Rare Casbane Diterpenoids from the Hainan Soft Coral *Sinularia depressa*

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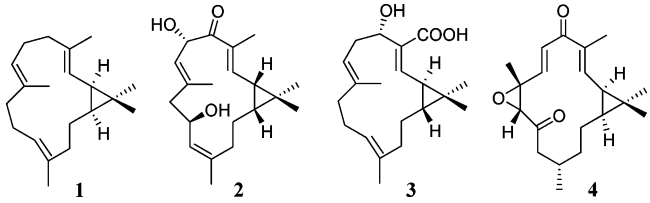
A series of nine casbane diterpenes, compounds **5**−**13**, exhibiting either *cis* or *trans* ring junctions were isolated from the Hainan soft coral *Sinularia depressa*. The structures of this group of compounds, the basic member of which was named depressin (**5**), were established by detailed spectroscopic analysis. In addition, the absolute configuration of the main metabolite, 10-hydroxydepressin (**7**), and of its epimer, 1-*epi*-10-hydroxydepressin (**8**), was determined by a combination of conformational analysis and the modified Mosher's method. A stereochemical relationship between all isolated molecules was investigated by analyzing their circular dichroism profiles. Antiproliferative and antibacterial activities of the depressins were also evaluated.

Soft corals of the genus *Sinularia* (Alcyoniidae) are a rich source of diterpene metabolites with intriguing structural features and various promising bioactivities.[1] In the course of our ongoing research focused toward the isolation of biologically active compounds from Chinese marine organisms,[2−6] we carried out a chemical investigation on the soft coral *Sinularia depressa*, collected off Lingshui Bay, Hainan Province, China. The soft coral was found to possess a secondary metabolite pattern characterized by the presence of casbane diterpenes. The casbane framework, which was found for the first time in casbene (**1**), isolated from an enzymatic preparation of castor bean seedlings,[7] is strictly related to the cembrane skeleton and differs from this in the presence of a dimethyl-cyclopropyl moiety rather than the isopropyl residue fused to the 14-membered ring. Casbane diterpenes are extremely rare in nature, being described so far only from some species of plants of the family Euphorbiaceae (i.e., agrostistachin, **2**,[8] and yuexiandajisu A, **3**[9]) as well as from the soft coral *Sinularia microclavata* (microclavatin, **4**[10]). The latter report represents the only example from the sea. In the majority of casbanes described in the literature the two rings forming the macrocyclic structure are *cis*-fused (**1**, **2**, **4**), whereas a very few molecules exhibit a corresponding *trans* junction (**3**).

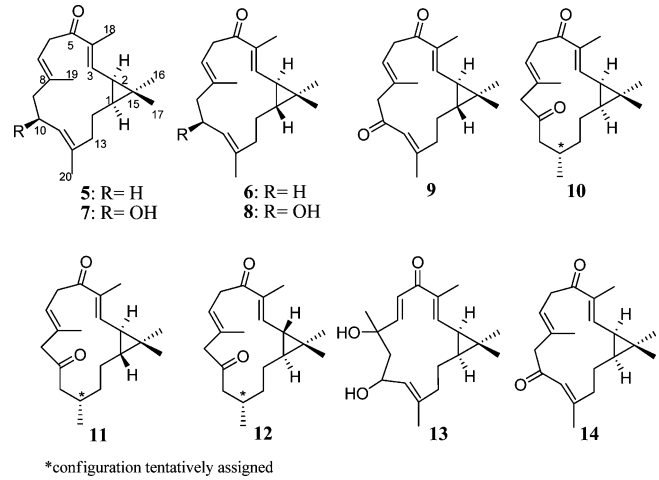


1 **2** **3** **4**

A total of nine unreported casbanes, compounds **5**−**13**, have been characterized in the course of this work. Five of these compounds show a *trans*-fused bicyclic system, whereas the remaining four molecules exhibit the *cis*-junction. This paper describes the isolation, the structure elucidation including the stereochemical analysis, and the bioactivity evaluation of compounds **5**−**13**.

Results and Discussion

The Et_2O-soluble portion of the acetone extract of the soft coral *S. depressa* was subjected to silica gel chromatography (light petroleum ether/acetone gradient). Selected casbane-containing fractions were subsequently purified on silica gel, Sephadex LH-20, and reversed-phase HPLC to afford nine pure metabolites, compounds **5**−**13**.



5: R= H
7: R= OH
6: R= H
8: R= OH
9 **10** **11** **12** **13** **14**

*configuration tentatively assigned

A preliminary ^{1}H NMR analysis showed that all molecules shared the same carbon skeleton, differing from each other in either the degree of oxidation or the configuration of one or more chiral centers. On the basis of these considerations and with the aim at simplifying the structure description, the compounds isolated were grouped and named according to both the extent of oxidation and the stereochemical relationship. Three *cis*/*trans* pairs of molecules, which were epimeric at C-1, were recognized: depressin (**5**) and 1-*epi*-depressin (**6**), 10-hydroxydepressin (**7**) and 1-*epi*-10-hydroxydepressin (**8**), and 10-oxo-11,12-dihydrodepressin (**10**) and 1-*epi*-10-oxo-11,12-dihydrodepressin (**11**). The remaining three co-occurring compounds were 1-*epi*-10-oxodepressin (**9**), 2-*epi*-10-oxo-11,12-dihydrodepressin (**12**), and 8,10-dihydroxy-*iso*-depressin (**13**). In addition, the epimer at C-1 of compound **9**, 10-oxodepressin (**14**), was obtained by oxidation of **7**. The elucidation of the structure of **5**−**13** is here reported according to this grouping.

