycosides (Table 3).

Structure determination

Compound (1) was obtained as colorless needles with the molecular formula of $\text{C}_{27}\text{H}_{28}\text{O}_{10}$ from the HR-ESI-MS peak at m/z 513.1757 $[\text{M} + \text{H}]^+$. Its UV spectrum showed characteristic peaks of gilvocarcin-type polyketide chromophore at λ_{max} 242, 274, 314 and 386 nm.^{4–8} The IR spectrum indicated that (1) possesses hydroxyl

(3398 cm^{-1}), carbonyl (1685 cm^{-1}), aromatic system (1619, 1589 cm^{-1}) and ether groups (1166 cm^{-1}). The ^1H NMR spectrum showed 28 proton signals including five aromatic protons at δ_{H} 8.47~6.93, two methoxy protons, two methyl protons and five oxygenated methine protons at δ_{H} 6.19~3.50 (Table 1, Supplementary Figure S1). The ^{13}C NMR spectrum displayed 27 carbon signals (Supplementary Figure S2) that were classified by DEPT (Supplementary Figure S3) and HMQC (Supplementary Figure S4) spectra as two methoxy carbons, two methyl carbons, five olefinic methine carbons, six oxygenated methine carbons, 11 olefinic quaternary carbons (four oxygenated) and one conjugated ester carbonyl carbon. These NMR data were very similar to those of gilvocarcin V,⁵ further indicating the nature of gilvocarcin-type

polyketide glycoside of (1). Compared with those of gilvocarcin V, the NMR differences of (1) embodied in the replacement of $-\text{CH}=\text{CH}_2$ signals by $-\text{CH}(\text{OH})\text{CH}_3$ signals that was further supported by $^1\text{H}-^1\text{H}$ COSY correlations between H_3-14 (δ_{H} 1.42) with $\text{H}-13$ (δ_{H} 4.93) and $\text{H}-13$ with $\text{HO}-13$ (δ_{H} 5.53) (Supplementary Figure S5). Furthermore, obvious downfield shifts for C-8, C-6a and H-7 and upfield shifts for C-7 and H-9 were observed between (1) and gilvocarcin V. These observations suggested that compound (1) is the hydrated derivative of gilvocarcin V at the C-8 side chain, which was further supported by the key HMBC correlations from H-7 (δ_{H} 7.96) to C-6 (δ_{C} 160.4), C-6a (δ_{C} 122.4), C-9 (δ_{C} 115.7) and C-13 (δ_{C} 168.1), from H-9 (δ_{H} 7.59) to C-8 (δ_{C} 150.5), C-10 (δ_{C} 157.5), C-10a (δ_{C} 122.5) and C-13, and from H_3-14 to C-8 (Figure 2, Supplementary Figure S6). Single crystal X-ray diffraction analysis further confirmed the structure (Figure 3), and its relative configuration was also resolved. As the absolute configuration of gilvocarcin V had been resolved by total synthesis,¹⁹ the absolute configuration of fucose moiety of compound (1) could be deduced from the

comparison of CD spectra between (1) and gilvocarcin V (3). Thus, the CD curves of (1) and gilvocarcin V (3) were measured in MeOH. Compound (1) exhibited CD cotton effects at 220 nm ($\Delta\epsilon$ +0.29), 243 nm ($\Delta\epsilon$ -2.3) and 300 nm ($\Delta\epsilon$ +0.29), similar to those of (3) at 219nm ($\Delta\epsilon$ +4.6), 254 nm ($\Delta\epsilon$ -1.5) and 303 nm ($\Delta\epsilon$ +0.25) (Figure 4), indicating D-fucose (that is, 1'R, 2'R, 3'R, 4'S, 5'R). According to the X-ray crystal structure, the absolute configuration of C-13 was therefore determined as S-. Consequently, the structure of gilvocarcin HE (1) was elucidated to be 4-[(2R,3R,4R,5S)-3,4-dihydroxy-5-(1S-hydroxyethyl) tetrahydrofuran-2-yl]-1-hydroxy-8-(1S-hydroxyethyl)-10,12-dimethoxy-6H-dibenzo[c,h]chromen-6-one.

