

Phenanthrenes from *Juncus articulatus* with Antibacterial and Biofilm Formation Inhibitory Activity

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Cite This: *J. Nat. Prod.* 2024, 87, 2068–2080



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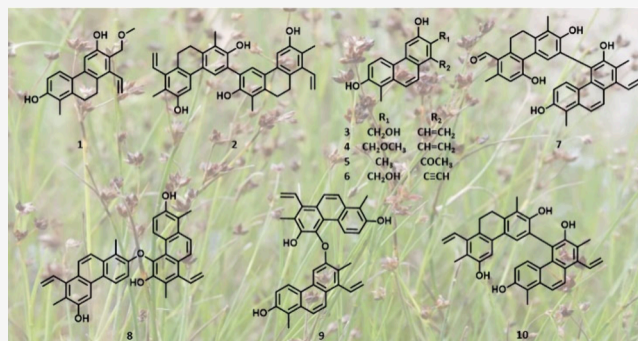


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ABSTRACT: Continuing our search for bioactive compounds in species from the Juncaceae family, *Juncus articulatus* was investigated. Ten previously undescribed phenanthrenes—articulins A–J (1–10)—and ten known compounds—juncuenin B, dehydrojuncuenin B, juncatrin B, ensifolins E, F, H, I, K, juncuenin D, and luzulin A (11–20)—along with other compounds, have been isolated and identified. The isolated compounds were evaluated for antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, methicillin-susceptible *Staphylococcus aureus* (MSSA), and methicillin-resistant *Staphylococcus aureus* (MRSA). Compounds 12 and 14 exhibited the most potent activity against planktonic and sessile MSSA and MRSA with minimum inhibitory concentration (MIC) values of 15.1 μM (12 for both bacterial strains) and 15.3 μM (14 for both bacterial strains). Compounds 15, 17, and 18 also exhibited activity against both strains, although to a lower extent, with MIC values ranging from 30.0 to 56.8 μM . The inhibition of biofilm formation of these compounds was observed at 15.1–114.3 μM . This study elucidates the phenanthrene composition of *J. articulatus* and the antibacterial effect of these compounds.



Since ancient times, bacterial infections caused high morbidities and mortality rates worldwide.^{1,2} Untreated infections or those where treatments fail due to ineffective antibiotic regimens or to unresponsive resistant bacteria may result in lethal conditions.^{3–6} Among these, sepsis is a major threat to healthcare systems with high economic costs.^{7–10} Another cause of therapeutic failures is the bacterial aggregates known as biofilms, whose structural matrices prevent antibacterial agents from reaching the cells.^{11–13} Consequently, the persistent bacterial communities become less sensitive to drugs and enhance the spread of antimicrobial tolerance.¹⁴ Under this scenario, considerable efforts are needed to develop effective therapeutic agents targeting planktonic, sessile-sensitive and resistant bacteria.

Plant-derived products are considered as promising sources for finding new bioactive compounds.^{15,16} Owing to biotic stress, plants produce biologically relevant secondary metabolites, many of which have antibacterial activity.¹⁷ The vast diversity of plants provide chemical entities with an array of chemical frameworks and pharmacophores that contribute to drug discovery pipelines.^{18–22} Among the natural compounds with outstanding pharmacological perspectives phenanthrenes have emerged as promising compounds with diverse biological properties, such as antiproliferative, anti-inflammatory, antioxidant, antialgal, and antimicrobial activities, including inhibition of MRSA growth.^{23–25}

Phenanthrenoids are the main bioactive components of the Juncaceae species, categorized primarily into monophenanthrenes and diphenanthrenes.²⁵ As a part of our ongoing research on isolating bioactive metabolites from the Juncaceae species, we investigated *Juncus articulatus* (*J. articulatus*), a rhizomatous perennial plant with terete culms that often roots at the nodes. It forms tufts or swards, often tinged with red, and ranges in height from 15 cm to about 50 cm. It grows in wet mud or sand in shallow along the margins of drains, still or slow-moving water, and wetlands.^{26–28} The plant may form dense mats that restrict water flow.²⁹ *J. articulatus* is native to Europe, Asia, North Africa, and Northern America and has been introduced in South Africa, Australia, and New Zealand.³⁰ This species has not been previously investigated from phytochemical or pharmacological perspective.

This study focuses on isolating and determining the structures of phenanthrenes from *J. articulatus* and evaluating their effects on pathogenic bacteria. Ten previously unde-

Received: May 16, 2024

Revised: July 31, 2024

Accepted: August 3, 2024

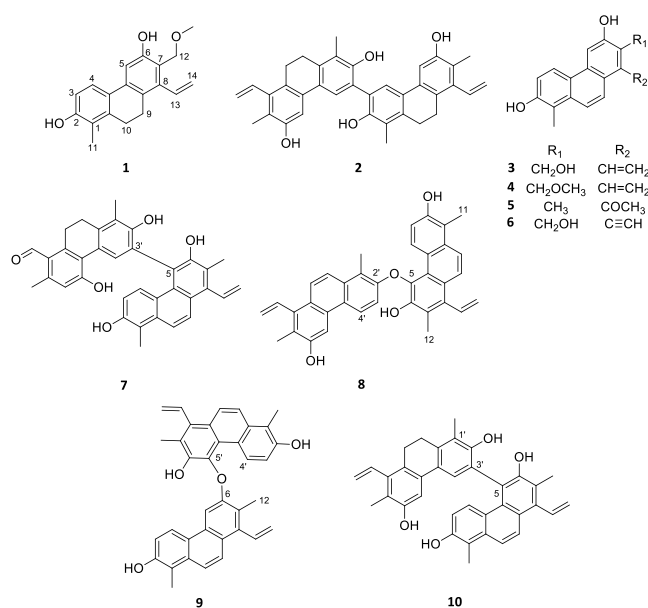
Published: August 9, 2024



scribed phenanthrenes—articulins A–J (1–10)—and ten known phenanthrenes (11–20) were described from the plant. The antibacterial activity of the isolated compounds was evaluated against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), methicillin-susceptible *Staphylococcus aureus* (MSSA), and methicillin-resistant *Staphylococcus aureus* (MRSA) as well as on sessile cells of both *Staphylococcus aureus* (*S. aureus*) strains.

RESULT AND DISCUSSION

The CHCl₃ fraction of the MeOH extract prepared from the air-dried whole plant of *J. articulatus* was fractionated using a silica gel column and Sephadex LH-20 gel chromatography and purified by high-performance liquid chromatography (HPLC).



Compound 1 was obtained as an amorphous solid. Its molecular formula was determined as C₁₉H₂₀O₃ by (–)-HRESIMS analysis (*m/z* 295.1342 [M–H][–], calcd for C₁₉H₁₉O₃ 295.1340). The ¹H NMR spectrum (Table 1) displayed signals of two *ortho*-coupled aromatic protons [δ_{H} 6.71 (1H, d, *J* = 8.4 Hz, H-3), 7.37 (1H, d, *J* = 8.4 Hz, H-4)], one aromatic proton as a singlet [δ_{H} 7.09 (1H, s, H-5)], a vinyl group [δ_{H} 6.86 (1H, dd, *J* = 17.8, 11.5 Hz, H-13), 5.28 (1H, d, *J* = 17.8 Hz, H-14a), and 5.58 (1H, d, *J* = 11.6 Hz, H-14b)], one methyl [δ_{H} 2.18 (3H, s, Me-11)], three aliphatic methylenes (including one oxygenated) [δ_{H} 2.70 (2H, m, H-9), 2.73 (2H, m, H-10) and 4.55 (2H, s, H-12)], and signals of protons belonging to one methoxy [δ_{H} 3.37 (3H, s, OCH₃-12)] and two hydroxy groups. In the ¹³C NMR JMOD (*J*-modulated spin–echo experiment) spectrum, 19 carbon signals were detected, including two methyl groups, three aliphatic methylenes, an olefinic methylene, four aromatic or olefinic methines, and nine aromatic quaternary carbons (Table 1). The compound was indicated as a 9,10-dihydrophenanthrene derivative based on two methylene signals at δ_{H} 2.73 and 2.70 (2 × 2H) in the ¹H NMR spectrum. In the ¹H–¹H COSY spectrum, correlations were observed between δ_{H} 6.71 and 7.37 d (H-3/H-4), and between δ_{H} 6.86 dd and 5.28 dd and 5.58 dd (H-13/H₂-14) (Figure 1).

