ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Expanding the Chemical Diversity of Secondary Metabolites Produced by Two Marine-Derived Enterocin- and Wailupemycin-Producing *Streptomyces* Strains

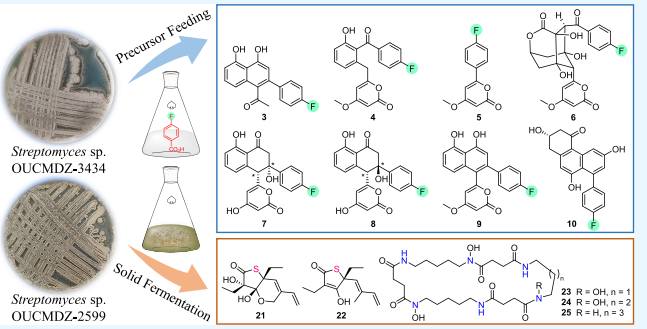
Kunyu Xia, Jiaxu Shang, Jiwen Sun, Weiming Zhu,* and Peng Fu*

Cite This: *ACS Omega* 2023, 8, 28886−28897

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: To expand the chemical diversity of secondary metabolites produced by two marine-derived enterocin- and wailupemycin-producing *Streptomyces* strains, OUCMDZ-3434 and OUCMDZ-2599, precursor feeding and solid fermentation strategies were used. Two new compounds, wailupemycins Q (**1**) and R (**2**), were isolated from the extracts of liquid and solid fermentation of OUCMDZ-3434. Furthermore, during the fermentation of OUCMDZ-3434, *p*-fluorobenzoic acid was added as the key biosynthetic precursor, which resulted in the isolation of eight new fluorinated enterocin and wailupemycin derivatives (**3**−**10**) and 10 previously reported analogues (**11**−**20**). From the solid fermentation extract of OUCMDZ-2599, a new sulfur-containing compound thiotetromycin B (**21**) and its known analogue thiotetromycin (**22**) were identified. Moreover, the solid fermentation strategy effectively activated the biosynthesis of siderophores (**23**−**25**) of strain OUCMDZ-2599. Compound **3** showed moderate antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* subsp. *aureus* with MIC values of 4 μg/mL. Compounds **23**−**25** were significantly capable of binding Fe(III).



INTRODUCTION

Microbial natural products have always been an important source for the discovery of lead compounds.[1] In the past two decades, there has been increasing research on natural products of marine-derived actinomycetes.[2] Over that period, a lot of spectacular compounds represented by salinosporamide A were reported.[3] However, like the terrestrial microbial natural product research, today, the research on natural products of marine microorganisms also encountered the problems of repeated isolation of common compounds and low utilization rate of microbial strains. To address these issues, some new effective strategies have been developed and widely applied, such as genome mining and heterologous expression.[4] In the meantime, the traditional approaches, such as fermentation optimization and biosynthetic precursor feeding, are still widely used to obtain new secondary metabolites from the existing strains on hand, which has been proven to be highly effective.[5] In recent years, we have been committed to utilizing various methods to discover new compounds from marine-derived actinomycetes.[6] During the process, by altering the fermentation conditions, we expanded the chemical diversity of wailupemycins produced by the marine algae-derived *Streptomyces* sp. OUCMDZ-3434 (Figure S1).[7] Meanwhile, it was found that the solid fermentation condition could stimulate the metabolic potential of this strain to produce hydroxamate siderophores.[7c] Our previous studies showed that the main secondary metabolites of strain OUCMDZ-3434 are enterocins and wailupemycins, two groups of polyketides produced by the genus *Streptomyces*. These compounds have been reported to have diverse structures and various bioactivities, which have attracted great attention.[8]

RESULTS AND DISCUSSION

In this work, we have continued to explore the remaining extracts of OUCMDZ-3434. Two new compounds, wailupemycins Q (**1**) and R (**2**), were isolated from the extracts of liquid and solid fermentation of OUCMDZ-3434, respectively (Figure 1). It is understood that benzoic acid is the key biosynthetic precursor of the enterocin and wailupemycin polyketides, and *p*-fluorobenzoic acid has been reported as a precursor, which can be integrated into the biosynthesis processes.[9] Thus, we also attempted to utilize the precursor-directed biosynthesis strategy to expand the chemical diversity

Received: June 13, 2023
Accepted: July 14, 2023
Published: July 29, 2023

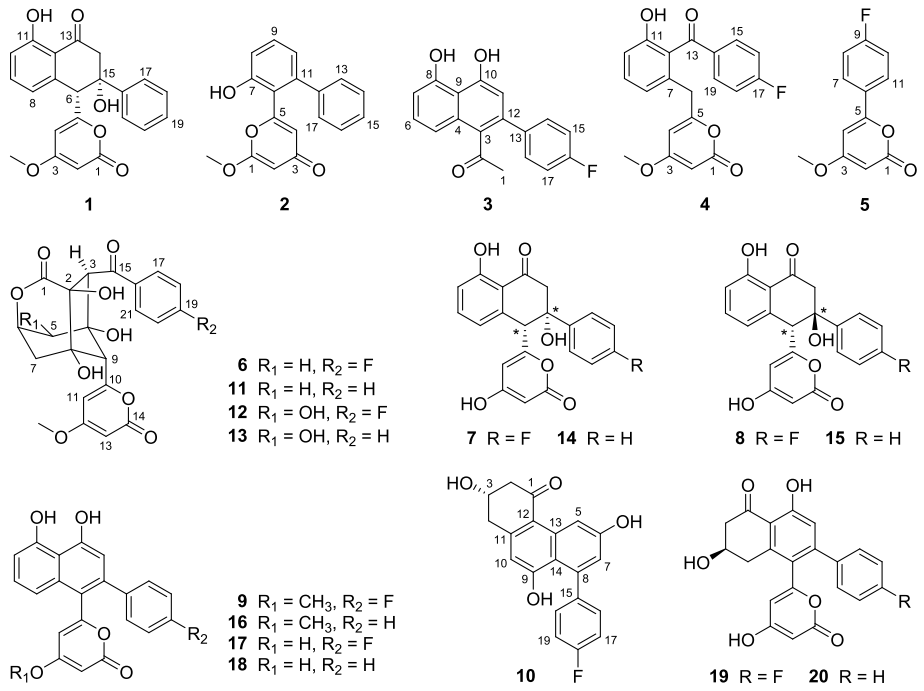


Figure 1. Chemical structures of the compounds isolated from OUCMDZ-3434.

of the enterocin and wailupemycin polyketides of OUCMDZ-3434. Eight new fluorinated enterocin and wailupemycin derivatives, wailupemycin S (**3**), 17-fluorowailupemycin O (**4**), wailupemycin T (**5**), 19-fluoro-5-deoxyenterocin (**6**), 19-fluorowailupemycin D (**7**), 19-fluorowailupemycin E (**8**), 19-fluoro-3-*O*-methylwailupemycin G (**9**), and wailupemycin U (**10**), and three known ones, 19-fluoroenterocin (**12**), 19-fluorowailupemycin G (**17**), and 19-fluorowailupemycin F (**19**), together with seven previously reported nonfluorinated enterocin and wailupemycin analogues (**11**, **13**−**16**, **18**, and **20**) were obtained (Figure 1 and Figure S2). While continuing to explore the metabolic potential of OUCMDZ-3434, we have also searched for other enterocin- and wailupemycin-producing bacteria. As a result, the marine-derived *Streptomyces* strain OUCMDZ-2599 was found out, which can produce a large amount of enterocin (Figure S2). Different fermentation conditions for OUCMDZ-2599 were investigated, and the differential natural products were observed (Figure S2). A new sulfur-containing compound thiotetromycin B (**21**), its known analogue thiotetromycin (**22**),[10] three hydroxamate siderophores (desferrioxamine X_7,[11] **23**; desmethylenylnocardamine,[12] **24**; terragine E,[13] **25**) as well as *R*- and *S*-wailupemycin K (**26** and **27**)[7b] were isolated from the solid fermentation extract of OUCMDZ-2599 (Figure 2 and Figure S2). Likewise, the solid fermentation strategy effectively activated the biosynthesis of siderophores of strain OUCMDZ-2599.

Wailupemycin Q (**1**) was obtained as a yellow amorphous powder. Its molecular formula was assigned as $C_{22}H_{18}O_6$ based on the HRESIMS analysis, being one CH_2 more than wailupemycin D (**14**). Its ^1H and ^{13}C NMR data (Table 1) showed similarities to those of wailupemycin D (Figure 1), except that 3-OH was replaced by 3-OCH_3 ($\delta_{C/H}$ 56.3/3.74).[14] The methoxyl group was confirmed by the HMBC correlation of 3-OCH_3 to C-3 (Figure 3). The structure of compound **1** was further determined by the key COSY correlations of H-8/H-9/H-10 and H-17/H-18/H-19/H-20/H-21, together with

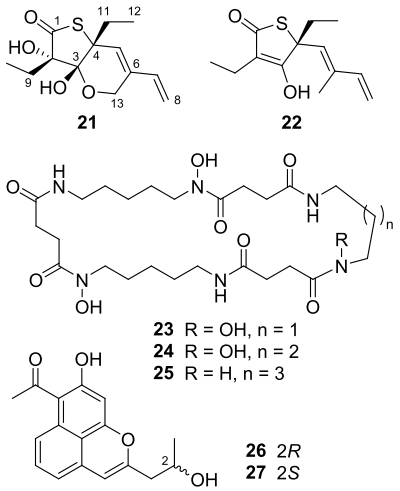


Figure 2. Chemical structures of the compounds isolated from OUCMDZ-2599.

the key HMBC correlations of H-2 to C-1/C-3/C-4, H-4 to C-5, H-6 to C-5/C-7/C-8/C-12/C-14/C-15/C-16, H-8 to C-12, H-9 to C-7/C-11, H-10 to C-12, and H_2-14 to C-13/C-15 (Figure 3). The relative configuration of compound **1** was established by the key NOESY correlations between H-6 and H-17/21, between H-14a and H-17/21, between 15-OH and 3-OCH_3, and between 15-OH and H-14b (Figure 4). To determine its absolute configuration, the predicted ECD spectrum was obtained by the TDDFT [B3LYP/6-31G(d)] method.[15] The measured ECD spectrum of compound **1** matches well with the calculated ECD curve of (6*R*, 15*S*)-**1** (Figure 5). Interestingly, compound **1** is not very stable. After 15 days in methanol, it turned into five compounds, including 3-*O*-methylwailupemycin G (**16**) and four stereoisomers, (6*S*, 15*S*)-**1** (**1a**), (6*S*, 15*R*)-**1** (**1b**), (6*R*, 15*R*)-**1** (**1c**), and (6*R*, 15*S*)-**1** (**1**). These compounds were separated by a Chiral MD column (Figure S3a), and their absolute configurations were

