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## Structurally Diverse Metabolites from the Soft Coral *Sinularia verruca* Collected in the South China Sea

Weiping Yuan<sup>†</sup>, Shimiao Cheng<sup>‡</sup>, Weitao Fu<sup>†</sup>, Min Zhao<sup>†</sup>, Xiubao Li<sup>§</sup>, Yuepiao Cai<sup>†</sup>, Jianyong Dong<sup>†</sup>, Kexin Huang<sup>†</sup>, Kirk R. Gustafson<sup>\*,†,⊥</sup>, and Pengcheng Yan<sup>\*,†,⊥</sup>

<sup>†</sup>School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, Zhejiang 325035, People's Republic of China

<sup>‡</sup>The Fifth Affiliated Hospital, Wenzhou Medical University, Lishui, Zhejiang 323000, People's Republic of China

<sup>§</sup>Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, Guangdong 510301, People's Republic of China

<sup>⊥</sup>Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702-1201, United States

### Abstract

Nineteen metabolites with diverse structures, including the rare pyrroloindoline alkaloid verrupyrroloindoline (**1**), the unprecedented highly fused benzosesquiterpenoid verrubenzospirolactone (**2**), the new asteriscane-type sesquiterpenoid 10-deoxocapillosanane D (**3**), and the two new cyclopentenone derivatives (4*S*\*,5*S*\*)-4-hydroxy-5-(hydroxymethyl)-2,3-dimethyl-4-pentylcyclopent-2-en-1-one (**4**) and (*S*)-4-hydroxy-5-methylene-2,3-dimethyl-4-pentylcyclopent-2-en-1-one (**5**), were isolated from a South China Sea collection of the soft coral *Sinularia verruca*. Eleven previously described marine metabolites (**7–15**, **18**, and **19**) were also obtained as well as three new EtOH-adduct artifacts (**6**, **16**, and **17**). The structures of the new compounds were elucidated by extensive spectroscopic analysis and by comparison with previously reported data. Compounds **4**, **5**, and **16** showed protection against the cytopathic effects of HIV-1 infection with EC<sub>50</sub> values of 5.8–34 μM, and **4**, **6**, and **16** exhibited inhibition against LPS-induced NO production with IC<sub>50</sub> values of 24–28 μM.

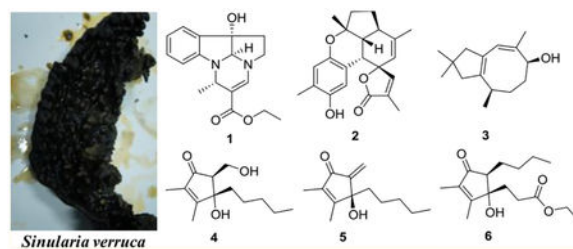
### Graphical Abstract

\*Corresponding Authors: Tel (K. R. Gustafson): +1-301-8465197. Fax: +1-301-8466851. gustafki@mail.nih.gov. (P. Yan): yanpc@wzmc.edu.cn; pengcheng.yan@nih.gov.

Supporting Information

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The authors declare no competing financial interest.



Soft corals of the genus *Sinularia* (family Alcyoniidae) are commonly distributed in the tropical coral reef systems of the world and are well known for being a rich source of biologically active secondary metabolites. In the past several decades, more than 50 *Sinularia* species have been chemically investigated, resulting in the discovery of hundreds of secondary metabolites including sesquiterpenoids, diterpenoids, polyhydroxylated steroids, cyclopentenones, and butenolides.<sup>1</sup> Many of these metabolites are structurally novel and display a wide range of significant ecological and pharmacological activities, such as antifouling, antifeedant, allelopathy, cytotoxic, antimicrobial, and anti-inflammatory properties.<sup>2–8</sup> In the course of our ongoing research on bioactive natural products from marine invertebrates of the South China Sea,<sup>9–11</sup> a previously unstudied soft coral, *Sinularia verruca* van Ofwegen, 2008, was collected. Detailed chemical examination of this specimen led to the isolation of 19 compounds with diverse structural scaffolds, including a rare pyrroloindoline alkaloid (**1**), a novel benzosesquiterpenoid (**2**), a new asteriscane-type sesquiterpenoid (**3**), and two new cyclopentenone derivatives (**4** and **5**). Eleven known compounds were also isolated from the chemically rich extract as well as three new EtOH-adduct artifacts (**6**, **16**, and **17**). All compounds were evaluated for *in vitro* antiviral activity against human immunodeficiency virus type 1 (HIV-1) and for inhibitory effects on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse peritoneal macrophages (PEMΦ). This paper reports details of the isolation, structure elucidation, and bioactivity assessment of these compounds.

## RESULTS AND DISCUSSION

Compound **1** had a molecular formula of C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> according to its HREASIMS data, implying nine degrees of unsaturation. The IR spectrum showed absorption bands attributed to hydroxy (3437 cm<sup>-1</sup>), carbonyl (1677 cm<sup>-1</sup>), and aromatic ring (1606 and 1466 cm<sup>-1</sup>) functionalities. The presence of an *ortho*-disubstituted benzene ring was revealed by <sup>1</sup>H NMR signals at δ<sub>H</sub> 7.31 (dd, *J* = 7.2, 1.2 Hz, H-3), 7.24 (td, *J* = 7.8, 1.2 Hz, H-5), 6.85 (t, *J* = 7.2 Hz, H-4), and 6.69 (d, *J* = 7.8 Hz, H-6) (Table 1) and <sup>13</sup>C NMR signals at δ<sub>C</sub> 150.9 (C, C-1), 131.1 (C, C-2), 131.0 (CH, C-5), 124.1 (CH, C-3), 120.3 (CH, C-4), and 111.1 (CH, C-6) (Table 2), as well as COSY correlations between H-3/H-4, H-4/H-5, and H-5/H-6 and HMBC correlations from H-3 to C-1 and from H-6 to C-2 (Figure 1). A disubstituted ethyl isopentenoate unit was established by HMBC correlations from methyl doublet H<sub>3</sub>-14 (δ<sub>H</sub> 1.41 d, *J* = 6.6 Hz) to methine carbon C-11 (δ<sub>C</sub> 46.3) and nonprotonated olefinic carbon C-12 (δ<sub>C</sub> 101.7), from H-11 (δ<sub>H</sub> 4.53 d, *J* = 6.6 Hz) to carbonyl carbon (3–15 (δ<sub>C</sub> 167.4) and olefinic methine carbon C-13 (δ<sub>C</sub> 142.6), from olefinic proton H-13 (δ<sub>H</sub> 7.13 s) to C-15 and C-11, and from the oxymethylene protons at δ<sub>H</sub> 4.13 (q, *J* = 7.2 Hz) to the carbonyl carbon

