

Identification of Cembrane Diterpenoids from *Sinularia* sp. That Reduce the Viability of Diffuse Pleural Mesothelioma Cell Lines

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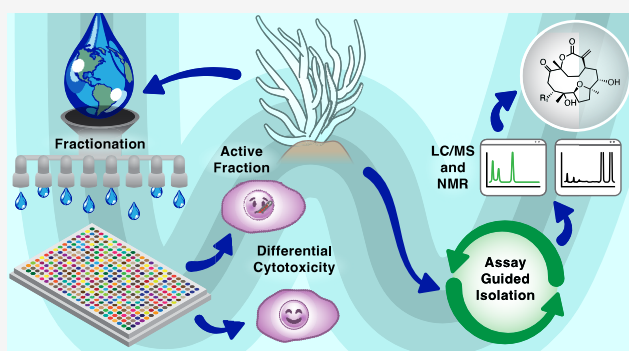
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ABSTRACT: Diffuse pleural mesothelioma (DPM) is a rare but aggressive late-onset cancer. A high-throughput screen of a natural product fraction library identified fractions derived from an aqueous extract of *Sinularia* sp. soft coral that reduced the viability of DPM cell lines. Bioassay-guided fractionation of the parent extract resulted in the identification of 13 cembrane diterpenoids, including nine new natural products sinulariolones B–J (1–4, and 7–11), as the active principles. The planar structures of the new compounds were established by the analysis of NMR spectroscopic and MS spectrometric data. Their relative and absolute configurations were determined using a combined approach, including NOESY interpretation, modified Mosher's method, ECD simulation, and single-crystal X-ray diffraction. All pure metabolites were tested for their effects on the viability of the DPM cell lines, and compounds 2, 3, 8, and 9 demonstrated low micromolar potency against these cancer cell lines.



Soft corals (on the order of Alcyonacea) are sessile benthic organisms. Anatomically, the majority of the organism is comprised of soft tissue, a proteinaceous hydroskeleton with embedded calcareous support structures (sclerites).^{1,2} These creatures constitute a dominant part of reef biomass, which is distributed throughout the world (mainly in tropical and subtropical waters). Members of the genus *Sinularia* (family Alcyoniidae), with more than 90 species,³ are globally distributed with an oceanic depth of about 12 m, including some of the most conspicuous soft coral species.³ Though abundant, this genus of more than 90 species³ is severely affected by coral bleaching events.⁴ Therefore, the annotation of their unique metabolites is of increasing importance.^{5,6} In addition, corals are a significant source of bioactive metabolites, especially terpenoids and steroids.^{7,8} *Sinularia* spp., along with other soft coral genera (e.g., *Sarcophyton*, *Eunicea*, *Clavularia*, *Nephthea*, and *Lobophytum*), are well-known to produce cembranoids,^{9,10} a class of diterpenoids featuring a 14-membered carbocyclic core structure, which is substituted with three methyl groups at positions 4, 8, and 12, along with an isopropyl unit at C-1 that can further close on the core structure to form different ring systems, such as γ -lactone, δ -lactone, and ϵ -lactone.^{10,11} The cembrane diterpenoids display diverse biological activities, including anticancer, anti-inflammatory, antibacterial, and hepato-protective properties.^{9,10,12}

Diffuse pleural mesothelioma (DPM) is a rare aggressive malignancy affecting the mesothelial cells in the pleural lining surrounding the lungs often related to asbestos exposure.¹³ Due to its long latency period, DPM is considered to be difficult to diagnose, stage, and treat.¹⁴ Despite the prohibition of asbestos use in some countries, the incidence of DPM has increased over the last 30 years due to the long time between first exposure to asbestos and diagnosis of DPM and the continued use of asbestos globally.^{15,16} The molecular mechanisms for DPM carcinogenesis remain incompletely understood but can involve alterations of NF2, RASSF1, LATS2, WT1, and p16 (CDKN2A), as well as BAP-1 tumor-suppressor genes, which regulate apoptosis, cell invasion, motility, cell division, chromatin remodeling, and DNA repair. All these processes are dysregulated in cancer.¹⁵ Current treatment options are often multimodal approaches, including debulking surgery consisting of pleurectomy-decortication often coupled with chemotherapy, monoclonal antibody treatment, and/or radiotherapy for early stage or epithelial

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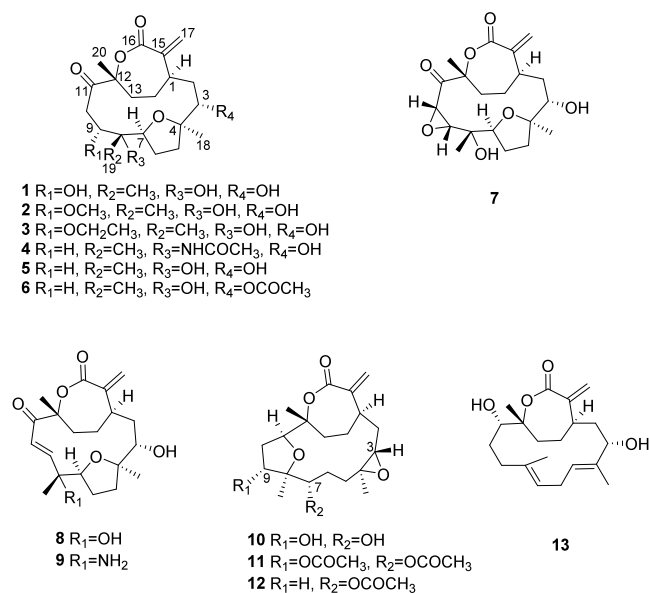
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DPM.^{17,18} Despite these treatments, DPM prognosis remains dismal, with a median survival of patients generally from 4 to 14 months.^{19,20} With only two FDA-approved anti-DPM drug regimens,^{21,22} there is a compelling need for more effective treatments.

In a recent DPM oriented high-throughput screening campaign using a fractionated natural product library, fractions from an extract of *Simularia* sp. soft coral were identified as active. This manuscript presents the bioassay-guided isolation, structure elucidation, and biological evaluation of the nine new cembranoids, namely, sinulariolones B–J (1–4 and 7–11), together with four known congeners (5, 6, 12, and 13), as potential pharmacophores for DPM chemotherapeutic applications.



RESULTS AND DISCUSSION

Despite the need for treatment options for DPM, few large-scale high-throughput drug discovery campaigns have been published directly assessing the effect of small molecules on the growth of mesothelioma cell lines.²³ Although some plant metabolites have been individually identified as reducing mesothelioma cell line growth, we are unaware of a high-throughput screening campaign purposefully built to probe natural product chemistry for agents which affect the viability of mesothelioma.²⁴ To address this knowledge gap, two patient-derived mesothelioma cell lines, MB24 (sarcomatoid) and MB52 (epithelial), were used in a viability screen against ~34,000 fractionated natural product extracts available as part of the NCI Program for Natural Products Discovery (NPNPD) screening library (Figure 1A).²⁵ The central aim of this screen was to identify natural product fractions that reduce the viability of either mesothelioma cell line or both that were representative of the histologic spectrum of DPM. Active fractions of interest would then be subjected to bioassay-guided fractionation to identify active metabolites for structural characterization and biological evaluation.

The resulting pure natural products were tested for DPM specificity by counter-screening against a patient-derived normal pleura mesothelial cell line, NP1.²⁶ Prior to initiating the complete screening campaign, a selected set of 20 384-well plates of NPNPD fractions was screened against all three cell

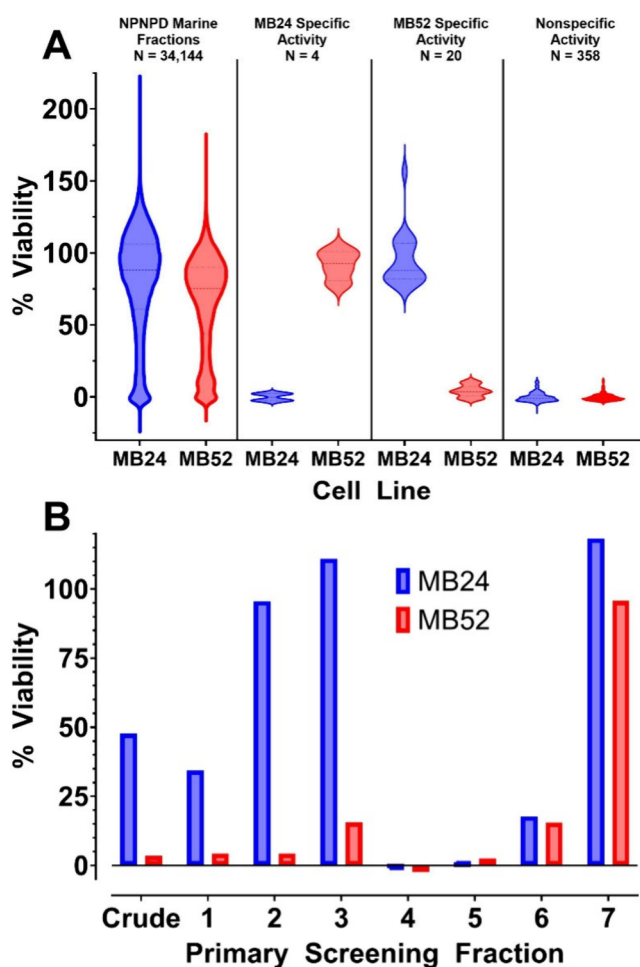


Figure 1. Primary screening outcomes. (A) Violin plot showing the distribution of all marine primary screening fractions and mesothelioma cell line-specific activity. (B) The behavior of all NPNPD fractions derived from the *Simularia* sp. extract demonstrated MB52-specific activity across multiple fractions.

lines at a concentration of 10 μ g/mL to assess the impact on viability for each cell line (Figure S1). As shown in Figure S1, the majority of tested fractions were inactive against any of the three cell lines, but there were samples that demonstrated differential specific activity against each of the cell lines, reducing the % viability by at least 50% while not reducing the viability of the other cell lines by more than 25%. The appearance of these discrete populations of bioactive fractions in this small subset demonstrated that this screening hypothesis should enable the identification of natural products with potential differential specificity against the DPM cell lines and a limited impact on the viability of the NP1 line.

