OL | Organic Letters

pubs.acs.org/OrgLett Letter

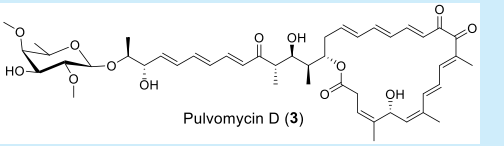
Structures and Biosynthetic Pathway of Pulvomycins B−D: 22-Membered Macrolides from an Estuarine *Streptomyces* sp.

Kyuho Moon,[⊥] Jinsheng Cui,[⊥] Eunji Kim, Evan Setiawan Riandi, So Hyun Park, Woong Sub Byun, Youngju Kal, Jun Young Park, Sunghoon Hwang, Daniel Shin, Jeongyoon Sun, Ki-Bong Oh, Sangwon Cha, Jongheon Shin, Sang Kook Lee, Yeo Joon Yoon, and Dong-Chan Oh*

Cite This: https://dx.doi.org/10.1021/acs.orglett.0c01249 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

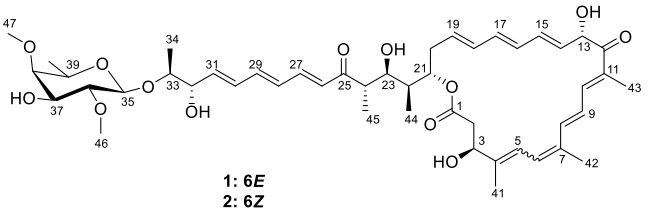
ABSTRACT: Pulvomycins B−D (**1**−**3**) were discovered from an estuarine *Streptomyces* strain along with the known pulvomycin (**4**). The 22-membered macrocyclic lactone structures of **1**−**3** were determined based on the interpretation of NMR, UV, and MS data, the modified Mosher's method, and Kishi's bidentate chiral solvent NMR spectroscopy. Genomic analysis of the bacterial strain revealed the biosynthetic gene cluster of pulvomycin and enabled us to propose the *trans*-acyltransferase polyketide biosynthetic pathway. Pulvomycin D displayed potent cytotoxic activity against various cancer cell lines.



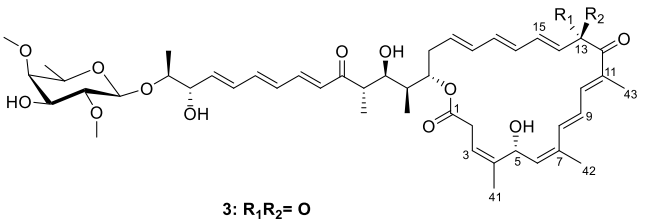
Pulvomycin is an interesting antibiotic discovered from *Streptomyces* sp. with a long history since its first report by J. T. Baker Chemical Company in the United States in 1957.[1] It was rediscovered in 1963[2] and 1976[3] and is also known as labilomycin.[2] The planar structure of pulvomycin was revised as a novel 22-membered macrocyclic lactone in 1985.[4] Pulvomycin has a unique mode of action preventing bacterial translational elongation factor Tu (EF-Tu)·GTP from complexing with aminoacyl-tRNA.[5] The stereochemistry of pulvomycin was determined by the X-ray crystallographic analysis of its complex with *Thermus thermophilius* EF-Tu in 2006.[6] A synthetic study on pulvomycin was reported,[7] but the total synthesis has not yet been accomplished. Surprisingly, despite the attractive structure and bioactivity of pulvomycin, neither additional members of this class nor its biosynthetic pathway have been reported, even though an early isotope labeling study proposed that pulvomycin is a polyketide-derived molecule in 1995.[8]

During our chemical studies of marine-derived actinomycetes, *Streptomyces* sp. HRS33, isolated from the estuary between the Yellow Sea and the Han River in the Republic of Korea, produced pulvomycin. A chemical examination of the strain identified three new members of the pulvomycin class, pulvomycins B−D (**1**−**3**), prompting the study of their structures and biosynthesis. Herein we report the structure elucidation with the comprehensive stereochemical determination, the bioactivity, and the biosynthetic pathway of pulvomycins.

