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Naphthomycin-derived macrolactams with two new carbon skeletons from endophytic *Streptomyces*†

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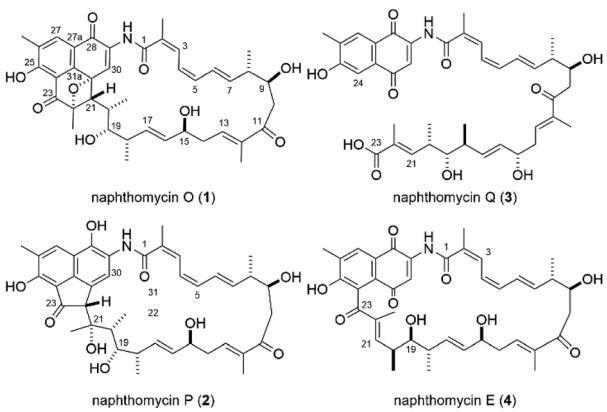
Chemical investigation of an endophytic *Streptomyces* strain led to the isolation of naphthomycin congeners **1–4**. Compound **1** contains a unique phenalene scaffold with a bridged oxetane ring, which was confirmed to be a result of an intramolecular [2 + 2] cycloaddition between the olefin and the ketone in the known compound **4** by chemical conversion. **2** features an unprecedented acenaphthene moiety in the ansamycin class of polyketides. Possible biosynthetic pathways are proposed for **1–3**. Compounds **1–3** were tested for cytotoxicity against selected human cancer cell lines and they showed moderate activity, with IC_{50} values ranging from 10.6 μM to 32.5 μM.

Introduction

Ansamycins generally comprise a rigid aromatic chromophore (naphthalene/benzene or the reduced quinone) connected to a polyketide chain, usually featuring as flexible macrocyclic lactam.[1] Based on the relatively conserved skeleton frameworks, structurally diversified ansamycins with a 3-amino-5-hydroxybenzoic acid (AHBA) moiety, which exhibit intriguing biological and pharmaceutical activities to serve as valuable antibiotics and anticancer agents, are predominantly generated from microorganisms.[1] Greatly inspired by the potential prospects of clinical applications exemplified by rifamycin-derived drugs, the knowledge to disclose the biosynthetic mechanisms within ansamycins has been continuously updated, relying on popular and extensive studies.[2] Undoubtedly, the extraordinary architectural complexity of ansamycins makes it quite challenging to afford diversity-oriented libraries through either semi-synthesis or total synthesis efforts.[3a–d] However, combinational biosynthesis originating from bioengineering[3e,f] and site-specific chemical modification and functionalization,[3g] whose targeting interferences are significantly and substantially depended on the deep exploration of related natural products, have emerged as alternative strategies. Recently, dozens of novel ansamycins with remarkable bioactive properties, such as divergolides,[4a] natalamycin,[4b] ansalactam,[4c] aminorifamycin,[4d] sporalactam,[4d] mccrearamycin[4e] and so forth, have been obtained from various terrestrial or marine natural resources.

Continued efforts on the screening of extract libraries from similar endophytes (mainly actinomycetes)[5] chemically and biologically highlighted an extract of a strain named *Streptomyces* sp. KIB-H2054 which was isolated from the plant *Campylotropis polyantha*, which had antibacterial activity against *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 8099. As a result, three new naphthomycin congeners, naphthomycins O–Q (**1–3**), which possess two new carbon skeletons, were obtained from this strain. Herein, the isolation, structure elucidation, and biological evaluation, together with the suggested biosynthetic origins of compounds **1–3**, are described.



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†Electronic supplementary information (ESI) available: Spectroscopic data, as well as the NMR and MS spectra of **1–3** and corresponding isomers **1a–3a**. See DOI: 10.1039/c8qo01107a

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Results and discussion

The molecular formula of naphthomycin O (**1**) was deduced from its HRESIMS data and was assigned as $C_{40}H_{47}NO_9$ (*m*/*z* 708.3142 $[M + Na]^+$, calcd 708.3143), corresponding to an unsaturation index of 18. The IR absorption bands of **1** at 3439 and 1634 cm^{-1} suggested the presence of hydroxyl and conjugated carbonyl groups, respectively. The ^1H and ^{13}C NMR data of **1** (Table S2†) showed 40 carbon resonances due to four carbonyl groups, nine olefinic groups, five oxygenated carbons including two quaternary carbons, and four tertiary and three secondary methyl groups. Careful analysis of the NMR and MS spectra showed that **1** seemed to be an analogue of naphthomycin E (**4**) which is a known compound and was isolated in this study too.

