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Lendenfeldaranes W–Y, new 24-homoscalaranes from a marine sponge *Lendenfeldia* species

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Three new scalarane-type sesterterpenoids, lendenfeldaranes W–Y (1–3), along with a known analogue, lendenfeldarane D (4), were isolated from a marine sponge identified as *Lendenfeldia* species. The structures of all isolates were determined based on spectroscopic methods. Scalarane 1 exhibited significant activity in enhancing alkaline phosphatase (ALP) activity.

1 Introduction

Sponges of the genus *Lendenfeldia* (phylum *Porifera*, class *Demospongiae*, subclass *Keratos*, order *Dictyoceratida*, family *Thorectidae*, subfamily *Phyllospongiinae*) are broadly distributed across shallow coral reefs in the Asia-Pacific region. These marine invertebrates have attracted significant scientific attention owing to their rich repertoire of secondary metabolites, many of which display noteworthy pharmacological potential. Among these, sesterterpenoids—particularly 26-carbon homoscalarane and 24-homoscalarane derivatives, represent the dominant chemical constituents of *Lendenfeldia* species. A variety of biological activities have been reported for these compounds, including anti-inflammatory,^{1–4} cytotoxic,^{5–11} antimicrobial,^{12–14} and anti-osteoporotic effects,¹⁵ understanding their promise as valuable leads in drug discovery and biomedical research.

In our previous work, we reported the isolation of a series of scalarane-type sesterterpenoids from a *Lendenfeldia* sponge collected in the coastal waters of Taiwan, together with an evaluation of their biological activities. Building on this research, we have now isolated three new 24-homoscalaranes, designated lendenfeldaranes W–Y (1–3), along with a known analogue, lendenfeldarane D (4) (ref. 9) (Fig. 1). The structures of compounds 1–3 were established through detailed spectroscopic analyses. Furthermore, their anti-osteoporotic potential was assessed by examining their ability to enhance ALP activity in MG63 osteoblast-like cells.

2 Results and discussion

Lendenfeldarane W (1) was obtained as an amorphous solid. The molecular formula was determined to be C₂₈H₄₄O₆ from the (+)-HRESIMS ion at *m/z* 499.30284 [M + Na]⁺ (calcd for C₂₈H₄₄O₆ + Na, 499.30301), corresponding to seven degrees of

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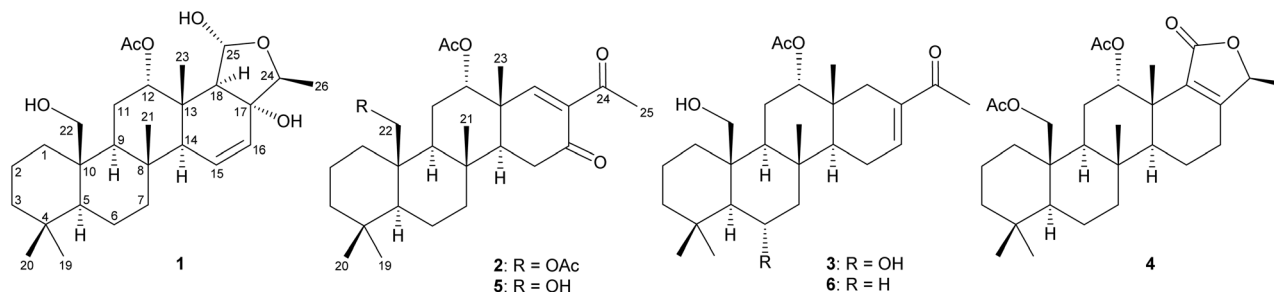


Fig. 1 Structures of lendenfeldaranes W–Y (1–3), lendenfeldarane D (4), felixin B (5), and felixin A (6).