The molecular formula $C_{20}H_{30}O$ of depressin (**5**) was deduced by the HREIMS molecular peak at *m*/*z* 286.2291 and implied six degrees of unsaturation. The ^{1}H NMR spectrum of **5** contained methyl singlets at δ_H 1.09 (s, 3H), 1.16 (s, 3H), 1.56 (s, 6H), and 1.87 (s, 3H), which were assigned to two tertiary and three vinyl methyl groups, immediately suggesting a diterpenoid framework. The presence of an α,β-unsaturated ketone group was inferred by

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Table 1. ^{1}H NMR Data (400 MHz, $CDCl_3$) for Compounds **5**−**14**[a]

proton	5	6	7	8	9	10	11	12	13	14
1	1.15, m	0.71, m	1.15, m	0.57, m	0.65, m	0.97, m	0.75, m	0.65, m	1.24, m	1.22, m
2	1.50, dd (10.2, 8.7)	1.08, m	1.50, dd (10.2, 8.4)	1.03, m	1.10, m	1.42, m	1.15, m	1.14, m	1.52, m	1.55, dd (10.2, 9.0)
3	6.37, d (10.2)	6.11, d (10.2)	6.30, d (10.5)	6.04, d (10.2)	6.12, d (9.9)	6.35, d (10.8)	6.05, d (10.5)	6.08, d (10.5)	6.39, d (10.5)	6.33, d (10.2)
6a	3.55, dd (13.8, 8.4)	3.71, dd (13.8, 11.1)	3.50, dd (13.8, 8.4)[b]	3.63, dd (14.3, 11.0)[b]	3.80, dd (13.5, 10.8)	3.85, dd (13.5, 10.8)	3.72, dd (12.0, 14.7)	3.63, dd (14.7, 10.5)	6.55, d (16.2)	3.78, dd (13.5, 10.2)
6b	2.97, dd (13.8, 5.7)	2.83, br d (13.8)	3.02, dd (13.8, 5.7)[c]	2.89, br d (14.3)[c]	2.91, d (13.5)	2.90, br d (13.5)	2.95, (overlapped)	3.05, dd (14.7, 3.0)		2.95, br d (13.5)
7	5.08, t (6.6)	5.21, br d (11.1)	5.11, t (6.6)	5.27, (overlapped)	5.35, br d (10.8)	5.42, dd (10.8, 1.2)	5.42, dd (12.0, 1.8)	5.42, dd (10.5, 3.0)	6.10, d (16.2)	5.35, br d (10.2)
9a	2.15, m	2.18, m	2.39, m[b]	2.48, br d (11.4)[b]	3.10, d (13.2)	3.07, d (11.1)	3.03, (overlapped)	3.03, s	2.40, dd (15.0, 3.6)	3.02, d (13.2)
9b	2.00, m	2.10, m	2.07, m[c]	2.06, m[c]	2.91, d (13.2)	2.80, d (11.1)	2.93, (overlapped)	3.03, s	1.91, m	2.85, d (13.2)
10a	2.17, m	2.28, m	4.38, td (9.3, 3.9)	4.31, td (9.6, 3.8)					4.85, m	
10b	1.96, m	2.07, m								
11	4.84, t (5.4)	4.88, br d (8.4)	5.06, d (9.3)	5.26, (overlapped)	6.03, s	2.60, dd (18.0, 9.0)	2.70, d (17.1)	2.35, m	5.67, d (10.2)	6.17, s
						2.12, d (18.0)	2.05, d (17.1)	2.35, m		
12						2.08, m	2.02, m	2.10, m		
13a	2.20, m	2.17, m	2.38, m[b]	2.33, m[c]	2.35, m	1.40, m	1.45, m	1.65, m	2.20, m	2.30, m
13b	1.75, m	1.93, m	1.80, m[c]	2.13, m[b]	1.98, m	0.80, m	1.25, m	1.30, m	1.78, m	1.88, m
14a	2.05, m	1.88, m	2.07, m[b]	1.99, m[c]	2.02, m	1.50, m	1.83, m	2.00, m	1.91, m	2.24, m
14b	0.80, m	1.03, m	1.19, m[c]	1.27, m[b]	1.18, m	0.60, m	1.20, m	1.07, m	1.40, m	0.80, m
16	1.16, s	1.16, s	1.18, s	1.15, s	1.17, s	1.16, s	1.12, s	1.13, s	1.27, s	1.16, s
17	1.09, s	1.11, s	1.06, s	1.11, s	1.11, s	1.15, s	1.13, s	1.15, s	1.15, s	1.08, s
18	1.87, s	1.80, s	1.83, s	1.77, s	1.84, s	1.83, s	1.82, s	1.83, s	1.87, s	1.92, s
19	1.56, s	1.59, s	1.66, s	1.71, s	1.61, s	1.79, s	1.61, s	1.64, s	1.45, s	1.56, s
20	1.56, s	1.59, s	1.63, s	1.59, s	2.17, s	0.95, d (7.2)	0.90, d (6.6)	0.88, d (6.6)	1.69, s	2.07, s

[a] The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b] Pro*R*. [c] Pro*S*.

Table 2. ^{13}C NMR Data (100 MHz, $CDCl_3$) for Compounds **5**−**14**[a]

