SHORT REPORT

A C G publications

Rec. Nat. Prod. 9:4 (2014) 628-632

records of natural products

Studies on the Chemical Constituents from Marine Bryozoan *Cryptosula pallasiana*

Xiang-Rong Tian[*1], Hai-Feng Tang[*2], Yu-Shan Li[3], Hou-Wen Lin[4], Xiu-Yun Zhang[1], Jun-Tao Feng[1] and Xing Zhang[1]

[1]*Research & Development Center of Biorational Pesticide, College of Plant Protection, Northwest A&F University, Yangling 712100, China;*
[2]*Institute of Materia Medica, School of Pharmacy, Fourth Military Medical University, Xi'an 710032, China*
[3]*School of Traditional Chinese Medicines, Shenyang Pharmaceutical University, Shenyang 110016, China*
[4]*Department of Pharmacy, Renji Hospital, Affiliated to School of Medicine, Shanghai Jiao-Tong University, Shanghai 200127, China*

(Received June 10, 2014; Revised September 9, 2014; Accepted September 20, 2014)

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(1*H*)-one (**2**), 7-bromo-2,4(1*H*,3*H*)-quinazolinedione (**3**), benzamide (**6**), phenylacetamide (**7**), 4(3*H*)-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 IC_{50} value of 11.87 μg/mL, while compounds **1** and **2** were inactivity.

Keywords: Marine bryozoan; *Cryptosula pallasiana*; cytotoxicity.

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*

[*] Corresponding author: E- Mail:tianxiangrong@163.com (X.-R. Tian); tanghaifeng71@163.com (H.-F. Tang) Phone/Fax: +86-29-84774748.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 06/01/2015 EISSN: 1307-6167

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 cytotoxcity 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 H_2O and extracted successively with AcOEt and BuOH, the AcOEt extract was further extracted by petroleum ether, CCl_4 and CH_2Cl_2. The extracts of CCl_4 and CH_2Cl_2 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(1*H*,3*H*)-quinazolinedione (**3**) [7], *p*-hydroxybenzaldehyde (**4**) [8], methylparaben (**5**) [9], benzamide (**6**) [10], phenylacetamide (**7**) [11], 4(3*H*)-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.

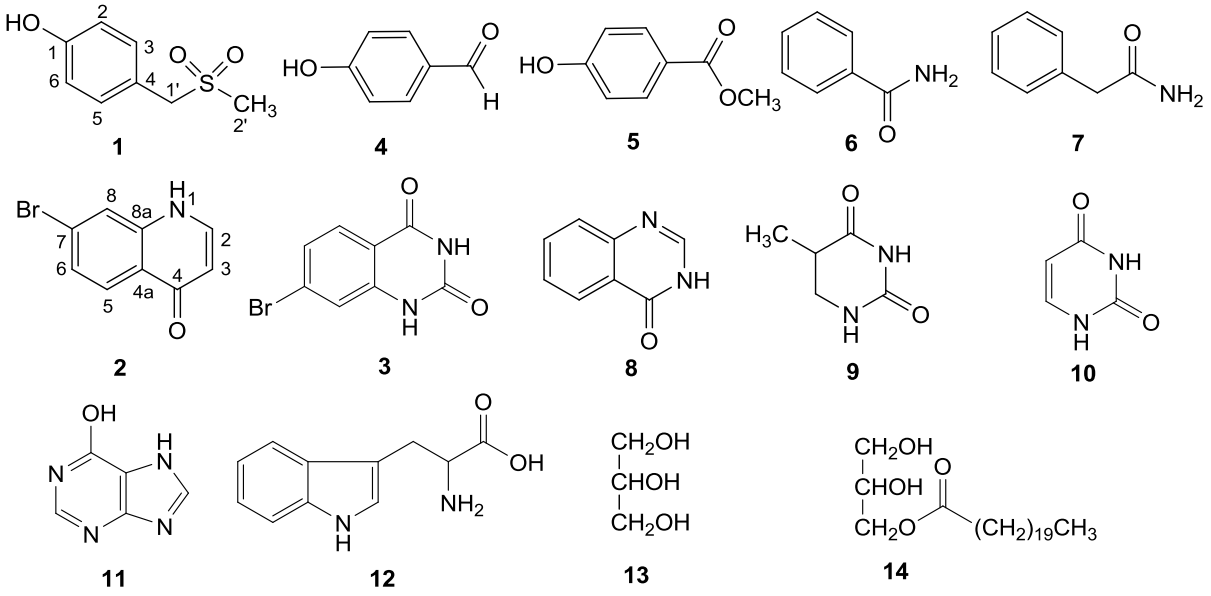

Figure 1. Structure of compounds **1–14** isolated from *C. pallasiana*.

