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BRIEF COMMUNICATION

Two novel quinomycins discovered by UPLC-MS from Stretomyces sp. HCCB11876

Zhijun Yang[1] · Lei Shao[1] · Meixia Wang[2] · Min Rao[3] · Mei Ge[3] · Yixin Xu[1]

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Abstract

Two novel quinomycins I (**1**) and J (**3**) were discovered by UPLC-MS, then the two novel compounds and five known quinomycins A(**2**), B(**4**), E(**5**), C(**6**) and monosulfoxide quinomycin (**7**) were isolated from the culture broth of *Streptomyces* sp. HCCB11876. The structures of these compounds were elucidated through MS and NMR spectroscopic analysis. Compounds **1**–**7** showed significant antibacterial and cytotoxic activities. The structure-activity relationship indicated that sulfoxide group in *N*-methylcysteine of quinomycins (**1**, **3** and **7**) would significantly decrease the antibacterial and cytotoxic activities. Moreover, the antibacterial and cytotoxic activities were decreased with the increase of carbon chain in amino-acid residues.

Quinomycins are cyclic otctapeptides that belong to a family of quinoxaline [1]. The class of compounds showed several biological activities, reportedly being antimicrobial, antiviral, insecticidal and antitumor [2–4]. Structure-activity relationship of quinoxalines showed that the core depsipeptide was neccesary to maintain the activities, and the types of chromophores, amino acid or disulphide cross-linkage also had effect on the bioactivities [5].

As a rapid, stable and effective qualitative method, UPLC-MS analysis has become a powerful tool for natural product analysis. For instance, carnosic acid had been directly detected in a new active packaging based on natural extract of rosemary by UPLC-MS [6]. Our group also discovered a new aminopeptidase inhibitor using UPLC-MS analysis [7]. In the course of bioactive screening and UPLC-MS/MS analysis for microbial metabolites, an streptomyces strain HCCB11876 was found to produce structural analogues with strong antimicrobial activities and cytotoxicity against cancer cell lines. Two of these structural analogues were not found through SciFinder molecular search, so we started chemical invesigation of the acinomycete strain. Two novel quinomycins named I (**1**) and J(**3**) (Fig. 1a), and five known quinomycin A(**2**) [8], quinomycin B(**4**) [9], quinomycin E(**5**) [9, 10], quinomycin C(**6**) [8] and monosulfoxide quinomycin (**7**) [11] were discovered. Details of the analysis, isolation, structure elucidation and the antimicrobial and cytotoxic activities of these compounds are presented here.

Strain HCCB11876 was isolated from a soil collected at Moutain Dabie, Anhui Province, China. The 16sRNA sequence data were submitted to GenBank with accession No. KT354239. Its sequence was similar to that of *Streptomyces collinus* TU365 (identities: 99%) and *Streptomyces lincolnensis* strain NRRL 2936 (identities: 98%). Accordingly, stain HCCB11876 was identified at the genus level as *streptomyces* sp.

The strain was cultured in seed culture medium consisting of 2.0% glucose, 2.0% glycerin, 2.0% soluble starch, 3.0% soybean powder, 0.02% KH_2PO_4, 0.02% $MgSO_4{\cdot}7H_2O$ and pH 7.5 at 28 °C for 2 days on a rotary shaker. Then, 1.0 ml of seed suspension was inoculated into 500-ml Erlenmeyer flask each with 50 ml of fermentation medium consisting of 2.2% soybean powder, 4.0% corn starch, 0.8% glucose,

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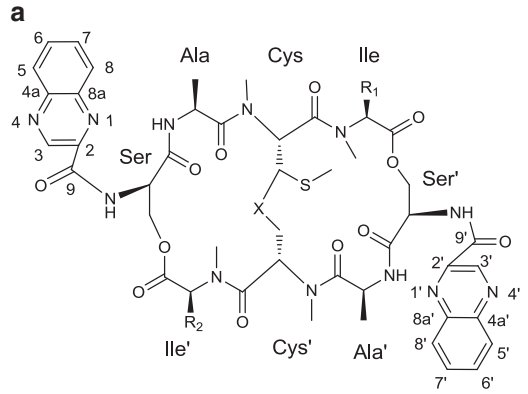
✉ Mei Ge
gemei@yeah.net

