RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2025, **15**, 44877

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

Chih-Yin Huang,[ab] Bo-Rong Peng,[c] Yueh-Wen Liu,[d] You-Ying Chen,[e] Jui-Hsin Su,[efg] Chia-Ching Liaw,[hijkl] Jih-Jung Chen,[j] Chung-Chih Tseng,[bm] Yu-Jen Wu,[n] Yuan-Bin Cheng,[g] Lun Kelvin Tsou,[o] Mingzi M. Zhang,[p] Zhi-Hong Wen,[eg] Li-Guo Zheng *[eq] and Ping-Jyun Sung *[egirstu]

Received 18th September 2025
Accepted 10th November 2025

DOI: 10.1039/d5ra07083j

rsc.li/rsc-advances

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.

[a]*Department of Orthopedics, Kaohsiung Armed Forces General Hospital, Kaohsiung 802301, Taiwan*
[b]*Institute of Medical Science and Technology, National Sun Yat-sen University, Kaohsiung 804201, Taiwan*
[c]*Research Center for Chinese Herbal Medicine, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan 333324, Taiwan*
[d]*Department of Cosmetics and Fashion Styling, Chien Shiu University, Kaohsiung 833301, Taiwan*
[e]*National Museum of Marine Biology and Aquarium, Pingtung 944401, Taiwan. E-mail: t0919928409@gmail.com; pjsung@nmmba.gov.tw*
[f]*Graduate Institute of Marine Biology, National Dong Hwa University, Pingtung 944401, Taiwan*
[g]*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804201, Taiwan*
[h]*National Research Institute of Chinese Medicine, MOHW, Taipei 112304, Taiwan*
[i]*Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807378, Taiwan*
[j]*Department of Pharmacy, School of Pharmaceutical Sciences, National Yang Ming Chian Tung University, Taipei 112304, Taiwan*
[k]*Department of Biochemical Science and Technology, National Chiayi University, Chiayi University, Chiayi 600048, Taiwan*
[l]*School of Chinese Medicine, National Yang Ming Chian Tung University, Taipei 112304, Taiwan*
[m]*School of Dentistry, College of Oral Medicine, National Defence Medical University, Taipei 114201, Taiwan*
[n]*Yu Jun Biotechnology Co., Ltd, Donggang, Pingtung 928003, Taiwan*
[o]*Institute of Biotechnology and Pharmaceutical Research, National Health Research Institute, Miaoli 350401, Taiwan*
[p]*Institute of Molecular and Genomics Medicine, National Health Research Institute, Miaoli 350401, Taiwan*
[q]*Doctoral Degree Program in Marine Biotechnology, National Sun Yat-sen University, Kaohsiung 804201, Taiwan*
[r]*Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung 404394, Taiwan*
[s]*Program in Pharmaceutical Biotechnology, Fu Jen Catholic University, New Taipei City 242062, Taiwan*
[t]*Department of Biochemistry and Molecular Medicine, National Dong Hwa University, Hualien 974301, Taiwan*
[u]*School of Medicine, Kaohsiung Medical University, Kaohsiung 807378, Taiwan*

1 Introduction

Sponges of the genus *Lendenfeldia* (phylum *Porifera*, class *Demospongiae*, subclass *Keratosa*, 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 $C_{28}H_{44}O_6$ from the (+)-HRESIMS ion at *m*/*z* 499.30284 $[M + Na]^+$ (calcd for $C_{28}H_{44}O_6$ + Na, 499.30301), corresponding to seven degrees of

