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Rhabdastrenones A–D from the sponge *Rhabdastrella globostellata*†

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Three new isomalabaricanes (**1–3**), a new α-pyrone derivative (**4**), together with four known isomalabaricane analogs rhabdastrellin G (**5**), isogeoditin A (**6**), stelliferin A (**7**), and (13*E*)-isogeoditin A (**8**) were isolated from the marine sponge *Rhabdastrella globostellata*. Their chemical structures were determined by HR-ESI-MS, 1D and 2D-NMR spectroscopic data analysis. The absolute configurations were identified by $Mo_2(OAc)_4$ induced ECD spectra and TD-DFT theoretical calculated ECD spectra. Compound **6** exhibited weak cytotoxic effects against HepG2 and SKMel2 cell lines with the IC_{50} values of 7.53 ± 0.70 and 9.93 ± 0.95 μM, respectively.

Introduction

Sponge natural products have been highlighted since the 1950s by the discovery of unusual nucleoside derivatives, which were successfully developed to be anti-leukemia drug, cytarabine. Studies on secondary metabolites from sponges were then speedily accelerated and hundreds of new compounds are being discovered annually in recent decades.[1,2] The sponge *Rhabdastrella globostellata* has been attracting a lot of attention for its production of a rare terpenoid group, called isomalabaricane analogs. These types of compounds display unique chemical structures with a *trans-syn-trans* 6/6/5 tricyclic nucleus and a polyunsaturated side chain.[3] Additionally, a series of isomalabaricanes exhibited extremely high cytotoxic activity against various cancer cell lines with IC_{50} values in the range of nanomolar concentrations.[4–7] Of these, rhabdastrellic acid A, stelletin A, and stelletin E have been accomplished through total syntheses, providing materials for studying biological mechanisms.[8,9] To date, over 70 isomalabaricane analogs have been isolated from *R. globostellata*.[6,7,10–18] It has been shown that chemical structures of isomalabaricanes mostly differ at the side chain by the oxidative degeneration, cyclization, and isomerization of double bonds.[3] In the present work, continuing investigation on chemical constituents of *R. globostellata*, we describe herein the isolation and determination of three new isomalabaricanes (**1–3**), a new α-pyrone derivative (**4**), together with four known isomalabaricane analogs (**5–8**) (Fig. 1). Cytotoxic activity of compounds (**1–8**) was screened on HepG2, LU1, SKMel2, and MCF7 human cancerous cells using the sulforhodamine B colorimetric assay.

Experimental

General experimental procedures

ECD spectra were acquired on an Applied Photophysics Chirascan spectrometer. Optical rotations were taken on a Jasco P2000 polarimeter. NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer. HR-ESI-MS were acquired on an Agilent 6530 Accurate-Mass QTOF LC/MS system. Semi-preparative HPLC was acquired on an Agilent 1260 Infinity II system (binary pump, autosampler, and DAD detector) using a YMC J'sphere ODS-H80 (20 × 250 mm, 4 μm) HPLC column and an isocratic mobile phase with a flow rate of 3 mL min^{-1}. Column chromatography was performed using silica gel (40–63 μm) or ODS (150 μm) as adsorbents. Thin-layer chromatography (TLC) was carried out on pre-coated plates (silica gel 60 F_{254} or RP-18 F_{254S}).

Sponge material

Sponge samples were collected at the Van Phong Bay, Nha Trang, Vietnam in May 2020 and taxonomically identified to be *Rhabdastrella globostellata* (Carter, 1883) by Dr Tran My Linh at the Institute of Marine Biochemistry, VAST. The voucher specimen (no. NCCT-B139) was kept at the Institute of Marine Biochemistry, VAST.

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† Electronic supplementary information (ESI) available: HR-ESI-MS, NMR, and ECD spectra of new compounds would be found. See DOI: 10.1039/d2ra01674e

