




Communication

# New Meroterpenes from South China Sea Soft Coral *Litophyton brassicum*

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**Abstract:** A chemical investigation of the extracts from the soft coral *Litophyton brassicum* led to the isolation and identification of four new meroterpenes, brassihydroxybenzoquinone A and B (1 and 2) and brassinaphthoquinone A and B (3 and 4), along with two known related meroterpenes (5 and 6). Their structures were elucidated using high-resolution electrospray ionization mass spectrometry (HRESIMS), nuclear magnetic resonance (NMR) spectroscopy, and a comparison with the literature data. All compounds were evaluated for antibacterial activity against six pathogenic bacterial strains and for cytotoxic activity against three cancer cell lines. In the cytotoxic assay, all compounds were inactive at 10  $\mu$ M against the A549, HeLa, and MDA-MB-231 cell lines. In the antibacterial assay, compounds 1 and 2 exhibited moderate inhibitory activity with minimum inhibitory concentrations (MIC) ranging from 8 to 64  $\mu$ g/mL.

**Keywords:** soft coral; *Litophyton brassicum*; meroterpenes; antibacterial activity



**Citation:** Chen, X.; Zhang, J.; Yang, J.; Li, B.; Li, T.; Ouyang, H.; Lin, W.; Hu, H.; Yan, X.; He, S. New Meroterpenes from South China Sea Soft Coral *Litophyton brassicum*. *Mar. Drugs* **2024**, *22*, 392. <https://doi.org/10.3390/md22090392>

Academic Editor: Yan-Bo Zeng

Received: 31 July 2024

Revised: 27 August 2024

Accepted: 28 August 2024

Published: 30 August 2024



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## 1. Introduction

The marine soft coral genus *Litophyton* belongs to the family Nephtheidae, order Alcyonacea, subclass Octocorallia. Currently, *Litophyton* comprises nearly 100 species, widely distributed throughout tropical and temperate waters such as the South China Sea, the Red Sea, and other regions of the Indo-Pacific Ocean [1]. Chemical investigations on *Litophyton* soft corals have revealed them to be prolific producers of bioactive secondary metabolites. Since the early 1970s, when novel cembrane diterpenes were first reported from *L. viridis* [2], numerous research groups worldwide have conducted chemical investigations on *Litophyton*, leading to significant discoveries. To date, nearly 200 secondary metabolites have been isolated and characterized from *Litophyton* corals over 50 years of research [1,3]. These compounds include sesquiterpenes, sesquiterpene dimers, diterpenes, norditerpenes, tetraterpenes, meroterpenes, steroids, ceramides, pyrimidines, and peptides [1]. A broad spectrum of pharmacological activities has been evaluated, including cytotoxic [4–7], antiviral [5], antibacterial [8], antifungal [9], antimalarial [10], anti-inflammatory [11], PTP1B inhibitory [12,13].

Meroterpenes, a class of compounds found in *Litophyton*, are relatively rare in the literature. For example, four meroterpenes isolated from the Red Sea soft coral *Nephthea* sp. were identified as potential SARS-CoV-2 main protease inhibitors [14]. Nine new meroditerpenoid-related metabolites were isolated from the Formosan soft coral *Nephthea chabrolii*, with proposed biosynthetic pathways [15]. Then, the next year, eight new meroditerpenoid-related metabolites were isolated from the organic extract of a Taiwanese soft coral *Nephthea chabrolii* [16]. It is noteworthy that the genus *Nephthea* was synonymized

with *Litophyton* in 2016 due to their identical characteristics [1]. As part of our ongoing efforts to discover bioactive marine natural products from soft corals, specimens of *Litophyton brassicum* were collected from the South China Sea. Herein, we report the isolation and identification of six meroterpenes. The antibacterial activities of these compounds were evaluated against six pathogenic bacteria, and their antiproliferative effects were tested on three cancer cell lines.

## 2. Results

The acetone extract of soft coral *Litophyton brassicum* was subjected to repeated silica gel and reversed-phase silica gel column chromatography, followed by semipreparative HPLC, to afford four new meroterpenes (1–4) and two known compounds (5 and 6) (Figure 1). The known compounds were identified as chabrolohydroxybenzoquinone G [16] and chabrolohydroxybenzoquinone B [15].