Pulvomycin B (**1**) was isolated as a yellow powder. The molecular formula of **1** was determined to be $C_{47}H_{66}O_{13}$ based on its HRFAB mass spectrometric data and ^{1}H and ^{13}C NMR data (Table S1). Even though the molecular formula of pulvomycin B (**1**) is identical to that of pulvomycin (**4**), a comprehensive spectroscopic analysis was required for the structure elucidation of **1** because its UV spectrum (λ_{max}: 278, 316, and 374 nm) displayed an additional absorption maximum at 374 nm compared with that of pulvomycin (λ_{max}: 278 and 316 nm), indicating a deviation in the conjugated double-bond systems.



1: 6*E*
2: 6*Z*



3: R_1R_2= O
4: R_1= OH, R_2= H

The analysis of the ^{1}H, ^{13}C, and HSQC NMR spectra enabled us to assign all of the $^{13}C-^{1}H$ one-bond correlations of **1**. The structural fragments were connected by the analysis of the COSY and HMBC data (Figure 1). First, the C-13 to C-24 chain structure bearing two aliphatic methyl groups (C-44

Received: May 15, 2020

https://dx.doi.org/10.1021/acs.orglett.0c01249
Org. Lett. XXXX, XXX, XXX−XXX

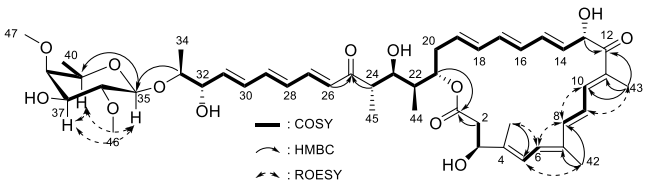


Figure 1. Key 2D NMR correlations of **1**.

and C-45) was assembled based on an array of COSY correlations among the protons in this spin system. The second spin system, from C-26 to C-34, was constructed by consecutive COSY correlations from H-26 to H_3-34. The COSY correlations from the anomeric proton H-35 to the methyl protons H_3-40 elucidated a sugar moiety. In addition, the H_2-2/H-3 COSY correlation secured the C-2−C-3 connectivity. The C-8−C-9−C-10 partial structure was deciphered based on the H-8/H-9 and H-9/H-10 COSY correlations. Olefinic methines C-5 and C-6 were connected by the H-5/H-6 three-bond coupling.

The analysis of HMBC data further connected these partial structures and constructed the gross structure of pulvomycin B (**1**), as shown in Figure 1. The geometries of the double bonds were determined from their ^{1}H−^{1}H coupling constants and ROESY correlations to be 4*E*, 6*E*, 8*E*, 10*E*, 14*E*, 16*E*, 18*E*, 26*E*, 28*E*, and 30*E*. The sugar was established as labilose by analyzing the one-bond C−H coupling constant of the anomeric position[9] and the ^{1}H−^{1}H vicinal coupling constants and ROESY NMR correlations. (See the Supporting Information.)

The relative configurations of the stereogenic centers at C-21, C-22, C-23, and C-24 were established as 21*S**, 22*S**, 23*S**, and 24*S** by *J*-based configuration analysis[10] (Figure S1). Although the combination of the observed coupling constants around C-32 and C-33 was most similar to that of the rotamer shown in Figure S1d, some of the *J* values were intermediate values, requiring an additional spectroscopic analysis for their relative configurations. A careful analysis of the ROESY correlations coupled to density functional theory (DFT) modeling supported the 32*S** and 33*S** configurations, whereas the other diastereomer (32*S**,33*R**) could not explain the observed ROESY correlations (Figure S2).

The absolute configurations of the stereogenic centers at C-3, C-13, C-32, and C-37, each bearing a secondary alcohol, were determined using the modified Mosher's method.[11] The hydroxy groups at C-3, C-13, C-32, and C-37 were esterified with *R*- and *S*-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (MTPA-Cl) to the *tetra*-*S*- and *R*-MTPA esters (**1a** and **1b**). The calculated $\Delta\delta_{S-R}$ values established the absolute configuration as 3*S*, 13*S*, 32*S*, and 37*S* (Figure 2). Possibly because of steric hindrance, the hydroxy group at C-23 was not derivatized by MTPA-Cl.

