Bromophenols Coupled with Methyl γ-Ureidobutyrate and Bromophenol Sulfates from the Red Alga *Rhodomela confervoides*

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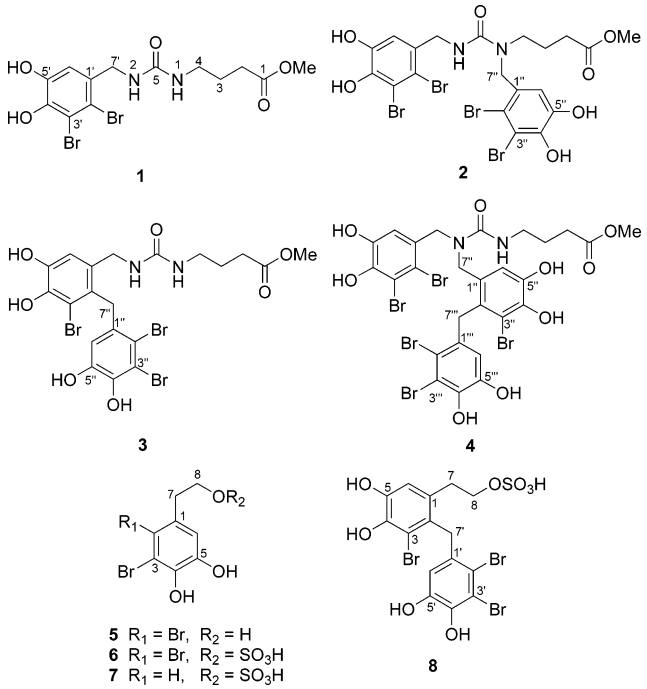
Four new bromophenols C−N coupled with methyl γ-ureidobutyrate (**1**−**4**), a phenylethanol bromophenol (**5**), and three phenylethanol sulfate bromophenols (**6**−**8**) have been isolated from polar fractions of an ethanolic extract of the red alga *Rhodomela confervoides*. On the basis of spectroscopic evidence including HRMS and 2D NMR data, the structures of the new compounds were determined as methyl *N*′-(2,3-dibromo-4,5-dihydroxybenzyl)-γ-ureidobutyrate (**1**), methyl *N*,*N*′-bis(2,3-dibromo-4,5-dihydroxybenzyl)-γ-ureidobutyrate (**2**), methyl *N*′-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-γ-ureidobutyrate (**3**), methyl *N*′-(2,3-dibromo-4,5-dihydroxybenzyl)-*N*′-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-γ-ureidobutyrate (**4**), 2,3-dibromo-4,5-dihydroxyphenylethanol (**5**), 2,3-dibromo-4,5-dihydroxyphenylethanol sulfate (**6**), 3-bromo-4,5-dihydroxyphenylethanol sulfate (**7**), and 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxyphenylethanol sulfate (**8**). The cytotoxicity of all compounds was evaluated against several human cancer cell lines including human colon cancer (HCT-8), hepatoma (Bel7402), stomach cancer (BGC-823), lung adenocarcinoma (A549), and human ovarian cancer (A2780). Among them, the phenylethanol and the phenylethanol sulfate bromophenols (**5**−**8**) showed moderate cytotoxicity against all tested cell lines.

Red algae of the family Rhodomelaceae (Ceramiales) are rich sources of bromophenols of several structural types and with various biological activities.[1−8] As part of our recently initiated program to assess systematically the chemical and biological diversity of seaweeds distributed along the gulf of the Yellow Sea,[9−11] 27 bromophenols of diverse structural types, including monoaryl, diaryl, and triaryl bromophenols,[9−11] and a unique bromophenol sulfoxide,[10] as well as unusual bromophenols coupled with pyroglutamic acid derivatives and C−N coupled deoxyguanosine nucleoside,[11] have been characterized from an EtOAc-soluble fraction of the alcoholic extract of *Rhodomela confervoides* (Huds.) Lamour. (Rhodomelaceae), collected at the coast of Qingdao, People's Republic of China. Continuing our investigation of the more polar fractions of the same material, we report herein the isolation and structural elucidation of four new bromophenols C−N coupled with methyl γ-ureidobutyrate (**1**−**4**), a phenylethanol bromophenol (**5**), and three phenylethanol sulfate bromophenols (**6**−**8**). This is the first report of bromophenols coupled with γ-ureidobutyrate derivatives and bromophenols with a basic structure of phenylethanol or its sulfate.



