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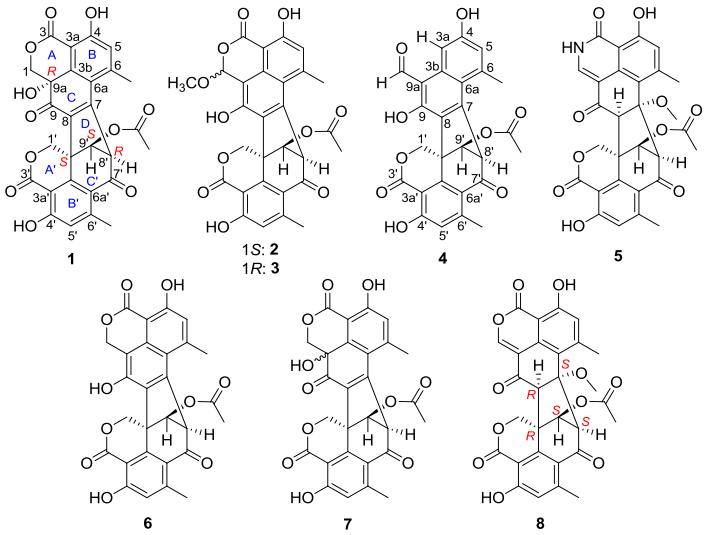
ABSTRACT: New polyketide-derived oligophenalenone dimers, *9a-epi*-bacillisporin E (**1**) and bacillisporins F−H (**2**−**5**) along with the known bacillisporin A (**6**), were isolated from the fungus *Talaromyces stipitatus*. Their structures and absolute configurations were determined on the basis of spectroscopic analyses, electronic circular dichroism (ECD) as well as GIAO NMR shift calculation followed by DP4 analysis. The antimicrobial activity of these compounds was evaluated against a panel of human pathogenic bacteria. Among them, bacillisporin H (**5**) exhibited antimicrobial activity together with modest cytotoxicity against Hela cells.

Fungi are considered as an important source of chemical diversity, such as highly elaborated polyketides.[1] These complex structures have attracted great interest because of their biological activities which may lead to new medicinal or agrochemical applications. Soil-inhabiting fungi constitute an extremely diverse fungal community and the soil fungus *Talaromyces stipitatus* ATCC 10500 (*syn*. *Penicillium stipitatum*) is known to produce a variety of structurally interesting aromatic polyketides such as tropolones[2] and oxaphenalenone dimers related to duclauxin (**8**), including complex spirobicyclic derivatives.[3-6] This strain was previously studied by Cox et *al.* for the biosynthesis of tropolones.[7] A recent study of *Penicillium mangini* by Huang and coworkers led to the structure revision of duclauxin (**8**) and derivatives, correcting a long-standing misrepresentation of the absolute configuration of C-9′.[8]

Duclauxin (**8**) is a heptacyclic bis(oxaphenalenone) heterodimer made of two functionalized 2-oxaphenalen-1-one units (corymbiferan skeleton) linked by two bonds which form the cyclopentane. Recently, Gao and coworkers characterized the *phn* biosynthetic gene cluster from the genome of *Penicillium herquei* and showed that the core skeleton of phenalenone polyketide was synthesized by the combined functions of non-reducing polyketides synthase (NR-PKS) (Phn A) and a flavin-dependent monooxygenase (Phn B).[9] Duclauxin (**8**) and related compounds such as bacillisporins A−E were isolated either from *Talaromyces* or *Penicillium* species and have been shown to inhibit the growth of several tumor cell lines,[10-12] while other analogues were efficient inhibitors of acetylcholinesterases.

In an effort to broaden our research in new bioactive polyketides, we investigated the chemistry of the fungus *Talaromyces stipitatus* ATCC 10500 by the one-strain many compounds (OSMAC) approach.[13] This led to the identification of five new oligophenalenone dimers named 9a-*epi*-bacillisporin E (**1**) and bacillisporins F−H (**2**−**5**) along with the known, bacillisporin A (**6**). Herein,

we report their isolation, structure elucidation and their antimicrobial activity. The absolute configuration of the isolated compounds was established by a combination of ECD calculations and the DP4 probability analysis based on NMR shift calculation.



