Polybrominated Diphenyl Ethers from the Indonesian Sponge *Lamellodysidea herbacea*[⊥]

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Four new (**1**−**4**) and 10 known polybrominated diphenyl ethers (**5**−**14**) have been isolated from the title sponge. The structures of the new entities were elucidated by interpretation of spectroscopic data and chemical transformations. These metabolites showed potent antimicrobial activity against *Bacillus subtilis* and moderate/weak cytotoxicity against NBT-T2 rat bladder epithelial cells. The major constituent **14** was treated under debromination conditions to give eight derivatives, which were subjected to a structure−activity relationship study. The results indicated that the presence of two phenolic hydroxyl groups and bromines at C-2 and/or C-5, as in **2**, is important for the exhibition of antibacterial activity.

Sponges of the family Dysideidae have been the subject of numerous chemical investigations and have yielded a number of unique, bioactive substances such as arenastatin,[1] dysidiolide,[2] dysiherbaine,[3] and dysidazirine,[4] to name a few. Among the species of the genus *Lamellodysidea* (formerly known as *Dysidea*), *L. herbacea* is the one studied most extensively. The majority of its metabolites can be grouped into three chemical classes: small peptides with a characteristic trichloromethyl group, sesquiterpenoids, and polybrominated diphenyl ethers (PBDEs). The genus *Lamellodysidea* is biologically characterized by the symbiotic presence of the filamentous cyanobacterium *Oscillatoria spongeliae*.[5,6] Faulkner and co-workers have reported that the PBDEs are produced by the associated cyanobacteria.[7] PBDEs have been found to exhibit a variety of bioactivities: antibacterial and antifungal properties,[8−11] brine shrimp toxicity,[10] antimicroalgal activity,[12] antiinflammatory activity,[13] and inhibition of a range of enzymes implicated in tumor development such as inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, and 15-lipoxygenase.[14] More recently, PBDEs have been reported to inhibit the assembly of microtubule protein, the maturation of starfish oocytes,[15] and also Tie2 kinase.[16] In this collaborative project on the studies of Indonesian marine organisms,[17] we have examined the constituents of *L. herbacea* collected at Sangiang Island, Indonesia, and have isolated four new PBDEs (**1**−**4**) along with known congeners (**5**−**14**). We also prepared 13 synthetic derivatives of these compounds for the study of their structure−activity relationships (SAR) against *B. subtilis* and NBT-T2 cells. We report herein the isolation and structure elucidation of the new compounds and the results of this SAR study.

The EtOAc-soluble portion of a crude extract from the sponge *L. herbacea* was partitioned between hexane and aqueous MeOH, and the latter layer was then extracted with CH_2Cl_2. The hexane extract was fractionated by silica gel flash chromatography followed by HPLC separation and recrystallization to give the new compounds **1**, **2**, and **4**, in addition to nine known substances (**5**, **7**−**14**). The major constituent **14** was also obtained from the CH_2Cl_2 extract by crystallization. The mother liquor portion was separated by HPLC to give the new compound **3** along with **6**, **13**, and **14**. The structures of the known compounds (**5**−**14**) were identified on the basis of the interpretation of their spectroscopic data and by comparison with literature values.[14,15,18−22]Mass spectrometry of compound **1** established its molecular formula as $C_{13}H_7Br_5O_3$. The ^{1}H NMR data showed the presence of *meta*-coupled protons (δ 6.81 and 7.48) and a methoxy group (δ 4.01) on ring B, as in **5**. An additional aromatic singlet at δ 7.65 suggested that **1** is a debromo analogue of **5**. The presence of a phenolic hydroxyl group was inferred from the low-field signal at δ 9.96 (brs) and the IR absorption band at 3350 cm^{-1}. HMBC correlations gave confirmation of the position of the methoxyl at C-2′ and the substitution pattern on ring A by the correlations H-4/C-2,3,5,6. Methylation of **1** furnished dimethyl ether **15**, which showed identical data with those reported.[21] Therefore, compound **1** was elucidated as 2,3,5-tribromo-6-(3′,5′-dibromo-2′-methoxyphenoxy)phenol. Comparison of the ^{13}C NMR data for the ring A portion of **1** with those of the demethyl analogue **13** showed good agreement ($\Delta\delta$ 0.0−0.3), except for C-6 ($\Delta\delta$ 7.5), which is probably influenced by additional hydrogen bonding in **13**.