Heteronuclear long-range correlations (HMBC) of H-3 and H₂-10 with C-4a, H-4 and H₂-9 with C-5a, H-4, H₂-9, and H₃-11 with C-1a, and H₂-10 with C-8a were used to establish a 9,10-dihydrophenanthrene skeleton for compound 1. Furthermore, HMBC cross-peaks of H-3, H-4, and H₃-11 with C-2 (δ_{C} 156.2), and H-5 and H₂-12 with C-6 (δ_{C} 156.2), showed two hydroxy groups at C-2 and C-6. Based on the H-13/C-8a/, H-13/C-7/, and H₂-14/C-8/ correlations, the vinyl moiety was placed at C-8, and its location at C-7 was confirmed by the strong cross-peak of the methoxy group (δ_{H} 3.37 s) and the methylene at δ_{C} 68.0 (Figure 1). In addition, the NOESY

Table 1. ¹H (500 MHz) and ¹³C (125 MHz) NMR Data of Compounds 1 and 3 in MeOH-*d*₄ and Compound 2 in CDCl₃ (δ in ppm)

position	1		2		3	
	δ_{C} , type	δ_{H} (<i>J</i> in Hz)	δ_{C} , type	δ_{H} (<i>J</i> in Hz)	δ_{C} , type	δ_{H} (<i>J</i> in Hz)
1	122.3, C		122.7, C		118.4, C	
1a	138.9, C		138.6, ^a C		133.9, C	
2	156.2, C		151.2, C		154.6, C	
3	113.9, CH	6.71 d (8.4)	120.6, C		117.0, CH	7.14 d (9.0)
4	123.4, CH	7.37 d (8.4)	123.8, CH	7.46 s	122.7, CH	8.30 d (9.0)
4a	127.7, C		126.8, C		124.6, C	
5a	138.0, C		133.5, C		133.6, C	
5	110.2, C	7.09 s	109.4, CH	7.06 s	106.3, CH	7.93 s
6	156.2, C		152.6, C		156.5, C	
7	120.6, C		120.8, C		125.4, ^b C	
8	140.5, C		138.6, ^a C		139.5, C	
8a	126.4, C		128.4, C		123.7, C	
9	26.5, CH ₂	2.70 m	25.8, CH ₂	2.86 m	125.4, ^b CH	7.90 d (9.5)
10	26.6, CH ₂	2.73 m	25.8, CH ₂	2.82 m	120.5, CH	7.68 d (9.5)
11	11.5, CH ₃	2.18 s	12.0, CH ₃	2.33 s	11.0, CH ₃	2.51 s
12	68.0, CH ₂	4.55 s	12.9, CH ₃	2.23 s	58.8, CH ₂	4.91 s
13	135.9, CH	6.86 dd (17.8, 11.5)	135.2, CH	6.77 dd (17.9, 11.4)	135.3, CH	7.20 dd (17.9, 11.5)
14	120.7, CH ₂	5.28 d (17.8)	120.5, CH ₂	5.22 dd (17.9, 1.9)	122.2, CH ₂	5.47 dd (17.9, 2.1)
		5.58 d (11.6)		5.62 dd (11.4, 1.9)		5.79 dd (11.5, 2.1)
12-OCH ₃	57.9, CH ₃	3.37 s				

^aOverlapping signals. ^bOverlapping signals.

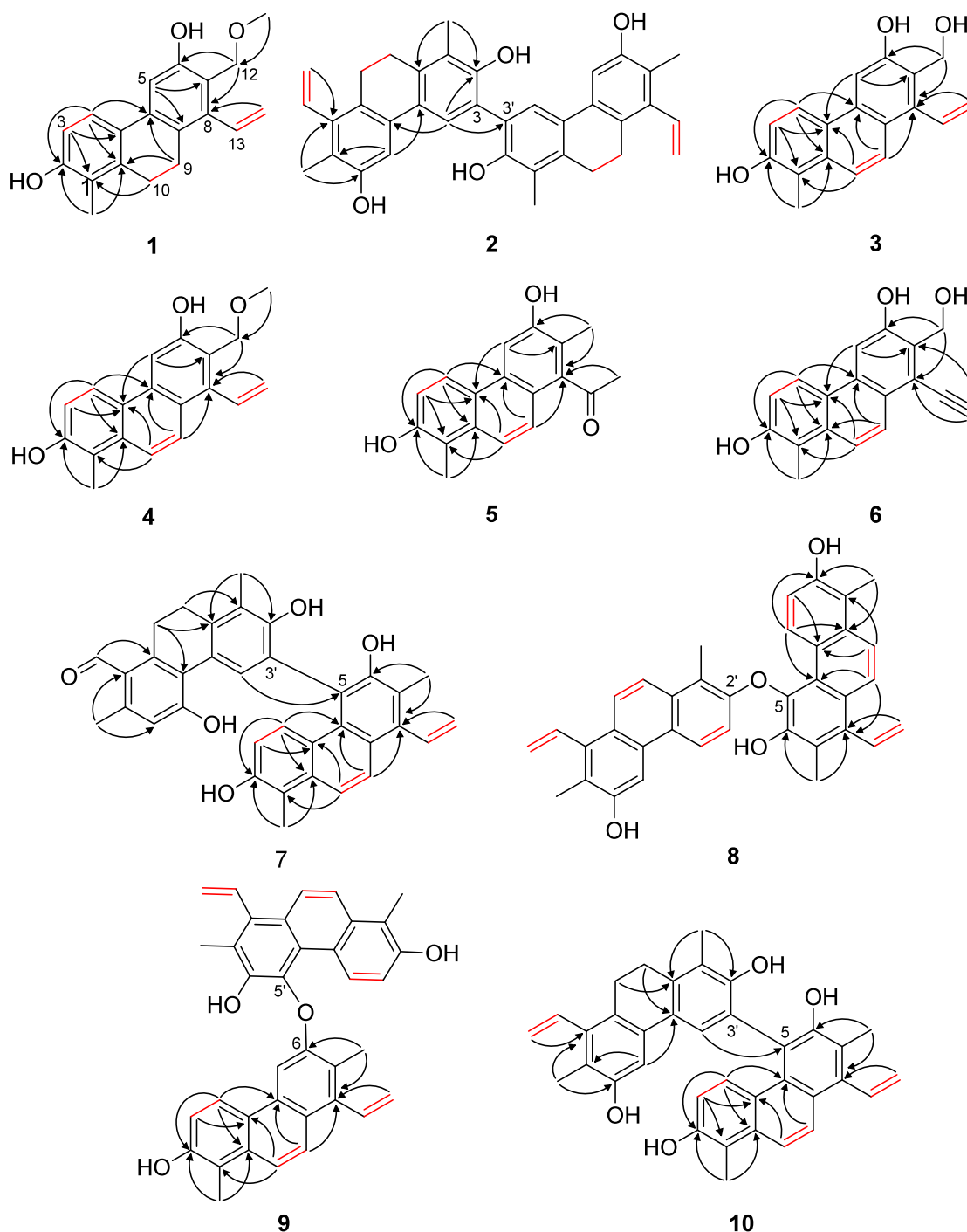


Figure 1. ^1H – ^1H COSY (—) and diagnostic HMBC ($\text{H} \rightarrow \text{C}$) correlations of compounds 1–10.

cross-peaks of H-4/H-5, H-9/H-13, H-10/H₃-11, and H₂-12/H₂-14 further confirmed the structure of articulin A (**1**) (Figure 2). Compound **1** differs from juncuenin B (**11**), a phenanthrene previously isolated from several Juncaceae species,²⁵ in having a methoxymethylene group in **1** instead of the methyl group (in **11**) at C-7. Finally, compound **1** was identified as 7-methoxymethylene-1-methyl-8-vinyl-9,10-dihydrophenanthren-2,6-diol and named articulin A.

Articulin B (**2**) was isolated as an amorphous solid. Analysis of ^1H NMR, JMOD and HSQC data of **2** (Table 1) revealed two aromatic protons (δ_{H} 7.46 and 7.06 s; δ_{C} 123.8 and 109.4), two aromatic methyl groups (δ_{H} 2.33 and 2.23 s; δ_{C}

12.0 and 12.9), a vinyl system at δ_{H} 6.77 dd/ δ_{C} 135.2 as well as 5.62 and 5.22 dd/ δ_{C} 120.5 (H-13, H₂-14), and two methylene groups (δ_{H} 2.86 and 2.82 m/ δ_{C} 2 × 25.8). In addition to these data, the presence of methylene signals (H₂-9 and H₂-10) indicated that this compound was a 9,10-dihydrophenanthrene derivative. Since the HRESIMS indicated the molecular formula $\text{C}_{36}\text{H}_{34}\text{O}_4$, (m/z 529.2378 [$\text{M}-\text{H}$][−], calcd for $\text{C}_{36}\text{H}_{33}\text{O}_4$, 529.2379) a dimeric nature of compound **2** was assumed. Therefore, compound **2** was considered as a dihydrophenanthrenoid dimer comprising two juncuenin B (**11**) monomers. In compound **2**, the methine group at C-3 of juncuenin B was replaced by a quaternary carbon (δ_{C} 120.6).