✉ Yixin Xu
Xuyx@sumhs.edu.cn

[1] School of Pharmacy, Shanghai University of Medine&Health Sciences, Shanghai, China

[2] Shanghai Normal University, Shanghai, China

[3] Shanghai Laiyi Center for Biopharmaceutical R&D, Shanghai, China



Quinomycin I(**1**) X=SO R_1= R_2=$CH(CH_3)CH_2CH_3$
Quinomycin J(**3**) X=SO R_1= R_2=$CH(CH_3)CH(CH_3)_2$
Monosulfoside quinomycin(**7**) X=SO R_1= R_2=$CH(CH_3)_2$
Quinomycin A(**2**) X=S R_1= R_2=$CH(CH_3)_2$
Quinomycin B(**4**) X=S R_1= R_2=$CH(CH_3)CH_2CH_3$
Quinomucin E(**5**) X=S R_1=$CH(CH_3)CH_2CH_3$ R_2=$CH(CH_3)CH(CH_3)_2$
Quinomycin C(**6**) X=S R_1= R_2=$CH(CH_3)CH(CH_3)_2$

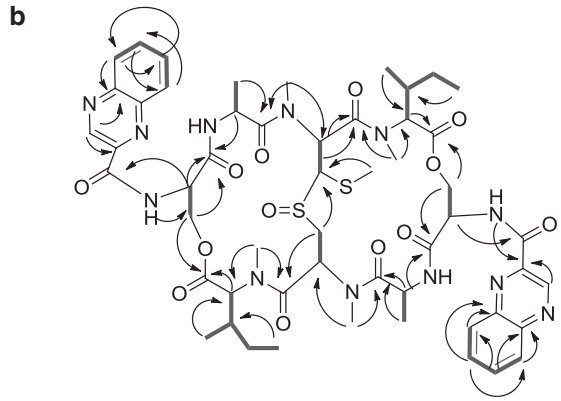


Fig. 1 **a** The structures of compounds **1**-**7**. **b** The ^{1}H-^{1}H COSY(▬) and key HMBCs(H ⟶ C)of **1**

0.02% KH_2PO_4, 0.1 % $MgSO_4{\cdot}7H_2O$, 0.2% NaCl and pH 7.5 at 28 °C for 7 days on a rotary shaker.

The whole broth was extracted with methanol for 10 h, then was centrifuged. The supernatant was extracted with ethyl acetate. The ethyl acetate extract was evaporated and dissolved in methanol, then analyzed by HPLC-DAD (Agilent SB C18, 4.6*250 mm, 5 μm, 10% acetonitrile in 0.05% Formic acid/H_2O for 5 min, gradient to 90% in 40 min, 1 ml min^{-1}) and UPLC-MS/MS (ACQUITY UPLC BEH C18, 2.1*100 mm, 1.7 μm, 5% acetonitrile in 0.2% formic acid/H_2O for 2 min, gradient to 95% in 10 min, 0.35 ml min^{-1}; MS conditions: capillary voltage 3.0 kV, sampling cone 35.0 V, source temperature 100 °C, desolvation temperature 350 °C, desolvation gas flow 600.0 L/Hr, collision energy 6.0 eV, Scan range *m/z* 100–2000, scan time 0.3 s, inter scan time 0.02 s).

