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Alkaloids and polyketides from the South China Sea sponge *Agelas* aff. *nemoechinata*†

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A chemical investigation of the South China Sea sponge *Agelas* aff. *nemoechinata* yielded three pairs of new enantiotopic pyrrole alkaloids nemoechines A–C (**1–3**), one new diterpene-adenine alkaloid nemoechine D (**8**), and one new polyketide nemoechioxide A (**10**), together with nine known analogues (**4–7**, **9**, **11–14**). Compounds **1–3** were initially obtained as racemates and were further separated by chiral HPLC chromatography to afford the three pairs of enantiomers. Their structures including absolute configurations of compounds **1–3** were elucidated on the basis of comprehensive spectroscopic analysis and quantum chemical calculation. Nemoechine A (**1**), possessing an unusual cyclopentane-fused imidazole ring system, represents the first monomeric precursor of nagelamide J. Compounds **8** and **9** showed cytotoxicity against HL-60 cell lines with IC_{50} values of 9.9 and 0.9 μM, respectively, and compound **9** was also cytotoxic against HeLa cell lines with IC_{50} value of 8.9 μM.

Introduction

Marine sponges of the genus *Agelas* (family Agelasidae), with recorded forty-six species,[1] are a rich source of pyrrole alkaloids and diterpenoid alkaloids.[2] These metabolites exhibit a broad bioactivity such as antileukemic,[3] antibacterial,[4] antimalarial,[5] and antifouling activities.[6] It should be noticed that previous investigations on the genus *Agelas*, since the isolation of oroidin from *A. oroides* in 1971,[7] mainly focus on those species collected from Okinawa, the Bahamas, and Caribbean islands, apart from only one report on the South China Sea sponge *A. mauritiana* by Hou-Wen Lin's group in 2012,[8] which implied a potential chemo-diversity of *Agelas* sponges in this area.

As part of our searching for new bioactive natural products from Xisha islands (Paracel islands) sponges,[9] the systematic chemical work on a *Agelas* aff. *nemoechinata* species has yielded diverse natural compounds including pyrrole alkaloids and rare *N*-methyladenine-containing diterpenes.[10] Herein, we reported the isolation, structural elucidation and the cytotoxic activity of fourteen metabolites structurally belonging to three structure families of pyrrole alkaloids (**1–7**), diterpene-adenine alkaloids (**8** and **9**), and polyketides (**10–14**), including three new pyrrole alkaloids nemoechines A–C (**1–3**), one new diterpene-adenine

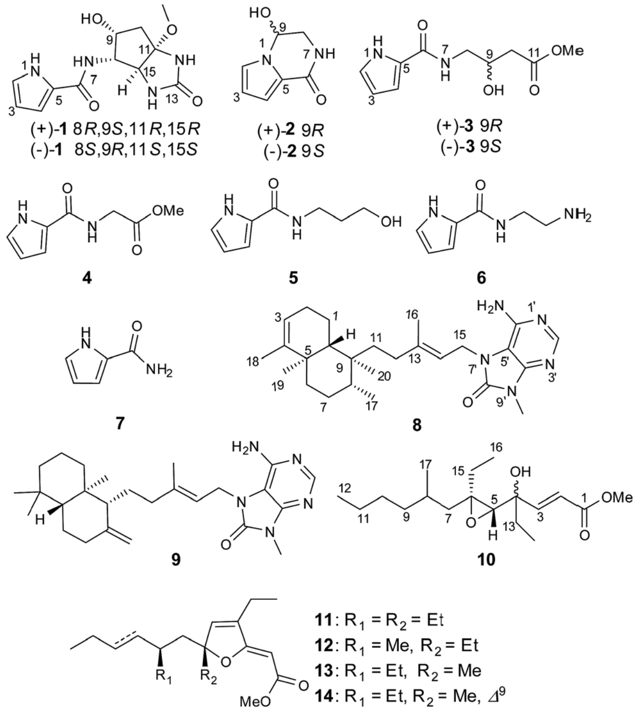

Fig. 1 Structures of compounds **1–14**.

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alkaloid nemoechine D (**8**) and one new polyketide nemoechioxide A (**10**) (Fig. 1). Compounds **1–3** were initially obtained in the form of racemates and were further separated by chiral HPLC chromatography to afford the three pairs of enantiomers. And compound **1** has an unusual skeleton distinguished by a bicyclic cyclopentene-fused imidazole moiety. Compounds **8** and **9** showed significant cytotoxicity against selected tumor cell lines.