The gross structure of **1** was elucidated by the analysis of 2D NMR data and by comparison with the NMR data of **4**. It was obvious that **1** shared the same side chain from C-1 to C-19 as **4**, however a different aromatic chromophore existed in **1**, instead of the naphthoquinone core in **4**. The HMBC correlations from H-27 (δ_H 7.99, s) to C-25, 26-Me, C-27a, C-28 and C-31a, from H-30 (δ_H 8.16, s) to C-23, C-28, C-29, C-31 and C-31a, from 26-Me (δ_H 2.34, s) to C-25, C-26 and C-27, from H-21 (δ_H 3.32, d) to C-22, C-23, C-31, and C-31a, as well as 22-Me (δ_H 1.82, s) to C-21, C-22 and C-23 allowed the aromatic chromophore in **1** to be assigned as an oxygen-bearing phenalene core (Fig. 1, left). In addition, the HMBC correlations of NH (δ_H 8.59, s) with C-1, C-2, C-28 and C-30, and of H-20 (δ_H 2.88, m) with C-21, C-22 and C-31 (Fig. 1A and Fig. S2†), verified that the side chain of **1** was connected to the oxygenated phenalene subunit at C-29 and C-21, respectively, in the form of a macrocyclic lactam. To sum up, only one oxygen atom and one unsaturation were not accounted for in **1**. The chemical shifts at C-22 (δ_C 88.0) and C-31 (δ_C 77.8) suggested that an ether linkage, which could balance the molecular weight of one oxygen atom and one unsaturation precisely, existed between C-22 and C-31.

Next, the relative configuration of **1** was discussed (Fig. 2A and Fig. S3†). The ROESY correlations of 2-Me/H-3, H-3/H-6, H-6/8-Me and 12-Me/H-14 indicated the geometries of olefins to be 2*Z*, 4*Z*, 6*E* and 12*E*, respectively. The ^1H–^1H coupling constant between H-16 and H-17 (15.4 Hz) indicated a *trans* double bond at C-16. The stereochemistry at C-8, C-9, C-15, C-18, C-19 and C-20 in **1** is suggested to be the same as that of

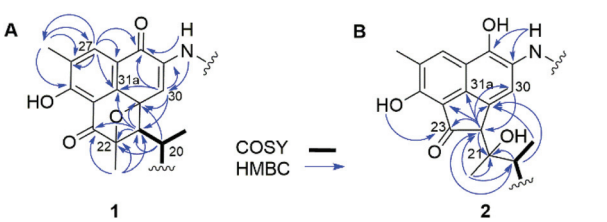


Fig. 1 Key 2D NMR correlations of naphthomycins O (**1**) (A) and P (**2**) (B).

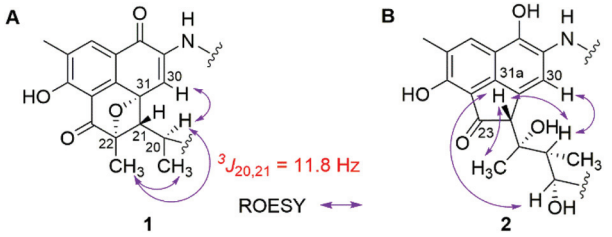


Fig. 2 Key ROESY correlations of naphthomycins O (**1**) (A) and P (**2**) (B).

naphthomycin E (**4**) on the basis of a common biosynthetic origin. The ROESY correlations between H-20 and 22-Me, together with the cross peak of H-20 and H-30, indicated that H-20 was approaching the benzocyclohexenone plane (Fig. 2A). Furthermore, the ^1H–^1H coupling constant between H-20 and H-21 (11.8 Hz) suggested that they were oppositely oriented. An additional ROESY correlation between 20-Me and 22-Me required the ether linkage between C-22 and C-31 to be located on the opposite side of the benzocyclohexenone ring. These data limited the relative configurations of C-21, C-22 and C-31 to being 21*S**, 22*S**, and 31*R**.

