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ARTICLE TYPE

Structural diversity of terpenoids in the soft coral *Sinularia flexibilis*, evidence by a collection from the South China Sea

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Eight new terpenoids, including six α -methylene- δ -lactone-bearing cembranoids (**1-6**), a 15-membered macrocyclic diterpenoid (**7**), and a biscembranoid (**8**), were isolated from South China Sea soft coral *Sinularia flexibilis*, along with five known analogues (**9-13**). Their structures including relative stereochemistry were elucidated by detailed spectroscopic analyses, chemical reactions and by comparison with literature data. Further, the structures of **8** and **10** were unambiguously confirmed by X-ray diffraction analyses. Compound **7**, named epoxyflexibilene, represents the second 15-membered macrocyclic diterpenoid being discovered from marine sources, whereas sinulaflexiolide L (**8**) is the third member of the extremely rare cembrane dimers connected through C-C single bond. The discovery of these new isolates showed the high chemical diversity and ecological complexity of the animal *S. flexibilis* collected in different locations. In a bioassay, compound **9** exhibited potent anti-tumor activity targeting the inositol-requiring 1/X-box-binding protein 1 (IRE1 /XBP1) signaling pathway.

Introduction

Soft corals of the genus *Sinularia* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea, family Alcyoniidae) are prolific in the South China Sea, which have been well recognized as a rich source of bioactive substances with intriguing structural features, including rearranged terpenoids and biscembranoids.¹ The genus *Sinularia* consists of approximately 90 species, of which more than 50 have been chemically investigated. *S. flexibilis*, standing as a popular species of them, has long been attractive thanks to its highly diverse constitutions. Many terpenoids with broad structural variations have been isolated in *S. flexibilis* collected from Taiwan,²⁻⁵ North Queensland,⁶ Philippine,⁷ Hainan,⁸ etc.. It has been suggested that such soft coral species in different habitat comprise different secondary metabolites. For instance, furanoditerpenes are isolated from this species collected from Philippine⁶, while a structurally unique sulfur-contained biscembranoid, namely thioflexibilolide A, was only isolated from an Taiwan soft coral *S. flexibilis*.⁵ The diverse structure features are probably related to the defensive system of the soft coral against their enemies in various marine ecological environment,⁹ and these diverse secondary metabolites were highly biologically focused on, which were found to be significant bioactive, such as cytotoxic,^{2,10} antimicrobial,¹¹ and anti-inflammatory.¹² Therefore, *S. flexibilis* is always attractive for its chemical and biological investigation based on different collected locations. With this aim, and in the course of our continuing research on bioactive secondary metabolites from the South China Sea soft corals,¹³⁻¹⁷ we collected the specimen *S.*

flexibilis off Yalong Bay, Hainan Island. Chemical investigation of Et₂O-soluble fraction of the acetone extract of the title animal led to the isolation of six new α -methylene- δ -lactone-bearing cembranoids, 9 α -hydroxy-flexibilide (**1**), 15(17)-dehydromanaarenolide E (**2**), 8-dehydroxy-15(17)-dehydromanaarenolide E (**3**), 15(17)-dehydromanaarenolide A (**4**), 15(17)-dehydromanaarenolide C (**5**), *epi*-flexilarin A (**6**), a new 15-membered ring macrocyclic diterpenoid epoxyflexibilene (**7**), and a new biscembranoid sinulaflexiolide L (**8**), together with five known related cembrane derivatives (**9-13**) (Fig. 1). Among them, epoxyflexibilene (**7**) represents the second 15-membered macrocyclic diterpenoid being discovered from marine sources,⁶ and sinulaflexiolide L (**8**) is the third member of the rare cembrane dimers connected through C-C single bond.^{3,8} This paper addresses the isolation, structure elucidation of these compounds and their bioassay results.

Results and discussion

Specimens of *S. flexibilis* were immediately frozen to -20 °C and stored at that temperature before they were exhaustively extracted by acetone. The Et₂O-soluble portion of the extract was subjected to repeated column chromatography (silica gel, Sephadex LH-20, and reversed-phase HPLC) to yield 13 cembrane derivatives (**1-13**). The known compounds were identified as flexibilide (**9**),⁶ sinuflexolide (**10**),² 11-acetylsinuflexolide (**11**),¹⁸ capillolide (**12**),¹⁹ and sinulaflexiolide A (**13**)⁸ by analysis of their NMR spectroscopic data, along with comparison with those reported.

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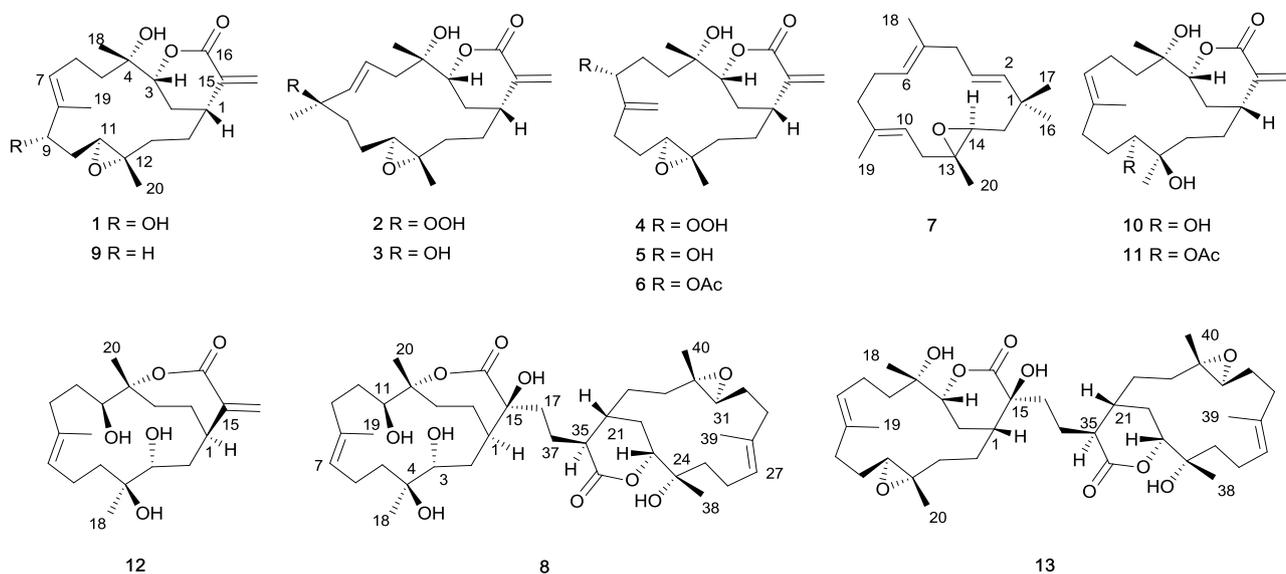


Fig. 1 Terpenoids from South China Sea soft coral *S. flexibilis*.