C-15, in combination with COSY couplings between H<sub>3</sub>-14 and H-11 and between a methyl triplet ( $\delta_{\text{H}}$  1.25 t,  $J = 7.2$  Hz) and the oxymethylene protons mentioned above. On the basis of the remaining degrees of unsaturation, **1** had to be tetracyclic. HMBC correlations from H-3 to nonprotonated carbon C-7 ( $\delta_{\text{C}}$  84.1), from H-10 ( $\delta_{\text{H}}$  4.75 s) to C-1, C-2, and C-9 ( $\delta_{\text{C}}$  49.8, CH<sub>2</sub>), from H<sub>2</sub>-8 ( $\delta_{\text{H}}$  2.61 m) to C-2, and from H<sub>2</sub>-9 to C-7, and a COSY correlation between H<sub>2</sub>-8 and H<sub>2</sub>-9, in combination with the chemical shifts of C-1, C-7, C-9, C-10, H-9, and H-10, allowed us to establish a 2,3,8,8a-tetrahydropyrrolo[2,3-*b*]indol-3a(1*H*)-ol moiety. Moreover, HMBC correlations from H-11 to C-1 and C-10 and from H-13 to C-9 and C-10 revealed that the ethyl isopentenoate unit was connected to the two N atoms of the fused indolin-3-ol and pyrrolidine moieties via C-11 and C-13, respectively. The relative configuration of 7*S*\*, 10*S*\*, and 11*S*\* was assigned based on NOESY correlations between H-6/H-11, H<sub>3</sub>-14/H-10, and H<sub>2</sub>-8/H-3 (Figure 2). Compound **1** was given the name verrupyrroloindoline. The ethyl ester is possibly an artifact because EtOH was used during the extraction process; however no evidence of a nonesterified precursor of **1** was observed during the isolation procedure. In either case, the free acid or ethyl ester form of **1** represents a rare natural alkaloid class with a 2a, 5a-diazacyclopenta[*jk*]fluorene skeleton.<sup>12</sup>

Compound **2** had a molecular formula of C<sub>22</sub>H<sub>24</sub>O<sub>4</sub> as determined by HRESIMS data, which implied 11 degrees of unsaturation. IR absorptions indicated the presence of hydroxy (3444 cm<sup>-1</sup>), carbonyl (1743 cm<sup>-1</sup>), and aromatic ring (1610, 1512, and 1450 cm<sup>-1</sup>) functionalities. The <sup>13</sup>C NMR spectrum exhibited 22 carbon signals including an ester carbonyl ( $\delta_{\text{C}}$  and 10 additional sp<sup>2</sup> carbons, which indicated that **2** was pentacyclic. Signals for two oxygenated aliphatic carbons, four methyls, two methylenes, and three aliphatic methines were also present in the <sup>13</sup>C NMR spectrum. A 1,2,4,5-tetrasubstituted benzene ring was established by HMBC correlations from H<sub>3</sub>-7' ( $\delta_{\text{H}}$  2.15 s) to C-4' ( $\delta_{\text{C}}$  147.4, C), C-5' ( $\delta_{\text{C}}$  124.5, C), and C-6' ( $\delta_{\text{C}}$  119.7, CH), from H-3' ( $\delta_{\text{H}}$  7.29 s) to C-1' ( $\delta_{\text{C}}$  146.4, C) and C-5', and from H-6' ( $\delta_{\text{H}}$  6.54 s) to C-2' ( $\delta_{\text{C}}$  116.8, C) and C-4', while an  $\alpha,\beta$ -unsaturated- $\gamma$ -lactone moiety was recognized by HMBC correlations from H<sub>3</sub>-13 ( $\delta_{\text{H}}$  1.96 s) to the carbonyl carbon C-12 ( $\delta_{\text{C}}$  174.0) and two olefinic carbons C-10 ( $\delta_{\text{C}}$  153.5, CH) and C-11 ( $\delta_{\text{C}}$  128.0, C) and from H-10 ( $\delta_{\text{H}}$  7.11, s) to the oxygenated nonprotonated carbon C-9 ( $\delta_{\text{C}}$  89.0) (Figure 1). COSY correlations between H-1 ( $\delta_{\text{H}}$  3.24 d,  $J = 4.8$  Hz)/H-2 ( $\delta_{\text{H}}$  1.80 dd,  $J = 12.0, 4.8$  Hz), H-2/H-6 ( $\delta_{\text{H}}$  2.56 m), H-6/H-5 ( $\delta_{\text{H}}$  1.99 m; 1.37 m), and H-5/H-4 ( $\delta_{\text{H}}$  2.10 m; 1.93 m) defined an extended proton spin system. HMBC correlations from H<sub>3</sub>-14 ( $\delta_{\text{H}}$  1.68 s) to the aliphatic methine carbon C-6 ( $\delta_{\text{C}}$  40.7) and two olefinic carbons C-7 ( $\delta_{\text{C}}$  143.1, C) and C-8 ( $\delta_{\text{C}}$  119.9, CH), from H<sub>3</sub>-15 ( $\delta_{\text{H}}$  1.33 s) to the other oxygenated nonprotonated carbon C-3 ( $\delta_{\text{C}}$  82.0), an aliphatic methine carbon C-2 ( $\delta_{\text{C}}$  50.1), and a methylene carbon C-4 ( $\delta_{\text{C}}$  39.0), and from H-1 to C-8 and two carbons (C-9 and C-10) of the  $\alpha,\beta$ -unsaturated- $\gamma$ -lactone moiety allowed us to establish the 5,6-membered ring system C/D, in which C-3 and C-7 were substituted by methyls, C-7/C-8 composed a double bond, and C-9 was a *spiro* carbon that was also a constituent of the  $\alpha,\beta$ -unsaturated- $\gamma$ -lactone. Direct linkage of the substituted benzene ring to C-1 was indicated by the chemical shift of H-1 and confirmed by HMBC correlations from H-1 to C-1', C-2', and C-3'. An ether linkage between C-3 and C-1' was supported by their chemical shifts ( $\delta_{\text{C}}$  82.0 and 146.4, respectively) and the remaining unsaturation equivalent, which thus required C-4' to be hydroxylated. The relative configurations of the stereogenic centers in **2** were assigned on

the basis of coupling constants and NOESY analysis. The coupling constant values  $J_{H-1/H-2}$  (4.8 Hz) and  $J_{H-2/H-6}$  (12.0 Hz) suggested the *cis* and *trans* fusions of rings B/D and C/D, respectively, while NOESY correlations between H<sub>3</sub>-15/H-1, H-1/H-2, and H-1/H-10 indicated that H<sub>3</sub>-15, H-1, H-2, and H-10 had the same facial orientation (Figure 2). Thus, compound **2** was established to be a highly fused benzosesquiterpenoid lactone, and it was named verrubenzospinolactone. It is interesting to note that **2** represents a novel skeleton that could be derived from capillobenzopyranol **12**<sup>13</sup> via a series of oxidation and cyclization reactions.