Data analysis following primary screening of ~34,000 marine fractions against the two DPM cell lines revealed that most samples did not significantly reduce viability in either cell line, resulting in a median reading of 81% viability (Figure 1A). Although there were fractions that exhibited apparent DPM cell line specificity, the majority of substances that reduced viability in one cell line also did so in the other cell line, with 358 substances showing nonspecific activity (Figure 1A). Following the primary screening, fractions derived from a *Simularia* sp. extract were chosen for follow-up due to observed reductions in viability of the MB52 cell line (Figure 1B). This broad activity was not seen with most fractions, in which the

Table 1. ^1H and ^{13}C NMR Spectroscopic Data of 1–4 in $\text{DMSO}-d_6$

No.	1		2		3		4	
	δ_{C} , type ^a	δ_{H} (J in Hz) ^b	δ_{C} , type ^a	δ_{H} (J in Hz) ^b	δ_{C} , type ^a	δ_{H} (J in Hz) ^b	δ_{C} , type ^a	δ_{H} (J in Hz) ^b
1	32.1, CH	3.06, td (11.4, 5.8)	32.3, CH	2.95, td (11.2, 6.0)	32.4, CH	2.98, m	32.1, CH	2.86, td (11.2, 6.0)
2	38.2, CH ₂	1.55, m, Ha	38.0, CH ₂	1.54, td (11.2, 2.6), Ha	38.2, CH ₂	1.54, m, Ha	38.1, CH ₂	1.58, m, Ha
		1.76, m, Hb		1.76, m, Hb		1.77, m, Hb		1.70, t (12.4), Hb
3	73.0, CH	3.32, m	72.9, CH	3.29, m	73.1, CH	3.31, m	72.7, CH	3.35, m
4	87.1, C		87.0, C		87.2, C		86.9, C	
5	37.3, CH ₂	1.55, m, Ha	37.2, CH ₂	1.63, m	37.4, CH ₂	1.60, m, Ha	36.9, CH ₂	1.59, m
		1.66, m, Hb				1.66, m, Hb		
6	24.9, CH ₂	1.80, m	25.2, CH ₂	1.72, m, Ha	24.9, CH ₂	1.76, m, Ha	24.9, CH ₂	1.57, m
				1.81, m, Hb		1.82, m, Hb		
7	78.5, CH	4.11, dd (7.8, 6.4)	78.5, CH	4.01, t (7.4)	78.5, CH	4.03, t (7.8)	81.3, CH	4.49, dd (7.6, 5.8)
8	74.8, C		75.1, C		75.0, C		57.8, C	
9	70.5, CH	3.87, m	80.4, CH	3.50, dd (7.6, 3.2)	80.3, CH	3.60, m	28.8, CH ₂	1.83, m, Ha
								2.39, ddd (14.0, 8.4, 3.0), Hb
10	43.1, CH ₂	2.68, m, Ha	40.4, CH ₂	2.77, dd (20.8, 3.4), Ha	41.1, CH ₂	2.75, dd (20.4, 3.2), Ha	33.7, CH ₂	2.57, ddd (20.8, 8.4, 3.0), Ha
		3.84, m, Hb		3.81, dd (20.8, 7.6), Hb		3.84, dd (20.4, 8.6), Hb		3.31, m, Hb
11	210.4, C		210.8, C		210.9, C		210.4, C	
12	91.2, C		91.2, C		91.3, C		90.7, C	
13	33.9, CH ₂	1.81, m, Ha	33.9, CH ₂	1.81, m, Ha	34.1, CH ₂	1.82, m, Ha	33.8, CH ₂	1.86, m, Ha
		2.17, dd (14.8, 5.2), Hb		2.18, dd (15.0, 5.8), Hb		2.18, dd (15.0, 5.0), Hb		2.27, dd (15.2, 6.2), Hb
14	30.2, CH ₂	1.05, td (11.4, 5.8), Ha	30.2, CH ₂	1.05, td (11.2, 4.4), Ha	30.3, CH ₂	1.04, m, Ha	30.2, CH ₂	1.06, m, Ha
		1.87, m, Hb		1.91, m, Hb		1.91, m, Hb		1.97, m, Hb
15	145.0, C		144.9, C		145.0, C		145.1, C	
16	167.7, C		167.6, C		167.8, C		167.6, C	
17	124.2, CH ₂	5.53, s, Ha	124.4, CH ₂	5.53, s, Ha	124.5, CH ₂	5.53, s, Ha	124.2, CH ₂	5.52, s, Ha
		6.14, s, Hb		6.14, s, Hb		6.14, s, Hb		6.11, s, Hb
18	17.2, CH ₃	0.95, s	17.3, CH ₃	0.95, s	17.5, CH ₃	0.95, s	17.0, CH ₃	0.93, s
19	20.2, CH ₃	0.92, s	20.6, CH ₃	0.92, s	20.6, CH ₃	0.93, s	20.4, CH ₃	1.02, s
20	28.9, CH ₃	1.34, s	28.7, CH ₃	1.38, s	28.9, CH ₃	1.37, s	29.0, CH ₃	1.34, s
1'			57.7, CH ₃	3.28, s	65.4, CH ₂	3.39, m	168.9, C	
				3.57, m				
2'					15.5, CH ₃	1.09, t (6.8)	23.5, CH ₃	1.77, s
3-OH		4.83, d (5.4)		4.85, d (5.2)		4.83, d (4.8)		4.84, d (5.4)
8-OH/ NH		4.35, s		4.36, s		4.19, s		7.52, s
9-OH		4.64, d (3.6)						

^aSpectrum recorded on 125 MHz. ^bSpectrum recorded on 600 MHz.

crude extract was generally inactive >80% of the time, and usually there were two or fewer active fractions from a single source organism. Given this uncommon fractional activity profile, the *Sinularia* sp. extract was designated for further chemical evaluation. Subsequent bioassay-guided isolation led to the identification of nine new (1–4 and 7–11) and four known (5, 6, 12, and 13) cembrane diterpenoids.

Sinulariolone B (1) was isolated as a white amorphous powder with a UV absorption maximum at 215 nm. Its molecular formula was deduced to be $\text{C}_{20}\text{H}_{30}\text{O}_7$ based on the presence of two ions, including a $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ ion at m/z 365.1965 (calculated for $\text{C}_{20}\text{H}_{29}\text{O}_6^+$, 365.1964) and an $[\text{M} + \text{Na}]^+$ ion at m/z 405.1888 (calculated for $\text{C}_{20}\text{H}_{30}\text{O}_7\text{Na}^+$, 405.1889) in the HRESIMS spectrum, accounting for six indices of hydrogen deficiency. IR bands at 3394 and 1710 cm^{-1} indicated the presence of hydroxyl and carbonyl functionalities, respectively. The ^1H NMR data of 1 in $\text{DMSO}-d_6$ (Table 1) displayed three exchangeable OH signals,

four sp^3 methines (three hydroxylated), an sp^2 exomethylene, six sp^3 methylenes, and three singlet methyls. Analyses of ^{13}C NMR and HSQC data (Table 1) denoted the presence of 20 carbon signals, including resonances corresponding to the functionalities described above and additional signals for three oxygen-bearing non-hydrogenated sp^3 carbons (δ_{C} 91.2, 87.1, and 74.8), two carbonyls (δ_{C} 210.4 and 167.7), and a non-hydrogenated sp^2 carbon (δ_{C} 145.0). The NMR data of 1 closely resembled those of the co-occurring known cembranoid sinulariolone (5),²⁷ except that the CH_2 –9 in 5 was hydroxylated in 1 (CH_2 –9, $\delta_{\text{H/C}}$ 3.87/70.5). The structural assignment was supported by the COSY spin system H-10 (δ_{H} 3.87 and 2.68)/H-9/9-OH (δ_{H} 4.64) and key HMBC correlations from H-9 to C-11 (δ_{C} 210.4), from H-7 (δ_{H} 4.11) to C-9, and from 9-OH to C-9 and C-10 (δ_{C} 43.1) (Figure 2A). The relative configuration of 1 was determined by the analysis of the NOESY data (Figure 2A). Briefly, NOESY correlations between H-1 (δ_{H} 3.06) and H-10b (δ_{H} 3.84), H-1