Result and discussion

Nemoechine A (**1**) was obtained as a colorless amorphous solid. The molecular formula of **1** was deduced to be $C_{12}H_{16}N_4O_4$ by its HRESIMS data, requiring seven degrees of unsaturation. The IR absorption bands at 3266, 1684, and 1649 cm^{-1}, together with UV absorption at λ_{max} 267 nm indicated the presence of a pyrrole amide moiety,[11] which was consistent with the typical ^{1}H and ^{13}C NMR (DEPT) signals (Table 1) at δ_H 11.51 (1H, br s, NH-1), 6.86 (2H, br s, H-2 and H-4), and 6.09 (1H, br s, H-3), and δ_C 160.2 (d, C-6), 121.3 (d, C-2), 126.1 (d, C-5), 110.8 (d, C-4), and 108.5 (d, C-3).[11] The consecutive ^{1}H–^{1}H COSY correlations from H-1 to H-5, and HMBC correlation of H-7 (δ_H 7.72, d, $J = 6.95$ Hz) with C-6 further improved the aforementioned assignment to be a pyrrole-5-formylamine core (Fig. 2). Considering the other amide carbonyl group signal at δ_C 160.7 (q, C-13) in ^{13}C NMR spectrum, compound **1** was deduced to have an additional bicyclic moiety for the unsaturation requirement. Detailed analyses of COSY and HMQC spectra disclosed a spin–spin coupling system of NH-14/CH-15/CH-8/(NH7) CH-9/CH$_2$-10. The HMBC correlations from H-14 (δ_H 6.93, s) to C-13 and C-15 (δ_C 62.9), from H-12 (δ_H 7.32, s) to C-11 (δ_C 94.7) and C-13, and from H$_2$-10 (δ_H 2.16, dd, J = 13.62, 5.10 Hz) to C-11 and C-15 suggested the bicyclic moiety to be a cyclopentane-fused 2-oxoimidazolidin through C-11 and C-15. The methoxyl group at C-11 was evident from the HMBC correlation from OMe (δ_H 3.07, s) to C-11, and the hydroxyl group at C-9 was deduced from COSY correlations of H-9/H-8 (H$_2$-10) (Fig. 1). Thus, the gross structure of nemoechine A (**1**) was assigned as depicted.

Table 1 NMR data (DMSO-d_6) of compound **1**[a]

Position	δ_C	δ_H (J in Hz)	HMBC (H → C)
1		11.51, br s	
2	121.3	6.86, br s	3, 4, 5
3	108.5	6.09, br s	2, 4, 5
4	110.8	6.86, br s	2, 3, 5
5	126.1		
6	160.2		
7		7.72, d (6.95)	6, 8
8	60.8	3.88, dd (11.36, 5.62)	6, 9, 10, 11, 15
9	69.3	4.06, dd (9.94, 5.04)	10, 15
10	44.2	2.16, dd (13.62, 5.10)	8, 11, 15
		2.09, dd (13.62, 5.10)	8, 11, 15
11	94.7		
12		7.32, s	11, 13, 15
13	160.7		
14		6.93, s	11, 13, 15
15	62.9	3.75, d (5.50)	8, 9, 11, 12
OMe	48.7	3.07, s	11

[a] Recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR.

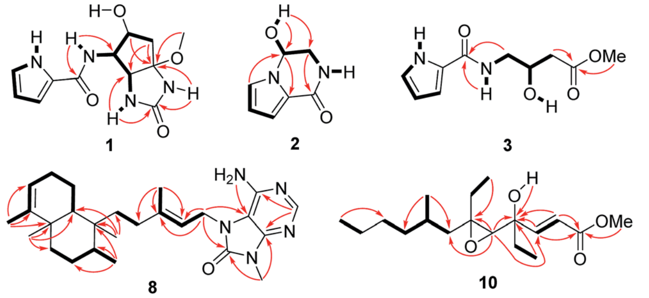

Fig. 2 Key COSY (**—**) and HMBC (→) correlations of **1–3**, **8** and **10**.

The relative configurations of the chiral cyclopantane-fused imidazole moiety in **1** were deduced from NOE differential spectra. Irradiations of H-OMe and H-15 generated respective enhancement of H-15 and H-7, and irritation of H-8 generated enhancement of H-14 and H-9 (Fig. 3 and SS9 in ESI†), indicating that the bicyclic moiety was *cis*-fused, and H-8 and H-9 were on the same side of cyclopentane ring, while OH-9, OMe-11 and H-15 were on the opposite side. Therefore, 8*S**,9*R**,11*S**,15*S** configuration was assigned to **1**, which was identical to that of the partial structure of nagelamide J from *Agelas* sp.[12] However, compound **1** was optically inactive, indicating **1** to be a racemate. The chiral HPLC separation of **1** yielded optical pure compounds (+)-**1** and (−)-**1**, showing opposite optical rotation (OR) values of respective +30.3 and −32.4, as well as mirror-like cotton effects curves (CEs) (Fig. 4).