carbon	5	6	7	8	9	10	11	12	13	14
1	35.2, CH	37.5, CH	35.2, CH	37.0, CH	37.2, CH	35.9, CH	38.8, CH	39.7, CH	34.6, CH	34.5, CH
2	27.6, CH	31.9, CH	28.6, CH	35.2, CH	31.6, CH	27.8, CH	32.6, CH	33.6, CH	27.9, CH	27.8, CH
3	143.1, CH	149.4, CH	142.9, CH	148.1, CH	148.1, CH	144.4, CH	148.3, CH	147.5, CH	145.9, CH	141.9, CH
4	136.6, qC	132.8, qC	136.2, qC	133.0, qC	133.9, qC	136.0, qC	134.6, qC	134.2, qC	137.7, qC	137.7, qC
5	199.9, qC	201.2, qC	200.0, qC	200.4, qC	199.6, qC	199.3, qC	200.2, qC	200.9, qC	195.1, qC	199.3, qC
6	39.4, CH_2	40.4, CH_2	39.8, CH_2	40.6, CH_2	40.6, CH_2	40.7, CH_2	41.0, CH_2	40.7, CH_2	128.4, CH	39.3, CH_2
7	119.4, CH	121.7, CH	120.5, CH	122.9, CH	125.6, CH	125.1, CH	126.1, CH	124.7, CH	143.6, CH	124.2, CH
8	137.1, qC	135.1, qC	134.5, qC	133.0, qC	130.1, qC	130.4, qC	131.4, qC	131.7, qC	83.0, qC	132.8, qC
9	39.0, CH_2	38.4, CH_2	46.7, CH_2	46.4, CH_2	56.6, CH_2	53.6, CH_2	56.0, CH_2	55.8, CH_2	43.4, CH_2	55.9, CH_2
10	23.9, CH_2	24.0, CH_2	67.5, CH	68.7, CH	198.3, qC	207.1, qC	209.1, qC	208.7, qC	67.6, CH	200.1, qC
11	124.4, CH	125.4, CH	127.8, CH	129.2, CH	122.9, CH	50.1, CH_2	49.5, CH_2	50.8, CH_2	124.5, CH	122.8, CH_2
12	135.9, qC	133.5, qC	140.8, qC	140.1, qC	158.2, qC	27.2, CH	27.9, CH	27.6, CH	139.5, qC	158.4, CH
13	39.9, CH_2	38.8, CH_2	39.2, CH_2	40.1, CH_2	41.1, CH_2	39.9, CH_2	35.7, CH_2	35.6, CH_2	37.1, CH_2	41.1, CH_2
14	26.3, CH_2	24.4, CH_2	25.8, CH_2	25.4, CH_2	24.0, CH_2	24.5, CH_2	27.5, CH_2	24.5, CH_2	22.9, CH_2	26.8, CH_2
15	25.4, qC	28.1, qC	25.3, qC	29.7, qC	28.3, qC	25.2, qC	29.5, qC	27.9, qC	27.2, qC	26.1, qC
16	29.0, CH_3	22.0, CH_3	29.0, CH_3	22.0, CH_3	21.6, CH_3	29.0, CH_3	21.7, CH_3	21.6, CH_3	29.1, CH_3	29.0, CH_3
17	15.8, CH_3	23.7, CH_3	15.7, CH_3	23.0, CH_3	23.3, CH_3	16.0, CH_3	23.8, CH_3	23.5, CH_3	16.3, CH_3	15.8, CH_3
18	11.6, CH_3	11.2, CH_3	11.5, CH_3	11.1 CH_3	11.1, CH_3	11.7, CH_3	11.6, CH_3	11.6, CH_3	11.5, CH_3	11.9, CH_3
19	15.6, CH_3	14.7, CH_3	18.5, CH_3	19.0, CH_3	15.6, CH_3	16.8, CH_3	16.4, CH_3	16.7, CH_3	26.0, CH_3	16.6, CH_3
20	15.3, CH_3	14.8, CH_3	18.3, CH_3	14.8, CH_3	17.8, CH_3	23.1, CH_3	22.4, CH_3	18.7, CH_3	18.8, CH_3	18.1, CH_3

[a] The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

both the ^{13}C NMR signal at δ_C 199.9 (C) and the IR band at 1655 cm^{-1}. Three trisubstituted double bonds were also evident by six sp^2 carbon signals in the ^{13}C NMR spectrum at δ 143.1 (CH), 137.1 (C), 136.6 (C), 135.9 (C), 124.4 (CH), and 119.4 (CH) and by three olefinic multiplets in the ^{1}H NMR spectrum at δ 4.84 (1H, t, J = 5.4 Hz, H-11), 5.08 (1H, t, J = 6.6 Hz, H-7), and 6.37 (1H, d, J = 10.2, H-3). The remaining two unsaturation degrees required by the molecular formula of **5** were thus attributed to two rings. The presence of both a quaternary carbon (δ 25.4, C-15) and a *gem*-dimethyl functionality [δ_H 1.16 (s, H_3-16), δ_H 1.09 (s, H_3-17); δ_C 29.0 (C-16), δ_C 15.8 (C-17)], together with the COSY correlation between two cyclopropyl protons [δ_H 1.15 (m, H-1), δ_H 1.50 (dd, J = 10.2 and 8.7 Hz, H-2)], indicated the presence of a substituted cyclopropyl ring. Consequently, the remaining ring was deduced to be a 14-membered macrocycle, according to a casbane carbon arrangement. A detailed analysis of 2D NMR spectra of **5** allowed the assignment of all proton and carbon atoms (Tables 1 and 2). The *E* geometry of all three double bonds $\Delta^{3(4)}$, $\Delta^{7(8)}$, and $\Delta^{11(12)}$ was deduced by the δ_C values of CH_3-18, CH_3-19, and CH_3-20 (<20 ppm).[11] The junction of the two rings at carbons C-1/C-2 was suggested to be *cis* on the basis of ^{13}C NMR chemical shifts of the geminal methyls C-16 and C-17 (δ_C 29.0 and 15.8, respectively), analogously with literature *cis*-fused casbane diterpenes, i.e., agrostistachin (**2**)[8] and microclavatin (**4**).[10] The proposed configuration was further confirmed by a diagnostic NOE interaction observed between H-1 and H-2.

Compound **6** was an isomer of **5** with the same molecular formula ($C_{20}H_{30}O$) as deduced by HREIMS. A detailed 2D NMR analysis revealed that the planar structure of **6** was the same as **5**, thus implying that the difference between the two compounds concerned the configuration. As the geometry of all three double bonds of **6** was inferred to be *E*, analogously with **5**, by both NOESY and ^{13}C NMR data (see Table 2), a different configuration at one of the two chiral carbons was thus expected. Analysis of the NOESY experiment of **6** showed the presence of interactions between H-1 and H_3-17, and between H-2 and H_3-16, indicating that H-1 and H-2 were oriented in opposite directions, consistent with a *trans* ring junction. This assignment was confirmed by the diagnostic ^{13}C NMR chemical shifts of the C-16 and C-17 methyl groups (δ_C 22.0 and 23.7, respectively), in accordance with those reported for the *trans*-fused yuexiandajisu A (**3**).[9] Due to the different ring junctions, the ^1H NMR values of H-1, H-2, and H-3 and the ^{13}C NMR chemical shifts of C-1, C-2, C-3, C-4, and C-15 were also significantly shifted compared to **5** (Tables 1 and 2). Detailed spectroscopic NMR analysis of compound **6** led us to assign all proton and carbon values. Surprisingly, the CD profiles of **5** and **6** were almost identical suggesting that C-2, near the chromophore, had the same configuration in both molecules. Consequently, the configuration of C-1 was assumed to be opposite, and thus **6** was named 1-*epi*-depressin.