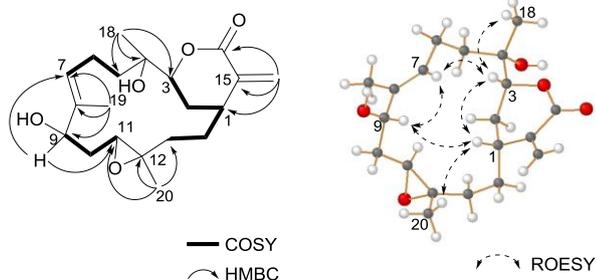


Fig. 2 H-¹H COSY, selected key HMBC (left) and ROESY (right) correlations of **1**.

Sinuflexolide (**10**) and 11-acetylsinuflexolide (**11**), exhibiting significant² and moderate¹⁸ cytotoxicity against the growth of human tumor cell lines, respectively, have only been reported for their relative configurations.^{2,18} With the aim to determine the absolute configurations of both **10** and **11**, a stereochemical study was conducted by using X-ray diffraction analysis and chemical reaction conversion. X-ray diffraction experiments with Cu-K α ($\lambda = 1.54178 \text{ \AA}$) radiation allowed the assignment of the absolute configurations to **10** as *1R, 3R, 4S, 11S, 12R*. The chemical conversion of **10** into **11** by simple acetylation in Ac₂O/pyridine indicated the absolute configuration of **11** is the same as **10**, which is also accordant with that of **9**.⁶

Compounds **1-6** showed IR absorptions indicative of the presence of hydroxyl groups (3360~3460 cm⁻¹) and α -methylene- δ -lactone moieties (1718~1731, 1611~1619 cm⁻¹). Their NMR spectra were reminiscent of those of flexibilide (**9**), which is most

frequently isolated in many soft corals belong to *Sinularia*. In fact, compounds **1-6** displayed the same structural unit extending from C-5 to C-1, C-9 to C-14 and C-1 to C-17, including two affiliated methyls at C-4 and C-12, an α,β -unsaturated- δ -lactone moiety, an epoxy group, and two oxygenated quaternary carbon atoms. The differences consist in either oxidative patterns, or isomerization and migration of double bonds.

9 α -hydroxy-flexibilide (**1**) was obtained as an optically active colorless oil. HRESIMS, ¹³C NMR and DEPT spectra established the molecular formula as C₂₀H₃₀O₅. A comparison of the NMR data of **1** with those of **9** revealed a close similarity with the exception of a methylene (δ_{H} 2.16; δ_{C} 35.8) in **9** replaced by a hydroxyl-bearing methine (δ_{H} 4.09; δ_{C} 75.3, CH) in **1**, which is in agreement with a plus of sixteen mass units in **1**. The location of the hydroxyl group was assigned at C-9 by a series of obvious proton connections of H-9/H-10/H-11 as established by a ¹H-¹H COSY experiment and HMBC correlations from H-9 (δ_{H} 4.09) to C-7, C-8, C-10, C-11 and C-19, respectively (Fig. 2). Due to the presence of 9-OH, ¹³C NMR data from C-7 to C-10 were downfield shifted, while that of C-11 was upfield shifted with respect to those of **9**.

The relative configuration of **1** was determined to be the same as that of **9** by ROESY experiment (Fig. 2). The correlations of H-3 (δ_{H} 3.97)/H-7 (δ_{H} 5.53), H-7/H-9 (δ_{H} 4.09), H-9/H-1 (δ_{H} 2.51), H-1/H-3 suggested that H-3, H-7, H-9 and H-1, are all co-facial, which could be arbitrarily assigned to be β -oriented. The cross-peaks of H-3/H₃-18 (δ_{H} 1.45), and H-1/H₃-20 (δ_{H} 1.36) were indicative of spatial proximity among these protons, implying that H₃-18 and H₃-20 were oriented in β face. Thus,

the structure of compound **4** was identified as 9 α -hydroxy-flexibilide.

On the basis of biogenetic considerations, the absolute configurations at C-1, C-3, C-4, C-11 and C-12 were tentatively suggested as *R, R, S, S,* and *S*, respectively. In consequence, the absolute configuration at C-9 was assigned as *S*.

The structure of compounds **2-6** were determined mainly by comparison of NMR, MS and optical rotation data with those of the known α -methylene- δ -lactone-bearing cembranoids, manaarenolide A, C, and E, previously reported from the same genus of Taiwan soft corals.²⁰

Table 1 ¹H NMR Assignments for **1-5** and **7**.^a

Position	1	2	3	4	5	7
1	2.51, m	2.35, m	2.38, m	2.56, m	2.60, m	
2	2.02, dd (14.0, 6.5)	2.02, m	2.02, m	2.02, m	2.02, m	5.40, d (15.8)
	1.39, m	1.37, m	1.38, m	1.37, m	1.38, m	
3	3.97, d (10.6)	4.03, d (11.1)	4.07, dd (11.1, 1.2)	3.95, d (10.7)	3.97, d (10.9)	5.46, dt (15.8, 6.3)
4						2.62, d (6.5)
5	1.88, m	2.51, d (15.6)	2.50, dt (15.4, 2.6)	1.68, m	1.67, m	
	1.63, m	2.38, dd (15.6, 10.5)	2.44, dd (15.4, 10.2)	1.58, m	1.58, m	
6	2.49, m	5.48, ddd	5.56, ddd	2.08, m	1.89, m	5.03, t (6.9)
	1.88, m	(16.2, 10.5, 2.5)	(15.3, 10.2, 2.6)	1.34, m	1.33, m	
7	5.53, t (8.1)	5.62, dd (16.2, 2.5)	5.71, dd (15.3, 2.6)	4.50, brs	4.32, brs	2.07, m
8						1.95, m
						2.13, m
						1.92, m
9	4.09, dd (11.1, 4.1)	2.18, m	1.85, m	2.43, m	2.30, m	
		1.67, m		2.11, m	2.19, m	
10	2.27, ddd	1.84, m	1.86, m	1.77, m	1.93, m	5.01, t (6.9)
	(14.2, 11.2, 3.3)	1.27, m	1.29, m	1.70, m	1.65, m	
	1.72, m					
11	2.50, m	2.86, d (7.3)	2.85, d (9.7)	2.93, dd (7.1, 4.0)	2.95, dd (8.9, 3.0)	2.14, m
12						1.92, m
						1.51, m
13	2.08, dt (14.1, 3.8)	2.08, m	2.13, m	2.09, m	2.08, m	
	1.16, dt (14.1, 3.8)	1.23, m	1.20, m	1.28, m	1.29, m	
14	1.91, m	1.83, m	2.20, m	1.90, m	1.90, m	2.79, dd (7.6, 3.2)
	1.50, m	1.25, m	1.48, m	1.26, m	1.27, m	
15						1.71, dd (14.3, 3.2)
						1.35, dd (14.3, 7.6)
16						1.10, s ^b
17	6.47, d (2.3)	6.58, d (2.5)	6.58, d (2.5)	6.50, s	6.48, d (2.4)	1.03, s ^b
	5.70, d (2.3)	5.71, d (2.5)	5.70, d (2.5)	5.70, s	5.70, d (5.7)	
18	1.45, s	1.52, s	1.54, s	1.39, s	1.39, s	1.61, s
19	1.74, s	1.39, s	1.32, s	5.35, s	5.37, s	1.54, s
				5.29, s	5.27, s	
20	1.36, s	1.25, s	1.23, s	1.32, s	1.33, s	1.25, s
OOH		7.9, brs		8.36, brs		