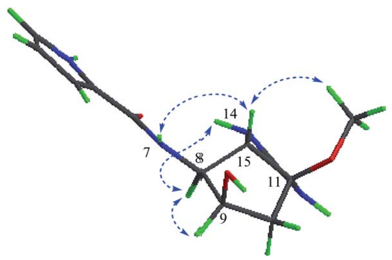

Fig. 3 Key NOSEY correlations of **1**.

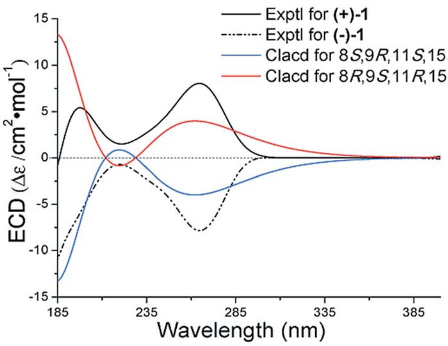

Fig. 4 Experimental CD spectrum of nemoechine A (**1**) overlaid with calculated spectra (redshifted by 15 nm) for the candidate stereostructures.

TDDFT/ECD calculations at RB3LYP/DGDZVP level indicated that the calculated ECD for 8*R*,9*S*,11*R*,15*R* configuration matched well with the experimental ECD spectrum of (+)-**1** showing a main positive CE at 264.5 nm, which was opposite to the experimental ECD spectrum of (−)-**1** and calculated ECD for 8*S*,9*R*,11*S*,15*S* configuration (Fig. 3). Accordingly, the absolute configurations of (+)-**1** and (−)-**1** were determined as 8*R*,9*S*,11*R*,15*R* and 8*S*,9*R*,11*S*,15*S* respectively.

Nemoechine B (**2**), a colorless oil, had molecular formula of $C_7H_8O_2N_2$ as deduced from HRESIMS and ^{13}C NMR data (Table 2). Its 1D NMR data suggested **2** as debromo-analogue of longamide isolated from *Agelas longissima*,[13] which was confirmed by COSY correlations of H-2/H-3/H-4 and H-7/H_2-8/H-9/H-OH, together with HMBC correlations from H-2 to C-9, from H-9 to C-5, from H_2-8 to C-6, and from H-OH to C-8 and C-9 (Fig. 2). Just as the natural occurring analogs longamides A and C and mukanadin C,[13,14] compound **2** was also obtained initially as enantisomers showing no optical activity and was chiral separated to obtain stable (+)-**2** ($[\alpha]_D^{25}$ 32.5, MeOH) and (−)-**2** ($[\alpha]_D^{25}$ −28.5, MeOH). A conformational search using Spartan software led to the conformers with respective quasiaxial OH-9 and quasiequatorial OH-9 conformations for both 9*S* and 9*R* configurations. Nevertheless, the small $J_{H\text{-}9/Ha\text{-}8}$ value of 3.32/3.48 Hz except for the 5.12 Hz for $J_{H\text{-}9/OH\text{-}9}$ of compound **2** indicated that OH-9 should be quasiequatorial-originated. And TDDFT/ECD calculations indicated that the calculated ECD spectra of the lower energy conformers 2a1 and 2a2 with quasiaxial OH-9 matched well with those of (+)- and (−)-**2** showing mirror-like CEs at 225 and 266 nm (Fig. 5, ESI†). Thus, the absolute configurations of 9*R* and 9*S* for respective (+)-**2** and (−)-**2**, identical to the synthetic analogue (*S*)-(-)-longamide B ($[\alpha]_D^{25}$−5.51, MeOH),[15] were finally determined.

Nemoechine C (**3**), a white amorphous solid without optical activity, had a molecular formula of $C_{10}H_{14}N_2O_4$, based on its HRESIMS and ^{13}C NMR data (Table 2). 1D NMR of compound **3** showed the typical pyrrole-2-carboxamido core and a butyric side chain containing two methylenes (δ_C 44.6, 40.0), one oxygenated methine (δ_C 66.7), and one carboxyl group (δ_C 171.7), as well as a methoxy (δ_C 51.2), indicating that compound **3** was an analogue of acanthamide A isolated from sponge *Acanthostylotella* sp.[16] In 2D NMR, two spin-coupling systems of H-1/H-2/H-3/H-4 and H-7/H_2-8/H-9/H_2-10(OH-9) evident from ^1H–^1H COSY spectrum of **3** indicated the partial structures of pyrrole core and side chain (Fig. 2), respectively. HMBC correlations from MeO and H_2-10 to C-11, and from H-7 and H_2-8 to C-6 supported the attachment of the methyl-esterified side chain to carboxamide moiety as depicted. Compound **3** was further purified by chiral HPLC chromatography to obtain (+)-**3** ($[\alpha]_D^{25}$ 10.1, MeOH) and (−)-**3** ($[\alpha]_D^{25}$ −12.1, MeOH). Absolute configurations of (+)-**3** and (−)-**3** were tentatively determined as 9*R* and 9*S* by comparing the OR values with those of the analogues (+)-(*R*)-debromodispacamide D ($[\alpha]_D^{25}$ 41.7, MeOH) and (−)-(*S*)-aspidostomide A ($[\alpha]_D^{25}$ −26.5, MeOH).[17]

