The Chemical and Chemo-ecological Studies on Weizhou Nudibranch *Glossodoris atromarginata*

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Abstract: A detailed chemical investigation of the nudibranch *Glossodoris atromarginata* collected from Weizhou Island, South China Sea, yielded a new spongian-type diterpene **1**, together with four known related compounds **2**–**5**. The structure of the new compound **1** was elucidated by detailed spectroscopic analysis and by the comparison of the spectroscopic data with the known diterpene isoagatholactone. In addition, evidence for the absolute stereochemistry of the known compound **2** was, for the first time, provided by the application of time-dependent density functional theory electronic circular dichroism (TDDFT-ECD) calculation.

Key Words: Nudibranch, *Glossodoris atromarginata*, spongian-type diterpene, TDDFT-ECD, absolute configuration

Introduction

Nudibranchs (phylum Mollusca, class Gastropoda, subclass Opistobranch) are a diverse group of marine gastropod molluscs of over 4700 known species found in practically all oceans and were able to yield a rich harvest of secondary metabolites, including diterpenes, cyanides, isothiocyanates and isocyanides, *etc*.[1, 2]. A large number of these metabolites exhibit broad biological properties, such as cytotoxic and ichthyotoxicity[3]. More interestingly, those metabolites not only exhibit extensive bioactivities but also play an important role in protecting shell-less nudibranchs from predating by the predators[4]. Moreover, some of those secondary metabolites can also be found in other marine invertebrates (*e.g.* sponges) suggesting the probable prey-predator relationship between them[5]. Hence, the investigation of chemical constituents, in particular, their distribution in different organs of nudibranchs, is extremely meaningful for a better understanding the potential chemo-ecological roles played by these tiny animals.

The sea slug *Glossodoris atromarginata* is a shell-less nudibranch belonging to the family chromodorididae. Although the title animal was discovered more than 200 years ago, to our knowledge, few chemical studies have been reported over the last decades[6,

7].

Our group has previously been engaged in searching for novel secondary metabolites from Chinese marine invertebrates[8]. In the course of our continued research efforts to explore chemically fascinating and biologically interesting secondary metabolites from the South China Sea, the nudibranch *G. atromarginata* was collected and chemically investigated, resulting in the isolation of a series of spongian-type diterpenes[9]. Recently, a newly collected nudibranch *G. atromarginata* from the same coast of Weizhou Island, Guangxi Autonomous Region, China, has been chemically investigated, leading to the isolation of a new spongian-type diterpene **1**, three known spongian-type diterpenes **2**−**4** and one known scalarane-type sesterteripene **5**. Herein, we describe the isolation, structural elucidation, anatomical distribution of these compounds and the potential chemo-ecological relationship of the nudibranch *G. atronmarginata* with its possible dietary sponges.

Results and Discussions

The 21 specimens of *G. atromarginata* were anatomized into mantle and inner organs, which were separately extracted with acetone, respectively. The mantle Et_2O-soluble portion (189.3 mg) of the acetone extract was chromatographed repeatedly over silica gel to yield pure compounds **1**−**5** (Figure 1), whereas the inner organs Et_2O-soluble extract (69.4 mg) afforded only compound **5**. Among them, known compounds were readily identified as 18-Nor-3,17-dihydroxyspongia-3,13(16),14-trien-2-one (**2**)[10], 17-hydroxy-3*β*,19-diacetoxyspongia-13(16),14-dien-2-one (**3**)[11], 17,19-dihydroxy-3β-acetoxyspongia-13(16),14-dien-2-one (**4**)[11], and 12-deacetoxy-12-oxodeoxoscalarin (**5**)[6b] by comparing their NMR spectroscopic data, mass spectra, and optical rotation with those reported in the literature.