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897

Table 1. ^{1}H and ^{13}C NMR Data for Compounds 1 and 7−9 in DMSO-d_6

	1[a]		7[b]		8[c]		9[d]	
no.	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
1	163.2, C		163.5, C		162.8, C		163.5, C	
2	88.0, CH	5.49, d (2.3)	89.3, CH	5.18, d (2.1)	88.8, CH	5.06, d (2.0)	88.2, CH	5.62, d (2.3)
3	170.4, C		169.8, C		169.9, C		170.5, C	
4	103.7, CH	6.14, d (2.3)	104.0, CH	6.09, d (2.1)	102.7, CH	5.73, d (2.1)	105.7, CH	6.06, d (2.2)
5	162.6, C		163.4, C		162.9, C		160.8, C	
6	54.1, CH	4.76, s	54.3, CH	4.73, s	55.3, CH	4.38, d (1.4)	119.3, C	
7	142.0, C		142.1, C		141.4, C		134.9, C	
8	119.3, CH	6.60, d (7.6)	119.3, CH	6.59, d (7.6)	120.6, CH	6.88, d (7.4)	116.1, CH	7.10, dd (8.6, 0.9)
9	137.1, CH	7.49, t (8.0)	137.1, CH	7.51, t (7.9)	137.0, CH	7.54, t (7.9)	128.9, CH	7.39, t (8.1)
10	116.2, CH	6.89, d (8.4)	116.2, CH	6.90, d (8.4)	116.4, CH	6.92, d (8.3)	109.3, CH	6.85, dd (7.7, 0.9)
11	161.5, C		161.5, C		161.4, C		154.5, C	
12	116.2, C		116.2, C		116.0, C		113.4, C	
13	203.5, C		203.4, C		204.0, C		156.0, C	
14	50.8, CH_2	3.64, d (17.2); 3.04, d (17.2)	51.0, CH_2	3.69, d (17.2); 3.00, d (17.2)	45.8, CH_2	3.94, dd (17.3); 2.94, dd (17.3, 1.5)	109.8, CH	6.75, s
15	75.4, C		75.3, C		74.3, C		140.2, C	
16	144.9, C		141.1, C, d (2.8)		140.7, C, d (3.0)		136.5, C, d (2.9)	
17	125.5, CH	7.48, d (7.1)	127.6, CH, d (8.2)	7.51, dd (8.8, 5.5)	127.5, CH, d (8.3)	7.47, dd (8.7, 5.5)	130.5, CH, d (8.1)	7.34, dd (8.8, 5.6)
18	128.0, CH	7.31, t (7.3)	114.7, CH, d (21.1)	7.15, t (8.9)	114.8, CH, d (21.0)	7.17, t (8.8)	115.2, CH, d (21.2)	7.23, t (8.9)
19	127.3, CH	7.22, t (7.2)	161.2, C, d (242.3)		161.5, C, d (242.3)		161.6, C, d (243.3)	
20	128.0, CH	7.31, t (7.3)	114.7, CH, d (21.1)	7.15, t (8.9)	114.8, CH, d (21.0)	7.17, t (8.8)	115.2, CH, d (21.2)	7.23, t (8.9)
21	125.5, CH	7.48, d (7.1)	127.6, CH, d (8.2)	7.51, dd (8.8, 5.5)	127.5, CH, d (8.3)	7.47, dd (8.7, 5.5)	130.5, CH, d (8.1)	7.34, dd (8.8, 5.6)
3-OH				11.64, brs		11.67, brs		
3-OCH_3	56.3, CH_3	3.74, s					56.4, CH_3	3.77, s
11-OH		12.37, brs		12.38, s		12.31, s		11.37, brs
13-OH								11.37, brs
15-OH		6.06, brs		6.12, brs		6.15, brs		

[a]Spectra were recorded at 400 MHz for ^{1}H and 150 MHz for ^{13}C. [b]Spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C. [c]Spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C. [d]Spectra were recorded at 500 MHz for ^{1}H and 150 MHz for ^{13}C.

determined by comparison of the calculated ECD curves with their measured ECD spectra (Figure S3c). We speculated that compound **1** underwent dehydration and enolization to generate compound **16**, which was then hydrated to produce four possible stereoisomers (Figure S3b). The results indicated that this type of natural products with different configurations might be formed through nonenzymatic reactions.

Wailupemycin R (**2**) was obtained as a yellow amorphous powder. Its molecular formula was determined as $C_{18}H_{14}O_4$ by HRESIMS, which is an isomer of wailupemycin P.[7c] Analysis of its 1D NMR data (Table 2) revealed that compound **2** also contains a 1,2,3-trisubstituted benzene unit and a monosubstituted phenyl group. The COSY and HMBC correlations (Figure 3) indicated that compound **2** has a similar structure to wailupemycin P, except that the 4-methoxy-2-pyrone unit was replaced by the 2-methoxy-4-pyrone unit. This could be confirmed by the key HMBC correlations of H-2 to C-1/C-3/C-4, H-4 to C-5/C-6, and 1-OCH_3 to C-1 (Figure 3).

Wailupemycin S (**3**) was obtained as a light purple powder. The HRESIMS analysis defined its molecular formula as $C_{18}H_{13}O_3F$. The ^{19}F NMR spectrum displayed a signal at δ_F −117.10. It is understood that carbon signals can be split by fluorine atoms. In the ^{13}C NMR spectrum, four carbon signals split into doublets by a fluorine were observed at δ_C 161.0 (J = 240.8 Hz), 113.2 (J = 21.1 Hz), 131.1 (J = 7.8 Hz) and 137.1 (J = 3.1 Hz). Thus, the presence of a *p*-fluorophenyl group was suggested. Two exchangeable protons (δ_H 9.37 and 10.21) could be assigned to two phenolic hydroxyl groups. Further comparison of its ^{1}H and ^{13}C NMR data with those of wailupemycin G[16] revealed that compound **3** contains the same 1,8-dihydroxynaphthalene moiety. This could be confirmed by the COSY and HMBC correlations (Figure 3). In addition, the 3-acetyl group ($\delta_{C/H}$ 32.5/2.08, δ_C 203.9) could be indicated by the HMBC correlations of H_3-1 to C-2/C-3 (Figure 3). The HMBC correlations of H-14/H-18 to C-12 revealed that the *p*-fluorophenyl group was attached to C-12 (Figure 3).

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897

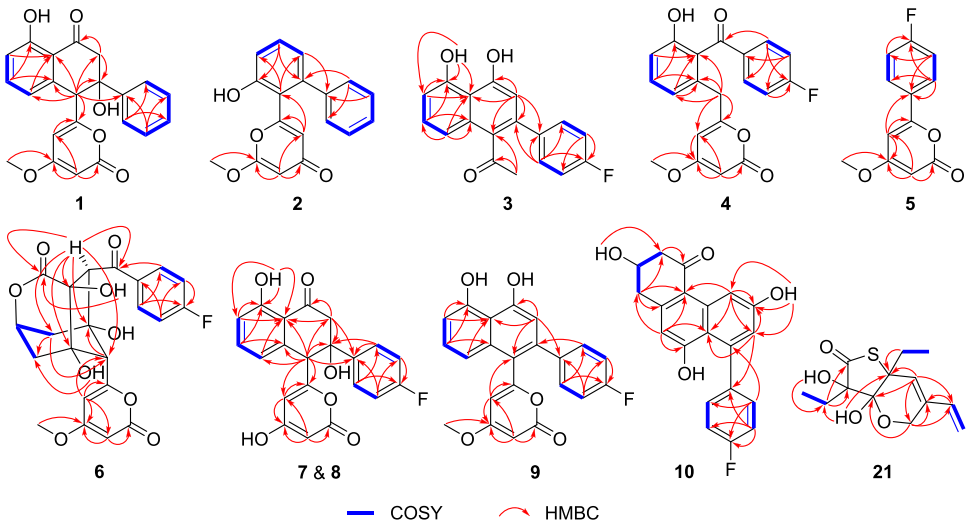


Figure 3. Key 2D NMR correlations for the structural assignment of compounds **1**−**10** and **21**.

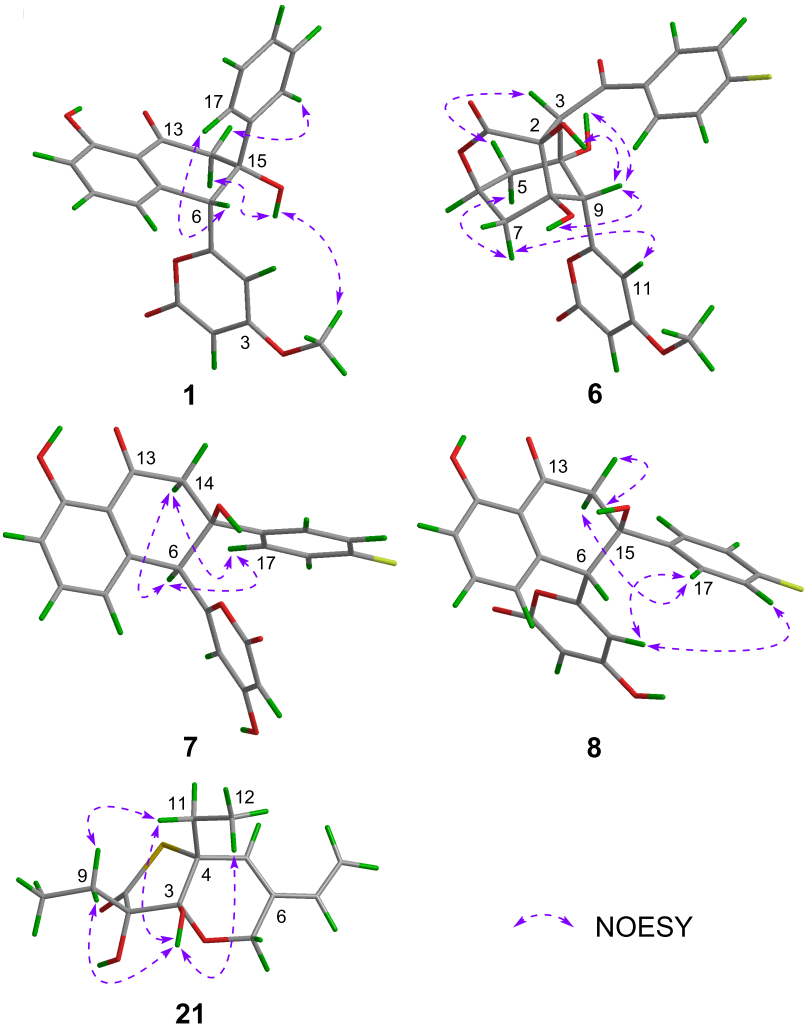


Figure 4. Key NOESY correlations of compounds **1**, **6**, **7**, **8**, and **21**.