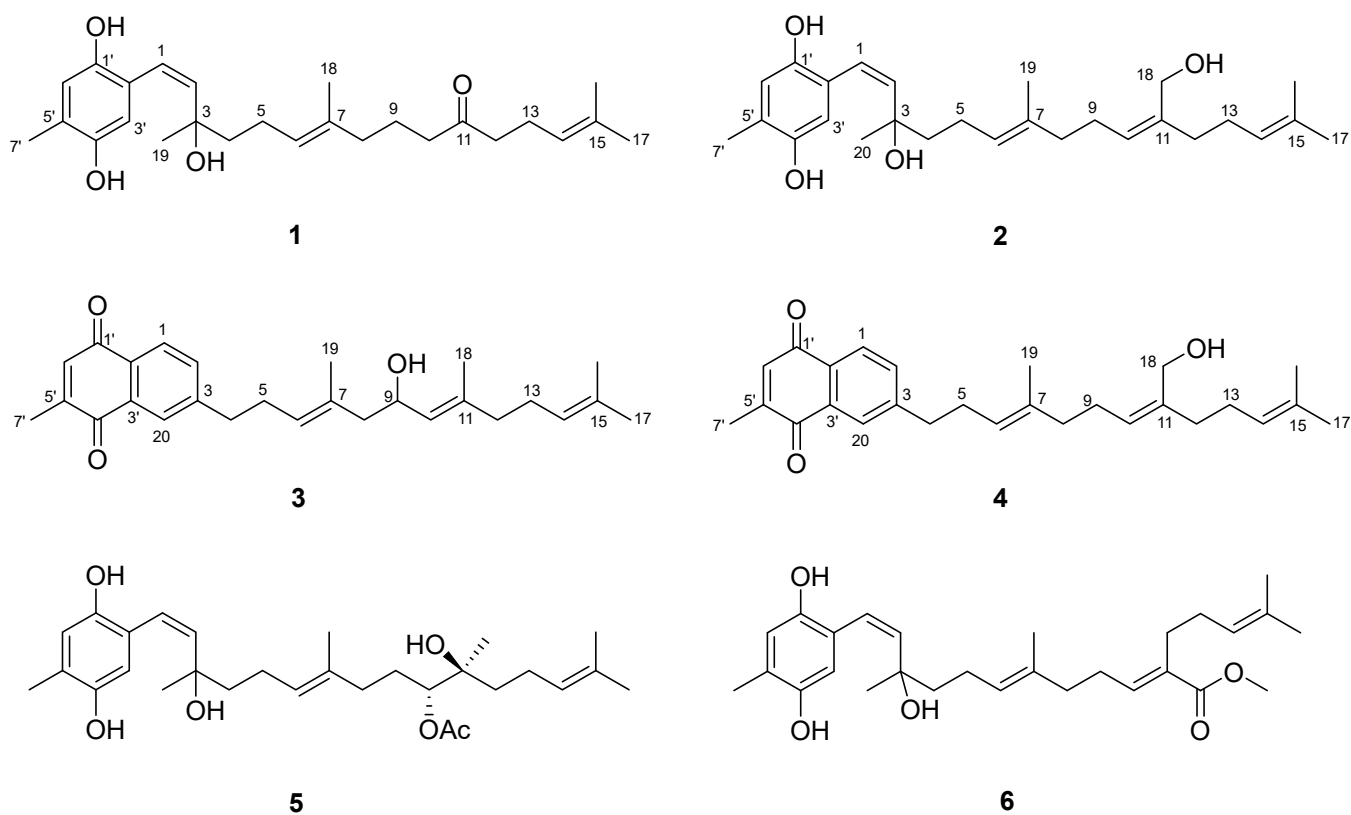


Figure 1. Chemical structures of compounds 1–6.

Compound 1 was obtained as an optically active colorless oil. Its molecular formula was determined to be  $C_{26}H_{38}O_4$  based on HRESIMS analysis [ $M-H_2O + H$ ] $^+ m/z$  397.2755, calcd. for  $C_{26}H_{37}O_3$ , 397.2742), indicating eight degrees of unsaturation. In the  $^1H$  NMR spectrum, two aromatic signals resonating at  $\delta_H$  6.55 (1H, br. s) and 6.42 (1H, br. s) indicated the presence of a 1, 2, 4, 5-tetrasubstituted benzene system. Four olefinic protons  $\delta_H$  6.25 (1H, d,  $J = 9.8$  Hz), 5.52 (1H, d,  $J = 9.8$  Hz), 5.09 (1H, tq,  $J = 7.2, 1.4$  Hz), and 5.05 (1H, tq,  $J = 7.4, 1.4$  Hz), indicated the presence of regular prenyl moieties. The  $^1H$ ,  $^{13}C$  NMR, DEPT, and HSQC spectra data confirmed the presence of 26 carbons, including a ketone carbonyl at  $\delta_C$  211.4, a benzene system, one disubstituted double bond [ $\delta_C$  122.6 (C-1),  $\delta_C$  129.9 (C-2)], two trisubstituted double bonds [ $\delta_C$  125.0 (C-6),  $\delta_C$  134.7 (C-7),  $\delta_C$  123.0 (C-14), and  $\delta_C$  132.8 (C-15)], an oxygenated quaternary carbon ( $\delta_C$  78.1), seven methylenes, and five methyls [ $\delta_C$  2.18 (C-7'),  $\delta_H$  1.60 (C-16),  $\delta_H$  1.66 (C-17),  $\delta_H$  1.55 (C-18), and  $\delta_H$  1.35 (C-19)]. The constitution of the side chain was elucidated initially by the  $^1H$ - $^1H$  COSY correlations of H-1/H-2, H<sub>2</sub>-4/H<sub>2</sub>-5/H-6, H<sub>2</sub>-8/H<sub>2</sub>-9/H<sub>2</sub>-10,