Six single peaks **1**–**6** (Fig. 2a and Fig. 2b) were selected for investigation. The six substances with single-protonated molecular ions at *m/z* ($[M + H]^+$) 1145.4612, 1101.4299, 1173.4900, 1129.4680, 1143.4832 and 1157.4990, respectively. Their possible formulae might be $C_{53}H_{68}N_{12}O_{13}S_2$, $C_{51}H_{64}N_{12}O_{12}S_2$, $C_{55}H_{72}N_{12}O_{13}S_2$, $C_{53}H_{68}N_{12}O_{12}S_2$, $C_{54}H_{70}N_{12}O_{12}S_2$ and $C_{55}H_{72}N_{12}O_{12}S_2$ according to information by MassLynx software. Because of the similar UV spectra and the same fragment ions (See Supporting Information Fig. S1–S6), like *m/z* 177 [quinoxaline-2-carbaldehyde + H_3O^+] or 198 [quinoxaline-2-carboxylic acid + Na^+], 1097/1053/1125/1081/1095/1109 $[M + H\text{-}SCH3]^+$, it was demonstrated that substances **1**–**6** were structural analogues. Database (SciFinder and DNP) search results suggested that $C_{51}H_{64}N_{12}O_{12}S_2$, $C_{53}H_{68}N_{12}O_{12}S_2$, $C_{54}H_{70}N_{12}O_{12}S_2$ and $C_{55}H_{72}N_{12}O_{12}S_2$ may be quinomycin A, quinomycin B, quinomycin E and quinomycin C, respectively, while $C_{53}H_{68}N_{12}O_{13}S_2$ and $C_{55}H_{72}N_{12}O_{13}S_2$ may be two new compounds, which have never been reported.

A crude ethyl acetate extract (5.0 g) was obtained from large-scale fermentation broth (10 l) by the above method. The extract was applied to a Sephadex LH-20 column, which was eluted with $CHCl_3$/CH_3OH (10:1) to yield an active fraction of 1.0 g. The fraction was subjected to semi-prep. RP- HPLC (YMC-Pack RP-C18 column, 20 × 250 mm, 65% acetonitrile in H_2O for 40 min, 6 ml min^{-1}) to yield three sub-fractions, Frs. 2.1–2.3. The Fr. 2.1 (15.9–17.2 min, 70 mg) was further purified by RP-HPLC (10 × 250 mm, 55% acetonitrile in H_2O for 50 min, 2 ml min^{-1}) to yield monosulfoxide quinomycin (**7**, 5.0 mg; t_R 32.8 min). The Fr. 2.2 (18.8–21.6 min, 300 mg) was further purified by RP-HPLC (10 × 250 mm, 60% acetonitrile in H_2O for 50 min, 2 ml min^{-1}) to yield quinomycin I (**1**, 3.5 mg; t_R 26.6 min), quinomycin A (**2**, 70 mg; t_R 31.6 min) and quinomycin J (**3**, 6.3 mg; t_R 33.4 min). The Fr. 2.3 (23.5–25.8 min, 200 mg) was then purified by RP-HPLC (10 × 250 mm, 62% acetonitrile in H_2O for 50 min, 2 ml min^{-1}) to yield quinomycin B (**4**, 5.6 mg; t_R 33.8 min), quinomycin E (**5**, 13.0 mg; t_R 35.6 min) and quinomycin C (**6**, 25.0 mg; t_R 38.3 min).

By comparing MS spectrum and NMR spectrum of five known compounds with reported data [8–11], five compounds were determined as quinomycin A, B, E, C and monosulfoxide quinomycin, respectively.

The physico-chemical properties of compounds **1** and **3** were as follows. Quinomycin I (**1**): white powder; HRESI-MS (positive) *m/z*: 1145.4602 ($[M + H]^+$, $C_{53}H_{69}N_{12}O_{13}S_2^+$; calc. 1145.4548); $[\alpha]_D^{22} = -187$ (*c* 0.6, $CHCl_3$); UV (CH_3OH): 210 (log ε 3.43), 243 (log ε 3.66), 325 (log ε 1.85); IR (neat) ν_{max} 3377, 2970, 1653, 1512, 1082, 748 cm^{-1}. Quinomycin J (**3**): white powder; HRESI-MS (positive) *m/z*: 1173.4886 ($[M + H]^+$, $C_{55}H_{73}N_{12}O_{13}S_2^+$; calc. 1173.4861); $[\alpha]_D^{22} = -173$ (*c* 0.5, $CHCl_3$); UV (CH_3OH): 210 (log ε 3.63), 244 (log ε 3.83), 326 (log ε 1.70); IR (neat) ν_{max} 3383, 2925 1651, 1512, 1080, 748 cm^{-1}.