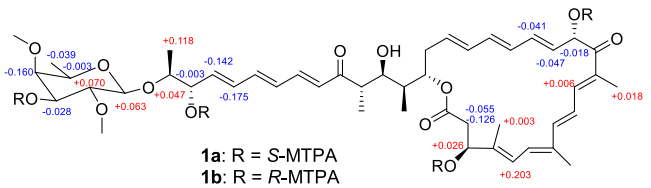


Figure 2. $\Delta\delta_{S-R}$ values (ppm) of the *tetra*-*S*- and *R*-MTPA esters (**1a** and **1b**) of **1** in CD_3CN.

To determine the remaining stereogenic center, we utilized the chemical shift behaviors of the adjacent carbons in Kishi's bidentate chiral solvents [(*R*,*R*)-,and (*S*,*S*)-*bis*-α-methylbenzylamine-*p*-Me (BMBA)].[12] The $\Delta\delta_{(R,R)-(S,S)}$ values of the ^{13}C chemical shifts for C-22 and C-24 enabled us to assign the absolute configuration of C-23 as *S* (Figure 3). Finally, the absolute configurations of pulvomycin B (**1**) were determined as 3*S*, 13*S*, 21*S*, 22*S*, 23*S*, 24*S*, 32*S*, 33*S*, 35*R*, 36*R*, 37*S*, 38*R*, and 39*R*.

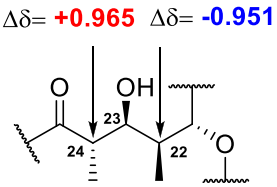


Figure 3. Chemical shift differences of the indicated carbons of pulvomycin B ($\Delta\delta_{(R,R)-(S,S)}$ in a 5:2 mixture of (*R*,*R*)- or (*S*,*S*)-BMBA and $CDCl_3$).

Pulvomycin C (**2**) was isolated as a yellow powder. Its molecular formula is identical to that of **1** based on high-resolution fast-atom bombardment mass spectroscopy (HRFABMS) and NMR data (Table S1). The UV spectrum of **2** was also quite similar to that of **1**, indicating the conservation of the triene, trienone, and tetraenone chromophores. Further investigation of its 1D and 2D NMR spectra identified that pulvomycin C (**2**) is a geometric isomer of **1** with 6*Z* configuration. (See the Supporting Information.)

Pulvomycin D (**3**), purified as a yellow powder, possessed the molecular formula $C_{47}H_{64}O_{13}$ based on its HRMS and NMR data (Table S1). The UV spectrum of **3** displayed only one absorption maximum (at 314 nm), whereas those of **1** and **2** exhibited three maxima, requiring a more comprehensive spectroscopic analysis for structure elucidation. A detailed examination of the ^{1}H−^{1}H COSY and HMBC spectra revealed that pulvomycin D (**3**) shares the same C-14 to C-34 fragment, including the C-44 and C-45 aliphatic methyl groups and the labilose sugar moiety. However, this compound was distinct from **1** and **2** by the substructure from C-1 to C-13. The COSY NMR correlations connected C-2−C-3, C-5−C-6, and C-8−C-9−C-10 substructures. The constructed chain spanning from C-2 to C-11 was flanked by ester carbon C-1 and ketone group C-12 based on the H-2/C-1 and H_3-43/C-12 HMBC cross-peaks. The last carbonyl carbon (C-13) at δ_C 200.1 was assigned adjacent to C-14 by the two-bond C−H correlation from H-14 to C-13. The connectivity between the two carbonyl carbons, C-12 and C-13, was confirmed by the $^3J_{C12H14}$ coupling. The macrolactone structure was also confirmed based on the H-21/C-1 HMBC correlation. The geometries of the olefins in **3** were determined as 3*Z*, 6*Z*, 8*E*, 10*E*, 14*E*, 16*E*, 18*E*, 26*E*, 28*E*, and 30*E* by their $^3J_{HH}$ and ROESY correlations. To determine the relative stereochemistry of **3**, its ROESY spectrum and *J*-based relative configurations[9] were considered, as shown for pulvomycin B (**1**), and this confirmed the configurations of 21*S**, 22*S**, 23*S**, 24*S**, 32*S** and 33*S** (Figure S31). The absolute configuration of pulvomycin D (**3**) was established based on the analysis of a combination of ^{1}H chemical shifts of bis-*S*- and *R*-MTPA esters (**3a** and **3b**) (Figure S36) and the sequences of the ketoreductase (KR) domains (Table S3 and Figure S48c).

