Studies on the Chemical Constituents from Marine Bryozoan

Cryptosula pallasiana

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Abstract: The aim of this study was to investigate the chemical constituents of marine bryozoan Cryptosula pallasiana inhabiting Huang Island of China. Three aromatic compounds, p-methylsulfonylmethyl-phenol (1), p-hydroxybenzaldehyde (4) and methylparaben (5), nine alkaloids, 7-bromoquinolin-4(1H)-one (2), 7-bromo-2,4(1H,3H)-quinazolinedione (3), benzamide (6), phenylacetamide (7), 4(3H)-quinazolinone (8), thymine (9), uracil (10), hypoxanthine (11) and tryptophan (12), together with two glycerol derivatives, glycerol (13) and monoheneicosanoin (14), were isolated for the first time from this marine bryozoan. Among the isolates, compounds 1 and 2 were new natural products, and their spectral traits were reported for the first time. The structures of the two compounds were elucidated by extensive spectroscopic analyses, including HR-ESI-MS, EI-MS, 1D- and 2D-NMR techniques. The isolated compounds 1–3 were evaluated for their cytotoxicity against HL-60 human myeloid leukemia cell line. The results showed that bromated alkaloid (3) appeared strong cytotoxicity against HL-60 cells with IC₅₀ value of 11.87 µg/mL, while compounds 1 and 2 were inactivity.

Keywords: Marine bryozoan; Cryptosula pallasiana; cytotoxicity. © 2014 ACG Publications. All rights reserved.

1. Animal Source

Animals of marine bryozoans have been proved to be a rich source of macrolides, alkaloids, sterols, as well as heteratom-contained compounds, which have been found to possess remarkable activities on antitumor cell lines, such as murine lymphocytic leukemia P388, human myeloid leukemia HL-60, human leukemia U937, and so on [1]. In the course of our continuing investigation of the new bioactive natural products from marine bryozoans [2–6], we focused on Cryptosula
C. pallasiana for its cytotoxic constituents. In this paper, three aromatic compounds (1, 4 and 5), nine alkaloids (2–3 and 6–12) and two glycerol derivatives (13 and 14) were identified (Figure 1). Among the isolates, compounds 1 and 2 were new natural products, and the representative compounds 1–3 were evaluated for their cytotoxicity against HL-60 cell line.

The samples of marine bryozoan C. pallasiana were collected in Huang Island, Qingdao City, Shandong Province, China, during March 2009, and were identified by one of the authors (H.-W. Lin). A voucher specimen (No: QD-0903-1) was deposited in the Marine Laboratory, Changzheng Hospital, Second Military Medical University.

2. Previous Studies

Previous chemical studies on C. pallasiana reported the isolation of alkaloids [5] and sterols [6].

3. Present Study

The fresh animals of marine bryozoan Cryptpsula pallasiana (20 kg) were extracted with 95% aq. EtOH, the resulting extract was then suspended in H₂O and extracted successively with AcOEt and BuOH, the AcOEt extract was further extracted by petroleum ether, CCl₄ and CH₂Cl₂. The extracts of CCl₄ and CH₂Cl₂ were subjected to column chromatography on Sephadex LH-20 gel, octadecylsilanized (ODS) silica gel, and reversed-phase preparative HPLC to afford compounds 1–14 (Figure 1). The detailed isolation procedures for compounds 1–14 were shown in the supporting information. The known compounds 3–14 were identified as 7-bromo-2,4(1H,3H)-quinazolininedione (3) [7], p-hydroxybenzaldehyde (4) [8], methylparaben (5) [9], benzamide (6) [10], phenylacetamide (7) [11], 4(3H)-quinazolinone (8) [12], thymine (9) [13], uracil (10) [14], hypoxanthine (11) [15], tryptophan (12) [16], glycerol (13) [17] and monoheneicosanoin (14) [18], respectively, by comparing their physical and spectroscopic data with those reported in the references.

