Caelestines A−D, Brominated Quinolinecarboxylic Acids from the Australian Ascidian *Aplidium caelestis*

Sheng Yin,[†] Glen M. Boyle,[‡] Anthony R. Carroll,[†] Michael Kotiw,[§] John Dearnaley,[§] Ronald J. Quinn,[†] and Rohan A. Davis*[,†]

Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia, Queensland Institute for Medical Research, Herston, QLD 4006, Australia, and Department of Biological and Physical Sciences, University of Southern Queensland, Toowoomba, QLD 4351, Australia

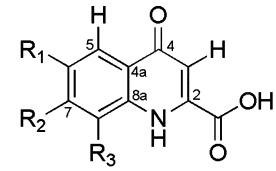
Received May 17, 2010

Four new brominated natural products, caelestines A−D (**1**−**4**), have been isolated from the Australian ascidian *Aplidium caelestis*. The structures of **1**−**4** were determined by analysis of their NMR and MS data. This is the first report of brominated quinolinecarboxylic acids from nature. Compound **1** has been previously synthesized but not spectroscopically characterized. Compounds **1**−**4** were tested against three mammalian cell lines (MCF-7, NFF, and MM96L) and a panel of microbial strains and showed only minor cytotoxicity.

2-Quinolinecarboxylic acid derivatives are relatively uncommon in nature. To date, about 20 simple quinolinecarboxylic acids have been isolated from insects,[1−4] bacteria,[5−7] and plants.[8−10] Xanthurenic acid, or 4,8-dihydroxy-2-quinolinecarboxylic acid, a metabolite of tryptophan, is present in the urine of vitamin B_6-deficient rats.[11] The 8-methoxy derivative of xanthurenic acid is a possible endogenous carcinogen.[12] Only seven quinolinecarboxylic acid derivatives have been reported from marine organisms. These include 4,5,8-trihydroxyquinolinecarboxylic acid from the Antarctic sponge *Dendrilla membranosa*,[13] xanthurenic acid and its derivatives, tridemnic acids A and B, which were isolated from the Canadian ascidian *Tridemnum* sp.,[14] distomadine B, which was isolated from the New Zealand ascidian *Pseudodistoma aureum*,[15] and perspicamides A and B, which were purified from the Australian ascidian *Botrylloides perspicuum*.[16] As part of our continuing efforts to discover new chemistry from Australian ascidians,[16−19] we undertook a detailed chemical analysis of the previously uninvestigated ascidian *Aplidium caelestis*. These studies resulted in the isolation of four new brominated natural products, caelestines A−D (**1**−**4**). The structures of **1**−**4** were determined by analysis of the NMR and MS data. Compound **1** has been previously synthesized but not spectroscopically characterized. This is the first report of brominated quinolinecarboxylic acids from nature. Caelestines A−D (**1**−**4**) were tested against three mammalian cell lines (MCF-7, NFF, and MM96L) and a panel of microbial strains and showed only minor cytotoxicity. In this paper we report the isolation, structure elucidation, and bioactivity of compounds **1**−**4**.

The freeze-dried and ground ascidian *A. caelestis* was exhaustively extracted with *n*-hexanes, $CHCl_3$/MeOH (4:1), and MeOH. The $CHCl_3$/MeOH extracts were all combined and chromatographed using reversed-phase C_{18} HPLC (MeOH/H_2O/0.1% TFA) to yield caelestines A−D (**1**−**4**).

Caelestine A (**1**) was isolated as a pale gum. The (+)-LRESIMS spectrum showed an isotopic cluster of $[M + H]^+$ ions in the ratio 1:1 at *m*/*z* 268/270, indicating the presence of one bromine atom. The $[M + H]^+$ ion in the (+)-HRESIMS spectrum at *m*/*z* 267.9597 allowed the molecular formula $C_{10}H_6BrNO_3$ to be assigned to **1**. The ^{1}H NMR data (Table 1) of **1** showed signals for two exchangeable protons [δ_H 11.98 (brs) and 11.14 (s)], a 1,2,4-trisubstituted benzene ring [δ_H 7.98 (d, J = 8.6 Hz), 7.50 (dd, J = 8.6, 1.8 Hz), and 8.17 (d, J = 1.8 Hz)], and an olefinic singlet (δ_H 6.63). The ^{13}C NMR spectrum together with the HSQC experiment