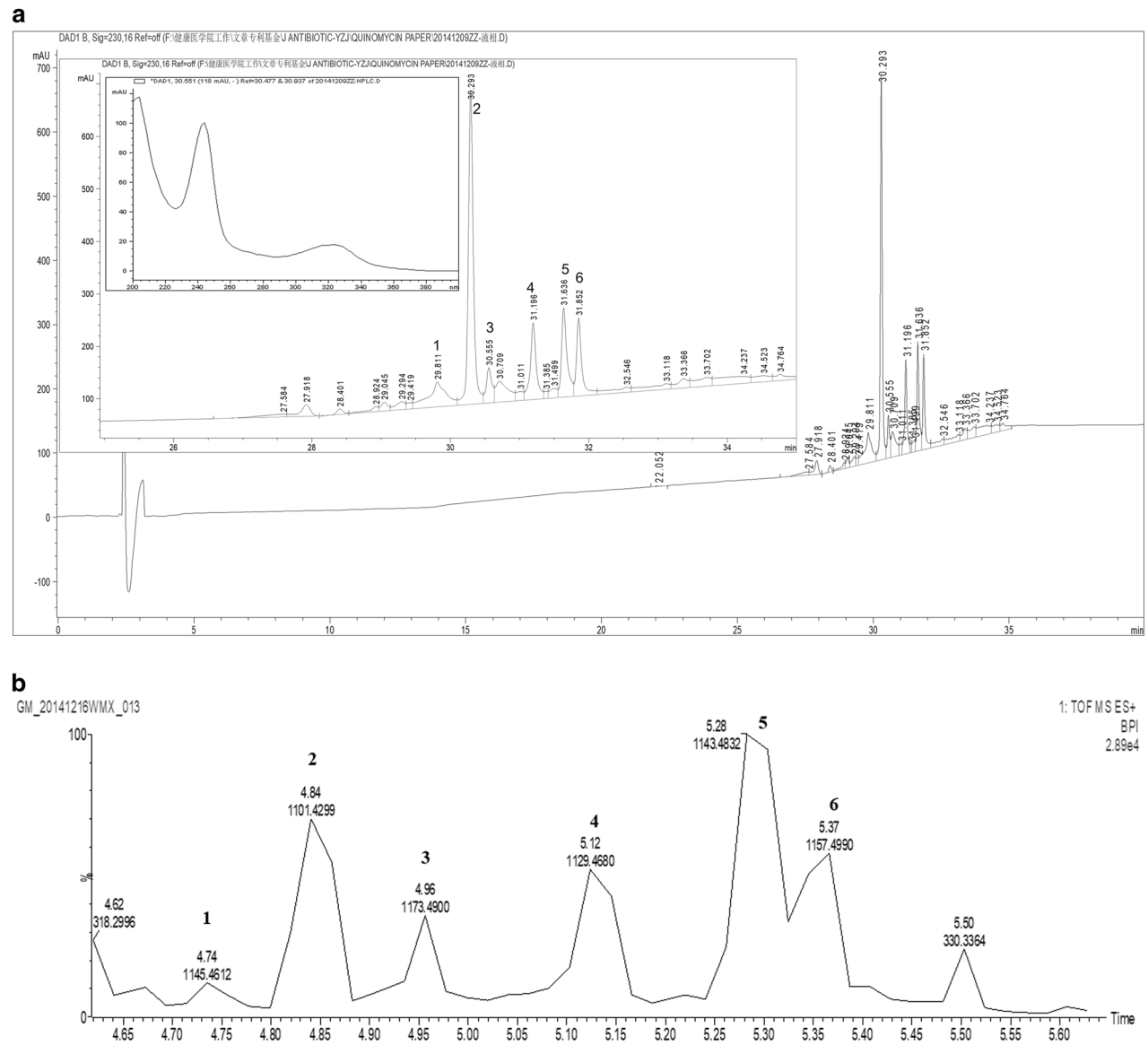


Fig. 2 **a** HPLC-DAD chromatogram of the sample. **b** UPLC-MS BPI chromatogram of the sample

