Tetrahedron 70 (2014) 6851–6858

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Novel cytotoxic nine-membered macrocyclic polysulfur cembranoid lactones from the soft coral *Sinularia* sp.

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ARTICLE INFO

Article history:
Received 11 March 2014
Received in revised form 4 July 2014
Accepted 10 July 2014
Available online 15 July 2014

Keywords:
Soft coral
Sinularia sp.
Trithio-cembranoid lactones
Cembranoids

ABSTRACT

One novel nine-membered macrocyclic polysulfur cembranoid lactone, sinulariaoid A (**1**); three new multioxygenated cembranoids, sinulariaoid B (**2**), sinulariaoid C (**3**), sinulariaoid D (**4**); and four known cembranoids, capilloloid (**5**), dihydrosinularin (**6**), sinularin (**7**), and dihydrosinuflexolide (**8**) were isolated from the soft coral *Sinularia* sp. collected off of Sanya Bay in the South China Sea. Their stereochemical structures were determined on the basis of extensive spectroscopic methods, including single crystal X-ray diffraction analysis. Sinulariaoid A (**1**) is the first reported nine-membered macrocyclic polysulfur cembranoid from soft coral. The cytotoxic activities of compounds **1**–**8** were determined in four human cancer cell lines (HepG2, HepG2/ADM, MCF-7, and MCF-7/ADM). Of these, sinulariaoid A (**1**) exhibited the most potent anticancer activity in vitro, and its cytotoxicity in HepG2/ADM was more potent than in the other three cell lines. Furthermore, it was found that sinulariaoid A (**1**) induced apoptosis, and its selective toxicity toward HepG2/ADM cells was not related to P-glycoproteins.

1. Introduction

Marine disulfides and multisulfide-containing compounds, especially cyclic polysulfide metabolites are a special, relatively rare, and important class of natural products.[1] It has been shown that many marine disulfides and multisulfides exhibit promising bioactivities, including antitumor,[2] antibiotic,[3,4] anti-inflammatory, and enzyme-inhibitory activities.[5] In particular, the disulfide or multisulfide moieties played an important role in their bioactivities. Thus far, cyclic polysulfides have mainly been found in tunicates,[6,7] red algae,[8–11] tropical mangroves of the genus *Bruguiera* (family Rhizophoraceae),[2,12–15] marine microorganisms, sponges, bryozoans, corals, mollusks, among others.[1] Although Cnidaria produces a large amount of various secondary metabolites,[16] sulfur-containing compounds are rarely reported from this phylum. To the best of our knowledge, there is only one report of a pregnane-type steroidal nucleus containing an unusual hexacyclic oxadithiino unit fused to ring A[17] and one symmetric dimer of a cembranoid containing a sulfide linkage[18] from the soft corals *Cladiella krempfi*[17] and *Sinularia flexibilis*, respectively.[18]

As part of our continuing studies on bioactive substances produced by marine microorganisms and their hosts from the South China Sea, one novel nine-membered macrocyclic polysulfur cembranoid lactone, sinulariaoid A (**1**); three new cembranoids, sinulariaoid B (**2**), sinulariaoid C (**3**), sinulariaoid D (**4**); and four known cembranoids, capilloloid (**5**),[19,20] dihydrosinularin (**6**),[21] sinularin (**7**),[21] and dihydrosinuflexolide (**8**)[22] were isolated from the host soft coral *Sinularia* sp. collected off of Sanya Bay in the South China Sea (Fig. 1). Their stereochemical structures were determined on the basis of extensive spectroscopic methods. The absolute stereochemical structure of **1** was elucidated by single crystal X-ray diffraction analysis. To the best of our knowledge, sinulariaoid A (**1**) is the only reported nine-membered macrocyclic polysulfur cembranoid from a soft coral. In addition, we found that sinulariaoid A (**1**) inhibited the proliferation of human cancer cell lines HepG2 and MCF-7 as well as their multidrug-resistant cancer cell lines MCF-7/ADR and HepG2/ADM. Multidrug resistance of cancer cells is believed to be a major cause of chemotherapy failure.[23] Interestingly, the present study indicated that the multidrug-

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http://dx.doi.org/10.1016/j.tet.2014.07.042
0040-4020/

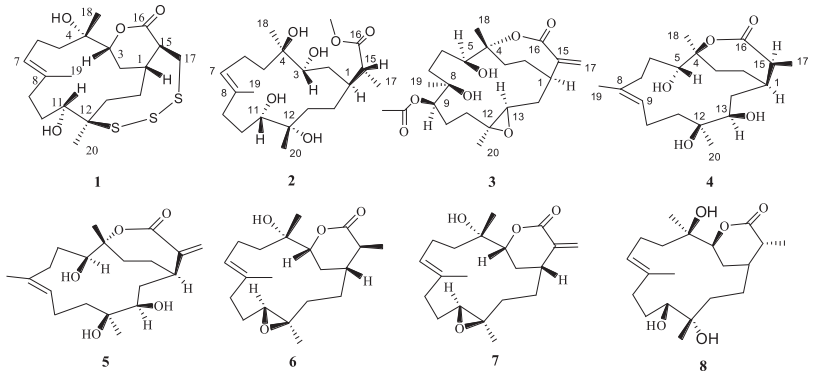

Fig. 1. Compounds **1**–**8** from the soft coral *Sinularia* sp.

resistant cells, HepG2/ADM, were more sensitive to sinulariaoid A (**1**) than their corresponding parent cells, HepG2, indicating that sinulariaoid A (**1**) could be a promising leading compound for the treatment of cancers, especially multidrug-resistant cancers.