The elemental composition of naphthomycin P (**2**) was determined to be $C_{40}H_{49}NO_9$ by HRESIMS (*m*/*z* 710.3302 $[M + Na]^+$, calcd for 710.3300). The ^1H and ^{13}C NMR spectra of **2** had some similarities with those of **4**. A careful comparison of the NMR data between **2** and **4** reached a conclusion that **2** also possesses the same side chain from C-1 to C-19, except for a different conjugated aromatic moiety. A series of HMBC correlations from 26-Me, H-27 and H-30 to the carbons in the naphthalene ring defined the naphthalene ring moiety (Fig. S6†). The HMBC correlations (Fig. 1B) from H-22 to C-23, C-24, C-30, C-31 and C-31a, and from H-30 to C-22, C-31 and C-31a established the acenaphthene moiety in **2**. Furthermore, the HMBC correlations from H-20 to C-21 and C-22, from 21-Me to C-20, C-21 and C-22, and from H-22 to C-20, C-21 and Me-21 (Fig. 1B) demonstrated the fragment of C-19–C-20–C-21–C-22 in the macrocyclic ring, which was linked to the acenaphthene moiety at C-22. Just like **1**, the stereochemistry of **2** at C-8, C-9, C-15, C-18, C-19 and C-20 was also suggested to be the same as that of **4**, and the geometries of the olefins of **2** were resolved as 2*Z*, 4*Z*, 6*E*, 12*E*, and 16*E*, respectively, based on the relevant ROESY correlations (Fig. S6†) and the corresponding proton coupling constant. Moreover, the ROESY correlations of H-22 with H-20 and H-30 together with the ROE effect of H-22 and 21-Me indicated that H-22 and 21-OH were in opposite configurations. Another ROESY correlation between H-20 and H-30 limited the two possible configurations of C-21 and C-22 to be 21*S**, 22*R** or 21*R**, 22*S** (Fig. 2B). Ultimately, the ROESY correlation between H-19 and H-22 indicated that the configurations at C-21 and C-22 could only be 21*S** and 22*R** on the basis of the energy-minimized modeling of the simplified structure **2** (Fig. S8†).

Naphthomycin Q (**3**) was found to possess the molecular formula $C_{40}H_{49}NO_{10}$ from the HRESIMS data (*m*/*z* 726.3247 $[M + Na]^+$, calcd for 726.3249). The ^1H NMR spectra of **3** were

similar to those of **4** except for the presence of one more singlet aromatic proton at δ_H 7.63 (H-24). The ^{13}C NMR spectrum of **3** revealed one significant up-shifted carbon C-23 (δ_C 172.2) when compared to that of **4** (δ_C 202.4). Taking the above NMR variations into consideration, it could be inferred that the carbon bond between C-23 and C-24 in **4** was cleaved to access a free carboxylic acid moiety at C-23 in **3**. In this way, the 18 Dalton molecular weight variation between **4** (MW: 685) and **3** (MW: 703) could be accounted for coincidently. This deduction was also supported by the HMBC correlations of H-24 with C-26, C-28, and C-31a and of 22-Me with C-21, C-22, and C-23 (Fig. S11†).

Small amounts of compounds **1a**, **2a**, and **3a** (Fig. S1†) were also isolated, which are double bond geometry isomers of **1**, **2**, and **3**, respectively. Careful analyses of the 1D and 2D NMR data of **1a–3a** (Tables S3, S5 and S7†) showed that the geometries of the triene system were 2*E*, 4*E*, and 6*E*, determined by ROESY experiments (Fig. S5, S10 and S14†). Although we have not yet provided direct evidence for the absolute configuration of all of the new compounds, we have pictured the molecules with the same stereochemistry as that of the known compound **4** due to co-isolation from the same strain and the shared biosynthetic machinery.