The molecular formula of **1** and **3** was determined as $C_{53}H_{68}N_{12}O_{13}S_2$ and $C_{55}H_{72}N_{12}O_{13}S_2$ based on positive HRESI-MS at *m/z* 1145.4602 $[M + H]^+$ and 1173.4886 $[M + H]^+$, 28 and 56 a.m.u higher than those of monosulfoxide quinomycin (**7**), respectively. The ^1H and ^{13}C NMR data in $CDCl_3$ for **1** and **3** are summarized in Table 1. All one bond ^1H-^{13}C connectivities were confirmed by a HMQC experiment.

Comparison of the ^1H and ^{13}C NMR spectroscopic data of compound **1** with the spectra of compound **7** revealed resonances for two more sp3 secondary carbon (δ C28.6, 28.0). The COSY interactions (Fig. 1b) of H-2/H-3/H-4/H-5 and H-3/H-*3*-CH_3 in *N*-methylisoleucine (Ile and Ile') residues and strong HMBC correlations (Fig. 1b) between H-2 and C-3, C-*3*-CH_3 and C-*N*-CH_3 indicate that the amino-acid residues were *N*-methylisoleucine instead of *N*-methylvaline in **7**. The other six amino-acid residues and two molecules of quinoxaline-2-carboxylic acid (QXA) were identified by COSY and HMBC data, and comparison of the ^1H and ^{13}C NMR data with those of compound **7**. Based on these results, the structure of **1** represents a novel compound named quinomycin I (Fig. 1a). The structure of compound **1** also can be elucidated by comparison of the MS and NMR data with those of compound **4**. The data of compound **1** and **4** were almost same except for one oxygen atom and chemical shifts of two C atom, so the S atom of *N*-methylcysteine (Cys′) in compound **1** was oxidized to sulfoxide, which was demonstrated by comparison of the ^{13}C NMR chemical shifts for C-$SOCH_2$ (δ C = 51.2, *S*-oxide-*N*-methylcysteine) and C-CHS (δ C = 71.9,

Table 1 ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data for **1** and **3** in CDCl3