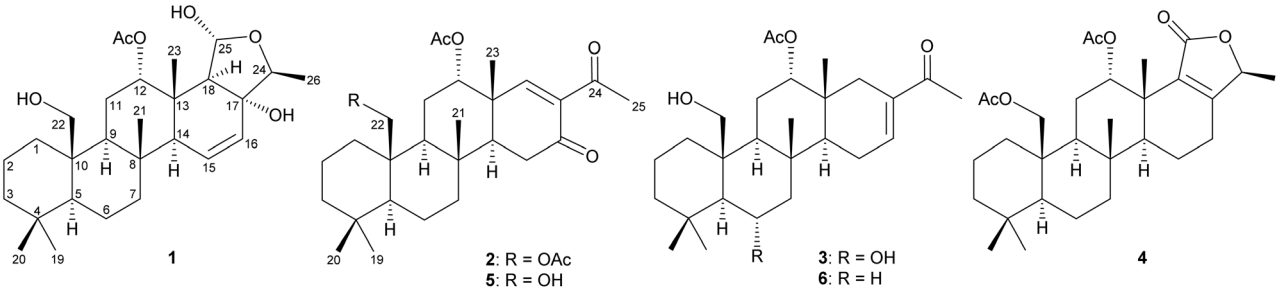

Fig. 1 Structures of lendenfeldaranes W–Y (**1–3**), lendenfeldarane D (**4**), felixin B (**5**), and felixin A (**6**).

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

	1		**2**		**3**	
Position	δ_H[a] (*J* in Hz)	δ_C[b], Mult.[c]	δ_H[a] (*J* in Hz)	δ_C[b], Mult.[c]	δ_H[a] (*J* in Hz)	δ_C[b], Mult.[c]
1α	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	
2α	1.47 ddddd (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	
3α	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
6α	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]			
7α	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]
11α	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)-Hα 2.43 dd (17.4, 14.4)-Hβ	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)-Hα 2.27 br d (17.4)-Hβ	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 $CDCl_3$. [b] Spectra recorded at 150 MHz in $CDCl_3$. [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/CH_2-22), four tertiary methyls (δ_C 33.9/CH_3-19; 21.9/CH_3-20; 17.3/CH_3-21; 16.6/CH_3-23), one secondary methyl (δ_C 15.7/CH_3-26), six aliphatic methylenes (δ_C 41.7/CH_2-3; 41.5/CH_2-7; 34.0/CH_2-1; 25.1/CH_2-11; 18.3/CH_2-2; 17.6/CH_2-6), 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-12, H-14/H-15/H-16, H-18/H-25, and H-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-15/C-8; H-5, H-9/C-10; H-15, H-18, H-25/C-13; and H-15, H-16, H-18, H-24, H-25, H_3-26/C-17, confirmed a 6/6/6/6/5 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-

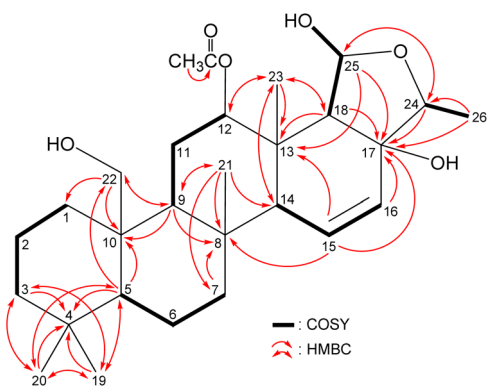

Fig. 2 Key COSY and HMBC correlations of **1**.

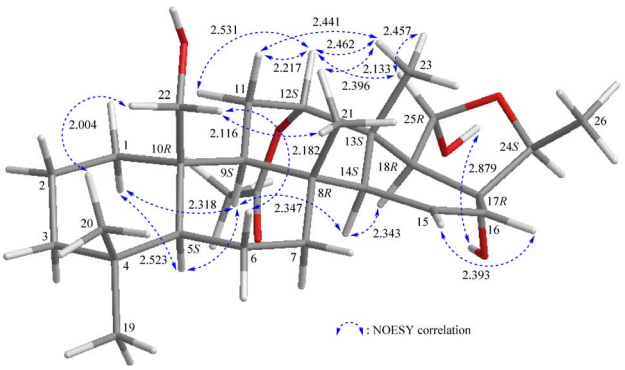

Fig. 3 Stereo-view of **1** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

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 ^{1}H 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 H_3-23 (δ_H 1.17), but not with the acetyl methyl H_3-25 (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 ^{1}H–^{1}H COSY crosslations of H-5/H-6/H_2-7 (Fig. 6).