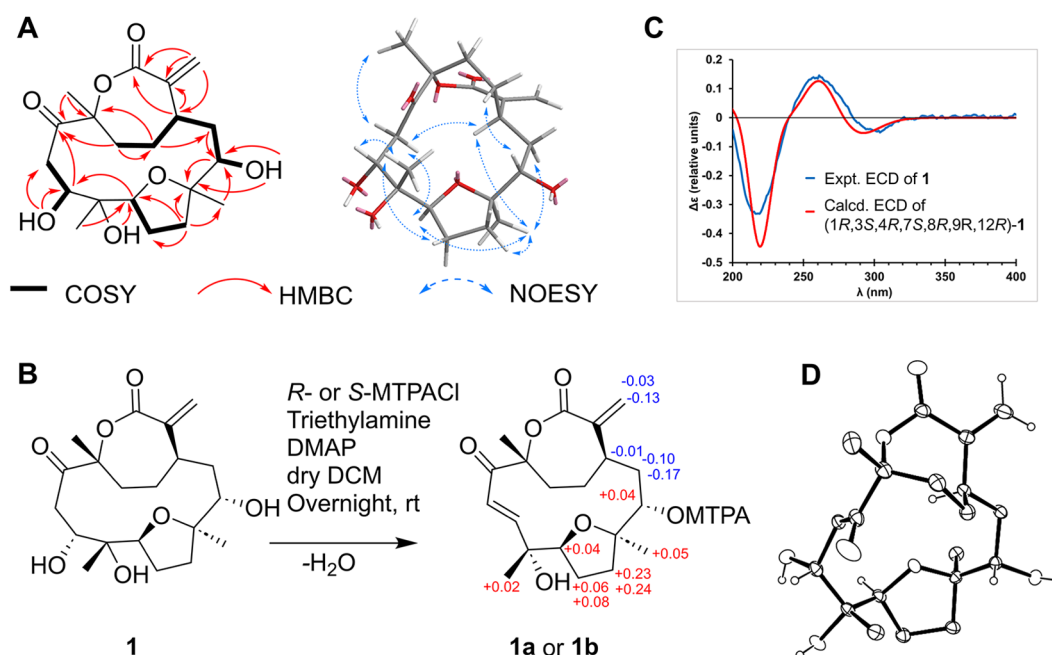


Figure 2. (A) Key COSY, HMBC, and NOESY correlations of **1**. (B) $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for the (S)- and (R)-MTPA esters (**1a** and **1b**), respectively. (C) Experimental (blue line) and calculated (red line) ECD spectra of **1**. (D) ORTEP drawing of the X-ray crystal structure of **1**.

and H₃-18 (δ_H 0.95), H-7 and H-10b, and H-7 and H₃-18 indicated that H-1, H-7, H-10b, and CH₃-18 shared the same α -orientation on the macrocyclic skeleton. Meanwhile, NOESY correlations between H-2b (δ_H 1.76) and H₃-19 (δ_H 0.92), H-3 (δ_H 3.32) and H₃-19, H-9 and H-10a (δ_H 2.68), H-9 and H₃-19, and H-10a and H₃-20 (δ_H 1.34) suggested that H-2b, H-3, H-9, H-10a, CH₃-19, and CH₃-20 were located on the opposite face of the cembranoid skeleton as β -orientated. Moreover, NOESY correlations between H-2a (δ_H 1.57) and H-17a (δ_H 5.53) and between H-3 (δ_H 3.32) and H-14b (δ_H 1.87) enabled the assignment of the orientation of the ϵ -lactone ring as depicted in Figure 1A. Thus, the relative configuration of **1** was deduced to be 1R*,3S*,4R*,7S*,8R*,9R*,12R*, consistent with that of **5**.

The absolute configuration of compound **1** was determined and cross-verified via multiple approaches (Figures 2B–D). First, the absolute configuration of the hydroxylated C-3 was established through the modified Mosher's method.²⁸ The reactions of **1** with (R)- and (S)-2-methoxy-2-trifluoromethyl-2-phenylacetic (MTPA) chlorides led to the (S)- and (R)-MTPA esters **1a** and **1b**, respectively (Figure 2B). It is worth noting the dehydroxylation of OH-9 formed an α,β -unsaturated ketone under both reaction conditions. The calculated $\Delta\delta_H$ values ($\delta_S - \delta_R$) for the protons neighboring C-3 led to the assignment of the 3S configuration (Figure 2B); therefore, the absolute configuration of **1** was deduced to be 1R,3S,4R,7S,8R,9R,12R. The stereochemical assignment was supported by the comparison of the experimental and calculated ECD spectra of **1** (Figure 2C). The calculated ECD spectrum for (1R,3S,4R,7S,8R,9R,12R)-**1** was simulated using time-dependent density functional theory (TDDFT) at the wB97XD/6-311+g(d,p) level in MeOH. The calculated ECD spectrum was in good agreement with the experimental one, which exhibited a strong negative Cotton effect at 220 nm ($\Delta\epsilon$ -1.3), a moderate positive Cotton effect at 264 nm ($\Delta\epsilon$ +0.1), and a weak negative Cotton effect at 302 nm ($\Delta\epsilon$

-0.04) (Figure 2C). Finally, the absolute configuration of **1** was confirmed by single-crystal X-ray diffraction analysis (Figure 2D, Table S7).

Simulariolones C–E (2–4) are close analogues of **1** and **5**²⁷ with minor modifications at C-8 and/or C-9 demonstrated by comparison of their NMR data (Table 1). Their molecular formulas were established as C₂₁H₃₂O₇ (**2**), C₂₂H₃₄O₇ (**3**), and C₂₂H₃₃NO₆ (**4**), respectively, based on their HRESIMS data. The NMR data of **2** were almost identical to those of **1** (Table 1) except for the resonances for an additional methoxy group OCH₃-1' at $\delta_{H/C}$ 3.28/57.7 and the deshielded C-9 (δ_C 80.3 in **2** vs δ_C 70.5 in **1**), indicating OH-9 in **1** was methylated in **2**. The assessment was supported by the HMBC correlation from H₃-1' to C-9 (Figure S2). Similar to **2**, compound **3** was determined as a 9-ethoxy (CH₂-1', $\delta_{H/C}$ 3.39 and 3.57/65.4; CH₃-2', $\delta_{H/C}$ 1.09/15.5) analogue of **1** based on COSY correlations from the methylene protons H₂-1' to H₃-2' and the HMBC correlation from H₂-1' to C-9 (δ_C 78.7) (Figure S2). The NMR data (Table 1) of **4** showed notable similarity to those of **5**²⁷ except for the presence of an acetamido group with characteristic chemical shifts (δ_{NH-8} 7.52, $\delta_{C-1'}$ 168.9, and $\delta_{H-2'/C-2'}$ 1.77/23.5)^{29,30} that replaced the OH-8 in **5**, supported by HMBC correlations from NH-8 to C-7 (δ_C 81.3)/C-8/C-9 (δ_C 28.8)/C-19 (δ_C 20.4)/C-1' (δ_C 168.9) and from H₃-2' to C-1', as well as the significantly shielded chemical shift of C-8 from δ_C 72.3 in **5** to δ_C 57.8 in **4** (Figure S2). Although nitrogen-containing cembrane diterpenoids are rare, some representatives containing the nitrogen group have previously been reported from the soft coral genera *Lobophytum* and *Sinularia*, while the biosynthetic pathway related to the addition of N-containing substituents in soft corals remains unclear.^{31,32} The relative configurations of 2–4 (1R*,3S*,4R*,7S*,8R*,9R*,12R* for **2** and **3**, and 1R*,3S*,4R*,7S*,8R*,12R* for **4**) were determined to be consistent with those of **1** and **5** based on their NOESY data (Figure S2). The ECD spectra of **1**–**4** demonstrated similar

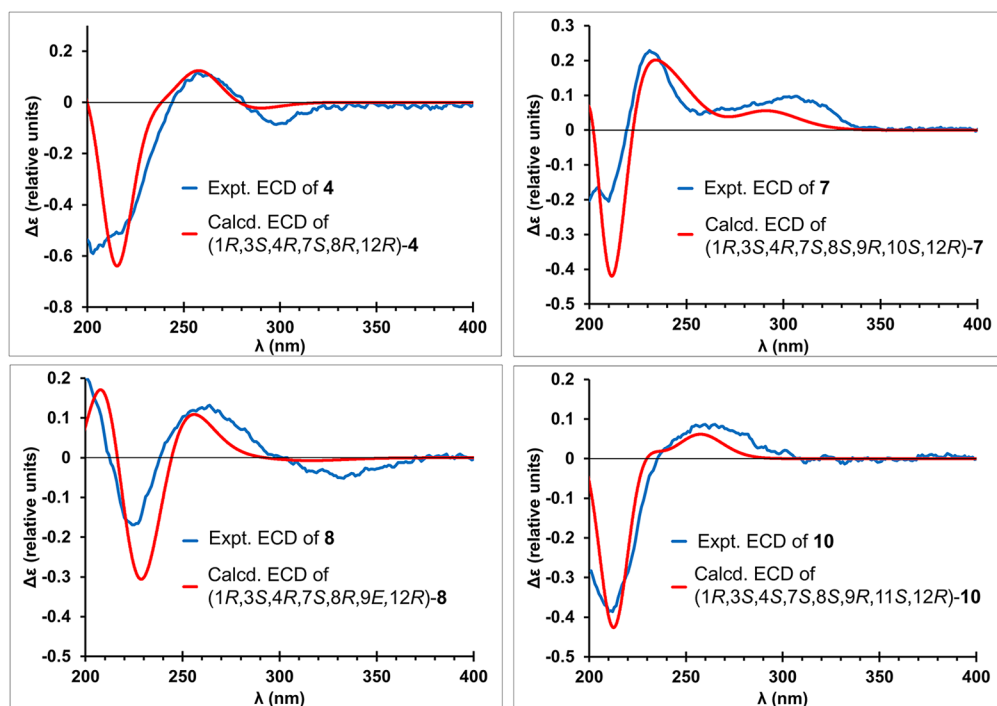


Figure 3. Experimental (blue line) and calculated (red line) ECD spectra of **4**, **7**, **8**, and **10**.