Compound **2** analyzed for $C_{12}H_6Br_4O_3$, indicating it to be a tetrabromodiphenyl ether without a methyl ether function. The ^{1}H NMR spectrum exhibited a pair of *meta*-coupled signals at δ 6.64 and 7.39, as in two other members of this compound series, and *ortho*-coupled resonances at δ 7.17 and 7.45. The presence of two phenolic hydroxyls was inferred by the IR spectrum (3444 cm^{-1}) and confirmed by methylation, giving the dimethyl ether **16**. The HMBC correlations H-6′/C-1′,2′,4′,5′ and H-4′/C-2′,6′ established the same substitution pattern on ring B as in **5**, while the correlations H-3/C-1,2,5 and H-4/C-2,5,6 indicated the ring A moiety to be 2,5-dibromo-6-phenoxyphenol. Compound **2** was elucidated as 2,5-dibromo-6-(3′,5′-dibromo-2′-hydroxyphenoxy)phenol.

The molecular formula of **3**, $C_{12}H_6Br_4O_3$, suggested that it is isomeric with **2**, but the substitution pattern is different. Ring B was found to contain one bromine atom as shown by 1,2,4-trisubstitution signals [δ 6.53 (d, J = 2.5 Hz), 6.80 (d, J = 8.5 Hz), 6.97 (dd, J = 8.5, 2.5 Hz)], as in **6**. Ring A was concluded to contain three bromine atoms (δ 7.74 s) and was elucidated as a 1-hydroxy-2,4,5-tribromo-6-phenoxyl moiety by HMBC correlations (H-3/C-1,2,4,5) and by comparing its ^{13}C NMR data with those reported for **17** and **18**.[10,23] Methylation of **3** gave **19**, having two methoxy groups. Therefore, **3** was deduced as 2,4,5-tribromo-6-(5′-bromo-2′-hydroxyphenoxy)phenol.

[⊥] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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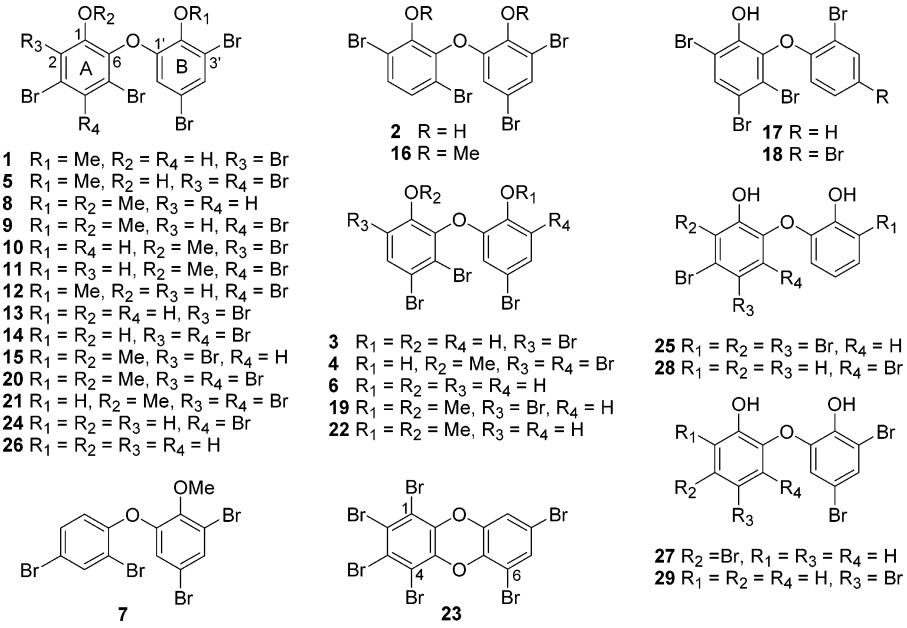
Chart 1