*p-Methylsulfonylmethyl-phenol (1):*Gray yellow amorphous powder; ^{1}H-NMR (CD_3OD, 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, H_2-1'), 2.80 (3H, s, H_3-2'); ^{13}C-NMR (CD_3OD, 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 $C_8H_{10}O_3NaS$, 209.0248); EI-MS *m/z* (rel. int.): 186 $[M]^+$ (9), 171 $[M - CH_3]^+$ (2), 107 $[M - SO_2CH_3]^+$

(100), 93 [M − $CH_2SO_2CH_3$]$^+$ (2), 77 [M − $CH_2SO_2CH_3$ − OH + H]$^+$ (26), 63 (7), 56 (6), 54 (12), 52 (13).

*7-Bromoquinolin-4(1H)-one (**2**):*Light yellow amorphous powder; ^{1}H-NMR (DMSO-d_6, 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); ^{13}C-NMR (DMSO-d_6, 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 IC_{50} 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 $C_8H_{10}O_3S$ on the basis of HR-ESI-MS *m/z* 209.0250 [M + Na]$^+$ (calcd. for $C_8H_{10}O_3NaS$, 209.0248). The ^{1}H-NMR spectrum revealed an AA′BB′ system for 2H doublets at δ_H 7.24 and 6.79 which indicated a *para*-disubstituted benzene ring. A tertiary methyl group (δ_H 2.80, s, 3H) and a methylene group (δ_H 4.28, s, 2H) in the ^{1}H-NMR spectrum indicated the tertiary methyl group linked with a heteratom. As determined by ^{13}C-NMR and DEPT experiments, the down-field signals corresponded to six aromatic carbons [δ_C 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 (δ_C 39.2) and a methylene carbon (δ_C 60.8). The NMR spectra results mentioned above suggested that **1** had a *p*-substituted phenol skeleton with a heteratom-linked methyl moiety. The presence of a fragment at *m/z* 171 [M − CH_3]$^+$ in the EI-MS due to loss of methyl group, and a strong fragment at *m/z* 107 [M − SO_2CH_3]$^+$ due to loss of methylsulfonyl group, as well as the weak fragment at *m/z* 93 [*M* − $CH_2SO_2CH_3$]$^+$ due to missing of methylsulfonylmethyl moiety, suggested the moiety of heteratom-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 C_9H_6BrNO, 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 δ_H 11.93. ^{1}H-NMR spectrum showed resonances for five aromatic protons for two spin systems, the first system composed of two resonances at δ_H 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 δ_H 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 *orth*- and *inter*-trisubstituted benzene ring. The COSY spectrum confirmed the above inference (Figure 2). The ^{13}C-NMR and DEPT spectra of **2** exhibited nine C-signals arising from a carbonyl group (δ_C 159.4), three aromatic quaternary C-atoms (δ_C 141.0, 124.9 and 124.6), five aromatic CH groups (δ_C 139.9, 127.3, 126.1, 120.5 and 109.3). Analysis of ^{13}C-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-bromoquinolin-4(1*H*)-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.

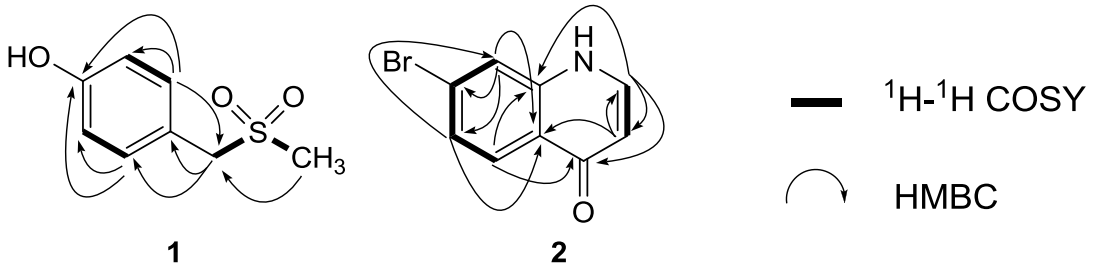

Figure 2. Important ^{1}H-^{1}H COSY and HMBC correlations for compounds **1** and **2**.

Two new natural products, *p*-methylsulfonylmethyl-phenol (**1**) and 7-bromoquinolin-4(1*H*)-one (**2**), as well as nine known compounds (**3–14**), were isolated from the animal of marine bryozoan *C. 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_2Cl_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.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (No. 31201551) and High Technology Research and Development Program Project of China (863 Project, 2007AA09Z401). The authors are grateful to Mrs. Hui-Min Wang, Mass Measurement Center, Shanghai Institute of Pharmaceutical Industry, and Dr. Ping-Hu Zhang, New Drug Screening Center, China Pharmaceutical University, for measuring MS and biological activities, respectively.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] A.J. Blackman and J.T. Walls (1995). Bryozoan secondary metabolites and their chemical ecology. In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry. Elsevier, Amsterdam, pp 73-112.

[2] X.R. Tian, H.F. Tang, Y.S. Li, H.W. Lin, N. Ma, W. Zhang and M.N. Yao (2009). Ceramides and cerebrosides from the marine bryozoan *Bugula neritina* inhabiting South China Sea, *J. Asian Nat. Prod. Res.* **11**, 1005-1012.