RESULTS AND DISCUSSION

9a-*epi*-bacillisporin E (**1**) was isolated as a colorless crystalline solid with an $[\alpha]^{20}_{D}$ of + 7.3 (CH_2Cl_2, *c* 0.1). The molecular formula $C_{28}H_{20}O_{11}$ indicating 19 degrees of unsaturation was deduced from the molecular peak at *m/z* 531.0822 $[M-H]^-$ in HREIMS. Its IR spectrum pointed out the presence of hydroxy (3421 cm^{-1}) and carbonyl (1670 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) of **1** showed signals for three methyl groups as singlets at δ_H 2.12 (3H, $COCH_3$), 2.60 (3H, H-6'), and 2.86 (3H, H-6), two oxymethylene [δ_H 4.87 (1H, d, *J* = 13.0 Hz, H-1), 4.31 (1H, d, *J* = 13.0 Hz, H-1); 4.83 (1H, d, *J* = 12.5 Hz, H-1'*β*), 4.74 (1H, d, *J* = 12.5 Hz, H-1'*α*)], one oxymethine [δ_H 5.67 (1H, s, H-9')], and two aromatic protons resonating at δ_H 6.80 (1H, s, H-5') and 6.91 (1H, s, H-5). ^{13}C NMR spectrum of **1** indicated the presence of nine sp^3 carbons: three methyls, two methylenes (oxygenated at δ_C 68.1, 71.1), two methines (including one oxygenated at δ_C 83.9), one oxygenated at δ_C 65.9 and one quaternary carbon at δ_C 47.9. ^{13}C NMR data of **1** also displayed two sp^2 methines (δ_C 121.3, 122.6), seventeen sp^2 carbons (including two

oxygenated at δ_C 163.1, 164.9, two lactones carbonyls at δ_C 167.3, 167.4, one ester carbonyl at δ_C 169.9 and two ketone carbonyls at δ_C 188.0, 191.2) (Table 1).

Table 1. ^{1}H and ^{13}C NMR data of compounds **1**−**4** (^{1}H 600 MHz and ^{13}C 150 MHz, 298K).

Pos.	**1**[a]		**2**			**3**[c,d]	**4**[e]	
	δ_C , type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)[b]	δ_H (J in Hz)[c]	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	71.1, CH_2	4.87,d (13.0) 4.31, d (13.0)	98.1, CH_2	6.66, s	6.55, s	6.57, s	-	-
3	167.4, C	-	168.4, C	-	-	-	-	-
3a	108.1, C	-	96.9, C	-	-	-	100.8, CH	7.47, s
3b	143.1, C	-	130.9, C	-	-	-	138.1, C	-
4	163.1, C	-	162.8, C	-	-	-	156.3, C	-
5	122.6, CH	6.91, s	119.2, CH	6.99, s	6.94, s	6.94, s	119.9, CH	6.85, s
6	148.1, C	-	146.8, C	-	-	-	139.2, C	-
6a	116.7, C	-	118.3, C	-	-	-	120.7, C	-
7	155.6, C	-	134.1, C	-	-	-	147.1, C	-
8	145.5, C	-	138.9, C	-	-	-	130.7, C	-
9	191.2, C	-	151.3, C	-	-	-	160.6, C	-
9a	65.9, C	-	109.7, C	-	-	-	111.7, C	-
CH_3-6	24.8, CH_3	2.86, s	24.3, CH_3	2.95, s	3.01, s	3.01, s	25.2, CH_3	2.96, s
CH_3-6'	23.9, CH_3	2.60, s	23.2, C	2.50 , s	2.57, s	2.55, s	23.8, CH_3	2.54, s
H-CO	-	-	-	-	-	-	193.5, CH	10.44, s
OH-4	-	11.24, s	-	11.78, s	-	-	-	-
OH-9	_	_	-	10.80, s	-	-	-	-
OCH_3	-	-	55.3, CH_3	3.47, s	3.578, s	3.66, s	-	-
1'α	68.1, CH_2	4.74, d (12.5)	68.8, CH_2	5.11, d (12.4)	5.19, d (12.4)	5.19, d (12.5)	68.8, CH_2	4.88, d (12.1)
1'β		4.83, d (12.5)		5.03, d (12.4)	5.07, d (12.4)	5.07, d (12.5)		5.13, d (12.1)
3'	167.3, C	-	167.2, C	-	-	-	167.7, C	-
3'a	104.1, C	-	103.9, C	-	-	-	103.5, C	-
3'b	135.8, C	-	146.3, C	-	-	-	145.9, C	-
4'	164.9, C	-	163.3, C	-	-	-	164.7, C	-
5'	121.3, CH	6.80, s	120.3, C	6.89, s	6.82, s	6.80, s	121.2, CH	6.75, s
6'	153.9, C	-	152.8, C	-	-	-	153.8, C	-
6'a	116.9, C	-	116.5, C	-	-	-	116.7, C	-
7'	188.0, C	-	190.9, C	-	-	-	189.8, C	-
8'	63.7, CH	4.71, s	61.4, CH	5.01, s	5.12, s	5.14, s	62.9, CH	5.15, s
9'	83.9, CH	5.67, s	85.5, CH	5.80, s	5.84, s	5.84, s	85.7, CH	5.79, s
9'a	47.9, C	-	47.9, C	-	-	-	48.0, C	-
OCOCH_3	20.9, CH_3	2.12, s	20.7, CH_3	1.97, s	2.05, s	2.05, s	20.8, CH_3	2.04, s
OCOCH_3	169.9, C	-	170.0, C	-	-	-	170.3, C	-
OH-4'	_	11.86, s	-	11.93, s	-	-	-	-