Compound **3** was assigned a molecular formula of C<sub>15</sub>H<sub>24</sub>O based on its HRESIMS data, requiring four degrees of unsaturation. The <sup>13</sup>C NMR spectrum showed 15 carbon signals including four olefinic carbons, indicating that **3** must be bicyclic. A 4,4-dimethylcyclopent-1-ene moiety was established by HMBC correlations from H<sub>3</sub>-12 ( $\delta_H$  1.04 s) and H<sub>3</sub>-13 ( $\delta_H$  1.03 s) to C-1 ( $\delta_C$  36.5, CH<sub>2</sub>), C-10 ( $\delta_C$  53.0, CH<sub>2</sub>), and C-11 ( $\delta_C$  29.2, C) and from H<sub>2</sub>-1 ( $\delta_H$  2.28 d,  $J = 15.6$  Hz; 1.99 d,  $J =$  Hz) and H<sub>2</sub>-10 ( $\delta_H$  2.21 d,  $J = 15.0$  Hz; 2.01 d,  $J = 15.0$  Hz) to two nonprotonated olefinic carbons C-2 ( $\delta_C$  142.4) and C-9 ( $\delta_C$  129.3). COSY correlations established a spin system from H-3 to H-6 and extended to H<sub>3</sub>-15. Additional HMBC correlations from H<sub>3</sub>-14 ( $\delta_H$  1.73 s) to oxygenated carbon C-6 ( $\delta_C$  71.5, CH) and two olefinic carbons C-7 ( $\delta_C$  136.7, C) and C-8 ( $\delta_C$  122.8, CH), from H-8 ( $\delta_H$  5.65 s) to C-10 and C-2, and from H<sub>3</sub>-15 ( $\delta_H$  0.99 d,  $J = 7.2$  Hz) to C-2 allowed connection of the structural subunits to define an asteriscanetype sesquiterpenoid. Accordingly, the planar structure of **3** was established as 6-hydroxyasterisca-2(9),7-diene. The relative configurations at C-3 and C-6 and the *Z* configuration of the C-7/C-8 double bond in **3** were suggested to be the same as those of the known analogues capillosananes B—D<sup>14</sup> from *Sinularia capillosa* based on their very similar NMR data. As such, compound **3** was assigned as 10-deoxocapillosanane D.

Compound **4** had a molecular formula of C<sub>13</sub>H<sub>22</sub>O<sub>3</sub> as determined by HRESIMS data, requiring three degrees of unsaturation. IR absorptions at 3393, 1693, and 1647 cm<sup>-1</sup> indicated the presence of hydroxy, carbonyl, and olefinic functionalities. The <sup>13</sup>C NMR spectrum showed 13 carbon signals including a conjugated ketone carbonyl ( $\delta_C$  206.1) and two nonprotonated olefinic carbons at  $\delta_C$  169.3 and 136.3 (Table 2), which accounted for two of the three degrees of unsaturation; therefore **4** was monocyclic. The <sup>1</sup>H NMR spectrum exhibited three methyl signals including two olefinic methyl singlets ( $\delta_H$  1.99 s, H<sub>3</sub>-13; 1.73 s, H<sub>3</sub>-12) and a methyl triplet ( $\delta_H$  0.85 t,  $J = 6.6$  Hz, H<sub>3</sub>-11) (Table 1). In addition, a tertiary oxygen-bearing carbon ( $\delta_C$  81.0, C-4), a secondary alcohol ( $\delta_C$  59.4, C-6;  $\delta_H$  4.05 dd,  $J = 10.8, 7.8$  Hz, H-6a; 3.88 br t,  $J = 8.4$  Hz, H-6b), an aliphatic methine ( $\delta_C$  59.7, C-5;  $\delta_H$  2.71 t,  $J = 7.2$  Hz, H-5), and four aliphatic methylenes were recognized from the <sup>1</sup>H and <sup>13</sup>C NMR data. An *n*-pentyl unit was established by COSY correlations between H<sub>3</sub>-11/H<sub>2</sub>-10 ( $\delta_H$  1.24 m), H<sub>2</sub>-7 ( $\delta_H$  1.72 m; 1.70 m)/H<sub>2</sub>-8 ( $\delta_H$  0.87 m), and H<sub>2</sub>-8/H<sub>2</sub>-9 ( $\delta_H$  1.24 m) and HMBC correlations (Figure 1) from H<sub>3</sub>-11 and H<sub>2</sub>-7 to the aliphatic methylene carbon C-9 ( $\delta_C$ ). Finally, the gross structure of **4** was established as 4-hydroxy-5-(hydroxymethyl)-2,3-dimethyl-4-pentylcyclopent-2-en-1-one based on HMBC correlations from H<sub>3</sub>-12 to the ketone carbonyl C-1 and the two olefinic carbons C-2 and C-3, from H<sub>3</sub>-13 to C-2, C-3, and the oxygenated nonprotonated carbon C-4, from H<sub>2</sub>-7 to C-3, C-4,

and the methine carbon C-5, and from the oxymethylene H<sub>2</sub>-6 to C-1, C-5, and C-4. COSY correlations between H-5 and H<sub>2</sub>-6 were observed, and an NOE correlation between H<sub>2</sub>-6 and H<sub>2</sub>-7 could establish their cis relationship (**4a** in Figure 2) in the cyclopent-2-enone nucleus, which is much more rigid than a typical five-membered ring system, such as a cyclopentane ring. In order to support this assignment, a set of 500 conformers of the *trans* isomer **4b** (Figure 2) were modeled, and the distance between H<sub>2</sub>-6 and H<sub>2</sub>-7 was determined to be 3.6–4.8 Å, which is greater than the typical limit for seeing NOE correlations. Compound **4** was found to be closely related to the previously reported metabolite (+)-sinularone **J**, which has a methoxy in place of the hydroxy at C-6.<sup>15</sup>

The closely related analogue **16** was also isolated and assigned a molecular formula of C<sub>15</sub>H<sub>26</sub>O<sub>3</sub> based on HRESIMS data. It provided NMR data (Tables 1 and 2) that were very similar to those of **4**, except there were additional signals for an ethoxy group ( $\delta_{\text{C}}$  66.8 (CH<sub>2</sub>), 15.2 (CH<sub>3</sub>);  $\delta_{\text{H}}$  3.54 2H, q,  $J$  = 6.6 Hz; 1.22 3H, t,  $J$  = 6.6 Hz). Linkage of the ethoxy group to C-6 in **16** was indicated by the deshielded chemical shift of C-6 ( $\delta_{\text{C}}$  66.8) in comparison with that of **4** (C-6,  $\delta_{\text{C}}$  59.4) and further confirmed by HMBC correlations from H<sub>2</sub>-6 ( $\delta_{\text{H}}$  3.96 dd,  $J$  = 9.6, 4.0 Hz; 3.52 dd,  $J$  = 10.2, 9.6 Hz) to the ketone carbon C-1 ( $\delta_{\text{C}}$  202.7) and to the oxymethylene carbon of the ethoxy group (Figure 1). The configurations at C-4 and C-5 in **16** were tentatively determined to be the same as those in **4** since it showed similar NOE correlations between H<sub>2</sub>-6 and H<sub>2</sub>-7 ( $\delta_{\text{H}}$  1.86 ddd,  $J$  = 13.2, 12.0, 4.2 Hz; 1.78 ddd,  $J$  = 13.2, 12.6, 5.4 Hz) and it had the same sign of specific rotation ( $[\alpha]_{\text{D}}^{25}$  + 7, MeOH).

The co-occurrence of **16** and **4** in the extract and the fact that EtOH was used during the extraction process suggest that compound **16** is likely a 6-*O*-ethyl artifact of **4**.