Compound **1** was obtained as an optically active white powder, $[\alpha]_D^{25}$ 17.5 (*c* 0.08, $CHCl_3$). The molecular formula was deduced as $C_{20}H_{28}O_3$ from its HR-EI-MS (*m/z* 316.2033, $[M]^+$, calcd. 316.2038) in combination with ^{13}C NMR (DEPT) experiments,

suggesting seven degrees of unsaturation. The ^{13}C NMR (DEPT) exhibits four methyls, six methylenes, four methines, and six quaternary carbons (visually determined by difference). According to the ^{1}H NMR and ^{13}C NMR data of **1** (Table 1), three degrees of unsaturation were attributed to two carbonyl carbon (δ_C217.0, qC;δ_C175.0, qC) and a trisubstituted olefinic bond (δ_H5.74, δ_C120.6, CH; δ_C129.8, qC;). Thus, the remaining four degrees indicated a tetracyclic ring system in the molecule. Detailed analysis of the ^{1}H-^{1}H COSY spectrum permitted identification of three spin systems from H_2-1 (δ_H 1.93, ddd, J = 13.7, 7.2, 3.8 Hz; δ_H 1.43, m) to H_2-2 (δ_H 2.56, ddd, J = 15.0, =7.2, 3.7 Hz; δ_H 2.41, ddd, J = 15.0, 6.9, 3.8 Hz), H-5 (δ_H 1.47, m) to H_2-7 (δ_H 2.62, dt, J = 13.7, 2.7, 2.7 Hz; δ_H 1.36, m), and H-9 (δ_H 1.35, dd, J = 11.4, 5.9 Hz) to H-12 (δ_H 5.74, m). The HMBC experiment showed the following correlations: H_2-2 to C-3 (δ_C 217.0, qC) and C-4 (δ_C 47.5, qC); H_3-18 (δ_H 1.08, s) to C-3, C-4 and C-5 (δ_C 55.6, CH); H_3-20 (δ_H 1.05, s) to C-1 (δ_C 38.8, CH_2), C-5 and C-9 (δ_C 53.4, CH); H_3-17 (δ_H 0.90, s) to C-7 (δ_C 39.2, CH_2), C-9, and C-14 (δ_C 53.9, CH); H_2-16 (δ_H 4.67, m) to C-12 (δ_C 120.6, CH), C-13 (δ_C 129.8, qC), C-14 and C-15 (δ_C 175.0, qC). The above structural features were reminiscent of previously reported isoagatholactone (**6**), a spongian-type diterpene isolated from the Sponge *Spongia officinalis* by Cimino *et al* [12]. A detailed comparison of the NMR data revealed that **1** should possess the same ring system as **6**, the only significant differences between these two compounds being the presence of two carbonyl groups, one at C-3 and the other at C-15 in **1**, instead of the single carbonyl group at C-16 in **6**. In fact, due to the presence of carbonyl group at C-3, the ^{13}C NMR data of C-2, C-3 and C-4 in **1**, relative to those in **6**, were obviously downfield shifted (δ_C 33.9, CH_2; δ_C 217.0, qC; δ_C 47.5, qC). Additionally, the chemical shift of C-12, C-13((δ_C 120.6, CH; δ_C 129.8, qC) and the splitting observed for H-14 (2.78 s), H_2-16 (4.67/4.67 m) both suggest the existence of a C-15 lactone in **1**, in contrast to the C-16 lactone in **6** (Table S1). The results of subsequent 2D NMR spectra, including ^{1}H-^{1}H COSY, HSQC, and HMBC, allowed the total assignment of NMR data for compound **1** and the determination of the its planar structure (Figure 2).

The relative configuration of **1** was determined on the basis of NOESY correlations, as shown in Figure 2. The remarkable NOESY correlations from H_3-20 to H_3-17 placed two methyl groups on the same face (β-orientation), and the obvious correlations from H-9 to H-5 and H-14 (δ_H 2.78, s) indicated the three protons at the α-orientation, which disclosed the relative configuration of **1** (5*R**, 8*R**, 9*R**, 10*R**, 14*S**).

In order to direct verification of the absolute configuration of compound **1**, the TDDFT-ECD calculation method was applied. Unfortunately, compound **1** had degraded when testing the experimental ECD evaluation. Since compound **1** and the co-occurring compound **2** belong to the same spongian-type diterpenes, on the basis of the biogenetic considerations, the absolute configuration of **1** was tentatively determined by the comparison with known spongian-type diterpenes **2** and **6** (Table S1-S3).