Table 1 ^1H and ^{13}C NMR data for lendenfeldaranes W–Y (1–3)

Position	1 δ_{H}^a (J in Hz)	δ_{C}^b , Mult. ^c	2 δ_{H}^a (J in Hz)	δ_{C}^b , Mult. ^c	3 δ_{H}^a (J in Hz)	δ_{C}^b , Mult. ^c
1 α	0.59 dddd (13.2, 13.2, 3.6, 1.2)	34.0, CH ₂	0.55 dddd (13.8, 13.8, 3.6, 1.2)	34.6, CH ₂	0.53 dddd (13.2, 13.2, 3.6, 1.2)	34.5, CH ₂
β	2.10 br d (13.2)		1.99 m		2.13 m	
2 α	1.47 dddd (18.0, 3.6, 3.6, 3.6, 3.6)	18.3, CH ₂	1.44 m ^d	18.2, CH ₂	1.45 m ^d	18.4, CH ₂
β	1.56 m ^d		1.56 m		1.54 m	
3 α	1.44 m	41.7, CH ₂	1.46 m	41.4, CH ₂	1.44 br d (13.2) ^d	41.6, CH ₂
β	1.21 dd (13.2, 3.6)		1.14 m		1.19 dd (13.2, 4.8)	
4		33.0, C		33.0, C		32.9, C
5	1.01 dd (13.2, 2.4)	57.0, CH	1.02 dd (12.0, 1.8)	57.1, CH	1.00 dd (12.0, 1.8)	56.6, CH
6 α	1.56 m ^d	17.6, CH ₂	1.60 m	17.7, CH ₂	4.60 m	68.4, CH
β	1.41 ddd (13.2, 13.2, 3.6)		1.44 m ^d			
7 α	1.06 ddd (13.2, 12.6, 3.6)	41.5, CH ₂	1.11 ddd (13.2, 13.2, 4.2)	40.8, CH ₂	1.40 m ^d 2.18 m	44.3, CH ₂
β	1.97 ddd (12.6, 3.6, 3.6)		1.80 ddd (13.2, 3.6, 3.6)			
8		36.7, C		37.2, C		39.1, C
9	1.35 br d (12.6)	52.5, CH	1.40 dd (13.2, 4.2)	52.7, CH	1.40 br d (13.8) ^d	53.0, CH
10		41.7, C		40.1, C		— ^e
11 α	1.88 ddd (15.0, 3.0, 2.4)	25.1, CH ₂	2.07 m	24.3, CH ₂	1.98 m	25.0, CH ₂
β	2.26 ddd (15.0, 12.6, 3.0)				2.17 m	
12	4.96 dd (3.0, 3.0)	74.1, CH	5.00 dd (3.0, 2.4)	76.0, CH	4.63 dd (3.0, 1.8)	77.6, CH
13		42.1, C		41.2, C		39.5, C
14	2.14 dd (3.0, 2.4)	52.0, CH	2.12 dd (14.4, 4.2)	48.8, CH	1.57 m	56.1, CH
15	5.98 dd (10.2, 2.4)	132.2, CH	2.55 dd (17.4, 4.2)-H α 2.43 dd (17.4, 14.4)-H β	34.9, CH ₂	2.26 m 2.34 m ^d	— ^e
16	5.71 dd (10.2, 3.0)	127.4, CH		197.4, C	6.64 dd (3.0, 3.0)	139.6, CH
17		80.2, C		136.7, C		— ^e
18	2.47 s	62.8, CH	7.31 s	163.5, CH	1.93 d (17.4)-H α 2.27 br d (17.4)-H β	35.2, CH ₂
19	0.89 s	33.9, CH ₃	0.89 s	33.7, CH ₃	0.88 s	33.8, CH ₃
20	0.78 s	21.9, CH ₃	0.84 s	21.8, CH ₃	0.78 s	21.9, CH ₃
21	1.08 s	17.3, CH ₃	1.01 s	15.8, CH ₃	1.24 s	16.9, CH ₃
22a	4.04 d (12.0)	62.6, CH ₂	4.63 d (12.0)	64.6, CH ₂	4.05 d (11.4)	62.9, CH ₂
b	3.88 d (12.0)		4.12 d (12.0)		3.92 dd (11.4, 1.2)	
23	0.84 s	16.6, CH ₃	1.17 s	18.6, CH ₃	0.92 s	20.8, CH ₃
24	4.41 q (6.6)	85.1, CH		197.8, C		199.4, C
25	5.28 d (3.0)	97.8, CH	2.44 s	30.7, CH ₃	2.34 s ^d	25.4, CH ₃
26	1.18 d (6.6)	15.7, CH ₃				
OAc-12	2.15 s	170.4, C 21.4, CH ₃	2.07 s	170.3, C 21.2, CH ₃	2.10 s	170.1, C 21.4, CH ₃
OAc-22			2.07 s	170.8, C 21.2, CH ₃		
OH-17	2.31 br s					
OH-25	2.73 br d (3.0)					