Results and Discussion

Compound **1** was obtained as a white powder, mp 155−158 °C, and showed absorption bands for amino, hydroxyl (3479 and 3298 cm^{-1}), and carbonyl (1707 cm^{-1}) functional groups and an aromatic ring (1595 and 1499 cm^{-1}) in its IR spectrum. The positive FABMS of **1** gave a molecular ion peak cluster diagnostic for a dibrominated molecule at *m*/*z* 439/441/443 (1:2:1) $[M + H]^+$, and the molecular formula was determined as $C_{13}H_{16}O_5Br_2N_2$ by HRFABMS. The ^1H NMR spectrum of **1** displayed three multiplets assignable to a 1,3-disubstituted propane unit at δ 2.32 (2H, t, $J = 7.5$ Hz, H_2-2), 1.74 (2H, quintet, $J = 7.5$ Hz, H_2-3), and 3.16 (2H, brt, $J = 7.5$ Hz, H_2-4) and three resonances characteristic of a 2,3-dibromo-4,5-dihydroxybenzyl unit[9−11] at δ 7.00 (1H, s, H-6′), 4.30 (2H, d, $J = 6.0$ Hz, H_2-7′), and 8.71 (2H, brs, exchangeable, O*H*-4 and O*H*-5), in addition to an ester methoxyl singlet at δ 3.59 (3H, s, OCH_3) and two exchangeable broad singlets attributed to amino protons at δ 5.80 (1H, brs, *N*-H) and 5.99 (1H, brs, *N*′-H). Besides the carbon signals associated with the above structural units (Table 2), the ^{13}C NMR and DEPT spectra of **1** exhibited two additional signals ascribed to an ester carbonyl carbon at δ 174.0 (C-1) and an oxygenated sp^2-hybridized quaternary carbon at δ 159.1 (C-5). To establish unambiguously the structure of **1**, $^1H-^1H$ COSY, HMQC, and HMBC experiments were carried out. The NMR signals of protons and corresponding carbons were readily assigned by the $^1H-^1H$ COSY and HMQC experiments (Tables 1 and 2). In the HMBC spectrum, long-range heteronuclear correlations

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**8**[a]

position	1	2	3	4	5	6	7	8
2	2.32 t (7.5)	2.35 t (7.5)	2.29 t (7.5)	2.24 t (7.0)			6.78 d (1.5)	
3	1.74 quintet (7.5)	1.91 quintet (7.5)	1.69 quintet (7.5)	1.69 quintet (7.0)				
4	3.16 brt (7.5)	3.35 t (7.5)	3.09 brt (7.5)	3.14 brt (7.0)				
6					6.91 s	6.79 s	6.63 d (1.5)	7.03 s
7					2.89 t (7.0)	3.00 t (7.0)	2.75 t (7.0)	2.79 t (6.5)
8					3.69 t (7.0)	4.09 t (7.0)	4.05 t (7.0)	4.13 t (6.5)
OMe	3.59 s	3.59 s	3.58 s	3.56 s				
N-H	5.80 brs		5.65 brs	5.92 brs				
N′-H	5.99 brs	6.45 t (5.5)	5.84 brs					
6′	7.00 s	7.02 s	6.97 s	6.71 s				6.38 s
7′	4.30 d (6.0)	4.37 d (5.5)	4.20 brs	4.25 s				4.10 s
6″		6.88 s	6.12 s	6.84 s				
7″		4.52 s	4.20 brs	4.35 s				
6‴				6.07 s				
7‴				4.02 s				

[a] Data were measured in acetone-d_6 for **1**−**5** and **8** and in methanol-d_4 for **6** and **7** at 500 MHz. Proton coupling constants (J) in Hz are given in parentheses.Assignments are based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**8**[a]

position	1	2	3	4	5	6	7	8
1	174.0 s	174.3 s	174.0 s	174.2 s	132.0 s	130.8 s	131.9 s	130.9 s
2	31.7 t	31.3 t	31.6 t	31.9 t	116.3 s	116.5 s	124.6 d	129.1 s
3	26.6 t	24.4 t	26.4 t	26.2 t	113.5 s	114.2 s	110.6 s	114.1 s
4	40.1 t	47.1 t	39.9 t	40.7 t	143.7 s	144.7 s	142.7 s	142.7 s
5	159.1 s	158.5 s	158.8 s	158.5 s	145.4 s	146.3 s	147.3 s	145.1 s
6					117.4 d	117.4 d	116.1 d	116.6 d
7					41.3 t	38.1 t	35.7 t	33.2 t
8					61.9 t	68.0 t	69.7 t	67.5 t
OMe	51.6 q	51.8 q	51.5 q	51.5 q				
1′	133.2 s	133.0 s	132.6 s	130.2 s				132.3 s
2′	114.8 s	114.6 s	128.3 s	114.6 s				116.4 s
3′	113.4 s	113.6 s	114.5 s	113.4 s				113.1 s
4′	144.2 s	144.1 s	142.8 s	144.3 s				143.9 s
5′	145.6 s	145.6 s	144.9 s	145.8 s				145.3 s
6′	115.7 d	115.6 d	115.6 d	113.9 d				116.3 d
7′	45.8 t	46.5 t	42.2 t	51.1 t				39.9 t
1″		130.9 s	132.1 s	130.0 s				
2″		114.6 s	116.2 s	129.0 s				
3″		113.8 s	113.4 s	114.9 s				
4″		144.4 s	143.4 s	143.1 s				
5″		145.9 s	145.3 s	145.2 s				
6″		114.5 d	114.9 d	115.1 d				
7″		52.1 t	39.5 t	47.9 t				
1‴				131.9 s				
2‴				116.2 s				
3‴				113.7 s				
4‴				143.5 s				
5‴				145.4 s				
6‴				114.6 d				
7‴				39.5 t				