[a] Recorded in $CDCl_3$; [b] in DMSO-d_6; [c] in CD_3OD; [d] ^{13}C was not obtained because of too low amount of **3**; [e] in C_5D_5N.

Analysis of 2D NMR data (COSY, HSQC and HMBC, Figure 1) revealed that **1** is an oxyphenalenone dimer closely related to the known compound bacillisporin E (**7**), although the relative and absolute configurations of the latter was undetermined. The only difference between

those two structures was the higher-field shift of the CH_2-1 (δ_H 4.87 and 4.31) of **1** compared with the bacillisporin E (**7**) signal (δ_H 4.90 and 4.82).[10]

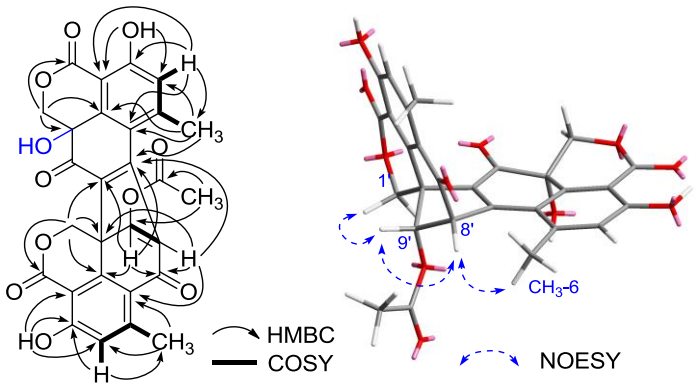


Figure 1. Key HMBC, COSY and NOESY correlations of **1**.

The relative configurations of C-8', C-9', and C-9'a of **1** were deduced from the proton coupling constant and NOESY correlations. Indeed, as described by Huang,[7] no coupling constant was observed between H-8′ and H-9′ suggesting a torsion angle of ca. 90° between these two protons. In addition, NOEs between H-8′ and H-9′/CH_3-6 as well as between H-9′ and H-1′α are in agreement with the recently revised configuration of duclauxin (**8**).

We used DP4 probability to determine the configuration at C-9a of **1**.[14] This approach based on theoretical NMR calculations has been proven to be a very powerful tool in natural products structure elucidation.[9,15] Taking both ^{1}H and ^{13}C chemical shifts calculations for the DP4 probability analysis (Figure S40), the relative configuration at C-9a was defined to be *R*.

Electronic circular dichroism (ECD) measurements were undertaken to determine the absolute configuration of **1**. Experimental and simulated spectra generated by time-dependent density functional theory (TDDFT) were then compared.[16] As observed in Figure 2, a good agreement was

obtained between both predicted and experimental spectra. Therefore, the absolute configuration of **1** was deduced to be 9a*R*, 8'*R*, 9'*S*, and 9'a*S*.