To determine the absolute configuration for compound **2**, energy minimization was carried out using the Macromodel method with OPLS_2003 force field. After, the energy-minimized structure was optimized under B3LYP/6-31 G(d) level. Further, ECD spectra were obtained by TDDFT calculations performed under B3LYP/6-311G(d,p) with IEFPCM (Polarizable Continuum Model using the Integral Equation Formalism variant) solvent model with acetonitrile. Finally, the Boltzmann averaged ECD spectrum of (5*R*, 8*S*, 9*R*, 10*R*)-**2** exhibited a good match with the experimental ECD spectrum of **2**. Hence, the absolute configuration of **2** was elucidated as 5*R*, 8*S*, 9*R*, 10*R*. It is worth pointing out that this represents the first time that confirmation of the absolute stereochemistry for compound **2** had been reported. In addition, the absolute configuration of compound **1** was tentatively assigned as 5*R*, 8*R*, 9*R*, 10*R*, 14*S*.

Conclusions

In this study, a new spongian-type diterpene **1**, together with three known related ones **2**-**4** and one known scalarane-type sesterterpene **5** were isolated from the nudibranch *G. atromarginata* collected from Weizhou Island. Among them, a new compound **1** was

elucidated by extensive NMR spectroscopic investigation. In addition, the absolute configuration of compound **2** has been confirmed for the first time as 5*R*, 8*S*, 9*R*, 10*R* by application of TDDFT-ECD calculations.

Among compounds **1**-**5**, compound **5** is a scalarane-type sesterterpene, while **1**-**4** belong to spongian-type diterpenes. Since spongian-type diterpenes normally originate from sponges of Genus *Spongia*, such as *Spongia officinalis* and *Spongia ceylonensis*[13], combining this research with previous investigation[14], the prey-predator relationship between *Spongia officinalis* and *G. atromarginata* could be illustrated. Meanwhile, given that scalarane-type sesterterpenes commonly isolated from several sponge species, like *Hyrtios erectus*[15], *Irciniafelix*[16], and *Carteriospongia foliascens*[17], it is reasonable to speculate that the above sponges also have potential dietary relationship with mollusk *G. atromarginata*. The high diversity of metabolites in this research reflects a varied sponge diet of the mollusk *G. atromarginata* (Figure 4). Unfortunately, no bioassay could be performed due to the limited quantities of isolated material.

Lastly, comparing this investigation with existing reports,[6,7] the diverse chemical constituents in *G. atromarginata* collected from different seas, partly demonstrate the environmental influence on the secondary metabolism in marine organisms and will provide additional inspiration for a more extensive examination of marine natural products.

Experimental Procedures

1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241MC polarimeter(Perkin-Elmer, Waltham, MA, USA). HR-EI-MS spectra were recorded on a Finnigan-MAT-95 mass spectrometer (Finnigan MAT, San Jose, CA, USA). Commercial silica gel (Qingdao Haiyang Chemical Group Co., Ltd., Qingdao, China, 200-300 and 500-600 mesh) was used for column chromatography, and precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co., Yantai, China, G60 F-254) were used for analytical TLC. Sephadex LH-20 (Pharmacia, USA) was also used for column chromatography. All solvents were of analytical grade.

2. NMR measurements

NMR spectra were measured on Bruker DRX-400, DRX-600 spectrometer (Bruker Biospin AG, Fällanden, Germany) with the residual $CHCl_3$ (δ_H 7.26 ppm), CH_3OH (δ_H 3.31 ppm) as the internal standard for ^1H NMR spectrometry and $CDCl_3$(δ_C 77.16 ppm), CD_3OD (δ_C 49.00 ppm) for ^{13}C NMR spectrometry. ^1H and ^{13}C NMR assignments were based on the ^1H-^1H COSY/ ^1H-^{13}C HSQC/ ^1H-^{13}C HMBC and ^1H-^1H NOESY experiments. For new compound **1**, the ^{13}C spectra was collected with the standard Bruker sequence "zgpg30", with the acquisition time 1.05 s and scan repetition number of 2048. The HSQC spectra was collected with the standard Bruker sequence "hsqcetgpsisp2", using time domain data of size as 2048 for F2 dimension and 156 for F1 dimension, scan repetition number of 2. The HMBC spectra was collected with the standard Bruker sequence "hmbcgpndqf", using time domain data of size as 2048 for F2 dimension and 220 for F1 dimension, scan repetition number of 16. The COSY spectra was collected with the standard Bruker sequence "cosygpmfppf", using time domain data of size as 2048 for F2 dimension and 320 for F1 dimension, scan repetition number of 1. The NOESY spectra collected with the standard Bruker sequence "noesyphpp" using time domain data of size as 2048 for F2 dimension and 320 for F1 dimension, scan repetition number of 8 and mixing time of 300 ms. Non-Uniform Sampling (NUS) was not used in the above acquisitions.