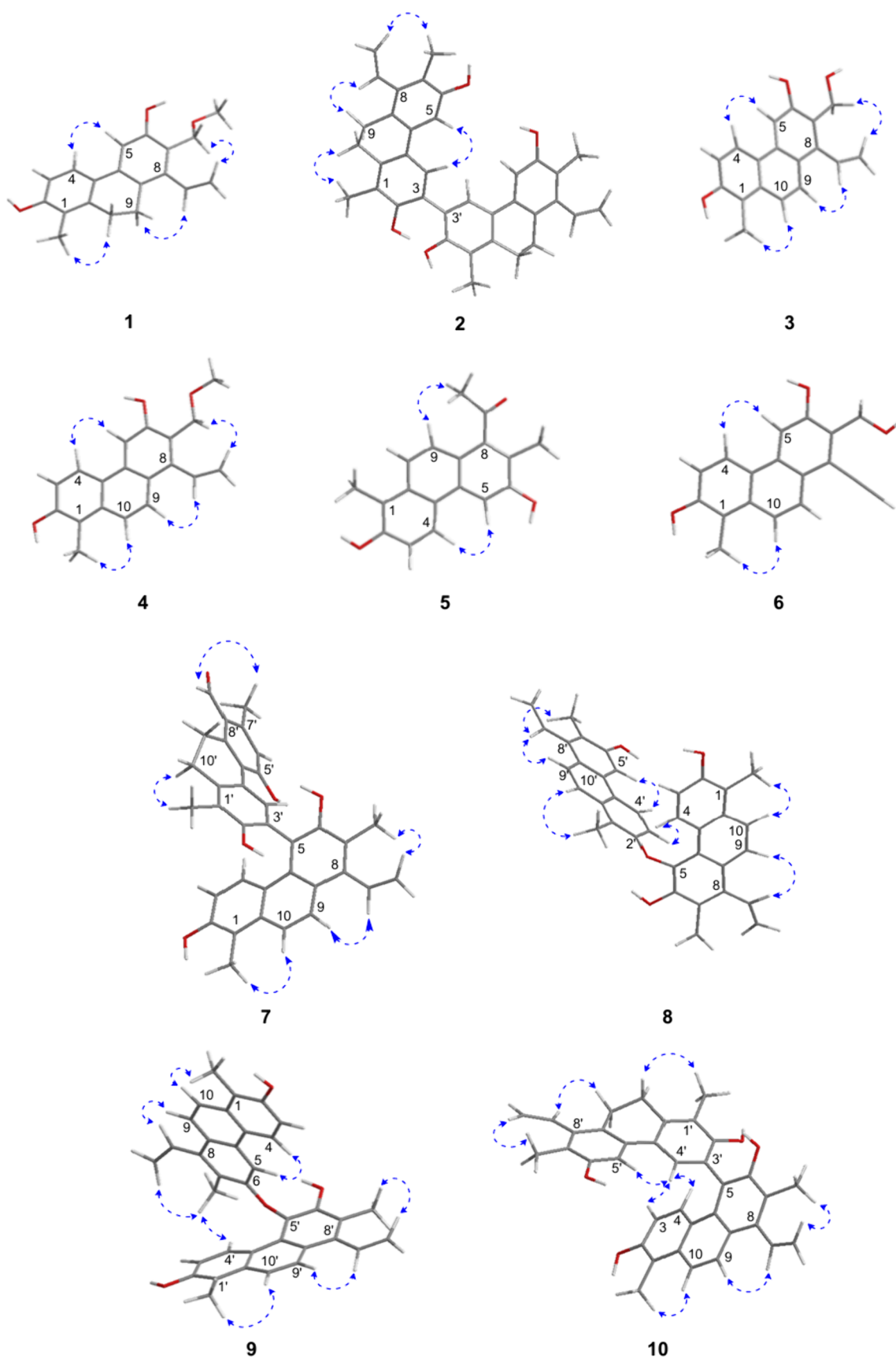


Figure 2. Key NOESY correlations ($\leftrightarrow$) for compounds 1–10.

Given that compound 2 showed only 18 carbon signals instead of 36 in the JMOD spectrum, it was proposed that compound

2 is a symmetrical dimer of two juncuenin B units linked via C-3 and C-3'. This hypothesis was further confirmed by analysis

Table 2. ¹H (500 MHz) and ¹³C (125 MHz) NMR Data of Compounds 4–6 in MeOH-*d*₄ (δ in ppm)

position	4		5		6	
	δ _C , type	δ _H (J in Hz)	δ _C , type	δ _H (J in Hz)	δ _C , type	δ _H (J in Hz)
1	118.5, C		118.7, C		118.8, C	
1a	134.0, ^a C		133.5, C		134.1, C	
2	154.7, C		154.6, C		154.9, C	
3	117.1, CH	7.15 d (9.0)	117.4, CH	7.16 d (9.0)	117.3, CH	7.17 d (9.0)
4	122.7, CH	8.30 d (9.0)	122.4, CH	8.28 d (9.0)	122.6, CH	8.30 d (9.0)
4a	124.6, C		124.5, C		124.4, C	
5a	134.1, ^a C		132.5, C		133.5, C	
5	106.3, CH	7.93 s ^b	106.7, CH	7.94 s	108.6, CH	8.00 s
6	156.4, C		156.0, C		156.2, C	
7	122.8, C		120.5, ^c C		130.8, C	
8	140.9, C		142.7, C		121.7, C	
8a	123.7, C		120.6, ^c C		126.5, C	
9	125.4, CH	7.92 d (9.4) ^b	123.4, CH	7.38 d (9.4)	125.7, CH	8.19 d (9.4)
10	120.6, CH	7.70 d (9.4)	121.5, CH	7.74 d (9.4)	121.9, CH	7.80 d (9.4)
11	11.0, CH ₃	2.52 s	11.0, CH ₃	2.51 s	11.0, CH ₃	2.53 s (3H)
12	68.3, CH ₂	4.74 s	13.0, CH ₃	2.26 s	60.4, CH ₂	5.12 s (2H)
13	135.3, CH	7.20 dd (17.9, 11.5)	211.0, C		80.7, C	
14	122.1, CH ₂	5.49 dd (17.9, 2.1)	33.4, CH ₃	2.64 s	87.9, CH	4.12 s
		5.79 dd (11.5, 2.1)				
12-OCH ₃	58.1, CH ₃	3.43 s				

^aInterchangeable signals. ^bOverlapping signals. ^cInterchangeable signals.

Table 3. ¹H (600 MHz) and ¹³C (150 MHz) NMR Data of Compounds 7 and 8 in MeOH-*d*₄ (δ in ppm)

position	7				8			
	unit A		unit B		unit A		unit B	
	δ _C , type	δ _H (J in Hz)	δ _C , type	δ _H (J in Hz)	δ _C , Type	δ _H (J in Hz)	δ _C , type	δ _H (J in Hz)
1	117.5, C		123.5, C		117.9, C		121.3, C	
1a	134.9, C		140.3, C		134.4, C		133.4, C	
2	153.4, C		152.8, C		154.0, C		154.0, C	
3	115.4, CH	6.53 d (9.4)	126.9, C		116.2, CH	6.82 d (9.4)	113.0, CH	6.42 d (9.4)
4	127.1, CH	7.54 d (9.4)	130.3, CH	7.90 s	127.1, CH	8.98 d (9.4)	125.6, CH	8.05 d (9.4)
4a	125.6, C		118.6, C		123.6, C		126.4, C	
5a	131.1, C		122.7, C		125.2, C		131.8, C	
5	126.9, CH		159.9, C		n.d. ^a		105.6, CH	7.74 s
6	153.4, C		118.5, CH	6.59 s	148.9, C		156.0, C	
7	123.5, C		143.2, C		124.9, C		124.2, C	
8	138.9, C		125.5, C		135.7, C		138.6, C	
8a	123.5, C		146.0, C		125.3, C		124.1, C	
9	125.8, CH	8.04 d (9.5)	26.5, CH	3.44 m 3.11 m	122.5, CH	8.06 d (9.6)	126.0, CH	8.02 d (9.6)
10	120.9, CH	7.73 d (9.5)	26.3, CH	2.96 m 2.75 m	121.4, CH	7.76 d (9.6)	120.0, CH	7.87 d (9.6)
11	11.2, CH ₃	2.47 s	12.4, CH ₃	2.35 s	11.2, CH ₃	2.47 s	11.9, CH ₃	3.03 s
12	14.5, CH ₃	2.45 s	20.8, CH ₃	2.53 s	14.2, CH ₃	2.46 s	13.6, CH ₃	2.32 s
13	136.9, CH	7.19 dd (17.9, 11.5)	193.9, C	10.51 s	136.5, CH	7.19 dd (17.9, 11.4)	136.3, CH	7.10 dd (17.9, 11.4)
14	121.8, CH ₂	5.85 dd (11.4, 2.1)			122.1, CH ₂	5.88 dd (11.3, 2.0)	121.6, CH ₂	5.81 dd (11.4, 2.0)
		5.41 dd (17.9, 2.1)				5.44 dd (17.8, 2.0)		5.37 dd (17.9, 2.0)