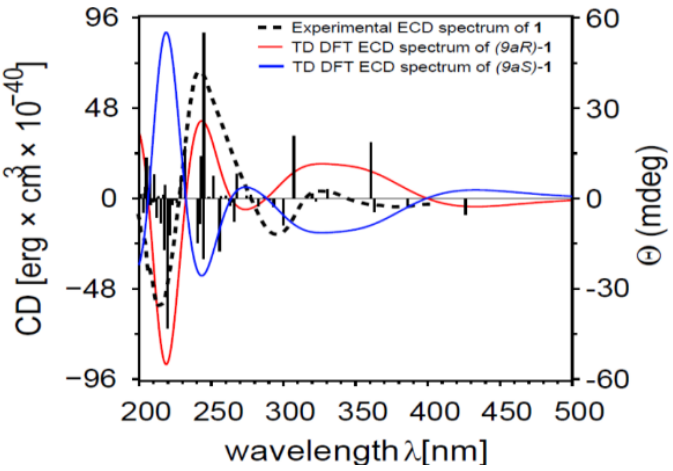


Figure 2. Comparison of the calculated and experimental ECD spectra of **1**.

Bacillisporin F (**2**) was isolated as a colourless crystalline solid with an $[\alpha]^{20}_{D}$ of + 43.5 (CH_2Cl_2, *c* 0.58). The molecular formula $C_{29}H_{22}O_{11}$ was deduced from the protonated ion at *m*/*z* 547.1255 $[M+H]^+$ in HREIMS. Its IR spectrum pointed out carbonyl groups at 1724 and 1676 cm^{-1}. The ^1H and ^{13}C NMR data of (**2**) in DMSO-d_6 (Table 1) indicated three exchangeable protons [δ_H 11.93 (OH-4'), 11.78 (OH-4), and 10.80 (OH-9)], four methyl groups [including a methoxy at δ_H 3.47 (3H, s) and δ_C 55.3], one methylene, five methines [two oxygenated at δ_H 6.99 (1H, s, H-1) and δ_C 98.1 (C-1); δ_H 5.80 (1H, s, H-9') and δ_C 85.5 (C-9'), respectively], eighteen sp^2 quaternary carbons including seven sp^2 oxygenated as well as one quaternary carbon. These data revealed that the structure of **2** was quite similar to bacillisporin A (**6**),[10] except the presence of an additional methoxy group signal for **2**. Key HMBC correlation from $O\underline{CH_3}$ to C-1 suggested the position of the methoxy to be on C-1 and HMBC correlations from H-1 to C-3, C-3b, C-9, and C-9a confirmed the planar structure of **2** (Figure 3).

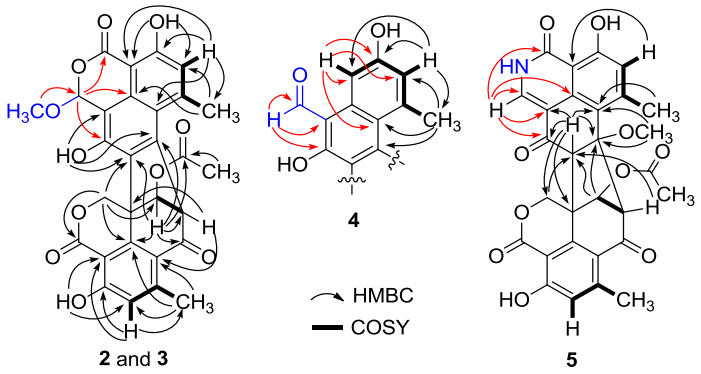


Figure 3. Key HMBC correlations of bacillisporins F−H (**2**−**5**).

Interestingly, 1-*epi*-bacillisporin F (**3**) was also isolated. ^{1}H NMR data revealed that the sole differences between compound **2** and **3** were the chemical shifts of the methoxy group in CD_3OD (δ_H 3.66 for **3** *versus* δ_H 3.58 for **2**) as well as the methine H-1 (δ_H 6.57 for **3** *versus* δ_H 6.55 for **2**), suggesting that **2** and **3** were epimers at C-1.