10-Hydroxydepressin (**7**), the main component of the depressin fraction, and 1-*epi*-10-hydroxydepressin (**8**) had the molecular formula $C_{20}H_{30}O_2$ and were the 10-hydroxy derivatives of **5** and **6**, respectively, as evidenced by careful analysis of their spectroscopic data (Tables 1 and 2). The geometry of the three double bonds in **7** and **8** was *E*, the same as in **5** and **6**. Compound **7** exhibited the *cis* junction at C-1/C-2, whereas in **8** the two rings were *trans*-fused, as indicated by the diagnostic ^{13}C NMR values of the geminal methyl groups C-16 (δ_C 29.0 in **7**, δ_C 22.0 in **8**) and C-17 (δ_C 15.7 in **7**, δ_C 23.0 in **8**). The $^1H-^1H$ COSY spectra showed the diagnostic correlation of a carbinolic signal (δ_H 4.38 in **7**, δ_H 4.31 in **8**) with two multiplets (δ_H 2.39 and 2.07 in **7**, δ_H 2.48 and 2.06 in **8**) and a doublet (δ_H 5.06 in **7**, δ_H 5.26 in **8**) that were attributed to an allylic methylene (H_2-9) and a vinylic proton (H-11), respectively. This result led us to locate unambiguously the secondary hydroxy function in the macrocyclic fragment of the molecule at C-10 in both molecules. Analysis of 2D NMR spectra confirmed the proposed planar structures and allowed the complete assignment as listed in Tables 1 and 2.

The CD curves of **7** and **8** were similar to those of **5** and **6**, thus suggesting a stereochemical correspondence between the two pairs of molecules. Due to the flexible nature of the 14-membered macrocyclic ring, the relative configuration at C-10 could not be deduced by 2D NOESY experiments. Furthermore, efforts to obtain crystals suitable for X-ray crystallographic analysis were unsuccessful. Thus, we decided first to determine the absolute configuration at C-10 in **7** and **8** by applying the modified Mosher method[12,13] and then to undertake a conformational analysis with the aim of relating this configuration to that of the ring junction and establishing the absolute configuration of both molecules.

The (*S*)- and (*R*)-MTPA esters of **7** and **8** were prepared by treatment with (*R*)- and (*S*)-MTPA chloride, respectively. The $\Delta\delta$ values observed for the signals of protons near the hydroxy group at C-10 for both esters of each compound are reported in Figure 1 and indicated the *S* configuration for the carbinol asymmetric center in **7** and **8**.

Models of the four possible diastereomers, (1*S*,2*S*,10*S*) and (1*R*,2*R*,10*S*) for compound **7** and (1*S*,2*R*,10*S*) and (1*R*,2*S*,10*S*) for compound **8**, were built in order to conduct a conformational study. A preliminary analysis showed a potential, albeit limited, flexibility for all these models, with the possible occurrence of a small number of main conformers endowed with similar conformational energy. In this case, NOESY spectra can not be safely assigned and integrated as arising from a single conformer. This, in turn, prevents traditional structural refinement approaches based on deriving distance restraints from NOE intensities to be applied in conformational sampling and a refinement strategy.

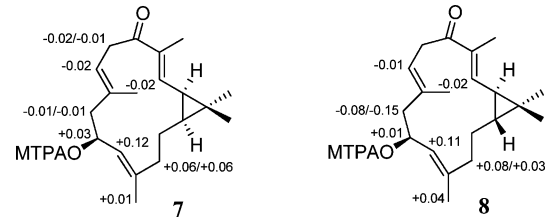

Figure 1. $\Delta\delta$ ($\delta S - \delta R$) values (in ppm) for the MTPA esters of **7** and **8**.

In view of this, we performed an exhaustive conformational search by generation of a large number of conformers ($N = 200$) for each model, by employing a simulated annealing (SA) protocol, followed by a derivation of ensemble $1/r^6$-averaged interprotonic distances. The SA protocol has been successfully applied in many restrained and unrestrained calculations on systems varying from small organic molecules up to peptides and proteins.[14]

For each molecule, predicted average distances of the two diastereomers were compared to distances derived from experimental peak volumes to check if all predicted short distances have a counterpart in the NOESY spectrum and *vice versa*. In particular, potentially diagnostic contacts, i.e., those giving rise to substantially different distance patterns between the two diasteromers, were monitored with special care.

In the case of **7**, only the (1*S*,2*S*,10*S*)-diastereomer was able to account for the whole experimental contact pattern, while the (1*R*,2*R*,10*S*)-diastereomer exhibited systematic violations in both the full ensemble containing all 200 calculated structures and the subsets only including the representative structures of lowest energy conformer clusters. For the (1*S*,2*S*,10*S*)-diastereomer, a subset including the representative structures of the two lowest energy conformer clusters, i.e., the two most two populated clusters (71 and 52 molecules, respectively) with an energy difference of 0.1 kcal/mol, fully accounts for the whole experimental NOE-derived contact pattern (see Supporting Information for details) (Figure 2). On these bases the absolute configuration of **7** was assigned as (1*S*,2*S*,10*S*).

A similar approach was also used for the two possible diastereomers of **8**. In this case, there is a pseudoenantiomeric relationship between the lowest energy conformers of the two diastereomers, where only the OH and H substituent groups on the asymmetric C-10 atom were swapped. Consequently, potentially diagnostic interactions are located mainly around either the hydroxy group, whose signals are not detected in the NOESY spectra due to fast exchange in chloroform, or the H-10 atom. In spite of this, a limited but clear-cut pattern of interactions differentiating the two possible diastereomers can be safely identified. In fact, in the case of (1*S*,2*R*,10*S*)-**8** neither the lowest energy cluster nor the most populated ones, singularly or in combination, fully accounted for the experimental NOE cross-peaks (see Supporting Information for details). On the contrary, in the case of the (1*R*,2*S*,10*S*)-**8** diastereomer, the observed pattern of 40 nontrivial NOE effects is fully justified by a simple mixture of the two most stable and populated conformer clusters (Figure 2), similar to that seen for (1*S*,2*S*,10*S*)-**7**. Even the structural relationship between the two conformers is similar to that detected in (1*S*,2*S*,10*S*)-**7**, their main difference consisting in a rotation around the C-7/C-8 bond. On these bases, the absolute configuration of **8** was determined as 1*R*,2*S*,10*S*.

Thus, compounds **7** and **8** were epimers at C-1. Due to the similarity of the CD curves of **5** and **6** with those of **7** and **8**, the absolute configurations of depressin (**5**) and 1-*epi*-depressin (**6**) were also defined as 1*S*,2*S* and 1*R*,2*S*, respectively.