[a] Data were measured in acetone-d_6 for **1**−**5** and **8** and in methanol-d_4 for **6** and **7** at 125 MHz. Assignments are based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

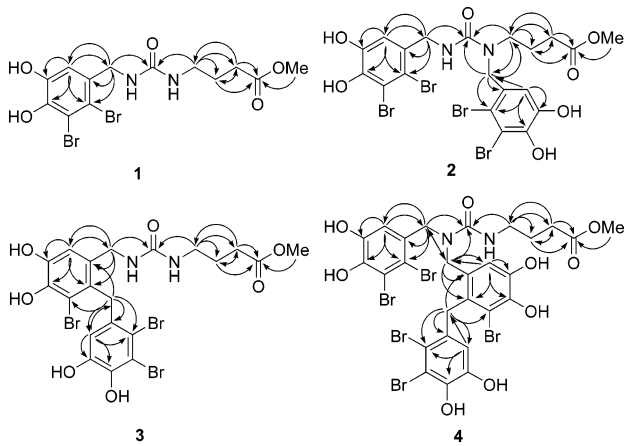

Figure 1. Major HMBC correlations of compounds **1**−**4** and **8**.

(Figure 1) from H-6′ to C-1′, C-2′, C-4′, C-5, and C-7′ and from H-7′ to C-1′, C-2′, and C-6′, in combination with chemical shift values of these carbons, confirmed the existence of the 2,3-dibromo-4,5-dihydroxybenzyl unit in **1**.[9] Meanwhile, HMBC correlations of C-1 with H_2-2, H_2-3, and the methoxyl protons, and C-4 with H_2-2 and H_2-3, as well as C-5 with H_2-4, together with homonuclear spin coupling correlations between H_2-4 and H−N and between H_2-3 and H_2-2 and H_2-4 in the ^{1}H−^{1}H COSY spectrum, demonstrated unequivocally the presence of a methyl γ-ureidobutyrate moiety in **1**. In addition, HMBC correlations from H_2-7′ to C-5, along with a ^{1}H−^{1}H COSY cross-peak between H-N′ and H_2-7′, revealed that C-5 connected across N′ to C-7′. Accordingly, **1** was determined as methyl *N*′-(2,3-dibromo-4,5-dihydroxybenzyl)-γ-ureidobutyrate.

Compound **2** was obtained as a brown gum. Its IR spectrum showed absorption bands similar to those of **1**. The negative FABMS of **2** exhibited a typical tetrabrominated molecular ion peak cluster at 715/717/719/721/723 (1:4:6:4:1) [M − H]$^-$, and the molecular formula was determined as $C_{20}H_{20}O_7Br_4N_2$ by HR-FABMS. The NMR spectroscopic features of **2** were similar to those of **1** except for the appearance of signals attributed to an additional 2,3-dibromo-4,5-dihydroxybenzyl unit (Tables 1 and 2) and the

presence of only one amino proton signal. A comparison of the NMR data of the methyl γ-ureidobutyrate moiety of **1** and **2** (Tables 1 and 2) indicated that H_2-4 and C-4 of **2** were shifted downfield by $\Delta\delta_H$ 0.19 and $\Delta\delta_C$ 7.0 ppm, respectively. These data provided evidence that **2** is a *N*- or *N*′-(2,3-dibromo-4,5-dihydroxybenzyl) derivative of **1**, which was confirmed by 2D NMR experiments, leading to the unambiguous assignment of the NMR spectroscopic data of **2** (Tables 1 and 2). In the HMBC spectrum of **2**, besides correlations similar to those of **1** that confirmed the presence of the methyl γ-ureidobutyrate moiety and two common 2,3-dibromo-4,5-dihydroxybenzyl units (Figure 1), three-bond correlations from H_2-4 to C-5 and C-7″ and from H_2-7″ to C-4 and C-5 proved unequivocally that the additional 2,3-dibromo-4,5-dihydroxybenzyl unit was located at N-1 of the methyl γ-ureidobutyrate moiety in **2**. Thus, the structure of **2** was determined as methyl *N*,*N*′-bis-(2,3-dibromo-4,5-dihydroxybenzyl)-γ-ureidobutyrate.