and H<sub>2</sub>-12/H<sub>2</sub>-13/H-14 (Figure 2). The spectroscopic data were similar to those of the known compound chabrolohydroxybenzoquinones E [16], except for the missing of a methyl group on C-11, and the  $\Delta^{10,11}$  double bond was reduced and oxidized to a keto group in **1**. In addition, the HMBC correlations from H<sub>2</sub>-9, H<sub>2</sub>-10, H<sub>2</sub>-12, and H<sub>2</sub>-13 to C-11 implied the keto group was on C-11. The smaller coupling constant ( $J_{1,2} = 9.8$  Hz) and the 1D NOE correlation (Figure 3) from H-6 to H<sub>2</sub>-8 suggested the *Z* configuration of the  $\Delta^{1,2}$  and *E* configuration of the  $\Delta^{6,7}$  double bonds, respectively. Comprehensive HMBC analysis allowed the complete assignment of the proton and carbon signals for **1** (Table 1 and Figure 2). As a result, the structure of **1** was elucidated as shown in Figure 1, named brassihydroxybenzoquinone A.

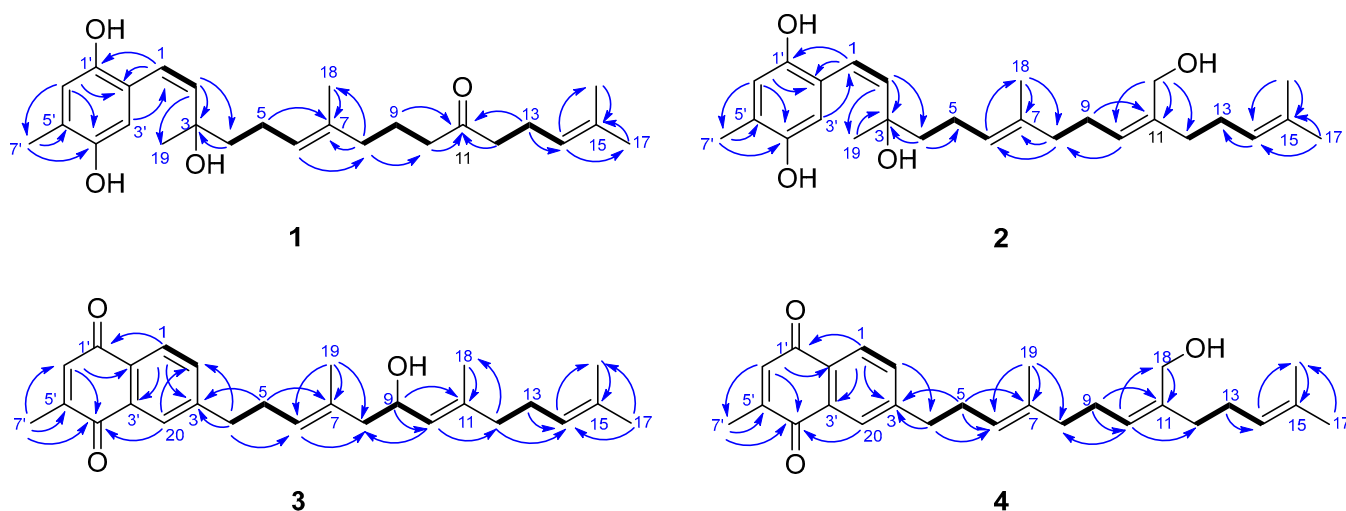


Figure 2. <sup>1</sup>H-<sup>1</sup>H COSY and key HMBC correlations of compounds 1–4.