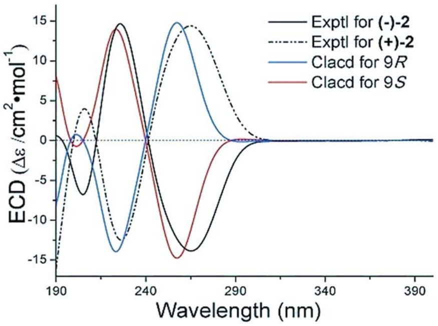

Fig. 5 Experimental CD spectrum of nemoechine B (**2**) overlaid with calculated spectra (redshifted by 10 nm) for the candidate stereostructures.

Nemoechine D (**8**) was obtained as a white amorphous solid with a molecular formula of $C_{26}H_{39}N_5O$ as determined by HRESIMS and ^{13}C NMR (Table 3). The isolated aza-aromatic

Table 2 ^1H and ^{13}C NMR data of compounds **2** and **3** (recorded in DMSO-d_6, δ in ppm, J in Hz)

	2[a]		**3**[b]	
Position	δ_C	δ_H	δ_C	δ_H
1				11.45, br s
2	122.1	7.04, m	121.3	6.84, td (1.47, 2.60)
3	108.9	6.16, m	108.5	6.07, dd (2.36, 3.60)
4	111.9	6.62, dd (3.45, 1.20)	110.1	6.77, ddd (1.52, 2.39, 3.74)
5	123.5		126.1	
6	159.2		160.7	
7		7.56, s		8.03, t (5.93)
8	46.4	3.58, m 3.32[c]	44.6	3.21, m
9	74.2	5.59, ddd (5.12, 3.48, 3.32)	66.7	4.01, br s
10			40.0	2.27, dd (8.96, 15.14) 2.51[c]
11			171.7	
9-OH		6.80, d (5.87)		5.13, br s
11-OMe			51.2	3.57, s

[a] Recorded at 500 MHz for ^1H and 125 MHz for ^{13}C NMR. [b] Recorded at 600 MHz for ^1H and 150 MHz for ^{13}C NMR. [c] Signals overlapped.

Table 3 ^1H and ^{13}C NMR data of compound **8** (recorded in DMSO-d_6, δ in ppm, J in Hz)[a]

Position	δ_C	δ_H
1	17.7	1.46, m
2	26.2	1.90, m, 1.47, m
3	120.2	5.09, br s
4	143.5	
5	37.5	
6	36.1	1.59, dt (2.88, 12.43), 1.05, dt (3.60, 12.39)
7	26.9	1.36, m, 1.34, m
8	35.5	1.38, m
9	38.1	
10	45.7	1.22, m
11	36.1	1.37, m, 1.25, m
12	32.4	1.80, td (4.38, 13.20), 1.69, m
13	140.2	
14	120.0	5.15, t (6.26)
15	38.1	4.56, d (6.53)
16	16.5	1.73, s
17	15.8	0.71, d (6.41)
18	17.7	1.50, s
19	19.6	0.91, s
20	18.1	0.64, s
2′	150.8	8.03, s
4′	146.9	
5′	104.3	
6′	147.6	
8′	152.3	
6′-NH_2		6.43, br s
9′-NMe	26.9	3.24, s

[a] Recorded at 600 MHz for ^1H and 150 MHz for ^{13}C NMR.

proton signal (δ_H 8.03, s) and five skeletal methyl signals (δ_H 1.73, s; 1.50, s; 0.91, s; 0.71, d, $J = 6.41$ Hz; 0.64, s) in ^1H NMR spectrum of **8** suggested it as a purine-labdane diterpenoid alkaloid. And **8** was further deduced to be constructed by the two identical partial structures of respective labdane moiety in ageloxime B and purine moiety in 8′-oxo-agelasine D.[8] The speculation was supported by analysis of 2D NMR of COSY, HMQC and HMBC spectra. Especially the HMBC correlations from NMe-9′ to C-4′ and C-8′, and from H_2-15 to C-13, C-14, C-8′ and C-5′ indicated that the diterpene moiety was attached to C-7′ of the 9′-methyl adenine (Fig. 1). In the NOESY spectrum (Fig. 2), NOE correlations of H_3-20 with H_3-19 and H_3-17, H-8 with H_b-11 (δ_H 1.25), H-10 with H_a-11 (δ_H 1.37), H_3-16 with H_2-15, and H_2-12 with H-14, together with the similar ^{13}C chemical shifts of the labdane moiety,[8] suggested that compound **8** had the same configuration as ageloxime B.