In the NOESY spectrum of **3** (Fig. 7), a correlation between H-6 (δ_H 4.60) and H_3-21 (δ_H 1.24) placed the C-6 hydroxy group on the α-face. H-16 (δ_H 6.64) showed a correlation with H_3-25 (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

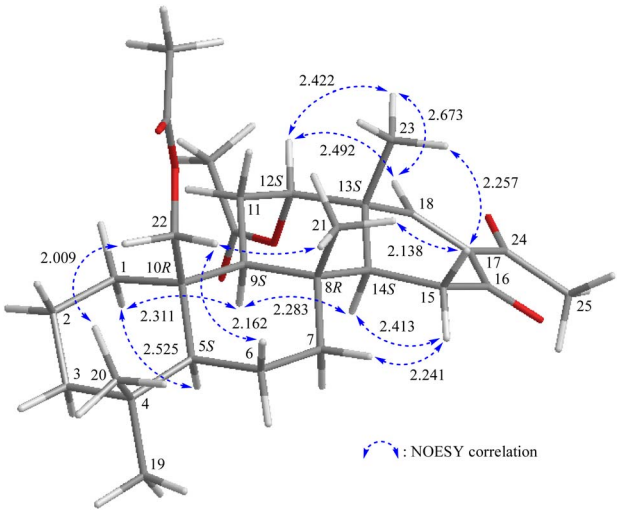

Fig. 5 Stereo-view of **2** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

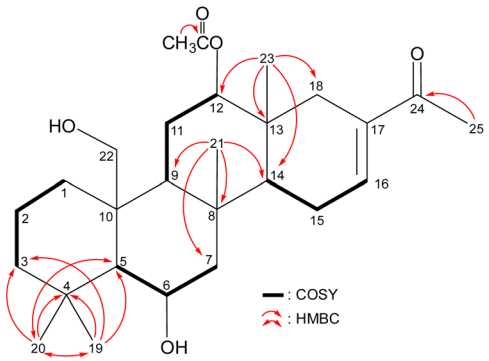

Fig. 6 Key COSY and HMBC correlations of **3**.

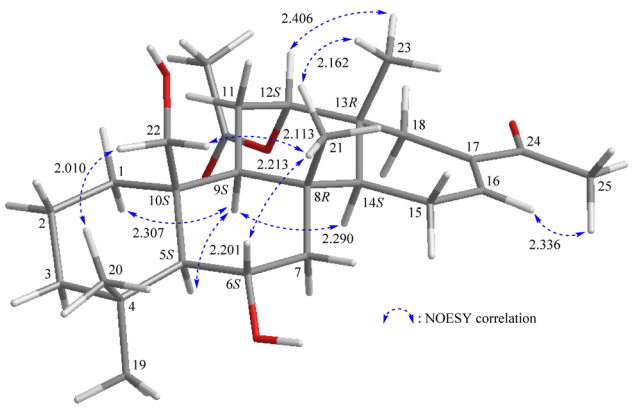

Fig. 7 Stereo-view of **3** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

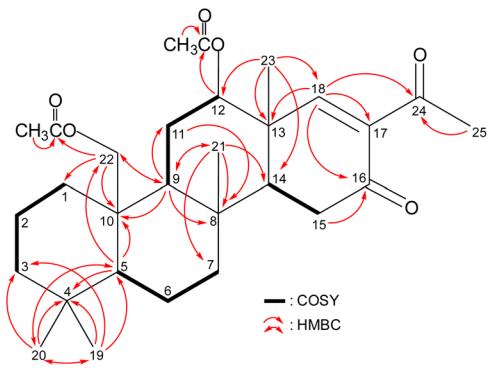

Fig. 4 Key COSY and HMBC correlations of **2**.

Table 2 The ALP activity was assessed after treating MG63 cells with homoscalaranes **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 ± 3.67[b]	159.90 ± 2.28
2	3.81 ± 1.91	30.02 ± 1.75
4	−5.65 ± 0.79	14.64 ± 0.34
Alendronate sodium	21.45 ±± 5.21[b]	95.14 ± 12.24
Control	2.53 ± 0.63	100.03 ± 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-homoscalaranes, 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 $60F_{254}$ (0.20 mm, Macherey-Nagel) and RP-18 F_{254}s (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 × 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 MeOH/CH_2Cl_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 → *n*-hexane/EtOAC) to give 14 fractions (A–N). Fraction G was further purified by silica gel CC (*n*-hexane/acetone, 8 : 1 → acetone) to yield subfractions G1–G15, and G10 was subjected to RP-HPLC (C18, MeOH/H_2O 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; [α] −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 $[M + Na]^+$; HRESIMS: *m*/*z* 499.30284 (calcd for $C_{28}H_{44}O_6$ + Na, 499.30301).