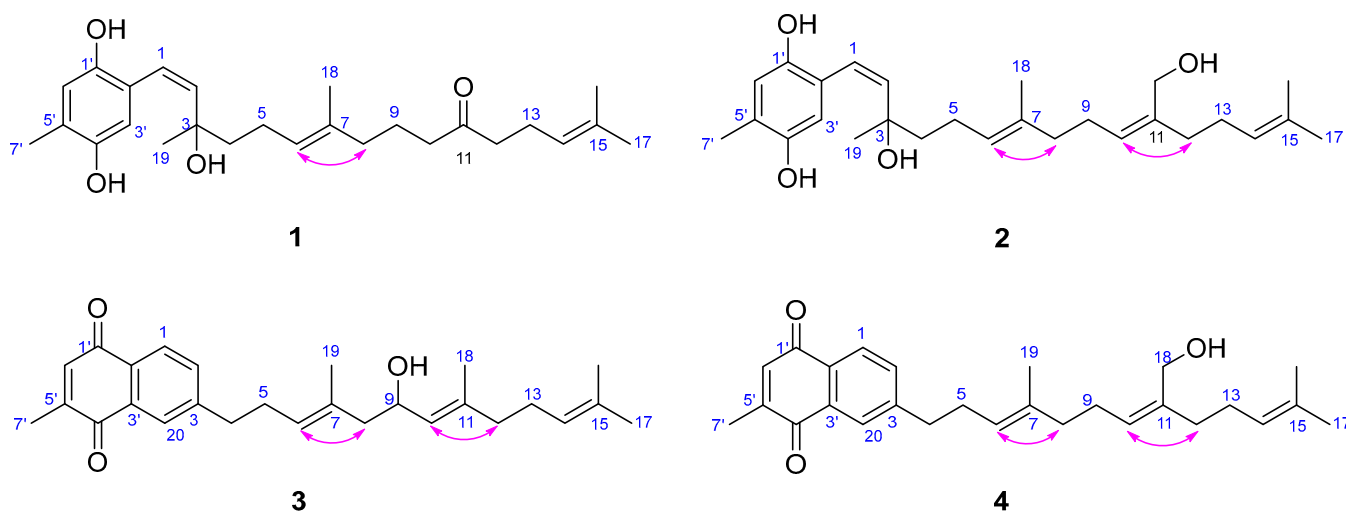


Figure 3. Key 1D NOEs of compounds 1–4.

Compound **2** (brassihydroxybenzoquinone B) was also obtained as a colorless oil. The molecular formula of C<sub>27</sub>H<sub>40</sub>O<sub>4</sub> derived by the HR-ESI-MS ion peak of [M-2H<sub>2</sub>O + H]<sup>+</sup> at *m/z* 393.2798 (calcd. for C<sub>27</sub>H<sub>37</sub>O<sub>2</sub>, 393.2793) gave 8 degrees of unsaturation. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **2** (Table 1) were also similar to those of chabrolohydroxybenzoquinones E [16], except that the oxygen-bearing methylene [ $\delta_C$  60.5 (C-18)/ $\delta_H$  4.10] attached at C-11 in **2** rather than the methyl group in chabrolohydroxybenzoquinones E, which was proven by the HMBC correlations from H<sub>2</sub>-18 to C-10 ( $\delta_C$  128.8), C-11 ( $\delta_C$  138.4), and C-12 ( $\delta_C$  35.3). The configuration of the  $\Delta^{1,2}$  double bond was also assigned as *Z*, based on the same method

as for **1**. The *E* geometry of  $\Delta^{6,7}$  and *Z* geometry of  $\Delta^{10,11}$  double bonds in **2** were supported by the 1D NOE enhancements of H-6/H<sub>2</sub>-8 and H-10/H<sub>2</sub>-12, respectively. We attempted to utilize the TDDFT-ECD calculation method to establish the absolute configuration of the C-3 position in compounds **1** and **2**. However, the experimental ECD spectrum did not exhibit a clear Cotton effect (Figures S36 and S37), which is likely due to the C-3 position being located on the side chain, leading to its flexibility. As far as we know, determining the absolute configuration of C-3 in compounds **1** and **2** remains a challenging task.