17-Fluorowailupemycin O (**4**) was obtained as a yellow amorphous powder. Its molecular formula was determined as $C_{20}H_{15}O_5F$ based on the HRESIMS analysis. Comparison of its NMR data (Table 2) with those of wailupemycin O[7c] revealed that H-17 in wailupemycin O was replaced by a fluorine atom (δ_F −107.15) in compound **4**. Thus, compound **4** was established as 17-fluorowailupemycin O, which was further confirmed by the 2D NMR correlations (Figure 3).

Wailupemycin T (**5**) was isolated as a white amorphous powder. Its molecular formula was assigned as $C_{12}H_9O_3F$

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897

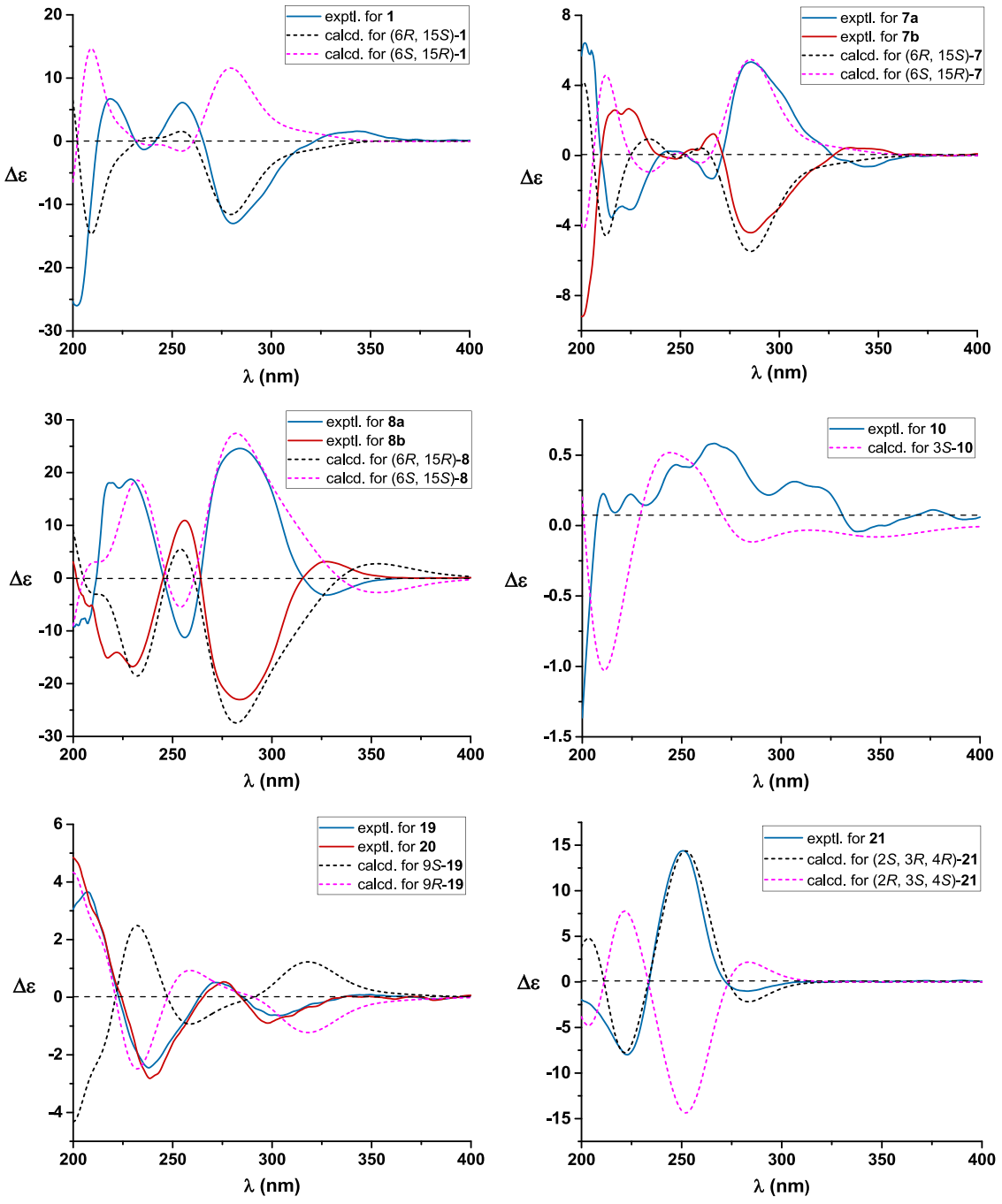


Figure 5. Experimental and calculated ECD curves of compounds **1**, **7**, **8**, **10**, and **19**–**21**.

according to the HRESIMS data. Comparison of its 1D NMR data (Table 2) with those of 6-phenyl-4-methoxy-2*H*-pyran-2-one[17] revealed that their structures were very similar, with the exception that the monosubstituted benzene was replaced by a *para*-disubstituted benzene unit in compound **5**. Further analysis of its NMR spectra suggested that a fluorine atom (δ_F −109.24) was introduced into the 4-position of the phenyl group. This was confirmed by the COSY correlation of H-7/H-8 and the HMBC correlations of H-7/H-8 to C-9 (Figure 3). Thus, the structure of compound **5** was determined as 6-(4-fluorophenyl)-4-methoxy-2*H*-pyran-2-one.

19-Fluoro-5-deoxyenterocin (**6**) was obtained as a white amorphous powder. The molecular formula was determined as $C_{22}H_{19}O_9F$ on the basis of its HRESIMS spectrum. It was thought to be an analogue of 5-deoxyenterocin (**11**)[18] due to the similar UV absorptions as well as their molecular formulas. Analysis of its ^{13}C NMR data (Table 3) together with the key COSY and HMBC correlations (Figure 3) suggested the presence of a *p*-fluorophenyl group. This was supported by the fluorine signal at δ_F −106.78 in the ^{19}F NMR spectrum. Comparison of its NMR data (Table 3) with those of 5-deoxyenterocin (**11**) indicated that H-19 in compound **11** was replaced by a fluorine atom. The relative configuration of compound **6** consistent with 5-deoxyenterocin (**11**) was determined by the key NOESY correlations of H-3/H-5a, H-5b/H-7b, H-7b/H-11, H-9/2-OH, and H-9/4-OH (Figure 4). The almost identical ECD curves of compounds **6** and **11**

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897

Table 2. ^{1}H and ^{13}C NMR Data for Compounds 2−5

	2[a]		3[b]		4[c]		5[d]	
no.	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
1	170.3, C		32.5, CH_3	2.08, s	167.1, C		162.7, C	
2	90.8, CH	5.52, d (1.9)	203.9, C		88.2, CH	5.28, d (2.3)	88.4, CH	5.71, d (2.1)
3	184.4, C		133.2, C		173.2, C		171.2, C	
4	117.7, CH	6.19, d (2.0)	134.3, C		103.6, CH	5.67, d (2.2)	98.0, CH	6.91, d (2.1)
5	161.1, C		117.3, CH	7.15, dd (8.2, 1.8)	164.0, C		158.2, C	
6	118.8, C		127.4, CH	7.17, t (7.2)	37.9, CH_2	3.71, s	127.4, C, d (3.1)	
7	157.4, C		107.6, CH	6.48, dd (7.0, 1.8)	135.3, C		128.1, CH, d (8.8)	7.95, dd (8.8, 5.4)
8	116.0, CH	6.96, dd (8.6, 1.1)	155.1, C		123.2, CH	6.92, dd (7.7, 0.9)	116.1, CH, d (22.0)	7.36, t (8.8)
9	132.9, CH	7.39, t (8.0)	116.7, C		131.9, CH	7.31, t (7.9)	163.6, C, d (248.1)	
10	122.3, CH	6.93, dd (7.6, 1.1)	150.2, C		115.9, CH	6.85, dd (8.3, 1.0)	116.1, CH, d (22.0)	7.36, t (8.8)
11	144.7, C		109.2, CH	7.13, s	156.2, C		128.1, CH, d (8.8)	7.95, dd (8.8, 5.4)
12	142.1, C		136.5, C		128.4, C			
13	129.5, CH	7.26, dd (7.7, 1.8)	137.1, C, d (3.1)		198.1, C			
14	129.5, CH	7.34, t (7.5)	131.1, CH, d, (7.8)	7.18, dd (8.8, 5.8)	135.6, C, d (2.2)			
15	128.5, CH	7.31, t (6.8)	113.2, CH, d (21.1)	7.08, t (8.8)	133.3, CH, d (9.3)	7.78, dd (8.9, 5.5)		
16	129.5, CH	7.34, t (7.5)	161.0, C, d (240.8)		116.5, CH, d (22.2)	7.14, t (8.8)		
17	129.5, CH	7.26, dd (7.7, 1.8)	113.2, CH, d (21.1)	7.08, t (8.8)	167.4, C, d (252.7)			
18			131.1, CH, d, (7.8)	7.18, dd (8.8, 5.8)	116.5, CH, d (22.2)	7.14, t (8.8)		
19					133.3, CH, d (9.3)	7.78, dd (8.9, 5.5)		
1-OCH_3	57.1, CH_3	3.69, s						
3-OCH_3					56.8, CH_3	3.69, s	56.6, CH_3	3.87, s
8-OH				9.37, brs				
10-OH				10.21, brs				

[a]Spectra were recorded in methanol-d_4 at 400 MHz for ^{1}H and 150 MHz for ^{13}C. [b]Spectra were recorded in DMSO-d_6 at 400 MHz for ^{1}H and 150 MHz for ^{13}C. [c]Spectra were recorded in methanol-d_4 at 500 MHz for ^{1}H and 150 MHz for ^{13}C. [d]Spectra were recorded in DMSO-d_6 at 400 MHz for ^{1}H and 150 MHz for ^{13}C.