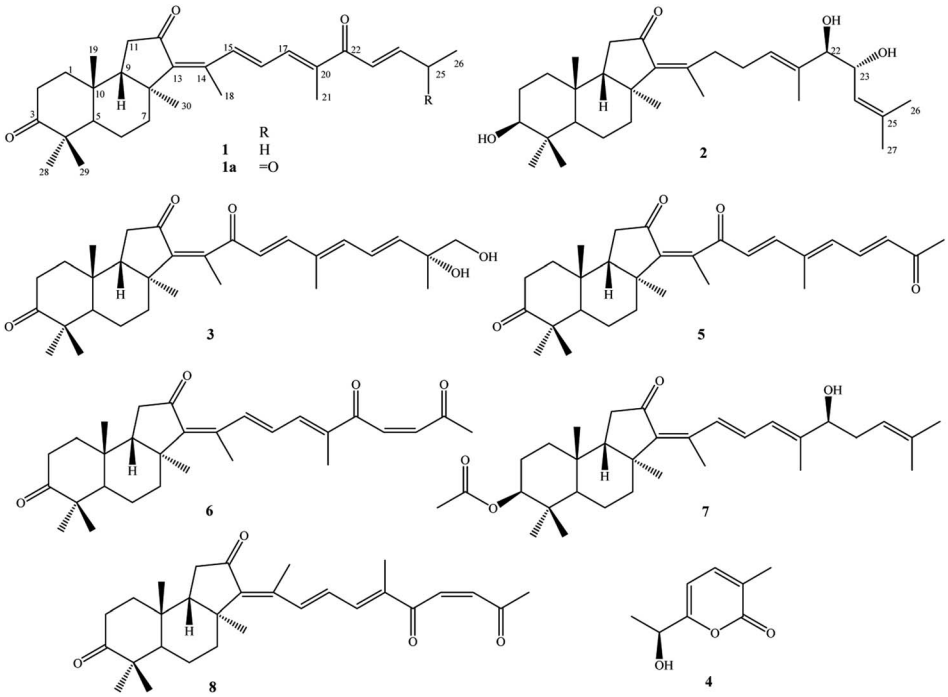

Fig. 1 Chemical structures of **1–8** isolated from the marine sponge *R. globostellata*.

Extraction and isolation

The fresh sponge (40 kg) was cut into small pieces and macerated in MeOH three times (50 L, 2 h in an ultrasonic bath, at room temperature each time) to produce methanol extract (420 g). This extract was suspended with water (3 L) and separated with dichloromethane to give dichloromethane extract (320 g). The dichloromethane extract was loaded on a silica gel column and then eluted with dichloromethane/methanol (0–100% volume of methanol) to give four fractions, D1–D4. Fraction D2 (200 g) was repeatedly subjected to a silica gel column and eluted with *n*-hexane/acetone (0–100% volume of acetone) to give four fractions, D2A–D2D. Fraction D2B was chromatographed on a reversed-phase C-18 column, eluting with acetone/water (2/1, v/v) to give five fractions, D2B1–D2B5. Fractions D2B2 and D2B4 were purified by semi-preparative HPLC using acetonitrile (ACN) in water (30% ACN) to obtain compounds **4** (7.3 mg, t_R 22.4) and **3** (15.2 mg, t_R 42.3), respectively. Fraction D2C was first separated on a reversed-phase C-18 column, eluting with acetone/water (3/2, v/v) and then purified by semi-preparative HPLC using ACN in water (60% ACN) to obtain compound **5** (6.2 mg, t_R 58.8). Fraction D3 (60 g) was loaded on a reversed-phase C-18 column and eluted with methanol/water (3/1, v/v) to give four fractions, D3A–D2D. Fraction D3C was further separated on a silica gel column, eluting with *n*-hexane/ethyl acetate (2/1, v/v) to give four fractions, D3C1–D3C4. The fraction D3C1 was purified by semi-preparative HPLC using ACN in water (70% ACN) to obtain compounds **8** (5.4 mg, t_R 48.5) and **6** (5.8 mg, t_R 50.0). Fraction D3C2 was purified by semi-preparative HPLC using ACN in water (95% ACN) to obtain compound **7** (5.7 mg, t_R 51.5). Fraction D3C3 was purified by semi-preparative HPLC using ACN in water (85% ACN) to obtain compound **1** (10.0 mg, t_R 59.4). Finally, fraction D3C4 was purified by semi-preparative HPLC using ACN in water (70% ACN) to obtain compound **2** (19.5 mg, t_R 43.7).

Rhabdastrenone A (1)

Pale yellow oil; $[\alpha]_D^{25}$: −32.4 (*c* 0.1, MeOH); UV (MeOH): λ_{max}(-log ε) 265(3.0), 354(4.4) nm; HR-ESI-MS: *m*/*z* 437.3040 $[M + H]^+$ (calcd. for $C_{29}H_{41}O_3$, 437.3056); ^{1}H- and ^{13}C-NMR data are shown in Table 1.

Rhabdastrenone B (2)

Pale yellow oil; $[\alpha]_D^{25}$: −25.2 (*c* 0.1, MeOH); UV (MeOH): λ_{max}(-log ε) 232(3.4), 339(4.1) nm; HR-ESI-MS: *m*/*z* 507.3219 $[M + {}^{35}Cl]^-$ (calcd. for $C_{30}H_{48}O_4{}^{35}Cl$, 507.3241) and *m*/*z* 509.3228 $[M + {}^{37}Cl]^-$ (calcd. for $C_{30}H_{48}O_4{}^{37}Cl$, 509.3212); ^{1}H- and ^{13}C-NMR data are shown in the Table 1.