Naphthomycin O (**1**) was characterized with the occurrence of an oxetane ring, which has rarely been encountered in the ansamycin class.[4] Naphthomycin O (**1**) is proposed to be derived from the major metabolite **4** through an intramolecular [2 + 2] cycloaddition of the C-21–C-22 olefin to the C-31 ketone (Scheme 1A). To confirm this conjecture, a solution of naphthomycin E (**4**) was treated with UV irradiation (254 nm), and the presence of **1** was detected by HPLC analysis (Fig. 3). This transformation not only confirmed the biosynthetic origin of the unique oxetane ring, but also supported the structure identification of **1**. To prove whether compound **1** was an artificial product during the isolation process or not, we conducted the HPLC analysis of fresh extract. We could

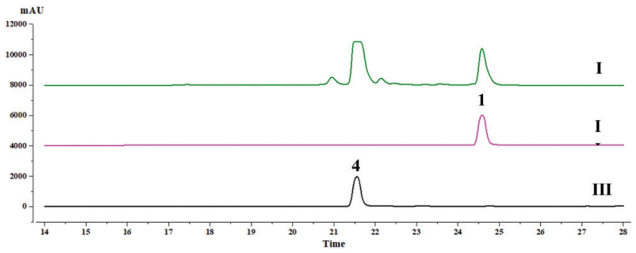
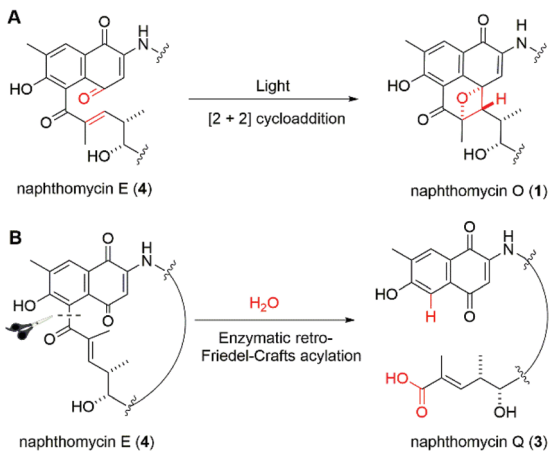


Fig. 3 HPLC analysis (detected at 236 nm) of the photocatalytic (254 nm UV-light) cycloaddition product of **4**. (I) The reaction mixture of **4**; (II) standard **1**; (III) standard **4**.

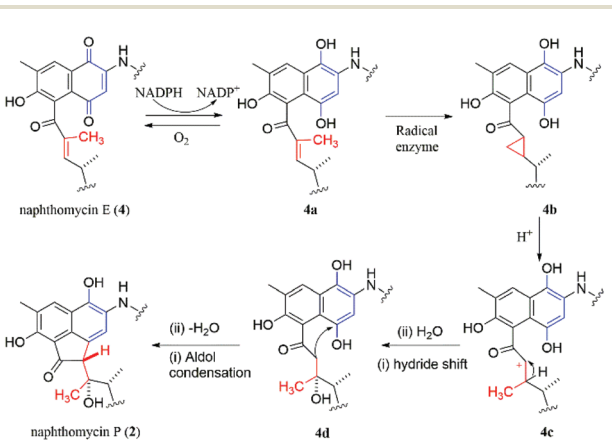
clearly detect the presence of **1**. In addition, we found that visible light could not convert **4** to compound **1** in the reactor and only ultraviolet light could do this pericyclic reaction.

Naphthomycin P (**2**) has a unique naphtho-cyclopentone moiety. Additionally, a methyl group, which is supposed to be at C-22 according to the biosynthetic route of naphthomycins,[6] was found to attach to C-21. It was recently reported that quinone moieties can be aromatized into phenols under reducing conditions in the biosynthesis of rifamycin SV.[2c] Hence, it was proposed that **2** results from a 1,4-naphthalenediol intermediate, and 22-Me is migrated through a cyclopropane intermediate **4b** because some ansamycins, such as sporalactam B and tolypomycin Y,[4d,7] were reported to encompass a cyclopropane unit previously. Cyclopropanation could be realized *via* a radical pathway initiated by radical related enzymes.[8] When the cyclopropane ring suffers in acidic conditions, a ring opening process is initiated, and subsequent aldol condensation and dehydration would generate the cyclopentone ring (Scheme 2).