(Figure S4) revealed the same absolute configurations. Thus, compound **6** was established as 19-fluoro-5-deoxyenterocin.

19-Fluorowailupemycin D (**7**) was isolated as a yellow amorphous powder. The HRESIMS analysis defined its molecular formula as $C_{21}H_{15}O_6F$. Its ^{1}H and ^{13}C NMR data (Table 1) showed similarities to those of wailupemycin D (**14**).[14] Analysis and comparison of NMR data with those of wailupemycin D (**14**) indicated that H-19 in compound **14** was replaced by a fluorine atom (δ_F −115.60) in compound **7**. The relative configuration was established by the key NOESY correlations of H-6/H-14a, H-6/H-17, and H-14a/H-17 (Figure 4). Considering the instability and changing process of compound **1** (Figure S3), we speculated that compound **7** may contain a pair of enantiomers. To confirm this deduction, compound **7** was analyzed on a chiral column, and two peaks were observed (Figure S5). They were further separated into two optically pure enantiomers, **7a** and **7b**, through a chiral HPLC separation. To determine their absolute configurations, the predicted ECD spectra were obtained by the TDDFT [B3LYP/6-31G(d)] method.[15] The measured ECD spectrum of compound **7a** matches well with the calculated ECD curve of (6*S*, 15*R*)-**7** (Figure 5). Thus, the absolute configurations of **7a** and **7b** were unambiguously established as (6*S*, 15*R*) and (6*R*, 15*S*), respectively.

The molecular formula of 19-fluorowailupemycin E (**8**) was also determined to be $C_{21}H_{15}O_6F$ by HRESIMS, which is an isomer of compound **7**. Comparison of its ^{1}H and ^{13}C NMR data (Table 1) with those of compound **7** suggested that these compounds may be stereoisomers at C-6 and C-15. The NOESY correlations of H-14a/15-OH, H-14b/H-17, and H-4/H-17 (Figure 4) revealed the same relative configuration as wailupemycin E (**15**).[16] Thus, the structure of compound **8** was determined as 19-fluorowailupemycin E. Like compound **7**, 19-fluorowailupemycin E (**8**) also contained a pair of enantiomers, **8a** and **8b**, which were separated on a chiral column (Figure S5). Their absolute configurations were determined as (6*S*, 15*S*) and (6*R*, 15*R*), respectively, by comparison of the measured ECD spectra with the calculated ECD curves (Figure 5).

Our research showed that wailupemycins D (**14**) and E (**15**) also contained the corresponding enantiomers, (6*S*, 15*R*)-**14** (**14a**)/(6*R*, 15*S*)-**14** (**14b**) and (6*S*, 15*S*)-**15** (**15a**)/(6*R*, 15*R*)-**15** (**15b**), respectively, which were observed and separated by the chiral HPLC (Figure S5). Their absolute

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897

Table 3. ^{1}H (400 MHz) and ^{13}C NMR (150 MHz) Data for Compounds 6, 10, and 21 in DMSO-d_6

	6		10		21	
no.	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
1	174.0, C		196.5, C		203.3, C	
2	79.7, C		49.7, CH_2	2.82, dd (15.7, 3.8); 2.58, dd (15.9, 8.2)	85.9, C	
3	60.8, CH	4.02, s	64.8, CH	4.17, m	99.1, C	
4	77.1, C		40.4, CH_2	3.13, dd (16.4, 3.9); 2.88, dd (16.2, 7.4)	56.8, C	
5	36.7, CH_2	2.22, dd (14.2, 3.2); 1.63, dd (14.2, 2.2)	108.2, CH	9.03, d (2.6)	127.3, CH	5.96, s
6	72.6, CH	4.84, ddd (4.5, 2.4, 2.1)	157.0, C		132.3, C	
7	40.1, CH_2	2.66, dd (15.0, 4.3); 2.08, dd (15.0, 2.2)	119.8, CH	6.68, d (2.6)	135.0, CH	6.34, dd (18.0, 11.1)
8	75.8, C		139.9, C		114.5, CH_2	5.19, d (18.0); 5.13, d (11.1)
9	54.3, CH	4.59, s	159.8, C		27.7, CH_2	1.88, m; 1.81, m
10	161.9, C		108.3, CH	6.38, s	7.1, CH_3	0.87, t (7.4)
11	104.6, CH	6.27, d (2.2)	147.3, C		29.6, CH_2	2.09, m; 1.88, m
12	170.7, C		117.5, C		9.6, CH_3	1.04, t (7.4)
13	87.9, CH	5.62, d (2.2)	136.7, C		57.4, CH_2	4.35, dd (15.8, 1.9); 4.29, d (15.7)
14	163.5, C		115.6, C			
15	193.7, C		141.0, C, d (2.1)			
16	136.2, C, d (2.1)		130.1, CH, d (7.8)	7.25, dd (8.3, 5.5)		
17	131.0, CH, d (9.8)	7.87, dd (8.7, 5.6)	113.5, CH, d (21.1)	7.13, t (8.8)		
18	115.5, CH, d (21.6)	7.35, t (8.8)	160.9, C, d (239.8)			
19	164.7, C, d (249.8)		113.5, CH, d (21.1)	7.13, t (8.8)		
20	115.5, CH, d (21.6)	7.35, t (8.8)	130.1, CH, d (7.8)	7.25, dd (8.3, 5.5)		
21	131.0, CH, d (9.8)	7.87, dd (8.7, 5.6)				
12-OCH_3	56.3, CH_3	3.83, s				
2-OH		5.87, s				5.26, d (1.9)
3-OH				5.12, d (3.8)		6.96, s
4-OH		5.92, s				
6-OH				9.99, s		
8-OH		5.50, s				
9-OH				10.56, brs		

configurations were determined by comparison of the measured ECD spectra with those of compounds **7** and **8** (Figure S4). The production of these stereoisomers may be caused by the nonenzymatic spontaneous reactions, like compound **1**.

19-Fluoro-3-*O*-methylwailupemycin G (**9**) was obtained as a yellow amorphous powder. Its molecular formula was assigned as $C_{22}H_{15}O_5F$ by HRESIMS. Comparison of its ^{1}H and ^{13}C NMR data (Table 1) with those of 3-*O*-methylwailupemycin G (**16**)[7b] revealed that compound **9** contains the same 3-methoxy-α-pyrone and 1,8-dihydroxynaphthalene moieties as compound **16**. Further analysis of the NMR data of compound **9** indicated that H-19 in compound **16** was replaced by a fluorine atom (δ_F −114.73).

Wailupemycin U (**10**) was isolated as a yellow amorphous powder. Its molecular formula was determined to be $C_{20}H_{15}O_4F$ by HRESIMS, indicating 13 degrees of unsaturation. Two sp^3 methylene, one sp^3 oxygenated methine, seven sp^2 methines, and ten sp^2 nonprotonated carbons were displayed in the ^{13}C and HSQC NMR spectra. Comparison of its ^{1}H NMR data (Table 3) and molecular formula with those of wailupemycin J[7b] revealed that the monosubstituted phenyl group of wailupemycin J was replaced by a *p*-fluorophenyl group. Further analysis of the NMR data of compound **10** and wailupemycin J indicated that C-1 in compound **10** was connected to the *para*-position of C-9 instead of the *ortho*-position. This assignment was confirmed by the key HMBC correlations of H-10 to C-4/C-9, H-5 to C-12, and H_2-4 to C-11/C-12, together with the COSY correlations of H_2-2/H-3/H_2-4 (Figure 3). The *S* configuration of compound **10** was determined by ECD calculations (Figure 5).

Two fluorinated compounds, 19-fluorowailupemycin G (**17**) and 19-fluorowailupemycin F (**19**), have been reported in other papers. However, they were only detected by LC−MS, and no materials were isolated.[8b,9] Here, we report the ^{1}H, ^{13}C, and ^{19}F NMR data for the first time. The structures of other known compounds (**11**,[18] **12**,[19] **13**,[18b] **14**,[14] **15**,[16] **16**,[7b] **18**,[7a] and **20**[16]) were determined by comparison of their NMR and specific rotation data with those reported.

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897

Thiotetromycin B (**21**) was obtained as a white powder. Its molecular formula was assigned to be $C_{13}H_{18}O_4S$ based on the HRESIMS data. Comparison of its ^1H and ^{13}C NMR spectra with those of thiotetromycin (**22**)[10b] suggested that compound **21** also contains a γ-thiolactone ring, with an evident difference that two sp^2 nonprotonated carbons of compound **22** were replaced by two sp^3 nonprotonated carbons. The signals of the two ethyl groups attached to C-2 and C-4, respectively, one oxygenated sp^3 methylene, two sp^2 methines, one sp^2 methylene, and one sp^2 nonprotonated carbon were also observed in the 1D and 2D NMR spectra of **21** (Table 3 and Figure 3). The methyl group linked to C-6 in compound **22** were replaced by the oxygenated methylene group ($\delta_{C/H}$ 57.4/4.35&4.29). The HMBC correlations of H_2-13 to C-3/C-6/C-7 and H-5 to C-3/C-4/C-7/C-13 demonstrated the presence of a pyran ring (Figure 3). The signals of two hydroxy groups (δ_H 5.26, 6.96) could be assigned by the HMBC correlations of 2-OH to C-9 and 3-OH to C-3/C-4 (Figure 3). The relative configuration of compound **21** was established by the key NOESY correlations of 3-OH/H_2-9, 3-OH/H_2-11, 3-OH/H_3-12, and H_2-9/H_2-11 (Figure 4). To determine its absolute configurations, the predicted ECD spectrum was obtained by the TDDFT [B3LYP/6-31G(d)] method. The measured ECD spectrum of compound **21** matches well with the calculated ECD curve of (2*S*, 3*R*, 4*R*)-**21** (Figure 5).