3. Biological material

The *Glossodoris atromarginata* (Figure 4) was collected manually along the coast of Weizhou Island, Guangxi Autonomous Region, China, in May 2009. Once obtained, the material was stored at -20 °C immediately until processed. A voucher specimen (No. WZ-55, 2009) was deposited at the Shanghai Institute of Materia Medica, CAS for inspection.

4. Extraction and isolation

The frozen specimens of *G. atromarginata* (21 individuals, dry weight 2.46 g) were sonicated in acetone (3×50ml) with 30min for each time to yield the mantle extract. Then dissected the mollusk and sonicated them repeatedly in acetone (3×30ml) with 30min for each time to yield the inner extract. Concentrating the mantle and inner

extract in vacuo and the aqueous residue was partitioned with ether (4×50ml) and butanol (4×50ml). After evaporation of the solvent, the mantle organs gave 189.3 mg of Et_2O-soluble extracts and 355.6 mg of *n*-BuOH-soluble extracts, while the internal organs provided 69.4 mg of Et_2O-soluble extracts and 77.8 mg of *n*-BuOH-soluble extracts, respectively. The mantle Et_2O-soluble portion (189.3 mg) was subjected to silica gel column chromatography (CC) using a petroleum ether (PE)/Et_2O gradient as eluent, fractions (A-D) were obtained based on the TLC method. Fraction B (24.4 mg) showed interesting purple and pink TLC spots after spraying with vanillin, and was subjected to silica gel CC (500-600 mesh, PE/Et_2O (8:2-7:3) to yield compound **1**(2.0 mg), compound **5** (2.5 mg). Fraction C (37.5 mg) also was subjected to silica gel CC (500-600 mesh, PE/ Et_2O (8:2-6:4) to yield compound **2** (2.0 mg). Fraction D (52.1mg) was subjected to silica gel CC (500-600 mesh, PE/ Et_2O (6:4-4:6) to yield compound **3** (2.5 mg), compound **4** (1.8 mg). In the above mentioned compounds, only compound **5** was also isolated from the internal organ Et_2O-soluble extract using the same chromatographic procedure as described for the mantle Et_2O-soluble extract.

Spongia-12-ene-3,15-dione (**1**): White powder; $[\alpha]_D^{25}$ 17.5 (c 0.08, $CHCl_3$); ^1H and ^{13}C NMR data ($CDCl_3$) see Table 1; HR-EI-MS *m/z* 316.2033 $[M]^+$(calcd. for $C_{20}H_{28}O_3$, 316.2038).

5. Elucidation of absolute configuration for compound 2 by TDDFT-ECD

ECD spectrum of compound **2** (2.0mg) was measured on a JASCO J-810 instrument. Compound **2** was dissolved in CH_3CN (xx). Data collection wavelength ranged 190nm to 400nm at room temperature. Linear data array is the average array of three measurement.

For the computational work, compound **2** was first subjected to energy minimization and conformational search using MacroModel which is integrated into Maestro V11.4 (Schrödinger Inc.)[18]. The OPLS3 force field with the implicit GB/SA

Octanol solvent model was employed. Only 1 conformer was generated under a 21 kJ/mol energy window. The above conformer was used as the initial file for optimization within the density functional theory (DFT) at basis set B3LYP/6-31G (d) level. After, executing the TDDFT calculation on the optimized geometry under B3LYP/6-311G (d, p) level with the IEFPCM solvent model with CH_3CN. All calculations in the present work were performed using the Gaussian 09[19].