The molecular formula of nemoechioxide A (**10**) was determined as $C_{18}H_{32}O_4$ by its HRESIMS and ^{13}C NMR data (Table 4). Four splitted aliphatic methyl groups including three triplets (δ_H 0.89, t, $J = 7.46$ Hz; 0.87, t, $J = 5.95$ Hz; 0.79, t, $J = 7.45$ Hz) and one doublet (δ_H 0.88, t, $J = 6.47$ Hz) in ^1H NMR spectrum of **10** were typical for polyketides.[9b] And compound **10** was further suggested as an lactone-opening derivative of plakortoxide A,[9b] based on the evidence that **10** had relatively upshifted oxygen-bearing quaternary carbon (δ_C 73.5) and carboxyl carbonyl (δ_C 166.2) and one extra methoxyl group (δ_C 51.4; δ_H 3.66). The COSY correlations of H-7/H-8/H-9(H_3-17), H_2-11/H_3-12, H_2-15/H_3-16, H_2-13/H_3-14, and H-2/H-3 (Fig. 1), together with HMBC correlations from OMe to C-1, from H-2 and H-3 to C-1 and C-4, from OH and H_3-14 to C-4, from H-5 to C-3, C-4, C-6 and C-7, from H_3-16 to C-6, from H_3-17 to C-7 and C-9, and from H_3-12 to C-10 completed the assignment of the structure. The large coupling constant of $J_{H-2/H-3} = 15.72$ Hz suggested the E geometry of double bond CH-2=CH-3. In NOE difference spectrum, irritation of H-5 (δ_H 2.75, 1H, s) generated enhancements of H_2-7 (δ_H 1.82, 1H, t, dd = 13.81, 4.90 Hz; 0.79, 1H, overlapped) and H-8 (δ_H 1.49, 1H, m), indicating that H-5 and ethyl moiety of –CH_2-15–CH_3-16 were located on opposite side of the epoxide ring. Nevertheless, the configurations of C-4 and C-8 remained to be determined due to the sample limit.

Table 4 ^1H and ^{13}C NMR data of compound **10** (recorded in DMSO-d_6, δ in ppm, J in Hz)[a]

Position	δ_C	δ_H
1	166.2	
2	119.0	5.99, d (15.72)
3	152.8	6.93, d (15.72)
4	73.5	
5	68.4	2.75, s
6	63.9	
7	42.3	1.82, dd (4.90, 13.81)
		0.79[b]
8	29.2	1.49, m
9	36.9	1.25, m
		1.09, m
10	28.7	1.25, m
11	22.4	1.25, m
12	14.0	0.87, t (5.95)
13	31.5	1.61, m
		1.66, m
14	7.4	0.79, t (7.45)
15	21.0	1.61, m
		1.86, m
16	9.3	0.89, t (7.46)
17	19.6	0.88, d (6.47)
1-OMe	51.4	3.66, s
4-OH		5.00, s

[a] Recorded at 600 MHz for ^1H and 150 MHz for ^{13}C NMR. [b] Signal overlapped.

Additionally, seven known compounds were isolated from *Agelas* aff. *nemoechinata*, including (**4**) glycine, *N*-(1*H*-pyrrol-2-ylcarbonyl)-, methyl ester (CAS no. 62 409-33-0),[18] (**5**) 1*H*-pyrrole-2-carboxamide, *N*-(3-hydroxypropyl)- (CAS no. 186 956-74-1),[19] (**6**) 1*H*-pyrrole-2-carboxamide, *N*-(2-aminoethyl)- (CAS no. 1 183 721-98-3),[20] (**7**) 1*H*-pyrrole-2-carboxamide (CAS no. 4551-72-8),[21] (**9**) 8′-oxo-agelasine D 8*H*-purin-8-one, 6-amino-7-[(2*E*)-5-[(1*R*,4a*R*,8a*R*)-decahydro-5,5,8*a*-trimethyl 2-methylene-1-naphthalenyl]-3-methyl-2-penten-1-yl]-7,9-dihydro-9-methyl- (CAS no. 1 360 451-92-8),[8] (**11**) acetic acid, 2-[(5*R*)-3,5-diethyl-5-[(2*S*)-2-ethylhexyl]-2(5*H*)-furanylidene]-, methyl ester, (2*Z*)-(CAS no. 206 443-26-7),[22] (**12**) acetic acid, 2-[(5*R*)-3,5-diethyl-5-[(2*S*)-2-methylhexyl]-2(5*H*)-furanylidene]-, methyl ester, (2*Z*)-(CAS no. 206 443-25-6),[22] (**13**) acetic acid, 2-[(5*R*)-3-ethyl-5-[(2*S*)-2-ethylhexyl]-5-methyl-2(5*H*)-furanylidene]-, methyl ester, (2*Z*)-(CAS no. 887 267-98-3),[23] (**14**) acetic acid, 2-[(5*R*)-3-ethyl-5-[(2*R*,3*E*)-2-ethyl-3-hexen-1-yl]-5-methyl-2(5*H*)-furanylidene]-, methyl ester, (2*Z*)-(CAS no. 767 314-30-7).[22] Their structures were determined by comparison of their spectroscopic data with those in the literature.