Rhabdastrenone C (3)

Pale yellow oil; $[\alpha]_D^{25}$: −27.0 (*c* 0.1, MeOH); UV (MeOH): λ_{max}(-log ε) 223(3.3), 328(4.0) nm; HR-ESI-MS: *m*/*z* 483.3093 $[M + H]^+$ (calcd. for $C_{30}H_{43}O_5$: 483.3110); ^{1}H- and ^{13}C-NMR data are shown in the Table 1.

Rhabdastrenone D (4)

Colorless oil; $[\alpha]_D^{25}$: −79.7 (*c* 0.1, MeOH); UV (MeOH): λ_{max}(log ε) 221(3.4), 296(4.1) nm; ECD (MeOH) θ(λ nm): −12.0(299) mdeg; HR-ESI-MS: *m*/*z* 155.0706 $[M + H]^+$ (calcd. for $C_8H_{11}O_3$, 155.0708); ^{1}H-NMR (CD_3OD, 500 MHz) δ_H (ppm): 7.35 (d, J = 6.5 Hz, H-4), 6.33 (d, J = 6.5 Hz, H-5), 4.53 (q, J = 6.5 Hz, H-7), 2.05 (s, H-9), 1.44 (d, J = 6.5 Hz, H-8); ^{13}C-NMR ($CDCl_3$, 125

Table 1 ^{1}H-NMR and ^{13}C-NMR spectral data for **1–3**

	1		**2**		**3**	
No.	δ_C [a,b]	δ_H [a,c] (mult., J in Hz)	δ_C [a,b]	δ_H [a,c] (mult., J in Hz)	δ_C [a,b]	δ_H [a,c] (mult., J in Hz)
1	31.4	1.52 (m)/2.15 (m)	33.3	1.37 (m)/1.52 (m)	31.3	1.50 (m)/2.13 (m)
2	33.4	2.38 (m)/2.73 (m)	29.0	1.68 (m)/1.79 (m)	33.6	2.38 (m)/2.72 (m)
3	218.9	—	79.3	3.29 (dd, 5.0, 11.5)	218.8	—
4	46.9	—	39.1	—	46.9	—
5	45.4	2.40 (dd, 2.0, 13.0)	46.4	1.66 (br d, 13.0)	45.4	2.40 (dd, 2.0, 13.0)
6	19.6	1.56 (m)/1.68 (m)	18.2	1.45 (m)/1.68 (m)	19.2	1.56 (m)/1.65 (m)
7	36.9	2.20 (m)/2.25 (m)	37.9	1.98 (m)/2.03 (m)	34.7	1.95 (m)/2.15 (m)
8	45.0	—	43.8	—	43.1	—
9	47.9	1.89 (m)	50.5	1.75 (m)	48.9	1.94 (m)
10	34.8	—	35.4	—	34.9	—
11	37.1	2.12 (m)/2.26 (m)	36.6	2.13 (m)/2.21 (m)	35.6	2.08 (m)/2.28 (m)
12	206.4	—	206.5	—	203.6	—
13	147.7	—	145.1	—	145.7	—
14	141.7	—	149.8	—	143.7	—
15	138.5	8.32 (d, 15.5)	34.7	2.51 (m)/2.67 (m)	200.8	—
16	129.8	6.97 (dd, 15.5, 11.0)	26.5	2.07 (m)/2.12 (m)	124.8	6.18 (d, 16.0)
17	138.6	7.20 (d, 11.0)	128.2	5.46 (t, 7.0)	147.8	6.93 (d, 16.0)
18	16.0	2.10 (s)	21.6	1.84 (s)	17.3	1.99 (s)
19	23.5	0.88 (s)	22.3	0.98 (s)	23.4	0.87 (s)
20	139.2	—	134.2	—	134.4	—
21	12.4	2.00 (s)	12.4	1.63 (s)	12.6	1.92 (s)
22	192.1	—	80.9	3.80 (d, 7.5)	138.3	6.33 (d, 11.0)
23	124.1	6.73 (d, 15.5)	70.1	4.29 (dd, 7.5, 8.5)	125.4	6.72 (dd, 11.0, 15.0)
24	149.2	6.92 (dt, 6.5, 15.5)	123.8	5.13 (d, 8.5)	142.3	5.96 (d, 15.0)
25	25.8	2.30 (m)	137.5	—	73.6	—
26	12.5	1.13 (t, 6.5)	18.5	1.68 (s)	69.9	3.49 (d, 10.5), 3.54 (d, 10.5)
27	—	—	25.9	1.71 (s)	24.4	1.32 (s)
28	29.2	1.12 (s)	29.2	1.02 (s)	29.2	1.13 (s)
29	19.4	1.05 (s)	15.9	0.82 (s)	19.4	1.07 (s)
30	24.6	1.42 (s)	24.8	1.30 (s)	24.9	1.46 (s)

[a] Measured in $CDCl_3$. [b] Measured in 125 MHz. [c] Measured in 500 MHz.