The relative stereochemistry of compound **2** and **3** was determined using the same methods as described above for **1** (Figure S41). According to the calculation of ^{1}H chemical shifts, 1*S* configuration was assigned for compound **2** and 1*R* for compound **3**. In order to further determine the absolute configurations of those two epimers, ECD spectra were measured and compared to those calculated. As shown in Figure 4, a very good agreement was observed between experimental and theoretical spectra, suggesting the same absolute configuration of compounds **2** and **3** except at C-1. Hence, the absolute configuration of **2** and **3** was respectively established as 1*S*, 8'*R*, 9'*S*, 9'a*S* and 1*R*, 8'*R*, 9'*S*, 9'a*S*.

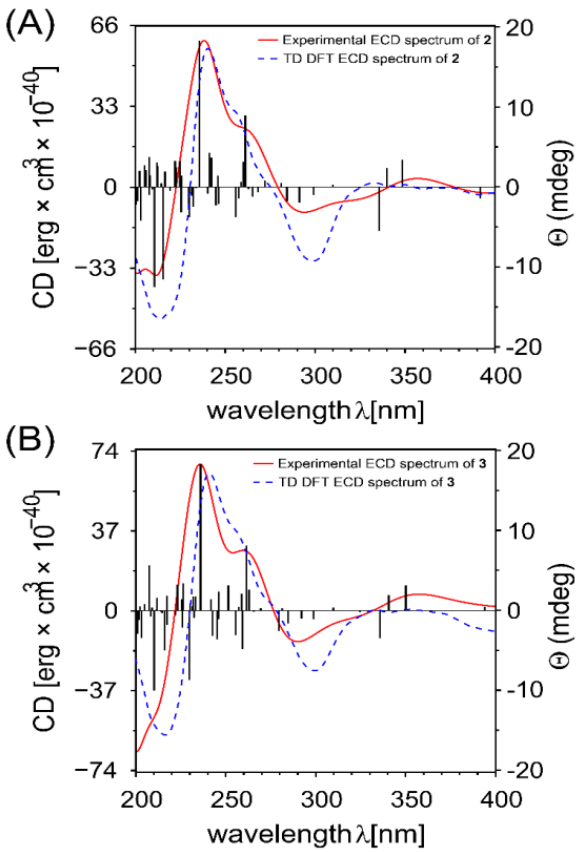


Figure 4. Comparison between calculated and experimental ECD spectra: (A) compound **2** and (B) compound **3**.

Bacillisporin G (**4**) gave an anion $[M-H]^-$ at *m*/*z* 487.1006 consistent with the molecular formula $C_{27}H_{20}O_9$ and indicative of eighteen degrees of unsaturation. The ^1H and ^{13}C NMR data (Table 1) of **4** showed characteristic signals of oligophenalenone derivatives such as bacillisporin A (**6**). However, the unobserved key signals of C-3 and CH_2-1 belonging to the lactone moiety suggested the absence of ring A. In addition, the chemical shifts of a proton at δ_H 10.44 (1H, s, HCO), and a carbonyl at δ_C 193.5 predicated the presence of an aldehyde group. Cross peak correlations from HMBC experiments of the aldehyde proton CHO to C-9 and C-9a indicated the presence of the aldehyde group on C-9a (Figure 3). The same coupling constants and NOEs as those for the

previously described bacillisporins were observed in **4,** leading thus to the fully consistent structure as shown in figure 3.

Bacillisporin H (**5**) was also produced by the fungus *T. stipitatus*. The $[M-H]^-$ ion peak at *m/z* : 544.1221 corresponded to the molecular formula $C_{29}H_{23}NO_{10}$. Analysis of 1D (Table 2) and 2D NMR data (Figure 3) showed that compound **5** is similar to compound **8** except for a low-field chemical shift for H-1 proton (δ_H 8.24). The assignment of the latter was determined by key HMBC correlations from H-1 to C-3, C-3b, and C-9a (Figure 3). The above analysis suggested a *δ*-lactam moiety for **5** instead of a *δ*-lactone, leading to the determination of the depicted structure shown. Interestingly, one nitrogenated bis-oxaphenalenone was recently reported by Cao et al.,[8] probably resulting from an azaphilone-like reactivity of duclauxin **8**, allowing it to react with nucleophilic amines.