Compound **4** was shown to have the same molecular formula, $C_{13}H_7Br_5O_3$, as **1**. A pair of *meta*-coupled protons (δ 6.77, 7.43) as in **1**, **2**, and **5** suggested that ring B is either 3′,5′-dibromo-2′-hydroxyphenyl or 3′,5′-dibromo-2′-methoxyphenyl. The presence of an aromatic singlet (δ 7.91) indicated that ring A of **4** contains three bromine atoms. Of the four possible sites for the proton, position 3 was considered more likely than 2, 4, and 5 because H-3 appears at a lower field as in **3** and related compounds.[24] A methoxy group (δ 3.86) can be placed at ring A by comparing chemical shifts of methyl ethers in this work and refs 14, 15, 17, and 22. In all compounds (**1**, **5**, **8**, **9**, **12**, **15**, **16**, **19**, **20**, and **22**) having a 3′,5′-dibromo-2′-methoxyphenoxy group (ring B), the methoxy group is observed in the range δ 3.92−4.03, while a methoxy at ring A (**8**−**11**, **15**, **16**, **19**−**22**) appears higher than δ 3.87. Scarcity of the sample precluded the running of the ^{13}C NMR spectrum for a more rigorous assignment of the ring A substitution, but the above evidence suggested that **4** is 2,4,5-tribromo-6-(3′,5′-dibromo-2′-hydroxyphenoxy)anisole.

In order to determine the effect of the substituents in PBDEs on antibacterial activity and cytotoxicity, methyl ethers **20**−**22** were prepared from **5**, **6**, and **14**. Furthermore, the major compound **14** was treated under debromination conditions using HBr and Na_2SO_3.[25] Eight products (**13**, **23**−**29**) were obtained with acetic acid as the reaction solvent, while only **23** was produced when MeOH was used instead of acetic acid. The structures of these compounds were characterized mainly by their ^{1}H NMR and EIMS data and by comparison with values reported in the literature.[22] Six products (**23**, **25**−**29**) were new compounds, of which **23** is an unprecedented hexabromodioxin. The structure of **23** was assigned by observing *meta*-coupled proton signals as in **14**, HMBC correlations (H-7/C-5a,6,8,9, H-9/C-5a,7,8,9a), and molecular ions corresponding to the loss of H_2O from **14**.

The results of the antibacterial assays carried out are shown in Table 2. In the standard disk diffusion assay, all compounds, except for **7**−**9**, **11**, **15**, **16**, **19**, **20**, and **22**, were active against the Gram-positive bacterium *B. subtilis* in the range of 1−10 μg/disk, while compounds **1**−**4**, **6**, **10**, **12**, **14**, **23**, **24**, **28**, and **29** were still active at the concentration of 0.1 μg/disk. Compound **2** was most active, giving clear zones of inhibition of 20, 20, 13, and 7 mm at 10, 5, 1, and 0.1 μg/disk, respectively. Among the derivatives, **24** showed inhibition zones of 7−16 mm at the concentrations of 0.1−10 μg/disk. These results point out that the presence of two phenolic hydroxyl groups as well as bromine atoms at C-2 and/or C-5 as in **2** is important for the resultant antibacterial activity.

Table 1. ^{13}C NMR Spectroscopic Data (125 MHz, acetone-d_6) of **1**−**3**

C#	1	2	3
1	150.8	150.2	149.5
2	115.1	111.6	115.1
3	123.4	131.9	133.8
4	127.9	125.2	111.7
5	117.0	116.7	120.2
6	139.0	140.6	141.9
1′	151.9	146.9	146.1
2′	146.5	145.2	147.1
3′	119.5	111.4	119.2
4′	129.7	129.6	127.1
5′	116.9	110.7	110.9
6′	117.5	116.8	117.5
OMe-2′	61.1		

In the cytotoxicity assay against NBT-T2 rat bladder epithelial cells, IC_{50} values of compounds **12** and **27** were obtained as 2.8 and 8.5 μg/mL, while compounds **2**, **3**, **5**, **6**, **13**, **14**, **23**−**26**, and **28**−**29** showed no significant activities (IC_{50} >15 μg/mL).