^an.d. not detected.

of ¹H–¹H COSY and HMBC data (Figure 1). The HMBC long-range correlations observed from H-11 and H-4 to oxycarbon C-2 and from H-11 to C-1 and C-1a indicated the C-2 and C-1 positions of a hydroxyl and a methyl group at ring A, respectively. The correlation from H-4 to a quaternary carbon at δ_C 120.6 highlighted the linking site at C-3. The HMBC correlations from H-5 to C-7 and C-4a, H₃-12 to C-6 and C-8, and H₂-14 to C-8 indicated the hydroxyl, methyl and

vinyl groups at ring C of the juncuenin B unit (Figure 1), respectively, thus establishing the structure of **2** as 1,1',7,7'-tetramethyl-8,8'-divinyl-9,9',10,10'-tetrahydro-[3,3'-biphenanthrene]-2,2',6,6'-tetraol, named articulin B. Analysis of NOESY data showed cross-peaks of H-4/H-5, H₂-9/H-13, H₂-10/H₃-11, and H₃-12/H-13 that further confirmed the structure of articulin B (**2**) (Figure 2).

Based on the protonated molecular peak in the HRESIMS at m/z 281.1173 $[M+H]^+$ (calcd for $C_{18}H_{17}O_3$, 281.1172), the molecular formula of $C_{18}H_{16}O_3$ was assigned to compound 3 (articulin C). The signals in the 1H NMR spectrum were similar to those of dehydrojuncuenin B (12), except for the replacement of a methyl group with an *O*-substituted methylene signal at δ_H 4.91 s.³¹ The methyl group at C-7 was oxidized into a hydroxymethyl side chain in compound 3 (Table 1). This assumption was substantiated by observing the HMBC correlations $H_2-12/C-6$, $H_2-12/C-7$, $H_2-12/C-8$, $H-5/C-7$, and $H_2-14/C-8$ (Figure 1). In addition, the NOESY cross-peaks of $H-4/H-5$, $H-9/H-13$, and $H-10/H_3-11$ further confirmed the structure of articulin C (3) (Figure 2).

The HRESIMS data provided the molecular formula of $C_{19}H_{18}O_3$ for articulin D (4) through a peak at m/z 293.1188 $[M-H]^-$ (calcd for $C_{19}H_{17}O_3$, 293.1183). The signals in the 1H NMR spectrum closely resembled those of articulin A (1) except for the replacement of its saturated H_2-9/H_2-10 structural part by two mutually coupled olefinic protons (δ_H 7.92 and 7.70, each 1H, $J = 9.4$ Hz) (Table 2). The double bond between C-9 and C-10 was supported by HMBC correlations between $H-9$ (δ_H 7.92) and C-1a, C-5a, and C-8 and between $H-10$ (δ_H 7.70) and C-1, C-4a, and C-8a (Figure 1). The $H-4/H-5$, $H-9/H-13$, and $H-10/H_3-11$ NOE cross-peaks confirmed the structure of compound 4 (Figure 2).

Articulin E (5) was obtained as a light-yellow amorphous solid. The HRESIMS suggested the molecular formula of $C_{18}H_{16}O_3$, through a peak at m/z 281.1175 $[M+H]^+$ (calcd for $C_{18}H_{17}O_3$, 281.1172). The signals in the 1H NMR spectrum closely resembled those of juncatrin A, isolated previously from *J. atratus*, except for the replacement of its saturated H_2-9/H_2-10 structural part by two olefinic protons (δ_H 7.74 and 7.38, each 1H, $J = 9.4$ Hz) (Table 2).³² The double bond between C-9 and C-10 was supported by HMBC correlations between $H-9$ (δ_H 7.38) and C-1a, C-5a, and C-8, and $H-10$ (δ_H 7.74) and C-1, C-4a, and C-8a (Figure 1). The NOESY cross-peaks of $H-4/H-5$, and $H-10/H_3-11$ further confirmed the structure of articulin E (5) (Figure 2).

The HRESIMS data provided the molecular formula of $C_{18}H_{14}O_3$ for compound 6 (articulin F) through a peak at m/z 277.0870 $[M-H]^-$ (calcd for $C_{18}H_{13}O_3$, 277.0870). Comparing its 1D NMR data with those of ensifolin H, a 9,10-dihydrophenanthrene described from *J. ensifolius* by our research group,³³ revealed two olefinic protons (δ_H 8.19 and 7.80, each 1H, $J = 9.4$ Hz) in the molecule instead of the saturated H_2-9/H_2-10 structural part (Table 2). This double bond was substantiated by HMBC correlations detected between $H-9$ (δ_H 8.19) and C-1a and C-5a, and between $H-10$ (δ_H 7.80) and C-1, C-4a, and C-8a (Figure 1). In addition, the NOESY cross-peaks of $H-4/H-5$, and $H-10/H_3-11$ further confirmed the structure of articulin F (6) (Figure 2).

Articulin G (7), obtained as a light-yellow amorphous powder, is a phenanthrene heterodimer with the molecular formula of $C_{35}H_{30}O_5$, determined by HRESIMS analysis (m/z 529.2021 $[M-H]^-$, calcd for $C_{35}H_{29}O_5$, 529.2021) and 35 carbon resonances detected in the ^{13}C JMOD NMR spectrum (Table 3). One of the building blocks of compound 7 was identified as dehydrojuncuenin B (12) by analysis of 2D NMR data and comparison with assignments reported in the literature.³¹ In the 1D NMR spectra, the absence of resonances for another vinyl group along with a singlet signal at δ_H 10.51 and a carbonyl carbon at δ_C 193.9 indicated the biosynthetic conversion of the vinyl moiety of the second monomeric

portion to an aldehyde moiety. Its position at C-8' (δ_C 125.5) was assigned by observing HMBC correlations from H_3-12' , $H-6'$, and H_2-9' to C-8' (Figure 1). Careful analysis of the 2D NMR spectra concluded that this part of the molecule is a derivative of ensifolin E.³³ Given the absence of H-5 in the dehydrojuncuenin B moiety and the correlation between a nonprotonated carbon at δ_C 126.9 (C-5) and the deshielded signal assigned to $H-4'$ (δ_H 7.90, 1H, s) of the other monomer, it was concluded that the monomers are connected through a C–C bond formed between C-5 of dehydrojuncuenin B and C-3' of the other portion. The significantly shielded signal of $H-3$ (δ_H 6.53) and $H-4$ (δ_H 7.54) compared to that of the corresponding monomer dehydrojuncuenin B [$H-3$ (δ_H 7.12) and $H-4$ (δ_H 8.25)] further confirmed the proposed structure. This phenomenon was likely caused by the anisotropic effect of the aromatic ring of monomer B, indicating that H-3 and H-4 are located in the shielding cone of this ring (Figure 3).

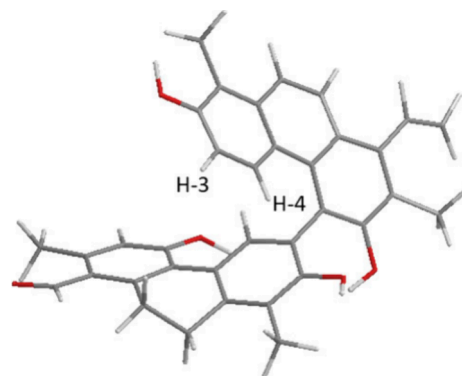


Figure 3. Calculated molecular structure of articulin G (7). It is shown that shielded H-3 and H-4 of the A monomer are situated within the shielding cone of the aromatic ring of the B monomer.

Articulin H (8) was obtained as an amorphous solid. Its molecular formula $C_{36}H_{30}O_4$ was established by HRMS analysis (m/z 525.2071 $[M-H]^-$, calcd for $C_{36}H_{29}O_4$, 525.2071). Its JMOD spectrum displayed 36 signals, suggesting that compound 8 is a phenanthrene dimer (Table 3). Analysis of the 1H NMR spectrum revealed that articulin H (8) comprises two dehydrojuncuenin B (12) monomers (Table 3). Unlike articulin B (2), compound 8 is not symmetrical as its 1D NMR data provided two sets of proton and carbon resonances assigned to the two subunits. The strong NOE from H_3-11' to $H-4$ indicated that the monomers are linked through an ether bond formed between the OH-2' group of one dehydrojuncuenin B monomer and the C-5 carbon of the other monomer (Figures 2 and 4).