https://dx.doi.org/10.1021/acs.orglett.0c01249
Org. Lett. XXXX, XXX, XXX−XXX

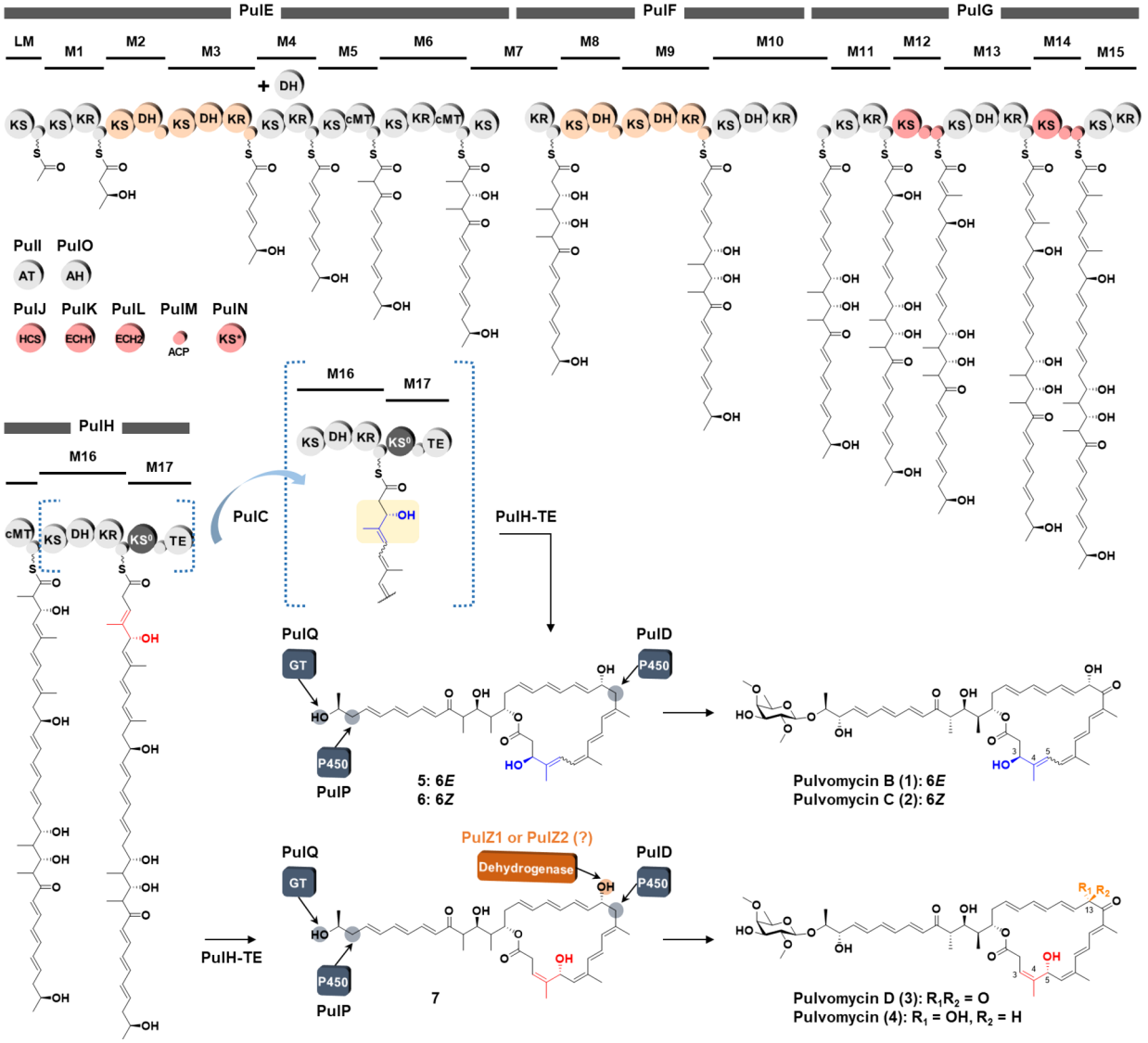


Figure 4. Proposed biosynthetic pathway of pulvomycins. The bimodules 2/3 and 8/9 are represented by light-orange circles. The putative *β*-branching enzymes and associated modules 12 and 14 are represented by light-red circles. Two discrete AT and AH domains, PulI and PulO, and an unknown trans-acting DH domain in module 4 are indicated. The proposed function of PulC is shown between dotted parentheses. The enzymes and locations putatively involved in the modification steps are indicated by blue-gray and dark-orange blocks with arrows. Domain abbreviations are as follows: ACP, acyl carrier protein; AH, acyl hydrolase; AT, *trans*-acyltransferase; cMT, C-methyltransferase; dehydrogenase, short-chain dehydrogenase; DH, dehydratase; ECH, enoyl-CoA hydratase; GT, glycosyltransferase; HCS, hydroxymethylglutaryl-CoA synthase; KR, ketoreductase; KS, ketosynthase; KS0, decarboxylating KS; KS*, KS-like enzyme; P450, cytochrome P450; TE, thioesterase.

The C-6 to C-19 fragment of pulvomycin D (**3**) is fully conjugated with 14 sp^2 carbons and a trienone from C-25 to C-31, which would likely display two different λ_{max}; however, the UV spectrum showed only one λ_{max} at 314 nm, corresponding to a tetraenone chromophore. The energy-minimized structure of the macrolide part of **3** clearly showed distortion by 80° between the two ketone groups at C-12 and C-13, breaking the coplanarity of the 14 conjugated sp^2 carbons into two trienone chromophores. This resulted in one λ_{max} at 314 nm from the three trienone moieties (Figure S38). The calculated UV spectrum based on the energy-minimized structure consistently displayed one UV absorption maximum (Figure S39).

The discovery of these new pulvomycin class metabolites and their intriguing structures led us to investigate the biosynthesis of the pulvomycin family. The *β*-branched methyl groups in pulvomycins suggested that a *trans*-acyltransferase polyketide synthase (*trans*-AT PKS)[13] is involved in their biosynthesis. The analysis of the draft genome sequence of *Streptomyces* sp. HRS33 using antiSMASH[14] showed that only one *trans*-AT PKS-coding gene cluster is present in the *Streptomyces* sp. HRS33 genome. The continuous 103.7 kb DNA sequence, designated the *pul* gene cluster, harbors 35 open reading frames (orf's) that putatively encode four PKSs that lack integral AT domains, 2 *trans*-ATs, a *β*-branching cassette, 6 enzymes involved in the biosynthesis and the attachment of sugar moiety, 3 modification enzymes, and others (Figure S47 and Table S2). On the basis of the detailed sequence analysis of the PKS and other genes in the *pul* cluster, we propose the *trans*-AT PKS biosynthetic pathway for pulvomycins. (See Figure 4 and the Supporting Information.)

The core structure of **3** and **4** bears an unusual *β*,*γ*-double bond at C-3/C-4. It is predicted that the *β*,*γ*-double bond is

https://dx.doi.org/10.1021/acs.orglett.0c01249
Org. Lett. XXXX, XXX, XXX−XXX

incorporated by module 16. Such shifted double bonds are found in some polyketides synthesized by *trans*-AT PKSs and are known to be formed through either direct β,γ-dehydration by the DH domain or a double-bond shift by the DH* domain.[15−17] Because PulH does not contain the double-bond-shifting DH* domains (Figure S48d), the β,γ-double bond at the C-3/C-4 position is proposed to be installed directly by the action of the module 16-encoded DH (Figure 4). One notable structural difference between **1**/**2** and **3**/**4** is the position of the double bonds and hydroxy groups across C-3, C-4, and C-5. A 3-hydroxyacyl-CoA dehydrogenase homologue encoded by *pulC* was predicted to be involved for the conversion of the pulvomycin D chain into the B chain. PulC belongs to the fadJ superfamily, and its N-terminal region shows homology to the C-terminal part of CorN, an enoyl-CoA hydratase-like enzyme from the corallopyronin cluster, which was proposed to remove the hydroxy group by dehydration during chain assembly.[18] Thus it is hypothesized that PulC is involved in the addition of water to the double bond at the β-position followed by the migration of the double bond at C-3/C-4 to C-4/C-5 along with the elimination of the hydroxy group at C-5. During this process, we assume that both the *cis* (6*E*) and *trans* (6*Z*) isomers, **5** and **6**, are simultaneously formed by PulC-mediated dehydration at C-5 due to the promiscuity of this enzyme.