Compound **3** was obtained as a brown powder, mp 176−178 °C. Its negative FABMS exhibited a characteristic tribrominated ion cluster at *m*/*z* 636/638/640/642 (1: 3:3: 1) $[M - H]^-$, and the molecular formula was determined as $C_{20}H_{21}O_7Br_3N_2$ by HR-FABMS. The IR and ^{1}H NMR spectra of **3** resembled those of **1** and **2**. As for **2** when compared to **1**, the NMR spectra of **3** showed signals assignable to an additional 2,3-dibromo-4,5-dihydroxybenzyl unit. However, the appearance of two amino proton signals at δ 5.84 and 5.65 (each 1H, brs, exchangeable) in the ^{1}H NMR spectrum of **3** indicated that the two 2,3-dibromo-4,5-dihydroxybenzyl analogue units of **3** must be connected to each other. This was confirmed by a detailed comparison of the ^{1}H NMR data of **1**−**3**. The data attributed to the methyl γ-ureidobutyrate moiety of **3** were in good agreement with those of **1**. The most significant differences between the NMR data of **2** and **3** were that the resonances for H-6″, H-7″, and C-7″ were upfield shifted from δ_H 6.88 and 4.52 and δ_C 52.1 in **2** to δ_H 6.12 and 4.20 and δ_C 39.5 in **3**, respectively. Taking into account the fact that the molecular formula of **3** showed the compound to have one bromine atom less than that of **2**, the above comparison suggested **3** is another derivative of **1**, this time with 2,3-dibromo-4,5-dihydroxybenzyl replacing a bromine atom. 2D NMR experiments of **3** confirmed these deductions and established the substitution position of the additional 2,3-dibromo-4,5-dihydroxybenzyl unit. The NMR signals of protons and corresponding carbons were assigned unambiguously from ^{1}H−^{1}H COSY and HMQC data (Tables 1 and 2). In the HMBC spectrum of **3** long-range correlations (Figure 1) from H-7″ to C-1′, C-2′, and C-3′, in combination with the chemical shift values of these carbons, confirmed unequivocally the bond between C-2′ and C-7″ in **3**. Thus, **3** was determined to be methyl *N*′-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-γ-ureidobutyrate.

Compound **4** was obtained as a brown gum and showed IR absorptions similar to those of **2** and **3**. The negative-mode FABMS of **4** exhibited a molecular ion peak cluster diagnostic of a pentabrominated molecule at *m*/*z* 915/917/919/921/923/925 (1:5: 10:10:5:1) $[M - H]^-$, and HRFABMS established its molecular formula as $C_{27}H_{25}O_9Br_5N_2$. The NMR spectra of **4** differed from those of **2** and **3** by showing signals for three tetrasubstituted benzyl units identical or similar to a 2,3-dibromo-4,5-dihydroxybenzyl unit (Tables 1 and 2). A detailed comparison of the ^{1}H NMR spectra of **1**−**4** indicated there to be only one amino proton signal at δ 5.92 (1H, brs, exchangeable, N-H) in the ^{1}H NMR spectrum of **4** and that the signals attributed to the methyl γ-ureidobutyrate moiety in **4** were in good agreement with those found in **1** and **3**. This indicated a lack of substitution at N-1 of the methyl γ-ureidobutyrate moiety in **4**. This deduction was confirmed by the cross-peak between *H*-N and H_2-4 seen in the ^{1}H−^{1}H COSY spectrum of **4**. In addition, in the ^{1}H NMR spectrum of **4**, two relatively deshielded aromatic proton signals at δ 6.84 (1H, s, H-6″) and 6.71 (1H, s, H-6′) and a relatively shielded aromatic proton signal δ 6.07 (1H, s, H-6‴) were assigned to the three tetrasubstituted benzyl units and indicated that two of them had to be located at N-2 of the methyl γ-ureidobutyrate moiety and that the remaining one was connected to one of these. The structure of **4** was finally established by interpretation of its HMQC and HMBC data. After the NMR signals of protons and protonated carbons (Tables 1 and 2) had been assigned unambiguously from the HMQC data, HMBC long-range correlations (Figure 1) from H-6′ to C-2′, C-4′, C-5′, and C-7′ and from H-7′ to C-1′, C-2′, and C-6′ in combination with a comparison of chemical shift values of these protons and carbons with those of the 2,3-dibromo-4,5-dihydroxybenzyl moiety of **1** (Tables 1 and 2) clearly showed the presence of a 2,3-dibromo-4,5-dihydroxybenzyl moiety in **4**. HMBC correlations from H-6″ to C-2″, C-4″, C-5″, and C-7″, from H-7″ to C-1″, C-2″, and C-6″, from H-6‴ to C-2‴, C-4‴, C-5‴, and C-7‴, and from H-7‴ to C-1‴, C-2‴, C-6‴, C-1″, C-2″, and C-3″, together with a comparison of the chemical shift values of these protons and carbons with those of the 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl moiety of **3** (Tables 1 and 2), indicated unambiguously that there is a 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl moiety in **4**. Furthermore, the location of both the moieties at N-2 of the basic structure methyl γ-ureidobutyate was confirmed by HMBC correlations from both H-7′ and H-7″ to C-5, from H-7′ to C-7″, and from H-7″ to C-7′. Consequently, **4** was determined as methyl *N*′-(2,3-dibromo-4,5-dihydroxybenzyl)-*N*′-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-γ-ureidobutyate.