MHz) δ_H (ppm): 166.7 (C-6), 165.5 (C-2), 142.0 (C-4), 124.4 (C-3), 102.3 (C-5), 67.2 (C-7), 21.5 (C-8), 16.5 (C-9).

ECD measurement of the molybdenum complex of compounds 2 and 3

Compounds **2** and **3** (each 0.5 mg) and $Mo_2(OAc)_4$ (1.0 mg) were dissolved in 1.0 mL anhydrous DMSO. The ECD spectrum of the obtained solution was measured immediately under wavelengths 250–500 nm. After 60 minutes, the stationary complex was formed, and the ECD spectrum of the solution was measured again and used to subtract the first ECD spectrum to obtain the $Mo_2(OAc)_4$ induced ECD spectrum of the compound.

Theoretical calculation of ECD spectra

Conformational searches and geometric equilibrium were carried out using the Spartan 18 program (Wavefunction Inc., Irvine, CA, USA). Possible conformations were optimized and subjected to TDDFT calculation on Gaussian 16 program (Gaussian Inc., Wallingford, CT, USA). The calculated ECD spectra were composed after correction based on the Boltzmann distribution of the stable conformers using the SpecDis v1.71 software (University of Wuerzburg, Wuerzburg, Germany). Stereoisomer **4a** (7*R*) was submitted to conformational searches at the molecular mechanics MMFF and equilibrium geometry was performed using a semi-empirical PM3 set. The initial stable conformers with relative energy lower than 40 kJ mol^{-1} were further optimized by DFT calculations at the B3LYP/6-31G(d,p) basic set. The solvent effects were taken by a polarizable continuum model (PCM) calculation with methanol as the solvent. The optimized conformers were then subjected to TDDFT calculations at the CAM-B3LYP/6-31G(d,p) level in the presence of methanol with a PCM. The ECD spectra at 30 excited states for each conformer were collected and summed to obtain the theoretical ECD spectra of each stereoisomer. The half-bands were taken at $\zeta = 0.3$ eV. ECD spectra of enantiomers **4b** (7*S*) were composed of a mirror image, which was of **4a** (7*R*).

Cytotoxic assay

LU1, HepG2, MCF7, and SKMel2 cell lines were obtained from Milan University, Italy and Long Island University, USA. The cells were maintained and cultured in DMEM supplemented with FBS, trypsin-EDTA, L-glutamine, sodium pyruvate, $NaHCO_3$, and penicillin/streptomycin at 37 °C in a humidified atmosphere (5% CO_2 and 95% air). Cytotoxic effects of compounds were determined using the sulforhodamine B (SRB)

assay as previously described.[19] In brief, the cells were incubated with/without compounds for three days in a 96-well culture plate. After incubation, cells were stained with sulforhodamine B and optical density (OD) was measured at 540 nm. The difference in OD between samples and vehicle well during experiments indicated the cell situation induced by the compounds. Results are expressed as the percentage of cell death in comparison with the vehicle as well. The dose–response curves of compounds were generated to determine IC_{50} values of the compounds corresponding to each cell line. Ellipticine was used as a positive control throughout the experiments.

Results and discussion

Compound **1** was obtained as pale yellow oil. Its molecular formula was determined to be $C_{29}H_{40}O_3$ by a protonated molecule at *m/z* 437.3040 $[M + H]^+$ (calcd. for $C_{29}H_{41}O_3$, 437.3056) in the HR-ESI-MS, indicating 10 degrees of unsaturation. The UV chromatogram of **1** showed two maxima absorptions at wavelengths of 265 and 354 nm, suggesting the n–π highly conjugated system. The ^{1}H-NMR spectrum of **1** contained signals of five olefinic protons [δ_H 8.32 (1H, d, J = 15.5 Hz), 7.20 (1H, d, J = 11.0 Hz), 6.97 (1H, dd, J = 15.5 and 11.0 Hz), 6.92 (1H, dt, J = 15.5 and 6.5 Hz), and 6.73 (1H, d, J = 15.5 Hz)], six tertiary methyl groups [δ_H 0.88, 1.05, 1.12, 1.42, 2.00, and 2.10 (each 3H, s)], and a primary methyl group [δ_H 1.13 (3H, t, J = 6.5 Hz)]. ^{13}C-NMR and HSQC spectra of **1** indicated signals of 29 carbons, which suggested **1** to be a nor-triterpene containing nine non-protonated carbons (three carbonyl, three olefinic, and three aliphatic carbons), seven methines (two aliphatic and five olefinic methines), six aliphatic methylenes, and seven methyl groups. Moreover, the ^{1}H- and ^{13}C-NMR data of **1** were close similarity to those of geoditin A (**1a**), an isomalabaricane nortriterpene, previously isolated from the marine sponges *Geodia japonica* and *Rhabdastrella* aff. *distincta*.[20,21] The difference with **1a** was the absence of the ketone functional group at C-25 (Fig. 1). This deduction was further confirmed by consecutive COSY correlations of H-23 (δ_H 6.73)/H-24 (δ_H 6.92)/H-25 (δ_H 2.30)/H-26 (δ_H 1.13). Additionally, the planar structure of **1** was well demonstrated by HMBC and COSY analysis as shown in Fig. 2. The stereochemistry of **1** was then elucidated by J coupling constant values from the ^{1}H-NMR and interactions between closed protons in the NOESY spectrum. Particularly, the *trans-syn-trans* configuration of the tricyclic system, a usual isomalabaricane feature, was demonstrated by NOESY correlations between H_3-19 (δ_H 0.88) and H_3-29 (δ_H 1.05)/H-9 (δ_H 1.89), and between H-5 (δ_H 2.40) and H_3-28 (δ_H 1.12)/H_3-30 (δ_H 1.42) (Fig. 3). A *Z*-geometric configuration at double bond C-13/C-14 was indicated by the NOESY correlation between H_3-30 (δ_H 1.42) and H_3-18 (δ_H 2.10). On the other hand, *E*-geometric configurations at C-15/C-16, C-17/C-20, and C-23/C-24 were confirmed by NOESY correlations between H-15 (δ_H 8.32) and H-17 (δ_H 7.20), H-16 (δ_H 6.97) and H_3-21 (δ_H 2.00), and between H-23 (δ_H 6.73) and H_2-25 (δ_H 2.30). The *E*-configurations of double bonds at C-15/C-16 and C-23/C-24 were also supported by J coupling constants of their protons ($J_{H\text{-}15/H\text{-}16}$ = 15.5 Hz and $J_{H\text{-}23/H\text{-}24}$ = 15.5 Hz). Consequently, the structure of **1** was established and named rhabdastrenone A.