The molecular formula of compound **5** was determined to be C<sub>13</sub>H<sub>20</sub>O<sub>2</sub> by HRESIMS, indicating four degrees of unsaturation. NMR data analyses revealed that **5** had a structure closely related to that of **4**. The only difference was loss of signals for an aliphatic methine and a secondary alcohol and the appearance of signals for an exocyclic double bond ( $\delta_{\text{C}}$  148.8 (C), 114.7 (CH<sub>2</sub>);  $\delta_{\text{H}}$  6.05 s, 5.53 s). HMBC correlations from the exomethylene protons to a ketone ( $\delta_{\text{C}}$  194.1, C-1) and a hydroxylated nonprotonated carbon ( $\delta_{\text{C}}$  78.7, C-4) revealed the exocyclic double bond was located at C-5/C-6. Thus, compound **5** was elucidated as 4-hydroxy-5-methylene-2,3-dimethyl-4-pentylcyclopent-2-en-1-one. The measured ECD spectrum of **5** was similar to the calculated ECD spectrum for the *S*-enantiomer, allowing the tentative assignment of the 4*S* configuration for **5** (Figure 3).

A molecular formula of C<sub>16</sub>H<sub>26</sub>O<sub>4</sub> was established for compound **6** by HRESIMS data, which required four unsaturation equivalents. The 2,3-dimethylcyclopent-2-enone nucleus was recognized from diagnostic NMR signals for conjugated ketone carbonyl C-1 ( $\delta_{\text{C}}$  204.9), two non-protonated olefinic carbons C-2 ( $\delta_{\text{C}}$  136.6) and C-3 ( $\delta_{\text{C}}$  166.7), and two olefinic methyls ( $\delta_{\text{C}}$  11.3, 8.0;  $\delta_{\text{H}}$  1.98, 1.70). An *n*-butyl group was established by COSY correlations between H<sub>3</sub>-9 ( $\delta_{\text{H}}$  0.94 t,  $J$  = 7.2 Hz)/H<sub>2</sub>-8 ( $\delta_{\text{H}}$  1.39 m), H<sub>2</sub>-8/H<sub>2</sub>-7 ( $\delta_{\text{H}}$  1.62 m; 1.51, m), and H<sub>2</sub>-7/H<sub>2</sub>-6 ( $\delta_{\text{H}}$  1.81 m; 1.39 m). In addition, an ethylpropanoate unit was constructed by a COSY correlation between two vicinal methylenes H<sub>2</sub>-10 ( $\delta_{\text{H}}$  2.05 m; 1.93 m) and H<sub>2</sub>-11 ( $\delta_{\text{H}}$  2.04 m) and the coupling between an oxymethylene ( $\delta_{\text{H}}$  4.11 q,  $J$  = 6.6 Hz) and the second methyl triplet ( $\delta_{\text{H}}$  1.24 t,  $J$  = 6.6 Hz), in combination with HMBC

correlations from H<sub>2</sub>-10, H<sub>2</sub>-11, and the oxymethylene protons to ester carbonyl C-12 ( $\delta_C$  173.1). HMBC correlations from H<sub>2</sub>-10 to olefinic carbon C-3, oxygenated nonprotonated carbon C-4 ( $\delta_C$  81.5), and methine C-5 ( $\delta_C$  58.9), along with a COSY correlation between H<sub>2</sub>-6 and the methine proton H-5 ( $\delta_H$  2.34 t,  $J$  = 6.6 Hz), allowed connection of the ethylpropanoate and *n*-butyl units to C-4 and C-5, respectively. This indicated that C-4 was hydroxylated. Thus, compound **6** was established as 4-hydroxy-4-(ethyl-propanoate)-2,3-dimethyl-5-butylcyclopent-2-en-1-one, probably an ethanolysis artifact formed from the co-occurring analogue sinularone D (**8**).<sup>16</sup> The absolute configurations at C-4 and C-5 were tentatively assigned to be identical to those in **8** based on biogenetic considerations and similar NOE correlations between H<sub>2</sub>-6 and H<sub>2</sub>-10 (Figure 2).

Eleven known compounds were also isolated from the *S. verruca* extract and identified as sinularones A (**7**),<sup>16</sup> D (**8**),<sup>16</sup> G (**9**),<sup>16</sup> and I (**10**),<sup>16</sup> (*S*)-5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5*H*)-one (**11**),<sup>17</sup> capillobenzopyranol (**12**),<sup>13</sup> flexibilisqui-nine (**13**),<sup>18</sup> 5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2-methylpentanoic acid methyl ester (**14**),<sup>19</sup> capillosanane I (**15**),<sup>14</sup> (*Z*)-*N*-(4-hydroxyphenethyl)-3-methyldodec-2-enamide,<sup>20</sup> and (*Z*)-*N*-(3-hydroxy-4-methoxyphenethyl)-3-methyl-dodec-2-enamide,<sup>20</sup> by comparison of their <sup>1</sup>H and <sup>13</sup>C NMR and MS spectroscopic data, as well as specific rotations, with those reported in the literature.

A final compound, **17**, was isolated, and its molecular formula determined to be C<sub>21</sub>H<sub>36</sub>O<sub>5</sub> on the basis of HRESIMS data. Therefore, compound **17** was isomeric with compound **10**, and its NMR data also corresponded very closely with the NMR data for **10**. HMBC correlations from H<sub>3</sub>-18 to the carbonyl carbon C-1 and two olefinic carbons C-2 and C-3 and from H<sub>3</sub>-19 to the two olefinic carbons and the acetal carbon C-4 disclosed the same  $\alpha,\beta$ -unsaturated-2,3-dimethyl- $\gamma$ -lactone unit as found in butenolide **10**. An ethoxy group [ $\delta_C$  58.3 (CH<sub>2</sub>), 15.2 (CH<sub>3</sub>);  $\delta_H$  3.40 and 3.14 (both 1H, dq,  $J$  = 12.0, 6.6 Hz), 1.17 (3H, t,  $J$  = 6.6 Hz)] and 12 aliphatic methylenes were also similar to those seen in the artifact sinularone I (**10**).<sup>16</sup> An HMBC correlation from the oxymethylene protons to the acetal carbon C-4 revealed the location of the ethoxy group at C-4 in **17**, instead of forming an ethyltridecanoate unit as in **10**. Thus, compound **17** was defined as 13-(2-ethoxy-3,4-dimethyl-5-oxo-2,5-dihydrofuran-2-yl)tridecanoic acid, which has not previously been reported in the chemical literature, and it was named isosinularone I. The absolute configuration at C-4 was tentatively assigned to be the same as that of **10** based on its similar specific rotation [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 6, MeOH).

All of the compounds obtained from the *S. verruca* extract were tested for anti-HIV-1 activity in a cell-based HIV screening assay.<sup>21–24</sup> Compounds **4**, **5**, and **16** were found to be moderately protective against the cytopathic effects of *in vitro* HIV-1 infection with EC<sub>50</sub> values of 34, 5.8, and 30  $\mu$ M, respectively, and maximum protection rates of 86%, 52%, and 75%, respectively. However, their IC<sub>50</sub> values for cytotoxicity against the CEM-SS host cells were determined to be 79, 6.3, and 66  $\mu$ M, indicating low therapeutic indices. The other compounds tested showed no detectable antiviral activity in this assay.

In addition, all compounds were evaluated for their *in vitro* anti-inflammatory activities.<sup>25</sup> In the primary assay, **4**, **6**, and **16** showed moderate inhibition against lipopolysaccharide-induced NO production in mouse peritoneal macrophages with IC<sub>50</sub> values of 28, 24, and 26  $\mu$ M, respectively, whereas no inhibitory effect (inhibition <50%) was observed for the other compounds at 30  $\mu$ M.