Naphthomycin Q (**3**) features an open polyketide chain. Since C-23 and C-24 are derived from one acetate unit on the basis of the biosynthetic pathway of naphthomycin,[1e] it is suggested that **3** is derived from **4** by a cleavage reaction between C-23 and C-24. This type of cleavage reaction was proposed to be a Baeyer–Villiger-style oxidation in the literature, just as reported in some ansamycins with an opening poly-



Scheme 1 (A) Chemical conversion of **1** from **4** *via* a photochemical [2 + 2] cycloaddition between the double bond and the ketone; (B) proposed biosynthetic pathways of **3**.



Scheme 2 Proposed biosynthetic pathway of **2**.

ketide chain, such as proansamycin B-M1,[9a] protorifamycin I-M1,[9a] streptovaricin U[9b] and ansalactam C.[4c] However, a phenolic hydroxyl group must be generated at C-24 after the Baeyer–Villiger-style oxidation process, rather than an aromatic proton in compounds **3** and **3a**. Therefore, we prefer retro-Friedel–Crafts acylation at C-23 and C-24 in this case, which could construct a free carboxylic acid at C-23 and an aromatic hydrogen at C-24 (Scheme 1B). As mentioned above, a biomimetic chemical transformation from **4** to **3** was planned *via* the key retro-Friedel–Crafts acylation reaction, with the aid of the Lewis acid $SnCl_4$ as the literature reported.[10] Unfortunately, neither **3** nor **3a** was detected (Fig. S15†). Consequently, the retro-Friedel–Crafts acylation of **4** is more likely to be enzymatic (Scheme 1B).

Previous studies demonstrated that ansamycins have diverse bioactivities such as antitumor[11] and antibacterial activities.[12] All isolates were evaluated for their cytotoxicity against the HL-60, SMMC-7721, A-549, MCF-7, and SW480 human tumor cell lines, as well as their antibacterial activity. Compounds **1** and **2** showed broad cytotoxicities towards all five cell lines (Table S1†). However, **3** only showed inhibitory activity against the HL-60 and A549 cells (Table S1†). Besides, compound **3** was found to have weak antibacterial activity against *E. coli* ATCC 8099 with an inhibition zone of 11 mm, while the positive control kanamycin showed an inhibition zone of 18 mm. However, **1** and **2** exhibited no antibacterial activity, presumably due to the loss of the naphthalene unit.

Experimental section

General experimental procedures

Optical rotations were recorded in MeOH using a JASCO P1020 digital polarimeter. UV spectra were acquired in MeOH with a Shimadzu UV-2401PC UV-VIS spectrophotometer. IR spectra were measured on a Bruker Tensor 27 FTIR spectrometer with KBr disks. NMR spectra were recorded in $CDCl_3$ or CD_3OD using a Bruker AV 600 MHz spectrometer with TMS as an internal standard. ESIMS spectra were recorded using a Waters Xevo TQ-S Ultrahigh Pressure Liquid Chromatography Triple Quadrupole Mass Spectrometer. HRESIMS data were obtained using an Agilent 1290 UPLC/6540 Q-TOF and a UPLC-IT-TOF mass instrument. Silica gel (200–300 mesh and 300–400 mesh, Qingdao Marine Chemical Inc., China), and Sephadex LH-20 (25–100 μm, Pharmacia Biotech Ltd, Sweden) were used for the chromatograph column. Semipreparative HPLC was conducted on a HITACHI Chromaster system, equipped with a DAD detector and a YMC-Triart C_{18} column (250 × 10 mm i.d., 5 μm), and had a flow rate of 3.0 mL min^{-1}. The HPLC analysis conditions were as follows: a HITACHI Chromaster system equipped with a DAD detector, a YMC-Triart C_{18} column (250 × 10 mm i.d., 5 μm) with a mobile phase of CH_3OH–H_2O, 0–20 min: 10%–100%, 20–24 min: 100%, and 24–28 min: 10%, and flow rate of 1 mL min^{-1}.