^a Bruker-DRX-400 spectrometer (400 MHz for ¹H NMR) in CDCl₃, chemical shifts (ppm) referred to CHCl₃ (δ_{H} 7.26) residual signal. ^b Proton coupling constants (*J*) in Hz are given in parentheses.

¹⁵ ^b Exchangeable.

15(17)-dehydromanaarenolide E (**2**), a white amorphous powder with a molecular formula of C₂₀H₃₀O₆ determined by HRESIMS (*m/z* 389.1927 [M+Na]⁺), has two mass units less than that of manaarenolide E. In fact, the only difference was at C-15 position, the linkage of a methyl group (δ_{H} 1.36; δ_{C} 16.2) in manaarenolide E was replaced by a terminal double bond (δ_{H} 6.58 and 5.70; δ_{C} 127.4) in **2**. The configuration of **2** was determined as (1*R*, 3*R*, 4*S*, 8*R*, 11*S*, 12*S*) by comparison of the NMR data with manaarenolide E (1*R*, 3*R*, 4*S*, 8*R*, 11*S*, 12*S*, 15*S*) and manaarenolide F (1*R*, 3*R*, 4*S*, 8*S*, 11*S*, 12*S*, 15*S*), which is also in accordance with the configuration of the related compounds **9** and **10** as discussed above, suggesting that **2** was the 15(17)-dehydro derivative of manaarenolide E.

The molecular weight of **3** [HRESIMS peak at *m/z* 373.2000 (M + Na)⁺, C₂₀H₃₀O₅], 8-dehydroxy-15(17)-dehydromanaarenolide E, was sixteen mass units less than that of

2. Careful comparison of ¹H and ¹³C NMR data of both compounds (Table 1 and 2) revealed that the only difference was the presence of a hydroxyl group at C-8 in **3** instead of a hydroperoxyl group in **2**. ¹H-¹H COSY, HSQC, and HMBC experiments allowed the unambiguous definition of the structure of **3**. Due to the reduction at 8-OOH, the chemical shift of C-8 was slightly downfield shifted from δ_{C} 73.3 to 83.9, which was further supported by chemical transformation. A reduction of **2** with triphenylphosphine yielded **3**, as an identical NMR spectroscopic data of the isolated product were observed. On the basis of above evidence, the structure of **3** was determined as the 8-dehydroxy derivative of 15(17)-dehydromanaarenolide E (**2**).

The molecular formula of compound **4**, 15(17)-dehydromanaarenolide A, was deduced to be C₂₀H₃₀O₆ by HRESIMS, ¹³C NMR and DEPT spectra. The diagnostic ¹H NMR signal appearing at δ_{H} 8.36 (s) suggested the presence of a

hydroperoxy group in **4**. A comparison of overall ^1H and ^{13}C NMR data (Table 1) of **4** with those of the model compound, manaarenolide A, revealed great similarities with the exception that the methyl group (δ_{H} 1.37; δ_{C} 17.1) in manaarenolide A replaced by a terminal double bond (δ_{H} 6.48 and 5.70; δ_{C} 128.4) in **4**. Therefore, the structure of **4** was identified as 15(17)-dehydromanaarenolide A.

Compound **5**, 15(17)-dehydromanaarenolide C, showed NMR spectroscopic data extremely similar to those of **4** (Tables 1 and 2), except for the upfield shifted (δ_{C} 72.7 δ_{H} 4.32) at C-7 with respect to that of **4** (δ_{C} 85.1 δ_{H} 4.50). Therefore, the hydroperoxy group attached at C-7 in **4** was assumed to be converted to a hydroxyl group in **5**, which was further supported by a loss of sixteen mass units than that of **4** as a HRESIMS peak at m/z 373.1985 ($\text{M} + \text{Na}$)⁺ observed in **5**. In fact, it also differs from another model compound, manaarenolide C, only by the presence of a double bond (δ_{H} 6.50 and 5.70; δ_{C} 128.3) in **5**, instead of a methyl group (δ_{H} 1.37; δ_{C} 17.3) at C-15/C-17 position. The configurations of **5** were elucidated to be the same as those of manaarenolide C by comparison of their ^{13}C NMR data. Accordingly, the structure of **5** was determined to be a 15(17)-dehydro derivative of manaarenolide C.

The molecular formula of compound **6** was established as $\text{C}_{22}\text{H}_{32}\text{O}_6$ by HRESIMS (m/z 415.2116 [$\text{M} + \text{Na}$]⁺). A literature investigation revealed that compound **6** is identical in all aspects, except for the optical rotation sign and magnitude, to a known cembranoid, flexilarin A, earlier reported from *S. flexibilis*.⁴ An opposite optical rotation observed of **6** {[α]_D²⁰ -38.0 (*c* 0.05, CH_2Cl_2)} as that of flexilarin A {[α]_D²⁰ +20.0 (*c* 0.20, CH_2Cl_2)}, indicated compound **6** to be an enantiomer of flexilarin A. In fact, we recorded twice the [α]_D value of compound **6** to confirm the correctness of our measurement. As a consequence, the structure of compound **6** was tentatively named as *epi*-flexilarin A.