Post-PKS tailoring steps are expected to occur in prepulvomycins **5**, **6**, and **7**. Two putative cytochrome P450s encoded by *pulD* and *pulP* were found in the *pul* cluster. Phylogenetic analysis of two P450 enzymes found in the *pul* cluster suggested that PulD and PulP are responsible for the formation of the C-12-carbonyl group and the C-32-hydroxy group, respectively (Figure S50).[19] Moreover, it is predicted that a *pulQ*-encoded glycosyltransferase appends a di-*O*-methyl-D-fucose, which is putatively biosynthesized by five enzymes, PulA, PulB, PulR, PulS, and PulT (Figure S47, Table S2),[20] on the macrolactone (Figure 4). These modification steps could convert **5**, **6**, and **7** to **1**, **2**, and **4**, respectively. Unlike **4**, pulvomycin D (**3**) has a carbonyl group at C-13 instead of a hydroxy group. A short-chain dehydrogenase encoded by *batM* is responsible for the reoxidation of hydroxy group in a late stage of the synthesis of kalimantacin.[21] Interestingly, two genes homologous to *batM*, *pulZ1*, and *pulZ2* are located ∼56 kb away from *pul* PKS genes (Figure S47). Either of these two or another gene is expected to generate the C-13 ketone for **3**.

Pulvomycins were initially evaluated in an antibacterial assay against pathogenic bacteria (Table S4). Pulvomycin exhibited antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *E. faecuium*, and *Salmonella enterica* (MIC = 1−2 μg/mL). In the antifungal assay, only pulvomycin B inhibited the pathogenic fungi *Candida albicans*, *Trichophyton rubum*, and *T. mentagrophytes* (MIC = 32 μg/mL) (Table S5).

The anticancer activity of pulvomycin has been limitedly studied. Only low cytotoxicity against HeLa, Yoshida sarcoma, and Ehrlich carcinoma was reported in 1964.[22] Thus the cytotoxicities of the pulvomycins were then tested against human cancer cell lines. Pulvomycin D (**3**), which bears an 1,2-diketone functional group, was the most potent inhibitor of colon cancer (HCT116), stomach cancer (SNU638), liver cancer (SK-Hep-1), and breast cancer (MDA-MB-231) cell lines (IC_{50} = 0.21−0.40 μM), whereas **1** displayed weak cytotoxicity (IC_{50} = 3.7−25 μM) (Table S6).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c01249.

Detailed experimental procedures, NMR spectral data, and genetic analysis for **1**−**4** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Dong-Chan Oh − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea;* orcid.org/0000-0001-6405-5535; Email: dongchanoh@snu.ac.kr

Authors

Kyuho Moon − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea; College of Pharmacy, Chonnam National University, Gwangju 61186, Republic of Korea*

Jinsheng Cui − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Eunji Kim − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Evan Setiawan Riandi − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

So Hyun Park − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Woong Sub Byun − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Youngju Kal − *Department of Chemistry, Dongguk University, Seoul 04620, Republic of Korea*

Jun Young Park − *Department of Chemistry, Dongguk University, Seoul 04620, Republic of Korea*

Sunghoon Hwang − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Daniel Shin − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Jeongyoon Sun − *Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Seoul 08826, Republic of Korea*

Ki-Bong Oh − *Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Seoul 08826, Republic of Korea*

Sangwon Cha − *Department of Chemistry, Dongguk University, Seoul 04620, Republic of Korea;* orcid.org/0000-0003-2819-3417

Jongheon Shin − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea;* orcid.org/0000-0002-7536-8462

Sang Kook Lee − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea;* orcid.org/0000-0002-4306-7024

https://dx.doi.org/10.1021/acs.orglett.0c01249
Org. Lett. XXXX, XXX, XXX−XXX

Yeo Joon Yoon − *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea;* orcid.org/0000-0002-3637-3103

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c01249

Author Contributions

⊥K.M. and J.C. contributed equally to this work.

Notes

The authors declare the following competing financial interest(s): The authors submitted a patent application for pulvomycin D and its anticancer activity.

■ ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (Ministry of Science and ICT/2018R1A4A1021703 and 2019R1A2B5B03069338) and by the Collaborative Genome Program of the Korea Institute of Marine Science and Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (MOF) (no. 20180430).

■ REFERENCES

(1) Zief, M.; Woodside, R.; Schmitz, H. *Antibiot. Chemother.* **1957**, *7*, 384−386.
(2) Akita, E.; Maeda, K.; Umezawa, H. *J. Antibiot., Ser. A* **1963**, *16*, 147−151.
(3) Schwartz, J. L.; Tishler, M.; Arison, B. H.; Shafer, H. M.; Omura, S. *J. Antibiot.* **1976**, *29*, 236−241.
(4) Smith, R. J.; Williams, D. H.; Barna, J. C. J.; McDermott, I. R.; Haegele, K.; Piriou, F.; Wagner, J.; Higgins, W. *J. Am. Chem. Soc.* **1985**, *107*, 2849−2857.
(5) Wolf, H.; Assmann, D.; Fischer, E. *Proc. Natl. Acad. Sci. U. S. A.* **1978**, *75*, 5324−5328.
(6) Parmeggiani, A.; Krab, I. M.; Okamura, S.; Nielsen, R. C.; Nyborg, J.; Nissen, P. *Biochemistry* **2006**, *45*, 6846−6857.
(7) Börding, S.; Bach, T. *Chem. Commun.* **2014**, *50*, 4901−4903.
(8) Priestley, N. D.; Gröger, S. *J. Org. Chem.* **1995**, *60*, 4951−4953.
(9) Pretsch, E.; Bühlmann, P.; Affolter, C. *Structure Determination of Organic Compounds-Tables of Spectral Data*; Springer: New York, 2000; p 153.
(10) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.
(11) Freire, F.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Org. Chem.* **2005**, *70*, 3778−3790.
(12) Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.* **2002**, *4*, 411−414.
(13) Helfrich, E. J.; Piel, J. *Nat. Prod. Rep.* **2016**, *33*, 231−316.
(14) Blin, K.; Wolf, T.; Chevrette, M. G.; Lu, X.; Schwalen, C. J.; Kautsar, S. A.; Suarez Duran, H. G.; de Los Santos, E. L. C.; Kim, H. U.; Nave, M.; Dickschat, J. S.; Mitchell, D. A.; Shelest, E.; Breitling, R.; Takano, E.; Lee, S. Y.; Weber, T.; Medema, M. H. *Nucleic Acids Res.* **2017**, *45*, W36−W41.
(15) Moldenhauer, J.; Götz, D. C.; Albert, C. R.; Bischof, S. K.; Schneider, K.; Süssmuth, R. D.; Engeser, M.; Gross, H.; Bringmann, G.; Piel, J. *Angew. Chem., Int. Ed.* **2010**, *49*, 1465−1467.
(16) Kusebauch, B.; Busch, B.; Scherlach, K.; Roth, M.; Hertweck, C. *Angew. Chem., Int. Ed.* **2010**, *49*, 1460−1464.
(17) Erol, O.; Schäberle, T. F.; Schmitz, A.; Rachid, S.; Gurgui, C.; El Omari, M.; Lohr, F.; Kehraus, S.; Piel, J.; Müller, R.; König, G. M. *ChemBioChem* **2010**, *11*, 1253−1265.
(18) Pogorevc, D.; Panter, F.; Schillinger, C.; Jansen, R.; Wenzel, S. C.; Müller, R. *Metab. Eng.* **2019**, *55*, 201−211.
(19) Greule, A.; Stok, J. E.; De Voss, J. J.; Cryle, M. J. *Nat. Prod. Rep.* **2018**, *35*, 757−791.
(20) Xu, Z.; Jakobi, K.; Welzel, K.; Hertweck, C. *Chem. Biol.* **2005**, *12*, 579−588.
(21) Mattheus, W.; Gao, L. J.; Herdewijn, P.; Landuyt, B.; Verhaegen, J.; Masschelein, J.; Volckaert, G.; Lavigne, R. *Chem. Biol.* **2010**, *17*, 149−159.
(22) Takeuchi, T.; Nitta, K.; Koyama, G.; Hori, M.; Umezawa, H. *J. Antibiot., Ser. A* **1964**, *17*, 124−126.