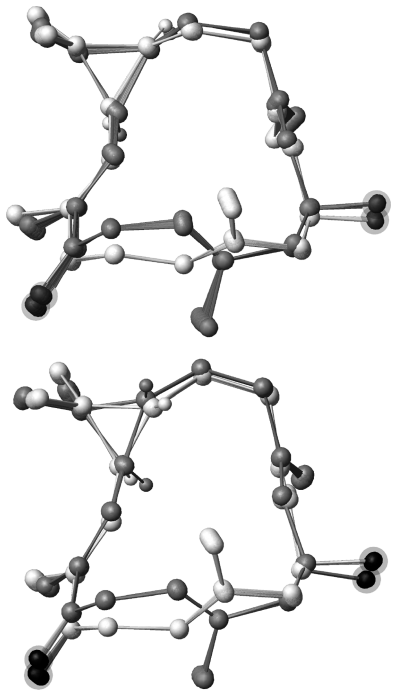

Figure 2. The two representative clusters for the (1*S*,2*S*,10*S*)-**7** (top) and (1*R*,2*S*,10*S*)-**8** (bottom) diastereomers are shown in ball-and-stick representations and colored in light (71 and 69 overlapped structures for (1*S*,2*S*,10*S*)-**7** and (1*R*,2*S*,10*S*)-**8**, respectively) or dark gray (52 and 58 overlapped structures for (1*S*,2*S*,10*S*)-**7** and (1*R*,2*S*,10*S*)-**8**, respectively). The oxygen atoms are in black with a transparent contour. For clarity, only hydrogen atoms on C-1 and C-2 atoms are evidenced to show the configuration of the cyclopropane ring.

The molecular formula ($C_{20}H_{28}O_2$) of 1-*epi*-10-oxodepressin (**9**) was established by HREIMS and contained an additional unsaturation degree with respect to **7** and **8**. Analysis of the spectroscopic data indicated the presence at C-10 in the macrocyclic ring of **9** of a second ketone group, which was α,β-unsaturated, as indicated by the ^{13}C NMR signal at δ_C 198.3, rather than the secondary hydroxy function present in **7** and **8**. Diagnostic HMBC correlations were observed between C-10 and both the isolated methylene H_2-9 (δ_H 2.91 and 3.10) and the olefinic proton H-11 (δ_H 6.03), according to the proposed arrangement. Compound **9** had the 1,2-*trans*-junction analogously with **6** and **8**, as indicated by the ^1H and ^{13}C NMR values (Tables 1 and 2). The CD curve of **9** was more complex with respect to those of **6** and **8** due to the presence of the second α,β-unsaturated ketone. However, a portion similar to that observed in the CD profiles of compounds **6** and **8** was recognizable in the curve of **9**, suggesting the same 1*R*,2*S* absolute configuration at the junction asymmetric centers. This was confirmed by oxidizing a sample of 1-*epi*-10-hydroxydepressin (**8**) by Dess-Martin reagent. The product obtained had spectroscopic and optical properties[15] identical with those of **9** (see Experimental Section and Supporting Information).

With the aim of confirming the stereochemical relationship between *Sinularia* metabolites, a sample of 10-hydroxydepressin (**7**) was oxidized under the same conditions as compound **8** to give the corresponding oxidized derivative **14**, which was fully characterized (Tables 1 and 2). The CD curve of non-natural compound **14** was identical with that of **9**. The two compounds were in the same *cis*−*trans* relationship as the corresponding hydroxy derivatives **7** and **8**, respectively.

Compounds **10**, **11**, and **12** exhibited the same molecular formula, $C_{20}H_{30}O_2$, as deduced by HREIMS. Analysis of their ^1H and ^{13}C NMR spectra (Tables 1 and 2) clearly revealed the presence in the three molecules of an identical oxidation pattern with two keto functional groups at C-5 and C-10 and only two *E*-double bonds ($\Delta^{3(4)}$ and $\Delta^{7(8)}$), with the third one having been reduced, in contrast to the other co-occurring depressins. The secondary methyl group was located at C-12 in all three compounds by analysis of their $^1H-^1H$ COSY spectra. The correlations observed in the HSQC and HMBC experiments confirmed the same planar structure and implied that they had to differ from each other in the relative configuration of one or two chiral centers. Analysis of their ^{13}C NMR spectra (Table 2) revealed that **10** had the 1,2-*cis* ring junction, whereas the other two isomers, **11** and **12**, were *trans*-fused. Comparison of their CD curves disclosed interesting stereochemical features. Compounds **10** and **11** had the same CD profile as depressins **5**−**8**, whereas compound **12** displayed an opposite CD curve. This implied that in compounds **10** and **11** the absolute configuration at the ring junction carbons was 1*S*,2*S* and 1*R*,2*S*, respectively, the same as the other co-occurring *cis* and *trans* molecules, whereas in **12** this configuration had to be opposite (1*S*,2*R*). At this point, having assessed the configuration of the ring junction, it remained to be defined the configuration of C-12. Due to the flexibility of the casbane macrocycle, the relative orientation of H_3-20 in compounds **10**−**12** could not be confidently assigned by NOE analysis. However, some considerations could be made by comparing the spectroscopic data of 10-oxo-11,12-dihydrodepressin (**10**) with literature models. In particular, the ^{13}C NMR value of C-20 (δ 23.1) of **10** was almost comparable with that of the structurally related microclavatin (**4**) (δ 21.8), the relative configuration of which has been secured by X-ray diffraction analysis.[10] This led us to tentatively assign to C-12 the same relative configuration as **4**. By biogenetic considerations, the same configuration at C-12 was assumed for 1-*epi*-10-oxo-11,12-dihydrodepressin (**11**). According to this assumption, due to the fact that **11** and **12** had opposite configurations in the *trans* ring junction moiety but are not enantiomers, the configuration of C-12 also had to be the same in **12**, 2-*epi*-10-oxo-11,12-dihydrodepressin.

The molecular formula ($C_{20}H_{30}O_3$) of 8,10-dihydroxy-*iso*-depressin (**13**) implied an additional oxygen atom with respect to **7** and **8**. The spectroscopic data of **13** revealed structural features similar to those of **7** including the secondary hydroxy group at C-10 and the 1,2-*cis*-junction, whereas the C-6/C-8 carbon fragment was different. The presence of an isolated disubstituted double bond $\Delta^{6(7)}$ was suggested by both ^1H NMR signals at δ_H 6.55 (1H, d, J = 16.2, H-6) and 6.10 (1H, d, J = 16.2, H-7) and the corresponding ^{13}C NMR signals at δ_C 128.4 (CH, C-6) and 143.6 (CH, C-7). The carbon spectrum of **13** also contained a signal at δ_C 83.0 (qC, C-8) attributable to a tertiary carbon linked to oxygen, thus indicating the presence a second hydroxy group located at C-8. Analysis of $^1H-^1H$ COSY, HSQC, and HMBC experiments confirmed this structural hypothesis. The geometry of the $\Delta^{6(7)}$ double bond was suggested to be *E* by the coupling constant value,[11] whereas the configurations at C-8 and C-10 were not determined. The CD profile was almost comparable with those of depressins **5**−**11**, and thus the configuration of the *cis* ring junction was assumed to be 1*S*,2*S*, the same as that of other *cis*-depressins.