^a Spectra recorded at 600 MHz in CDCl₃. ^b Spectra recorded at 150 MHz in CDCl₃. ^c Multiplicity was deduced by ^{13}C , HSQC, and HMBC spectra. ^d Signals overlapped. ^e — signals were not observed.



unsaturation. The IR spectrum showed absorptions at 3419 and 1735 cm^{-1} , indicating the presence of hydroxy and ester carbonyl groups.

The ^1H NMR data for **1** (Table 1) displayed four tertiary methyl singlets at δ_{H} 0.78 (H_3 -20), 0.84 (H_3 -23), 0.89 (H_3 -19), and 1.08 (H_3 -21); one secondary methyl doublet at δ_{H} 1.18 (3H, d, J = 6.6 Hz, H_3 -26); two olefinic protons at δ_{H} 5.98 (1H, dd, J = 10.2, 2.4 Hz, H-15) and 5.71 (1H, dd, J = 10.2, 3.0 Hz, H-16); and three oxymethine protons at δ_{H} 5.28 (1H, d, J = 3.0 Hz, H-25), 4.96 (1H, dd, J = 3.0, 3.0 Hz, H-12), and 4.41 (1H, q, J = 6.6 Hz, H-24). In addition, an oxymethylene group was evident from the diastereotopic geminal protons at δ_{H} 4.04 (1H, d, J = 12.0 Hz, H-20a) and 3.88 (1H, d, J = 12.0 Hz, H-20b). The ^{13}C NMR and HSQC spectra revealed 28 carbon signals (Table 1), comprising one 1,2-disubstituted double bond (δ_{C} 132.2/CH-15; 127.4/CH-16), one acetal carbon (δ_{C} 97.8/CH-25), one oxygenated quaternary carbon (δ_{C} 80.2/C-17), two oxymethines (δ_{C} 85.1/CH-24; 74.1/CH-12), one oxymethylene (δ_{C} 62.6/CH₂-22), four tertiary methyls (δ_{C} 33.9/CH₃-19; 21.9/CH₃-20; 17.3/CH₃-21; 16.6/CH₃-23), one secondary methyl (δ_{C} 15.7/CH₃-26), six aliphatic methylenes (δ_{C} 41.7/CH₂-3; 41.5/CH₂-7; 34.0/CH₂-1; 25.1/CH₂-11; 18.3/CH₂-2; 17.6/CH₂-6), four aliphatic methines (δ_{C} 62.8/CH-18; 57.0/CH-5; 52.5/CH-9; 52.0/CH-14), four non-oxygenated quaternary carbons (δ_{C} 41.7/C-10; 36.7/C-8; 33.0/C-4; 42.1/C-13), and one acetoxy group (δ_{C} 21.4/acetate methyl; 170.4/acetate carbonyl).

Analysis of the NMR data indicated that two degrees of unsaturation were attributed to one acetoxy group and a 1,2-disubstituted olefin, while the remaining five degrees of unsaturation defined a pentacyclic homoscalarane skeleton. This inference was supported by the ^1H - ^1H COSY correlations of **1** (Fig. 2), which established six partial spin systems: H_2 -1/ H_2 -2/ H_2 -3, H_5 / H_2 -6/ H_2 -7, H_9 / H_2 -11/ H_2 -12, H_4 -14/ H_5 -15/ H_2 -16, H_8 -18/ H_2 -25, and H_3 -24/ H_3 -26. Key 2J - and 3J -HMBC correlations from protons to quaternary carbons, such as H_2 -3, H_5 -C-4; H_2 -7, H_9 , H_5 -C-8; H_5 , H_9 -C-10; H_5 , H_8 , H_2 -C-13; and H_5 , H_8 , H_2 -C-17, confirmed a 6/6/6/5 fused pentacyclic 24-homoscalarane framework.

