Bioorganic & Medicinal Chemistry Letters 20 (2010) 7152–7154

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Highly brominated metabolites from marine red alga *Laurencia similis* inhibit protein tyrosine phosphatase 1B

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ARTICLE INFO

Article history:
Received 20 July 2010
Revised 27 August 2010
Accepted 31 August 2010
Available online 19 September 2010

Keywords:
Brominated metabolites
Laurencia similis
Protein tyrosine phosphatase 1B

ABSTRACT

Five new highly brominated metabolites, 3′,5′,6′,6-tetrabromo-2,4-dimethyldiphenyl ether (**1**), 1,2,5-tribromo-3-bromoamino-7-bromomethylnaphthalene (**2**), 2,5,8-tribromo-3-bromoamino-7-bromomethylnaphthalene (**3**), 2,5,6-tribromo-3-bromoamino-7-bromomethylnaphthalene (**4**) and 2′,5′,6′,5,6-pentabromo-3′,4′,3,4-tetramethoxybenzo-phenone (**5**) were isolated from the red alga *Laurencia similis*. Their structures were elucidated by spectroscopic methods including one- and two- dimensional NMR as well as HREIMS analysis. Compounds **1** and **5** showed strong inhibitory activities against protein tyrosine phosphatase 1B (PTP1B) with IC_{50} of 2.97 and 2.66 μM, respectively.

Laurencia (family Rhodomelaceae, order Ceramiales) are widely distributed along the coast in tropical and subtropical areas around the world. The secondary metabolites of this genus[1–3] have been reported to possess a variety of biological activities, such as antimicrobial,[4] antifeedant,[5] anthelmintic,[6,7] and cytotoxic activities.[8,9] Within the context of our searching for biologically active and new structurally metabolites with potential application in human or animal health from Chinese marine algal species, we have examined the chemical constituents of a sample of *Laurencia similis*,[10] which was collected from Hainan coastlines of People's Republic of China. These efforts resulted in the identification of five new highly brominated metabolites: 3′,5′,6′,6-tetrabromo-2,4-dimethyldiphenyl ether (**1**), 1,2,5-tribromo-3-bromoamino-7-bromomethylnaphthalene (**2**), 2,5,8-tribromo-3-bromoamino-7-bromomethylnaphthalene (**3**), 2,5,6-tribromo-3-bromoamino-7-bromomethylnaphthalene (**4**) and 2′,5′,6′,5,6-pentabromo-3′,4′,3,4-tetramethoxybenzophenone (**5**) (Fig. 1). This report describes the isolation and structural determination of the compounds **1**–**5**, as well as their inhibitory effects on protein tyrosine phosphatase 1B (PTP1B).

Compound **1** was obtained as colorless oil. The molecular formula of **1** was established as $C_{14}H_{10}Br_4O$ based on positive HREIMS at *m*/*z* 513.7420 (calcd for $C_{14}H_{10}{}^{79}Br_2{}^{81}Br_2O$ 513.7424), indicating eight degrees of unsaturation. The IR spectrum showed the characteristic absorption bands for an aromatic ring at 1600 and 1434 cm^{-1}. The positive EIMS spectrum exhibited a characteristic quasi-molecular ion peak cluster at *m*/*z* 510/512/514/516/518 $[M+H]^+$ with an intensity ratio of 1:4:6:4:1, indicating the presence of four Br-atoms. The ^1H NMR spectrum in acetone-d_6 showed signals for two singlet methyl groups at δ 2.45 (3H, s, CH_3-8) and 2.52 (3H, s, CH_3-7), four singlet aromatic protons signals at δ 7.12 (1H, s, H-3), 7.79 (1H, s, H-5), 7.84 (1H, s, H-4′) and 8.41 (1H, s, H-2′). The ^{13}C NMR and DEPT spectra showed 14 signals attributed to two methyl carbons at δ 17.0 (C-7) and 21.4 (C-8), four aromatic methine carbons at δ 116.4 (C-4′), 118.6 (C-5), 125.3 (C-2′) and 129.8 (C-3), and eight aromatic quaternary carbons at δ 113.5