METHODS

General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter (JASCO Corporation, Tokyo, Japan). IR spectra were recorded using a Bruker model. UV spectra were recorded on Beckman DU 640 spectrophotometer (Beckman, Brea, CA, USA). ^1H , ^{13}C NMR and DEPT spectra and 2D-NMR were recorded on a JEOL JNM-ECP 600 spectrometer (JEOL Ltd., Tokyo, Japan) using TMS as internal standard, and chemical shifts were recorded as δ values. ESI-MS was measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer (Waters, Milford, MA, USA). Semi-preparative HPLC was performed using an ODS column (YMC-pack ODS-A, 10 \times 250 mm², 5 μm , 4 ml min⁻¹).

Strain

The *Streptomyces* sp. QD01-2 was isolated from a 3-m-deep soil sample collected at Huiquan Square near Qingdao First Beach, Shandong province of China. It was identified according to its morphological characteristics and

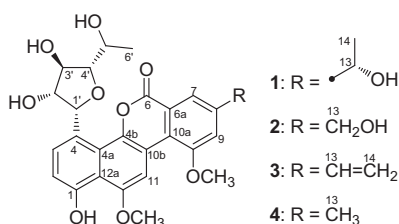


Figure 1 The structures of compounds 1–4.

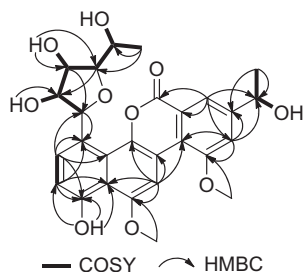


Figure 2 $^1\text{H}-^1\text{H}$ COSY and HMBC correlations of (1).

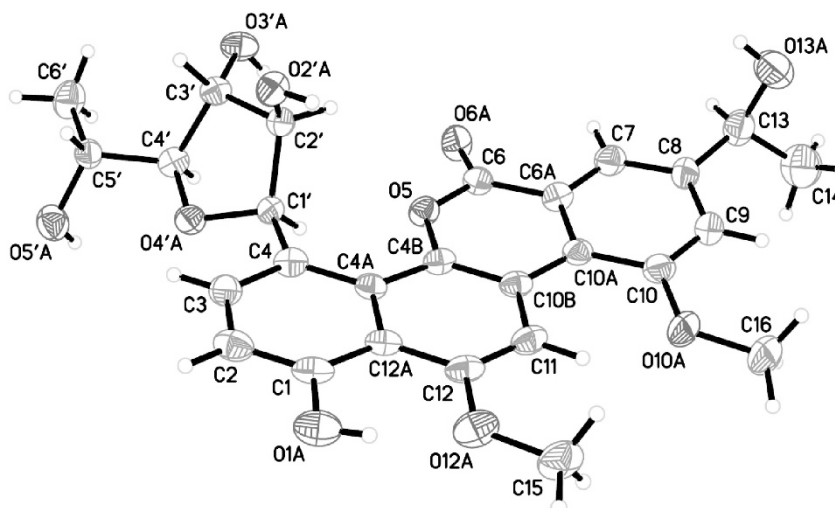


Figure 3 Final X-ray drawing of (1). A full color version of this figure is available at *The Journal of Antibiotics* journal online.

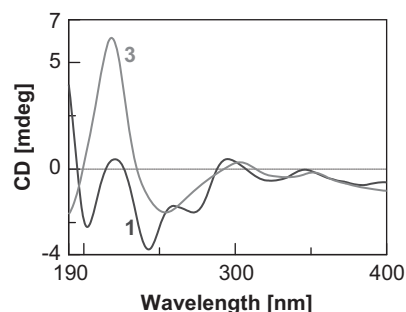


Figure 4 The CD curve of (1) (blue) and (3) (red). A full color version of this figure is available at *The Journal of Antibiotics* journal online.

16S rRNA sequences. The voucher specimen is deposited in our laboratory at -80°C . The working strain was prepared on a Gause's Synthetic Agar medium and stored at 4°C .

Fermentation

The *Streptomyces* sp. QD01-2 was incubated on a rotary shaker (180 r.p.m.) at 28°C for 8 days in 500 ml \times 200 conical flasks each containing 150 ml of a liquid medium composed of glucose (3 g), beef extract (0.45 g), amidulin (1.5 g), yeast extract (1.5 g), corn steep liquor (0.15 g), NaCl (0.075 g), MgSO_4 (0.075 g), KH_2PO_4 (0.075 g), CaCO_3 (0.3 g), adjusting its pH to 7.0.