The cytotoxicities of compounds **1–14** were evaluated against four tumor cell lines, HeLa (cervical carcinoma), HL-60 (promyelocytic leukemia), K562 (erythroleukemia) and P388 (leukaemia), using the MTT method with adriamycin as the positive control.[24] Diterpene alkaloids **8** and **9** showed cytotoxity against HL-60 cell lines with IC_{50} values of respective 9.9 and 0.9 μM, respectively, and compound **9** was also cytotoxic against HeLa cell lines with IC_{50} value of 8.9 μM. And it was the first time to reveal the cytotoxicity feature of 8′-oxo-agelasine D (**9**) since the report of its lack of antimicrobial and antileishmanial activities.[8] All of the compounds tested were inactive against the P388 and K562 cell lines (IC_{50} value > 10 μM).

Conclusions

In conclusion, three kinds of metabolites including five pyrrole alkaloids (**1–7**), two pyrimidine diterpenes (**8** and **9**), and five polyketides (**10–14**) were obtained from a Xisha sponge *Agelas* aff. *nemoechinata*. The new compounds (**1–3**, **8**, and **10**), especially an unusual cyclopentene-fused imidazole ring system in **1** enriched chemo-diversity of *Agelas* genus. And the cyctotoxity feature of new pyrimidine diterpene (**8**) and the known analogue (**9**) was unveiled for a pharmaceutical potential. Most interestingly, almost all pyrrole alkaloids from *Agelas* sponge were brominated, while in present study the compounds (**1–7**) of the same family from the affinis species in Xisha islands were all non-brominated. That could imply a potential habitat-specificity of the *Agelas* genus in Xisha islands.

Experimental section

General experimental procedures

Optical rotations were measured on a Jasco P-1020 digital polarimeter. UV spectra were measured on a Beckman DU640 spectrophotometer. CD spectra were obtained on a Jasco J-810 spectropolarimeter. IR spectra were recorded on a Nicolet NEXUS 470 spectrophotometer using KBr discs. 1D and 2D NMR spectra were recorded on a JEOL JNMECP 600 and Bruker AVANCE III 500 spectrometers using residual solvent signals (for DMSO-d_6: δ_H 2.50 and δ_C 39.52 ppm) as reference standards. HRESIMS was performed on a Micromass Q-TOF Ultima Global GAA076 LC-MS spectrometer. Semi-preparative HPLC utilized an ODS column [YMC-Pack ODS-A, 10 × 250 mm, 5 μm, 1.5 mL min^{-1}]. Chiral HPLC utilized chiral analytical columns [Daicel Chiralpack IC: 5 μm, 4.6 mm × 250 mm]. Silica gel (200–300 mesh, Qingdao, China) was used for column chromatography, and precoated silica gel plates (GF254, Qingdao, China) were used for TLC. The cell lines for *in vitro* cytotoxicity studies were procured from Capital Medical University, Beijing, China.

Animal material

The sponge *Agelas* aff. *nemoechinata* was collected from Xisha islands (Paracel islands) in the South China Sea, in June 2009, at a depth of about 20 m. The sponge was identified by one of the authors (N. J. D. V.). A voucher specimen (no. XS 2009-26) was deposited in the school of Medicine and Pharmasy, Ocean University of China, People's Republic of China.