trends of Cotton effects (Figure S3A) with a highly negative Cotton effect at 212–217 nm, a weak positive Cotton effect at 260–262 nm, and a weak negative Cotton effect at 300–304 nm, suggesting the same absolute configuration of the cembrane diterpenoid skeleton as that of (1*R*,3*S*,4*R*,7*S*,8*R*,9*R*,12*R*)-**1**, which was further supported by comparison of the experimental and calculated ECD spectra of the representative analogue **4** (Figure 3).

The HRESIMS spectrum of sinulariolone F (**7**) showed two ion peaks at m/z 363.1801 $[M - H_2O + H]^+$ and 403.1726 $[M + Na]^+$, suggesting the molecular formula as $C_{20}H_{28}O_7$. Interpretation of the NMR data (Table 2 and Figure 4) revealed that the structure of **7** was almost identical to that of **1**, except for the absence of a methylene at C-10 and the presence of an epoxy group at positions C-9 ($\delta_{H/C}$ 3.44/65.6) and C-10 ($\delta_{H/C}$ 4.10/55.2), which accounted for an extra index of hydrogen deficiency. The structural assignment was supported by HMBC correlations from H-10 to C-9 and C-11 (δ_C 208.7) and from H-7 (δ_H 3.85) and H₃-19 (δ_H 1.23) to C-9 (Figure 4). Comparison of key NOESY correlations of **1** and **7** (Figures 1 and 4) indicated the same relative configuration for the core structures of **1** and **7** (1*R**,3*S**,4*R**,7*S**,8*S**,12*R**). Further analysis of NOESY correlations of H-10/H-9, H-10/H-13b (δ_H 2.75), H-10/H-14b (δ_H 2.24), H-9/H₃-19, and H-9/H-6a (δ_H 1.65) enabled the assignment of the 9*R**,10*S** configuration in **7** (Figure 4). The experimental ECD spectrum of **7**, with Cotton effect maxima at 210 (−), 232 (+), and 303 (+) nm, was in good agreement with the one calculated for (1*R*,3*S*,4*R*,7*S*,8*S*,9*R*,10*S*,12*R*)-**7** using TDDFT at the mPW1PW91/6–311g(d,p) level in MeOH, as shown in Figure 3, confirming the stereochemistry of **7**.

Both sinulariolones G (**8**) and H (**9**) were obtained as optically active white powders. Their molecular formulas were established as $C_{20}H_{28}O_6$ (**8**) and $C_{20}H_{29}NO_5$ (**9**), respectively, deduced from their HRESIMS and NMR data. The NMR data

of **8** (Table 2) resembled those of **5**²⁷ except for the resonances accounting for a *trans*- Δ^9 double bond (CH-9, δ_H 6.84, d, J = 15.2 Hz, δ_C 150.7; CH-10, δ_H 7.20, d, J = 15.2 Hz, δ_C 126.7), supported by HMBC correlations from H-9 and H-10 to C-8 (δ_C 73.8) and C-11 (δ_C 202.4) and from H-7 (δ_H 3.79) to C-9 (Figure 4). Similarly, compound **9** was elucidated as an NH₂-8 (δ_H 3.79) analogue of **8**, as evidenced by the significantly shielded C-8 (δ_C 73.8 in **8** vs δ_C 56.2 in **9**) and key HMBC correlations from CH₃-19 (δ_H 1.08) to C-7 (δ_C 88.4), C-8, and C-9 (δ_C 151.9) (Figure S4). Comparison of key NOESY correlations and ECD spectra of **8** and **9** with those of **1** (Figures 1, 4, and S4) suggested a 1*R*,3*S*,4*R*,7*S*,8*R*,9*E*,12*R* configuration for the cembranoid skeleton, which was further supported by a good match of the comparison between the experimental and calculated ECD spectra of **8** (Figures 3 and S3).

Sinulariolone I (**10**) was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{20}H_{30}O_6$ based on the HRESIMS ion peak at m/z 367.2114 $[M + H]^+$ (calculated for $C_{20}H_{31}O_6^+$, 367.2121), implying six indices of hydrogen deficiency. The ¹H NMR spectrum of **10** in DMSO-*d*₆ indicated the presence of two exchangeable OH signals, five sp³ methines (four oxygen-bearing), an sp² exomethylene, six sp³ methylenes, and three methyl groups. The ¹³C NMR spectrum displayed a total of 20 carbon signals, including 15 resonances accounting for the aforementioned structural units and additional signals for three oxygenated non-hydrogenated sp³ carbons (δ_C 88.0, 85.0, and 60.0), a carbonyl (δ_C 168.6), and a non-hydrogenated sp² carbon (δ_C 145.4). The NMR data of **10** and the co-occurring known analogue 9-acetoxy-5,8:12,13-diepoxycembr-15(17)-en-16,4-olide (**12**)³³ showed high similarity, with the major differences that the C-9 position in **12** was hydroxylated in **10** (OH-9, δ_H 4.49) and the acetate group at C-7 in **12** was hydrolyzed in **10** (OH-7, δ_H 4.79), which were supported by the COSY spin systems OH-7/H-7 (δ_H 3.37)/H₂-6 (δ_H 1.59 and 1.42)/H₂-5 (δ_H 1.95 and 1.04)

Table 2. ¹H and ¹³C NMR Spectroscopic Data (δ in ppm, J in Hz) of 7–11 in DMSO-*d*₆

No.	7		8		9		10		11	
	δ _C type ^a	δ _H (J in Hz) ^b	δ _C type ^a	δ _H (J in Hz) ^c	δ _C type ^a	δ _H (J in Hz) ^b	δ _C type ^a	δ _H (J in Hz) ^b	δ _C type ^a	δ _H (J in Hz) ^b
1	31.5, CH	2.54, m	32.5, CH	3.17, m	32.8, CH	3.08, m	35.3, CH		35.5, CH	2.99, m
2	37.5, CH ₂	1.64, m	38.6, CH ₂	1.35, t (12.4), Ha 1.93, m, Hb	38.6, CH ₂	1.34, m, Ha 1.93, m, Ha	32.8, CH ₂	2.98, m 1.19, m, Ha	32.8, CH ₂	1.24, m, Ha 2.09, m, Hb
3	71.5, CH	3.41, m	70.7, CH	3.14, m	70.6, CH	3.13, m	60.1, CH	1.98, m, Hb	59.2, CH	3.18, dd (10.4, 3.8)
4	86.0, C		86.0, C		85.9, C		60.0, C	3.03, m	59.7, C	
5	37.4, CH ₂	1.56, m, Ha, 1.95, ddd (12.8, 9.4, 3.4), Hb	37.1, CH ₂	1.52, m, Ha 2.11, m, Hb	37.3, CH ₂	1.55, m, Ha 2.14, m, Hb	34.8, CH ₂	1.04, m, Ha 1.95, m, Hb	33.7, CH ₂	0.83, m, Ha 1.94, m, Hb
6	26.7, CH ₂	1.65, m, Ha 1.82, m, Hb	27.8, CH ₂	1.55, m, Ha 1.80, m, Hb	27.1, CH ₂	1.57, m, Ha 1.82, m, Hb	29.0, CH ₂	1.42, m, Ha 1.59, dd (14.6, 10.6), Hb	26.9, CH ₂	1.66, m, Ha 1.75, m, Hb
7	83.4, CH	3.85, dd (9.4, 5.0)	87.7, CH	3.79, dd (10.8, 7.4)	88.4, CH	3.74, dd (10.8, 3.4)	77.2, CH	3.37 m	76.5, CH	5.03, d (9.0)
8	71.4, C		73.8, C		56.2, C		85.0, C		82.7, C	
9	65.6, CH	3.44, d (4.2)	150.7, CH	6.84, d (15.2)	151.9, CH	6.87, d (15.2)	75.4, CH	4.03, td (8.4, 4.0)	75.3, CH	4.85, t (8.8)
10	55.2, CH	4.10, d (4.2)	126.7, CH	7.20, d (15.2)	126.8, CH	7.16, d (15.2)	35.1, CH ₂	1.89, m, Ha 2.07, m, Hb	32.1, CH ₂	2.06, m, Ha 2.30, m, Hb
11	208.7, C		202.4, C		202.3, C		79.0, CH	4.15, dd (9.4, 5.2)	79.7, CH	4.34, dd (9.6, 5.4)
12	89.4, C		90.3, C		90.4, C		88.0, C		87.4, C	
13	32.0, CH ₂	2.14, m, Ha 2.75, dd (15.6, 6.2), Hb	34.8, CH ₂	1.88, m, Ha 2.09, m, Hb	34.7, CH ₂	1.89, m, Ha 2.09, m, Hb	29.2, CH ₂	1.70, m, Ha 2.13, dd (16.4, 5.0), Hb	29.0, CH ₂	1.78, m, Ha 2.16, dd (16.6, 5.6), Hb
14	30.0, CH ₂	1.14, td (12.8, 6.4), Ha 2.24, m, Hb	29.7, CH ₂	1.04, m, Ha 1.80, m, Hb	29.6, CH ₂	1.04, m, Ha 1.84, m, Hb	32.4, CH ₂	1.18, m, Ha 2.35, m, Hb	32.4, CH ₂	1.34 m, Ha 2.35, m, Hb
15	144.3, C		145.6, C		145.3, C		145.4, C		145.0, C	
16	167.0, C		167.8, C		167.8, C		168.6, C		168.4, C	
17	124.8, CH ₂	5.56, s, Ha 6.08, s, Hb	123.9, CH ₂	5.46, s, Ha 6.09, s, Hb	124.0, CH ₂	5.47, s, Ha 6.09, s, Hb	123.0, CH ₂	5.45, s, Ha 6.04, s, Hb	123.6, CH ₂	5.51, s, Ha 6.09, s, Hb
18	18.3, CH ₃	0.96, s	20.0, CH ₃	1.05, s	20.1, CH ₃	1.05, s	16.2, CH ₃	1.09, s	15.9, CH ₃	1.12, s
19	25.2, CH ₃	1.23, s	24.5, CH ₃	1.14, s	24.1, CH ₃	1.08, s	11.0, CH ₃	0.97, s	12.5, CH ₃	1.19, s
20	27.8, CH ₃	1.56, s	28.0, CH ₃	1.43, s	28.1, CH ₃	1.43, s	28.9, CH ₃	1.11, s	28.5, CH ₃	1.13, s
1'									170.7, C	
2'									20.6, CH ₃	1.95, s
3'									169.5, C	
4'									20.5, CH ₃	2.04, s
3-OH		4.85, d (5.4)		4.76, br s						
7-OH								4.79, d (5.2)		
8-OH		5.50, s		5.17, s		8.38, br s				
9-OH								4.49, d (3.6)		