Position		δ_H	δ_C		Position	δ_H[a]	δ_C[b]
QXA/QXA'	2/2′*		144.3, 144.2	QXA/QXA'	2/2′*		144.3, 144.1
	3/3′*	9.65 (s, 1 H)/9.63 (s, 1 H)	143.7, 143.6		3/3′*	9.65 (s, 1 H)/9.63 (s, 1 H)	143.7, 143.5
	4a/4a'*		142.6, 142.3		4a/4a'*		142.3, 142.1
	5/5′*	7.86–7.93 (m, 2 H)	132.2, 132.0		5/5′*	7.82–7.99 (m, 2 H)	132.2, 132.0
	6/6′*	7.86–7.93 (m, 1 H) 7.81–7.84 (m, 1 H)	131.2 131.1		6/6′*	7.82–7.99 (m, 2 H)	131.2, 131.0
	7/7′*	8.18–8.21 (m, 2 H)	129.8, 129.7		7/7′*	8.18–8.20 (m, 2 H)	129.8, 129.6
	8/8′*	7.99 (d, 7.7, 1 H) 7.86–7.93 (m, 1 H)	129.4 129.2		8/8′*	7.99–8.00 (d, 7.5, 1 H) 7.82–7.99 (m, 1 H)	129.4 129.1
	8a/8a'*		140.2, 140.0		8a/8a'*		140.3, 140.1
	9/9′*		164.0, 164.2		9/9′*		164.0, 164.1
Ser	1		167.2	Ser	1*		167.2
	2	4.97–4.99 (m, 1 H)	52.4		2	4.81–4.87 (m, 1 H)	53.3
	3	4.78 (d, 11.8, 1 H) 4.62–4.65 (m, 1 H)	64.8		3	4.81–4.87 (m, 2 H)	65.0
	2-NH	8.83 (d, 6.2, 1 H)			2-NH	8.83 (d, 6.0, 1 H)	
Ser'	1		167.4	Ser'	1		167.4
	2	4.84–4.88 (m, 1 H)	53.3		2	4.96–4.98 (m, 1 H)	52.4
	3	4.69–4.72 (m, 2 H)	65.1		3	4.71–4.73 (m, 2 H)	64.9
	2-NH	8.66 (d, 7.2, 1 H)			2-NH	8.72 (d, 6.9, 1 H)	
Ala/Ala'	1		173.1(Ala), 174.3(Ala')	Ala/Ala'	1		172.8 (Ala), 174.0 (Ala')
	2*	4.84–4.88 (m, 2 H)	46.3, 46.3		2*	4.81–4.87 (m, 2 H)	46.3, 46.3
	3*	1.37–1.41 (m, 6 H)	17.7, 17.1		3*	1.37 (d, 7.0, 6 H)	17.6, 17.2
	2-NH*	6.81 (d, 5.6,1 H) 6.68 (d, 5.8, 1 H)			2-NH*	6.81 (d, 5.2,1 H) 6.73 (d, 5.1, 1 H)	
Cys	1		167.8	Cys	1		167.9
	2	6.31 (d, 10.7, 1 H)	54.4		2	6.31 (d, 10.5, 1 H)	54.5
	3	5.14 (d, 10.0 1 H)	72.6		3	5.12 (d, 9.9 1 H)	72.5
	S-Me	2.51 (s, 3 H)	19.1		*S*-Me	2.51 (s, 3 H)	19.0
	N-Me	3.05 (s, 3 H)	31.9		*N*-Me	3.05 (s, 3 H)	31.7
Cys'	1		168.4	Cys'	1		168.4
	2	5.72 (d, 10.9, 1 H)	49.6		2	5.70 (d, 8.4, 1 H)	49.6
	3	4.26 (d, 15.0, 1 H) 3.32 (t, 11.6, 1 H)	51.1		3	4.25 (dd, 14.6,5.2, 1 H) 3.33 (t, 13.7, 1 H)	51.1
	N-Me	3.16 (s, 3 H)	30.2		*N*-Me	3.16 (s, 3 H)	30.3
Ile	1		171.4	Methyl-Ile	1		171.5
	2	5.51 (d, 10.5, 1 H)	59.5		2	5.50 (d, 10.5, 1 H)	59.8
	3	2.20–2.35 (m, 1 H)	38.1		3	2.20–2.25 (m, 1 H)	37.4
	4	1.75–1.78 (m, 2 H)	28.7		4	1.76–1.77 (m, 1 H)	28.6
	5	0.90 (dd, 17.4, 6.5, 3 H)	15.9		5	0.98 (d,7.0, 3 H)	21.7
	3-Me	1.09 (d, 11.5, 3 H)	20.3		*3*-Me	0.77 (d, 7.0, 3 H)	9.9
	N-Me	3.05 (s, 3 H)	30.8		*4*-Me	0.89 (d, 7.0, 3 H)	21.7
					N-Me	3.05 (s, 3 H)	30.8
Ile'	1		170.5	Methyl-Ile'	1		170.7
	2	5.14 (d, 9.9, 1 H)	62.4		2	5.35 (d, 10.6, 1 H)	59.6
	3	2.20–2.35 (m, 1 H)	37.3		3	2.20–2.25 (m, 1 H)	37.7
	4	1.62–1.66 (m, 2 H)	28.0		4	1.76–1.77 (m, 1 H)	28.6
	5	0.86 (t, 6.5, 3 H)	14.9		5	0.89 (d, 7.0, 3 H)	15.9
	3-Me	0.98 (d, 6.5, 3 H)	21.7		*3*-Me	0.77 (d, 7.0, 3 H)	9.9
	N-Me	3.05 (s, 3 H)	31.2		*4*-Me	0.89 (d, 7.0, 3 H)	15.8
					N-Me	3.05 (s, 3 H)	31.2