Strain isolation and cultivation. The strain designated KIB-H2054 was isolated from *Campylotropis polyantha*, which was collected in Hailuogou, Sichuan Province, China, in 2015. It was identified as *Streptomyces* sp. by a 16S rRNA gene sequence (GenBank accession no. MH542663) and showed a 99.0% identity to the *Streptomyces nitrosporeus* strain NRRL B-1316 (GenBank accession no. NR044140.1). This strain was grown on ISP2 agar plates (glucose 4 g, malt extract 10 g, yeast extract 4 g, and agar 20 g in 1 L of water, pH 7.2) for five days at 30 °C. Then, it was inoculated into 250 mL baffled Erlenmeyer flasks containing 50 mL of sterile seed medium (tryptone soy broth, 30 g L^{-1}) and cultivated for two days at 30 °C on a rotary shaker (220 rpm). After that, aliquots (12.5 mL) of the culture were transferred into 1000 mL baffled Erlenmeyer flasks filled with 250 mL of production medium (pH 7.0) consisting of 0.1% tryptone (w/v), 3% glucose (w/v), 0.5% beef extract (w/v), 0.25% $CaCO_3$ (w/v), 0.5% NaCl (w/v), and 0.1% minor elements concentrate (v/v) (ferrous sulphate heptahydrate (1.0 g), copper sulphate pentahydrate (0.45 g), zinc sulphate heptahydrate (1.0 g), manganese sulphate tetrahydrate (0.1 g), and potassium molybdate (0.1 g), made up to 1 L with distilled water and clarified by the addition of a few drops of concentrated hydrochloric acid), and cultured on a rotary shaker (220 rpm) at 30 °C for five days.

Extraction and isolation of compounds. The fermentation broth (20 L) was centrifuged (4000 rpm, 15 min), and the supernatant was extracted with EtOAc (10 L × 3). The EtOAc extract was subsequently evaporated *in vacuo* to afford 5.0 g of oily crude extract. The mycelium was extracted with acetone (1 L × 2) and was then concentrated *in vacuo* to remove the acetone to yield the aqueous concentrate. This aqueous concentrate was finally extracted with EtOAc (1 L × 3) to give 1.0 g of solid crude extract after removing the EtOAc. Both extracts revealed an identical set of metabolites based on HPLC and TLC analyses and therefore both extracts were combined for further purification.

The crude extract (6.0 g) was fractionated by a silica gel using a PE/EA gradient (20 : 1 to 0 : 1) as the eluent to afford nine fractions (F1–F9). F5 was sequentially subjected to Sephadex LH-20 column elution with MeOH to collect four fractions (F5A–F5D). F5B was further purified by semipreparative HPLC (YMC-Triart C_{18} column, 250 × 10 mm i.d., 5 μm, 3.0 mL min^{-1}, CH_3CN : H_2O = 52 : 48, 0.1% acetic acid) to afford **1** (t_R = 22.5, 6.7 mg), **1a** (t_R = 23.5 min, 4.5 mg), **2** (t_R = 21.4 min, 11.4 mg), **2a** (t_R = 20.3 min, 3.1 mg), and **4** (t_R = 15.3 min, 60 mg). F6 was subjected to Sephadex LH-20 column elution with MeOH to collect two fractions (F6A and F6D). F6C was purified by semipreparative HPLC (YMC-Triart C_{18} column, 250 × 10 mm i.d., 5 μm, 3.0 mL min^{-1}, CH_3CN : H_2O = 50 : 50, 0.1% acetic acid) to afford **3** (t_R = 18.0 min, 24.2 mg) and **3a** (t_R = 18.7 min, 13.7 mg).

Photocatalytic reaction of naphthomycin E (4). Compound **4** (5 mg, 7.3×10^{-3} mmol) and methanol (0.02 mL) were dissolved in methylbenzene (1.0 mL). This reaction mixture was placed in a UV photochemical reactor for 30 min then the mixture was concentrated and the crude product was analyzed by HPLC (A: water; B: methanol; 0 min 10% B; 20 min 100% B; 24 min 100% B; 24.1 min 10% B; 28 min 10% B; flow rate was 1.0 mL min^{-1}).