The HREIMS spectrum of **7** exhibited a molecular peak at m/z 288.2466, consistent with the molecular formula $\text{C}_{20}\text{H}_{32}\text{O}$, implying five degrees of unsaturation. The ^{13}C NMR and DEPT data of **7** showed the presence of two trisubstituted double bonds,

one 1,1-disubstituted double bond and one trisubstituted epoxide, which accounted for four degrees of unsaturation. The remaining degree of unsaturation was assigned to be one ring in the molecule. The ^1H NMR spectrum of **7** displayed methyl singlets at δ_{H} 1.02 (s, 3H), 1.10 (s, 3H), 1.54 (s, 3H), 1.61 (s, 3H), 1.25 (s, 3H), which were assigned to two tertiary, two vinyl and a hydroxyl-bearing methyl groups. Analysis of the ^1H - ^1H COSY spectra readily identified four spin-spin systems: H-2 (δ_{H} 5.40) to H-3 (δ_{H} 5.46), H-3 to H-4 (δ_{H} 2.62); H-6 (δ_{H} 5.03) to H-7 (δ_{H} 2.07 and 1.95), H-7 to H-8 (δ_{H} 2.13 and 1.92); H-10 (δ_{H} 5.01) to H-11 (δ_{H} 2.14 and 1.92), H-11 to H-12 (δ_{H} 1.51) and H-14 (δ_{H} 2.79) to H-15 (δ_{H} 1.71 and 1.35). In the HMBC spectrum of **7**, the cross-peaks from Me-16 (δ_{H} 1.10) and Me-17 (δ_{H} 1.13) to C-1 (δ_{C} 35.6), C-2 (δ_{C} 140.9) and C-15 (δ_{C} 42.6) determined the position of these two tertiary methyl group; the correlations from Me-18 (δ_{H} 1.61) to C-4 (δ_{C} 41.4), C-5 (δ_{C} 134.5) and C-6 (δ_{C} 123.4), and from Me-19 (δ_{H} 1.54) to C-8 (δ_{C} 39.1), C-9 (δ_{C} 134.6) and C-10 (δ_{C} 125.4) indicated the position of the two vinyl methyl groups; finally, the position of the epoxide ring located at C-13/14 was supported by the cross-peaks from Me-20 (δ_{H} 1.25) to C-13 (δ_{C} 59.5), C-14 (δ_{C} 58.8) and C-15. On the basis of the above evidences, the molecular framework of **7** could be established as an extremely unusual 15-membered macrocyclic diterpenoid. Checking the literature revealed that there was only one natural product, flexibilene, which was previously isolated from the same soft coral species,^{6,21,22} possessing the same skeleton. In fact, only difference between **7** and flexibilene was that the trisubstituted double bond at $\Delta^{13/14}$ in flexibilene was replaced by an epoxy group (δ_{H} 2.79; δ_{C} 59.5 and 58.8) in **7**. Furthermore, on the basis of the ^{13}C NMR chemical shift for CH_3 -20 (δ_{C} 18.2, < 20 ppm),²³ the configuration of the epoxide ring was defined as *trans*. In addition, the *E* geometries for three olefins at $\Delta^{2/3, 5/6, 9/10}$, the same as that of flexibilene, were further secured by either the large coupling constant observed between H-2 and H-3 ($J = 15.8$ Hz) or ^{13}C NMR chemical shift for CH_3 -18 (δ_{C} 17.7, < 20 ppm) and CH_3 -19 (δ_{C} 15.6, < 20 ppm).²⁴ Thus, structure of **7**, named epoxyflexibilene, was unambiguously determined.

Table 2 ^{13}C NMR Assignments for **1-5** and **7**.^a

position	1	2	3	4	5	7
1	33.7, CH	34.5, CH	34.6, CH	34.5, CH	34.7, CH	35.6, C
2	27.6, CH ₂	26.2, CH ₂	26.2, CH ₂	26.9, CH ₂	25.7, CH ₂	140.9, CH
3	84.5, CH	83.5, CH	83.7, CH	82.1, CH	82.0, CH	125.0, CH
4	73.9, C	72.6, C	72.6, C	72.3, C	72.6, C	41.4, CH ₂
5	38.4, CH ₂	42.4, CH ₂	41.7, CH ₂	30.0, CH ₂	30.6, CH ₂	134.5, C
6	22.4, CH ₂	122.5, CH	120.9, CH	27.8, CH ₂	26.8, CH ₂	123.4, CH
7	128.9, CH	135.7, CH	139.2, CH	72.7, C	85.1, C	22.1, CH ₂
8	138.1, C	83.9, C	73.3, C	146.8, C	142.0, C	39.1, CH ₂
9	75.3, CH	33.0, CH ₂	38.4, CH ₂	32.2, CH ₂	31.7, CH ₂	134.6, C
10	33.3, CH ₂	22.7, CH ₂	23.0, CH ₂	23.4, CH ₂	24.0, CH ₂	125.4, CH
11	60.8, CH	65.5, CH	65.8, CH	64.0, CH	63.6, CH	24.3, CH ₂
12	59.1, C	59.4, C	59.4, C	59.3, C	59.2, C	37.2, CH ₂
13	34.5, CH ₂	34.8, CH ₂	34.8, CH ₂	34.3, CH ₂	34.4, CH ₂	59.5, C
14	33.4, CH ₂	29.7, CH ₂	29.5, CH ₂	28.8, CH ₂	30.4, CH ₂	58.8, CH
15	139.9, C	139.2, C	139.2, C	139.9, C	139.7, C	42.6, CH ₂
16	167.2, C	166.0, C	166.0, C	167.2, C	167.0, C	26.6, CH ₃ ^b
17	128.4, CH ₂	127.4, CH ₂	127.3, CH ₂	128.3, CH ₂	128.4, CH ₂	30.0, CH ₃ ^b
18	24.7, CH ₃	25.2, CH ₃	25.2, CH ₃	24.6, CH ₃	24.6, CH ₃	17.7, CH ₃
19	10.6, CH ₃	25.1, CH ₃	31.2, CH ₃	110.0, CH ₂	112.7, CH ₂	15.6, CH ₃
20	15.5, CH ₃	15.3, CH ₃	15.2, CH ₃	15.6, CH ₃	15.6, CH ₃	18.2, CH ₃

^a Bruker-DRX-400 spectrometer (100 MHz for ^{13}C NMR) in CDCl_3 , chemical shifts (ppm) referred to CDCl_3 (δ_{C} 77.0).

^b Exchangeable.

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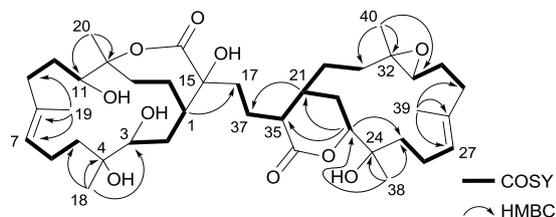


Fig. 3 ^1H - ^1H COSY, selected key HMBC correlations of **8**.