^aSpectrum recorded on 125 MHz. ^bSpectrum recorded on 600 MHz. ^cSpectrum recorded on 500 MHz.

and OH-9/H-9 (δ_H 4.03)/H₂-10 (δ_H 2.07 and 1.89)/H-11 (δ_H 4.15) and key HMBC correlations from H₃-19 (δ_H 0.97) to C-7 (δ_C 77.2), C-8 (δ_C 85.0), and C-9 (Figure 4). The relative configuration of **10** was assigned based on NOESY data analysis (Figure 4). Briefly, NOESY correlations of H-1 (δ_H 2.98)/H₃-18, H-5b (δ_H 1.95)/H₃-18, H-11/H-10a (δ_H 1.89), and H-11/H₃-19 suggested that H-1, H-5b, H-10a, H-11, CH₃-

18, and CH₃-19 shared the same α-orientation on the cembranoid skeleton. Meanwhile, NOESY correlations of H-3 (δ_H 3.03)/H-5a (δ_H 1.03), H-3/H-7 (δ_H 3.37), H-3/H-14b (δ_H 2.35), H-5a/H-7, H-7/H-9, H-7/H-14b (δ_H 2.35), H-9/H-10b (δ_H 2.07), H-10b/H-13a (δ_H 1.70), and H-10b/H₃-20 (δ_H 1.11) indicated that H-3, H-5a, H-7, H-9, H-10b, and CH₃-20 were located on the opposite side of the macrocyclic

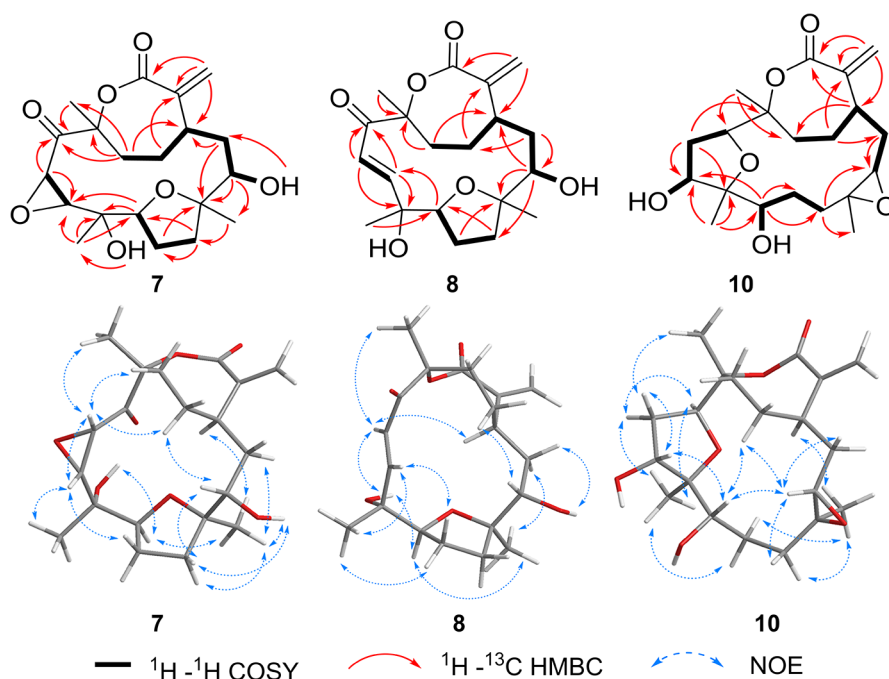


Figure 4. Key COSY, HMBC, and NOESY correlations of 7, 8, and 10.

skeleton as β -orientated, as well as the orientation of the ϵ -lactone ring as depicted in Figure 4. Thus, the relative configuration of compound 10 was deduced to be $1R^*,3S^*,4S^*,7S^*,8S^*,9R^*,11S^*,12R^*$. Comparison of the experimental ECD spectrum of 10 with the calculated one of $(1R,3S,4S,7S,8S,9R,11S,12R)$ -10 with a good match (Figure 3) confirmed the absolute configuration of 10.

Sinulariolone J (11) was isolated as a white amorphous powder. Its molecular formula was deduced as $C_{24}H_{34}O_8$ based on the HRESIMS data. The NMR data (Table 2) of 11 were highly reminiscent of those of 10, except for the resonances for two additional acetoxy groups ($CO-1'$, δ_C 170.7; CH_3-2' , δ_H 1.95, δ_C 20.6 and $CO-3'$, δ_C 169.5; CH_3-4' , δ_H 2.04, δ_C 20.5) in 11. Key HMBC correlations from H-7 (δ_H 5.03) to C-1' and from H-9 (δ_H 4.85) to C-3' placed the acetoxy groups to C-7 and C-9, respectively (Figure S4). Compound 11 was assigned the same relative and absolute configurations as 10 based on their highly similar NOESY and ECD spectral features (Figures S3 and S4). Thus, compound 11 was elucidated as (+)-(1*R*,3*S*,4*S*,7*S*,8*S*,9*R*,11*S*,12*R*)-sinulariolone J.

Four previously reported cembrane diterpenoids, sinulariolone (5),²⁷ sinulariolone acetate (6),²⁷ 9-acetoxy-5,8:12,13-diepoxycebr-15(17)-en-16,4-olide (12),³³ and sandensolide (13),³⁴ were coisolated from the same extract, and the structures were confirmed by comparison of their spectroscopic data (Table S9, Figure S5) with those reported in the literature.

Following bioassay-guided isolation of compounds 1–13, the dose–response bioactivity of four new metabolites, 2, 3, 8, and 9, was assessed against both DPM cell lines, MB24 and MB52, as well as NP1, the normal pleura cell line (Table 3). Complete dose–response curves for compounds listed in Table 3 are included as figures in the Supporting Information (Figures S6–S9). The remaining compounds had LD_{50} values of $>50 \mu M$ and were therefore not reported.