Table 2. ^{1}H NMR (500 MHz, 298 K) and ^{13}C NMR (125 MHz, 298 K) data of **5** in $CDCl_3$

Pos.	δ_C, type	δ_H (*J* in Hz)	Pos.	δ_C, type	δ_H (*J* in Hz)
1	134.3, CH	8.24, s	1'α	72.1, CH_2	5.14, d (12.1)
3	167.8, C	-	1'β		5.47, d (12.1)
3a	108.3, C	-	3'	168.4, C	-
3b	136.1, C	-	3'a	106.1, C	-
4	162.6, C	-	3'b	143.8, C	-
5	118.0, CH	6.97, s	4'	168.4, C	-
6	149.2, C	-	5'	120.3, CH	6.59, s
6a	117.3, C	-	6'	150.1, C	-
7	89.4, C	-	6'a	122.0, C	-
8	64.6, CH	4.40, s	7'	191.9, C	-
9	194.3, C	-	8'	68.5, CH	4.56, s
9a	112.2, C	-	9'	79.8, CH	5.60, s
CH_3-6	22.3, CH_3	2.84, s	9'a	51.9, C	-
CH_3-6'	22.4, CH_3	2.12, s	$CO\underline{CH_3}$	20.8, CH_3	2.21, s
OH-4	-	13.26, s	$\underline{CO}CH_3$	170.4, C	-
OCH_3	51.7, CH_3	3.06, s	-	-	-

Duclauxin and related compounds have been reported to display antimicrobial and cytotoxic activities.[4,10,11] Accordingly, we evaluated the cytotoxic properties of compounds **1, 2**, and **4**–**6** against Hela cell line as well as a panel of human pathogenic bacteria. The scarcity of **3** prevented

us from evaluating its biological activity. No effect was observed on the growth of *Escherichia coli* (IC_{50} > 100 μg/mL, data not show) for all isolated compounds. Similar to previous antimicrobial activities described for bacillisporins,[17] bacillisporin A (**6**) displayed antimicrobial activity against Gram positive with MIC values from 2.4 to 9.5 μg/mL. Also of note is the effect of compound **5** against *Staphylococcus aureus* with CMI value at 5.0 μM with a modest cytotoxicity against Hela cell line with IC_{50} value > 50 μg/mL.

Table 3. Cytotoxic and antibacterial activities (IC_{50} in μM and MIC values in µg/mL) of compounds **1**, **2** and **4**−**6**

compound	*S. aureus*	*S. haemolyticus*	*E. faecalis*	Hela
1	29.3±0.3	> 30	> 30	> 100
2	15.6±0.5	> 30	> 30	> 50
4	> 50	> 50	> 50	> 50
5	5.0 ± 0.9	20.4 ± 6.5	>30	49.5 ± 10.3
6	5.2 ± 0.9	9.5 ± 0.4	2.4± 0.1	NT
Tetracycline	0.05 ± 0.005	29.5± 0.3	0.4 ± 0.1	NT
Cisplatine	NT	NT	NT	10.6 ± 6.6

NT: Not tested

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were determined using a Perkin Elmer 341 Polarimeter. IR spectra were taken on a Shimadzu FTIR-8400S Infrared spectrophotometer. UV spectra were recorded on a Kontron Uvikon 9X3W Double Beam UV/Vis spectrophotometer (Bioserv, France). The CD spectra were finished on Jasco J-810 spectropolarimeter system. Mass spectra were recorded on an API Q-STAR PULSAR i of Applied Biosystem. For the CID spectra, the collision energy was 40 eV and the collision gas was nitrogen. The NMR experiments were recorded on Bruker Avance III HD

300 MHz, 400 MHz, 500 MHz and 600 MHz spectrometers (Wissembourg, France) equipped with a BBFO Plus Smartprobe and a triple resonance TCI cryoprobe, respectively. Chemical shifts are expressed in δ (ppm), and are referenced to the residual non-deuterated solvent signals. Preparative HPLC was performed on an Agilent system and an Agilent PrepHT XDB-C18 column (21.2 × 150 mm i.d.; 5 µm; USA). Column chromatography (CC) was performed using silica gel (Geduran Si 60, 40−63 µm, Merck, Germany and Lichroprep RP-18, 40−63 µm, Merck KGaA, Germany) and sephadex LH-20 (Sigma-Aldrich Lipophilic Sephadex, Germany). Silica gel-precoated plates (F254, 20 × 20 cm, Merck KGaA, Germany) were used for TLC and PTLC.