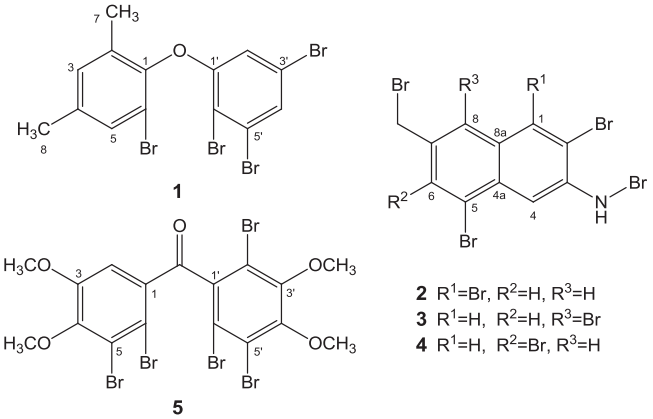

Figure 1. Chemical structures of compounds **1**–**5**.

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doi:10.1016/j.bmcl.2010.08.144

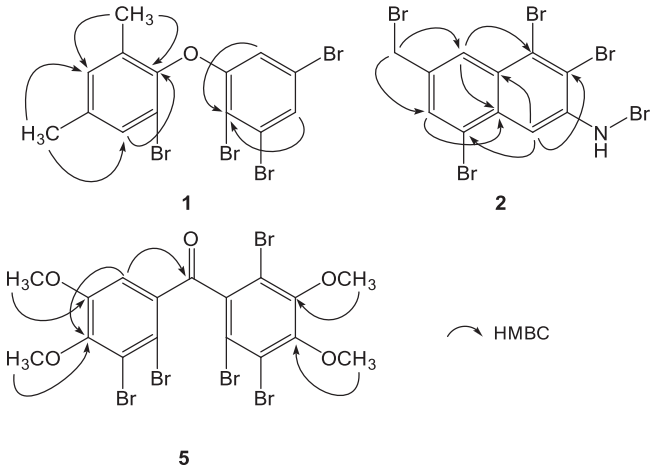

Figure 2. Key HMBC correlations of compounds **1**, **2** and **5**.

(C-3′), 120.3 (C-6′), 121.1 (C-2), 122.1 (C-5′), 125.5 (C-4), 129.9 (C-6), 139.1 (C-1), and 140.8 (C-1′). Detailed 2D NMR analyses (Fig. 2) and comparison of the NMR data (Table 1) with those of 3,3′,4,5,5′,6-hexabromo-2,2′-diacetoxydiphenyl ether[11] suggested that both compounds shared the same skeleton. The HMBC spectrum showed correlations from H-3/C-1, C-5, C-7, C-8, H-5/C-1, C-3, C-8, C-4, C-6, C*H*$_3$-7/C-1, C-2, C-3, C*H*$_3$-8/C-3, C-4, C-5, confirmed the presence of the benzyl unit. The remaining correlations signals of the HMBC spectrum of H-2′/C-1′, C-3′, C-4′, C-6′ and H-4′/C-2′, C-3′, C-5′, C-6′, indicated the presence of 3′,5′,6′-tribromobenzol unit. The above evidence allowed the elucidation of **1** as: 3′,5′,6′,6-tetrabromo-2,4-dimethyldiphenyl ether.

Compound **2** was obtained as colorless oil with a molecular formula of $C_{11}H_6Br_5N$ determined by HREIMS (*m*/*z* found 550.6361 $[M+H]^+$, calcd 550.6376). The IR spectrum showed the characteristic absorption bands for an aromatic ring at 1584 and 1460 cm^{-1}. The positive EIMS spectrum exhibited a characteristic quasi-molecular ion peak cluster at *m*/*z* 546/548/550/552/554/556 $[M+H]^+$ with an intensity ratio of 1:5:10:10:5:1, indicating that **2** was polyhalogenated. Furthermore, quasi-molecular fragments ion peaks found in the EIMS at 310/312/314 (1:2:1) $[M-3Br]^+$, 231/233 (1:1) $[M-4Br]^+$ and 152 $[M-5Br]^+$, indicated the presence of five bromine atoms. The ^{1}H NMR spectrum (Table 1) of **2** in acetone-d_6 showed four signals attributed to one methylene at δ 4.64 (2H, s, H_2-9), three singlet aromatic methine signals at δ 7.93 (1H, s, H-4), 7.79 (1H, s, H-8) and 6.82 (1H, s, H-6). The ^{13}C NMR and DEPT spectra revealed the presence of 11 signals attributed to one methylene carbon at δ 32.2 (C-9) and three aromatic methine carbons at δ 114.5 (C-6), 116.8 (C-8) and 123.7 (C-4), and seven aromatic quaternary carbons at δ 113.0 (C-1), 113.7 (C-5), 116.4 (C-2), 118.0 (C-4a), 128.7 (C-8a), 135.0 (C-7) and 136.9 (C-3). Comparison of the NMR data of **2** with those of 1-bromo-7-bromomethylnaphthalene[12] suggested both compounds shared the same bromomethylnaphthalene substructure. The HMBC (Fig. 2) correlations H-4/C-2, C-3, C-4a, C-5, H-6/C-5, C-7, C-9, H-8/C-4a, C-8a, H-9/C-6, C-7, C-8 led to the establishment of the structure of **2** as: 1,2,5-tribromo-3-bromoamino-7-bromomethylnaphthalene.