## EXPERIMENTAL SECTION

### General Experimental Procedures.

Optical rotations, UV, and IR spectra were measured using a PoLAAR 3005 digital polarimeter, a TU 1901 spectrometer, and a Bruker Equinox 55 spectrometer, respectively. <sup>1</sup>H and <sup>13</sup>C NMR spectra were acquired with a Bruker Avance-600FT NMR spectrometer using TMS as an internal standard. HRESIMS data were obtained using a Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemistry Co. Ltd.), Sephadex LH-20 (GE Healthcare Biosciences AB), and ODS (50  $\mu$ m, YMC). Silica gel GF<sub>254</sub> (Qingdao Marine Chemistry Co. Ltd.) was used for TLC analysis. HPLC separation was performed using an Agilent 1100 series instrument equipped with a VWD G1314A detector and a YMC-Pack C<sub>18</sub> column (10  $\mu$ m, 250  $\times$  10 mm).

### Animal Material.

Specimens of the soft coral *Sinularia verruca* van Ofwegen, 2008, were collected from the inner coral reef of Ximao Island, Hainan Province, China, in November 2011, at a depth of 8 m and frozen immediately after collection. The specimen was identified by one of the authors (X.L.). A voucher specimen (HS201102) was deposited at the Laboratory of Marine Natural Products Chemistry, Wenzhou Medical University, China.

### Extraction and Isolation.

The frozen soft coral *S. verruca* (wet weight: 1.20 kg) was homogenized and extracted with 95% EtOH at room temperature (rt). The concentrated extract was partitioned between H<sub>2</sub>O and EtOAc. Evaporation of EtOAc *in vacuo* yielded a dark residue of 22.5 g. The EtOAc fraction (22.0 g) was subjected to silica gel vacuum column chromatography, eluting with a gradient of EtOAc/petroleum ether (1:20, 1:10, 1:5, 1:3, and 1:2), to obtain eight fractions (A1–A8). Fraction A3 (700.2 mg) was chromatographed on a Sephadex LH-20 column, eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1), to afford four fractions (A3a–A3d). Fraction A3b (100.5 mg) was further separated on an ODS column, eluting with MeOH/H<sub>2</sub>O (75:25, 80:20), to afford **8** (6.4 mg) and **11** (26.1 mg). Fraction A3d (60.0 mg) was purified by semipreparative HPLC, using MeOH/H<sub>2</sub>O (75:25) as eluent, to yield **5** (6.0 mg) and **15** (22.5 mg). Fraction A4 (1.1 g) was subjected to a Sephadex LH-20 column, eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1), to obtain three fractions (A4a–A4c). Fraction A4c (798.1 mg) was further separated on an ODS column, eluting with MeOH/H<sub>2</sub>O (70:30, 75:25, 80:20, 85:15, and 90:10), to afford seven fractions (A4c1–A4c7). Fraction A4c2 (40.0 mg) was purified by HPLC (MeOH/H<sub>2</sub>O, 60:40) to yield **9** (3.5 mg) and **1** (1.5 mg). Fraction A4c3 (19.8 mg) was purified by HPLC (MeOH/H<sub>2</sub>O, 62:38) to afford **6** (3.3 mg). Fraction A4c4 (30.2 mg) was purified by HPLC (MeOH/H<sub>2</sub>O, 70:30) to yield **13** (14.0 mg) and **14** (2.2 mg). Fraction A4c5 (70.3 mg) was

purified by HPLC (MeOH/H<sub>2</sub>O, 70:30) to obtain (*Z*)-*N*-(3-hydroxy-4-methoxyphenethyl)-3-methyldodec-2-enamide (5.0 mg), **3** (6.3 mg), and **7** (2.6 mg). Fraction A4c6 (90.2 mg) was purified by HPLC (MeOH/H<sub>2</sub>O, 80:20) to obtain **17** (5.2 mg), **12** (11.3 mg), and (*Z*)-*N*-(4-hydroxyphenethyl)-3-methyldodec-2-enamide (23.5 mg). Fraction A6 (1.5 g) was subjected to a Sephadex LH-20 column, using CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1) as eluent, to obtain three fractions (A6a–A6c). Fraction A6c (580.1 mg) was fractionated on an ODS column, eluting with MeOH/H<sub>2</sub>O (65:35, 70:30, 75:25, and 80:20), to afford five fractions (A6c1–A6c5). Fraction A6c2 (19.3 mg) was purified by HPLC (MeOH/H<sub>2</sub>O, 70:30) to yield **16** (2.6 mg) and **2** (2.0 mg). Following the same protocol as for fraction A6c2, **4** (1.2 mg) and **10** (1.3 mg) were separated from fraction A6c4 (10.5 mg) using MeOH/H<sub>2</sub>O (80:20) as a mobile phase.

*Verrupyrroloindoline (1)*: colorless oil;  $[\alpha]_{\text{D}}^{25} - 22$  (*c* 0.06, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 205 (3.96), 242 (3.55), 288 (3.76); IR (KBr)  $\nu_{\text{max}}$  3437, 2932, 2873, 1677, 1607, 1466, 1383, 1237, 1148, 1105, 1048, 756 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2; HRESIMS *m/z* 323.1369 [M + Na]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>Na, 323.1372).

*Verrubenzospirolactone (2)*: colorless oil;  $[\alpha]_{\text{D}}^{25} + 14$  (*c* 0.10, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 203 (4.68), 296 (3.37); IR (KBr)  $\nu_{\text{max}}$  3444, 2929, 2862, 1743, 1647, 1610, 1512, 1450, 1413, 1381, 1186, 1049 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2; HRESIMS *m/z* 375.1567 [M + Na]<sup>+</sup> (calcd for C<sub>22</sub>H<sub>24</sub>O<sub>4</sub>Na, 375.1572).

*10-Deoxocapillosanane D (3)*: colorless oil;  $[\alpha]_{\text{D}}^{25} - 230$  (*c* 0.04, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 240 (3.73); IR (KBr)  $\nu_{\text{max}}$  3432, 2925, 1645, 1459, 1385, 1051, 997 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2; HRESIMS *m/z* 203.1791 [M + H - H<sub>2</sub>O]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>23</sub>, 203.1800).

*(4*S*\*,5*S*\*)-4-Hydroxy-5-(hydroxymethyl)-2,3-dimethyl-4-pentylcy-clopent-2-en-1-one (4)*: colorless oil;  $[\alpha]_{\text{D}}^{25} + 19$  (*c* 0.05, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 232 (3.68); IR (KBr)  $\nu_{\text{max}}$  3393, 2923, 2855, 1693, 1647, 1382, 1048 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2; HRESIMS *m/z* 249.1465 [M + Na]<sup>+</sup> (calcd for C<sub>13</sub>H<sub>22</sub>O<sub>3</sub>Na, 249.1467).

*(S)-4-Hydroxy-5-methylene-2,3-dimethyl-4-pentylcyclopent-2-en-1-one (5)*: colorless oil;  $[\alpha]_{\text{D}}^{25} + 6$  (*c* 0.10, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 259 (3.72); ECD (MeOH) 257 (  $\epsilon$  +2.53) nm; IR (KBr)  $\nu_{\text{max}}$  3454, 2927, 2861, 1694, 1631, 1386, 1033 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2; HRESIMS *m/z* 231.1359 [M + Na]<sup>+</sup> (calcd for C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>Na, 231.1361).