Fermentation, extractions and purification. *Talaromyces stipitatus* ATCC 10500 was grown at 27 °C on polished rice (using Roux flasks containing 50 g rice mixed with 50 mL distilled water) or PDA medium during 21 days and 14 days respectively. Following this fermentation, the mycelium and the broth were extracted by ethyl acetate (3 times / 6 L). The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 20.2 g and 2.4 g of crude extracts, respectively.

The EtOAc extract of *T. stipitatus* obtained from the rice-based growth was subjected to a flash column chromatography (CC) over silica gel with a gradient elution using C_6H_{12}−CH_2Cl_2 (2:1 to CH_2Cl_2 100%, v/v) and CH_2Cl_2−MeOH gradient (80:1 to 5:1, v/v) to yield six fractions (Fr.1−Fr.6). Fr.4 was separated by a silica gel CC with a C_6H_{12}−EtOAc gradient (3:1−1:1 to MeOH 100%, v/v) to give four sub-fractions (Fr.4A−Fr.4D). Fr.4A was further separated by a silica gel CC with a C_6H_{12}−EtOAc−AcOH gradient (12:1:0.12−2:1:0.1, v/v) and gave six sub-fractions (Fr.4A1−Fr.4A6). From Fr.4A5, compound **5** (13.4 mg) and **6** (17.0 mg) were isolated by a silica gel CC eluted with C_6H_{12}−EtOAc−AcOH (3:1:0.1). **6** was further purified by preparative HPLC (gradient from 45% to 52% MeCN in H_2O for 32 min; flow rate: 20.0 mL/min; t_R = 28.5 min).

Sub-fraction Fr.4A6 was purified by preparative TLC eluted with (C_6H_{12}−EtOAc−AcOH, 3:1:0.1) to yield compound 1 (3.8 mg).

The Fr.3 was subjected to a silica gel CC with a gradient elution using C_6H_{12}−EtOAc−AcOH (9:1:0.1 to 1:1:0.1, v/v) to yield four fractions (Fr.3A−Fr.3D). Sub-fraction 3B were further purified by preparative HPLC to give **2** (3.4 mg, gradient from 40% to 55% MeCN in H_2O for 43 min; flow rate: 20.0 mL/min; **2**: t_R = 32.8 min) and **3** (1 mg).

The crude ethyl acetate extract of *T. stipitatus* obtained from growth on PDA was subjected to a silica gel column chromatography eluted with C_6H_{12}−EtOAc (10:1 to 2:1, v/v) and further purified with preparative HPLC to give compound **4** (2.1 mg, gradient from 40% to 62% MeCN in H_2O for 37 min; flow rate: 20.0 mL/min; t_R = 25.9 min).

9a-*epi*-Bacillisporin E (1): pale yellow oil; $[\alpha]_D^{20}$ +7.3 (*c* 0.1, CH_2Cl_2); UV (MeCN) λ_{max} nm (log ε): 235 (5.5), 280 (3.6), 320 (3.3), 350 (3.1) ; IR (NaCl) ν_{max} cm^{-1}: 3421 (br), 2924, 2851, 1670, 1635, 1269, 1223, 1126, 1018; CD (*c* 2.8×10^{-4} M, CH_2Cl_2) λ_{max} (Δε) 350 (+1.6) nm; ^1H and ^{13}C NMR data ($CDCl_3$) see Table 1; (−) ESI-MS: *m/z* 531.0950 $[M-H]^-$ (calcd. for $C_{28}H_{19}O_{11}$, 531.0927).

Bacillisporin F (2): pale yellow semisolid; $[\alpha]_D^{20}$ +43.5 (*c* 0.58, CH_2Cl_2); UV (MeCN) λ_{max} nm (log ε): 228 (4.9), 275 (4.4), 320 (2.9), 355(2.8); IR (NaCl) ν_{max} cm^{-1}: 2958, 2929, 2872, 2858, 1724, 1676, 1460, 1273, 1124, 1072, 742; ^1H and ^{13}C NMR data (pyridine-d_5) see Table 1; (−) ESI-MS: *m/z* 545.1139 $[M-H]^-$, (+) ESI-MS: *m/z* 547.1255 $[M+H]^+$ (calcd. for $C_{29}H_{23}O_{11}$, 547.1240).