The oxymethylene unit (δ_{C} 62.6) correlated with the methylene protons at δ_{H} 4.04 and 3.88 in the HSQC spectrum, and these protons showed 2J - and 3J -HMBC correlations to C-10 (δ_{C} 41.7), C-1 (δ_{C} 34.0) and C-9 (δ_{C} 52.5), indicating a hydroxymethyl

substituent at C-10 (Fig. 2). Further HMBC correlations, H_3 -19/C-3, C-4, C-5, C-20; H_3 -20/C-3, C-4, C-5, C-19; H_3 -21/C-7, C-8, C-9, C-14; H_3 -23/C-12, C-13, C-14, C-18; and H_3 -26/C-17, C-24, established the position of Me-19, Me-20, Me-21, Me-23, and Me-26 at C-4, C-4, C-8, C-13, and C-24, respectively.

An acetoxy substituent was placed at C-12, an oxymethine center, based on the chemical shifts of H-12 (δ_{H} 4.96, dd, J = 3.0, 3.0 Hz) and C-12 (δ_{C} 74.1), which closely matched those reported for felixin D (δ_{H} 4.91, dd, J = 3.2, 2.4 Hz; δ_{C} 74.6),⁶ a known 24-homoscalarane analogue possessing an identical functional group. Although no HMBC correlation was observed between H-12 and the acetate carbonyl, the substitution pattern was confirmed by comparison. The hydroxy group at C-25 was deduced from the COSY correlation between the hydroxy proton (δ_{H} 2.73, d, J = 3.0 Hz, OH-25) and the acetal proton at δ_{H} 5.28 (br d, J = 3.0 Hz, H-25). Formation of a cyclic ether linkage between C-24 and C-25 was evidenced by the HMBC correlation from H-25 (δ_{H} 5.28) to the oxymethine carbon at C-24 (δ_{C} 85.1). The chemical shift of C-25 (δ_{C} 97.8) was consistent with its assignment as an acetal carbon.

Of the six oxygen atoms in the molecular formula, five were accounted for by an acetal (including one hydroxy group), an additional hydroxy group, and an acetoxy substituent. The remaining oxygen atom was assigned as a hydroxy group attached to C-17, supported by the downfield chemical shift of the oxygenated quaternary carbon (δ_{C} 80.2).

The stereochemistry of **1** was determined by analysis of NOE correlations in the NOESY spectrum (Fig. 3). Following the established convention for scalarane-type sesterterpenoids, H-5 and the hydroxymethyl group at C-10 were assigned to the α - and β -faces, respectively, based on the absence of an NOE correlation between H-5 and H_2 -22.^{16,17} In the NOESY spectrum of **1**, H-9 showed correlations with H-5 and H-14, but not with H_3 -21 and H_2 -22, indicating that H-9 and H-14 reside on the α face, whereas Me-21 and the C-10 hydroxymethyl group are positioned on the β -face. Correlations of H_3 -23 with both H_3 -21 and H-12 established the β -orientations of Me-23 and H-12. H-18 correlated with H-14, but not with H_3 -23, placing H-18 on the α -face, while H-25 correlated with H-12 and H_3 -23, supporting its β -orientation. Additionally, a correlation between H-

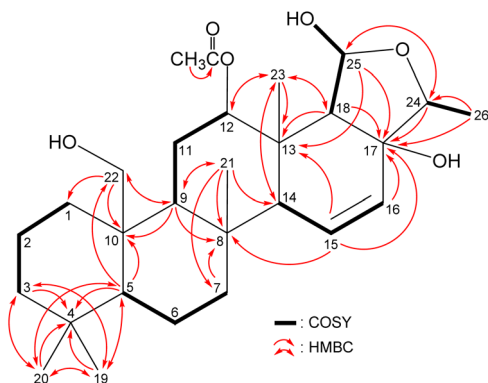


Fig. 2 Key COSY and HMBC correlations of **1**.