Compound **5** was obtained as a brown gum. Its IR spectrum showed absorption bands for hydroxyl (3382 cm^{-1}) and aromatic (1600 and 1498 cm^{-1}) functionalities. The EIMS contained a molecular ion peak cluster at *m*/*z* 310/312/314 (1:2:1) $[M]^+$, consistent with the molecular formula $C_8H_8Br_2O_3$. In the ^{1}H NMR spectrum of **5**, three signals at δ 6.91 (1H, s, H-6), 2.89 (2H, t, J = 7.0 Hz, H_2-7), and 3.69 (2H, t, J = 7.0 Hz, H_2-8), together with the molecular composition, showed **5** to be a dibromodihydroxyphenylethanol derivative. This deduction was supported by the ^{13}C NMR data of **5** that contained eight carbon signals, including six for a dibromodihydroxyphenyl moiety and two methylenes for a 2-substituted ethanol unit (Table 2). To determine unambiguously the substitution pattern of **5**, a HMBC measurement was made. HMBC correlations from H-6 to C-2, C-4, C-5, and C-7 and from H-7 to C-1, C-2, and C-6, along with the oxygenated character of C-4 and C-5 and the brominated character of C-2 and C-3 indicated by their chemical shifts, established unequivocally that **5** is 2,3-dibromo-4,5-dihydroxyphenylethanol.

Compound **6** was obtained as a brown gum and had IR spectroscopic absorption bands characteristic of hydroxyl groups (3440 cm^{-1}), an aromatic functionality (1600 and 1502 cm^{-1}), and a sulfate group (1225 and 987 cm^{-1}). The negative ESIMS of **6** exhibited a dibrominated molecular ion peak cluster at *m*/*z* 389/391/393 (1:2:1) $[M - H]^-$, and the molecular formula of $C_8H_8Br_2O_6S$ was determined by HRESIMS. The NMR spectroscopic data of **6** closely resembled those of **5**, except that the resonances associated with H_2-8 and C-8 were significantly deshielded in **6**, by $\Delta\delta_H$ 0.4 ppm and $\Delta\delta_C$ 6.1 ppm, respectively (Tables 1 and 2). On considering this molecular composition, **6** was assigned as 2,3-dibromo-4,5-dihydroxyphenylethanol sulfate.

Compound **7** was obtained as a brown gum and showed IR absorptions similar to those of **6**. The negative ESIMS of **7** gave a monobrominated molecular ion peak cluster at *m*/*z* 311/313 (1:1) $[M - H]^-$. HRESIMS measurement established the molecular formula of **7** as $C_8H_9BrO_6S$. The ^{1}H NMR spectrum of **7** was similar to that of **6** except for the presence of two *meta*-coupled proton signals at δ 6.78 (1H, d, J = 1.5 Hz, H-2) and 6.63 (1H, d, J = 1.5 Hz, H-6), in place of the single aromatic proton (H-6) in **6**. These data indicated **7** to be 3-bromo-4,5-dihydroxyphenylethanol sulfate,

Table 3. Cytotoxicity Data of Compounds **5**−**8**[a]

	IC$_{50}$ value (μM)[b]				
compound	A549	A2780	Bel7402	BGC-823	HCT-8
5	19.7	19.9	19.4	20.2	15.4
6	14.7	9.4	14.8	14.0	14.6
7	18.5	20.8	20.4	19.1	18.8
8	14.5	>16.9	13.5	15.1	12.1
5-Fu[c]	1.4	5.0	4.2	5.4	4.2

[a] Compounds **1**−**4** were inactive against all cell lines tested (IC$_{50}$ > 10 μg/mL). [b] For cell lines used, see Experimental Section. [c] 5-Fu = 5-fluorouracil (positive control).

a deduction supported by the ^{13}C NMR data of **7** (Tables 1 and 2). In the HMBC spectrum of **7**, correlations from H-2 to C-3, C-4, C-6, and C-7, from H-6 to C-2, C-4, C-5 and C-7, and from H$_2$-7 to C-1, C-2, C-6, and C-8, in combination with the chemical shifts of these carbons, confirmed that **7** is 3-bromo-4,5-dihydroxyphenylethanol sulfate.