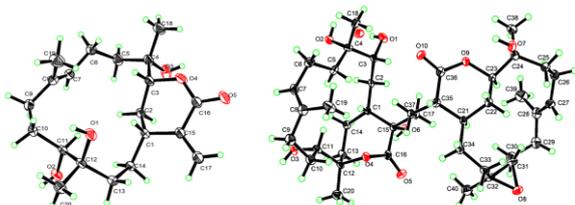


Fig. 4 Perspective drawing of X-ray structures of **10** (left) and **8** (right).

5 Sinulaflexiolide L (**8**) was obtained as colorless crystals, mp 240–242 °C. Analysis of HREIMS, ^{13}C NMR and DEPT data revealed a molecular formula of $\text{C}_{40}\text{H}_{64}\text{O}_{10}$. Its ^1H -NMR spectrum (Table 3) showed signals attributable to two lactonic carbonyl groups (δ_{C} 183.4, 173.8), a lactonic methine [δ_{C} 84.2 (CH) and δ_{H} 4.26 (1H, d, $J = 9.9$ Hz)], a lactonic quaternary carbon (δ_{C} 88.2), two trisubstituted double bonds [δ_{C} 128.5 (CH), 133.5 (C); 126.5 (CH), 133.4 (C) and δ_{H} 5.42 (1H, d, $J = 9.5$ Hz); 5.28 (1H, t, $J = 7.1$ Hz)], three tertiary hydroxyls [δ_{C} 75.1 (C),

73.9 (C) and 80.6 (C)], two secondary hydroxyls [δ_{C} 75.4 (CH), 15 66.9 (CH) and δ_{H} 4.35 (1H, d, $J = 7.9$ Hz), 4.68 (1H, t, $J = 8.8$ Hz)], an epoxymethine [δ_{C} 63.0 (CH) and δ_{H} 3.02 (1H, t, $J = 5.7$ Hz)], an epoxydic quaternary carbon (δ_{C} 59.0), three methines and sixteen methylenes. The presence of six tertiary methyl groups in the molecule was deduced by ^1H NMR signals at δ_{H} 1.88 (3H, s 20 H_3 -19), 1.69 (3H, s H_3 -18), 1.65 (3H, s H_3 -20), 1.61 (3H, s H_3 -38), 1.54 (3H, s H_3 -39) and 1.43 (3H, s H_3 -40), respectively. Based on the above observations, **8** was suggested to be a biscembranoid similar to those previously reported from soft coral of the same genus, such as sinuflexlin³ and sinulaflexiolide 25 A (**13**).⁸ All NMR resonances of **8** were assigned by analysis of 2D NMR spectra as reported in Table 3. Selected ^1H - ^1H COSY and HMBC correlations of compound **8** are reported in Figure 3. Fortunately, a single-crystal of **8** suitable for X-ray diffraction analysis was obtained from the methanol solution. The X-ray 30 result (Figure 4) clearly indicated that compound **8** could biogenetically derive from two different cembranoid units, **9** and **12**, probably through an *oxo*-Diels-Alder reaction and a subsequent hydrolysis as suggested for another biscembranoid, sinuflexlin³ deriving from two units of **9**.

35 With the biogenetic consideration, and by analogy to **9**, the absolute configurations of **8** at C-21, C-23, C-24, C-31 and C-32 were tentatively assigned as *R*, *R*, *S*, *S*, and *S*, respectively, and consequently, the absolute configuration of **8** was suggested as (1*R*, 3*R*, 4*S*, 11*S*, 12*R*, 15*S*, 21*R*, 23*R*, 24*S*, 31*S*, 32*S*, 35*S*).

40 Table 3 ^1H (400 MHz)^a and ^{13}C (100 MHz)^b NMR Spectroscopic Data of Compound **8** in Pyr- d_5 .

Position	δ_{C} , type	δ_{H} , mult. (J in Hz)	Position	δ_{C} , type	δ_{H} , mult. (J in Hz)
1	38.6, CH	2.63, m	21	32.6, CH	2.37, m
2	27.9, CH ₂	2.34, m; 1.64, m	22	28.0, CH ₂	2.24, m; 1.73, m
3	75.4, CH	4.35, d (7.9)	23	84.2, CH	4.26, d (9.9)
4	75.1, C		24	73.9, C	
5	39.8, CH ₂	2.14, m; 1.80, m	25	40.2, CH ₂	1.93, m
6	23.8, CH ₂	2.50, m; 2.30, m	26	23.2, CH ₂	2.22, m; 1.95, m
7	128.5, CH	5.42, d (9.5)	27	126.5, CH	5.28 t (7.1)
8	133.4, C		28	133.5, C	
9	36.5, CH ₂	2.26, m	29	35.9, CH ₂	2.07, m
10	25.3, CH ₂	1.69, m	30	25.6, CH ₂	1.86, m; 1.62, m
11	66.9, CH	4.68, d (8.8)	31	63.0, CH	3.02, t (5.7)
12	88.2, C		32	59.0, C	
13	32.4, CH ₂	2.14, m; 1.84, m	33	35.2, CH ₂	1.97, m; 1.32, m
14	28.6, CH ₂	2.51, m; 1.92, m	34	31.8, CH ₂	1.85, m; 1.05, m
15	80.6, C		35	48.1, CH	2.42, m
16	183.4, C		36	173.8, C	
17	36.2, CH ₂	2.54, m; 2.46, m	37	36.0, CH ₂	2.33, m
18	25.2, CH ₃	1.69, s	38	25.0, CH ₃	1.61, s
19	16.2, CH ₃	1.88, s	39	16.1, CH ₃	1.54, s
20	21.9, CH ₃	1.65, s	40	16.0, CH ₃	1.43, s
3-OH		5.24, brs	15-OH		7.32, brs
4-OH		6.38, brs ^c	24-OH		5.11, brs ^c
11-OH		6.49, brs			

^a Bruker-DRX-400 spectrometer (400 MHz for ^1H NMR) in Pyr- d_5 , chemical shifts (ppm) referred to pyridine (δ_{H} 8.72, 7.57, 7.20) residual signal. Proton coupling constants (J) in Hz are given in parentheses.

^b Bruker-DRX-400 spectrometer (100 MHz for ^{13}C NMR) in Pyr- d_5 , chemical shifts (ppm) referred to pyridine (δ_{C} 149.9, 135.5, 123.5).

^c Exchangeable.