Compounds **3** and **4** were obtained as colorless oil which shared the same molecular formula of $C_{11}H_6Br_5N$ with **2** determined by HREIMS (*m*/*z* found 550.6340 and 550.6371 $[M+H]^+$, respectively, calcd 550.6376). Comparison of NMR spectra of **3** and **4** with those of **2** revealed that **3** and **4** possessed similar structure, except for the position of bromated substitution. Detailed 2D NMR (Fig. 2) spectra analysis led to the elucidation of **3** and **4** as 2,5,8-tribromo-3-bromoamino-7-bromomethyl naphthalene and 2,5,6-tribromo-3-bromoamino-7-bromomethylnaphthalene, respectively.

Compound **5** was obtained as a yellowish amorphous solid displayed a prominent pseudomolecular ion at *m*/*z* 692.6759 in HRMS, indicating a molecular formula of $C_{17}H_{14}Br_5O_5$ (calcd for $C_{17}H_{14}{}^{79}Br_5O_5$, 692.6758). The IR spectrum showed an absorption bands for a conjugated carbonyl group at 1633 cm^{-1} as well as the characteristic absorption bands for an aromatic ring at 1565 and 1452 cm^{-1}. The positive ESIMS spectrum exhibited a characteristic brominated quasi-molecular ion peak cluster at *m*/*z* 693/695/697/699/701/703 (1:5:10:10:5:1) $[M+H]^+$. The ^{1}H NMR spectrum of **5** in $CDCl_3$ showed five signals at δ 7.38 (1H, s, H-2), 3.94 (3H, s, OCH_3-3), 3.93 (3H, s, OCH_3-3′), 3.92 (3H, s, OCH_3-4) and 3.83 (3H, s, OCH_3-4′), attributed to one aromatic and four methoxyl protons, respectively. The ^{13}C NMR and DEPT spectra (Table 1)

Table 1
NMR data of compounds **1**–**5**[a]