*(4*S*,5*S*)-4-Hydroxy-4-(ethylpropanoate)-2,3-dimethyl-5-butylcy-clopent-2-en-1-one (6)*: colorless oil;  $[\alpha]_{\text{D}}^{25} + 14$  (*c* 0.08, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 229 (3.77); IR (KBr)  $\nu_{\text{max}}$  3446, 2930, 2866, 1776, 1707, 1650, 1461, 1384, 1165, 1018 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Tables 1 and 2; HRESIMS *m/z* 305.1726 [M + Na]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>26</sub>O<sub>4</sub>Na, 305.1729).

*Sinularone D (8)*: colorless oil;  $[\alpha]_{\text{D}}^{25} - 5$  (*c* 0.10, MeOH); lit.  $[\alpha]_{\text{D}}^{23} - 3$  (*c* 0.09, MeOH).<sup>16</sup>



(4*S*\*,5*S*\*)-4-Hydroxy-5-(ethoxymethyl)-2,3-dimethyl-4-pentylcyclopent-2-en-1-one (**16**): colorless oil;  $[\alpha]_{\text{D}}^{25} + 7$  (*c* 0.10, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 233 (3.63); IR (KBr)  $\nu_{\text{max}}$  3440, 2928, 2865, 1700, 1651, 1458, 1382, 1110, 1042  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  3.96 (1H, dd,  $J = 9.6, 4.0$  Hz, H-6a), 3.54 (2H, q,  $J = 6.6$  Hz), 3.52 (1H, dd,  $J = 10.2, 9.6$  Hz, H-6b), 2.84 (1H, dd,  $J = 10.2, 4.0$  Hz, H-5), 1.98 (3H, s, H<sub>3</sub>-13), 1.86 (1H, ddd,  $J = 13.2, 12.0, 4.2$  Hz, H-7a), 1.78 (1H, ddd,  $J = 13.2, 12.6, 5.4$  Hz, H-7b), 1.71 (3H, s, H<sub>3</sub>-12), 1.22 (3H, t,  $J = 6.6$  Hz), 1.23 (2H, m, H-9), 1.23 (2H, m, H-10), 0.85 (1H, m, H-8a), 0.85 (3H, t,  $J = 6.6$  Hz, H<sub>3</sub>-11), 0.80 (1H, m, H-8b);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  202.7 (C, C-1), 168.2 (C, C-3), 136.1 (C, C-2), 81.3 (C, C-4), 66.8 (CH<sub>2</sub>, C-6), 66.8 (CH<sub>2</sub>), 59.4 (CH, C-5), 36.6 (CH<sub>2</sub>, C-7), 32.1 (CH<sub>2</sub>, C-9), 25.2 (CH<sub>2</sub>, C-8), 22.4 (CH<sub>2</sub>, C-10), 15.2 (CH<sub>3</sub>), 14.0 (CH<sub>3</sub>, C-11), 11.3 (CH<sub>3</sub>, C-13), 7.8 (CH<sub>3</sub>, C-12); HRESIMS  $m/z$  277.1776  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{15}\text{H}_{26}\text{O}_3\text{Na}$ , 277.1780).

Isosinularone I (**17**): colorless oil;  $[\alpha]_{\text{D}}^{25} + 6$  (*c* 0.08, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 204 (4.24), 228 (sh); IR (KBr)  $\nu_{\text{max}}$  3448, 2926, 2861, 1727, 1636, 1383, 1277, 1120, 1074  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  3.40 (1H, dq,  $J = 12.0, 6.6$  Hz), 3.14 (1H, dq,  $J = 12.0, 6.6$  Hz), 2.34 (2H, m, H<sub>2</sub>-16), 1.98 (1H, ddd,  $J = 13.8, 12.0, 3.6$  Hz, H-5a), 1.86 (3H, s, H<sub>3</sub>-19), 1.84 (3H, s, H<sub>3</sub>-18), 1.67 (1H, m, H-5b), 1.62 (2H, m, H<sub>2</sub>-15), 1.30 (2H, m, H-6), 1.25 (16H, H<sub>2</sub>-7-H<sub>2</sub>-14), 1.17 (3H, t,  $J = 6.6$  Hz);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  178.4 (C, C-17), 172.0 (C, C-1), 156.6 (C, C-3), 126.9 (C, C-2), 110.1 (C, C-4), 35.8 (CH<sub>2</sub>, C-5), 34.1 (CH<sub>2</sub>, C-16), 29.5 (CH<sub>2</sub>, C-7), 29.5 (CH<sub>2</sub>, C-8), 29.5 (CH<sub>2</sub>, C-9), 29.5 (CH<sub>2</sub>, C-10), 29.4 (CH<sub>2</sub>, C-11), 29.4 (CH<sub>2</sub>, C-12), 29.2 (CH<sub>2</sub>, C-13), 29.1 (CH<sub>2</sub>, C-14), 24.9 (CH<sub>2</sub>, C-15), 22.6 (CH<sub>2</sub>, C-6), 10.9 (CH<sub>3</sub>, C-19), 8.4 (CH<sub>3</sub>, C-18); HRESIMS  $m/z$  367.2495  $[\text{M} - \text{H}]^-$  (calcd for  $\text{C}_{21}\text{H}_{35}\text{O}_5$ , 367.2484).

### Conformational Modeling.

The electrostatic potential of molecule **4b** was calculated at the Hartree–Fock level with the 6–31G\* basis set using Gaussian09. The general AMBER force field (gaff) and the ff99SB force field were used for **4b** and  $\text{CHCl}_3$  molecules, respectively. Molecule **4b** was solvated in a box of  $\text{CHCl}_3$  molecules with a shell of 10 Å. Energy minimization and molecular dynamic (MD) simulation were performed with the AMBER 11 packages. First, in order to remove bad contacts, the whole system was subjected to 500 steps of minimization without any restriction. Then, the system was heated gradually from 0 to 300 K in the NVT ensemble for 100 ps and equilibrated at 300 K for 100 ps prior to MD simulation. The 500 ps of MD simulation was accomplished at 300 K under a constant pressure of 1 bar. The particle mesh Ewald method was employed to deal with the long-range electrostatic interactions under periodic boundary conditions. The SHAKE method was used to constrain hydrogen atoms, and the time step was set to 2 fs. Finally, Ptraj, a module of AMBER 11 packages, was used to monitor the distance between H<sub>2</sub>-6 and H<sub>2</sub>-7 in **4b**.

### ECD Calculation.

The MD simulations were accomplished for a pair of enantiomers of **5** following the same protocol as depicted for **4b** except that methanol was used as solvent for **5**. The lowest energy conformers were optimized by CAM-B3LYP/6–311++G\*\* in methanol. The solvent

effects were included using the polarizable continuum model. The theoretical ECD spectra were obtained according to Boltzmann weighting of each conformer.