The antimicrobial activity of compounds **1**−**27** was evaluated against *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 11775, *Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 6538, methicillin-resistant *Staphylococcus aureus* subsp. *aureus* ATCC 43300, *Candida albicans* ATCC 10231, and *Candida glabrata* ATCC 2001. Compounds **3**, **26**, and **27** exhibited antibacterial activity against *Staphylococcus aureus* ATCC 6538, methicillin-resistant *Staphylococcus aureus* subsp. *aureus* ATCC 43300, and *Bacillus subtilis* ATCC 6051 with MIC values of 4−32 μg/mL (Table 4).

Table 4. Antibacterial Activity of Compounds 3, 26, and 27 (MIC and MBC Values, μg/mL)

	B. subtilis ATCC 6051		*S. aureus* ATCC 6538		MRSA ATCC 43300	
compounds	MIC	MBC	MIC	MBC	MIC	MBC
3	16	32	4	32	4	>32
26	16	>32	32	>32	32	>32
27	16	>32	32	>32	32	>32
ciprofloxacin	0.063	0.25	0.125	0.25	1	4
vancomycin	0.25	0.5	1	2	2	8

The Fe(III)-binding properties of compounds **23**−**25** were assessed by the chrome azurol S (CAS) assay.[7c,12] The results suggested that all the three compounds can form an Fe(III) complex (Figure 6). Compound **24** showed stronger binding activity than the positive control, deferoxamine B mesylate (DFB) (Figure 6).

EXPERIMENTAL SECTION

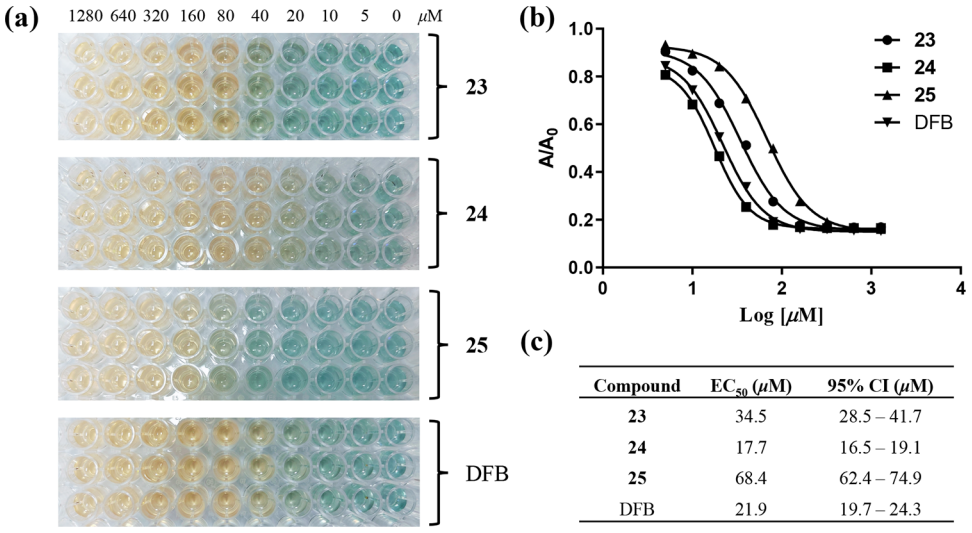
General Experimental Procedures. Optical rotations were recorded with a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Thermofisher NanoDrop OneC microvolume UV−Vis spectrophotometer. ECD spectra were measured on a JASCO J-815 spectrometer. IR spectra were obtained on a Nicolet Nexus 470 spectrophotometer in KBr discs. NMR spectroscopic data were recorded on an Agilent DD2 500, Bruker AVENCE NEO 400, or JEOL JNM-ECP 600 spectrometer, and chemical shifts were referenced to the corresponding residual solvent signals ($\delta_{H/C}$ 3.31/49.00 for methanol-d_4 and $\delta_{H/C}$ 2.50/39.52 for DMSO-d_6). High-resolution ESI-TOF mass spectra were recorded using a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Low-resolution LC/ESI-MS data were measured using a Waters ACQUITY SQD 2 UPLC/MS system (diode array detector, full wavelength scanned from 200 to 500 nm) with a reversed-phase C_{18} column (ACQUITY UPLC BEH C_{18}, 2.1 × 50 mm, 1.7 μm) at a flow rate of 0.4 mL/min. Semipreparative HPLC was performed using an ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 μm), a C_{18}-PFP column (ACE C_{18}-PFP, 10 × 250 mm, 5 μm), or a phenyl column (YMC-pack Ph, 10 × 250 mm, 5 μm). Chiral separation was carried out on a Chiral MD column (4.6 × 250 mm, 5 μm) or a Chiral INC column (4.6 × 250 mm, 5 μm). TLC was performed on plates precoated with silica gel GF254 (10−40 μm, Marine Chemical Factory of Qingdao). Silica gel (200−300 or 100−200 mesh, Marine Chemical Factory of Qingdao) was used for vacuum-liquid chromatography (VLC). RP-18 silica gel (YMC ODS-A, 50 μm), Toyopearl HW-40F (TOSOH Corporation), and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography (CC). Natural seawater was collected from Huanghai Sea in the Jiaozhou Bay (Qingdao, China) without treatment. All solvents and reagents were purchased from Tianjin Kemiou Chemical Reagent Co. Ltd., Sinopharm Chemical Reagent Co. Ltd., J&K Scientific, Energy Chemical, and Aladdin.

Collection and Phylogenetic Analysis of Strains OUCMDZ-3434 and OUCMDZ-2599. The actinomycete *Streptomyces* sp. OUCMDZ-3434 was isolated and identified as previously described.[7a,b] The actinomycete *Streptomyces* sp. OUCMDZ-2599 was isolated from a soil sample collected from the Yellow River Delta Natural Reserve (N 37°45.539′, E 119°10.046′), Shandong Province of China. The sample (2 g) was dried over 24 h in an incubator at 35 °C, and the dried soil was added to 10 mL of sterile natural seawater. The soil suspension (100 μL) was then deposited on an agar plate that was prepared from soluble starch (20 g), KNO_3 (1 g), $K_2HPO_4{\cdot}3H_2O$ (0.5 g), $MgSO_4{\cdot}7H_2O$ (0.5 g), NaCl (0.5 g), $FeSO_4{\cdot}7H_2O$ (0.01 g), agar (20 g), and 1 L of natural seawater and incubated at 28 °C for 8 days. Bacterial colonies were selected and streaked to purity using the same agar media. It was identified by the 16S rRNA gene sequence (GenBank accession no. OQ318184).

Cultivation and Extraction of OUCMDZ-3434 and OUCMDZ-2599. Details of the method to yield the extracts of OUCMDZ-3434 grown on a liquid and solid medium were reported previously (extract A, 34.0 g; extract B, 30.0 g).[7c]

To obtain new fluorinated enterocin and wailupemycin analogues, *p*-fluorobenzoic acid was used as a precursor in the fermentation. The microbial strain OUCMDZ-3434 was cultured in 500 mL Erlenmeyer flasks containing 150 mL of liquid medium prepared by dissolving 20 g of soluble starch, 1 g of KNO_3, 0.5 g of $K_2HPO_4{\cdot}3H_2O$, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.5 g of NaCl, and 0.01 g of $FeSO_4{\cdot}7H_2O$ in 1 L of seawater and shaken for 4 days at 28 °C and 180 rpm: this was used as a seed culture. The seed culture (5 mL) was transferred to an Erlenmeyer flask (500 mL) containing 150 mL of the culture medium prepared by dissolving 20 g of glucose, 10 g of soluble starch, 10 g of yeast extract, 10 g of peptone, 0.5 g of KH_2PO_4,

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897



Compound	EC_{50} (μM)	95% CI (μM)
23	34.5	28.5 – 41.7
24	17.7	16.5 – 19.1
25	68.4	62.4 – 74.9
DFB	21.9	19.7 – 24.3

Figure 6. CAS assay results for compounds **23**–**25** and desferrioxamine B mesylate (DFB, positive control). (a) Concentration-dependent CAS liquid well-plate assay. (b) Dose–response curves for the iron binding activity. (c) EC_{50} values and 95% confidence intervals.

0.5 g of $MgSO_4$, 3 g of beef extract, 2 g of $CaCO_3$, and 0.133 g of *p*-fluorobenzoic acid in 1 L of seawater. The culture was shaken for 8 days at 28 °C and 180 rpm. Cultivation of 20 L was performed and extracted with equal volumes of EtOAc three times to give the extract (extract C, 12.7 g).

To accumulate the unstable metabolites detected in extract C, a repeated fermentation of 6 L according to the above conditions was conducted, and another crude extract was obtained (extract D, 3.6 g).

The microbial strain OUCMDZ-2599 was cultured on a solid medium. The seed broth was cultured in 500 mL Erlenmeyer flasks, each flask containing 150 mL of medium that was prepared by dissolving soluble starch (20 g), KNO_3 (1 g), $K_2HPO_4{\cdot}3H_2O$ (0.5 g), $MgSO_4{\cdot}7H_2O$ (0.5 g), NaCl (0.5 g), and $FeSO_4{\cdot}7H_2O$ (0.01 g) in 1 L of seawater and shaken for 4 days at 28 °C and 180 rpm. The seed culture (10 mL) was transferred to an Erlenmeyer flask (1 L) containing 100 g of dried rice and 80 mL of ISP2 liquid medium (4 g of glucose, 4 g of yeast extract, and 10 g of malt extract powder, 500 mL of seawater, 500 mL of tap water, pH 7.3). A total of 100 Erlenmeyer flasks were used in this fermentation. The culture was incubated at room temperature for 55 days. After incubation in static cultivation, the whole culture was extracted with equal volumes of EtOAc three times. The extract was dried *in vacuo* to yield the extract (extract E, 41.7 g).

Purification. The extract A (34.0 g) was subjected to a silica gel VLC column (70 × 160 mm) using step gradient elution with CH_2Cl_2–petroleum ether (30–100%) and then with MeOH–CH_2Cl_2 (0–50%) to give nine fractions (Fr.1–Fr.9). Fr.3 (619.4 mg) was fractionated into eight fractions (Fr.3.1–Fr.3.8) by an RP-silica gel column (18 × 130 mm) eluting with a stepwise gradient of MeOH–H_2O (10–100%). Fr.3.5 (56.7 mg) was subjected to a Sephadex LH-20 column eluting with MeOH, and further purified by semipreparative HPLC on an ODS column (YMC-pack ODS-A, 10 × 250 mm, 3.0 mL/min) eluting with 38% MeCN to afford compound **1** (3.4 mg, t_R = 28.0 min).