![Figure 1. Structure of compounds 1–14 isolated from C. pallasiana.](image)

p-Methylsulfonylmethyl-phenol (1): Gray yellow amorphous powder; ¹H-NMR (CD₃OD, 500 MHz) δ: 7.52 (2H, dt, J = 8.1, 2.9 Hz, H-3 and H-5), 6.79 (2H, dt, J = 8.1, 2.9 Hz, H-2 and H-6), 4.27 (2H, s, H₂-1'), 2.80 (3H, s, H₃-2'); ¹³C-NMR (CD₃OD, 125 MHz) δ: 159.4 (C-1), 133.2 (C-2 and C-6), 120.4 (C-4), 116.6 (C-3 and C-5), 60.8 (C-2'), 30.2 (C-1'); HR-ESI-MS m/z 209.0250 [M + Na]⁺ (calcd. for C₃H₃NONaS, 209.0248); El-MS m/z (rel. int.): 186 [M]⁺ (9), 171 [M - CH₃]⁺ (2), 107 [M - SO₂CH₃]⁺
(100), 93 [M – CH₂SO₂CH₃]⁺ (2), 77 [M – CH₂SO₂CH₃ – OH + H]⁺ (26), 63 (7), 56 (6), 54 (12), 52 (13).

7-Bromoquinolin-4(1H)-one (2): Light yellow amorphous powder; ¹H-NMR (DMSO-d₆, 500 MHz) δ: 11.93 (1H, s, NH), 7.98 (1H, d, J = 8.6 Hz, H-5), 7.91 (1H, d, J = 7.5 Hz, H-2), 7.77 (1H, s, H-8), 7.45 (1H, dd, J = 8.6, 1.9 Hz, H-6), 6.05 (1H, d, J = 7.5 Hz, H-3); ¹³C-NMR (DMSO-d₆, 125 MHz) δ: 176.3 (C-4), 141.0 (C-8a), 139.9 (C-2), 127.3 (C-5), 126.1 (C-6), 124.9 (C-7), 124.6 (C-4a), 120.5 (C-8), 109.3 (C-3); ESI-MS (positive mode) m/z 224 [M + H]⁺, 226 [M + 2 + H]⁺, 246 [M + Na]⁺, 248 [M + 2 + Na]⁺, 469 [2M + Na]⁺, 471 [2M + 2 + Na]⁺. EI-MS m/z (rel. int.): 225 [M + 2]⁺ (22), 223 [M]⁺ (22), 187 (9), 185 (9), 167 (20), 143 [M – HBr]⁺ (6), 121 (23), 107 (21), 83 (100), 56 (47).

Bioactivity Test: The human-cancer line used in this study was purchased from ATCC (American Type Culture Collection). The cytotoxic activity of compounds 1–3 was determined against the HL-60 (human myeloid leukemia) cancer line with the MTT assay method according to a reported procedure [6]. Adriamycin was used as a positive control and exhibited IC₅₀ value of 2.50 µg/mL against the HL-60 cancer cell line.

Compound 1, was obtained as a gray yellow amorphous powder and assigned to possess a molecular formula C₈H₁₀O₃S on the basis of HR-ESI-MS m/z 209.0250 [M + Na]⁺ (calcd. for C₈H₆O₂NaS, 209.0248). The ¹H-NMR spectrum revealed an AA′BB′ system for 2H doublets at δH 7.24 and 6.79 which indicated a para-disubstituted benzene ring. A tertiary methyl group (δH 2.80, s, 3H) and a methylene group (δH 4.28, s, 2H) in the ¹H-NMR spectrum indicated the tertiary methyl group linked with a heteratom. As determined by ¹³C-NMR and DEPT experiments, the down-field signals corresponded to six aromatic carbons [δC 159.4 (linked with a hydroxyl group), 133.2 (two carbons), 120.4 and 116.6 (two carbons)], and the up-shield region showed a methyl carbon (δC 39.2) and a methylene carbon (δC 60.8). The NMR spectra results mentioned above suggested that 1 had a p-substituted phenol skeleton with a heteroatom-linked methyl moiety. The presence of a fragment at m/z 171 [M – CH₃]⁺ in the EI-MS due to loss of methyl group, and a strong fragment at m/z 107 [M – SO₂CH₃]⁺ due to loss of methylsulfonyl group, as well as the weak fragment at m/z 93 [M – CH₂SO₂CH₃]⁺ due to missing of methylsulfonylmethyl moiety, suggested the moiety of heteroatom-linked methyl to be methylsulfonylmethyl group. The HMBC spectrum showed correlations between H-1’ of the methylsulfonylmethyl unit to C-4, C-3 or C-5 of the phenol skeleton, which suggested the methylsulfonylmethyl group was located at C-4. Other 2D-NMR correlations confirmed the proposed structure shown in Figure 2. Accordingly, the structure of compound 1 was unambiguously established as p-methylsulfonylmethyl-phenol.