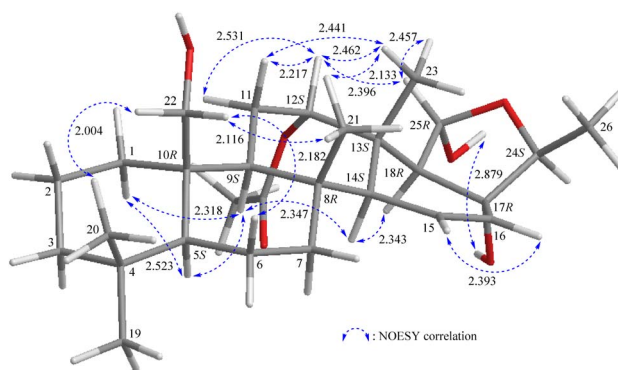


Fig. 3 Stereo-view of **1** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

15 and H-16 confirmed the *Z*-geometry of the C-15/16 double bond. The hydroxy proton at C-25 (OH-25) showed correlation with OH-17, indicating the α -orientation of the hydroxy group at C-17. Taken together, these data established the absolute configuration of **1** as 5*S*, 8*R*, 9*S*, 10*R*, 12*S*, 13*S*, 14*S*, 17*R*, 18*R*, 24*S*, 25*R*. Notably, compound **1** represents the first reported example of a 17-hydroxy scalarane derivative.

Lendenfeldarane X (**2**) was assigned a molecular formula of $C_{29}H_{42}O_6$ based on its (+)-HRESIMS ion at m/z 509.28714 (calcd for $C_{29}H_{42}O_6 + Na$, 509.28736), corresponding to nine degrees of unsaturation. Analysis of the 1H and ^{13}C NMR data (Table 1) indicated that **2** belongs to the 24-homoscalarane class, closely resembling the known analogue felixin B (**5**) (Fig. 1), originally isolated from the Formosan marine sponge *Ircinia felix*.⁶ The key structural difference between **2** and **5** lies in the substitution at C-10: in **5**, a hydroxymethyl group is present (δ_H 4.03, 1H, d, J = 11.6 Hz; 3.89, 1H, d, J = 11.6 Hz/ δ_C 63.0, CH_2 -22; δ_C 41.8, C-10),⁶ whereas in **2** this functionality is replaced by an acetoxymethyl group (δ_H 4.63, 1H, d, J = 12.0 Hz; 4.12, 1H, d, J = 12.0 Hz/ δ_C 64.6, CH_2 -22; δ_C 40.1, C-10). Detailed interpretation of the 2D NMR spectroscopic data of **2** corroborated this substitution, thereby establishing its planar structure (Fig. 4).

NOESY correlations of **2** established the configurations of the stereogenic centers in rings A–D, which were consistent with those of **1** and **5** (Fig. 5). The olefinic proton H-18 (δ_H 7.31) exhibited correlations with H-12 (δ_H 5.00) and H₃-23 (δ_H 1.17), but not with the acetyl methyl H₃-25 (δ_H 2.44), supporting an *s-cis* diene configuration for C-18/17/24. Based on these data, the stereogenic carbons were assigned as 5*S*, 8*R*, 9*S*, 10*R*, 12*S*, 13*S*, 14*S*. Thus, the structure of lendenfeldarane X (**2**) was established.

Lendenfeldarane Y (**3**) was obtained as an amorphous powder with the molecular formula $C_{27}H_{42}O_5$, established by (+)-HRESIMS at m/z 469.29239 (calcd for $C_{27}H_{42}O_5 + Na$, 469.29245), indicating seven degrees of unsaturation. IR absorptions at 3443, 1731, and 1664 cm^{-1} revealed hydroxy, ester carbonyl, and α,β -unsaturated ketone groups. NMR data of **3** closely resembled those of felixin A (**6**) (ref. 6) (Fig. 1), except for an additional oxymethine signal (δ_C 68.4/ δ_H 4.60, 1H, m, CH-6), consistent with a C-6 hydroxy substitution, further confirmed by 1H – 1H COSY crossrelations of H-5/H-6/H₂-7 (Fig. 6).