Experimental Section

General Experimental Procedures. UV spectra were obtained on a Hitachi U-2001 spectrophotometer and FTIR spectra on a JASCO FTIR 300 spectrometer. NMR spectra were recorded on a JEOL α500 FT NMR spectrometer in acetone-d_6, $CDCl_3$, or CD_3OD. Chemical shifts were referenced to TMS or solvent signals (acetone-d_6: δ_C 206.7; $CDCl_3$: δ_C 77.2; CD_3OD: δ_C 49.2). Multiplicities of ^{13}C NMR data were determined by DEPT experiments. ESIMS were recorded on an ESITOFMS QSTAR mass spectrometer (PE Biosystem), while EIMS were measured on a Hitachi M-2500 instrument. HPLC separations were carried out on a Tosoh CCPE pump equipped with a Tosoh UV-8011 detector and a Shodex RI-101 refractive index detector or on a Hitachi L-6000 pump outfitted with a Waters R403 RI monitor and a Hitachi L-4000 UV detector. Columns used for HPLC were silica gel (250 × 10 mm, Mightysil Si-60) or reversed-phase silica gel (250 × 10 mm, Mightysil RP18 GP). Merck silica gel 60 (0.063−0.20 mm) was used for initial column chromatography. Analytical TLC was performed on commercial silica gel 60 F_{254} plates and visualized with iodine vapor.

Animal Material. A specimen of the sponge *Lamellodysidea herbacea* was collected by hand using scuba in Sangiang Island, West Java, Indonesia, in August 2004. Voucher specimens have been deposited at the Departement of Chemistry, Biology, and Marine Science, University of the Ryukyus (Code No. 04C35) and also at

Table 2. Antibacterial Activity against *B. subtilis* (inhibition zone in mm)

compound	concentration (μg/disk)			
	0.1	1	5	10
1	6	11	14	14
2	7	13	20	20
3	7	8	16	17
4	6	7	10	13
5	0	10	10	13
6	7	13	16	18
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	6	9	9	9
11	0	0	0	0
12	6	9	10	10
13	0	7	8	10
14	6	7	12	12
15	0	0	0	0
16	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0
22	0	0	0	0
23	7	12	13	14
24	7	10	13	16
25	0	0	8	13
26	0	0	16	18
27	0	0	7	13
28	6	8	10	14
29	6	6	8	11

Naturalis, National Museum of Natural History, The Netherlands (No. RMNH Por 2653).

Extraction and Isolation. An air-dried sample of the sponge (1.8 kg) was extracted at room temperature with MeOH (4 × 2.5 L). The MeOH extract was concentrated, and the residue was partitioned between water and EtOAc. The organic extract (47.71 g) was further partitioned between hexane and aqueous MeOH (50%) to afford a hexane-soluble fraction (3.89 g). The aqueous MeOH layer was extracted with CH_2Cl_2 to give a CH_2Cl_2 fraction (35.21 g). Both the hexane and CH_2Cl_2 fractions showed strong activity against the Gram-positive bacterium *B. subtilis* and weak toxicity against NBT-T2 cells. Bioassay-guided fractionation of the hexane-soluble portion was carried out by flash column chromatography over Si gel 60 using stepwise gradient elution with hexane−EtOAc−MeOH to yield nine fractions. The first fraction (0.75 g) was purified by silica HPLC (hexane−EtOAc) to give 16 subfractions. Compound **7** (24.8 mg) was isolated from the second subfraction by silica HPLC (hexane−CH_2Cl_2). The fifth subfraction was similarly purified to give compound **8** (69.7 mg). The last subfraction gave compound **9** (7.3 mg). Compound **1** (111.1 mg) was obtained from the third fraction (0.46 g) by fractional crystallization from hexane−acetone. The fourth fraction (0.39 g) was washed with CH_2Cl_2 and then recrystallized with the same solvent to afford compound **10** (5.2 mg). The fifth fraction (0.43 g), showing strong activity against *B. subtilis*, was separated by reversed-phase HPLC (RP18, MeOH) to give 10 subfractions. The second subfraction (4.5 mg) was recrystallized from hexane−$CHCl_3$ to give compound **2** (4.2 mg) as a white solid. Repeated recrystallization of the fourth, fifth, sixth, and seventh subfractions using the same solvent system afforded compounds **4** (0.2 mg), **5** (17.0 mg), **11** (5.3 mg), and **12** (50.6 mg), respectively. The mother liquor of the sixth fraction (0.62 g) was washed with CH_2Cl_2, and the residue of the CH_2Cl_2 solution was recrystallized from hexane−acetone to give compound **13** (10.7 mg). The eighth fraction (0.34 g) was similarly recrystallized to afford **14** (69.4 mg). The residue of the initial CH_2Cl_2 fraction (35.21 g) was also washed with a small amount of CH_2Cl_2, and the residue was recrystallized from hexane−acetone to afford **14** (12.57 g). Separation of the CH_2Cl_2-soluble portion using ODS VFC gave three fractions. The fraction eluted with 60% aqueous MeOH was purified by recrystallization and HPLC (Si60, CH_2Cl_2) to afford compounds **3** (2.2 mg) and **6** (0.7 mg).