4.3.2 Lendenfeldarane X (2). Amorphous powder; [α] 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 $[M + Na]^+$; HRESIMS: *m*/*z* 509.28714 (calcd for $C_{29}H_{42}O_6$ + Na, 509.28736).

4.3.3 Lendenfeldarane Y (3). Amorphous powder; [α] 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 $[M + Na]^+$; HRESIMS: *m*/*z* 469.29239 (calcd for $C_{27}H_{42}O_5$ + Na, 469.29245).

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

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**.

Acknowledgements

We thank Ms. Hsiao-Ching Yu and Ms. Chao-Lien Ho (High Valued Instrument Center, National Sun Yat-sen University) for assistance with MS (MS 006500) and NMR (NMR 001100) data acquisition under NSTC 113-2740-M-110-002. This work was partially supported by the National Museum of Marine Biology & Aquarium and NSTC, Taiwan, (grants NSTC 112-2320-B-291-002-MY3 and 114-2320-B-291-001), awarded to P.-J. Sung.

Notes and references

1 B.-R. Peng, K.-H. Lai, Y.-C. Chang, Y.-Y. Chen, J.-H. Su, Y. M. Huang, P.-J. Chen, S. S.-F. Yu, C.-Y. Duh and P.-J. Sung, Sponge-derived 24-homoscalaranes as potent anti-inflammatory agents, *Mar. Drugs*, 2020, **18**, 434.

2 P. Francis and K. Chakraborty, Anti-inflammatory scalarane-type sesterterpenes, erectascalaranes A–B, from the marine sponge *Hyrtios erectus* attenuate pro-inflammatory cyclooxygenase-2 and 5-lipoxygenase, *Med. Chem. Res.*, 2021, **30**, 886–896.

3 B.-R. Peng, K.-H. Lai, G.-H. Lee, S. S.-F. Yu, C.-Y. Duh, J.-H. Su, L.-G. Zheng, T.-L. Hwang and P.-J. Sung, Scalarane-type sesterterpenoids from the marine sponge *Lendenfeldia* sp. alleviate inflammation in human neutrophils, *Mar. Drugs*, 2021, **19**, 561.

4 B.-R. Peng, L.-G. Zheng, L.-Y. Chen, M. El-Shazly, T.-L. Hwang, J.-H. Su, M.-H. Lee, K.-H. Lai and P.-J. Sung, Nor-24-homoscalaranes, neutrophilic inflammatory mediators from the marine sponge *Lendenfeldia* sp, *Pharmaceuticals*, 2023, **16**, 1258.

5 F. Cao, Z.-H. Wu, C.-L. Shao, S. Pang, X.-Y. Liang, N. J. de Voogd and C.-Y. Wang, Cytotoxic scalarane sesterterpenoids from the South China Sea sponge *Carteriospongia foliascens*, *Org. Biomol. Chem.*, 2015, **13**, 4016–4024.

6 Y.-Y. Lai, M.-C. Lu, L.-H. Wang, J.-J. Chen, L.-S. Fang, Y.-C. Wu and P.-J. Sung, New scalarane sesterterpenoids from the Formosan sponge *Ircinia felix*, *Mar. Drugs*, 2015, **13**, 4296–4309.

7 Y.-Y. Lai, L.-C. Chen, C.-F. Wu, M.-C. Lu, Z.-H. Wen, T.-Y. Wu, L.-S. Fang, L.-H. Wang, Y.-C. Wu and P.-J. Sung, New cytotoxic 24-homoscalarane sesterterpenoids from the sponge *Ircinia felix*, *Int. J. Mol. Sci.*, 2015, **16**, 21950–21958.