Extraction and isolation

The fermented whole broth (80 l) was extracted three times with an equal volume of EtOAc to give an ethyl acetate solution. The ethyl acetate solution was concentrated *in vacuo* to give a crude extract (32 g). The crude extract was then subjected to vacuum liquid chromatography using step gradient elution with CH_2Cl_2 -MeOH (100 : 0, 100 : 1, 50 : 1, 30 : 1, 20 : 1, 10 : 1) to give six fractions (fractions 1–6) based on TLC properties. Fraction 4 (2 g) was washed with MeOH to give insoluble yellow amorphous powder. The yellow amorphous powder was further separated by semi-preparative HPLC (68% MeOH- H_2O) to yield (1) (4 mg, t_{R} 10.2 min) and (2) (2 mg, t_{R} 6.4 min). Fraction five (5 g) was washed with MeOH to give insoluble yellow amorphous powder that was further separated by semi-preparative HPLC (60% CH_3CN - H_2O) to yield (3) (10 mg, t_{R} 11.5 min) and (4) (18 mg, t_{R} 9.1 min).

X-ray structure determination of (1)

Compound (1) was obtained as a colorless monoclinic crystal with molecular formula $\text{C}_{27}\text{H}_{32}\text{O}_{12}$. Space group $P2(1)/c$, $a = 16.9509(17) \text{ \AA}$, $b = 4.8840(4) \text{ \AA}$, $c = 31.013(3) \text{ \AA}$, $\alpha = 90.00^{\circ}$, $\beta = 90.00^{\circ}$, $\gamma = 90.00^{\circ}$, $V = 2567.5(4) \text{ \AA}^3$, $Z = 4$, $D_{\text{calcd}} = 1.419 \text{ mg m}^{-3}$, $\mu = 0.112 \text{ mm}^{-1}$, $F(000) = 1160$, crystal size $0.45 \times 0.15 \times 0.12 \text{ mm}^3$, $T = 298(2) \text{ K}$. A total of 2661 unique reflections ($2\theta < 50^{\circ}$) were collected on a CCD area detector diffractometer with graphite monochromated MoK α radiation ($\lambda = 0.71073 \text{ \AA}$). The structure was solved by direct methods (SHELXS-97) and expanded using Fourier techniques (SHELXL-97). The final cycle of full-matrix least squares refinement was based on 2661 unique reflections ($2\theta < 50^{\circ}$) and 356 variable parameters and converged with unweighted and weighted agreement factors of $R_1 = 0.1077$, $R_w = 0.1515$ and $R = 0.0611$ for $I > 2\sigma(I)$ data. Crystallographic data (excluding structure factors) for structure (1) have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 860606. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Antimicrobial assay

The antimicrobial activities against *E. coli*, *B. subtilis*, *A. aerogenes*, *P. aeruginosa*, *S. aureus* and *C. albicans* were evaluated by an agar dilution method.²⁰ The tested strains were cultivated on LB agar plates for bacteria and on YPD agar plates for *C. albicans* at 37°C . The four compounds and positive controls were dissolved in MeOH at different concentrations from 50 to $0.05 \mu\text{g ml}^{-1}$ by the continuous 2-fold dilution methods. A $10 \mu\text{l}$ quantity of test solution was absorbed by a paper disk (5 mm diameter) and placed on the assay plates. After 12 h incubation, zones of inhibition (mm in diameter) were recorded. The MICs were defined as the lowest concentration at which no microbial growth could be observed. Ciprofloxacin was used as the positive control for *E. coli*, *B. subtilis*, *S. aureus*, *P. aeruginosa* and *A. aerogenes* with MIC values of 0.9, 1.2, 0.3, 1.9 and $3.7 \mu\text{M}$, respectively. Ketoconazole was used as the positive control for *C. albicans* with MIC values of $0.5 \mu\text{M}$.

Cytotoxicity assay

The four compounds were evaluated for cytotoxic effects on MCF-7, K562 and P388 cell lines using the MTT method.²¹ In the MTT assay, cell lines were grown in broth RPMI-1640 supplemented with 10% FBS under a humidified

atmosphere of 5% CO_2 and 95% air at 37°C . Two hundred microliter of cell suspensions at a concentration of $5 \times 10^4 \text{ cells ml}^{-1}$ were plated in 96-well microtiter plates and incubated for 24 h. Then, $2 \mu\text{l}$ of the test solutions (in DMSO) were added to each well and further incubated for 72 h. The MTT solution ($20 \mu\text{l}$, 5 mg ml^{-1} in IPMI-1640 medium) was then added to each well and incubated for 4 h. Old medium containing MTT ($150 \mu\text{l}$) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader (Molecular Devices, Sunnyvale, CA, USA) at 540 nm. The IC_{50} values were obtained using the Bliss method.

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