Compound 1: white solid; UV (MeOH) λ_{max} (log ϵ) 214 (4.94) nm; IR (KBr) ν_{max} 3350, 1620, 1475 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 4.01 (3H, s, OMe-2′), 6.81 (1H, d, J = 2.5 Hz, H-6′), 7.48 (1H, d, J = 2.5 Hz, H-4′), 7.65 (1H, s, H-4), 9.96 (1H, brs, OH-1); ^{13}C NMR, see Table 1; EIMS *m*/*z* 605.6 (10), 607.6 (51), 609.6 (100), 611.6 (97), 613.6 (48), 615.6 (10) [M]$^+$; HRESIMS *m*/*z* 634.6116 [M + Na]$^+$ (634.6149 calcd for $C_{13}H_7{}^{79}Br_2{}^{81}Br_3O_3Na$).

Compound 2: white solid; UV (MeOH) λ_{max} (log ϵ) 208.5 (4.87) nm; IR (KBr) ν_{max} 3444, 1574, 1474 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.64 (1H, d, J = 2.0 Hz, H-6′), 7.17 (1H, d, J = 9.0 Hz, H-4), 7.39 (1H, d, J = 2.0 Hz, H-4′), 7.45 (1H, d, J = 9.0 Hz, H-3); ^{13}C NMR, see Table 1; EIMS *m*/*z* 513.7 (16), 515.7 (70), 517.7 (100), 519.7 (66), 521.7 (18); HRESIMS *m*/*z* 540.7031 [M + Na]$^+$ (540.7009 calcd for $C_{12}H_6{}^{79}Br_2{}^{81}Br_2O_3Na$).

Compound 3: white solid; UV (MeOH) λ_{max} (log ϵ) 211 (4.83) nm; IR (KBr) ν_{max} 3444, 1698, 1487 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 6.53 (1H, d, J = 2.5 Hz, H-6′), 6.80 (1H, d, J = 8.5 Hz, H-3′), 6.97 (1H, dd, J = 8.5, 2.5 Hz, H-4′), 7.74 (1H, s, H-3); ^{13}C NMR, see Table 1; EIMS *m*/*z* 513.7 (16), 515.7 (70), 517.7 (100), 519.7 (66), 521.7 (18) [M]$^+$; HREIMS *m*/*z* 519.6970 (519.6988 calcd for $C_{12}H_6{}^{79}Br^{81}Br_3O_3$).

Compound 4: white solid; UV (MeOH) λ_{max} (log ϵ) 211.5 (4.90) nm; IR (KBr) ν_{max} 3368, 1540, 1477 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 3.86 (3H, s, OMe-1), 6.77 (1H, d, J = 2.0 Hz, H-6′), 7.43 (1H, d, J = 2.0 Hz, H-4′), 7.91 (1H, s, H-3); EIMS *m*/*z* 605.6 (10), 607.6 (51), 609.6 (100), 611.6 (98), 613.6 (48), 615.6 (10) [M]$^+$; HREIMS *m*/*z* 609.6274 (609.6271 calcd for $C_{13}H_7{}^{79}Br_3{}^{81}Br_2O_3$).

Methylation of 5. To a solution of **5** (1.2 mg) in MeOH (1.1 mL) was added dropwise 10% $TMSCHN_2$ in hexane. The solution was allowed to stand at room temperature (15 min) and concentrated to dryness under a stream of nitrogen to yield the methyl derivative **20**: white solid; ^{1}H NMR ($CDCl_3$) δ 3.86 (3H, s), 4.00 (3H, s), 6.97 (1H, d, J = 2.5 Hz), 7.50 (1H, d, J = 2.5 Hz).