The abundant production and accumulation of cembrane terpenes (**9**, **10**, **12** and **13**, 4 analogues reported in this paper) in this specimen of *S. flexibilis* is intriguing. The highest production of flexibilide (**9**) in the title animalis not only widely distributed in most colonies of *S. flexibilis*, but also well-known for its antifouling, allelopathy and cytotoxic activity.⁹ Thus, all the compounds related to **9** (**1-9** and **13**) were tested against a panel of tumor cell lines including HL-60, A-549 and HCT-116, as well as X-box binding protein 1 (XBP1), a novel targeting as anti-tumor strategy.²⁵ The result revealed that only flexibilide (**9**) showed significant activity. In particular, **9** exhibited potent anti-tumor activity targeting the inositol-requiring 1/X-box-binding protein 1 (IRE1 /XBP1) signaling pathway, with an IC₅₀ value of 4.10 μg/mL.

Experimental

General Experimental Procedures.

Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. IR spectra were recorded on a Nicolet-Magna FT-IR 750 spectrometer. HRESIMS spectra were recorded on a Q-TOF Micro LC-MS-MS mass spectrometer. The NMR spectra were measured on a Bruker DRX-400 spectrometer with the residual CHCl₃ (δ_{H} 7.26 ppm, δ_{C} 77.0 ppm) or pyridine (δ_{H} 8.72, 7.57, 7.20 ppm, δ_{C} 149.9, 135.5, 123.5 ppm) as internal standard. Chemical shifts are expressed in δ (ppm) and coupling constants (*J*) in Hz. ¹H and ¹³C NMR assignments were supported by ¹H-¹H COSY, HSQC, HMBC and ROESY experiments. Commercial Silica gel (Qing Dao Hai Yang Chemical Group Co., 200-300 and 400-600 mesh) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography. Reversed phase HPLC (Agilent 1100 series liquid chromatography using a VWDG1314A detector at 210 nm and a semi-preparative ODS-HG-5 [5 μm, 10 mm (i.d.)×25 cm] column) was also employed.

Biological Material.

The soft corals *S. flexibilis* was collected by scuba at Yalong Bay, Hainan Province, China, in February 26, 2006, at a depth of -15 to -20 m, and identified by Professor R.-L. Zhou of South China Sea Institute of Oceanology, Chinese Academy of Sciences. The voucher sample is deposited at the Shanghai Institute of Materia Medica, CAS, under registration No. YAL-19.

Extraction and Separation.

The lyophilized bodies of *S. flexibilis* (220 g, dry weight) were minced into pieces and exhaustively extracted with Me₂CO at room temperature (3×1 L). The solvent-free Me₂CO extract was partitioned between Et₂O and H₂O. The organic phase was evaporated under reduced pressure to give a dark brown residue (10 g), which was subjected to Si gel column chromatography (CC) and eluted with petroleum ether (PE) in Et₂O (0-100%, gradient) to yield 9 fractions (A-I). Fraction B was chromatographed over Sephadex LH-20 eluting with PE/CHCl₃/MeOH (2:1:1), followed by silica gel CC (PE/Me₂CO, 200:1 to 50:1) to afford **7** (7.3 mg). Fraction E eluted with PE/Et₂O (6:4) to yield **9** (2001.7 mg). Fraction F was divided into

six subfractions (F1-F6) by silica gel CC (PE/Me₂O, 7:3 to 5:5), each of which was chromatographed over Sephadex LH-20 eluting with PE/CHCl₃/MeOH (2:1:1), followed by RP-HPLC (MeOH/H₂O, 58:42; MeOH/H₂O, 65:35; MeOH/H₂O, 68:32, respectively) to afford **2** (3.1 mg), **4** (2.3 mg), **6** (1.5 mg), **11** (3.9 mg). Fraction G eluted with silica gel CC (CHCl₂/MeOH, 400:1 to 90:1) to give seven subfractions (G1-G7), each of which was separated by a column of Sephadex LH-20 eluting with PE/CHCl₃/MeOH (2:1:1) and RP-HPLC (MeOH/H₂O, 49:51; MeOH/H₂O, 75:25, respectively) to yield **1** (1.4 mg), **5** (2.0 mg), **10** (2.8 mg), **12** (9.0 mg), **13** (2.4 mg). Fraction H was chromatographed over Sephadex LH-20 eluting with PE/CHCl₃/MeOH (2:1:1), followed by RP-HPLC (MeOH/H₂O, 54:46) to afford **3** (1.9 mg). Finally, fraction I eluted with silica gel CC (CHCl₂/MeOH, 9:1 to 8:1) and was further purified by RP HPLC (MeOH/H₂O, 50:50) to afford **8** (2.0 mg).

9 α -hydroxy-flexibilide (1): colorless oil; [α]_D²⁰ -84.0 (c 0.10, EtOH); IR (KBr): ν_{max} /cm⁻¹ 3584, 1731, 1615, 1453; ¹H and ¹³C NMR data, see Table 1 and 2; HRESIMS *m/z* 373.1998 [M+Na]⁺ (calcd. for C₂₀H₃₀O₅ 373.1991).

15,17-dedihydrimanaarenolide E (2): white amorphous power; [α]_D²⁰ -8.7 (c 0.15, EtOH); IR (KBr): ν_{max} /cm⁻¹ 3384, 1720, 1631, 1611; ¹H and ¹³C NMR data, see Table 1 and 2; HRESIMS *m/z* 389.1927 [M+Na]⁺ (calcd for C₂₀H₃₀O₆ 389.1940).

8-dehydroxy-15,17-dedihydrimanaarenolide E (3): white amorphous power; [α]_D²⁰ -21.0 (c 0.10, EtOH); IR (KBr): ν_{max} /cm⁻¹ 3418, 1723, 1628, 1611; ¹H and ¹³C NMR data, see Table 1 and 2; HRESIMS *m/z* 373.2000 [M+Na]⁺ (calcd for C₂₀H₃₀O₅ 373.1991).

15,17-dedihydrimanaarenolide A (4): a white amorphous power; [α]_D²⁰ -73.0 (c 0.10, EtOH); IR (KBr): ν_{max} /cm⁻¹ 3360, 1722, 1618; ¹H and ¹³C NMR data, see Table 1 and 2; HRESIMS *m/z* 389.1934 [M+Na]⁺ (calcd for C₂₀H₃₀O₆ 389.1940).

15,17-dedihydrimanaarenolide C (5): a white amorphous power; [α]_D²⁰ -35.0 (c 0.22, EtOH); IR (KBr): ν_{max} /cm⁻¹ 3404, 1720, 1618; ¹H and ¹³C NMR data, see Table 1 and 2; HRESIMS *m/z* 373.1985 [M+Na]⁺ (calcd for C₂₀H₃₀O₅ 373.1991).