Compounds **5**−**13** were tested for cytotoxicity against human tumor cell lines HepG2 and SW-1990. The activity against *Staphylococcus aureus* and *Escherichia coli* was also evaluated for **5**−**8**. 10-Hydroxydepressin (**7**) showed cytotoxic activity against tumor cell lines HepG2 and SW-1990 with IC_{50} values of 61 and 37 μM, respectively, and antimicrobial activity against *S. aureus* and *E. coli* at 17 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. UV spectra were recorded on a Varian Cary 300 Bio spectrometer. IR spectra were recorded on a Nicolet-Magna FT-IR 750 spectrometer. NMR spectra were measured on Bruker DRX-400, Bruker DRX-600, and Varian Mercury 300 spectrometers with the residual $CHCl_3$ (δ_H 7.26 ppm; δ_C 77.0 ppm) as

an internal standard. NOESY spectra were obtained with different mixing times (0.180, 0.250, and 0.400 s). EIMS and HREIMS data were obtained on a Finnigan-MAT-95 mass spectrometer. ESIMS and HRESIMS spectra were recorded on a Q-TOF Micro LC-MS-MS mass spectrometer. Reversed-phase HPLC (Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm and a semipreparative ODS-HG-5 [5 μm, 10 mm (i.d.) × 25 cm] column was also employed. Commercial Si gel (Qing Dao Hai Yang Chemical Group Co., 200−300 and 400−600 mesh) was used for column chromatography (CC), and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical and preparative TLC.

Collection and Extraction of the Animal Material. Specimens of *Sinularia depressa* were collected at Lingshui Bay, Hainan Province, China, in July 2004, at a depth of 20 m and were frozen immediately after collection. A voucher specimen of *S. depressa* (LS-338) is available for inspection at the Shanghai Institute of Materia Medica, CAS. The frozen material (510 g dry weight) was cut into small pieces and extracted exhaustively with acetone at room temperature (3 × 1.5 L). The organic extract was evaporated to give a residue, which was partitioned between Et_2O and H_2O. The Et_2O solution was concentrated under reduced pressure to give a dark brown residue (9.6 g).

Purification of Compounds 5−13. The Et_2O extract was fractionated by gradient Si gel column chromatography eluting with a step gradient (0−100% acetone in light petroleum ether) to yield three casbane-containing fractions [A (900 mg), B (1.2 g), C (500 mg)]. An aliquot of fraction A (50.0 mg) was further purified by Si gel CC (light petroleum ether/Et_2O, 95:5) to yield **5** (5.6 mg) and **6** (6.2 mg). An aliquot of fraction B (61.0 mg) was subjected to a RP-HPLC purification [semipreparative ODS-HG-5 (5 μm, 250 × 10 mm), MeCN/H_2O, 70:30, 2.0 mL/min], obtaining **9** (4.7 mg; t_R 16.7 min), **11** (5.5 mg; t_R 19.2 min), **12** (4.3 mg; t_R 22.3 min), and **10** (5.0 mg; t_R 25.5 min). Fraction C was fractionated by Si gel CC (light petroleum ether/Et_2O, 6:4 and 5:5) to give a mixture, which was further purified on a Sephadex LH20 column (light petroleum ether, $CHCl_3$, CH_3OH, 2:1:1), yielding the main component, **7** (134.5 mg), along with **8** (1.6 mg) and **13** (1.1 mg).

Depressin (5): colorless oil; $[\alpha]_D^{25}$ −80.0 (*c* 0.26, $CHCl_3$); CD (*c* 2.10 × 10^{-3} M, *n*-hexane) λ ($\Delta\varepsilon$) 204 (7.4), 249 (−2.1); UV (EtOH) λ_{max} (log ε) 271 (3.56); IR (KBr) ν_{max} 1655 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HREIMS *m/z* 286.2291 (calcd for $C_{20}H_{30}O$, 286.2297).

1-*epi*-Depressin (6): colorless oil; $[\alpha]_D^{25}$ +34.0 (*c* 0.25, $CHCl_3$); CD (*c* 1.05 × 10^{-3} M, *n*-hexane) λ ($\Delta\varepsilon$) 205 (5.1), 236 (0.2); UV (MeOH) λ_{max} (log ε) 274 (5.0) nm; IR (KBr) ν_{max} 1653 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HREIMS *m/z* 286.2306 (calcd for $C_{20}H_{30}O$, 286.2297).

10-Hydroxydepressin (7): colorless oil; $[\alpha]_D^{25}$ −218 (*c* 0.55, $CHCl_3$); CD (*c* 9.93 × 10^{-4} M, *n*-hexane) λ ($\Delta\varepsilon$) 202 (27.6), 252 (−17.7); UV (EtOH) λ_{max} (log ε) 270 (4.76); IR (KBr) ν_{max} 3388, 1650 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HREIMS *m/z* 302.2240 (calcd for $C_{20}H_{30}O_2$, 302.2246).

1-*epi*-10-Hydroxydepressin (8): colorless oil; $[\alpha]_D^{25}$ +4.5 (*c* 0.54, $CHCl_3$); CD (*c* 9.93 × 10^{-4} M, *n*-hexane) λ ($\Delta\varepsilon$) 206 (31.9), 232 (−10.5); UV (EtOH) λ_{max} (log ε) 270 (4.82); IR (KBr) ν_{max} 3399, 1651 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HRESIMS *m/z* 325.2140 (calcd for $C_{20}H_{30}O_2Na$, 325.2144).

1-*epi*-10-Oxodepressin (9): colorless oil; $[\alpha]_D^{25}$ +11.0 (*c* 0.27, $CHCl_3$);[15] CD (*c* 1.00 × 10^{-3} M, *n*-hexane) λ ($\Delta\varepsilon$) 201 (2.1), 241 (2.1), 265 (−1.1); IR (KBr) ν_{max} 1668 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HREIMS *m/z* 300.2089 (calcd for $C_{20}H_{28}O_2$, 300.2089).