Compound **2** was obtained as pale yellow oil. Its molecular formula was determined to be $C_{30}H_{48}O_4$ by chlorinated adduct ion at *m/z* 507.3219 $[M + {}^{35}Cl]^-$ (calcd. for $C_{30}H_{48}O_4{}^{35}Cl$, 507.3241) and *m/z* 509.3228 $[M + {}^{37}Cl]^-$ (calcd. for $C_{30}H_{48}O_4{}^{37}Cl$, 509.3212), showing seven degrees of unsaturation. The ^{1}H-NMR spectra of **2** showed signals of two olefinic protons [δ_H 5.46 (1H, t, J = 7.0 Hz) and 5.13 (1H, d, J = 8.5 Hz)], three carbinol protons [δ_H 4.29 (1H, dd, J = 7.5 and 8.5 Hz), 3.80 (1H, d, J = 7.5 Hz), and 3.29 (1H, dd, J = 5.0 and 11.5 Hz)], and eight singlet methyl groups [δ_H 1.84, 1.71, 1.68, 1.63, 1.30, 1.02, 0.98, and 0.82 (each 3H, s)]. The ^{13}C-NMR spectrum of **2** revealed signals of 30 carbons including one carbonyl carbon (δ_C 206.5), six olefinic carbons (δ_C 149.8, 145.1, 137.5, 134.2, 128.2, and 123.8), three oxygenated carbons (δ_C 80.9, 79.3, and 70.1), and others 20 sp^3-hybridized carbons (δ_C 12.4–50.5). The aforementioned data suggested **2** to be a tricyclic isomalabaricane-type triterpene. The HMBC correlations between H_3-28 (δ_H 1.02)/H_3-29 (δ_H 0.82) and C-3 (δ_C 79.3) indicated the presence of the hydroxy group at C-3. The location of the ketone functional group at C-12 was confirmed by COSY cross-peaks of H-9 (δ_H 1.75)/H_2-11 (δ_H 2.13 and 2.21), following the HMBC correlation between H_2-11 and C-12 (δ_C 206.5). The connection between the side chain and the tricyclic system *via* the double bond C-13/C-14 was indicated by

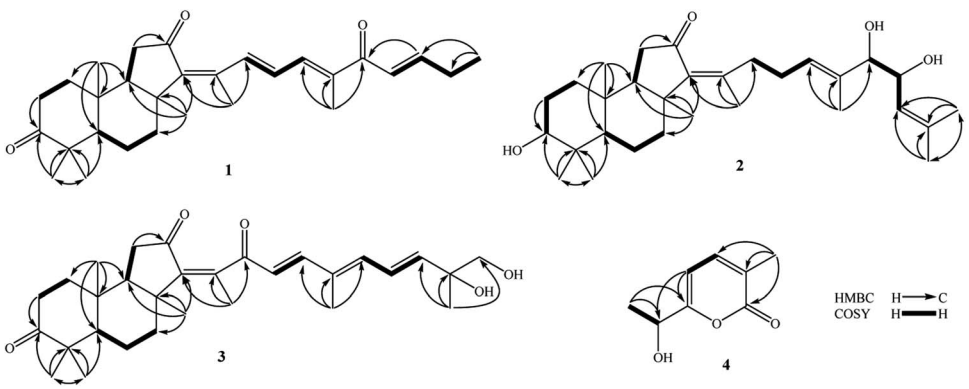

Fig. 2 Key HMBC and COSY correlations of compounds **1–4**.