In the NOESY spectrum of **3** (Fig. 7), a correlation between H-6 (δ_H 4.60) and H₃-21 (δ_H 1.24) placed the C-6 hydroxy group on

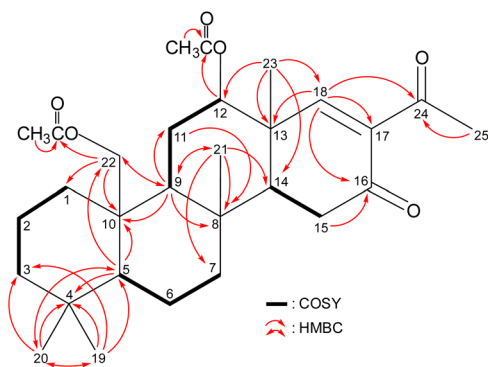


Fig. 4 Key COSY and HMBC correlations of **2**.

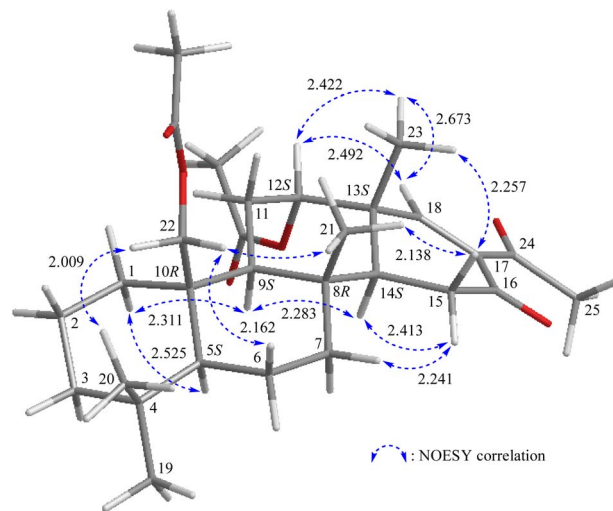


Fig. 5 Stereo-view of **2** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

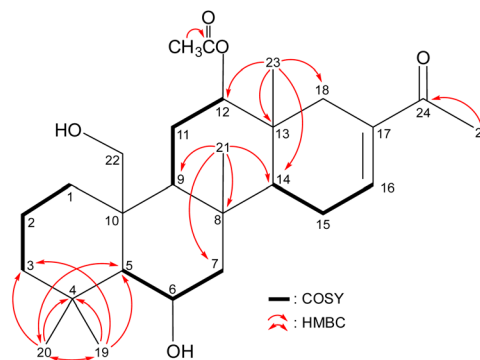


Fig. 6 Key COSY and HMBC correlations of **3**.

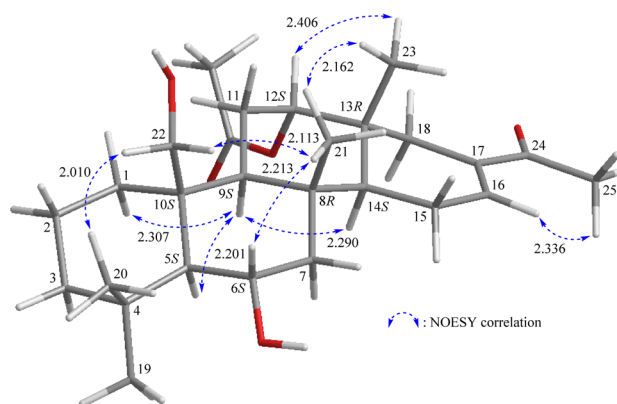


Fig. 7 Stereo-view of **3** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

the α -face. H-16 (δ_H 6.64) showed a correlation with H₃-25 (δ_H 2.34), consistent with an *s-trans* α,β -unsaturated ketone. The NOESY data of **3** were comparable to those of **2** and **6**, indicating



Table 2 The ALP activity was assessed after treating MG63 cells with homosclaranes **1**, **2**, and **4** and alendronate sodium (positive control) at concentration of 10 μM for 72 h^a