No.	1		2		3		4		5	
	δ_H	δ_C mult.	δ_H	δ_C mult.	δ_H	δ_C mult.	δ_H	δ_C mult.	δ_H	δ_C mult.
1	—	139.1 qC	—	113.0 qC	7.63 (s)	115.4 CH	7.61 (s)	115.4 CH	—	132.3 qC
2	—	121.1 qC	—	116.4 qC	—	116.5 qC	—	116.9 qC	7.38 (s)	115.8 CH
3	7.12 (s)	129.8 CH	—	136.9 qC	—	135.4 qC	—	135.3 qC	—	153.0 qC
4	—	125.5 qC	7.93 (s)	123.7 CH	7.70 (s)	123.5 CH	7.78 (s)	122.9 CH	—	152.1 qC
4a			—	118.0 qC	—	118.3 qC	—	118.6 CH	—	—
5	7.79 (s)	118.6 CH	—	113.7 qC	—	112.7 qC	—	112.1 CH	—	115.4 qC
6	—	129.9 qC	6.82 (s)	114.5 CH	6.98 (s)	114.2 CH	—	110.8 qC	—	117.7 qC
7	2.52 (s)	17.0 CH_3	—	135.0 qC	—	134.5 qC	—	133.7 qC	—	—
8	2.45 (s)	21.4 CH_3	7.79 (s)	116.8 CH	—	110.8 qC	6.56 (s)	113.8 CH	—	—
8a	—	—	—	128.7 qC	—	127.5 qC	—	127.9 qC	—	—
9	—	—	4.64 (s)	32.2 CH_2	2.24 (d, 9.0)	34.6 CH_2	—	30.8 CH_2	—	—
NH	—	—	—	—	8.46 (br s)	—	8.37 (br s)	—	—	—
1′	—	140.8 qC	—	—	—	—	—	—	—	139.1 qC
2′	8.41 (s)	125.3 CH	—	—	—	—	—	—	—	117.6 qC
3′	—	113.5 qC	—	—	—	—	—	—	—	151.8 qC
4′	7.84 (s)	116.4 CH	—	—	—	—	—	—	—	151.1 qC
5′	—	122.2 qC	—	—	—	—	—	—	—	115.6 qC
6′	—	120.3 qC	—	—	—	—	—	—	—	122.3 qC
3′-OCH_3	—	—	—	—	—	—	—	—	3.93 (s)	60.8 CH_3
4′-OCH_3	—	—	—	—	—	—	—	—	3.83 (s)	56.5 CH_3
3-OCH_3	—	—	—	—	—	—	—	—	3.94 (s)	61.2 CH_3
4-OCH_3	—	—	—	—	—	—	—	—	3.92 (s)	61.1 CH_3
C=O	—	—	—	—	—	—	—	—	—	190.2 qC

[a] Compounds **1**—**2** were measured in acetone-d_6; **3**–**5** were measured in $CDCl_3$, respectively, at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

Table 2
Inhibitory activity of compounds **1**–**5** against PTP1B

Compounds	1	2	3	4	5	HD
IC_{50} (μg/mL)	2.97	102	65.3	69.8	2.66	0.80

HD: positive control.

displayed 17 carbon signals consisting of one carbonyl carbon at δ 190.2 (C=O), four methoxyls at δ 56.5 (OCH_3-4′), 60.8 (OCH_3-3′), 61.1 (OCH_3-4) and 61.2 (OCH_3-3), and 12 sp^2 carbons including one aromatic methine carbon at δ 115.8 (CH-2), four oxygenated quaternary carbons at δ 151.1 (C-4′), 151.8 (C-3′), 152.1 (C-4) and 153.0 (C-3), five brominated quaternary carbons at δ 115.4 (C-5), 115.6 (C-5′), 117.6 (C-2′), 117.7 (C-6) and 122.3 (C-6′), and two quaternary carbons at δ 132.3 (C-1) and 139.1 (C-1′). The protonated carbons were assigned by the HMQC experiment and the oxygenated and brominated quaternary carbons were distinguished by their chemical shifts at lower (δ >140 ppm) or higher (δ <120 ppm) fields, respectively. Detailed comparison of the above NMR data with those of Phenstatin,[13] Phenstatin acetate[14] and 2,2′,3-tribromo-3,4,4′,5-tetrahydroxy-6′-methoxymethyldiphenylmerhane[15] in the literatures indicated that **5** was a tetramethoxybenzophenone. In addition, HMBC spectrum (Fig. 2) showed the following correlations: from OCH_3-3 to C-3, from OCH_3-3′ to C-3′ and C-2′ (a weak correlation), from OCH_3-4 to C-4 and C-5 (a weak correlation), and from OCH_3-4′ to C-4′ and C-5′ (a weak correlation). On the basis of the above spectral data and the HRMS calculation, the structure of **5** was determined as: 5,6,2′,5′,6′-pentabromo-3,4,3′,4′-tetramethoxybenzophenone.

Compounds **1**–**5** were assayed for their inhibitory activity against PTP1B, and the results are presented in Table 2. Of the compounds tested, **1** and **5** showed strongest inhibitory activity with IC_{50} of 2.97 and 2.66 μM, respectively, and the other three new compounds showed moderate inhibitory activity on PTP1B.

Acknowledgments

This work was partially supported by the Program for New Century Excellent Talents in University (XL, NCET-09-0423), Ministry of Education of the People's Republic of China; National Natural Science Foundation of China (JCQ, NSFC-31000149); and supported by Key Projects in the National Science & Technology Pillar Program during the Eleventh Five-Year Plan Period (2006BAD08A08); Basic Research Program (20060544), Science and Technology Department of Jilin Province; International Technology Cooperation Project (06GH07), Changchun, China.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.144.