### Anti-HIV-1 Activity Assay.

The XTT-tetrazolium primary NCI screen for anti-HIV activity was performed as described previously.<sup>21–24</sup> The CEM-SS human lymphocytic target cell line was maintained in RPMI 1640 medium (Gibco) without phenol red and supplemented with 5% fetal bovine serum, 50  $\mu\text{g}/\text{mL}$  gentamicin, and 2 mM L-glutamine (complete medium). Exponentially growing cells were pelleted and resuspended in complete medium at a concentration of  $2 \times 10^5$  cells/mL. Frozen virus stock solution (Haitian variant HIV-1<sub>RF</sub>,  $3.54 \times 10^6$  SFU/mL) was thawed immediately prior to use and resuspended in complete medium to yield  $1.2 \times 10^5$  SFU/mL. Each test compound was dissolved in 100% DMSO and then diluted to the desired initial concentrations in complete medium.

Uninfected CEM-SS cells were plated in 50  $\mu\text{L}$  of complete medium at a density of  $1 \times 10^4$  cells. Diluted HIV-1 virus was then added in a volume of 50  $\mu\text{L}$  to yield a multiplicity of infection of 0.6. Appropriate cell, virus, and positive control (AZT) were incorporated in each experiment, and the final volume in each microtiter well was 200  $\mu\text{L}$ . Quadruplicate and duplicate wells were used for virus-infected and uninfected cells, respectively. Plates were incubated for 6 days at 37 °C in an atmosphere containing 5% CO<sub>2</sub>. Subsequently, aliquots of cell-free supernatant were removed from each well. Cellular growth then was estimated on the remaining contents of each well using the XTT assay as previously described.<sup>23,24</sup>

### Assay for Inhibition of Nitric Oxide Production.

A previously established protocol<sup>25</sup> was followed except that 30 mM dexamethasone in DMSO was used as the positive control and each test compound (30 mM in DMSO) was diluted to 1–30  $\mu\text{M}$  before the experiment.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

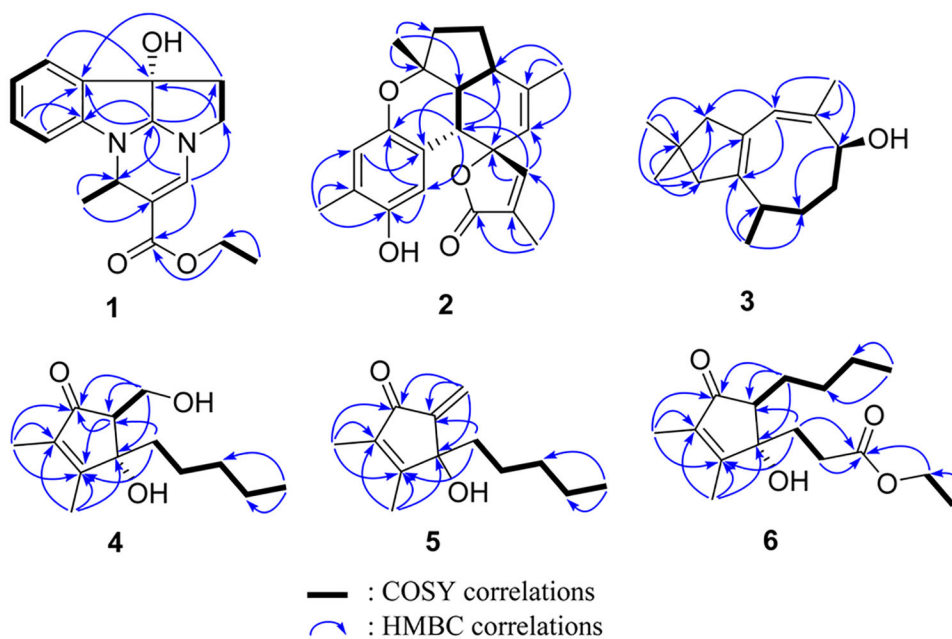
### ACKNOWLEDGMENTS

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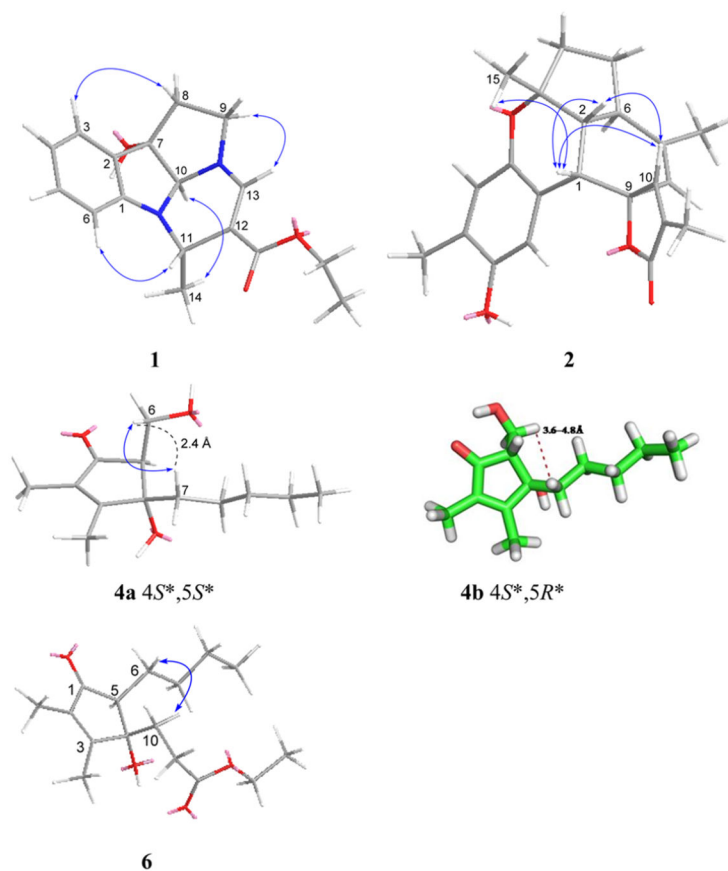
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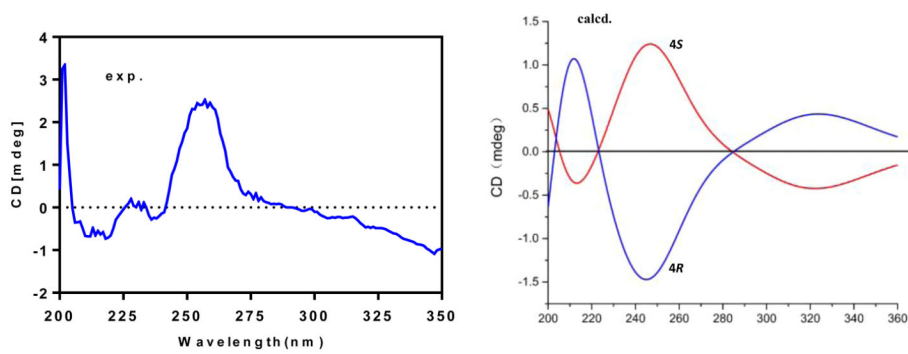


**Figure 1.**  
Key COSY and HMBC correlations for 1–6.



**Figure 2.**

Key NOE correlations and computer-generated models for **1**, **2**, **4**, and **6**. Structures **1**, **2**, **4a**, and **6** were generated using MM2 force field calculations, while **4b** was produced by MD simulation and represents a higher energy conformer of the alternative 4*S*\*,5*R*\*-diastereomer that is not supported by the NOE data.