The extract B (30.0 g) was separated into eight fractions (Fr.1–Fr.8) on a silica gel VLC column (70 × 150 mm) using step gradient elution with CH_2Cl_2–petroleum ether (0–100%) and then with MeOH–CH_2Cl_2 (0–50%). Fr.4 (2.6 g) was subjected to an RP-silica gel column (25 × 130 mm) eluting with a stepwise gradient of MeOH–H_2O (10–100%) to yield nine fractions (Fr.4.1–Fr.4.9). Fr.4.8 (47.2 mg) was subjected to a Sephadex LH-20 column eluting with MeOH–CH_2Cl_2 (50%) and further purified by semipreparative HPLC on an ODS column (YMC-pack ODS-A, 10 × 250 mm, 3.0 mL/min) eluting with 50% MeCN to yield compound **2** (1.4 mg, t_R = 9.8 min).

The extract C (12.7 g) was subjected to a silica gel VLC column (50 × 140 mm) and eluted with a step gradient of CH_2Cl_2–petroleum ether (0–100%) and then MeOH–CH_2Cl_2 (0–50%), and seven fractions (Fr.1–Fr.7) were obtained. Fr.3 (493.4 mg) was fractionated by a silica gel column (20 × 80 mm) using step gradient elution with EtOAc–petroleum ether (5–100%) to give six fractions (Fr.3.1–Fr.3.6). Fr.3.3 (9.6 mg) was separated by a Sephadex LH-20 column eluting with MeOH–CH_2Cl_2 (50%) and further purified by semipreparative HPLC on an ODS column (YMC-pack ODS-A, 10 × 250 mm, 3.0 mL/min) eluting with 41% MeCN to yield compound **3** (2.0 mg, t_R = 26.2 min). Fr.4 (1.4 g) was fractionated into seven fractions by an RP-silica gel column (25 × 130 mm) eluting with a stepwise gradient of MeOH–H_2O (10–100%). Fr.4.2 (128.0 mg) was subjected to a Sephadex LH-20 column eluting with MeOH and further purified by semipreparative HPLC on a phenyl column (YMC-pack Ph, 10 × 250 mm, 3.0 mL/min) eluting with 38% MeCN to yield compounds **5** (1.0 mg, t_R = 21.0 min) and **4** (10.0 mg, t_R = 23.7 min). Fr.4.4 (66.4 mg) was subjected to a Sephadex LH-20 column eluting with MeOH–CH_2Cl_2 (50%) and further purified by HPLC on a C_{18}-PFP column (ACE C_{18}-PFP, 10 × 250 mm, 3.0 mL/min) eluting with 40% MeCN (0.5‰ TFA) to yield compounds **16** (1.9 mg, t_R = 35.5 min) and **9** (3.6 mg, t_R = 39.3 min). Fr.5 (846.5 mg) was separated into nine fractions (Fr.5.1–Fr.5.9) by an RP-silica gel column (18 × 130 mm) eluting with a stepwise gradient of MeOH–H_2O (10–100%). Fr.5.4 (356.1 mg) was subjected to a Sephadex LH-20 column eluting with MeOH and further purified by semipreparative HPLC on a C_{18}-PFP column (ACE C_{18}-PFP, 10 × 250 mm, 3.0 mL/min) eluting with 30% MeCN to yield compounds **11** (7.6 mg, t_R = 10.3 min) and **6** (9.4 mg, t_R = 12.7 min). Fr.6 (1.7 g) was separated into four fractions

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886–28897

(Fr.6.1−Fr.6.4) on an RP-silica gel column (25 × 130 mm) eluting with a stepwise gradient of MeOH−H_2O (10−100%). Fr.6.1 (404.0 mg) was applied to a Toyopearl HW-40F column eluting with MeOH to give five fractions (Fr.6.1.1−Fr.6.1.5). Fr.6.1.3 (65.2 mg) was further purified by HPLC on a C_{18}-PFP column (ACE C_{18}-PFP, 10 × 250 mm, 3.0 mL/min) eluting with 34% MeCN (0.5‰TFA) to yield compounds **15** (6.2 mg, t_R = 15.5 min) and **8** (8.4 mg, t_R = 19.3 min). Fr.6.2 (664.1 mg) was fractionated into five fractions (Fr.6.2.1 − Fr.6.2.5) on a Sephadex LH-20 eluting with MeOH. Fr.6.2.2 (92.7 mg) was further purified by semipreparative HPLC on a C_{18}-PFP column (ACE C_{18}-PFP, 10 × 250 mm, 3.0 mL/min) eluting with 36% MeCN (0.5‰ TFA) to yield compounds **14** (7.3 mg, t_R = 20.2 min) and **7** (9.1 mg, t_R = 24.7 min). Fr.7 (5.9 g) was separated on an RP-silica gel column (25 × 130 mm) eluting with a stepwise gradient of MeOH−H_2O (10−100%) to give five fractions (Fr.7.1−Fr.7.5). Fr.7.3 (1.1 g) was subjected to a Sephadex LH-20 column eluting with MeOH to give four fractions (Fr.7.3.1−Fr.7.3.4). Fr.7.3.2 (200.0 mg) was purified by HPLC on a C_{18}-PFP column (ACE C_{18}-PFP, 10 × 250 mm, 3.0 mL/min) eluting with 28% MeCN to afford compounds **13** (48.0 mg, t_R = 8.1 min) and **12** (34.0 mg, t_R = 9.7 min). Fr.7.5 (1.4 g) was separated into 10 fractions (Fr.7.5.1 − Fr.7.5.10) on a Sephadex LH-20 column eluting with MeOH−CH_2Cl_2 (50%). Fr.7.5.8 (48.2 mg) was further separated by semipreparative HPLC on a C_{18}-PFP column (ACE C_{18}-PFP, 10 × 250 mm, 3.0 mL/min) eluting with 45% MeCN (0.5‰ TFA) to give compounds **18** (2.4 mg, t_R = 13.1 min) and **17** (3.2 mg, t_R = 14.9 min). Fr.7.5.10 (27.0 mg) was purified on a phenyl column (YMC-pack Ph, 10 × 250 mm, 3.0 mL/min) eluting with 40% MeCN (0.5‰ TFA) to yield compound **10** (2.1 mg, t_R = 14.0 min).

The extract D (3.6 g) was separated into 10 fractions (Fr.1−Fr.10) on a silica gel VLC column (25 × 180 mm) using step gradient elution with CH_2Cl_2−petroleum ether (0−100%) and then MeOH−CH_2Cl_2 (0−10%). Fr.8 (448.6 mg) was subjected to a Sephadex LH-20 column eluting with MeOH to give five fractions (Fr.8.1−Fr.8.5). Fr.8.3 (11.5 mg) was further purified by HPLC on an ODS column (YMC-pack ODS-A, 10 × 250 mm, 2.5 mL/min) eluting with 45% MeCN (0.5‰ TFA) to yield compounds **20** (3.1 mg, t_R = 11.9 min) and **19** (3.5 mg, t_R = 13.0 min).

The extract E (41.7 g) was subjected to a silica gel VLC column (70 × 160 mm) using step gradient elution with CH_2Cl_2−petroleum ether (0−100%) and then with MeOH−CH_2Cl_2 (0−50%) to give five fractions (Fr.1−Fr.5). Fr.3 (2.8 g) was fractionated into 11 fractions (Fr.3.1−Fr.3.11) by an RP-silica gel column (25 × 130 mm) eluting with a stepwise gradient of MeOH−H_2O (10−100%). Fr.3.10 (181.2 mg) was subjected to a Sephadex LH-20 column eluting with MeOH−CH_2Cl_2 (50%) and further purified by HPLC on an ODS column (YMC-pack ODS-A, 10 × 250 mm, 3.0 mL/min) eluting with 52% MeCN to afford compounds **21** (2.0 mg, t_R = 15.0 min), **22** (11.0 mg, t_R = 20.0 min), and the racemic **26**/**27** (6.5 mg, t_R = 17.9 min). Compounds **26**/**27** were further separated by a Chiral INC column (4.6 × 250 mm, 5 μm, 0.8 mL/min) eluting with *i*-PrOH−*n*-hexane (10%) to give the optically pure compounds **27** (1.2 mg, t_R = 22.5 min) and **26** (1.4 mg, t_R = 23.7 min). Fr.5 (4.2 g) was subjected to an RP-silica gel column (25 × 130 mm) eluting with a stepwise gradient of MeOH−H_2O (10−100%) to yield seven fractions (Fr.5.1−Fr.5.7). Fr.5.2 (2.5 g) was fractionated into 10 fractions (Fr.5.2.1−Fr.5.2.10) by another RP-silica gel column (25 × 130 mm) eluting with a stepwise gradient of MeOH−H_2O (10−100%). Fr.5.2.4 (150.3 mg) was subjected to a Sephadex LH-20 column eluting with MeOH and further purified by HPLC on an ODS column (YMC-pack ODS-A, 10 × 250 mm, 3.0 mL/min) eluting with 16% MeCN to yield compound **23** (2.4 mg, t_R = 16.0 min). Fr.5.2.5 (364.1 mg) was separated into six fractions (Fr.5.2.5.1−Fr.5.2.5.6) on a Sephadex LH-20 column eluting with MeOH. Fr.5.2.5.3 (89.0 mg) was further purified by semipreparative HPLC on an ODS column (YMC-pack ODS-A, 10 × 250 mm, 3.0 mL/min) eluting with 16% MeCN to afford compounds **24** (10.3 mg, t_R = 19.5 min) and **25** (8.3 mg, t_R = 22.2 min).

*Wailupemycin Q (**1**).* Yellow amorphous powder; $[\alpha]_D^{26}$ −173.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.39), 262 (3.78), 332 (3.41) nm; ECD (0.66 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 219 (+6.71), 236 (−1.31), 255 (+6.09), 280 (−13.00), 344 (+1.59) nm; IR (KBr disk) ν_{max} 3335, 2929, 1690, 1643, 1565, 1252 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 379.1169 $[M + H]^+$ (calcd for $C_{22}H_{19}O_6$, 379.1176).