2. Results and discussion

Sinulariaoid A (**1**) was obtained as colorless columnar crystals. The HREIMS of **1** exhibited a molecular ion peak at *m*/*z* 432.1457 and established a molecular formula of $C_{20}H_{32}O_4S_3$, implying 5° of unsaturation. Elemental analysis also confirmed the presence of sulfur (C 55.02, H 7.15, S 22.75%; calcd for $C_{20}H_{32}O_4S_3$, C 55.52, H 7.45, S 22.23%). The IR spectrum of **1** revealed the presence of hydroxyl groups and isolated ester functionalities based on the absorptions at 3372 and 1695 cm^{-1}. The UV spectrum indicated that there was an isolated carbon–carbon double bond (λ_{max} 262.0 nm (log ε 3.16)). NMR data indicated the presence of an isolated carbon–carbon double bond at δ_H 5.26 (1H, t, *J*=5.6 Hz) and δ_C 129.3 (d) and 133.0 (s); a lactone group at δ_C 171.9 (s); two oxygen-bearing methines at δ_H 4.34 (1H, dd, *J*=2.8, 11.2 Hz), 4.10 (1H, br d, *J*=10.0 Hz) and δ_C 85.0 (d) and 69.6 (d); one oxygenated quaternary carbon at δ_C 72.9 (s); one sulfur-bearing quaternary carbon at δ_C 56.2 (s); and one sulfur-bearing methylene at δ_H 4.16 (1H, dd, *J*=2.8, 11.2 Hz), 3.23 (1H, dd, *J*=2.8, 14.8 Hz), and δ_C 42.1 (t). Based on the above data, it appeared that compound **1** contained a sulfur-bearing cembrane skeleton with three rings.

The planar structure of **1** was determined by a detailed analysis of 1D and 2D NMR spectra. The HMQC experiment allowed us to assign all of the protons to their corresponding carbon atoms (Tables 1 and 2), and the ^1H–^1H COSY spectrum revealed the

Table 1
^1H NMR data of compounds **1**–**4**[a] in pyridine-d_5 (δ_H (mult., *J*, Hz))

Pos.	1	2	3	4
1	3.09 (dq, 2.8, 7.8)	2.62 (m)[b]	3.26 (m)	3.26 (dq, 2.8, 7.2)
2	2.28 (br t, 12.8)	1.71 (d, 6.8)	2.12 (m)[b]	1.34 (m)
	2.32 (t, 4.8, 15.0)	2.31 (d, 12.0)	2.22 (m)	2.12 (m)
3	4.34 (dd, 2.8, 11.2)	4.28 (br d, 10.4)	1.93 (m)[b]	1.72 (t, 3.2, H-a)
			2.04 (m)[b]	2.34, t, (5.2, H-b)
4				
5	1.87 (t, 4.0)	1.92 (t, 3.2)	4.30 (dd, 4.4, 8.4)	4.51 (br d, 7.2)
	1.99 (m)	2.51 (dd, 2.8, 9.8)		
6	2.13 (m)	2.59 (dd, 2.8, 9.8)	1.76 (m)	1.66 (t, 8.0)
		2.71 (m)	1.93 (m)[b]	2.23 (dt, 2.0, 14.0)
7	5.26 (t, 5.6)	5.94 (t, 5.6)	1.58 (m)	2.22 (d, 12.8)
			1.95 (m)[b]	2.37 (t, 12.8)[b]
8				
9	2.15 (m)[b]	2.34 (m)[b]	5.2 (d, 8.8)	5.17 (t, 8.8)
	2.63 (br t, 13.2)	2.62 (m)[b]		
10	1.64 (m)[b]	1.87 (dd, 2.8, 16.0)	1.70 (m)	1.67 (d, 12.4)
	1.92 (m)[b]	2.34 (dd, 4.8, 16.0)	2.09 (dd, 4.4, 11.6)[b]	2.06 (br s)
11	4.10 (br d, 10.0)	4.07 (br d, 11.2)	1.19 (dd, 8.0, 11.2)	2.05 (m)
			2.09 (dd, 4.4, 11.6)	2.18 (m)
12				
13	1.95 (m)[b]	1.68 (m)	3.43 (dd, 2.4, 7.3)	3.81 (d, 6.8)
	2.67 (dd, 2.8, 8.6)	2.21 (t, 3.2)		
14	1.49 (t, 7.6)	1.95 (m)	1.93 (m)[b]	1.65 (t, 13.2)
	2.43 (dt, 4.8, 15.0)		2.29 (ddd, 2.4, 5.2, 11.6)	2.32 (d, 6.8)
15	2.67 (d, 2.8, 8.6)	2.61 (m)		2.37 (t, 2.8)
16				
17	3.23 (dd, 2.8, 14.8)	1.19 (d, 6.4)	6.04 (s)	1.37 (d, 7.6)
	4.16 (dd, 2.8, 11.2)		6.75 (s)	
18	1.57 (s)	1.54 (s)	1.34 (s)	1.52 (s)
19	1.62 (s)	1.79 (s)	1.23 (s)	1.55 (s)
20	1.41 (s)	1.57 (s)	1.31 (s)	1.60 (s)
–OMe		3.59 (s)		
–OAc			2.04 (s)	

[a] At 400 MHz, the middle signal at δ_H 7.580.
[b] Overlapped signals.