Compound **8** was obtained as a brown gum and showed IR absorptions similar to those of **6** and **7**. The negative ESIMS of **8** gave a [M − H]$^-$ ion peak cluster at *m*/*z* 589/591/593/595 (1:3:3:1) consistent with the presence of three bromines in the molecule, a deduction supported by HRESIMS. The ^{1}H NMR spectroscopic data of **8** were similar to those of **6** except for the appearance of signals ascribed to an additional tetrasubstituted benzyl unit at δ 6.38 (1H, s, H-6′) and 4.10 (2H, s, H$_2$-7′). The presence of the additional tetrasubstituted benzyl unit was confirmed by the ^{13}C NMR data of **8** that showed resonances for 15 carbon atoms, two aromatic methines, 10 aromatic quaternary carbons (four oxygenated), and three methylenes (one oxygenated) (Table 2). Further, comparison of the ^{13}C NMR spectroscopic data of **8** with those for **3** indicated that the chemical shifts of the aromatic carbons of **8** were in good agreement with those of **3** (Table 2), as were those for C-7′. These data therefore showed **8** to be 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxyphenylethanol sulfate, a deduction supported by the HMBC data of **8**.

In the negative ESIMS of compounds **6**−**8** a strong fragment ion peak attributable to HSO_4^- at *m*/*z* 97 suggested that these phenylethanol sulfate bromophenols were obtained as their acid form rather than the salt form. This was supported by the simultaneous measurement of the positive ESIMS, which gave neither a [M + H]$^+$ nor a [M + Na or K]$^+$ for compounds **6**−**8**.

The 2,3-dibromo-4,5-dihydroxybenzyl moiety found in **1**−**4** has been isolated in alcoholic, aldehyde, and ether forms from the same alga.[9−11] Even though the γ-ureidobutyric acid subunit of **1**−**4** has not been obtained from this plant material as yet, it has been reported as a red alga metabolite (grateloupine) from *Grateloupia filicina*.[12] Biogenetically, **1**−**4** may be formed reasonably from the coupling reaction of the possible precursors 2,3-dibromo-4,5-dihydroxybenzaldehyde and/or 2,3-dibromo-4,5-dihydroxybenzyl alcohol with γ-ureidobutyric acid, and **5**−**8** may originate from tyrosine and/or phenylalanine metabolism. To exclude the artificial formation of **1**−**4** in the isolation procedure, experiments simulating the isolation conditions were carried out. γ-Ureidobutyric acid was synthesized by following the reported method.[12] The coupling reaction was not observed after refluxing solutions of γ-ureidobutyric acid with 2,3-dibromo-4,5-dihydroxybenzaldehyde or 2,3-dibromo-4,5-dihydroxybenzyl alcohol in different solvents with silica gel for 28 h. Meanwhile, the hydrolysis of **6** did not occur by refluxing aqueous methanol or acetone solutions of **6** with silica gel for 16 h.

Compounds **1**−**8** were assessed for their cytotoxicity against human colon cancer (HCT-8), hepatoma (Bel7402), stomach cancer (BGC-823), lung adenocarcinoma (A549), and human ovarian cancer (A2780) cell lines. The results showed the phenyethanol-derived bromophenols **5**−**8** to be moderately cytotoxic (Table 3).

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4 micro melting point apparatus. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. 1D- and 2D-NMR spectra were obtained at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on an Inova 500 MHz spectrometer in acetone-d_6 and methanol-d_4, with solvent peaks being used as references. EIMS, HREIMS, FABMS, and HRFABMS data were measured employing a Micromass Autospec-Ultima ETOF spectrometer, and ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo ionspray source) spectrometer. HRESIMS data were measured using a AccuToFCS JMS-T100CS spectrometer. Column chromatography was performed over normal-phase silica gel (200−300 mesh), Bio-Beads SX3 (200−400 mesh), RP-18 reversed-phase silica gel (43−60 μm), and Sephadex LH-20. HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector with an Alltima (250 × 22 mm) preparative column packed with C$_{18}$ RP-material (10 μm). TLC was carried out using glass precoated silica gel GF$_{254}$ plates (Qingdao Marine Chemical Factory, Qingdao, People's Republic of China). Plates were visualized under UV light (254 nm) or by spraying with 3% $FeCl_3$ in EtOH.