Conflicts of interest

The authors declare no competing financial interest.

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Reference

[1] a) W. Zhang, Y.-W. Guo, *Shengtai Xuebao*,**2007**, *27* (3), 1192; b) G. Cimino, *Phytochem. Rev.***2010**, *9* (4), 547; c) A. Putz, G. M. Koenig, H. Waegele, *Nat. Prod. Rep.* **2010**, *27* (10), 1386.

[2] L. J. Dean, M. R. Prinsep, *Nat. Prod. Rep.***2017**, *34*, 1359.

[3] G. Cimino, M. Ciavatta, A. Fontana, M. Gavagnin, Metabolites of marine opisthobranchs: chemistry and biological activity. In: Tringali C (ed) Bioactive compounds from natural sources – isolation, characterization and biological properties. Taylor & Francis, London, pp. 579–637.

[4] a) E. Manzo, M. L. Ciavatta, M. Gavagnin, E. Mollo, Y.-W. Guo, G. Cimino, *J. Nat. Prod.***2004**, *67*, 1701; b) M. Gavagnin, E. Mollo, T. Docimo, Y.-W. Guo, G. Cimino, *J. Nat. Prod*. **2004**, *67*, 2104.

[5] J.-R. Wang, W.-F. He, Y.-W. Guo, *J. Asian Nat. Prod. Res.* **2013**, *15* (2), 185.

[6] a) E. D. De Silva, P. J. Scheuer, **1982**, *17*, 167; b) A. Fontana, P. Cavaliere, N. Ungur, L. D'Souza, P. S. Parameswaram, G. Cimino, *J. Nat. Prod.* **1999**, *62* (10), 1367.

[7] a) M. J. Somerville, E. Mollo, G. Cimino, W. Rungprom, M. J. Garson, *J. Nat.*

Prod. **2006,** *69* (7), 1086; b) K. W. Yong, I. W. Mudianta, K. L. Cheney, E. Mollo, J. T. Blanchfield, M. J. Garson, *J. Nat. Prod.* **2015**, *78* (3), 421.

[8] a)M.Carbone, Y. Li, C. Irace,E. Mollo, F. Castelluccio, A. D. Pascale, G. Cimino, R. Santamaria, Y.-W. Guo, M. Gavagnin,*Org. Lett.* **2011**, *13* (10), 2516; b) S.-C. Mao,M. Gavagnin, E. Mollo, Y.-W. Guo, *Syst. Ecol.* **2011**, *39* (4), 408; c) R.-Y. Huang, W.-T. Chen,T. Kurtán, A. Mándi, J. Ding, J. Li, X.-W. Li,Y.-W. Guo, *Future Med.Chem.* **2016**, *8* (1), 17.

[9] M. Carbone, M. Gavagnin, M. Haber, Y.-W. Guo, A. Fontana, E. Manzo, G. Genta-Jouve, M. Tsoukatou, W. B. Rudman, G. Cimino, *PloS one* **2013**, *8* (4), e62075.

[10] S. M. Parrish, W. Y. Yoshida, T. P. Kondratyuk, E.-J. Park, J. M. Pezzuto, M. Kelly, P. G. Williams, *J. Nat. Prod.* **2014**,*77* (7), 1644.

[11] A. Fontana, E. Mollo, D. Ricciardi, I. Fakhr, G. Cimino, *J. Nat. Prod.* **1997**, *60*, 444.

[12] G. Cimino, D. Derosa, S. Destefan, L. Minale, *Tetrahedron*,**1974**, *30*, 645.

[13] A. H. El-Desoky, H. Kato, I. Kagiyama, Y. Hitora, F. Losung, R. E. P. Mangindaan,N. J. de Voogd, S. Tsukamoto,*J. Nat. Prod.***2017**, *80*(1), 90.

[14] G.-Y. Han,D.-Y. Sun. L.-F. Liang, L.-G. Yao, K.-X. Chen. and Y.-W. Guo, *Fitoterapia*, **2018**, *127*, 159.