Compound 9 (articulin I) presented the molecular formula $C_{36}H_{30}O_4$ compatible with its molecular peak at m/z 525.2074 $[M-H]^-$ (calcd for $C_{36}H_{29}O_4$, 525.2071). The 36 carbon signals in the ^{13}C NMR JMOD spectrum suggested that compound 9 is a phenanthrene dimer. Analysis of its 1H NMR spectrum and 1H – 1H COSY correlations, and comparison of spectral data with literature values showed two dehydrojuncuenin B (12) units in 9 (Table 4). Given the absence of proton signal at C-5' in the 1H NMR spectrum, and the strong NOE correlation between H_3-12 and $H-4'$, the monomers must be attached through an ether bond between C-6 and C-5' (Figure 2).

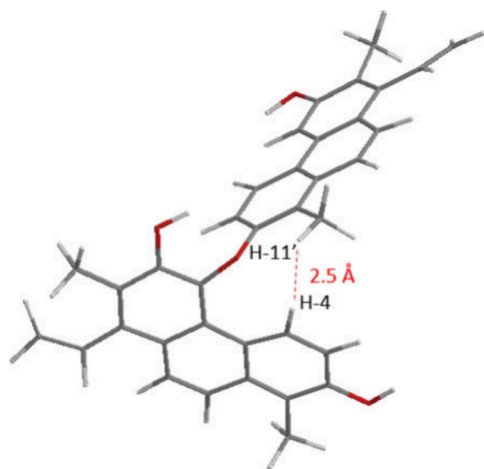
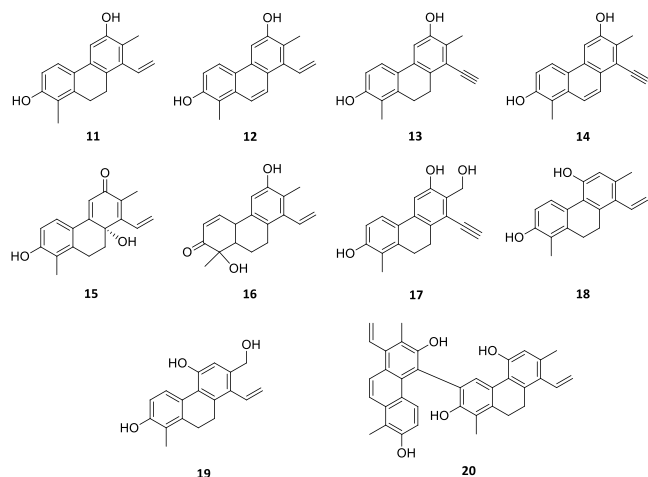


Figure 4. Energy-minimized 3D structure of articulin H (8) and atom distance between H-4 and H-11'.

The molecular formula of compound **10** (articulin J) is $C_{36}H_{32}O_4$ based on HRESIMS analysis (m/z 527.2224 $[M-H]^-$, calcd for $C_{36}H_{31}O_4$, 527.2222). Analysis of 1H NMR, ^{13}C NMR JMOD, and HSQC data of this compound revealed a heterodimeric phenanthrenoid structure comprising a dihydrophenanthrene and a phenanthrene unit (Table 4). The 1H and ^{13}C resonances of the phenanthrene part closely resembled those of unit A of articulin G (7), and the dihydrophenanthrene part could be elucidated as a 3-substituted juncuenin B (11). The C-5–C-3' connection of the two monomers was proved by observing HMBC correlations of H-4' (δ_H 7.19 s) and H₃-12 (δ_H 2.44 s) with C-5 (δ_C 118.9) (Figure 1) and NOESY correlations of H-4' (δ_H 7.19 s) with H-3 (δ_H 6.53 d, J = 9.4 Hz) and H-4 (δ_H 7.57 d, J = 9.4 Hz) (Figure 2). The anisotropic effect of the aromatic ring of monomer B, which caused shielding of H-3 and H-4 signals, can be observed similarly in articulins I (9) and J (10), as mentioned for 7.

In addition to the ten new articulins A–J (1–10), ten known phenanthrenes were also isolated, including juncuenin B (11),³¹ dehydrojuncuenin B (12),³¹ juncatrin B (13),³² juncuenin D (15),³¹ luzulin A (16),³⁴ ensifolins E (18), F (19), H (17), I (14),³³ ensifolin K (20),³³ as well as apigenin and luteolin,³⁵ 1,3-*O*-diferuloylglycerol,³⁶ (+)-*S*-dehydrovomifoliol,³⁷ vanillin,³⁸ and *p*-hydroxybenzaldehyde.³⁸



Given that juncuenin B (11) and dehydrojuncuenin B (12) were isolated in high amounts, they are considered the main

phenanthrenes of *J. articulatus*, and the isolated phenanthrenes are mainly derivatives of these two compounds. Compounds 1 and 2, juncatrin B (13), juncuenin D (15), luzulin A (16), and ensifolin H (17) derive from juncuenin B (11), while compounds 3–10 and ensifolin I (14) originate from dehydrojuncuenin B (12). In articulins H (8) and I (9), two dehydrojuncuenin B (12) units are connected through an ether bridge, while articulin J (10) is the dimer originating from a juncuenin B (11) and a dehydrojuncuenin B (12) unit.

Considering that the MeOH extract of *J. articulatus* was previously reported to inhibit the growth of MRSA,³⁹ the isolated compounds were tested for their inhibitory effects on the growth of planktonic *E. coli*, *P. aeruginosa*, MSSA, and MRSA as well as on sessile cells of both *S. aureus* strains. Compounds 2, 3, 6, 9, 12–15, and 17–20 inhibited the growth of MSSA and MRSA with minimum inhibitory concentration (MIC) values below 64 $\mu\text{g/mL}$. Compounds 12, 14, 15, 17, and 18 exerted activity with MIC values ranging from 4 to 16 $\mu\text{g/mL}$ (Table 5). Compounds 12 and 14 were the most potent, exhibiting MIC values of 15.1 and 15.3 μM , respectively. The activity of these phenanthrenes partly explain the inhibition of MRSA growth observed for *J. articulatus*.³⁹ None of the target metabolites inhibited Gram-negative bacteria. Previous studies demonstrated that dehydrojuncuenin B (12) inhibited MRSA growth with an MIC value of 25 $\mu\text{g/mL}$, while juncuenin D (15) did the same at 12.5 $\mu\text{g/mL}$,⁴⁰ with the latter being more active than compound 12, contrary to the observations in this work. To our knowledge, this is the first report of the antibacterial activity of compounds 13, 17–20, and, particularly of compound 14. The latter phenanthrene inhibited the proliferation of cervical cancer (HeLa), doxorubicin-sensitive colonic adenocarcinoma (COLO 205), and multidrug-resistant colonic adenocarcinoma (COLO 320/MDR-LRP) cell lines, with IC_{50} values ranging from 18.2 to 24.1 μM . Compounds 17–20 showed IC_{50} values ranging from 12.3 to 93.7 μM .³³ In addition, compounds 14 and 17–20 exhibited strong synergism with doxorubicin.³³ However, compound 13 demonstrated antiproliferative effects against SiHa cells with an IC_{50} value of 25.3 μM .³²

Although, a clear correlation between structures and activities was not observed a preliminary structure–activity relationship analysis revealed that the double bond at C-9–C-10 increased the antibacterial activity, as observed when comparing compounds 1 and 4, 11, and 12, and 13 and 14. The hydroxylation of the CH_3 -11 in compound 19 was detrimental to the antibacterial effect compared to the activity observed for compound 18 (Table 5).

The monomeric phenanthrenes 12, 14, 15, 17, and 18 showed lower MIC values, ranging from 4 to 16 $\mu\text{g/mL}$, than the dimers 7, 8, 9, and 20, which displayed MIC values equal to or higher than 32 $\mu\text{g/mL}$ (Table 5). This tendency was observed when comparing the MIC values of diphenanthrene 12 (MIC = 15.1 μM) and its monomers 8 and 9 (MIC = 121.5 and 60.5 μM , respectively). Similarly, diphenanthrene 20 showed an MIC value of 60.5 μM , formed by combining of compounds 12 and 18, with the latter having an MIC value of 30.0 μM . The presence of an alkyne bond in compounds 6 and 14 instead of an alkene bond in compounds 3 and 12 had no impact on the activity.