Plant Material. As described in a previous report.[9]

Extraction and Isolation. The preliminary separation procedure has been described in a previous report.[9] The EtOAc fraction (594.6 g) was chromatographed over silica gel (1200 g), eluting with a gradient of increasing MeOH (0−100%) in $CHCl_3$, and separated into 24 fractions (I−XXIV) on the basis of TLC analysis. Fraction XIII (8.70 g) obtained from column chromatography of the EtOAc-soluble portion over silica gel was further chromatographed over Sephadex LH-20 (100 g), by eluting with petroleum ether−$CHCl_3$−MeOH (5: 5: 1), to yield five subfractions, ma$_1$−ma$_5$, and then eluting with $CHCl_3$−MeOH (1: 1) to give six subfractions, mb$_6$−mb$_{11}$. Subfractions ma$_8$ (1.60 g) and mb$_5$ (0.22 g) were decolored by column chromatography over Bio-Beads SX3 using $CHCl_3$−EtOAc (1: 1) as eluent to yield the corresponding decolored subfractions ma$_8$ (0.76 g) and mb$_5$ (41 mg). Reversed-phase preparative HPLC of subfraction ma$_8$ with MeOH−H_2O−AcOH (60:40:0.1) as mobile phase yielded **1** (30 mg) and **2** (11 mg) and of subfraction mb$_5$ gave **4** (8 mg). Fraction XIV (11.80 g) was subject to MPLC over C$_{18}$ reversed-phase silica gel (200 g), eluting with a gradient of increasing MeOH (0%−100%) in H_2O, to yield subfractions n$_1$−n$_8$. Subfraction n$_4$ (0.22 g) was decolored by column chromatography over Sephadex LH-20 eluting with $CHCl_3$−MeOH (1: 1) to give the corresponding decolored subfraction (68 mg), which was further purified by reversed-phase HPLC using MeOH−H_2O−AcOH (60:40:0.1) as mobile phase, to yield **3** (14 mg). Since fractions XVIII−XXIV did not show resolvable spots by TLC, these fractions were pooled to give a combined fraction (59.10 g) that was subjected to MPLC over C$_{18}$ reversed-phase silica gel (500 g). Elution with a gradient of increasing MeOH (0%−100%) in H_2O gave subfractions r$_1$−r$_9$. Fractions r$_1$ (0.17 g), r$_3$ (0.21 g), and r$_5$ (2.80 g) were decolored using Sephadex LH-20 column chromatography eluted with MeOH. The corresponding decolored fractions were purified by reversed-phase HPLC, using a mobile phase of MeOH−H_2O (10:90) for r$_1$, to yield **7** (9 mg), MeOH−H_2O (23:77), for r$_3$, to yield **5** (8 mg) and **6** (16 mg), and MeOH−H_2O (50:50), for r$_5$, to yield **8** (11 mg).

Methyl *N*′-(2,3-dibromo-4,5-dihydroxybenzyl)-γ-ureidobutyrate (1): white amorphous powder, mp 155−158 °C; IR (KBr) ν_{max} 3479, 3298, 2952, 1707, 1626, 1595, 1499, 1421, 1281, 1163, 1039, 854 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Tables 1 and 2; FABMS *m*/*z* 443/441/439 (44:84:42) [M + H]$^+$, 363 (15), 361 (26), 359 (14), 283 (8), 281 (18), 279 (9), 185 (39), 161 (33), 86 (100); HRFABMS *m*/*z* 438.9421 [M + H]$^+$ (calcd for $C_{13}H_{17}O_5{}^{79}Br_2N_2$ 438.9504).

Methyl *N*,*N*′-bis-(2,3-dibromo-4,5-dihydroxybenzyl)-γ-ureidobutyrate (2): brown gum; IR (KBr) ν_{max} 3172, 2924, 2850, 1716, 1624, 1589, 1539, 1499, 1404, 1363, 1275, 1171, 1022, 858 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Tables 1 and 2; FABMS *m*/*z* 723/721/719/717/715 (5:20:35:23:5) [M − H]$^-$, 643 (8), 641 (25), 639 (27), 637 (9), 563 (9), 561 (19), 559 (10), 459 (34), 439 (30); 367 (100), 357 (42), 355 (43), 347 (25), 325 (23), 311(26); HRFABMS *m*/*z* 714.7912 [M − H]$^-$ (calcd for $C_{20}H_{19}O_7{}^{79}Br_4N_2$ 714.7926).

Methyl *N*′-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-γ-ureidobutyrate (3): brown powder; mp 176−178 °C; IR (KBr) ν_{max} 3396, 2924, 2852, 1716, 1614, 1437, 1385, 1275, 1174, 1092, 864 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Tables 1 and 2; FABMS *m*/*z* 642/640/638/636 (12:34: 33:10) [M − H]$^-$, 562 (8), 560 (22), 558 (22), 556 (7), 401 (12), 399 (22), 397 (10), 265 (22), 263 (39); 261 (19), 173 (88), 171 (100), 81(32), 79 (33); HRFABMS *m*/*z* 636.8792 [M − H]$^-$ (calcd for $C_{20}H_{20}O_7{}^{79}Br_3N_2$, 636.8821).