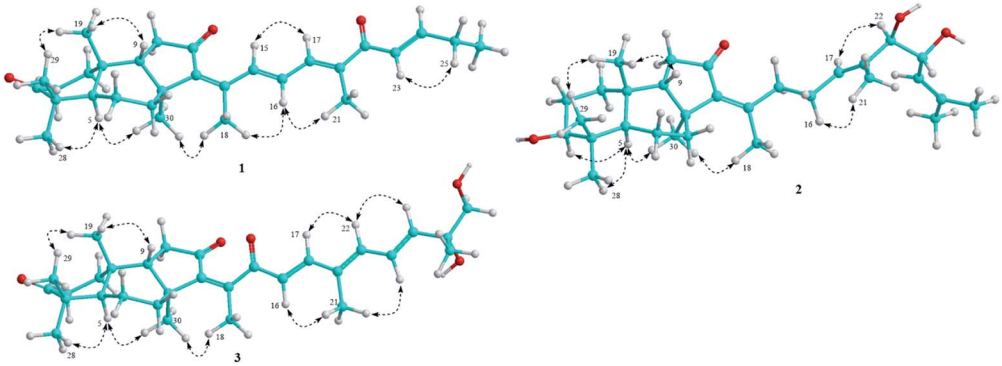

Fig. 3 Important NOESY correlations of compounds **1–3**.

HMBC correlations between H_3-18 (δ_H 1.84) and C-13 (δ_C 145.1)/C-14 (δ_C 149.8)/C-15 (δ_C 34.7). Continuously, COSY cross-peaks of H_2-15 (δ_H 2.51 and 2.67)/H_2-16 (δ_H 2.07 and 2.12)/H-17 (δ_H 5.46) and HMBC correlations between H_3-21 (δ_H 1.63) and C-17 (δ_C 128.2)/C-20 (δ_C 134.2)/C-22 (δ_C 80.9) indicated the location of another double bond at C-17/C-20. The COSY cross-peaks of H-22 (δ_H 3.80)/H-23 (δ_H 4.29)/H-24 (δ_H 5.13) and HMBC correlations between H_3-26 (δ_H 1.68)/H_3-27 (δ_H 1.71) and C-24 (δ_C 123.8)/C-25 (δ_C 137.5) indicated the presence of hydroxy groups at C-22, C-23, and the location of the third double bond at C-24/C-25 (Fig. 2). In the NOESY spectrum of **2**, correlations between H_3-19 (δ_H 0.98) and H_3-29 (δ_H 0.82)/H-9 (δ_H 1.75), and between H-5 (δ_H 1.66) and H_3-28 (δ_H 1.02)/H_3-30 (δ_H 1.30) demonstrated the *trans-syn-trans* configuration of the tricyclic system. The NOESY correlation between H-3 (δ_H 3.29) and H-5 (δ_H 1.66) indicated α,axial-orientation of H-3 (β,equatorial-orientation of hydroxy group) (Fig. 3). The axial orientation of H-3 was also agreed by axial–axial coupled protons between H-3 and H_{ax}-2 ($J_{H\text{-}3/H\text{-}2ax} = 11.5$ Hz). Geometric configurations of double bonds at C-13/C-14 and C-17/C-20 were determined to be 13*Z*,17(20)*E* by NOESY correlations between H_3-30 (δ_H 1.30) and H_3-18 (δ_H 1.84), H_2-16 (δ_H 2.07 and 2.12) and H_3-21 (δ_H 1.63). Relative configuration between hydroxy groups at C-22 and C-23 was determined to be *threo*-configuration by *J* coupling constant value between H-22 and H-23 ($J_{H\text{-}22/H\text{-}23} = 7.5$ Hz) as previously described for 1,2-dioxygenated compounds (*erythro*: $J \sim 3$ Hz and *threo*: $J \sim 8$ Hz).[22] Additionally, negative chirality pattern in the $Mo_2(OAc)_4$ induced ECD spectrum of **2** [positive Cotton effect at 266 nm (+1.1 mdeg) and negative Cotton effect at 355 nm (−0.80 mdeg)] indicated the (22*R*,23*R*) absolute configurations, as previously described (ESI†).[23] Consequently, the structure of **2** was established and named rhabdastrenone B.