*Wailupemycin R (**2**).* Yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 221 (4.12), 243 (4.00) nm; IR (KBr disk) ν_{max} 3406, 2926, 1653, 1565, 1254, 703 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 295.0961 $[M + H]^+$ (calcd for $C_{18}H_{15}O_4$, 295.0965).

*Wailupemycin S (**3**).* Light purple powder; UV (MeOH) λ_{max} (log ε) 220 (4.16), 256 (3.90), 345 (3.17) nm; IR (KBr disk) ν_{max} 3444, 2921, 1652, 1510, 1354, 1222, 1162, 1024 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; ^{19}F NMR (376 MHz, DMSO-d_6) δ −117.10; HRESIMS *m*/*z* 297.0924 $[M + H]^+$ (calcd for $C_{18}H_{14}O_3F$, 297.0921).

*17-Fluorowailupemycin O (**4**).* Yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 212 (4.14), 257 (3.85) nm; IR (KBr disk) ν_{max} 3300, 3192, 2929, 2856, 1698, 1564, 1251, 701 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; ^{19}F NMR (376 MHz, methanol-d_4) δ −107.15; HRESIMS *m*/*z* 355.0972 $[M + H]^+$ (calcd for $C_{20}H_{16}O_5F$, 355.0976).

*Wailupemycin T (**5**).* White amorphous powder; UV (MeOH) λ_{max} (log ε) 220 (3.85), 315 (3.74) nm; IR (KBr disk) ν_{max} 3441, 2926, 1725, 1642, 1563, 1510, 1455, 1221, 1010 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; ^{19}F NMR (376 MHz, DMSO-d_6) δ −109.24; HRESIMS *m*/*z* 221.0606 $[M + H]^+$ (calcd for $C_{12}H_{10}O_3F$, 221.0608).

*19-Fluoro-5-deoxyenterocin (**6**).* White amorphous powder; $[\alpha]_D^{28}$ −36.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.40), 252 (4.04) nm; ECD (1.12 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 208 (+9.94), 247 (−4.59), 278 (+0.96), 325 (−1.31) nm; IR (KBr disk) ν_{max} 3460, 3327, 1744, 1693, 1642, 1563, 1462, 1336, 1220, 1135, 1086, 1005 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; ^{19}F NMR (376 MHz, DMSO-d_6) δ −106.78; HRESIMS *m*/*z* 447.1077 $[M + H]^+$ (calcd for $C_{22}H_{20}O_9F$, 447.1086).

*19-Fluorowailupemycin D (**7**).* Yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 210 (4.30), 262 (3.82), 335 (3.43) nm; IR (KBr disk) ν_{max} 3385, 3076, 1699, 1640, 1570, 1257, 1026, 996 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ^{19}F NMR (376 MHz, DMSO-d_6) δ −115.60; HRESIMS *m*/*z* 383.0922 $[M + H]^+$ (calcd for $C_{21}H_{16}O_6F$, 383.0925). **7a**: $[\alpha]_D^{27}$ +112.0 (*c* 0.1, MeOH); ECD (1.31 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 215 (−3.54), 245 (+0.24), 266 (−1.33), 286 (+5.34), 344 (−0.64) nm. **7b**: $[\alpha]_D^{27}$ −118.4 (*c* 0.1, MeOH); ECD (1.31 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 217 (+2.59), 248 (−0.20), 266 (+1.21), 286 (−4.41), 341 (+0.41) nm.

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897

*19-Fluorowailupemycin E (**8**)*. Yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 212 (4.36), 260 (3.92), 292 (3.67), 331 (3.57) nm; IR (KBr disk) ν_{max} 3417, 3078, 1697, 1640, 1569, 1240, 1026, 997 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ^{19}F NMR (376 MHz, DMSO-d_6) δ −115.06; HRESIMS m/z 405.0752 $[M + Na]^+$ (calcd for $C_{21}H_{15}O_6FNa$, 405.0745). **8a**: $[\alpha]_D^{27}$ +427.4 (c 0.1, MeOH); ECD (1.31 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 229 (+18.76), 256 (−11.20), 284 (+24.57), 329 (−3.17) nm. **8b**: $[\alpha]_D^{27}$ −441.5 (c 0.1, MeOH); ECD (1.31 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 229 (−16.75), 256 (+10.94), 284 (−23.02), 329 (+3.08) nm.

*19-Fluoro-3-O-methylwailupemycin G (**9**)*. Yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 209 (4.33), 247 (4.28), 340 (3.83) nm; IR (KBr disk) ν_{max} 3384, 2932, 1683, 1562, 1409, 1212, 1023, 760, 670 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ^{19}F NMR (376 MHz, DMSO-d_6) δ −114.73; HRESIMS m/z 379.0977 $[M + H]^+$ (calcd for $C_{22}H_{16}O_5F$, 379.0976).

*Wailupemycin U (**10**)*. Yellow amorphous powder; $[\alpha]_D^{27}$ +22.5 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.64), 332 (3.12), 363 (3.26) nm; ECD (1.48 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 266 (+0.58), 338 (−0.04) nm; IR (KBr disk) ν_{max} 3415, 2929, 1681, 1604, 1382, 1210, 1028 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; ^{19}F NMR (376 MHz, DMSO-d_6) δ −117.59; HRESIMS m/z 339.1026 $[M + H]^+$ (calcd for $C_{20}H_{16}O_4F$, 339.1027).

*Wailupemycin D (**14**)*. Yellow amorphous powder. **14a**: $[\alpha]_D^{27}$ +123.4 (c 0.1, MeOH); ECD (1.37 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 222 (−2.04), 240 (+0.20), 257 (−1.61), 284 (+3.70), 346 (−0.45) nm. **14b**: $[\alpha]_D^{27}$ −142.5 (c 0.1, MeOH); ECD (1.37 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 219 (+2.30), 239 (−0.42), 257 (+2.20), 284 (−4.82), 344 (+0.55) nm.

*Wailupemycin E (**15**)*. Yellow amorphous powder. **15a**: $[\alpha]_D^{27}$ +374.0 (c 0.1, MeOH); ECD (1.37 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 230 (+13.39), 257 (−8.37), 284 (+19.73), 328 (−2.76) nm. **15b**: $[\alpha]_D^{27}$ −379.6 (c 0.1, MeOH); ECD (1.37 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 230 (−14.01), 256 (+9.20), 284 (−21.20), 328 (+2.97) nm.

*19-Fluorowailupemycin G (**17**)*. Yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 207 (4.32), 248 (4.26), 339 (3.75) nm; IR (KBr disk) ν_{max} 3398, 2922, 1683, 1564, 1386, 1203, 1025, 843, 761 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.59 (brs, 1H), 7.38 (t, J = 8.0 Hz, 1H), 7.33 (dd, J = 8.5, 5.6 Hz, 2H), 7.23 (t, J = 8.8 Hz, 2H), 7.07 (d, J = 8.5 Hz, 1H), 6.83 (d, J = 7.6 Hz, 1H), 6.73 (s, 1H), 5.88 (d, J = 2.1 Hz, 1H), 5.30 (d, J = 2.1 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.9, 163.8, 161.6 (d, J = 243.2 Hz), 160.9, 156.3, 154.7, 140.1, 136.7 (d, J = 2.5 Hz), 134.9, 130.5 (d, J = 8.2 Hz), 128.8, 119.3, 115.9, 115.2 (d, J = 21.3 Hz), 113.6, 109.7, 109.2, 105.9, 89.3; ^{19}F NMR (376 MHz, DMSO-d_6) δ −114.84; HRESIMS m/z 365.0828 $[M + H]^+$ (calcd for $C_{21}H_{14}O_5F$, 365.0820).

*19-Fluorowailupemycin F (**19**)*. Yellow amorphous powder; $[\alpha]_D^{27}$ −4.1 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.08), 283 (3.80) nm; ECD (1.31 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 207 (+3.65), 238 (−2.45), 272 (+0.51), 303 (−0.63) nm; IR (KBr disk) ν_{max} 3424, 2946, 2830, 1684, 1641, 1565, 1206, 723 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 11.82 (s, 1H), 7.31 (dd, J = 8.6, 5.5 Hz, 2H), 7.24 (t, J = 8.8 Hz, 2H), 6.87 (s, 1H), 5.88 (d, J = 2.1 Hz, 1H), 5.28 (brs, 1H), 5.27 (d, J = 2.1 Hz, 1H), 4.29 (m, 1H), 3.08 (dd, J = 16.8, 3.4 Hz, 1H), 3.01 (dd, J = 16.8, 3.3 Hz, 1H), 2.82 (dd, J = 16.8, 6.0 Hz, 1H), 2.73 (dd, J = 16.8, 6.5 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 204.8, 169.7, 163.4, 162.1, 162.0 (d, J = 244.0 Hz), 159.5, 148.3, 142.9, 135.6 (d, J = 3.0 Hz), 130.3 (d, J = 8.4 Hz), 123.1, 116.3, 115.6, 115.4 (d, J = 21.0 Hz), 105.6, 89.4, 64.3, 46.3, 35.8; ^{19}F NMR (376 MHz, DMSO-d_6) δ −113.77; HRESIMS m/z 383.0931 $[M + H]^+$ (calcd for $C_{21}H_{16}O_6F$, 383.0925).

*Wailupemycin F (**20**)*. Yellow amorphous powder; $[\alpha]_D^{27}$ −5.5 (c 0.1, MeOH); ECD (1.37 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 207 (+4.64), 239 (−2.82), 276 (+0.53), 298 (−0.90) nm.

*Thiotetromycin B (**21**)*. White powder; $[\alpha]_D^{28}$ +34.7 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.90), 249 (3.86) nm; ECD (1.85 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 222 (−7.95), 251 (+14.38), 282 (−0.99) nm; IR (KBr disk) ν_{max} 3396, 2976, 2935, 2880, 2851, 1702, 1460, 1383, 1185, 1076, 974, 909 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; HRESIMS m/z 269.0849 $[M - H]^-$ (calcd for $C_{13}H_{17}O_4S$, 269.0853).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04199.

HRESIMS and NMR spectra, details for calculations and bioassays (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Weiming Zhu − *Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China; Laboratory for Marine Drugs and Bioproducts, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, People's Republic of China;* orcid.org/0000-0002-7591-3264; Email: weimingzhu@ouc.edu.cn

Peng Fu − *Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China; Laboratory for Marine Drugs and Bioproducts, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, People's Republic of China;* orcid.org/0000-0002-7768-4004; Email: fupeng@ouc.edu.cn

Authors

Kunyu Xia − *Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China*

Jiaxu Shang − *Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China*

Jiwen Sun − *Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c04199

Notes

The authors declare no competing financial interest.