[15] a) S. E. Sameh,M. A. Ahmed, M. E. Ali, M. A. Abdulrahman, A. H. Hashim,A. A. Safwat,*Mar*. *Drugs*, **2016**, *14*(7), 130;b) K. Wirongrong, M. Chulabhorn,T. Pittaya,R. Somsak, P. Hunsa,*Mar*. *Drugs*,**2018**, *16*(12), 474.

[16] Y.-Y. Lai, M.-C. Lu, L.-H. Wang, J.-J. Chen, L.-S. Fang, Y.-C. Wu, P.-J. Sung, *Mar*. *Drugs*, **2015**, *13*(7), 4296.

[17] F. Cao, Z.-H.Wu, C.-L.Shao, S. Pang, X.-Y. Liang,N. J. de Voogdc, C.-Y. Wang, *Org*. *Biomol*. *Chem*.**2015**, *13*, 4016.

[18] Schrödinger Release 2018-4: MacroModel, Schrödinger, LLC, New York, NY, 2018.

[19] Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.

Table1. ^{1}H and ^{13}C NMR chemical shifts(δ in ppm) for compound **1** in $CDCl_3$[a].

position	δ_C		position	δ_H (mult, J in Hz)	
1	38.8	CH_2	1a	1.93	(ddd, 13.7, 7.2, 3.8)
			1b	1.43	(m)
2	33.9	CH_2	2a	2.56	(ddd, 15.0, 7.2, 3.7)
			2b	2.41	(ddd, 15.0, 6.9, 3.8)
3	217.0	C			
4	47.5	C			
5	55.6	CH		1.47	(m)
6	19.2	CH_2	6a	1.55	(m)
			6b	1.58	(m)
7	39.2	CH_2	7a	1.36	(m)
			7b	2.62	(dt, 13.7, 2.7, 2.7)
8	34.3	C			
9	53.4	CH		1.35	(dd, 11.4, 5.9)
10	36.9	C			
11	22.7	CH_2	11a	2.14	(m)
			11b	2.08	(m)
12	120.6	CH		5.74	(m)
13	129.8	C			
14	53.9	CH		2.78	(s)

15	175.0	C			
16	69.8	CH_2	16a	4.67	(m)
			16b	4.67	(m)
17	14.7	CH_3		0.90,	3H (s)
18	21.5	CH_3		1.08,	3H (s)
19	26.5	CH_3		1.11,	3H (s)
20	15.1	CH_3		1.05,	3H (s)

[a]Bruker-DRX-600 spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C NMR) in $CDCl_3$, chemical shifts (ppm) referred to $CHCl_3$ (δ_H 7.26 ppm) and to $CDCl_3$ (δ_C 77.16 ppm); assignments were deduced from analysis of 1D and 2D NMR spectra. Since the coupling constants of most signals of proton can not be extracted directly. The simulation chemical shift and coupling constants was added in Figure S1