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Further reading

17. *Algal material*:The marine red alga *L. similis* was collected from Hainan coastlines of People's Republic of China, in May 2006 and identified by Professor L. P. Ding at the Institute of Oceanology, Chinese Academy of Sciences (IOCAS). A voucher specimen (No. 2006037) was deposited in the Herbarium of Marine Organisms at IOCAS.
18. *Extraction and isolation*: The air-dried and ground L. similis (1.72 kg) was extracted with 95% EtOH at room temperature for 3 × 48 h. The extract was concentrated to give a brown residue then partitioned between H_2O and EtOAc. The EtOAc-soluble portion was subjected to column chromatography over silica gel. The subsequent fractions were further purified by a combination of Si gel and Sephadex LH-20 column chromatography, to yield compounds **1**–**5**.
19. 3′,5′,6,6′-Tetrabromo-2,4-dimethyldiphenyl ether (**1**): colorless oil; IR (KBr) ν_{max} 1600, 1434; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 510/512/514/516/518 (1:4:6:4:1) $[M+H]^+$, 351/353/355 (1:2:1), 272:274 (1:1); HREIMS *m*/*z* 513.7420 $[M+H]^+$ (calcd for $C_{14}H_{10}{}^{79}Br_2{}^{81}Br_2O$, 513.7424).
20. 1,2,5-Tribromo-3-bromoamino-7-bromomethylnaphthalene (**2**): colorless oil; IR (KBr) ν_{max} 1584, 1460; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 546/548/550/552/554/556 (1:5:10:10:5:1) $[M+H]^+$, 470/472/474 (2:3:2), 389/391/393/395 (1:3:3:1), 310/312/314 (1:2:1), 231/233 (1:1); HREIMS *m*/*z* 550.6361 $[M+H]^+$ (calcd for $C_{11}H_6{}^{79}Br_3{}^{81}Br_2N$, 550.6376).
21. 2,5,8-Tribromo-3-bromoamino-7-bromomethylnaphthalene (**3**): colorless oil; IR (KBr) ν_{max} 1580, 1456; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 546/548/550/552/554/556 (1:5:10:10:5:1) $[M+H]^+$, 470/472/474 (2:3:2), 389/391/393/395 (1:3:3:1), 310/312/314 (1:2:1), 231/233 (1:1); HREIMS *m*/*z* 550.6340 $[M+H]^+$ (calcd for $C_{11}H_6{}^{79}Br_3{}^{81}Br_2N$, 550.6376).
22. 2,5,6-Tribromo-3-bromoamino-7-bromomethylnaphthalene (**4**): colorless oil; IR (KBr) ν_{max} 1589, 1460; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 546/548/550/552/554/556 (1:5:10:10:5:1) $[M+H]^+$, 470/472/474 (2:3:2), 389/391/393/395 (1:3:3:1), 310/312/314 (1:2:1), 231/233 (1:1); HREIMS *m*/*z* 550.6371 $[M+H]^+$ (calcd for $C_{11}H_6{}^{79}Br_3{}^{81}Br_2N$, 550.6376).
23. 2′,5,5′,6,6′-Pentabromo-3,3′,4,4′-tetramethoxybenzophenone (**5**): amorphous solid; IR (KBr) ν_{max} 1633, 1565, 1452; ^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 693/695/697/699/701/703 (1:5:10:10:5:1) $[M+H]^+$; HRESIMS *m*/*z* 692.6759 $[M+H]^+$ (calcd for $C_{17}H_{14}{}^{79}Br_5O_5$, 692.6758).
24. *PTP1B assay*: The inhibitory effect of isolated compounds on PTP1B enzyme activity was measured as previously described.[16] Briefly, 2 mM *p*-NPP and PTP1B (0.1 μg) in a buffer, containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) with or without compounds **1**–**5**, were added into 96-well to a final volume of 200 μL. Following incubation at 37 °C for 30 min, the reaction was terminated with 10 M NaOH. The amount of produced *p*-nitro phenol was calculated by measuring the absorbance at 405 nm.