*unable to be assigned

N,*S*-dimethylcysteine) of **1** with those for corresponding C-atoms (δ (C) 51.2 and 72.5, respectively) of the model compound monosulfoxide quinomycin [11].

The structure of compound **3** was similar with compound **7**, except for two *N*,β-dimethylleucine residues instead of *N*-methylvaline residues, which was determined by the MS and

Table 2 Biological activities of compounds **1–7**

Compound	Antibacterial *MIC* (μg/mL)					Cytotoxicity CC_{50} (μg/mL)		
	Bacillus subtilis	*Micrococcus luteus*	*Staphylococcus aureus*	*MRSA*	*Enterococcus faecalis*	A549	PANC-1	MDA-MB-231
1	1.00	0.50	2.00	16.00	1.00	105.4	145.3	95.6
2	0.01	0.01	0.06	0.50	0.06	7.2	15.5	22.8
3	2.00	1.00	2.00	32.00	4.00	125.6	186.5	113.4
4	0.03	0.03	0.12	1.00	0.12	17.8	23.2	35.4
5	0.12	0.12	0.25	2.00	0.25	35.4	47.7	56.5
6	0.06	0.06	0.25	2.00	0.25	55.0	68.4	70.0
7	0.25	0.25	0.50	4.00	0.50	32.6	63.5	69.8
Penicillin	2.00	2.00	4.00	>256	32.00	-	-	-
5-Fluorouracil	-	-	-	-	-	75.0	65.0	47.0

1D-, 2D-NMR data. Its structure was almost same as compound **6** except for one oxygen atom. The S atom of *N*-methylcysteine (Cys′) in compound **3** should be oxidized to sulfoxide, which was supported by comparison of the ^{13}C NMR chemical shifts for C-$SOCH_2$ (δ C = 51.1, *S*-oxide-*N*-methylcysteine) and C-CHS (δ C = 72.5, *N*,*S*-dimethylcysteine) of **3** with those for corresponding C-atoms (δ (C) 51.2 and 72.5, respectively) of the model compound monosulfoxide quinomycin [11]. Based on these results, compound **3** is a novel compound named quinomycin J (Fig. 1a).

Compounds **1–7** were evaluated for antibacterial and cytotoxic activities (Table 2). All compounds exhibited antibacterial and cytotoxic activities. Compound **2**, **4**, **5** and **6** showed most potent antibacterial activities with MIC values of 0.06–0.25 ml min^{-1} against susceptible *Staphylococcus aureus* and 0.50–2.00 ml min^{-1} against *MRSA*. They also displayed significant cytotoxicity with CC_{50} values less than 70.0 ml min^{-1} against A549, MDA-MB-231 and PANC-1 cell lines. While, the MICs of compounds **1**, **3** and **7** were 0.25–4.00 ml min^{-1} against susceptible *Staphylococcus aureus* and they were 4.00–32.00 ml min^{-1} against *MRSA*. They also exhibited moderate cytotoxicity with CC_{50} values of 32.6–186.5 ml min^{-1} against A549, MDA-MB-231 and PANC-1 cell lines.

Based on the analysis of antibacterial or cytotoxic potencies with the structural characteristics of **1–7**, it was found that the S atom substituted with sulfoxide in *N*-methylcysteine (**1**, **3** and **7**) would significantly decrease the antibacterial or cytotoxic activities. Moreover, the antibacterial or cytotoxic activities (e.g. MICs **5 > 6 > 4 > 2**) were decreased with the increase of carbon chain in amino-acid residues.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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