Epi-flexilarin A (6): a white amorphous power; [α]_D²⁰ -38.0 (c 0.05, CH₂Cl₂); IR (KBr): ν_{max} /cm⁻¹ 3400, 1718, 1618, 1456; ¹H NMR data, see Table 1; HRESIMS *m/z* 415.2116 [M+Na]⁺ (calcd for C₂₂H₃₂O₆ 415.2097).

Epoxyflexibilene (7): colorless oil; [α]_D²⁰ +30.4 (c 0.31, CHCl₃); IR (KBr): ν_{max} /cm⁻¹ 1442, 1380, 965; ¹H and ¹³C NMR data, see Table 1 and 2; HREIMS *m/z* 288.2466 (calcd. for C₂₀H₃₂O 288.2466).

Sinulaflexilide L (8): colorless crystals; [α]_D²⁰ -18.0 (c 0.10, EtOH); IR (KBr): ν_{max} /cm⁻¹ 3495, 2925, 1737, 1697, 1344, 1081; ¹H and ¹³C NMR data, see Table 3; HRESIMS *m/z* 727.4396 [M+Na]⁺ (calcd for C₄₀H₆₄O₁₀ 727.4397).

Acetylation of Sinuflexolide (10).

To a dry pyridine (0.5 mL) of **10** (1.0 mg) was added 1 drop of Ac₂O. The mixture was stirred at room temperature overnight, and then stopped by adding 1 drop of H₂O. The crude acetylated products, after evaporating the solvent *in vacuo*, were purified on silica gel column chromatography (PE/Me₂CO in gradient), to

afford the expected di-acetate **11**. Physical and spectroscopic data of this product were found to be in full agreement with those of the natural product **11**.

5 Reduction of 15,17-didehydromanaarenolide E (2).

2 (1.0 mg) was stirred with 4 mg of triphenylphosphine in 2.5 mL of ether for 4 h at room temperature. After evaporation of excess reagent, the residue was separated by short column chromatography on silica gel (CH₂Cl₂-MeOH in gradient) to give a reduced product of **2**. Physical and spectroscopic data of this product were found to be in full agreement with those of the natural product **3**.

X-ray Crystallographic Studies of Sinuflexolide (10).

Colorless block crystals of **10** were obtained by recrystallization in Me₂CO-H₂O mixtures. Crystal data were obtained on a Bruker APEX- II CCD diffractometer with monochromated Cu K_α radiation ($\lambda = 1.54178 \text{ \AA}$). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters.

Crystal data for 10. C₂₀H₃₂O₅ ($M_r = 352.46$), orthorhombic system, space group $P2_12_12_1$ with $a = 10.2341(15) \text{ \AA}$, $b = 10.4857(16) \text{ \AA}$, $c = 10.2341(15) \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 117^\circ$, $\gamma = 90^\circ$, $V = 978.8(3) \text{ \AA}^3$, $T = 296 \text{ K}$, $Z = 4$, $D_{\text{calcd}} = 1.196 \text{ mg/m}^3$, $\lambda = 1.54178 \text{ \AA}$, $F(000) = 384$. Crystal size: $0.200 \times 0.300 \times 0.400 \text{ mm}^3$. Independent reflections 2978 [$R(\text{int}) = 0.0828$]. The final indices were $R_1 = 0.0528$, $wR_2 = 0.1362$ [$I > 2\sigma(I)$]. The absolute configuration was determined on the basis of a Flack parameter of $-0.1(2)$. Crystallographic data for **10** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under reference number CCDC 1040484. The data can be obtained free of charge at <http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi>, or from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44-1223-336-033; e-mail: data_request@ccdc.cam.ac.uk

40 X-ray Crystallographic Studies of Sinulaflexiolide L (8).

Colorless block crystals of **8** were obtained by recrystallization in MeOH-H₂O mixtures. Crystal data were obtained on a Bruker APEX- II CCD diffractometer with monochromated Mo K_α radiation. The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters.

Crystal data for 8. C₄₀H₆₄O₁₀ H₂O ($M_r = 722.93$), orthorhombic system, space group $P2_12_12_1$ with $a = 17.9396(13) \text{ \AA}$, $b = 8.5245(7) \text{ \AA}$, $c = 24.5138(19) \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 99^\circ$, $\gamma = 90^\circ$, $V = 3704.8(5) \text{ \AA}^3$, $T = 100 \text{ K}$, $Z = 2$, $D_{\text{calcd}} = 1.296 \text{ mg/m}^3$, $\lambda = 0.71073 \text{ \AA}$, $F(000) = 1576$. Crystal size: $0.120 \times 0.150 \times 0.230 \text{ mm}^3$. Independent reflections 6362 [$R(\text{int}) = 0.0945$]. The final indices were $R_1 = 0.0526$, $wR_2 = 0.0932$ [$I > 2\sigma(I)$]. Crystallographic data for **8** reported in this paper have been

deposited at the Cambridge Crystallographic Data Centre, under reference number CCDC 1040659.

60 Cytotoxicity Bioassays.

The cytotoxicities of compounds **1-9** and **13** against human promyelocytic leukemia cell lines HL60, human lung adenocarcinoma A-549, human colon carcinoma HCT-116 cell lines, and IRE1 /XBP1 signaling pathway were evaluated by using the MTT,²⁶ SRB²⁷ and B16-F10-XBP1-DBD-Luc²⁸ methods, respectively, according to the protocols described in previous literature.

Conclusion

In conclusion, eight new and five known terpenoids belonging to three different structural classes were isolated from the Hainan soft coral *S. flexibilis*. The first structural class, including six new (**1-6**) and three known cembranoids (**9-11**), all possesses a typical common α -methylene- δ -lactone group. Since these cembranoids are mainly isolated from soft corals of genus *Sinularia*,^{2-6,8,18,20,29} they are commonly regarded as chemotaxonomic markers of this genus. Further, the absolute configurations of two known compounds, sinuflexolide (**10**)² and its acetylated derivative, 11-acetylsinuflexolide (**11**),¹⁸ both previously isolated from the same species of Taiwan origin, have been unambiguously determined firstly by Cu-K_α X-ray diffraction analysis [Flack parameters: $-0.1(2)$] on the crystal of **10** and then successively by chemical conversion of **10** to **11** in the present work, as a completion for their full structural elucidation. Epoxyflexibilene (**7**), represents the second example of 15-membered ring macrocyclic diterpenoid that have ever discovered from marine organism. In fact, the first 15-membered diterpene, flexibilene, a formal precursor of **7**, was found from the same species but three different locations, Kisser Island,²¹ the Great Barrier Reef,⁶ and Indian Ocean.²² Interestingly, this is the first time that such kind of macrocyclic diterpenoid was isolated from the South China Sea collection. The third structural class, exemplified by sinulaflexiolide L (**8**), displays the extremely rare dimeric cembrane skeleton formally connected through C-C single bond of two cembrane monomers. It may be worth to point out that up to now, there are only two biscembranoids, sinuflexlin from Taiwan *S. flexibilis*³ and sinulaflexiolide A (**13**) from Hainan *S. flexibilis*,⁸ possessing the same skeleton, were reported. Compound **8** is the third member of this unusual biscembranoids family.