**Table 1.** <sup>1</sup>H NMR and <sup>13</sup>CNMR data for compounds **1** to **4** at 600 MHz in CDCl<sub>3</sub>.

| Position | 1                       |                       | 2                       |                       | 3                       |                       | 4                       |                       |
|----------|-------------------------|-----------------------|-------------------------|-----------------------|-------------------------|-----------------------|-------------------------|-----------------------|
|          | $\delta_H$ Mult (J, Hz) | $\delta_C$ , Type     | $\delta_H$ Mult (J, Hz) | $\delta_C$ , Type     | $\delta_H$ Mult (J, Hz) | $\delta_C$ , Type     | $\delta_H$ Mult (J, Hz) | $\delta_C$ , Type     |
| 1'       |                         | 146.8, C              |                         | 146.8, C              |                         | 185.1, C              |                         | 185.2, C              |
| 2'       |                         | 119.7, C              |                         | 119.7, C              |                         | 130.5, C              |                         | 130.4, C              |
| 3'       | 6.42, s                 | 112.6, CH             | 6.42, s                 | 112.6, CH             |                         | 132.3, C              |                         | 132.0, C              |
| 4'       |                         | 147.6, C              |                         | 147.6, C              |                         | 186.1, C              |                         | 186.1, C              |
| 5'       |                         | 124.7, C              |                         | 124.7, C              |                         | 148.1, C              |                         | 148.1, C              |
| 6'       | 6.55, s                 | 118.3, CH             | 6.55, s                 | 118.3, CH             | 6.81, br.s              | 135.9, CH             | 6.81, q (1.6)           | 135.9, CH             |
| 7'       | 2.18, s                 | 16.1, CH <sub>3</sub> | 2.17, s                 | 16.1, CH <sub>3</sub> | 2.18, br.s              | 16.4, CH <sub>3</sub> | 2.18, d (1.6)           | 16.6, CH <sub>3</sub> |
| 1        | 6.25, d (9.8)           | 122.6, CH             | 6.25, d (9.8)           | 122.6, CH             | 7.96, d (7.9)           | 126.5, CH             | 7.96, d (7.9)           | 126.4, CH             |
| 2        | 5.52, d (9.8)           | 129.9, CH             | 5.52, d (9.8)           | 129.9, CH             | 7.52, dd (7.9, 1.8)     | 134.0, CH             | 7.52, dd (7.9, 1.8)     | 134.1, CH             |
| 3        |                         | 78.1, C               |                         | 78.1, C               |                         | 148.8, C              |                         | 149.1, C              |
| 4        | 1.63, m; 1.69, m        | 41.1, CH <sub>2</sub> | 1.68, m; 1.62, m        | 41.0, CH <sub>2</sub> | 2.80, t (7.6)           | 36.1, CH <sub>2</sub> | 2.77, t (7.6)           | 36.3, CH <sub>2</sub> |
| 5        | 2.10, m                 | 22.8, CH <sub>2</sub> | 2.10, m                 | 22.8, CH <sub>2</sub> | 2.40, q (7.4)           | 29.5, CH <sub>2</sub> | 2.35, q (7.5)           | 29.4, CH <sub>2</sub> |
| 6        | 5.09, tq (7.2, 1.4)     | 125.0, CH             | 5.10, tq (5.7, 1.4)     | 124.86, CH            | 5.26, m                 | 126.6, CH             | 5.15, td (7.2, 1.4)     | 123.2, CH             |
| 7        |                         | 134.7, C              |                         | 134.9, C              |                         | 133.6, C              |                         | 136.5, C              |
| 8        | 1.93, t (7.2)           | 39.1, CH <sub>2</sub> | 1.98, t (7.5)           | 40.0, CH <sub>2</sub> | 2.13, m                 | 48.2, CH <sub>2</sub> | 1.99, m                 | 40.0, CH <sub>2</sub> |
| 9        | 1.64, m                 | 21.9, CH <sub>2</sub> | 2.14, m; 2.10, m        | 26.3, CH <sub>2</sub> | 4.42, m                 | 66.1, CH              | 2.14, m                 | 26.4, CH <sub>2</sub> |
| 10       | 2.32, t (7.4)           | 42.3, CH <sub>2</sub> | 5.28, t (7.3)           | 128.8, CH             | 5.15, d (8.2)           | 127.3, CH             | 5.27, t (7.3)           | 128.6, CH             |
| 11       |                         | 211.4, C              |                         | 138.4, C              |                         | 138.5, C              |                         | 138.6, C              |
| 12       | 2.40, t (7.4)           | 43.0, CH <sub>2</sub> | 2.11, m                 | 35.3, CH <sub>2</sub> | 2.00, t (7.7)           | 39.7, CH <sub>2</sub> | 2.11, m                 | 35.4, CH <sub>2</sub> |
| 13       | 2.23, q (7.4)           | 22.7, CH <sub>2</sub> | 2.10, m                 | 27.2, CH <sub>2</sub> | 2.07, m                 | 26.5, CH <sub>2</sub> | 2.11, m                 | 27.2, CH <sub>2</sub> |
| 14       | 5.05, tp (7.2, 1.4)     | 123.0, CH             | 5.10, tq (5.7, 1.4)     | 124.3, CH             | 5.07, tq (6.9, 1.4)     | 124.1, CH             | 5.10, tq (5.4, 1.2)     | 124.3, CH             |
| 15       |                         | 132.8, C              |                         | 131.9, C              |                         | 131.8, C              |                         | 131.2, C              |
| 16       | 1.60, s                 | 17.8, CH <sub>3</sub> | 1.68, s                 | 25.9, CH <sub>3</sub> | 1.59, s                 | 17.8, CH <sub>3</sub> | 1.60, s                 | 17.9, CH <sub>3</sub> |
| 17       | 1.66, s                 | 25.8, CH <sub>3</sub> | 1.60, s                 | 17.9, CH <sub>3</sub> | 1.67, s                 | 25.8, CH <sub>3</sub> | 1.68, s                 | 25.9, CH <sub>3</sub> |
| 18       | 1.55, s                 | 15.8, CH <sub>3</sub> | 4.10, s                 | 60.5, CH <sub>2</sub> | 1.66, s                 | 16.6, CH <sub>3</sub> | 4.11, s                 | 60.5, CH <sub>2</sub> |
| 19       | 1.35, s                 | 26.2, CH <sub>3</sub> | 1.57, s                 | 16.1, CH <sub>3</sub> | 1.59, s                 | 16.7, CH <sub>3</sub> | 1.52, s                 | 16.2, CH <sub>3</sub> |
| 20       |                         |                       | 1.35, s                 | 26.2, CH <sub>3</sub> | 7.91, d (1.8)           | 126.5, CH             | 7.90, d (1.8)           | 126.6, CH             |