Considering biofilms as protective barriers for bacteria against the controlling effects of antibiotics, new molecules targeting their establishment or eradicating preformed biofilms are greatly needed. Therefore, we investigated the antibiofilm

Table 4. ¹H (600 MHz) and ¹³C (150 MHz) NMR Data of Compounds 9 and 10 in MeOH-*d*₄ (δ in ppm)

position	9			10		
	unit A	unit B		unit A	unit B	
	δ _C , type	δ _H (J in Hz)	δ _C , type	δ _H (J in Hz)	δ _C , type	δ _H (J in Hz)
1	118.2, C		118.0, C		117.5, C	124.6, C
1a	133.3, C		134.4, C		135.0, C	139.1, C
2	154.2, C		154.0, C		153.4, C	153.3, C
3	117.0, CH	6.83 d (9.3)	116.3, CH	6.86 d (9.6)	115.3, CH	n.d.
4	121.9, CH	7.36 d (9.3)	126.9, CH	8.97 d (9.6)	127.4, CH	7.19 s
4a	124.6, C		123.6, C		125.6, C	
5a	131.5, C		125.0, C		131.2, C	
5	103.4, CH	7.22 s	137.8, C		118.9, C	6.78 s
6	155.5, C		148.7, C		153.5, C	
7	124.9, C		125.2, C		123.5, C	
8	139.1, C		135.8, C		138.8, C	
8a	125.1, C		125.5, C		125.2, C	
9	125.1, CH	7.96 d (9.8)	125.6, CH	8.12 d (9.8)	125.8, CH	8.04 d (9.5)
10	121.3, CH	7.67 d (9.8)	121.5, CH	7.77 d (9.8)	120.9, CH	7.74 d (9.5)
11	10.9, CH ₃	2.41 s	11.2, CH ₃	2.47 s	11.2, CH ₃	2.48 s
12	14.4, CH ₃	2.85 s	14.3, CH ₃	2.49 s	14.5, CH ₃	2.44 s
13	136.5, ^a CH	7.25 dd ^{c,d} (18.3, 11.6)	136.2, ^a CH	7.22 dd ^{c,d} (18.3, 11.6)	136.9, CH	7.20 dd (17.4, 11.4)
14	122.2, ^b CH ₂	5.48 dd ^{e,f} (18.3, 2.0)	122.1, ^b CH ₂	5.49 dd ^{e,f} (18.3, 2.0)	121.8, CH ₂	5.40 dd (17.4, 2.2)
		5.91 dd ^{g,h} (11.6, 2.0)		5.91 dd ^{g,h} (11.6, 2.0)		5.86 dd (11.4, 2.2)

^aInterchangeable signals. ^bInterchangeable signals. ^cInterchangeable signals. ^dInterchangeable signals. ^eInterchangeable signals. ^fOverlapping signals, n.d. not detected. ^gInterchangeable signals. ^hOverlapping signals, n.d. not detected.

Table 5. Antibacterial Activity of Phenanthrenes Isolated from *J. articulatus*^a

Compound	MIC							
	<i>E. coli</i>		<i>P. aeruginosa</i>		MSSA		MRSA	
	(μg/mL)	(μM)	(μg/mL)	(μM)	(μg/mL)	(μM)	(μg/mL)	(μM)
1	>128	>413	>128	>413	128	413.0	128	413.0
2	>64	>121	>64	>121	32	60.5	32	60.5
3	>64	>229	>64	>229	32	114.3	32	114.3
4	>128	>435	>128	>435	64	217.5	64	217.5
5	>128	>457	>128	>457	128	457.0	128	457.0
6	>64	>230	>64	>230	16	57.5	32	115.0
7	>64	>121	>64	>121	32	60.5	64	121.0
8	>128	>243	>128	>243	64	121.5	64	121.5
9	>64	>121	>64	>121	32	60.5	32	60.5
10	NT	NT	NT	NT	NT	NT	NT	NT
11	>128	>481	>128	>481	128	481.0	128	481.0
12	>128	>484	>128	>484	4	15.1	4	15.1
13	>128	>485	>128	>485	32	121.3	32	121.3
14	>128	>488	>128	>488	4	15.3	4	15.3
15	>128	>454	>128	>454	16	56.8	16	56.8
16	>128	>458	>128	>458	64	229.0	64	229.0
17	>128	>457	>128	>457	16	57.1	16	57.1
18	>128	>480	>128	>480	8	30.0	8	30.0
19	>128	>454	>128	>454	32	113.5	32	113.5
20	>128	>242	>128	>242	32	60.5	32	60.5
ciprofloxacin	0.0078	0.02	0.125	0.37	0.125–0.25	0.37–0.74	0.125–0.25	0.37–0.74
vancomycin	256	176.7	2560	1766.7	1	0.69	2	1,38

^aMIC: Minimum Inhibitory Concentration. MSSA: methicillin-susceptible *Staphylococcus aureus*. MRSA: methicillin-resistant *Staphylococcus aureus* (MRSA). NT: not tested.

Table 6. Effects of Isolated Compounds on Biofilm Formation and Disruption of Preformed Biofilms of MSSA and MRSA

Compound	MBPC ^a				MBEC ^b			
	MSSA		MRSA		MSSA		MRSA	
	(μg/mL)	(μM)	(μg/mL)	(μM)	(μg/mL)	(μM)	(μg/mL)	(μM)
2	64	121.0	128	242.0				
3	32	114.3	32	114.3				
6	32	115.0	32	114.3				
9	64	121.0	64	121.0				
12	4	15.1	4	15.1	>128	>484	>128	>484
14	4	15.3	4	15.3	>128	>488	>128	>488
15	32	113.5	32	113.5				
17	32	114.3	32	114.3				
18	16	60.0	16	60.0				
19	64	227.0	64	227.0				
20	32	60.5	32	60.5				
ciprofloxacin	0.25–0.5	0.75–1.51	0.5	1.51	>128 ^c	>386.7	>128 ^c	>386.7
vancomycin	1	0.69	2	1.38	>128	>88.3	>128	>88.3

^aMBPC: minimum biofilm prevention concentration. ^bMBEC: minimum biofilm eradication concentration. ^cThese values are in agreement to those found by Cruz et al. (2018).⁴¹

properties of the isolated phenanthrenes, which showed effectiveness against planktonic *S. aureus*. The activity of the investigated phenanthrenes against sessile cells was evaluated by interfering with biofilm formation or eradication. Most of the tested compounds were observed to effectively prevent MSSA and MRSA biofilm formation, with compounds **12** and **14** being the most active, with a minimum biofilm prevention concentration (MBPC) value of 15.1 μM for **12**, and 15.3 μM for **14**, respectively (Table 6). In some cases, the active concentrations were higher than those observed for the inhibition of planktonic *S. aureus*. Notably, that this is the

first time the antibiofilm activity of the known phenanthrenes **11–20**, including compounds **12** and **15** previously studied, has been described. The most potent compounds (**12** and **14**) with biofilm prevention effects were further evaluated for their ability to eradicate preformed biofilms; however, no effect was observed in this assay (Table 6).

Our research led to the isolation of 20 phenanthrenes (**1–20**), including 10 new ones (**1–10**), and 6 other compounds from *J. articulatus*. Previously a disc diffusion assay established that the CHCl₃ extract of the plant exhibited antibacterial activity against MRSA with a 9.3 mm inhibition zone.³⁹ The

antibacterial activity of the compounds isolated from this extract was tested against *E. coli*, *P. aeruginosa*, MSSA, and MRSA (Table S5). Compounds 15, 17, 18, and particularly 12 and 14, demonstrated the ability to inhibit the growth of sessile MSSA and the resistant MRSA strain. Furthermore, these compounds inhibited biofilm formation by these pathogens. We have provided preliminary SAR conclusions based on the identified target activities. Generally, phenanthrenes with a double bond at C-9–C-10 demonstrated stronger activity than dihydrophenanthrenes. Moreover, monophenanthrenes showed more potent effectiveness than diphenanthrenes.

EXPERIMENTAL SECTION

General Experimental Procedures. A Shimadzu UV-1800 spectrophotometer was used to obtain UV spectra. NMR spectra were recorded in MeOH-*d*₄ and CDCl₃ on a Bruker Avance DRX 500 spectrometer (Bruker, Germany) at 500 and 600 MHz (¹H) and 125 and 150 MHz (¹³C). The signals of the deuterated solvents (δ_{H} 4.78 and 3.31, and δ_{C} 49.2 for CD₃OD, and δ_{H} 7.26 and δ_{C} 77.16 for CDCl₃) were used as reference. High-resolution MS spectra were acquired on a Thermo Scientific Q-Exactive Plus Orbitrap mass spectrometer equipped with ESI ion source in either positive or negative ionization mode. The data were acquired and processed using MassLynx software. HPLC was performed out on a Shimadzu HPLC using normal (Phenomenex, Kinetex Luna silica, 3 μ m, 100 Å, 150 × 4.6 mm) and RP (Phenomenex, Kinetex Phenyl-Hexyl, 5 μ m, 100 Å, 150 × 4.6 mm) columns. Vacuum liquid chromatography (VLC) was performed on silica gel (GF₂₅₄, 15 μ m, Merck, Darmstadt, Germany). Sephadex LH-20 (25–100 μ m, Sigma-Aldrich, Budapest, Hungary) was used for gel filtration. Preparative thin-layer chromatography (prep. TLC) was performed on silica gel plates (TLC silica gel 60 F₂₅₄, Merck, Darmstadt, Germany) and on reversed-phase (RP) silica gel plates (TLC silica gel 60 RP-18 F₂₅₄, Merck, Darmstadt, Germany). All solvents used for column chromatographies were of analytical grade (VWR Ltd., Szeged, Hungary).