Compound 2, was obtained as a light yellow amorphous powder. The appearance of isotopic clusters at m/z 223 [M]⁺ and 225 [M + 2]⁺ in the ratio of 1:1 in the EI-MS spectrum, and the pseudomolecular ion peaks at m/z 246 [M + Na]⁺ and 248 [M + 2 + Na]⁺ in the ratio of 1:1 in the positive ESI-MS spectrum, indicated a mono-brominated compound with the molecular formula C₇H₆BrNO, with the help of NMR spectra. The odd numbered molecular weight suggested the occurrence of one nitrogen atom while the presence of three nitrogens or more were excluded, which was confirmed by the observation of a downfield nitrogen proton signal at δH 11.93. ¹H-NMR spectrum showed resonances for five aromatic protons for two spin systems, the first system composed of two resonances at δH 7.91 (1H, d, J = 7.45 Hz) and 6.05 (1H, d, J = 7.45 Hz), the second spin system composed of three proton resonances at δH 7.98 (1H, d, J = 8.65 Hz), 7.77 (1H, s) and 7.45 (dd, J = 8.60, 1.85 Hz), indicating an ABX system for ortho- and inter-trisubstituted benzene ring. The COSY spectrum confirmed the above inference (Figure 2). The ¹³C-NMR and DEPT spectra of 2 exhibited nine C-signals arising from a carbonyl group (δC 159.4), three aromatic quaternary C-atoms (δC 141.0, 124.9 and 124.6), five aromatic CH groups (δC 139.9, 127.3, 126.1, 120.5 and 109.3). Analysis of ¹³C-NMR, HSQC, COSY, and HMBC spectra allowed to deduce the skeleton of 2 to be mono-brominated quinolinone derivate. The position of the carbonyl group was determined to be C-4 on the basis of H-2 attached to C-3 and C-4, H-3 attached to C-4a, and H-5 attached to C-4 in the HMBC spectrum (Figure 2). Similarly, the position of the bromine atom was deduced to be C-7 based on the key cross-peaks H-8 with C-7 and C-6, and H-6 with C-8 in the HMBC experiment. Therefore, the structure of 2 was elucidated as 7-bromoquinolinin-4(1H)-one, which had been reported as a
synthetic alkaloid [19], but was reported as a new natural product about spectral traits for the first time.

![Figure 2. Important $^1$H-$^1$H COSY and HMBC correlations for compounds 1 and 2.](image)

Two new natural products, $p$-methylsulfonylmethyl-phenol (1) and 7-bromoquinolin-4(1H)-one (2), as well as nine known compounds (3–14), were isolated from the animal of marine bryozoan Cryptosula pallasiana. To the best of our knowledge, 1 and 2 are rare types of heteratom-contained compounds in marine bryozoans. All of the known compounds were isolated for the first time from this species, and compounds 5, 8 and 14 were isolated for the first time from a marine source. Since the CCl$_4$ and CH$_2$Cl$_2$ extracts of marine bryozoans were reported to have antitumor activity [20], we evaluated cytotoxicity for representative compounds 1–3 against human myeloid leukemia HL-60, the factor responsible for evaluation the antitumor activity, using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) method. Both of the new natural products 1 and 2 showed inactive to this cell line, while the known bromated alkaloid (3) appeared strong cytotoxicity against HL-60 cells with IC$_{50}$ value of 11.87 µg/mL.

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**Supporting Information**

Supporting Information accompanies this paper on [http://www.acgpubs.org/RNP](http://www.acgpubs.org/RNP)

**References**


Chemical constituents from *Cryptosula pallasiana* 632


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