Retro-Friedel–Crafts acylation reaction of naphthomycin E (4), with the aid of a Lewis acid, $SnCl_4$. Compound **4** (5 mg, 7.2×10^{-3} mmol) was dissolved in THF (1.0 mL). This solution was degassed with a stream of nitrogen. The reaction was then placed in an ice bath, and $SnCl_4$ was added in a dropwise manner. The ice bath was removed and the solution was refluxed at 58 °C. A small amount of the reaction solution was taken at 10, 20, 35, 50, 80 and 110 minutes. The solution was hydrolyzed by adding water, dried and evaporated to remove water and THF, and the crude product was analyzed by HPLC (A: water; B: methanol; 0 min 10% B; 20 min 100% B; 24 min 100% B; 24.1 min 10% B; 28 min 10% B; flow rate was 1.0 mL min^{-1}).

Cytotoxicity assay. Five human tumor cell lines, HL-60, SMMC-7721, A-549, MCF-7, and SW480 were used in this cytotoxicity assay, all of which were obtained from ATCC (Manassas, VA, USA). All of the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. The cytotoxicity assay was conducted by a 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymenthoxyphenyl)-2-(4-sulfopheny)-2*H*–tetrazolium (MTS) assay. Firstly, cells were seeded into each well of a 96-well cell culture plate. After cell attachment overnight, the test compound (40 μM) was added. After incubating for two days, the cells were submitted to the MTS-based assay. Formazan crystals that were formed lastly in the viable cells were measured at 490 nm using a microplate reader (BioTek Instruments, Inc. USA). Compounds with a growth inhibition rate of 50% were further evaluated at the concentrations of 0.064, 0.32, 1.6, 8.0, and 40 μM, using cisplatin as the positive controls. All of the experiments were carried out in triplicate.

Antibacterial assay. *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 8099 from the stock were inoculated into a sterile LB broth of 5 mL, each incubated for 18–24 h at 37 °C. From the overnight culture, 0.1 mL of each organism was taken and put into 9.9 mL of sterile distilled water to obtain a 10^{-2} inoculum concentration of the organism. From the diluted inoculum, 0.2 mL was put into the prepared sterile LB agar and cooled to about 40–45 °C, and was then poured into a sterile dish and allowed to solidify for about 45–60 min. Six filter papers of 6 mm diameter were placed on the LB agar evenly. 10 μL of the compounds with a concentration of 2 mg mL^{-1} was added to the filter paper, including the controls. The studies were done in triplicate to confirm the results obtained. The plates were left on the bench for about 2 h to allow the extract to diffuse properly into the LB agar. The plates were incubated for 18–24 h at 37 °C.

Naphthomycin O (1). Pale yellow solid; $[\alpha]_D^{23.2}$ = −317.4 (*c* 0.06, methanol); UV (methanol) λ_{max} (log ε) 195.5 (1.75), 231.0 (1.85), 277.0 (1.81), 316.5 (1.59), 374.5 (0.90) nm; IR (KBr) ν_{max} 3439, 2968, 2928, 1634, 1518, 1459, 1383 cm^{-1}; for ^1H, ^{13}C, and 2D NMR data, see Table S2;† ESIMS $[M + Na]^+$ *m*/*z* 708; HRESIMS $[M + Na]^+$ *m*/*z* 708.3142 ($C_{40}H_{47}NO_9$, calcd $[M + Na]^+$ 708.3143).

1a: Pale yellow solid; $[\alpha]_D^{23.2}$ = −102.0 (*c* 0.10, methanol); UV (methanol) λ_{max} (log ε) 195.0 (1.62), 229.0 (1.78), 300.0 (1.58) nm; IR (KBr) ν_{max} 3441, 2973, 2927, 1632, 1570, 1503, 1384 cm^{-1}; for ^1H, ^{13}C, and 2D NMR data, see Table S3;† ESIMS $[M + Na]^+$ *m*/*z* 708; HRESIMS $[M + Na]^+$ *m*/*z* 708.3125 ($C_{40}H_{47}NO_9$, calcd $[M + Na]^+$ 708.3143).