10-Oxo-11,12-dihydrodepressin (10): colorless oil; $[\alpha]_D^{25}$ +155 (*c* 0.13, $CHCl_3$); CD (*c* 8.28 × 10^{-4} M, *n*-hexane) λ ($\Delta\varepsilon$) 202 (43.0), 234 (−26.6); 268 (9.9); IR (KBr) ν_{max} 1709, 1647 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HREIMS *m/z* 302.2247 (calcd for $C_{20}H_{30}O_2$, 302.2246).

1-*epi*-10-Oxo-11,12-dihydrodepressin (11): colorless oil; $[\alpha]_D^{25}$ −74.0 (*c* 0.24, $CHCl_3$); CD (*c* 1.32 × 10^{-3} M, *n*-hexane) λ ($\Delta\varepsilon$) 204 (7.1), 232 (−0.7), 268 (3.4); IR (KBr) ν_{max} 1709, 1653 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HREIMS *m/z* 302.2250 (calcd for $C_{20}H_{30}O_2$, 302.2246).

2-*epi*-10-Oxo-11,12-dihydrodepressin (12): colorless oil; $[\alpha]_D^{25}$ +133 (*c* 0.28, $CHCl_3$); CD (*c* 1.16 × 10^{-3} M, *n*-hexane) λ ($\Delta\varepsilon$) 204 (−7.6), 235 (2.6), 267 (−1.7); IR (KBr) ν_{max} 1709, 1655 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HREIMS *m/z* 302.2257 (calcd for $C_{20}H_{30}O_2$, 302.2246).

8,10-Dihydroxy-*iso*-depressin (13): colorless oil; $[\alpha]_D^{25}$ −39.0 (*c* 0.20, $CHCl_3$); CD (*c* 1.42 × 10^{-3} M, *n*-hexane) λ ($\Delta\varepsilon$) 203 (0.06), 217 (0.8), 262 (−2.3); IR (KBr) ν_{max} 1668 cm^{-1}; ^1H and ^{13}C NMR in Tables 1 and 2; HRESIMS *m/z* 341.2096 (calcd for $C_{20}H_{30}O_3Na$, 341.2093).

Preparation of MTPA Esters. (*R*)- and (*S*)-MTPA-Cl (10 μL) and a catalytic amount of DMAP were separately added to two different aliquots of alcohol **7** (1.0 mg each) in dry CH_2Cl_2 (0.5 mL), and the resulting mixtures were allowed to stand at room temperature for 12 h. After the evaporation of the solvent, the mixtures were purified on preparative TLC (SiO_2, light petroleum ether/Et_2O, 8:2), affording pure (*S*)- and (*R*)-MTPA esters of **7**, respectively. The MTPA esters of **8** were prepared following the same procedure.

(*S*)-MTPA ester of 7: selected ^1H NMR values ($CDCl_3$, 400 MHz) δ 6.27 (1H, d, J = 10.5 Hz, H-3), 5.65 (1H, ddd, J = 14.0, 10.0, 4.0 Hz, H-10), 5.17 (1H, t, J = 6.6 Hz, H-7), 5.06 (1H, d, J = 9.3 Hz, H-11), 3.53 (3H, s, OMe), 3.50 (1H, m, H-6a), 3.02 (1H, dd, J = 13.8, 5.7, H-6b), 2.41 (1H, m, H-13a), 2.35 (1H, m, H-9a), 2.18 (1H, m, H-9b), 1.85 (1H, m, H-13b), 1.85 (3H, s, H_3-18), 1.72 (3H, s, H_3-20), 1.64 (3H, s, H_3-19), 1.18 (3H, s, H_3-16), 1.05 (3H, s, H_3-17).

(*R*)-MTPA ester of 7: selected ^1H NMR values ($CDCl_3$, 400 MHz) δ 6.26 (1H, d, J = 10.5 Hz, H-3), 5.62 (1H, ddd, J = 14.0, 10.0, 4.0 Hz, H-10), 5.19 (1H, t, J = 6.6 Hz, H-7), 4.94 (1H, d, J = 9.3 Hz, H-11), 3.54 (3H, s, OMe), 3.52 (1H, m, H-6a), 3.03 (1H, dd, J = 13.8, 5.7 Hz, H-6b), 2.45 (1H, m, H-9a), 2.35 (1H, m H-13a,), 2.28 (1H, m, H-9b), 1.85 (1H, m, H-13b), 1.85 (3H, s, H_3-18), 1.71 (3H, s, H_3-20), 1.66 (3H, s, H_3-19), 1.18 (3H, s, H_3-16), 1.05 (3H, s, H_3-17).

(*S*)-MTPA ester of 8: selected ^1H NMR values ($CDCl_3$, 400 MHz) δ 6.02 (1H, d, J = 10.2 Hz, H-3), 5.66 (1H, ddd, J = 13.4, 9.8, 3.8 Hz, H-10), 5.35 (1H, br d, J = 8.3 Hz, H-7), 5.28 (1H, d, J = 9.3 Hz, H-11), 3.61 (1H, dd, J = 14.3, 10.9 Hz, H-6a), 3.53 (3H, s, OMe), 2.92 (1H, br d, J = 14.3 Hz, H-6b), 2.44 (1H, br d, J = 11.4 Hz, H-9a), 2.20 (1H, dd, J = 11.4, 10.5 Hz, H-9b,), 2.32 (1H, m, H-13a), 2.16 (1H, m, H-13b), 1.99 (1H, m, H-14b), 1.26 (1H, m, H-14a), 1.78 (3H, s, H_3-18), 1.66 (3H, s, H_3-19), 1.62 (3H, s, H_3-20), 1.17 (3H, s, H_3-16), 1.10 (3H, s, H_3-17), 1.04 (1H, m, H-2), 0.56 (1H, m, H-1).