Compound **3** was obtained as pale yellow oil. Its molecular formula was determined to be $C_{30}H_{42}O_5$ by a protonated molecule at *m*/*z* 483.3093 $[M + H]^+$ (calcd. for $C_{30}H_{43}O_5$: 483.3110) in the HR-ESI-MS, indicating 10 degrees of unsaturation. The ^{1}H-NMR and HSQC spectra of **3** showed signals of five olefinic protons [δ_H 6.93 (1H, d, $J = 16.0$ Hz), 6.72 (1H, dd, $J = 11.0$ and 15.0 Hz), 6.33 (1H, d, $J = 11.0$ Hz), 6.18 (1H, d, $J = 16.0$ Hz), and 5.96 (1H, d, $J = 15.0$ Hz)], a hydroxymethylene group [δ_H 3.54 and 3.49 (each 1H, d, $J = 10.5$ Hz)], and seven singlet methyl groups [δ_H 1.99, 1.92, 1.46, 1.32, 1.13, 1.07, and 0.87 (each 3H, s)]. The ^{13}C-NMR and HSQC spectra of **3** indicated signals of 30 carbons including three carbonyl carbons (δ_C 218.8, 203.6, 200.8), eight olefinic carbons (δ_C 147.8, 145.7, 143.7, 142.3, 138.3, 134.4, 125.4, and 124.8), two oxygenated carbons (δ_C 73.6 and 69.9), and others 17 sp^3-hybridized carbons (δ_C 12.6–48.9). The NMR spectral data also suggested **3** to be a tricyclic isomalabaricane-type triterpene. Similar with **1**, the HMBC correlations between H_3-28 (δ_H 1.13)/H_3-29 (δ_H 1.07) and C-3 (δ_C 218.8), H_2-11 (δ_H 2.08 and 2.28) and C-12 (δ_C 203.6) indicated the presence of ketone functional groups at C-3 and C-12. However, the HMBC correlation between H_3-18 (δ_H 1.99) and C-13 (δ_C 145.7)/C-14 (δ_C 143.7)/C-15 (δ_C 200.8) suggested that **3** contained an additional ketone functional group at C-15. Furthermore, COSY interaction of H-16 (δ_H 6.18)/H-17 (δ_H 6.93), HMBC correlations between H_3-21 (δ_H 1.92) and C-17 (δ_C 147.8)/C-20 (δ_C 134.4)/C-22 (δ_C 138.3), and COSY consecutive interactions of H-22 (δ_H 6.33)/H-23 (δ_H 6.72)/H-24 (δ_H 5.96) confirmed the conjugated 16,20(22),23-triene side chain. Later, the HMBC correlations between H_3-27 (δ_H 1.32) and C-24 (δ_C 142.3)/C-25 (δ_C 73.6)/C-26 (δ_C 69.9) suggested the presence of hydroxy groups at C-25 and C-26 (Fig. 2). The NOESY correlations between H_3-19 (δ_H 0.87) and H_3-29 (δ_H 1.07)/H-9 (δ_H 1.94), and between H-5 (δ_H 2.40) and H_3-28 (δ_H 1.13)/H_3-30 (δ_H 1.46) demonstrated the *trans-syn-trans* configuration of the tricyclic system. The *Z*-geometric configuration at double bond C-13/C-14 was indicated by the NOESY correlation between H_3-30 and H_3-18. Meanwhile, *E*-geometric configurations at C-16/C-17, C-20/C-22, and C-23/C-24 were confirmed by NOESY correlations between H-16 (δ_H 6.18) and H_3-21 (δ_H 1.92), H_3-21 and H-23 (δ_H 6.72), and between H-22 (δ_H 6.33) and H-24 (δ_H 5.96) (Fig. 3). Absolute configuration at C-25 was determined to be 25*R* by negative chirality pattern in the $Mo_2(OAc)_4$ induced ECD spectrum of **3** [positive Cotton effect at 290 nm (+1.48 mdeg) and negative Cotton effect at 331 nm (−1.18 mdeg)], as previously described (ESI†).[23] Consequently, the structure of **3** was established and named rhabdastrenone C.

Compound **4** was obtained as a colorless oil. Its molecular formula was determined to be $C_8H_{10}O_3$ by a protonated molecule at *m*/*z* 155.0706 $[M + H]^+$ (calcd. for $C_8H_{11}O_3$, 155.0708) in