Compound **3** (brassinaphthoquinone A) was a yellow oil. The molecular formula of C<sub>27</sub>H<sub>34</sub>O<sub>3</sub>, giving 11 degrees of unsaturation, was established by the HR-ESI-MS ion peak at *m/z* 389.2484 [M-H<sub>2</sub>O + H]<sup>+</sup> (calcd. for C<sub>27</sub>H<sub>33</sub>O<sub>2</sub>, 389.2480). From the <sup>1</sup>H NMR spectrum of **3**, the resonances of three aromatic protons  $\delta_H$  7.96 (1H, d, *J* = 9.8 Hz), 7.90 (1H, d, *J* = 1.8 Hz), and 7.52 (1H, dd, *J* = 9.8, 1.8 Hz) indicated the presence of a 1, 2, 4-trisubstituted benzene system. One additional aromatic proton 6.81 (1H, br.s) was also observed. From the <sup>1</sup>H and <sup>13</sup>C NMR spectral data (Table 1), together with the HSQC data, 27 signals were assigned to two carbonyls, seven sp<sup>2</sup> quaternary olefinic carbons, seven sp<sup>2</sup> methine, an oxygenated sp<sup>3</sup> methine [ $\delta_C$  66.1 (C-9)/ $\delta_H$  4.42], five sp<sup>3</sup> methylene, and five methyls. The NMR data of **3** closely resembled those of chabrolonaphthoquinone A [15], a meroditerpenoid with a naphthoquinone moiety obtained from the Formosan soft coral *Nephthea chabrolii*. The differences were one more methyl group (C-18) on **3** rather than the carboxyl group on chabrolonaphthoquinone A, and C-9 was hydroxylated on **3**. The different side chain was elucidated by the <sup>1</sup>H-<sup>1</sup>H COSY correlations of H<sub>2</sub>-8/H-9/H-10 and the HMBC correlations from H-9 to C-7, C-10, and C-11. The key 1D NOE correlations (Figure 3) from H-6 to H<sub>2</sub>-8 and from H-10 to H<sub>2</sub>-12 established the *E* configuration of the  $\Delta^{6,7}$  and  $\Delta^{10,11}$  double bonds, respectively. Thus, the structure of **3** was elucidated, as shown in Figure 2.

Brassinaphthoquinone B (**4**) was also isolated as a yellow oil with a molecular formula of C<sub>27</sub>H<sub>34</sub>O<sub>3</sub> on the basis of HR-ESI-MS ion peak at *m/z* 389.2471 [M-H<sub>2</sub>O + H]<sup>+</sup> (calcd. for C<sub>27</sub>H<sub>33</sub>O<sub>2</sub>, 389.2480), which is the structural isomer of **3**. The <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1) of **4** were extremely similar to those of **3**, except that the absence of hydroxyl group at C-9 in **4**, and the oxygenated sp<sup>3</sup> carbon [ $\delta_C$  60.5 (C-18)/ $\delta_H$  4.11] was a methylene in **4** rather than a methine in **3**. It was deduced by analysis of the <sup>1</sup>H-<sup>1</sup>H COSY correlations of

H<sub>2</sub>-8/H<sub>2</sub>-9/H-10 and the HMBC correlations from H<sub>2</sub>-18 to C-10, C-11, and C-12. The 1D NOE enhancements between H-6/H<sub>2</sub>-8 and H-10/H<sub>2</sub>-12 indicated the *E* geometry of the  $\Delta^{6,7}$  and  $\Delta^{10,11}$  double bonds, respectively.

Compounds 1–6 were evaluated for cytotoxicity and antibacterial activities. In the cytotoxic assay, all compounds were inactive at 10  $\mu$ M against the cell lines of A549, HeLa, and MDA-MB-231. An evaluation of the antibacterial activity against six pathogenic bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Vibrio parahaemolyticus*, and *Vibrio harveyi*) showed that compounds 1 and 2 displayed moderate inhibitory activity (MIC 8–64  $\mu$ g/mL) (Table 2). Interestingly, compound 2 may have potential as an antibiotic agent for controlling aquatic pathogens in the future.

**Table 2.** Inhibitory effects of 1–6 on six kinds of pathogenic bacteria.

| Compounds               | MIC ( $\mu$ g/mL) |                    |                   |                            |                |                      |
|-------------------------|-------------------|--------------------|-------------------|----------------------------|----------------|----------------------|
|                         | <i>S. Aureus</i>  | <i>B. Subtilis</i> | <i>V. Harveyi</i> | <i>V. Parahaemolyticus</i> | <i>E. coli</i> | <i>P. Aeruginosa</i> |
| 1                       | 32                | 32                 | >64               | 32                         | 64             | 16                   |
| 2                       | >64               | 8                  | 16                | 16                         | 16             | 16                   |
| 3                       | >64               | >64                | >64               | >64                        | >64            | >64                  |
| 4                       | >64               | >64                | >64               | >64                        | >64            | >64                  |
| 5                       | >64               | 32                 | >64               | 64                         | 32             | 32                   |
| 6                       | >64               | >64                | >64               | >64                        | >64            | >64                  |
| Penicillin <sup>a</sup> | <0.5              | <0.5               | <0.5              | <0.5                       | <0.5           | <0.5                 |

<sup>a</sup> Positive control.