(*R*)-MTPA ester of 8: selected ^1H NMR values ($CDCl_3$, 400 MHz) δ 6.01 (1H, d, J = 10.2 Hz, H-3), 5.65 (1H, ddd, J = 13.2, 9.7, 3.7 Hz, H-10), 5.36 (1H, br d, J = 8.3 Hz, H-7), 5.17 (1H, d, J = 9.3 Hz, H-11), 3.60 (1H, dd, J = 14.3, 11.0 Hz, H-6a), 3.53 (3H, s, OMe), 2.93 (1H, br d, J = 14.3 Hz, H-6b), 2.52 (1H, br d, J = 11.4 Hz, H-9a), 2.35 (1H, dd, J = 11.4, 10.4 Hz, H-9b,), 2.24 (1H, m, H-13a), 2.13 (1H, m, H-13b), 2.01 (1H, m, H-14a), 1.25 (1H, m, H-14b), 1.78 (3H, s, H_3-18), 1.68 (3H, s, H_3-19), 1.58 (3H, s, H_3-20), 1.15 (3H, s, H_3-16), 1.09 (3H, s, H_3-17), 1.04 (1H, m, H-2), 0.55 (1H, m, H-1).

Oxidation of Compounds 7 and 8. Compounds **7** (6.0 mg) and **8** (1.1 mg) were separately treated with Dess-Martin reagent in CH_2Cl_2 (1 mL). The reaction mixtures were stirred at room temperature for 18 h. The excess Dess-Martin reagent was destroyed by adding isopropyl alcohol, and the mixtures were washed with $NaHCO_3$ solution. The organic phases were separated and concentrated under vacuum, and the residues were purified by TLC chromatography on Si gel CC (light petroleum ether/diethyl ether, 7:3) to give **14** (4.6 mg, from **7**) and a compound with spectroscopic data identical with those of natural **9** (0.4 mg, from **8**).

10-Oxodepressin (14): colorless oil; $[\alpha]_D^{25}$ −76 (*c* 0.4, $CHCl_3$); ^1H and ^{13}C NMR in Tables 1 and 2; ESIMS $[M + Na]^+$ *m/z* 323.

Synthetic 1-*epi*-10-oxodepressin: colorless oil; $[\alpha]_D^{25}$ +210 (*c* 0.02, $CHCl_3$);[15] ^1H and ^{13}C NMR identical to those of **9** in Tables 1 and 2; ESIMS $[M + Na]^+$ *m/z* 323.

Conformational Analysis. Structures were obtained by unrestrained SA/EM.[16] Calculations were performed with Sander module of AMBER10,[17] using AMBER GAFF parametrization[18] and RESP charges,[19] fitted from *ab initio* 6-31G* calculations performed with GAMESS.[20] Starting structures for all molecules were built with Ghemical 2.95.[21] Conformational sampling was obtained by unrestrained simulated annealing. The starting structure of each molecule underwent 200 SA cycles of 100 000 MD steps, where system temperature was linearly raised from 10 to 1200 K (steps 1 to 5000), then kept constant at 1200 K (steps 5001 to 50 000), and finally, linearly decreased down to 10 K (steps 50 001 to 100 000). A time step of 1 fs, with no constraints or restraints on bond lengths, a nonbonded cutoff of 16 Å, and a 0.05 fs time constant for heat bath coupling were used, with all other parameters set at their default values. *trans*-Alkene bonds were forced into a *trans* orientation (ω = 180°) by torsional constraints

with a force constant of 30 kcal mol^{-1} operating for deviations higher than 20° from the *trans* form. Final structures were energy minimized (EM) with 2000 steps of steepest descent followed by a conjugate gradient method, down to a gradient norm value less than 10^{-3} kcal mol^{-1} $Å^{-1}$ (thus leading to the final SA/EM structure set), both with the same energy setup used for SA. The MOLMOL[22] program was used for structural analysis and to obtain clusters of conformers by superposition of heavy atoms, using a 0.5 Å cutoff to include structures in the same cluster.

The inverse of the sixth power ($1/r_{ij}^6$) of each possible interprotonic distance was computed either on single structures, or as an average over the whole ensemble of 200 structures, or as a Boltzmann-weighted average, at 300 K, of the representative structures of the selected conformer clusters (see also Supporting Information). Distances involving one or more methyl groups were computed and stored as single distances, by averaging all possible distances involving methyl H atoms for each bundle structure. The inverse of the sixth root of these average values provided the ensemble-averaged distances, directly related to observed peak volumes. Experimental NMR-NOESY peaks were integrated with the CCPNMR 1.0 rel. 15 program.[23]

By using reference peak volumes corresponding to fixed-distance geminal interactions (-3.06×10^{-7} for the H-9a/H-9b geminal peak of **7**, distance 1.75 Å; -4.42×10^{-7} for H-14a/H-14b in **8**, distance 1.76 Å), conversion factors were calculated for all peaks, to be used in the translation of peak volumes into average *experimental* distances.

Antiproliferative Assays. The inhibition of cancer cell proliferation by the compounds **5**−**12** was measured using the MTT assay.[24] Cell suspensions were plated in 96-well plates at a density of 1×10^4 cells mL^{-1}. The test compound solutions (10 μL in DMSO) at various concentrations were added to each well, and the culture was incubated for 72 h at 37 °C in a 5% CO_2 incubator. After the treatment, 20 μL of the MTT solution (5 mg/mL) was added to each well and cells were incubated at 37 °C in 5% CO_2. After incubation for 4 h the medium was removed and 150 μL of DMSO was added. The absorbance was measured at 570 nm. Dose−response curves were generated, and the concentration of each compound required to inhibit cell proliferation by 50% (IC_{50}) was calculated from the linear portion of the log dose response curves.

Antibacterial Assays. Antibacterial assays were performed by the broth macrodilution method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) document M27-P (NCCLS, 1992)[25] and of the Clinical and Laboratory Standard Institute (CLSI) document M7-A7 (CLSI, 2007).[26] The medium used to prepare the 10× drug dilutions, and the inoculum suspension was liquid LB (Luria−Bertani medium: 10 g/L Bactotryptone, 5 g/L Bactoyeast, and 10 g/L NaCl, pH 7.5).[27,28] The bacteria suspensions were adjusted with the aid of a spectrophotometer to a cell density of 0.5 McFarland (2×10^8 cfu/mL) standard at 530 nm and diluted to 1:4000 (50 000 cfu/mL) in LB medium. The bacteria suspension (0.9 mL) was added to each test tube that contained 0.1 mL of eleven 2-fold dilutions (512−0.05 μg/mL final) of each tested compound.

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Supporting Information Available: ^1H and ^{13}C NMR, $^1H-^1H$ COSY, HSQC, HMBC, and CD spectra of compounds **5**−**14** and computational analysis details are available free of charge via the Internet at http://pubs.acs.org.

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