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897

■ ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (Nos. 22277112, 41876172, and U1906213).

■ REFERENCES

(1) Pham, J. V.; Yilma, M. A.; Feliz, A.; Majid, M. T.; Maffetone, N.; Walker, J. R.; Kim, E.; Cho, H. J.; Reynolds, J. M.; Song, M. C.; Park, S. R.; Yoon, Y. J. A Review of the Microbial Production of Bioactive Natural Products and Biologics. *Front. Microbiol.* **2019**, *10*, 1404.

(2) (a) Yang, C.; Qian, R.; Xu, Y.; Yi, J.; Gu, Y.; Liu, X.; Yu, H.; Jiao, B.; Lu, X.; Zhang, W. Marine Actinomycetes-Derived Natural Products. *Curr. Top. Med. Chem.* **2019**, *19*, 2868−2918. (b) Wang, C.; Lu, Y.; Cao, S. Antimicrobial Compounds from Marine Actinomycetes. *Arch. Pharmacal Res.* **2020**, *43*, 677−704.

(3) (a) Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. Salinosporamide A: A Highly Cytotoxic Proteasome Inhibitor from a Novel Microbial Source, a Marine Bacterium of the New Genus *Salinospora*. *Angew. Chem., Int. Ed.* **2003**, *42*, 355−357. (b) Fenical, W. Marine Microbial Natural Products: The Evolution of a New Field of Science. *J. Antibiot.* **2020**, *73*, 481−487.

(4) (a) Romsdahl, J.; Wang, C. C. C. Recent Advances in the Genome Mining of *Aspergillus* Secondary Metabolites (Covering 2012−2018). *Med. Chem. Commun.* **2019**, *10*, 840−866. (b) Kang, H.-S.; Kim, E.-S. Recent Advances in Heterologous Expression of Natural Product Biosynthetic Gene Clusters in *Streptomyces* Hosts. *Curr. Opin. Biotechnol.* **2021**, *69*, 118−127.

(5) (a) Reen, F. J.; Romano, S.; Dobson, A. D. W.; O'Gara, F. The Sound of Silence: Activating Silent Biosynthetic Gene Clusters in Marine Microorganisms. *Mar. Drugs* **2015**, *13*, 4754−4783. (b) Wang, C.; Monger, A.; Wang, L.; Fu, P.; Piyachaturawat, P.; Chairoungdua, A.; Zhu, W. Precursor-Directed Generation of Indolocarbazoles with Topoisomerase IIα Inhibitory Activity. *Mar. Drugs* **2018**, *16*, 168.

(6) (a) Yin, S.; Liu, Z.; Shen, J.; Xia, Y.; Wang, W.; Gui, P.; Jia, Q.; Kachanuban, K.; Zhu, W.; Fu, P. Chimeric Natural Products Derived from Medermycin and the Nature-Inspired Construction of Their Polycyclic Skeletons. *Nat. Commun.* **2022**, *13*, 5169. (b) Zhao, S.; Xia, Y.; Liu, H.; Cui, T.; Fu, P.; Zhu, W. A Cyclohexapeptide and Its Rare Glycosides from Marine Sponge-Derived *Streptomyces* sp. OUCMDZ-4539. *Org. Lett.* **2022**, *24*, 6750−6754. (c) Ma, R.; Cheng, S.; Sun, J.; Zhu, W.; Fu, P. Antibacterial Gilvocarcin-Type Aryl-*C*-Glycosides from a Soil-Derived *Streptomyces* Species. *J. Nat. Prod.* **2022**, *85*, 2282−2289.

(7) (a) Chen, Z.; Hao, J.; Wang, L.; Wang, Y.; Kong, F.; Zhu, W. New α-Glucosidase Inhibitors from Marine Algae-Derived *Streptomyces* sp. OUCMDZ-3434. *Sci. Rep.* **2016**, *6*, 20004. (b) Liu, H.; Chen, Z.; Zhu, G.; Wang, L.; Du, Y.; Wang, Y.; Zhu, W. Phenolic Polyketides from the Marine Alga-Derived *Streptomyces* sp. OUCMDZ-3434. *Tetrahedron* **2017**, *73*, 5451−5455. (c) Xia, K.; Luo, H.; Ma, R.; Zhang, R.; Zhu, W.; Fu, P. Aromatic Polyketides and Hydroxamate Siderophores from a Marine-Algae-Derived *Streptomyces* Species. *J. Nat. Prod.* **2021**, *84*, 1550−1555.

(8) (a) Piel, J.; Hertweck, C.; Shipley, P. R.; Hunt, D. M.; Newman, M. S.; Moore, B. S. Cloning, Sequencing and Analysis of the Enterocin Biosynthesis Gene Cluster from the Marine Isolate '*Streptomyces maritimus*': Evidence for the Derailment of an Aromatic Polyketide Synthase. *Chem. Biol.* **2000**, *7*, 943−955. (b) Kalaitzis, J. A.; Izumikawa, M.; Xiang, L.; Hertweck, C.; Moore, B. S. Mutasynthesis of Enterocin and Wailupemycin Analogues. *J. Am. Chem. Soc.* **2003**, *125*, 9290−9291. (c) Kirsch, S.; Bach, T. Total Synthesis of (+)-Wailupemycin B. *Angew. Chem., Int. Ed.* **2003**, *42*, 4685−4687. (d) Koser, L.; Lechner, V. M.; Bach, T. Biomimetic Total Synthesis of Enterocin. *Angew. Chem., Int. Ed.* **2021**, *60*, 20269−20273.

(9) Kalaitzis, J. A.; Cheng, Q.; Thomas, P. M.; Kelleher, N. L.; Moore, B. S. *In Vitro* Biosynthesis of Unnatural Enterocin and Wailupemycin Polyketides. *J. Nat. Prod.* **2009**, *72*, 469−472.

(10) (a) Omura, S.; Iwai, Y.; Nakagawa, A.; Iwata, R.; Takahashi, Y.; Shimizu, H.; Tanaka, H. Thiotetromycin, a New Antibiotic. Taxonomy, Production, Isolation, and Physicochemical and Biological Properties. *J. Antibiot.* **1983**, *36*, 109−114. (b) Omura, S.; Nakagawa, A.; Iwata, R.; Hatano, H. Structure of a New Antibacterial Antibiotic, Thiotetromycin. *J. Antibiot.* **1983**, *36*, 1781−1782. (c) Chamber, M. S.; Thomas, E. J. Total Synthesis of (5*S*)-Thiolactomycin: Revision of the Absolute Configuration of the Natural Product. *J. Chem. Soc., Chem. Commun.* **1989**, *1*, 23−24.

(11) Mike, L. A.; Tripathi, A.; Blankenship, C. M.; Saluk, A.; Schultz, P. J.; Tamayo-Castillo, G.; Sherman, D. H.; Mobley, H. L. T. Discovery of Nicoyamycin A, an Inhibitor of Uropathogenic *Escherichia coli* Growth in Low Iron Environments. *Chem. Commun.* **2017**, *53*, 12778−12781.

(12) Lee, H.-S.; Shin, H. J.; Jang, K. H.; Kim, T. S.; Oh, K.-B.; Shin, J. Cyclic Peptides of the Nocardamine Class from a Marine-Derived Bacterium of the Genus *Streptomyces*. *J. Nat. Prod.* **2005**, *68*, 623−625.

(13) Wang, G.-Y.-S.; Graziani, E.; Waters, B.; Pan, W.; Li, X.; McDermott, J.; Meurer, G.; Saxena, G.; Andersen, R. J.; Davies, J. Novel Natural Products from Soil DNA Libraries in a Streptomycete Host. *Org. Lett.* **2000**, *2*, 2401−2404.

(14) Piel, J.; Hoang, K.; Moore, B. S. Natural Metabolic Diversity Encoded by the Enterocin Biosynthesis Gene Cluster. *J. Am. Chem. Soc.* **2000**, *122*, 5415−5416.

(15) (a) Fu, P.; Yang, C.; Wang, Y.; Liu, P.; Ma, Y.; Xu, L.; Su, M.; Hong, K.; Zhu, W. Streptocarbazoles A and B, Two Novel Indolocarbazoles from the Marine-Derived Actinomycete Strain *Streptomyces* sp. FMA. *Org. Lett.* **2012**, *14*, 2422−2425. (b) Fu, P.; Kong, F.; Li, X.; Wang, Y.; Zhu, W. Cyanogramide with a New Spiro[indolinone-pyrroloimidazole] Skeleton from *Actinoalloteichus cyanogriseus*. *Org. Lett.* **2014**, *16*, 3708−3711.

(16) Xiang, L.; Kalaitzis, J. A.; Nilsen, G.; Chen, L.; Moore, B. S. Mutational Analysis of the Enterocin Favorskii Biosynthetic Rearrangement. *Org. Lett.* **2002**, *4*, 957−960.

(17) Endo, Y.; Minowa, A.; Kanamori, R.; Araya, H. A Rare α-Pyrone from Bitter Tooth Mushroom, *Sarcodon scabrosus* (Fr.) Karst. *Biochem. Syst. Ecol.* **2012**, *44*, 286−288.

(18) (a) Kang, H.; Jensen, P. R.; Fenical, W. Isolation of Microbial Antibiotics from a Marine Ascidian of the Genus *Didemnum*. *J. Org. Chem.* **1996**, *61*, 1543−1546. (b) Cowled, M. S.; Vuong, D.; Crombie, A.; Lacey, E.; Karuso, P.; Piggott, A. M. The Chemical Gymnastics of Enterocin: Evidence for Stereodivergence in Nature. *Org. Biomol. Chem.* **2020**, *18*, 5879−5890.

(19) Kawashima, A.; Seto, H.; Kato, M.; Uchida, K.; Ōtake, N. Preparation of Fluorinated Antibiotics Followed by ^{19}F NMR Spectroscopy. I. Fluorinated Vulgamycins. *J. Antibiot.* **1985**, *38*, 1499−1505.

https://doi.org/10.1021/acsomega.3c04199
ACS Omega 2023, 8, 28886−28897