Plant Material. *J. articulatus* L. (whole plant, 2.6 kg) was collected during the flowering period in June 2020 from the sandy area of the dried lakebed near Barcs, Hungary (GPS coordinates: 45° 58' 30.817" N, 17° 32' 17.764" E). Botanical identification of the plant material was conducted by Dragica Purger (Department of Pharmacognosy, University of Pécs, Hungary). A voucher specimen (No. 892) was deposited at the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Extraction and Isolation. The air-dried whole plant of *J. articulatus* (2.6 kg) was ground and percolated with MeOH (20 L) at room temperature. The MeOH extract was concentrated (350 g) under reduced pressure. The extract was dissolved in 50% aqueous MeOH after evaporation, and solvent–solvent partitions were performed using *n*-hexane (8 × 0.5 L), CHCl₃ (10 × 0.5 L), and EtOAc (9 × 0.5 L).

The concentrated CHCl₃-soluble fraction (20.5 g) was separated via VLC on silica gel using a gradient system of cyclohexane–EtOAc–MeOH [from 98:2:0 to 1:1:0 (1500 mL/eluent) and finally with MeOH; the volume of collected fractions was 100 mL]. The fractions were combined into 12 main fractions (Fr. 1–12) based on TLC analysis using UV light at 254 and 366 nm, and visualized with vanillin sulfuric acid reagent followed by heating at 110 °C for 1 min.

Almost all fractions (Fr. 1–11) were purified by Sephadex LH-20 gel chromatography using CH₂Cl₂–MeOH (1:1) as the eluent. Fraction 12 was separated via VLC on silica gel with a gradient system of CHCl₃–MeOH [from 1:0 to 0:1 (200 mL/eluent)]; the volume of the collected fractions was 10 mL] yielding 12 fractions (Fr. 12/1–12/12). The fractions were combined based on their TLC patterns. Fraction 12/12 was pure and yielded luteolin (306.4 mg). Fraction 2/2 was separated by normal-phase (NP)-HPLC under gradient conditions, using cyclohexane–EtOAc (from 80:20 to 55:45 in 10 min; flow rate 1 mL/min) as the mobile phase, yielding vanillin (*t*_R =

9.5 min, 33.2 mg). Fraction 2/3 was purified by RP-HPLC under gradient conditions using MeOH–H₂O (from 82:18 to 85:15 in 10 min; flow rate 1 mL/min) as the mobile phase, yielding compounds 10 (0.8 mg, *t*_R = 6.4 min) and 20 (2.9 mg, *t*_R = 6.9 min). Fraction 3/3 was separated by prep. TLC on silica gel using cyclohexane–EtOAc–EtOH (60:30:3) as a solvent system. Subfraction 3/3/1 was further purified by RP-HPLC using MeOH–H₂O gradient solvent system (from 8:2 to 1:0 in 9 min; flow rate 1 mL/min) as the mobile phase, yielding compound 8 (0.9 mg, *t*_R = 6.8 min). Fraction 4/3 was purified by RP-HPLC under gradient conditions using MeOH–H₂O (from 5:5 to 1:0 in 10 min; flow rate 1 mL/min) as the mobile phase, yielding two subfractions. Fraction 4/3/2 was pure, resulting compound 1 (7.6 mg, *t*_R = 8.8 min). Fraction 4/4 was purified by NP-HPLC under isocratic conditions using cyclohexane–EtOAc (82:18 for 16 min; flow rate 1 mL/min) as the mobile phase. Subfraction 4/4/1 was further purified by RP-HPLC using MeCN–H₂O solvent system (from 7:3 to 85:15 in 6 min; flow rate 1 mL/min) as the mobile phase, to yield compound 18 (1.2 mg, *t*_R = 2.5 min), and compound 9 (1.5 mg, *t*_R = 4.2 min). Fraction 5/2 was purified by NP-HPLC using a cyclohexane–EtOAc solvent system (from 75:25 to 1:1 in 6 min; flow rate 1 mL/min) as the mobile phase, yielding compound 15 (3.3 mg, *t*_R = 10.3 min). Subfraction 5/2/1 was further separated by RP-HPLC under gradient conditions using MeCN–H₂O (from 4:6 to 1:0 in 8 min; flow rate 1 mL/min) as the mobile phase, yielding *p*-hydroxybenzaldehyde (3.4 mg, *t*_R = 2.16 min). Fraction 5/3 was separated by prep. TLC on silica gel using cyclohexane–EtOAc–EtOH (60:30:3) as the solvent system, yielding compound 11 (100.6 mg). Fraction 6/5 was purified by prep. TLC on silica gel using cyclohexane–EtOAc–EtOH (60:30:3) as the solvent system. Subfraction 6/5/1 was further purified by RP-HPLC using a MeOH–H₂O gradient (from 8:2 to 1:0 in 4 min; flow rate 1 mL/min) as the mobile phase, yielding compound 2 (1.4 mg, *t*_R = 7.10 min). Fraction 6/6 was separated by RP-HPLC using a MeOH–H₂O gradient (from 75:25 to 8:2 in 8 min; flow rate 1 mL/min), yielding compound 13 (3.4 mg, *t*_R = 4.8 min). Fraction 7/4 was purified by RP-HPLC using a gradient of MeCN–H₂O (from 4:6 to 8:2 in 10 min; flow rate 1 mL/min) as the mobile phase to yield compound 16 (4.2 mg, *t*_R = 6.7 min). Furthermore, fraction 7/5 was purified by the same RP-HPLC method used for fraction 7/4, yielding compound 7 (0.8 mg, *t*_R = 11.8 min). Fraction 7/6 was separated by prep. TLC on silica gel using cyclohexane–EtOAc–EtOH (60:30:3) as the solvent system to yield compound 12 (135.7 mg).

After gel filtration of fraction 9 on Sephadex LH-20, fraction 9/6 was pure and yielded compound 14 (5.8 mg). Fraction 9/2 was separated by prep. TLC on silica gel using CHCl₃–MeOH (95:5) as the mobile phase, and subfraction 9/2/1 was further purified by RP-HPLC using MeOH–H₂O gradient solvent system (from 1:1 to 1:0 in 7 min; flow rate 1 mL/min) to yield dehydrovomifolol (1.5 mg, *t*_R = 3.5 min). Fraction 9/5 underwent further separation by RP-HPLC under gradient conditions using MeCN–H₂O (from 4:6 to 1:0 in 9 min; flow rate 1 mL/min) as the mobile phase, yielding compounds 17 (3.9 mg, *t*_R = 3.7 min) and 5 (0.9 mg, *t*_R = 4.8 min). Fraction 10/4 was separated by prep. TLC on RP silica gel using MeOH–H₂O (7:3) as the mobile phase, yielding two subfractions. Furthermore, subfraction 10/4/2 was further purified by RP-HPLC under gradient conditions, using MeOH–H₂O (from 7:3 to 85:15 in 10 min; flow rate 1 mL/min) as the mobile phase, yielding compounds 3 (10.8 mg, *t*_R = 3.76 min) and 4 (1.3 mg, *t*_R = 5.9 min). Fraction 10/5 was purified by RP-HPLC using MeOH–H₂O solvent system (from 65:35 to 75:25 in 9 min; flow rate 1 mL/min), and compound 6 (3.1 mg, *t*_R = 4.15 min) was isolated. In addition, fraction 11/5 was purified by RP-HPLC using a gradient solvent system comprising MeOH–H₂O (from 65:35 to 8:2 in 8 min; flow rate 1 mL/min), yielding apigenin (1.0 mg, *t*_R = 4.1 min) and compound 19 (1.1 mg, *t*_R = 4.62 min). Fraction 12/4 was separated by prep. TLC on RP silica gel using MeOH–H₂O (8:2) as the mobile phase, and subfraction 12/4/1 underwent further purification through RP-HPLC using an isocratic MeOH–H₂O (48:52) eluent, at a flow rate of 1 mL/min, yielding 1,3-O-diferuloylglycerol (1.9 mg, *t*_R = 21.50 min).