The outcomes of this study have identified nine new cembrane diterpenoids, namely, sinulariolones B–J (1–4 and

Table 3. LD_{50} Values of Compounds 2, 3, 8, and 9 against DPM Cell Lines

Compound	MB24 (μM)	MB52 (μM)	NP1 (μM)
2	11.4	4.7	5.2
3	13.3	7.1	7.8
8	14.2	7.9	15.7
9	27.4	12.5	15.9

7–11), together with four previously reported analogues (5, 6, 12, and 13), some with interesting bioactivities against human cell line models of mesothelioma. In addition, we have introduced a mesothelioma-specific screening platform that can be used to potentially reveal additional new pharmacophores, which may be useful in the context of mesothelioma. DPM continues to be a globally increasing disease with no specific chemotherapeutic options³⁵ and the work presented here makes progress in potentially identifying new opportunities to treat this recalcitrant surface cancer with substances from natural product source organisms.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Rudolph research analytical AUTOPOL IV automatic polarimeter using a cell of 0.25 dm path length at 25 °C, and UV spectra were measured with an HP 8453 UV–vis spectrophotometer. ECD experiments were performed on a J-1500 CD spectrophotometer, and IR spectra were recorded with a Bruker ALPHA II FT-IR spectrometer. NMR spectra were obtained with: (a) a Bruker Avance III NMR spectrometer equipped with a 3 mm CP TCI probe operating at a frequency of 600 MHz for the 1H nucleus and 150 MHz for the ^{13}C nucleus with nonuniform sampling (NUS) set to 25% for 1H – 1H and 1H – ^{13}C detection experiments, except for the NOESY experiment with NUS set to 40%; (b) a Bruker Avance III HD spectrometer equipped with a 5 mm CPP TCI probe, operating at a frequency of 600 MHz for the 1H nucleus and 150 MHz for the ^{13}C nucleus with NUS set to 35% for 1H – ^{13}C detection experiments and 40% for 1H – 1H detection experiments; or (c) a Bruker Avance III HD spectrometer equipped with a 5 mm CPP TCI probe

operating at a frequency of 500 MHz for the ^1H nucleus and 125 MHz for the ^{13}C nucleus with NUS set to 32% for ^1H – ^1H and ^1H – ^{13}C detection experiments, except for the NOESY experiment with 40%, using in all cases the standard Bruker pulse sequences. Spectra were calibrated to residual solvent signals at δ_{H} 2.50 and δ_{C} 39.5 for DMSO- d_6 , δ_{H} 7.26 and δ_{C} 77.2 for CDCl_3 , and δ_{H} 1.94 and δ_{C} 1.3 for CD_3CN . HRESIMS data were acquired on a 6230 Accurate-Mass TOF LC/MS system (1260 Infinity II) equipped with a dual AJS ESI source and a 6545 Accurate-Mass Q-TOF LC/MS system (1260 Infinity II), whereas low-resolution mass spectra were recorded with an Agilent InfinityLab 1260 LC/MSD System. HPLC separations were performed on a Gilson HPLC system equipped with a 322 pump, a 172-diode array detector, and a GX-281 liquid handler. HPLC fractions were dried on a SP Genevac system. Widespore C4 silica (40 μm , 275 Å) material was purchased from JT Baker. All chemicals were purchased from Sigma-Aldrich and TCI Chemicals. All solvents required for isolation and analytical experiments were purchased from Sigma-Aldrich. Ultrapure water was produced by a Hydro purification system.

Collection, Extraction, and Isolation. The soft coral *Simularia* sp. was collected at a depth of 10 m in Vanuatu in December 2000 by Dr. Patrick L. Colin and the Coral Reef Research Foundation for the National Cancer Institute. The specimen was taxonomically identified by Dr. Phil Alderslade, and a voucher specimen (OCDN7777) was deposited at the Smithsonian Institution. The coral (wet weight 1.1 kg) was extracted in H_2O , followed by a MeOH/DCM overnight soak according to the National Cancer Institute's standard marine extraction procedure³⁶ to give the aqueous extract C21254 (208.2 g) and the organic solvent extract C21255 (8.8 g). Given the lower availability of the organic solvent extract, C21254 was used for the bioassay-guided isolation of the active principle.

A portion of the *Simularia* sp. aqueous extract C21254 (5.0 g) was reconstituted in 50 mL of ultrapure water and was kept at +4 °C for 2 h. The extract was then subjected to ethanol precipitation at 50% (v/v) final ethanol concentration, which proceeded overnight at +4 °C. Soluble components post-ethanol precipitation were separated from insoluble precipitant by centrifugation, and ethanol was removed from the sample supernatant under reduced pressure. The EtOH supernatant (2.8 g) was prefractionated on 40 g of widepore C₄ material (column dimensions: 70 × 35 cm) eluted with 100% water to yield 1.8 g of fraction 1, water/MeOH (9:1) to yield 69.7 mg of fraction 2, water/MeOH (8:2) to yield 117.4 mg of fraction 3, water/MeOH (7:3) to yield 223.4 mg of fraction 4, water/MeOH (6:4) to yield 278.2 mg of fraction 5, water/MeOH (4:6) to yield 199.6 mg of fraction 6, and MeOH/MeCN (1:1) to yield 112.0 mg of fraction 7. Fractions 3–6 were found to be active in the DPM assay and further purified on a C₈ Kinetex column [100 Å, 5 μm , 150 × 21.2 mm (Phenomenex)] at a flow rate of 10 mL/min. Fractions were collected in 30 s increments. The gradient used for the combined fractions 3 and 4 was water/MeCN (98:2) [0.1% FA] to water/MeCN (30:70) [0.1% FA] over 30 min. Subfractions 28 and 29 were combined and further fractionated with the same column and flow rate and a gradient from water/MeCN (98:2) [0.1% FA] to water/MeCN (60:40) [0.1% FA] over 30 min, followed by fractionation on an Onyx Monolithic C₁₈ column [100 × 10 mm (Phenomenex)] at a flow rate of 4 mL/min eluting with a gradient from water/MeCN (95:5) [0.1% FA] to water/MeCN (90:10) [0.1% FA] over 50 min to give compound 10 (0.5 mg, 0.010% crude organic extract weight). Subfractions 30–34 were combined and further purified with preparative HPLC using the previously mentioned C₈ Kinetex column at a flow rate of 10 mL/min with a gradient from water/MeCN (98:2) [0.1% FA] to water/MeCN (60:40) [0.1% FA] over 90 min to give compounds 1 (33.5 mg, 0.670% crude organic extract weight) and 9 (2.3 mg, 0.046% crude organic extract weight). Subfractions 35–39 were combined and further purified with the same C₈ Kinetex column and flow rate and a gradient from water/MeCN (95:5) [0.1% FA] to water/MeCN (85:15) [0.1% FA] over 50 min to give compound 7 (3.3 mg, 0.066% crude organic extract weight). Subfractions 40–60 were combined and further fractionated with the same column and flow rate and a gradient from water/

MeCN (90:10) [0.1% FA] to water/MeCN (75:25) [0.1% FA] over 50 min, followed by fractionation on an Onyx Monolithic C₁₈ column [100 × 10 mm (Phenomenex)] at a flow rate of 4 mL/min eluting with a gradient from water/MeCN (95:5) [0.1% FA] to water/MeCN (85:15) [0.1% FA] over 80 min to give compound 4 (1.3 mg, 0.026% crude organic extract weight).

Fraction 5 from the initial C₄ fractionation was fractionated with identical chromatographic conditions as combined fractions 3 and 4, and its subfractions 43–47 were combined and further purified with the same column and flow rate and a gradient from water/MeCN (98:2) [0.1% FA] to water/MeCN (60:40) [0.1% FA] over 80 min, followed by fractionation with the same column and a gradient from water/MeCN (90:10) [0.1% FA] to water/MeCN (75:25) [0.1% FA] over 50 min, which resulted in the isolation of compounds 5 (39.8 mg, 0.796% crude organic extract weight) and 8 (2.6 mg, 0.052% crude organic extract weight). A fraction that was fractionated with the same Onyx Monolithic C₁₈ column and flow rate eluting with a gradient from water/MeCN (90:10) [0.1% FA] to water/MeCN (75:25) [0.1% FA] over 80 min to give compound 3 (1.5 mg, 0.030% crude organic extract weight), and a fraction that was subjected to fractionation with an Onyx Monolithic C₁₈ [100 × 10 mm] (Phenomenex) column at a flow rate of 4 mL/min eluting with a gradient from water/MeCN (95:5) [0.1% FA] to water/MeCN (85:15) [0.1% FA] over 80 min to give a fraction that was fractionated with the same C₈ Kinetex column and flow rate and a gradient from water/MeCN (85:15) [0.1% FA] to water/MeCN (70:30) [0.1% FA] over 80 min to give compound 6 (9.8 mg, 0.196% crude organic extract weight). Subfractions 48–60 were combined and further purified with the previously mentioned C₈ Kinetex column, a flow rate of 10 mL/min, and a gradient from water/MeCN (85:15) [0.1% FA] to water/MeCN (65:35) [0.1% FA] over 80 min to give compounds 11 (0.9 mg, 0.018% crude organic extract weight), and 12 (2.3 mg, 0.046% crude organic extract weight).

Fraction 6 from the initial C₄ fractionation was fractionated with the C₈ Kinetex column [100 Å, 5 μm , 150 × 21.2 mm (Phenomenex)] at a flow rate of 10 mL/min and a gradient from water/MeCN (98:2) [0.1% FA] to water/MeCN (2:98) [0.1% FA] over 30 min. Subfractions 6–16 were combined and purified with an X-Bridge Protein BEH C₄ OBD column [300 Å, 5 μm , 100 × 10.0 mm] (Waters) at a flow rate of 4 mL/min with a gradient from water/MeCN (98:2) [0.1% FA] to water/MeCN (70:30) [0.1% FA] over 90 min, followed by fractionation with the same C₈ Kinetex column and flow rate and a gradient from water/MeCN (90:10) [0.1% FA] to water/MeCN (80:20) [0.1% FA] over 90 min to give compound 2 (5.4 mg, 0.108% crude organic extract weight). Subfractions 44–47 were combined and further purified with an X-Bridge Protein BEH C₄ OBD column [300 Å, 5 μm , 100 × 10.0 mm] (Waters) at a flow rate of 4 mL/min with a gradient from water/MeCN (98:2) [0.1% FA] to water/MeCN (70:30) [0.1% FA] over 90 min to give compound 13 (7.1 mg, 0.142% crude organic extract weight).