### 3. Materials and Methods

#### 3.1. General Chemical Experimental Procedures

NMR spectra were recorded on a Bruker AVANCE NEO 600 spectrometer (Bruker-Biospin AG, Fällanden, Germany). <sup>1</sup>H chemical shifts were referenced to the residual CDCl<sub>3</sub> (7.26 ppm), and <sup>13</sup>C chemical shifts were referenced to the CDCl<sub>3</sub> (77.2 ppm) solvent peaks. High-resolution electrospray ionization mass spectra (HRESIMS) were performed on an ultra-high-performance liquid chromatograph (UPLC) and TIMS-QTOF high-resolution mass spectrometry (Waters, MA, USA). The purification was performed by reversed-phase high-performance liquid chromatography using a Shimadzu LC-20AT system (Shimadzu Corporation, Tokyo, Japan). The solvents used for HPLC were all Fisher HPLC grade. A Cosmosil C<sub>18</sub>-MS-II column (250 mm  $\times$  20.0 mm, id, 5  $\mu$ m, Cosmosil, Nakalai Tesque Co., Ltd., Kyoto, Japan) was used for the preparative HPLC separation. Column chromatography was performed using silica gel (300–400 mesh, Qingdao Ocean Chemical Co., Ltd., Qingdao, China) and C<sub>18</sub> reversed-phase silica gel (75  $\mu$ m, Nakalai Tesque Co., Ltd., Kyoto, Japan).

#### 3.2. Animal Material

Soft coral *Litophyton brassicum* was sampled off the coast of Xisha Islands, South China Sea, 12 m underwater, with a wet weight of 5.07 kg, and it was frozen immediately after collection. The specimens (XSSC201906) were deposited at the Li Dak Sum Yip Yio Chin Kenneth Li Marine Biopharmaceutical Research Center, Health Science Center, Ningbo University, China.

#### 3.3. Extraction and Isolation

The soft coral samples were vacuum freeze-dried with a freeze-dryer, crushed in a pulverizer, and fully soaked in acetone at room temperature for 2 days each time, followed by ultrasonic extraction for 1 h, repeated soaking ultrasonic extraction for 4–5 times. The extract was filtered to remove the sample residue, and the extract was concentrated under reduced pressure. The extract was partitioned three times with Et<sub>2</sub>O and water (1:1, v:v), and the Et<sub>2</sub>O layer extract was concentrated under reduced pressure to obtain 80 g brown residue.

The 80 g of the extract was separated by gradient elution on a normal-phase silica gel column, yielding 11 fractions (Fr.A–Fr.K). Fr.G (1.8 g) was eluted with MeOH/H<sub>2</sub>O (75:15 to 100:0, *v/v*) on reversed-phase column chromatography to obtain five subfractions (Fr.G.1–Fr.G.5). Purification of Fr.G.3 by semi-preparative HPLC (MeCN/H<sub>2</sub>O, 72:18, 2 mL/min) gave compounds **1** (6.8 mg, *t<sub>R</sub>* = 62 min) and **3** (5.8 mg, *t<sub>R</sub>* = 71 min). Separation of Fr.H (1.54 g) on a reversed-phase column with MeOH/H<sub>2</sub>O (75:15~100:0, *v/v*) afforded seven subfractions (Fr.H.1~Fr.H.6). Fr.H.5 was purified by semipreparative reversed-phase HPLC (MeCN/H<sub>2</sub>O, 75: 15, 2 mL/min) to provide compounds **2** (5.7 mg, *t<sub>R</sub>* = 60 min) and **4** (7.8 mg, *t<sub>R</sub>* = 40 min). Separation of Fr.J (1.4334 g) on a reversed-phase column with MeOH/H<sub>2</sub>O (80:20~100:0, *v/v*) provided seven subfractions (Fr.J.1~Fr.J.6). Fr.J.6 was purified by semipreparative reversed-phase HPLC (MeCN/H<sub>2</sub>O, 80:20, 2 mL/min) to provide compounds **5** (6.7 mg, *t<sub>R</sub>* = 53 min) and **6** (8.8 mg, *t<sub>R</sub>* = 62 min).

Brassihydroxybenzoquinone A (**1**): colorless oil;  $[\alpha]_D^{25} -17.40$  (c 0.5, MeOH); UV (MeOH): 221 (3.38), 270 (2.51), 330 (2.57); IR (KBr)  $\nu = 3400, 3100, 1766, 1651, 1261, 1079, 888, 630$  cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Table 1; HRESIMS *m/z* 397.2755 [M-H<sub>2</sub>O + H]<sup>+</sup> (calcd. for C<sub>26</sub>H<sub>37</sub>O<sub>3</sub>, 397.2742).

Brassihydroxybenzoquinone B (**2**): colorless oil;  $[\alpha]_D^{25} -18.67$  (c 0.5, MeOH); UV (MeOH): 220 (3.27), 270 (2.48), 330 (2.55); IR (KBr)  $\nu = 3574, 3154, 1436, 1261, 1037, 951, 710$  cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Table 1; HRESIMS *m/z* 393.2798 [M-2H<sub>2</sub>O + H]<sup>+</sup> (calcd. for C<sub>27</sub>H<sub>37</sub>O<sub>2</sub>, 393.2793).