Compounds	ALP activity (king unit per mgprot)	MTT (% control)
1	20.04 \pm 3.67 ^b	159.90 \pm 2.28
2	3.81 \pm 1.91	30.02 \pm 1.75
4	−5.65 \pm 0.79	14.64 \pm 0.34
Alendronate sodium	21.45 \pm 5.21 ^b	95.14 \pm 12.24
Control	2.53 \pm 0.63	100.03 \pm 2.28

^a Data are expressed with the mean standard error of the mean (SEM) ($n = 3$). The significance was determined with Student's t -test. ^b $p < 0.01$ and comparison with untreated cells.

the same stereochemical framework, and the stereogenic centers of **3** were assigned as 5*S*, 6*S*, 8*R*, 9*S*, 10*S*, 12*S*, 13*R*, 14*S*.

Compounds **1**, **2**, and **4** were evaluated for osteogenic activity in MG63 cells at 10 μM (72 h). Compound **1** enhanced ALP activity (20.04 KU per mgprot) and cell viability (159.90%), whereas compound **2** reduced viability to 30.02%. Compound **4** was the most cytotoxic, lowering viability to 14.64% (Table 2).

3 Conclusions

Marine sponges of the genus *Lendenfeldia* are well known as rich sources of scalarane-type sesterterpenoids with diverse structures and notable biological activities.^{16,17} In this study, three new 24-homosclaranes, lendenfeldaranes W–Y (**1–3**), along with a known analogue, lendenfeldarane D (**4**),⁹ were isolated from *Lendenfeldia* sp. In MG63 osteoblast-like cells, compound **1** significantly enhanced ALP activity and cell viability, showing effects comparable to or exceeding those of alendronate sodium, whereas compound **4** was cytotoxic and suppressed osteogenic differentiation. These results underscore the osteogenic potential of scalarane derivatives and support further investigation of sponge-derived sesterterpenoids as candidates for bone regenerative agents.

4 Experimental

4.1 General experimental procedures

Optical rotations were measured on a JASCO P-1010 digital polarimeter using the sodium D line (589 nm) with a 10 mm cell. IR spectra were obtained with a Thermo Scientific Nicolet iS5 FT-IR spectrophotometer. NMR spectra were recorded on a 600 MHz Jeol ECZ NMR spectrometer using the residual CHCl_3 (δ_{H} 7.26 ppm) and CDCl_3 (δ_{C} 77.0 ppm) as internal standards for ^1H and ^{13}C NMR, respectively; coupling constants (J) are presented in Hertz (Hz). ESIMS and HRESIMS were acquired on a Thermo Fisher Orbitrap Exploris 120 (positive SI). Crude extracts were fractionated by silica gel CC (230–400 mesh, Merck). TLC was performed on silica gel 60F₂₅₄ (0.20 mm, Macherey-Nagel) and RP-18 F₂₅₄s (0.16–0.20 mm, Merck) plates, visualized under UV and with 10% H_2SO_4 /heat. Final purification used RP-HPLC (Luna C18(2), 5 μm , 100 Å, 250 \times 21.2 mm) on a Hitachi L-7110 pump with L-2400 PDA detector.

4.2 Animal material

A specimen of the genus *Lendenfeldia* was collected by SCUBA diving along the southern coast of Taiwan in April 2019. The material was preserved as a voucher (specimen no. 2019-04-SP) at the National Museum of Marine Biology & Aquarium (NMMBA), Taiwan. Species-level identification was confirmed by Professor Yusheng M. Huang (National Penghu University of Science and Technology).

4.3 Extraction and isolation

The freeze-dried sponge (wet/dry: 2900/213 g) was extracted with $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (1 : 1, v/v) at room temperature. The crude extract (33.7 g) was partitioned between EtOAc and water, and the EtOAc layer (7.93 g) was fractionated by silical gel CC (n -hexane \rightarrow n -hexane/EtOAc) to give 14 fractions (A–N). Fraction G was further purified by silica gel CC (n -hexane/acetone, 8 : 1 \rightarrow acetone) to yield subfractions G1–G15, and G10 was subjected to RP-HPLC (C18, $\text{MeOH}/\text{H}_2\text{O}$ 80 : 20, 5.0 mL min^{−1}) to afford **3** (0.7 mg, R_{t} = 20.9 min), **2** (1.6 mg, R_{t} = 39.1 min), **4** (1.0 mg, R_{t} = 47.4 min), and **1** (1.2 mg, R_{t} = 53.4 min), respectively.