[3] X.R. Tian, H.F. Tang, Y.S. Li, H.W. Lin, N. Ma and W. Zhang (2010). Sterols from marine bryozoan *Bugula neritina*, *Biochem. Syst. Ecol.* **38**, 435-437.

[4] X.R. Tian, H.F. Tang, J.T. Feng, Y.S. Li, H.W. Lin, X.P. Fan and X. Zhang (2014). Neritinaceramides A-E, new ceramiedes from the marine bryozoan *Bugula neritina* inhabiting South China Sea and their cytotoxicity, *Mar. Drugs.* **12**, 1987-2003.

[5] X.R. Tian, H.F. Tang, Y.S. Li, H.W. Lin, X.Y. Tong and N. Ma (2010). Alkaloids from marine bryozoan *Cryptpsula pallasiana*, *Biochem. Syst. Ecol.* **38**, 1250-1252.

[6] X.R. Tian, H.F. Tang, Y.S. Li, H.W. Lin, X.L. Chen, N. Ma, M.N. Yao and P.H. Zhang (2011). New Cytotoxic Oxygenated Sterols from the marine bryozoan *Cryptosula pallasiana*, *Mar. Drugs.* **9**, 162-183.

[7] H. Niwa, Y. Yoshida and K. Yamada (1988). A brominated quinazoli-nedione from the marine tunicate *Pyura sacciformis*, *J. Nat. Prod.* **51**, 343-344.
[8] K. Toyoda, Y. Yaoita and M. Kikuchi (2006). Constituents of the leaves and roots of *Ligularia stenocephala* Matsum. et Koidz, *J. Nat. Med.* **60**, 329-330.
[9] J.K. Tian, F. Sun and Y.Y. Cheng (2006). Chemical constituents from the roots of *Ranunculus ternatus*, *J. Asian Nat. Prod. Res.* **8**, 35-39.
[10] D. Wu, C. Zhang, M. Zhang, J. Zhang and Z. Wang (2008). Study on chemical constituents of the flower buds of *Tussilago farfara*, *Chin. Pharm. J.* **43**, 260-263.
[11] X. Peng, D. Xiao, S. Deng, W. Ma and H. Wu (2004). Studies on the chemical constituents of the marine sponge *Cinachyrella australiansis* from the South China Sea, *China J. Mar. Drugs.* **23**, 5-7.
[12] S.M. Roopan, T. Maiyalagan and F.N. Khan (2008). Solvent-free syntheses of some quinazolin-4(3H)-ones derivatives, *Can. J. Chem.* **86**, 1019-1025.
[13] J.H. Goldstein and A.R. Tarpey (1971). Carbon-13 nuclear magnetic resonance spectral of uracil, thymine and the 5-halouracils, *J. Am. Chem. Soc.* **92**, 3573-3578.
[14] D.E. Paul, R.B. Dumlop, A.L. Pollard, K. Seidman and A.D. Cardin (1973). Carbon-13 nuclear magnetic resonance of 5-subsituted uracils, *J. Am. Chem. Soc.* **95**, 4398-4403.
[15] M.T. Chenon, R.J. Pugmire, D.M. Grant, R.P. Panzica and L.B. Townsend (1975). Carbon-13 magnetic resonance. XXVI. Quantitative determination of the tautomeric populations of certain purines, *J. Am. Chem. Soc.* **97**, 4436-4642.
[16] M.D. Kemple, P. Yuan, K.E. Nollet, J.A. Fuchs, N. Silva and F.G. Prendergast (1994). ^{13}C NMR and fluorescence analysis of tryptophan dynamics in wild-type and two single-trip variants of *Escherichia coli* thioredoxin, *Biophys. J.* **66**, 2111-2126.
[17] B.L. Zhang, S. Buddrus, M. Trierweiler and G.J. Marin (1998). Characterization of glycerol from different origins by 2H- and 13C-NMR studies of site-specific natural isotope fractionation, *J. Agric. Food. Chem.* **46**, 1374-1380.
[18] J.S. Kim, S.S. Kang, K.H. Son, H.W. Chang, H.P. Kim and K.H. Bae (2002). Constituents from the roots of *Hemerocallis fulva*, *Saengyak Hakhoechi.* **32**, 105-109.
[19] S. Rotzoll, H. Reinke, C. Fischer and P. Langer (2009). Synthesis of novel halogenated 4(1H)-quinolones by thermolysis of arylaminomethylene-1,3-dioxane-4,6-diones, *Synthesis* **1**, 69-78.
[20] H.W. Lin, G.L. Liu, Y.H. Yi, X.S. Yao and H.M. Wu (2004). Studies on antineoplastic constituents from marine bryozoan *Bugula neritina* inhabiting South China Sea: Isolation and structural elucidation of a novel macrolide, *Acad. J. Sec. Mil. Med. Univ.* **25**, 473-478.