Naphthomycin P (2). Pale yellow solid; $[\alpha]_D^{23.2}$ = +278.6 (*c* 0.05, methanol); UV (methanol) λ_{max} (log ε) 308.0 (1.99), 226.5 (2.16), 194.5 (1.97) nm; IR (KBr) ν_{max} 3439, 3030, 2965, 2926, 2857, 1633, 1531, 1439, 1373, 1318, 1274 cm^{-1}; for ^1H, ^{13}C, and 2D NMR data, see Table S4;† ESIMS $[M + Na]^+$ *m*/*z* 710; HRESIMS $[M + Na]^+$ *m*/*z* 710.3302 ($C_{40}H_{49}NO_{10}$, calcd $[M + Na]^+$ 710.3300).

2a: Pale yellow solid; $[\alpha]_D^{23.2}$ = +16.4 (*c* 0.11, methanol); UV (methanol) λ_{max} (log ε) 293.0 (1.67), 228.0 (1.88), 194.0 (1.71) nm; IR (KBr) ν_{max} 3431, 3030, 2969, 2928, 2882, 1638, 1529, 1439, 1378, 1318, 1278 cm^{-1}; for ^1H, ^{13}C, and 2D NMR data, see Table S5;† ESIMS $[M + Na]^+$ *m*/*z* 710; HRESIMS $[M + Na]^+$ *m*/*z* 710.3296 ($C_{40}H_{49}NO_{10}$, calcd $[M + Na]^+$ 710.3300).

Naphthomycin Q (3). Pale yellow solid; $[\alpha]_D^{23.2}$ = −72.5 (*c* 0.12, methanol); UV (methanol) λ_{max} (log ε) 348.0 (1.25), 307.5 (1.59), 278.5 (1.61), 215.0 (1.69) nm; IR (KBr) ν_{max} 3428, 3036, 2966, 2928, 2876, 1656, 1587, 1501, 1330 cm^{-1}; for ^1H, ^{13}C, and 2D NMR data, see Table S6;† ESIMS $[M + Na]^+$ *m*/*z* 726; HRESIMS $[M + Na]^+$ *m*/*z* 726.3247 ($C_{40}H_{47}NO_{10}$, calcd $[M + Na]^+$ 726.3249).

3a: Pale yellow solid; $[\alpha]_D^{23.2}$ = −76.9 (*c* 0.12, methanol); UV (methanol) λ_{max} (log ε) 347.0 (1.33), 308.5 (1.59), 278.5 (1.51), 215.0 (1.62), nm; IR (KBr) ν_{max} 3429, 3034, 2964, 2927, 2875, 1656, 1587, 1502, 1330 cm^{-1}; for ^1H, ^{13}C, and 2D NMR data, see Table S7;† ESIMS $[M + Na]^+$ *m*/*z* 726; HRESIMS $[M + Na]^+$ *m*/*z* 726.3242 ($C_{40}H_{47}NO_{10}$, calcd $[M + Na]^+$ 726.3249).

Conclusions

In summary, our discovery of naphthomycins O–Q (**1–3**) has enriched the members of ansamycins with two different skeletons. Naphthomycin O (**1**) contains a unique phenalene scaffold with a bridged oxetane ring, which is proposed to be derived from the intermolecular [2 + 2] cycloaddition of the olefin to the ketone of **4**. Our successfully biomimetic synthesis of **1** from **4** verified this biosynthetic origin. Naphthomycin P (**2**) has a unique naphtho-cyclopentone, which is hypothesized to undergo an enzyme involved process with a 1,4-naphthalenediol-cyclopropane intermediate. Naphthomycin Q (**3**), possessing an opening polyketide side chain, indicates the possibility of the enzymatic retro-Friedel–Crafts acylation of **4**. The identification of structurally cyclized (**1**), rearranged (**2**), and cleaved (**3**) naphthomycins highlights the distinguishing feature of biosynthetic versatility in the ansamycin class of polyketides, which also reflects their inherent fascination and attraction in nature.

Conflicts of interest

There are no conflicts to declare.

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