4.3.1 Lendenfeldarane W (1). Amorphous powder; $[\alpha]_{\text{D}}^{25}$ −81 (c 0.09, CHCl_3); IR (KBr) ν_{max} 3419, 1735 cm^{−1}; ^1H (600 MHz, CDCl_3) and ^{13}C NMR (150 MHz, CDCl_3) data see Table 1; ESIMS: m/z 499 $[\text{M} + \text{Na}]^+$; HRESIMS: m/z 499.30284 (calcd for $\text{C}_{28}\text{H}_{44}\text{O}_6 + \text{Na}$, 499.30301).

4.3.2 Lendenfeldarane X (2). Amorphous powder; $[\alpha]_{\text{D}}^{25}$ 96 (c 0.08, CHCl_3); IR (KBr) ν_{max} 1738, 1682 cm^{−1}; ^1H (600 MHz, CDCl_3) and ^{13}C NMR (150 MHz, CDCl_3) data see Table 1; ESIMS: m/z 509 $[\text{M} + \text{Na}]^+$; HRESIMS: m/z 509.28714 (calcd for $\text{C}_{29}\text{H}_{42}\text{O}_6 + \text{Na}$, 509.28736).

4.3.3 Lendenfeldarane Y (3). Amorphous powder; $[\alpha]_{\text{D}}^{25}$ 162 (c 0.05, CHCl_3); IR (KBr) ν_{max} 3443, 1731, 1663 cm^{−1}; ^1H (600 MHz, CDCl_3) and ^{13}C NMR (150 MHz, CDCl_3) data see Table 1; ESIMS: m/z 469 $[\text{M} + \text{Na}]^+$; HRESIMS: m/z 469.29239 (calcd for $\text{C}_{27}\text{H}_{42}\text{O}_5 + \text{Na}$, 469.29245).

4.3.4 Lendenfeldarane D (4). Amorphous powder; $[\alpha]_{\text{D}}^{25}$ 135 (c 0.08, CHCl_3) (ref. 9 $[\alpha]_{\text{D}}^{25}$ 38 (c 0.05, CHCl_3)); IR (KBr) ν_{max} 1740, 1672 cm^{−1}; ESIMS: m/z 523 $[\text{M} + \text{Na}]^+$.

4.4 ALP activity assay and cell viability assays

The osteogenic activity of compounds **1**, **2**, and **4** was evaluated in MG63 human osteoblast-like cells obtained from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan; BCRC 60279). ALP activity was measured following treatment with test compounds according to establish protocols with minor modifications.¹⁸ Cell viability was assessed by MTT assay: MG63 cells (1×10^3 per well) were seeded in 96-well plates, incubated 24 h, and treated with alendronate (0.01 μM) or compounds (10 μM) for 72 h. MTT solution (10 μL , 5 mg mL^{−1}) and medium (90 μL) were added for 4 h, and formazan crystals were dissolved in 100 μL DMSO. Absorbance at 570 nm was measured as an indicator of viability.¹⁹



Author contributions

C.-Y. Huang, B.-R. Peng, Y.-W. Liu, Y.-Y. Chen, J.-H. Su, C.-C. Liaw, J.-J. Chen, C.-C. Tseng, Y.-J. Wu, Y.-B. Cheng, L. K. Tsou, M. M. Zhang, Z.-H. Wen: methodology, analysis, investigation, data curation, and draft preparation. L.-G. Zheng and P.-J. Sung: conceptualization, resources, supervision, project administration, visualization, draft review & editing, and funding acquisition.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The datasets supporting this article have been uploaded as part of the supplementary information (SI). Supplementary information: HRESI-MS, 1D, and 2D-NMR spectra of 1–3. See DOI: <https://doi.org/10.1039/d5ra07083j>.

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