**Figure 3.**  
Experimental ECD and calculated ECD spectra for **5**.

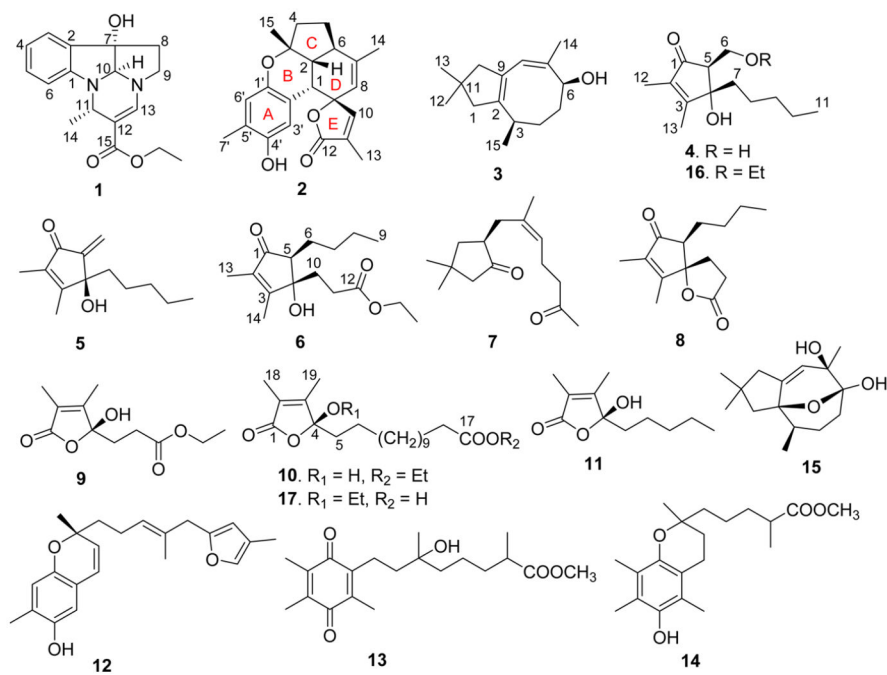


Chart 1.

Table 1.

<sup>1</sup>H NMR Data for Compounds 1–6 (CDCl<sub>3</sub>, 600 MHz)<sup>a</sup>

no.	1	2	3	4	5	6
1		3.24, d (4.8)	2.28, d (15.6)			
2		1.80, dd (12.0, 4.8)	1.99, d (15.6)			
3	7.31, dd (7.2, 1.2)		2.22, m			
4	6.85, t (7.2)	2.10, m 1.93, m	1.78, m 1.43, m			
5	7.24, td (7.8, 1.2)	1.99, m 1.37, m	1.78, m 1.59, m	2.71, t (7.2)		2.34, t (6.6)
6	6.69, d (7.8)	2.56, m	4.83, br s	4.05, dd (10.8, 7.8)	6.05, s 5.53, s	1.81, m 1.39, m
7				3.88, br t (8.4)		
				1.72, m 1.70, m	1.87, m 1.82, m	1.62, m 1.51, m
8	2.61, m	4.88, s	5.65, s	0.87, m	0.96, m 0.87, m	1.39, m
9	3.73, m 2.88, dd (18.6, 9.6)			1.24, m	1.22, m	0.94, t (7.2)
10	4.75, s	7.11, s	2.21, d (15.0) 2.01, d (15.0)	1.24, m	1.23, m	2.05, m 1.93, m
11	4.53, q (6.6)			0.85, t (6.6)	0.83, t (6.6)	2.04, m
12			1.04, s	1.73, s	1.81, s	
13	7.13, s	1.96, s	1.03, s	1.99, s	2.00, s	1.70, s
14	1.41, d (6.6)	1.68, s	1.73, s			1.98, s
15		1.33, s	0.99, d (7.2)			
3'		7.29, s				
6'		6.54, s				
7'		2.15, s				
OEt	4.13, q (7.2)					4.11, q (6.6)
	1.25, t (7.2)					1.24, t (6.6)

<sup>a</sup>The coupling constants (*J*) are in parentheses and reported in Hz; chemical shifts are given in ppm.



Table 2.

<sup>13</sup>C NMR Data for Compounds 1–6 (CDCl<sub>3</sub>, 150 MHz)<sup>a</sup>

no.	1	2	3	4	5	6
1	150.9, C	36.6, CH	36.5, CH <sub>2</sub>	206.1, C	194.1, C	204.9, C
2	131.1, C	50.1, CH	142.4, C	136.3, C	139.4, C	136.6, C
3	124.1, CH	82.0, C	36.9, CH	169.3, C	166.3, C	166.7, C
4	120.3, CH	39.0, CH <sub>2</sub>	31.0, CH <sub>2</sub>	81.0, C	78.7, C	81.5, C
5	131.0, CH	25.1, CH <sub>2</sub>	33.3, CH <sub>2</sub>	59.7, CH	148.8, C	58.9, CH
6	111.1, CH	40.7, CH	71.5, CH	59.4, CH <sub>2</sub>	114.7, CH <sub>2</sub>	24.3, CH <sub>2</sub>
7	84.1, C	143.1, C	136.7, C	36.2, CH <sub>2</sub>	37.0, CH <sub>2</sub>	30.9, CH <sub>2</sub>
8	33.9, CH <sub>2</sub>	119.9, CH	122.8, CH	25.1, CH <sub>2</sub>	24.1, CH <sub>2</sub>	23.0, CH <sub>2</sub>
9	49.8, CH <sub>2</sub>	89.0, C	129.3, C	32.0, CH <sub>2</sub>	31.9, CH <sub>2</sub>	13.9, CH <sub>3</sub>
10	78.4, CH	153.5, CH	53.0, CH <sub>2</sub>	22.4, CH <sub>2</sub>	22.4, CH <sub>2</sub>	30.1, CH <sub>2</sub>
11	46.3, CH	128.0, C	29.2, C	14.0, CH <sub>3</sub>	13.9, CH <sub>3</sub>	31.0, CH <sub>2</sub>
12	101.7, C	174.0, C	29.6, CH <sub>3</sub>	7.7, CH <sub>3</sub>	8.2, CH <sub>3</sub>	173.1, C
13	142.6, CH	10.4, CH <sub>3</sub>	29.6, CH <sub>3</sub>	11.3, CH <sub>3</sub>	10.7, CH <sub>3</sub>	8.0, CH <sub>3</sub>
14	21.7, CH <sub>3</sub>	20.2, CH <sub>3</sub>	17.2, CH <sub>3</sub>			11.3, CH <sub>3</sub>
15	167.4, C	23.8, CH <sub>3</sub>	22.7, CH <sub>3</sub>			
1'		146.4, C				
2'		116.8, C				
3'		115.4, CH				
4'		147.4, C				
5'		124.5, C				
6'		119.7, CH				
7'		15.6, CH <sub>3</sub>				
OEt	14.5, CH <sub>3</sub>					14.2, CH <sub>3</sub>
	59.2, CH <sub>2</sub>					60.9, CH <sub>2</sub>

<sup>a</sup>The assignments were based on HMQC, HMBC, and COSY spectra.