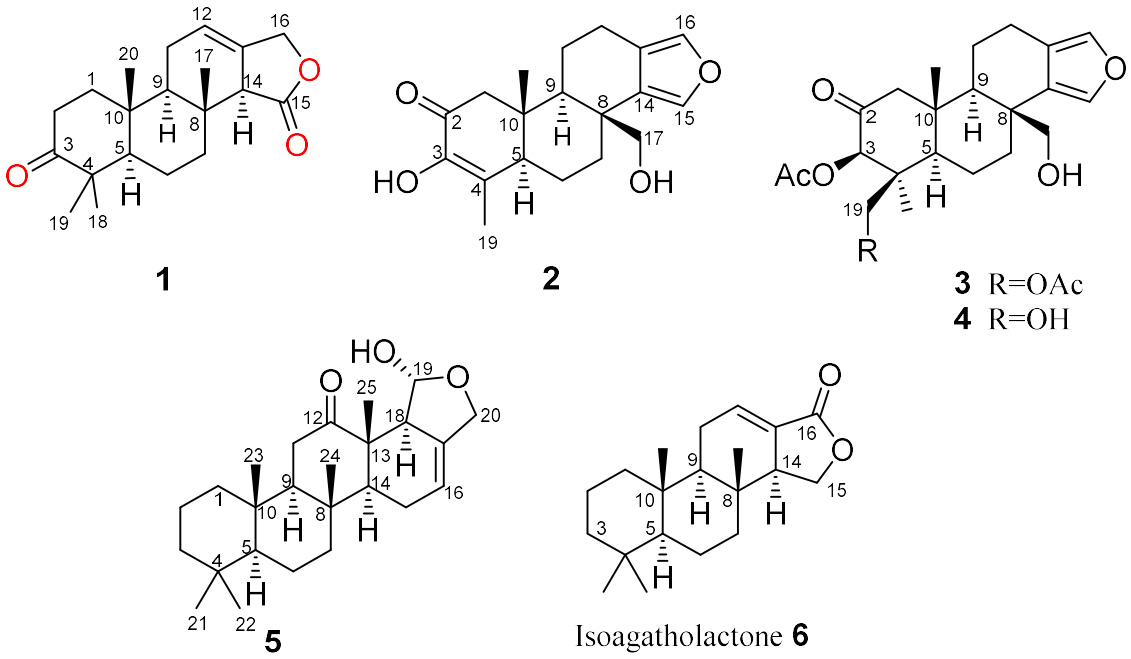
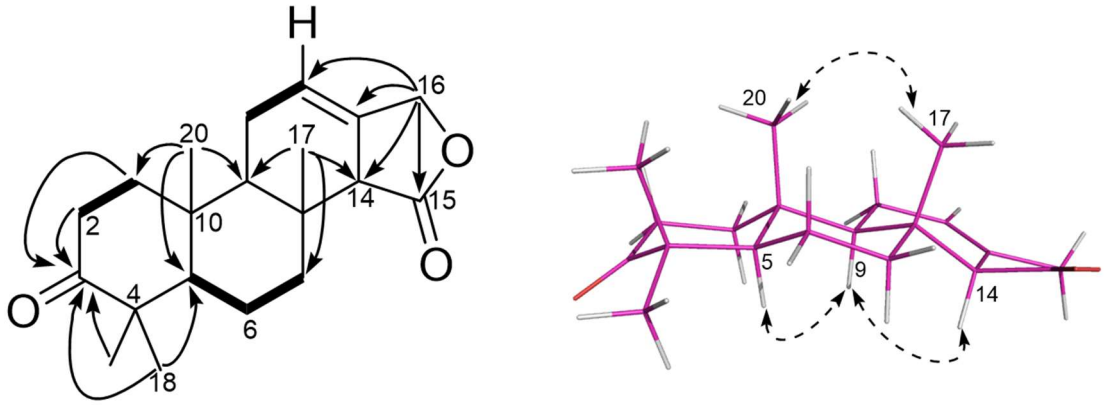


Figure 1. Structures of compounds **1**−**6**.



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328 **Figure2**. Key ^{1}H-^{1}H COSY (▬), ^{1}H-^{13}C HMBC (↘) and ^{1}H-^{1}H NOESY(⇠⇢)

329 correlations of compound **1**.