Simulariolone B (1). White amorphous powder; $[\alpha]_{\text{D}}^{25} = -19.7$, $[\alpha]_{546} = -24.4$, $[\alpha]_{633} = -15.9$ (c 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (2.76) nm; ECD (864 μM , MeOH) λ ($\Delta\epsilon$) 220 (–1.3), 264 (+0.1), 302 (–0.04) nm; IR (film) 3394, 2975, 2939, 1710, 1629, 1451, 1376, 1250, 1123, 1037 cm^{-1} ; ^1H and ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 1; (+)-HRESIMS m/z [$\text{M} - \text{H}_2\text{O} + \text{H}$]⁺ 365.1965 (calculated for $\text{C}_{20}\text{H}_{29}\text{O}_6$ ⁺, 365.1964), m/z [$\text{M} + \text{Na}$]⁺ 405.1888 (calculated for $\text{C}_{20}\text{H}_{30}\text{O}_7\text{Na}^+$, 405.1889).

Preparation of MTPA Esters of Compound 1a. To a solution of 1 (1.0 mg) in dry DCM (400 μL) were successively added (R)- (–)-MTPA chloride (2.8 μL , 6.0 equiv), triethylamine (3.6 μL , 10.0 equiv), and DMAP (1 granule). The mixture was stirred overnight at room temperature. After stirring for 16 h, the reaction mixture was concentrated under a N_2 stream, and the residue was purified by reversed-phase HPLC (Onyx monolithic C₁₈ column, 10 mm × 100 mm; 3.8 mL/min) with a gradient from water/MeCN (98:2) [0.1% FA] to water/MeCN (2:98) [0.1% FA] over 40 min to give the (S)-MTPA ester of 1 (compound 1a, 0.8 mg, yield: 80.0%). The (R)-MTPA esters were prepared from (S)-(+)-MTPA chloride in a similar fashion (compound 1b, 0.7 mg, yield: 70.0%).

(S)-MTPA Ester of Compound 1 (1a). White amorphous powder; ^1H and ^{13}C NMR data, see Table S10; (+)-LRESIMS m/z $[\text{M} + \text{Na}]^+$ 603.3 (calculated for $\text{C}_{30}\text{H}_{35}\text{F}_3\text{O}_8\text{Na}^+$, 603.2).

(R)-MTPA Ester of Compound 1 (1b). White amorphous powder; ^1H and ^{13}C NMR data, see Table S10; (+)-LRESIMS m/z $[\text{M} + \text{Na}]^+$ 603.3 (calculated for $\text{C}_{30}\text{H}_{35}\text{F}_3\text{O}_8\text{Na}^+$, 603.2).

Sinulariolone C (2). White amorphous powder; $[\alpha]_{\text{D}}^{23} = -31.2$, $[\alpha]_{546} = -37.3$, $[\alpha]_{633} = -28.2$ (c 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (2.91) nm; ECD (700 μM , MeOH) λ ($\Delta\epsilon$) 217 (−0.4), 260 (+0.2), 304 (−0.1) nm; IR (film) 3410, 2977, 2936, 1713, 1629, 1452, 1373, 1251, 1229, 1086 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS m/z $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ 379.2130 (calculated for $\text{C}_{21}\text{H}_{31}\text{O}_6^+$, 379.2121), m/z $[\text{M} + \text{Na}]^+$ 419.2041 (calculated for $\text{C}_{21}\text{H}_{32}\text{O}_7\text{Na}^+$, 419.2046).

Sinulariolone D (3). White amorphous powder; $[\alpha]_{\text{D}}^{23} = -32.5$, $[\alpha]_{546} = -39.7$, $[\alpha]_{633} = -24.7$ (c 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (2.77) nm; ECD (500 μM , MeOH) λ ($\Delta\epsilon$) 218 (−0.6), 262 (+0.2), 303 (−0.1) nm; IR (film) 3464, 3978, 1714, 1628, 1451, 1373, 1250, 1082, 1055 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS m/z $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ 393.2279 (calculated for $\text{C}_{22}\text{H}_{33}\text{O}_6^+$, 393.2277), m/z $[\text{M} + \text{Na}]^+$ 433.22196 (calculated for $\text{C}_{22}\text{H}_{34}\text{O}_7\text{Na}^+$, 433.22192).

Sinulariolone E (4). White amorphous powder; $[\alpha]_{\text{D}}^{23} = -36.6$, $[\alpha]_{546} = -38.5$, $[\alpha]_{633} = -31.1$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (2.94) nm; ECD (491 μM , MeOH) λ ($\Delta\epsilon$) 212 (−0.5), 260 (+0.1), 300 (−0.1) nm; IR (film) 3355, 2934, 1710, 1657, 1547, 1451, 1373, 1255, 1136, 1056 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS m/z $[\text{M} + \text{H}]^+$ 408.2384 (calculated for $\text{C}_{22}\text{H}_{34}\text{NO}_6^+$, 408.2386).

Sinulariolone F (7). White amorphous powder; $[\alpha]_{\text{D}}^{23} = +26.9$, $[\alpha]_{546} = +33.8$, $[\alpha]_{633} = +22.1$ (c 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (2.95) nm; ECD (870 μM , MeOH) λ ($\Delta\epsilon$) 210 (−0.2), 232 (+0.2), 303 (+0.1) nm; IR (film) 3407, 2921, 1703, 1601, 1372, 1231, 1141, 1012, 947, 918, 806, 759, 629 cm^{-1} ; ^1H and ^{13}C NMR data in DMSO- d_6 , see Table 2; ^1H NMR data in CD_3CN , see Table S8; (+)-HRESIMS m/z $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ 363.1801 (calculated for $\text{C}_{20}\text{H}_{27}\text{O}_6^+$, 363.1808), m/z $[\text{M} + \text{Na}]^+$ 403.1726 (calculated for $\text{C}_{20}\text{H}_{28}\text{O}_7\text{Na}^+$, 403.1733).

Sinulariolone G (8). White amorphous powder; $[\alpha]_{\text{D}}^{23} = +31.7$, $[\alpha]_{546} = +39.5$, $[\alpha]_{633} = +28.8$ (c 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (3.19) nm; ECD (700 μM , MeOH) λ ($\Delta\epsilon$) 227 (−0.4), 265 (+0.1), 333 (−0.1) nm; IR (film) 3400, 2935, 1686, 1626, 1372, 1233, 1138, 1096, 1054, 1005 cm^{-1} ; ^1H and ^{13}C NMR data in DMSO- d_6 , see Table 2; ^1H NMR data in CD_3CN , see Table S8; (+)-HRESIMS m/z $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ 347.1856 (calculated for $\text{C}_{20}\text{H}_{27}\text{O}_5^+$, 347.1858), m/z $[\text{M} + \text{Na}]^+$ 387.1776 (calculated for $\text{C}_{20}\text{H}_{28}\text{O}_6\text{Na}^+$, 387.1784).

Sinulariolone H (9). White amorphous powder; $[\alpha]_{\text{D}}^{23} = +16.4$, $[\alpha]_{546} = +26.1$, $[\alpha]_{633} = +12.3$ (c 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (2.79) nm; ECD (634 μM , MeOH) λ ($\Delta\epsilon$) 226 (−0.1), 268 (+0.1), 334 (−0.01) nm; IR (film) 3376, 2973, 2935, 1694, 1612, 1452, 1353, 1232, 1139, 1096, 1079, 1053, 1005 cm^{-1} ; ^1H and ^{13}C NMR data in DMSO- d_6 , see Table 2; ^1H NMR data in CD_3CN , see Table S8; (+)-HRESIMS m/z $[\text{M} + \text{H}]^+$ 364.2121 (calculated for $\text{C}_{20}\text{H}_{30}\text{NO}_5^+$, 364.2124).

Sinulariolone I (10). White amorphous powder; $[\alpha]_{\text{D}}^{23} = -2.5$, $[\alpha]_{546} = -5.7$, $[\alpha]_{633} = -5.7$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (2.72) nm; ECD (683 μM , MeOH) λ ($\Delta\epsilon$) 212 (−0.4), 267 (+0.1) nm; IR (film) 3408, 2921, 1693, 1615, 1457, 1353, 1240, 1100, 1069, 1051 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 2; (+)-HRESIMS m/z $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ 349.2003 (calculated for $\text{C}_{20}\text{H}_{29}\text{O}_5^+$, 349.2015), m/z $[\text{M} + \text{H}]^+$ 367.2114 (calculated for $\text{C}_{20}\text{H}_{31}\text{O}_6^+$, 367.2121).