Extraction and isolation

The frozen specimen (5.0 kg, wet weight; 2.2 kg, dry weight) was mined and extracted with MeOH three times (each time, 5 days) at room temperature. The combined solutions were concentrated in vacuum, and the concentrated extract was finally desalted by redissolving with MeOH three times to yield a residue (310.5 g). The crude extract was subjected to silica gel vacuum liquid chromatography (VLC), eluting with a gradient of petroleum/acetone (from 10 : 0 to 1 : 1, v/v) to obtain seven fractions (F1–F7). Fraction F2 (petroleum/acetone 10 : 1, 8.7 g, 0.4% of dry material weight) was chromatographed on a silica gel column using petroleum ether-acetone (20 : 1, v/v) as eluent to give three subfractions (SF2-1–SF2-3). Subfraction SF2-2 (1.66 g, 0.07% of dry material weight) was further separated on an ODS reversed-phase silica column using MeOH/H_2O (1 : 1, 3 : 2, 4 : 1 and 10 : 0, v/v) to give five fractions (SF2-2-1–SF2-2-5). Subfraction SF2-2-1 (0.26 g, 0.01% of dry material weight) was purified by HPLC (ODS; 5 um, 250 × 10 mm; MeOH–H_2O, 78 : 22, v/v; 1.5 mL min^{-1}) to afford compounds **10** (2.1 mg), **11** (4.0 mg), **12** (4.2 mg), **13** (3.9 mg), **14** (3.5 mg). Similarly, fractions F5 (CH_3Cl/MeOH 20 : 1, 8.0 g, 0.36% of dry material weight) was subjected to CC on silica gel repeatedly and further purified by HPLC to give compounds **3** (2.5 mg), **4** (6.0 mg), **5** (1.5 mg), **7** (30 mg), **8** (8.0 mg), and **9** (8.6 mg). And compounds **1** (2.2 mg), **2** (7.2 mg), and **6** (2.5 mg) were obtained from S6 (CH_3Cl/MeOH 1 : 1, 5.2 g, 0.24% of dry material weight). Chiral separations of partial **1** [Daicel Chiralpack IC, 5 μm, 4.6 mm × 250 mm; hexane/isopropyl alcohol 70 : 30, 1.5 mL min^{-1}], **2** [Daicel Chiralpack IC, 5 μm, 4.6 mm × 250 mm; hexane/isopropyl alcohol 75 : 25, 1.5 mL min^{-1}], and **3** [Daicel Chiralpack IC, 5 μm, 4.6 mm × 250 mm; hexane/isopropyl alcohol 70 : 30, 1.5 mL min^{-1}] were performed on Agilent analytical HPLC system to afford optically pure (+)-**1** (0.9 mg), (−)-**1** (1.1 mg), (+)-**2** (1.0 mg), (−)-**2** (1.1 mg), (+)-**3** (1.0 mg), and (−)-**3** (1.3 mg).

Computational section

The quantum chemical calculations were performed by using the density functional theory (DFT) as carried out in the Gaussian 09.[25] The initial structures of compounds **1** and **2** were built with Spartan 10 software and all trial structures were firstly minimized based on molecular mechanics calculations. Conformational search was performed by Spartan 10 software using MMFF force filed, and conformers occurring within a 10 kcal mol^{-1} energy window from the global minimum were chosen for geometry optimization in the gas phase with the DFT method at the B3LYP/6-31G(d,p) levels. The stable conformations of **1** and **2** were calculated for ECD spectra using TD-DFT method with the basis set RB3LYP/DGDZVP.[26] Solvent effects of methanol solution were evaluated at the same DFT level by using the SCRF/PCM method. Electronic transitions were expanded as Gaussian curves with a FQHM (full width at half maximum) for each peak of 0.6 and 0.32 eV for respective **1** and

2. The ECD spectra were combined after Boltzmann weighting according to their population contribution (Fig. S1–S4 in ESI†).

(±)-Nemoechine A (1). Colorless amorphous solid; UV (MeOH) (log ε) $\lambda_{\max}$ 267 (2.70), 217 (2.92); IR (KBr) $\nu_{\max}$ 3266, 2922, 2360, 1684, 1649, 1559, 1521, 1380, 1068, 894, 628, 604 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 303.2 [M + Na]$^+$; HRESIMS *m*/*z* 303.1061 [M + Na]$^+$ (calcd for $C_{12}H_{16}O_4N_4Na$, 303.1064). (+)-**1** [=*N*-((3a*R*,4*R*,5*S*,6a*R*)-5-hydroxy-6*a*-methoxy-2-oxooctahydrocyclopenta[*d*]imidazol-4-yl)-1*H*-pyrrole-2-carboxamide]: $[\alpha]_D^{25}$ 30.3 (*c* 0.08, MeOH); CD (*c* 0.0008 M, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 197.5 (5.4), 264.5 (8.0) nm; (−)-**1** [=*N*-((3a*S*,4*S*,5*R*,6a*S*)-5-hydroxy-6*a*-methoxy-2-oxooctahydrocyclopenta[*d*]imidazol-4-yl)-1*H*-pyrrole-2-carboxamide]: $[\alpha]_D^{25}$ −32.4 (*c* 0.0867, MeOH); CD (*c* 0.0008 M, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 264.5 (−7.8) nm.