Partial Methylation of 14. Compound **14** (3.1 mg) was treated with diluted $TMSCHN_2$ solution, and the resulting mixture was separated by HPLC (silica, hexane−CH_2Cl_2, 1:2) to give **5** (0.2 mg, 6%), **20** (0.4 mg, 12%), **21** (1.0 mg, 31%), and recovery of **14** (1.3 mg, 42%). Compound **21**: ^{1}H NMR (acetone-d_6) δ 3.84 (3H, s), 6.84 (1H, d, J = 2.0 Hz), 7.40 (1H, d, J = 2.0 Hz).

Methylation of 1, 2, 3, and 6. Each of these samples was similarly treated with $TMSCHN_2$ as for **5** to give compounds **15**, **16**, **19**, and **22**, respectively. Compound **15**: ^{1}H NMR ($CDCl_3$) δ 3.82 (3H, s), 3.99 (3H, s), 6.50 (1H, d, J = 2.5 Hz), 7.40 (1H, d, J = 2.5 Hz), 7.76 (1H, s). Compound **16**: ^{1}H NMR (acetone-d_6) δ 3.82 (3H, s), 4.01 (3H, s), 6.69 (1H, d, J = 2.0 Hz), 7.49 (1H, d, J = 2.0 Hz), 7.50 (1H, d, J = 9.0 Hz), 7.56 (1H, d, J = 9.0 Hz). Compound **19**: ^{1}H NMR (acetone-d_6) δ 3.82 (3H, s), 3.92 (3H, s), 6.68 (1H, d, J = 2.5 Hz), 6.96 (1H, d, J = 8.5 Hz), 7.09 (1H, dd, J = 8.5, 2.5 Hz,), 7.94 (1H, s). Compound **22**: ^{1}H NMR (acetone-d_6) δ 3.81 (3H, s), 3.93 (3H, s), 6.46 (1H, d, J = 2.5 Hz), 6.85 (1H, d, J = 8.5 Hz), 6.98 (1H, d, J = 8.5, 2.5 Hz), 7.07 (1H, d, J = 8.5 Hz), 7.40 (1H, d, J = 8.5 Hz).

Treatment of 14 with HBr and Na_2SO_3 in MeOH.[25] Hydrobromic acid (47%, 3.0 mL) was added to a stirred solution of **14** (50.1 mg) and sodium sulfite (93 mg, 10 equiv) in MeOH (10 mL). After the solution was stirred under reflux for 1 h, it was then basified with aqueous KOH to pH 10−11. The resulting mixture was extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated to give a residue. The residue was purified by HPLC (RP18, MeOH−H_2O, 10:1) to give **23** (19.9 mg, 40%) and **14** (1.2 mg, 2%). Compound **23**: ^{1}H NMR (CD_3OD) δ 7.28 (1H, d, J = 2.0 Hz), 6.94 (1H, d, J = 2.0 Hz); ^{13}C NMR (CD_3OD) δ 159.2, 148.8, 147.6, 145.0, 130.1, 124.9, 120.7, 120.4, 119.5, 113.1, 110.5, 108.4; EIMS *m*/*z* 651.5 (6), 653.5 (23), 655.5 (100), 657.5 (87), 659.5 (63), 661.5 (32), 663.5 (4) [M]$^+$.

Debromination of 14 in AcOH. Hydrobromic acid (47%, 2.5 mL) was added to a stirred solution of **14** (51 mg) and sodium sulfite (95 mg, 10 equiv) in AcOH (16 mL). The mixture was stirred under reflux for 1 h, neutralized with aqueous KOH, and partitioned between EtOAc and water. The organic layer was taken, and the product was purified by HPLC (RP18, MeOH) to afford **13** (4.5 mg, 10%), **23** (12.5 mg, 25%), and **24** (0.6 mg, 1%). Compound **13**: ^{1}H NMR (acetone-d_6) δ 6.73 (1H, d, J = 2.0 Hz), 7.39 (1H, d, J = 2.0 Hz), 7.62 (1H, s); ^{13}C NMR (acetone-d_6) δ 151.0, 146.5, 144.8, 139.4, 129.7, 127.8, 123.4, 116.9, 116.5, 114.9, 111.5, 111.1; HMBC H-4/C-3,5,6, H-4′/2′,3′,5′,6′, H-6′/C-1′,2′,4′,5′; EIMS *m*/*z* 591.6 (9), 593.6 (49), 595.6 (99), 597.6 (100), 599.6 (51), 601.6 (12) [M]$^+$. Compound **24**:[12,15] ^{1}H NMR (acetone-d_6) δ 6.65 (1H, d, J = 2.5 Hz), 7.24 (1H, d, J = 2.5 Hz), 7.38 (1H, s).