Methyl *N*′-(2,3-dibromo-4,5-dihydroxybenzyl)-*N*′-[3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl]-γ-ureidobutyrate (4): brown gum; IR (KBr) ν_{max} 3406, 2922, 2854, 1709, 1624, 1539, 1460, 1406, 1369, 1267, 1173, 1036, 858 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Tables 1 and 2; FABMS *m*/*z* 925/923/919/921/917/915 (2:10:24:27:12:3) [M − H]$^-$, 562 (8), 560 (22), 558 (22), 556 (7), 401 (12), 399 (22), 397 (10), 265 (22), 263 (39); 261 (19), 173 (88), 171 (100), 81-(32), 79 (33); HRFABMS *m*/*z* 914.7346 [M − H]$^-$ (calcd for $C_{27}H_{24}O_9{}^{79}Br_5N_2$ 914.7398).

2,3-Dibromo-4,5-dihydroxyphenylethanol (5): brown gum; UV (MeOH) λ_{max} (log ϵ) 211 (4.10), 292 (3.23) nm; IR (KBr) ν_{max} 3382, 2933, 1716, 1600, 1572, 1498, 1471, 1406, 1273, 1178, 1043, 858 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Tables 1 and 2; EIMS *m*/*z* 314/312/310 (12:24:13) [M]$^+$, 283 (48), 281 (100), 279 (50), 232 (12), 230 (9), 216 (20), 214 (21), 203 (24), 201 (26), 149 (13), 136 (8), 135 (14), 133 (6), 123 (8), 107 (7), 105 (9), 89 (9), 80 (14), 77 (23), 75 (12), 65 (9), 63 (15); HREI *m*/*z* 309.8822 (calcd for $C_8H_7{}^{79}Br_2O_3$, 309.8840).

2,3-Dibromo-4,5-dihydroxyphenylethanol sulfate (6): brown gum; UV (MeOH) λ_{max} (log ϵ) 210 (4.39), 292 (3.29) nm; IR (KBr) ν_{max} 3440, 2958, 1600, 1502, 1473, 1406, 1225,1061, 987, 910, 858 cm^{-1}; ^{1}H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) data, see Tables 1 and 2; ESIMS *m*/*z* 393/391/389 (51:100:56) [M − H]$^-$, 311 (21), 309 (19), 131 (10), 97 (72); HRESIMS *m*/*z* 388.8340 (calcd for $C_8H_7{}^{79}Br_2O_6S$, 388.8330).

3-Bromo-4,5-dihydroxyphenylethanol sulfate (7): brown gum; UV (MeOH) λ_{max} (log ϵ) 209 (4.27), 287 (3.32) nm; IR (KBr) ν_{max} 3437, 2964, 1633, 1514, 1487, 1435, 1228, 1065, 985, 908, 839 cm^{-1}; ^{1}H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) data, see Tables 1 and 2; ESIMS *m*/*z* 313/311 (98, 100) [M − H]$^-$, 233 (5), 231 (10), 97 (58); HRESIMS *m*/*z* 310.9219 (calcd for $C_8H_8{}^{79}BrO_6S$, 310.9225).

3-Bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxyphenyethanol sulfate (8): brown gum; UV (MeOH) λ_{max} (log ϵ) 209 (4.75), 290 (3.68) nm; IR (KBr) ν_{max} 3346, 2964, 1685, 1610, 1560, 1491, 1473, 1406, 1217, 1180, 1061, 978, 858 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz) data, see Tables 1 and 2; ESIMS *m*/*z* 595/593/591/589 (37, 100, 100, 35) [M − H]$^-$, 513 (20), 511 (38), 509 (17), 433 (6), 431 (13), 429 (10), 97 (20); HRESIMS *m*/*z* 590.7784 (calcd for $C_8H_8{}^{79}Br_2{}^{81}BrO_8S$, 590.7783).

Cells and Culture Conditions. Human lung adenocarcinoma (A549), human hepatoma (Bel7402), human stomach cancer (BGC-823), human colon cancer (HCT-8), and human ovarian cancer (A2780) cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were maintained in RRMI1640 supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

Cell Proliferation Assay. A549, Bel7402, BGC-823, HCT-8, and A2780 cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, the compounds were added to the cells. After 96 h of drug treatment, cell viability was determined by measuring the metabolic conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into purple formazan crystals by active cells.[13,14] MTT assay results were read using a MK 3 Wellscan (Labsystem Drogon) plate reader at 570 nm. All compounds were tested at five concentrations and were dissolved in 100% DMSO to give a final DMSO concentration of 0.1% in each well. Each concentration of the compounds was tested in three parallel wells. IC_{50} values were calculated using Microsoft Excel software.

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Supporting Information Available: MS, 1D and 2D NMR spectra of compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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