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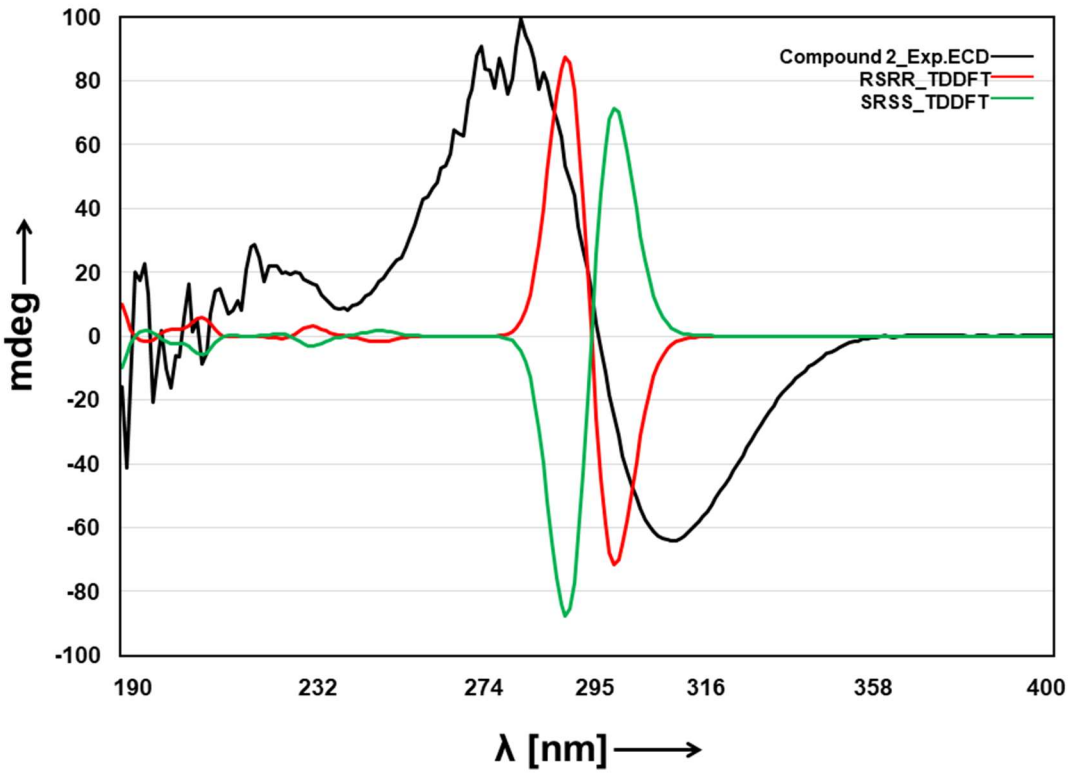

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334 **Figure3**. Experimental ECD spectrum (black line) of compound **2** and calculated ECD

335 spectrum for (5*R*, 8*S*, 9*R*, 10*R*)-**2** (red line) and (5*S*, 8*R*, 9*S*, 10*S*)-**2** (green line).

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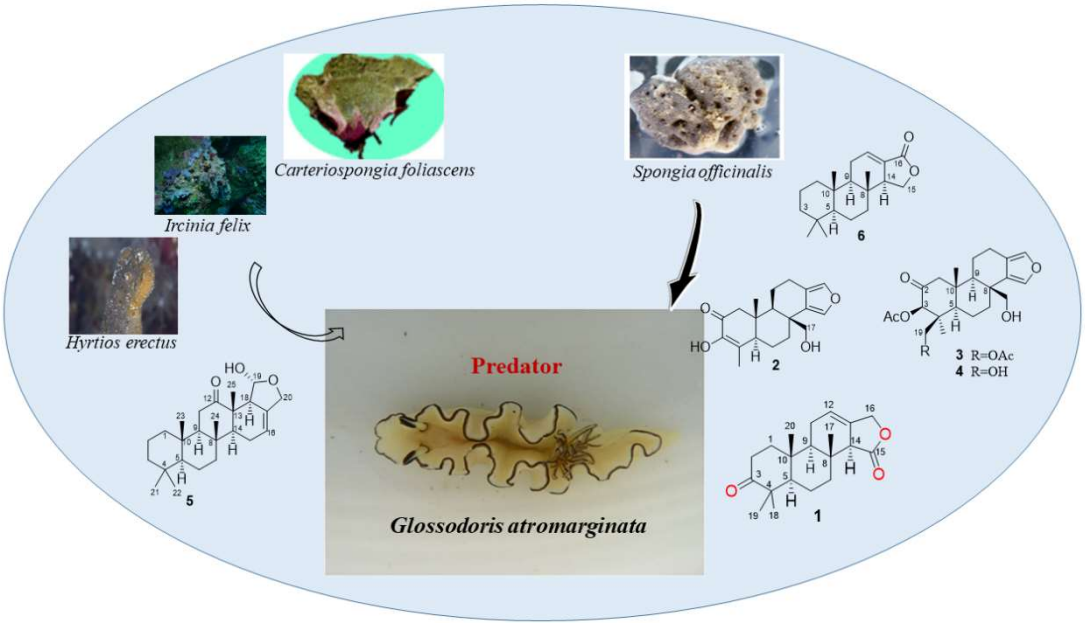

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339 **Figure 4**. The potential prey-predator relationship between *G. atromarginata* and other

340 marine organisms.