1-*epi*-Bacillisporin F (3): pale yellow amorphous solid; $[\alpha]_D^{20}$ +43 (*c* 0.58, CH_2Cl_2); UV (MeCN) λ_{max} nm (log ε): 208 (5.3), 238 (5.3), 280 (5.4), 322 (5.5); IR (NaCl) ν_{max} cm^{-1}:2958,

2870, 1720, 1675, 1260; ^{1}H and ^{13}C NMR data (pyridine-d_5) see Table 1; (−) ESI-MS: *m/z* 545.1139 $[M-H]^-$, (+) ESI-MS: *m/z* 547.1255 $[M+H]^+$ (calcd. for $C_{29}H_{23}O_{11}$, 547.1240).

Bacillisporin G (4): pale yellow semisolid; $[\alpha]_D^{20}$ +30.4 (*c* 0.1, CH_2Cl_2); UV (MeCN) λmax nm (log ε): 235 (6.1), 282 (5.6), 373 (5.2); IR (NaCl) νmax cm−1: 2360, 2341, 1751, 1670, 1615, 1269, 1223, 1045, 1018, 818; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 2; (−) ESI-MS: *m/z* 487.1006 $[M-H]^-$ (calcd. for $C_{27}H_{19}O_9$, 487.1029).

Bacillisporin H (5): pale yellow oil; $[\alpha]_D^{20}$ −10.2 (*c* 0.04, CH_2Cl_2); UV (MeCN) λ_{max} nm (log ε): 215 (3.6), 238 (3.8), 315 (3.2), 355(2.8), 375(2.8), 450 (2.9); IR (NaCl) ν_{max} cm^{-1}: 2956, 2922, 2852, 2359, 2341, 1734, 1675, 1446, 1261, 800, 739; ^{1}H and ^{13}C NMR data (pyridine-d_5) see Table 1; (−) ESI-MS: *m/z* 544.1221 $[M-H]^-$ (calcd. for $C_{29}H_{22}NO_{10}$, 544.1244).

Computational Methods. All calculations have been performed using Gaussian 09, Revision D.01[18] with the B3LYP functional at the 6-31g(d) level. After a frequency calculation at the same level of theory in order to check that the studied structure was not presenting any negative (imaginary) frequencies, the shielding constants have been calculated with the GIAO method at the 6-311+g(d,p) level. ECD calculations were performed for 150 exited states at the 6-31g(d) level in acetonitrile using the Polarizable Continuum Model. SpecDis 1.64 was used for the process of the quantum chemical ECD calculations.[19]

Growth Inhibition Assays On HeLa Cells. The cytotoxicity was determined by the MTT assay. Briefly, HeLa cells were seeded in 96-well tissue culture plates at 2500 cells/well and incubated overnight. The exponentially growing cells were then exposed to different drug concentrations for four doubling times. Cellular viability was determined by exposing cells to the MTT tetrazolium salt for 4 h at 37 °C, and the formation of formazan was measured at 560 nm by a microplate reader. The concentration inhibiting cell growth by 50% compared with untreated

controls was determined from the curves plotting survival as a function of dose. All values are averages of at least three independent experiments each done in duplicate. Cisplatin® was used as a control compound.

Bacterial Strains and Culture Conditions. References strains of *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus haemolyticus* (MNHN26), *Enterococcus faecalis* (CIP 103014) were cultivated for 24 h in Luria Bertani medium (LB) at 37 °C.

Antimicrobial Assay. Briefly, pre-cultures of the tested micro-organisms were made by inoculating 10 mL of LB medium and incubated for 24 h at 37 °C. A culture suspension were made by 1/1000 dilution (OD600 0.01) from preculture and seeded in 96-well microtitration plates. Two microliters of two-fold serial dilutions of each compound (10 mg/mL) was prepared in 200 µL of medium. The plates were incubated at 37 °C for bacteria. After 24 h, the optical density of the bacterial suspension of each well was measured at 595 nm using a multiplate reader. The MIC which is the minimal concentration of a compound resulting in a 90% decrease in the number of microbial cultures compared to control (DMSO only) were determined by curve fitting for bacteria. Tetracycline was used as positive control.

ASSOCIATED CONTENT

Supporting Information. Experimental details and copies of 1D and 2D NMR spectra of **1**–**5**, CD calculations of **1**–**3** and DP4 probability of **1-3** are disclosed. This supporting information is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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