The discovery of new compounds **1-8** from *S. flexibilis* collected in the South China Sea is not only an addition of a diverse and complex series of terpenoids to this species, but also provides the evidence of the powerful adaptive capacity of this animal towards various marine ecological environment by the production of diverse secondary metabolites. Further studies would be intriguing and challenging on the understanding of this chemical ecological influence in a genetic point of view, and exploring the biological role of these second metabolites in the life cycle of the title animal. Chemical methods, such as total synthesis or structure modifications, could also be interesting to be involved in the structure determination and structure-activity relationship study of these structurally intriguing and biologically

active terpenoids.

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- 1 W.-T. Chen, Y. Li and Y.-W. Guo, *Acta Pharma. Sinica B* 2012, **2**, 227-237.
- 2 C.-Y. Duh, S.-K. Wang, H.-K. Tseng, J.-H. Sheu and M.-Y. Chiang, *J. Nat. Prod.* 1998, **61**, 844-847.
- 25 3 C.-Y. Duh, S.-K. Wang, H.-K. Tseng and J.-H. Sheu, *Tetrahedron Lett.* 1998, **39**, 7121-7122.
- 4 Y.-S. Lin, C.-H. Chen, C.-C. Liaw, Y.-C. Chen, Y.-H. Kuo and Y.-C. Shen, *Tetrahedron* 2009, **65**, 9157-9164.
- 5 B.-W. Chen, C.-H. Chao, J.-H. Su, C.-Y. Huang, C.-F. Dai, Z.-H. Wen and J.-H. Sheu, *Tetrahedron Lett.* 2010, **51**, 5764-5766.
- 30 6 R. Kazlauskas, P. T. Murphy, R. J. Wells, P. Schonholzer and J. C. Coll, *Aust. J. Chem.* 1978, **31**, 1817-1824.
- 7 P. P. Guerrero, R. W. Read, M. Batley and G. C. Janairo, *J. Nat. Prod.* 1995, **58**, 1185-1191.
- 35 8 T. Wen, Y. Ding, Z.-W. Deng, L.-P. Van Ofwegen, P. Proksch and W.-H. Lin, *J. Nat. Prod.* 2008, **71**, 1133-1140.
- 9 J. C. Coll, *Chem. Rev.* 1992, **92**, 613-631.
- 10 T.-R. Su, J.-J. Lin, C.-C. Chiu, J. Y. F. Chen, J.-H. Su, Z.-J. Cheng, W.-I. Hwang, H.-H. Huang and Y.-J. Wu, *Electrophoresis* 2012, **33**, 1139-1152.
- 40 11 T. L. Aceret, J. C. Coll, Y. Uchio and P. W. Sammarco, *Comp. Biochem. Phys. C* 1998, **120**, 121-126.
- 12 P. J. Buckle, B. A. Baldo and K. M. Taylor, *Agents Actions* 1980, **10**, 361-367.
- 45 13 L.-F. Liang, X.-J. Wang, H.-Y. Zhang, H.-L. Liu, J. Li, L.-F. Lan, W. Zhang and Y.-W. Guo, *Bioorg. Med. Chem. Lett.* 2013, **23**, 1334-1337.
- 14 S. Qin, H. Huang and Y.-W. Guo, *J Asian Nat. Prod. Res.* 2008, **10**, 1075-1079.
- 50 15 Y. Li, H. Huang and Y.-W. Guo, *Nat. Prod. Res.* 2008, **22**, 1359-1364.
- 16 Y. Li, A.-H. Gao, H. Huang, J. Li, E. Mollo, M. Gavagnin, G. Cimino, Y.-C. Gu and Y.-W. Guo, *Helv. Chim. Acta* 2009, **92**, 1341-1348
- 55 17 Y. Li, M. Carbone, R. M. Vitale, P. Amodeo, F. Castelluccio, G. Sicilia, E. Mollo, M. Nappo, G. Cimino, Y.-W. Guo and M. Gavagnin, *J. Nat. Prod.* 2010, **73**, 133-138.
- 18 C.-C. Su, B.-S. Wong, C. Chin, Y.-J. Wu and J.-H. Su, *Int. J. Mol. Sci.* 2013, **14**, 4317-4325.
- 60 19 J.-Y. Su, R.-L. Yang, Y.-Y. Kuang and L.-M. Zeng, *J Nat. Prod.* 2000, **63**, 1543-1545.
- 20 J.-H. Su, A. F. Ahmed, P.-J. Sung, C.-H. Chao, Y.-H. Kuo and J.-H. Sheu, *J Nat. Prod.* 2006, **69**, 1134-1139.
- 21 A. S. R. Anjaneyulu, K. S. Sagar and G.V. Rao, *J. Nat. Prod.* 1997, **60**, 9-12.

- 22 M. Herin, M. Colin and B. Tursch, *Bull. Soc. Chim. Belg.* 1976, **85**, 801-803.
- 23 B. N. Ravi and D. John Faulkner, *J. Org. Chem.* 1978, **43**, 2127-2131.
- 70 24 T. Iwagawa, R. Nakashima, K. Takayama, H. Okamura, M. Nakatani, M. Doe and K. Shibata, *J. Nat. Prod.* 1999, **62**, 1046-1049.
- 25 A. C. Koong, V. Chauhan and L. Romero-Ramirez, *Cancer Biol. Ther.* 2006, **5**, 756-759.
- 75 26 M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker and M. R. Boyd, *Cancer Res.* 1988, **48**, 589-601.
- 27 P. A. Skehan, R. Storeng, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J Natl Cancer Inst.* 1990, **82**, 1107-1112.
- 80 28 L.-X. Fan, A.-H. Gao, Y.-B. Zhou and J. Li, *China Biotech.* 2012, **32**, 73-80.
- 29 A. J. Weinheimer, J. A. Matson, M. B. Hossain and D. Vanderhelm, *Tetrahedron Lett.* 1977, **34**, 2923-2926.

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