the HR-ESI-MS, indicating four degrees of unsaturation. The ^{1}H-NMR spectrum of **4** contained signals including a pair of *ortho* coupled olefinic protons [δ_H 7.35 and 6.33 (each, 1H, d, $J = 6.5$ Hz)], a carbinol group [δ_H 4.53 (1H, q, $J = 6.5$ Hz)], and two methyl groups [δ_H 2.05 (3H, s) and 1.44 (3H, d, $J = 6.5$ Hz)]. The ^{13}C-NMR spectrum of **4** showed eight carbons including five sp^2-hybridized carbons [one carbonyl (δ_C 165.5) and four olefinic carbons (δ_C 166.7, 142.0, 124.4, 102.3)] and three sp^3-hybridized carbons [one oxygenated methine (δ_C 67.2) and two methyl groups (δ_C 21.5 and 16.5)]. The COSY spectrum of **8** revealed two pairs of coupled protons, including H_3-8 (δ_H 1.44)/H-7 (δ_H 4.53) and H-4 (δ_H 7.35)/H-5 (δ_H 6.33). Continuously, a carbon backbone of **4** was established by HMBC correlations between H_3-8 (δ_H 1.44)/H-5 (δ_H 6.33) and C-6 (δ_C 166.7)/C-7 (δ_C 67.2), H_3-9 (δ_H 2.05) and C-2 (δ_C 165.5)/C-3 (δ_C 124.4)/C-4 (δ_C 142.0) (Fig. 2). The carbon chemical shift value of C-7 (δ_C 67.2) suggested the presence of a hydroxy group at C-7. Meanwhile, de-shielded carbon signals of C-2 (δ_C 165.5) and C-6 (δ_C 166.7) suggested the formation of a lactone bridge between C-2 and C-6. This deduction was also supported by the molecular formula $C_8H_{10}O_3$ of **4**. Absolute configuration at C-7 was determined to be *S*-configuration by ECD analysis, which showed a similar Cotton effect with that of TD-DFT calculated ECD spectrum of 7*S*-isomer (ESI†). Therefore, the structure of **4** was determined to be (*S*)-6-(α-hydroxyethyl)-3-methylpyran-2-one and named as rhabdastrenone D.

Other compounds were determined to be rhabdastrellin G (**5**),[24] isogeoditin A (**6**),[21] stelliferin A (**7**),[25] and (13*E*)-isogeoditin A (**8**)[21] by the consistency of their NMR spectral data with those reported in the literature (Fig. 1).

Cytotoxic activities of **1–4**, **7** and **8** were evaluated against four typical human cancer cell lines including LU1, HepG2, MCF7, and SKMel2 using the SRB assay.[19] With the exception of compounds **4** and **8**, other compounds (**1–3**, **6** and **7**) exhibited cytotoxic activity with IC_{50} values ranging from 7.53 ± 0.70 to 44.46 ± 1.47 μM (Table 2). Rhabdastrellin G (**5**) was previously reported to show cytotoxic effects on LU1, HepG2, MCF7, and SKMel2 with IC_{50} values from 75.39 ± 4.83 to 84.82 ± 6.67 μM.[24] Based on IC_{50} values (in range of 1–10 μM),[26] only compound **6** exhibited weak cytotoxic effects on HepG2 (IC_{50}: 7.53 ± 0.70 μM) and SKMel2 (IC_{50}: 9.93 ± 0.95 μM) cell lines. Other experiments showed inactivity (IC_{50} > 10 μM). Additionally, our results suggest that (13*Z*)-isomalabaricane (**1–3**, and **6**) processed higher cytotoxic activity than (13*E*)-isomalabaricane (**8**).

Table 2 Cytotoxic effects of **1–8** against several human cancer cell lines

	IC_{50} (μM)			
Comp	LU1	HepG2	MCF7	SKMel2
1	19.44 ± 1.48	29.35 ± 2.52	21.39 ± 1.04	12.91 ± 1.03
2	25.64 ± 2.34	21.43 ± 1.94	24.21 ± 1.37	21.70 ± 1.01
3	40.01 ± 1.52	36.82 ± 4.55	23.31 ± 2.43	34.01 ± 2.21
4	>100	>100	>100	>100
6	10.21 ± 1.68	7.53 ± 0.70	10.18 ± 1.60	9.93 ± 0.95
7	37.12 ± 3.46	44.46 ± 1.47	42.80 ± 1.08	27.63 ± 2.01
8	>100	>100	>100	>100
[a]Pos	2.20 ± 0.20	2.07 ± 0.24	1.95 ± 0.16	1.18 ± 0.16

[a] Ellipticine was used as a positive control.

Conclusions

Our investigation on cytotoxic constituents of the sponge *R. globostellata* collected at the Van Phong Bay, Vietnam, resulted in seven isomalabaricanes (comprising three new ones) and a new α-pyrone derivative. The absolute configurations of new compounds were proved by $Mo_2(OAc)_4$ induced ECD spectra and TD-DFT theoretical calculated ECD spectra. Isogeoditin A (**6**) showed weak cytotoxic effects on HepG2 and SKMel2 cell lines with IC_{50} values of 7.53 ± 0.70 and 9.93 ± 0.95 μM, respectively.

Author contributions

PV Kiem, NX Nhiem, BH Tai contributed to research idea and writing; DT Trang, DTT Hang, DT Dung contributed to isolation; NT Cuc, PH Yen, PTT Huong, NT Mai, LT Huyen contributed to structure elucidation and cytotoxic evaluation.

Conflicts of interest

There are no conflicts to declare.

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