Articulin A (1). Light-yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 213 (4.35), 282 (4.08), 320 (3.82) nm; ^1H and ^{13}C NMR data, see Table 1; (–)-HRESIMS m/z 295.1340 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{19}\text{H}_{19}\text{O}_3$, 295.1342).

Articulin B (2). Light-yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 240 (4.12), 321 (3.46) nm; ^1H and ^{13}C NMR data, see Table 1; (–)-HRESIMS m/z 529.2378 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{36}\text{H}_{33}\text{O}_4$, 529.2384).

Articulin C (3). Yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 228 (4.08), 264 (4.19), 348 (2.81), 365 (2.85) nm; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS m/z 281.1173 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{18}\text{H}_{17}\text{O}_3$, 281.1172).

Articulin D (4). Light-yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 228 (4.14), 267 (4.23), 350 (2.80), 367 (2.84) nm; ^1H and ^{13}C NMR data, see Table 2; (–)-HRESIMS m/z 293.1188 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{19}\text{H}_{17}\text{O}_3$, 293.1183).

Articulin E (5). Light-yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 229 (3.85), 262 (3.95), 347 (2.53), 364 (2.56) nm; ^1H and ^{13}C NMR data, see Table 2; (+)-HRESIMS m/z 281.1175 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{18}\text{H}_{17}\text{O}_3$, 281.1172).

Articulin F (6). Yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 230 (4.44), 269 (4.63), 360 (3.91), 375 (3.86) nm; ^1H and ^{13}C NMR data, see Table 2; (–)-HRESIMS m/z 277.0870 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{18}\text{H}_{13}\text{O}_3$, 277.0870).

Articulin G (7). Light-yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 230 (3.66), 270 (3.74), 367 (2.26) nm; ^1H and ^{13}C NMR data, see Table 3; (–)-HRESIMS m/z 529.2021 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{35}\text{H}_{29}\text{O}_5$, 529.2021).

Articulin H (8). Light-yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 224 (4.09), 266 (4.06), 365 (2.51) nm; ^1H and ^{13}C NMR data, see Table 3; (–)-HRESIMS m/z 525.2071 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{36}\text{H}_{29}\text{O}_4$, 525.2071).

Articulin I (9). Light-yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 225 (4.21), 267 (4.29), 348 (2.78), 366 (2.82) nm; ^1H and ^{13}C NMR data, see Table 4; (–)-HRESIMS m/z 525.2074 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{36}\text{H}_{29}\text{O}_4$, 525.2071).

Articulin J (10). Yellow amorphous solid; UV (MeOH) $\lambda_{\max}$ (log ϵ) = 223 (3.92), 270 (3.83), 367 (2.48) nm; ^1H and ^{13}C NMR data, see Table 4; (–)-HRESIMS m/z 527.2224 $[\text{M}-\text{H}]^-$ (calcd. for $\text{C}_{36}\text{H}_{31}\text{O}_4$, 527.2222).

Dehydrojuncuenin B (12). ^1H NMR (500 MHz, $\text{MeOH}-d_4$) δ 8.25 d (1H, d, J = 8.9 Hz, H-4), 7.85 (1H, s, H-5), 7.83 (1H, d, J = 9.5 Hz, H-9), 7.57 (1H, d, J = 9.5 Hz, H-10), 7.12 (1H, d, J = 8.9 Hz, H-3), 7.00 (1H, dd, J = 17.9, 11.4 Hz, H-13), 5.73 (1H, dd, J = 11.4, 2.2 Hz, H-14a), 5.30 (1H, dd, J = 17.9, 2.2 Hz), 2.48 (3H, s, 11- CH_3), and 2.32 (3H, s, 12- CH_3); ^{13}C NMR (125 MHz, $\text{MeOH}-d_4$) δ 155.7 (C-6), 154.0 (C-2), 138.5 (C-8), 136.3 (C-13), 133.4 (C-1a), 131.9 (C-5a), 125.4 (C-9), 124.8 (C-4a), 123.7 (C-7), 123.6 (C-8a), 122.3 (C-4), 121.4 (C-14), 120.1 (C-10), 118.3 (C-1), 116.8 (C-3), 105.4 (C-5), 13.6 (C-12), and 11.0 (C-11).

Antibacterial Activity. Compounds were tested against medically relevant bacterial strains, including *E. coli* ATCC 25922 and *P. aeruginosa* PAO1, selected as models for Gram-negative bacteria. Furthermore, MSSA (ATCC 6538) and MRSA (ATCC 33592) were used as models for Gram-positive strains. All cells were grown at 37 °C in Luria–Bertani media for 16 h with orbital shaking. Overnight cultures were then utilized and dissolved in Müller–Hinton media.

Planktonic Bacteria Susceptibility Assays. Bacterial suspensions adjusted to 1×10^6 CFU/mL were exposed, in triplicate, to varying concentrations of each target compound in wells containing Müller–Hinton medium. The compounds dissolved in filtered EtOH were added to achieve final concentrations ranging from 1 to 128 $\mu\text{g}/\text{mL}$. Plates were subsequently incubated at 37 °C with constant stirring for 24 h. Absorbance at 595 nm was measured using a Synergy HTX multimodal microplate reader (BioTek Instruments, Inc., USA) at the end of the incubation period. Ciprofloxacin and vancomycin were simultaneously used as positive controls. Moreover, MIC values were interpreted as the lowest concentrations exhibiting 90% or greater growth inhibition than the negative control comprising medium and 1% EtOH. Notably, no difference in growth was

observed between these cells and viability control bacteria grown solely in Müller–Hinton medium. The results obtained from a minimum of three independent experiments were averaged.

Reversion of Biofilm Formation and Eradication of Preformed Biofilm Assays. To determine whether the active compounds inhibit *S. aureus* biofilm formation and eradicate preformed biofilms, preexposure and postexposure experiments were performed, as previously described, with some modifications.^{42,43} Briefly, 96-well plates containing Luria–Bertani media and bacterial inoculum corresponding to 1×10^6 CFU/mL cells harvested from exponentially growing cultures were incubated with compounds 2, 3, 6, 9, 12, 14, 15, and 17–20 at 1 to 128 $\mu\text{g}/\text{mL}$. This incubation was at 37 °C for 24 h under static conditions to allow biofilm formation. Similarly, to determine the biofilm eradication ability of compounds 12 and 14, which showed the highest effect in the previous experiment, the formed biofilms were treated with both molecules and incubated for an additional 24 h at 37 °C. Ciprofloxacin and vancomycin simultaneously served as positive controls. Following the treatment period, the supernatants were carefully discarded. For biofilm fixation, 200 μL per well of pure EtOH was added for 20 min. The cells were then stained with 200 μL of 0.1% crystal violet (H_2O – MeOH 4:1) for 5 min and washed four times with sterile phosphate-buffered saline (PBS) solution to remove detached cells. The stained bacteria were suspended in 100 μL of EtOH, and absorbance was measured at 595 nm using a Synergy HTX multimodal microplate reader (BioTek Instruments, Inc., USA). Both MBPC and minimum biofilm eradication concentration (MBEC) were determined as the lowest concentrations inducing at least 90% growth inhibition compared to the negative control containing only the dissolution solvent. No difference was observed in the growth of the former cells compared to the viability control group containing only Luria–Bertani broth. At least three independent experiments were conducted, and the results were averaged.

■ ASSOCIATED CONTENT

Data Availability Statement

The NMR data for compounds 1–10 have been deposited in the Natural Products Magnetic Resonance Database (NP-MRD; www.np-mrd.org) and can be found at NP0333257 (1), NP0333258 (2), NP0333259 (3), NP0333260 (4), NP0333261 (5), NP0333262 (6), NP0333263 (7), NP0333264 (8), NP0333265 (9), and NP0333266 (10).

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jnatprod.4c00577>.

NMR, HRESIMS, and UV spectra for compounds 1–10 (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by the UNKP-21-3-SZTE-250 New National Excellence Program, the National Research, Development and Innovation Fund (NKFI), Hungary (grant number K135845), Ministry of Innovation and Technology of Hungary (grant number TKP2021-EGA-32), Catholic University of Córdoba, CONICET (PIP- 2021-2023), and by FONCyT (PICT 2017-1381, PICT 2019-2019-00721, PICT-2020-SERIEA-01627, PICT-2021-CAT-II-00045). A.S. acknowledges receipt of a Scholarship from the Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación. The authors would also like to acknowledge the networking contribution by the COST Action CA21145 – “European Network for diagnosis and treatment of antibiotic-resistant bacterial infections”. M.C.C. is staff member of CONICET.

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