Sinulariolone J (11). White amorphous powder; $[\alpha]_{\text{D}}^{23} = -23.6$, $[\alpha]_{546} = -4.4$, $[\alpha]_{633} = -26.7$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (3.03) nm; ECD (769 μM , MeOH) λ ($\Delta\epsilon$) 212 (−0.7), 266 (+0.2) nm; IR (film) 2979, 1742, 1716, 1457, 1373, 1238, 1136, 1101, 1054, 937 cm^{-1} ; ^1H and ^{13}C NMR data in DMSO- d_6 , see Table 2; ^1H NMR data in CD_3CN , see Table S8; (+)-HRESIMS m/z $[\text{M} +$

$\text{Na}]^+$ 473.2140 (calculated for $\text{C}_{24}\text{H}_{34}\text{O}_8\text{Na}^+$, 473.2151), $[\text{M} - \text{OCOCH}_3]^+$ 391.2115 (calculated for $\text{C}_{22}\text{H}_{31}\text{O}_6^+$, 391.2121).

Single-Crystal X-ray Structure Determination of Sinulariolone B (1). A suitable colorless crystal of sinulariolone B (1) was obtained by slow evaporation from a mixture of diethyl ether/petroleum ether/acetone 5:2:2 (v/v/v). Crystal data: $\text{C}_{26}\text{H}_{32}\text{O}_8$, crystallizes in the monoclinic space group $\text{P}2_1$ (systematic absences 0k0: $k = \text{odd}$) with $a = 8.99740(10)$ Å, $b = 11.36950(10)$ Å, $c = 10.33180(10)$ Å, $\alpha = 90^\circ$, $\beta = 108.5290(10)^\circ$, $\gamma = 90^\circ$, $V = 1002.114(18)$ Å³, $Z = 2$, and $d_{\text{calc}} = 1.327$ g/cm³. X-ray intensity data were collected on a Rigaku XtaLAB Synergy-S diffractometer (CrysAlisPro 1.171.43.142a: Rigaku Oxford Diffraction, Rigaku Corporation, Oxford, UK) equipped with an HPC area detector (HyPix-6000HE) and employing confocal multilayer optic-monochromated Cu-K α radiation ($\lambda = 1.54184$ Å) at a temperature of 100 K. Preliminary indexing was performed from a series of 60 0.5° rotation frames with exposures of 0.5 s for $\theta = \pm 47.664^\circ$ and 2 s for $\theta = 113.250^\circ$. A total of 8292 frames (129 runs) were collected employing ω scans with a crystal-to-detector distance of 34.0 mm, rotation widths of 0.5°, and exposures of 1 s.

Rotation frames were integrated using CrysAlisPro, producing a list of unaveraged F^2 and $\sigma(F^2)$ values. A total of 28,990 reflections were measured over the ranges $9.028 \leq 2\theta \leq 148.946^\circ$, $-11 \leq h \leq 11$, $-14 \leq k \leq 14$, and $-12 \leq l \leq 12$, yielding 4062 unique reflections ($R_{\text{int}} = 0.0592$). The intensity data were corrected for Lorentz and polarization effects and for absorption using SCALE3 ABSPACK (SCALE3 ABSPACK v1.0.7: an Oxford Diffraction program; Oxford Diffraction Ltd.: Abingdon, UK, 2005, minimum and maximum transmission 0.54704 and 1.00000, respectively). The structure was solved by dual space methods using SHELXT (v2018/2).³⁷ Refinement was by full-matrix least-squares based on F^2 using SHELXL (v2019/3).³⁷ All reflections were used during refinement. The weighting scheme used was $w = 1/[\sigma^2(F_o^2) + (0.0560P)^2 + 0.2271P]$, where $P = (F_o^2 + 2F_c^2)/3$. Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were refined by using a riding model. Refinement converged to $R1 = 0.0330$ and $wR2 = 0.0883$ for 3984 observed reflections for which $F > 4\sigma(F)$ and to $R1 = 0.0339$, $wR2 = 0.0890$, and $\text{GOF} = 1.037$ for all 4062 unique, nonzero reflections and 262 variables. The maximum Δ/σ in the final cycle of least-squares was 0.000, and the two most prominent peaks in the final difference Fourier were +0.17 and −0.28 e/Å³. The final report was created with Olex2,³⁸ compiled on 2022.04.07 svn.rca3783a0 for OlexSys. The crystallographic data for 1 have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 2448109), and copies can be obtained free of charge from <https://www.ccdc.cam.ac.uk/structures/>.

Computational Methods. 3D structures of the molecules were drawn and subjected to conformational analysis using MMFF94 as a force field and the GMMX methodology. Geometrical optimization and energy calculation of conformers occurring in an energy window ($\Delta\epsilon$) of 0–3 kcal/mol were done by implementation of B3LYP/6-31G(d,p) using the gas phase in Gaussian 16 software. The optimized structures (Tables S1–S6) were used to calculate the thermochemical parameters estimated at 298 K and 1 atm. Calculations considering the solvent (MeOH for ECD) were carried out starting from DFT-optimized structures. Optimized conformers were then subjected to DFT calculations on Gaussian 16 using wB97XD/6-311+g(d,p) for compound 1, wB97XD/6-311g(d,p) for compounds 4 and 11, mPW1PW91/6-311g(d,p) for compound 7, mPW1PW91/6-311+g(d,p) for compound 8, and B3LYP/6-311g(d,p) for compound 13, all in MeOH, to obtain the ECD spectra. The obtained values were Boltzmann averaged and compared with the experimental values. All quantum mechanical calculations were carried out using the Gaussian 16 software on a Linux operating system in the Biowulf cluster.³⁹ Obtained ECD spectra with a half-band of 0.3 eV and UV shifts of 2 nm for compound 10 and 8 nm for all of the other compounds were Boltzmann averaged and scaled using the SpecDis program spectra and compared with experimental spectra obtained in MeOH.

High-Throughput Screening Campaign. Two patient-derived cell lines, MB24 (sarcomatoid) and MB52 (epithelial), were obtained

from MesoBank, U.K. (N.P.),⁴⁰ and were maintained in M199 Media (Gibco) supplemented with 10% defined fetal bovine serum (Cytiva Hyclone), 1X GlutaMax (Gibco), 1X Penicillin/Streptomycin (Gibco). The NP1 cell line was maintained as previously published.⁴¹ For high-throughput screening, 1500 cells/well of each DPM cell line were independently plated into 384-well plates (SpectraPlate TC, Revvity) and allowed to adhere overnight. The following day, library substances from the NPND fractionated marine organic library were added to each well at a final concentration of 10 µg/mL and 0.4% DMSO. After a 72 h incubation with substances, viability was assessed using the previously published XTT assay⁴² using a PerkinElmer Envision plate reader (Revvity Inc., Waltham, MA). The XTT absorbance readings from each substance well were normalized to the plate based on background controls, media only as a low control, and untreated cells as a high control, according to the following formula:

$$\% \text{Viability} = 100 * \frac{(\text{Value}_{\text{Substance}} - \text{Value}_{\text{LowControl}})}{(\text{Value}_{\text{HighControl}} - \text{Value}_{\text{LowControl}})}$$

Assay actives, “hits”, were then identified using our previously published source fraction pool (SFP) methodology.⁴³ Those substances whose standard normalized % viability measures were ≤ −3 (three standard deviations below the mean for the SFP) in either cell line were prioritized for further study.

Dose–Response LD₅₀ Determinations. Compounds 1–13 were identified through bioassay-guided fractionation using DPM cell lines. Compounds 2, 3, 8, and 9 were then used for LD₅₀ determinations in all three cell lines to evaluate the DPM-specific viability. Cells were plated at a density of 4000 cells/well in 96-well plates and allowed to adhere overnight. After adhering, cells were treated with compounds 2, 3, 8, and 9 at 12 concentrations, ranging from 200 to 2 µM. After a 72 h incubation with the four compounds, viability was assessed using cell-titer glo (CTG, Promega, Madison, WI) on a PerkinElmer Envision plate reader (Revvity Inc., Waltham, MA). Nonlinear regression curve-fitting algorithms from GraphPad Prism software were used to determine the LD₅₀ values.

■ ASSOCIATED CONTENT

Data Availability Statement

The NMR data for the following compounds have been deposited in the Natural Products Magnetic Resonance Database (NP-MRD; www.np-mrd.org) and can be found at NP0350992 (sinulariolone B), NP0350993 (sinulariolone C), NP0350994 (sinulariolone D), NP0350995 (sinulariolone E), NP0350996 (sinulariolone F), NP0350997 (sinulariolone G), NP0351000 (sinulariolone H), NP0351004 (sinulariolone I), NP0351013 (sinulariolone J), NP0351014 ((S)-MTPA ester of sinulariolone B) and NP0351015 ((R)-MTPA ester of sinulariolone B).

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jnatprod.5c00786>.

NMR, HRESIMS, ECD, and IR spectra of 1–4 and 7–11; physical, spectroscopic and spectrometric data of the co-occurring known membrane diterpenoids 5, 6, 12, and 13 as well as molecular modeling data of 1–4, 7, 8, 10, and 13; and LD₅₀ viability curves for compounds 2, 3, 8, and 9 (PDF)

Accession Codes

Deposition Number 2448109 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via the joint Cambridge Crystallographic Data Centre (CCDC) and Fachinformationszentrum Karlsruhe Access Structures service.

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Notes

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