Brassinaphthoquinone A (**3**): yellow oil;  $[\alpha]_D^{25} -23.47$  (c 0.5, MeOH); UV (MeOH): 204 (3.39), 237 (3.23), 270 (3.05); IR (KBr)  $\nu = 3582, 3088, 1765, 1692, 1450, 1139, 916, 749$  cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Table 1; HRESIMS *m/z* 389.2484 [M-H<sub>2</sub>O + H]<sup>+</sup> (calcd. for C<sub>27</sub>H<sub>33</sub>O<sub>2</sub>, 389.2480).

Brassinaphthoquinone B (**4**): yellow oil;  $[\alpha]_D^{25} -23.73$  (c 0.5, MeOH); UV (MeOH): 204 (3.34), 245 (3.13), 270 (2.90); IR (KBr)  $\nu = 3583, 2928, 1759, 1692, 1470, 1138, 915, 749$  cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, Table 1; HRESIMS *m/z* 389.2480 [M-H<sub>2</sub>O + H]<sup>+</sup> (calcd. for C<sub>27</sub>H<sub>33</sub>O<sub>2</sub>, 389.2481).

### 3.4. Antibacterial Assays

All isolated compounds were tested for antibacterial activities according to established methods [17]. Six bacterial strains were selected: *S. aureus* [CMCC (B) 26003], *B. subtilis* [CMCC (B) 63501], *V. harveyi* 1708B04 (accession number: MZ333451), *V. parahaemolyticus* (accession number: OL636376), *P. aeruginosa* [CMCC (B) 10104], and *E. coli* [CMCC (B) 44102], with penicillin G serving as the positive control. Compounds **1–6** were dissolved in DMSO and tested at concentrations of 128, 64, 32, 16, 8, 4, and 2 µg/mL. Briefly, bacteria were grown in Mueller–Hinton (MH) medium for 24 h at 28 °C with agitation (180 rpm), then diluted with sterile MH medium to match the 0.5 McFarland standard. One hundred microliters of each bacterial suspension and 100 µL of MH medium containing 0.002% 2,3,5-triphenyltetrazolium chloride, along with test or control compounds, were incubated. Inhibition data were recorded optically.

### 3.5. Cytotoxic Activity Assays

MDA-MB-231, HeLa, and A549 cells were cultured in DMEM (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% FBS (Gibco, Thermo Fisher Scientific, Inc.). These cell lines were obtained from the Shanghai Cell Bank, Chinese Academy of Sciences. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. MTT assays were performed as described by Zhang et al. (2011) [18]. Briefly, cells were seeded into 96-well plates at a density of 5 × 10<sup>3</sup> cells/well and incubated for 12 h, followed by exposure to various test compounds at different concentrations for 48 h. Subsequently, the cells were stained with 20 µL of MTT solution (5 mg/mL) for 4 h. The medium and MTT solution were then removed, and 150 µL of DMSO was added to dissolve the formazan crystals. The plates were shaken at low speed for 10 min. Absorbance was measured at 490 nm using a microplate reader.

#### 4. Conclusions

In summary, the chemical study of the soft coral *Litophyton brassicum* from the South China Sea led to the identification of four novel meroterpenes, brassihydroxybenzoquinone A and B and brassinaphthoquinone A and B, along with two known related meroterpenes. The structures of these compounds were elucidated using HRESIMS and NMR spectroscopy and corroborated with the existing literature. Compounds **1** and **2** exhibited moderate antibacterial activity, with minimum inhibitory concentrations (MIC) ranging from 8 to 64 µg/mL. The isolation of these novel terpenoids from marine soft corals underscores the rich chemical diversity of the *Litophyton* genus. These newly characterized compounds, with their unique structures, hold promise for developing innovative antimicrobial therapies, particularly amid escalating antibiotic resistance. Exploring their antimicrobial properties is, therefore, a critical endeavor for public health.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/md22090392/s1>, Figures S1–S35: HRESIMS, IR, 1D and 2D NMR spectra of all new compounds **1–4**; Figures S36 and S37: Experimental ECD spectra of compounds **1** and **2**.

**Author Contributions:** X.C., J.Z., J.Y., and B.L. performed the extraction, isolation, and structure determination of the compounds and wrote the manuscript. H.O., H.H., and X.Y. performed the cytotoxic and antimicrobial bioassays. T.L., W.L., and S.H. collected the soft coral by SCUBA. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the Fujian Provincial Key Laboratory of Innovative Drug Target Research (FJ-YW-2022KF01), the Li Dak Sum Yip Yio Chin Kenneth Li Marine Biopharmaceutical Development Fund of Ningbo University, the Ningbo Key Science and Technology Development Program (2021Z046), and the National 111 Project of China (D16013).

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available in the Supplementary Materials file associated with this article.

**Acknowledgments:** We thank Zhenhua Long, Daning Li, and Da Huo of the Xisha Marine Science Comprehensive Experimental Station, South China Sea Institute of Oceanology, Chinese Academy of Sciences, for their assistance. We thank Xisha Marine Environment National Observation and Research Station. We would also like to thank the MS Center of the Institute of Drug Discovery Technology, Ningbo University.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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