(±)-Nemoechine B (2). Colorless oil; UV (MeOH) (log ε) $\lambda_{\max}$ 269 (2.10), 230 (2.45); IR (KBr) $\nu_{\max}$ 3278, 2927, 2365, 1641, 1556, 1471, 1338, 1099, 1061, 848, 749, 695 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 153.07 [M + H]$^+$; HRESIMS *m*/*z* 153.0659 [M + H]$^+$ (calcd for $C_7H_9O_2N_2$, 153.0659).(+)-**2** [=(*R*)-4-hydroxy-3,4-dihydropyrrolo[1,2-*a*]pyrazin-1(2*H*)-one]: $[\alpha]_D^{25}$ 32.5 (*c* 0.193, MeOH); CD (*c* 0.0008 M, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 206 (4.0), 226.5 (−12.5), 265 (14.4) nm; (−)-**2** [=(*S*)-4-hydroxy-3,4-dihydropyrrolo[1,2-*a*]pyrazin-1(2*H*)-one]: $[\alpha]_D^{25}$ −28.5 (*c* 0.167, MeOH); CD (*c* 0.0008 M, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 205 (−6.8), 225.5 (14.7), 265 (−13.9) nm.

(±)-Nemoechine C (3). White, amorphous solid; UV (MeOH) (log ε) $\lambda_{\max}$ 265 (2.83), 206 (2.78); IR (KBr) $\nu_{\max}$ 3257, 2931, 2361, 1731, 1633, 1564, 1526, 1439, 1328, 1200, 1027, 739 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 249.0 [M + Na]$^+$; HRESIMS *m*/*z* 249.0851 [M + Na]$^+$ (calcd for $C_{10}H_{14}O_4N_2Na$, 249.0846). (+)-**3** [=methyl (*R*)-3-hydroxy-4-(1*H*-pyrrole-2-carboxamido)butanoate]: $[\alpha]_D^{25}$ 10.1 (*c* 0.0467,MeOH); CD (*c* 0.0008 M, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 199.1 (−7.8), 232.8 (3.4), 263.3 (−9.3), 302.0 (4.3), 347.3 (3.6) nm; (−)-**3** [=methyl (*S*)-3-hydroxy-4-(1*H*-pyrrole-2-carboxamido)butanoate]: $[\alpha]_D^{25}$ −12.1 (*c* 0.0533, MeOH); CD (*c* 0.0008 M, MeOH) $\lambda_{\max}$ ($\Delta\varepsilon$) 199.1 (−7.8), 232.8 (3.4), 263.3 (−9.3), 302.0 (4.3), 347.3 (3.6) nm.

Nemoechine D (8). [=6-Amino-9-methyl-7-((*E*)-3-methyl-5-((1*S*,2*R*,4a*R*,8a*R*)-1,2,4*a*,5-tetramethyl-1,2,3,4,4*a*,7,8,8*a*-octahydronaphthalen-1-yl)pent-2-en-1-yl)-7,9-dihydro-8*H*-purin-8-one]: white, amorphous solid; $[\alpha]_D^{25}$ −20.1 (*c* 0.533, MeOH); UV (MeOH) (log ε) $\lambda_{\max}$ 273 (3.24), 218 (2.51); IR (KBr) $\nu_{\max}$ 3315, 3188, 2923, 1709, 1635, 1510, 1441, 1368, 1164, 858 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 438.3 [M + H]$^+$; HRESIMS *m*/*z* 438.3239 [M + H]$^+$ (calcd for $C_{26}H_{40}ON_5$, 438.3227).

Nemoechinoxide A (10). [=Methyl (*E*)-4-((2*S*,3*S*)-3-ethyl-3-(2-methylhexyl)oxiran-2-yl)-4-hydroxyhex-2-enoate]: colorless oil; $[\alpha]_D^{25}$ 62.2 (*c* 0.133, MeOH); UV (MeOH) (log ε) $\lambda_{\max}$ 210 (2.48); IR (KBr) $\nu_{\max}$ 3508, 2958, 2929, 2357, 1726, 1461, 1308, 1271, 1197, 1169, 1018, 928, 840, 568, 482 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; ESIMS *m*/*z* 235.0 [M + Na]$^+$; HRESIMS *m*/*z* 335.2197 [M + Na]$^+$ (calcd for $C_{18}H_{32}O_4Na$, 335.2193).

Cytotoxicity assay

The cytotoxicity of compounds **1–14** against HeLa, HL-60, K562 and P388 cell lines were determined by MTT method with Adriamycin as a positive control.[23] The IC_{50} value of each compound was calculated by Reed and Muench's method.

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