8 A. M. Alahdal, H. Z. Asfour, S. A. Ahmed, A. O. Noor, A. M. Al-Abd, M. A. Elfaky and S. S. Elhady, Anti-*Helicobacter*, antitubercular and cytotoxic activities of scalaranes from the Red Sea sponge *Hyrtios erectus*, *Molecules*, 2018, **23**, 978.

9 B.-R. Peng, K.-H. Lai, Y.-Y. Chen, J.-H. Su, Y. M. Huang, Y.-H. Chen, M.-C. Lu, S. S.-F. Yu, C.-Y. Duh and P.-J. Sung, Probing anti-proliferative 24-homoscalaranes from a sponge *Lendenfeldia* sp, *Mar. Drugs*, 2020, **18**, 76.

10 O.-S. Kwon, D. Kim, C.-K. Kim, J. Sun, C. J. Sim, D.-C. Oh, S. K. Lee, K.-B. Oh and J. Shin, Cytotoxic scalarane sesterterpenes from the sponge *Hyrtios erectus*, *Mar. Drugs*, 2020, **18**, 253.

11 L.-G. Zheng, Y.-Y. Chen, C.-C. Liaw, Y.-C. Lin, S.-Y. Chien, Y.-H. Lo, Y.-C. Tsai, H.-C. Hu, Y.-Y. Chen, T.-L. Hwang, T.-M. Ou and P.-J. Sung, Isolation of 24-homoscalarane sesterterpenoids from a sponge of the genus *Lendenfeldia*, *Phytochem. Lett.*, 2024, **61**, 177–181.

12 H. N. Kamel, Y. B. Kim, J. M. Rimoldi, F. R. Fronczek, D. Ferreira and M. Slattery, Scalarane sesterterpenoids: semisynthesis and biological activity, *J. Nat. Prod.*, 2009, **72**, 1492–1496.

13 C. Hertzer, S. Kehraus, N. Böhringer, F. Kaligis, R. Bara, D. Erpenbeck, G. Wörheide, T. F. Schäberle, H. Wägele and G. M. König, Antibacterial scalarane from *Doriprismatica stellata* nudibranchs (Gastropoda, Nudibranchia), egg ribbons, and their dietary sponge *Spongia* cf. *agaricina* (Demospongiae, Dictyoceratida), *Beilstein J. Org. Chem.*, 2020, **16**, 1596–1605.

14 A.-Y. Shin, H.-S. Lee and J. Lee, Isolation of scalimides A–L: β-alanine-bearing scalarane analogs from the marine sponge *Spongia* sp, *Mar. Drugs*, 2022, **20**, 726.

15 A. H. El-Desoky, H. Kato, I. Kagiyama, Y. Hitora, F. Losung, R. E. P. Mangindaan, N. J. de Voogd and S. Tsukamoto, Ceylonins A–F, spongian diterpene derivatives that inhibit RANKL-induced formation of multinuclear osteoclasts, from the marine sponge *Spongia ceylonensis*, *J. Nat. Prod.*, 2017, **80**, 90–95.

16 M. A. González, Scalarane sesterterpenoids, *Curr. Bioact. Compd.*, 2010, **6**, 178–206.

17 H.-B. Yu, H.-Y. Chen, S. Duan, Y.-P. Zhu, B. Hu, Y. He, S.-T. Cheng, B.-H. Jiao and X.-Y. Liu, Bioactive scalarane-type sesterterpenoids from marine sources, *Chem. Biodivers.*, 2022, **19**, e202200049.

18 Y. Wang, B. Kong, X. Chen, R. Liu, Y. Zhao, Z. Gu and Q. Jiang, BMSC exosome-enriched acellular fish scale scaffolds promote bone regeneration, *J. Nanobiotechnol.*, 2022, **20**, 444.

19 H.-M. Kuo, C.-C. Tseng, N.-F. Chen, M.-H. Tai, H.-C. Hung, C.-W. Feng, S.-Y. Cheng, S.-Y. Huang, Y.-H. Jean and Z.-H. Wen, MSP-4, an antimicrobial peptide, induces apoptosis via activation of extrinsic Fas/FasL- and intrinsic mitochondria-mediated pathways in one osteosarcoma cell line, *Mar. Drugs*, 2018, **16**, 8.