Similar treatment of **14** (55 mg) with the above procedure using a larger amount of sodium sulfite (15 equiv) and extended reflux (6 h) resulted in the formation of **13** (10.5 mg, 21%), **25** (0.1 mg, 0.2%), and **26** (1.0 mg, 2%) and recovery of unreacted **14** (1.2 mg, 2%). Compound **25**: ^{1}H NMR (acetone-d_6) δ 6.45 (1H, t, J = 8.0 Hz), 6.74 (1H, s), 7.18 (1H, dd, J = 8.0, 2.0 Hz), 7.66 (1H, dd, J = 8.0, 2.0 Hz); EIMS m/z 513.7 (18), 515.7 (64), 517.7 (100), 519.7 (68), 521.7 (16) $[M]^+$. **26**: ^{1}H NMR (acetone-d_6) δ 6.64 (1H, d, J = 2.5 Hz), 7.24 (1H, d, J = 2.5 Hz), 7.37 (1H, d, J = 2.5 Hz), 7.38 (1H, d, J = 2.5 Hz); EIMS m/z 513.7 (17), 515.7 (69), 517.7 (100), 519.7 (66), 521.7 (16) $[M]^+$.

Further treatment of **14** (60 mg) using the above procedure with larger amounts of hydrobromic acid (10 mL) and sodium sulfite (20 equiv) gave compounds **27** (2.4 mg, 6%), **28** (0.2 mg, 0.6%), and **29** (2.2 mg, 6%). Compound **27**: ^{1}H NMR (acetone-d_6) δ 6.67 (1H, d, J = 2.0 Hz), 6.75 (1H, d, J = 8.5 Hz), 6.93 (1H, dd, J = 2.0, 8.5 Hz), 7.03 (1H, d, J = 2.0 Hz), 7.12 (1H, d, J = 2.0 Hz); EIMS m/z 435.8 (34), 437.8 (100), 439.8 (100), 441.8 (34) $[M]^+$. **28**: ^{1}H NMR (CD_3-OD) δ 6.48 (1H, dd, J = 8.0, 2.0 Hz), 6.62 (1H, td, J = 8.0, 2.0 Hz), 6.87 (2H, m), 7.05 (1H, d, J = 2.5 Hz), 7.22 (1H, d, J = 2.5 Hz); EIMS m/z 357.8 (51), 359.8 (100), 361.8 (51) $[M]^+$. **29**: ^{1}H NMR (CD_3OD) δ 6.74 (1H, d, J = 8.5 Hz), 6.79 (1H, d, J = 2.0 Hz), 6.80 (1H, d, J = 2.0 Hz), 6.97 (1H, dd, J = 2.0, 8.5 Hz), 7.07 (1H, d, J = 2.0 Hz); EIMS m/z 435.8 (31), 437.8 (100), 439.8 (98), 441.8 (36) $[M]^+$.

Cytotoxicity Assay. Compounds **2**, **3**, **5**, **6**, **13**, **14**, and **23**−**29** were evaluated for their cytotoxicity against NBT-T2 rat bladder epithelial cells as described previously.[26] IC_{50} values were obtained by using the MTT method.

Agar-Plate Diffusion Assay. Paper disks were impregnated with isolated compounds ranging from 0.1 to 10 μg/disk and placed on agar plates inoculated with *B. subtilis*. The plates were checked for inhibition zones after incubation at 37 °C for 24 h. Prior to and after the testing, all materials were sterilized at 121 °C for 20 min. Acetone was used to dissolve the compounds.

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