1 R_1 = H R_2 = Br R_3 = H
2 R_1 = Br R_2 = Br R_3 = H
3 R_1 = Br R_2 = H R_3 = OMe
4 R_1 = Br R_2 = Br R_3 = OMe

revealed 10 carbon resonances, which included two carbonyl signals (δ_C 177.2 and 163.4), four sp^2 methines (δ_C 110.5, 127.0, 127.0, and 121.8), and four quaternary sp^2 carbons (δ_C 139.5, 126.0, 141.1, and 124.5). The aforementioned data were similar to those of 4-oxo-1,4-dihydroquinoline-2-carboxylic acid[10] except that one of the aromatic protons in 4-oxo-1,4-dihydroquinoline-2-carboxylic acid was replaced with a bromine atom in **1**. This was supported by the ABX substitution pattern of the benzene ring and the presence of the bromine atom in the molecular formula of **1**. Detailed analysis of the 2D NMR data (HSQC and HMBC) of **1** allowed the bromine atom to be positioned at C-7. In particular the intensity of the HMBC correlations assisted in the unambiguous assignment of the brominated benzenoid system (see Supporting Information). Strong $^3J_{CH}$ HMBC correlations from the aromatic proton at δ_H 7.98 to C-4 (δ_C 177.2), C-7 (δ_C 126.0), and C-8a (δ_C 141.1) placed this proton at C-5, while the proton at δ_H 8.17 was positioned at C-8 due to HMBC correlations between H-8 and C-4 ($^4J_{CH}$, weak correlation), C-6 ($^3J_{CH}$), and C-4a ($^3J_{CH}$) (Figure 1). The proton at δ_H 7.50 and bromine atom were placed at C-6 and C-7, respectively, on the basis of the ABX substitution pattern of the benzene ring. Strong HMBC correlations ($^3J_{CH}$) from both H-3 and H-6 to C-4a further supported this assignment. Thus caelestine A (**1**) was assigned as 7-bromo-4-oxo-1,4-dihydroquinoline-2-carboxylic acid. Compound **1** has previously been synthesized and shown to be an antagonist toward the *N*-methyl-D-aspartate (NMDA) receptor.[20] No spectroscopic data have been previously reported for **1**.

Caelestine B (**2**) was isolated as a pale gum. The (+)-LRESIMS spectrum showed a cluster of pseudomolecular ions in the ratio 1:2:1 at *m*/*z* 346/348/350, indicating the presence of two bromine atoms. The molecular formula of $C_{10}H_5Br_2NO_3$ was determined by (−)-HRESIMS data at *m*/*z* 343.8547 $[M - H]^-$, which showed it had one more bromine atom than **1**. The ^{1}H NMR spectrum of **2** was similar to **1** except for the replacement of the ABX benzene ring system in **1** by two aromatic singlets in **2**. This indicated that the additional bromine atom in **2** was also substituted on the benzene ring. 2D NMR experiments (HMBC, HSQC) were employed to

* To whom correspondence should be addressed. Tel: +61-7-3735-6043. Fax: +61-7-3735-6001. E-mail: r.davis@griffith.edu.au.
[†] Eskitis Institute.
[‡] Queensland Institute for Medical Research.
[§] University of Southern Queensland.

10.1021/np100329w
Published on Web 08/12/2010

Table 1. ^{1}H NMR (600 MHz) Data for Caelestines A−D (**1**−**4**)

	1	**2**	**3**		**4**		
no.	δ_H[a]	δ_H[a]	δ_H[a]	δ_H[b]	δ_H[a]	δ_H[b]	δ_H[c]
3	6.63, s	6.66, s	6.71, brs	6.97, s	N.O.	6.95, s	6.65, s
5	7.98, d (8.6)	8.27, s	7.74, d (1.6)	7.92, s	8.15, s	8.30, s	8.11, s
6	7.50, dd (8.6, 1.8)						
7			7.47, d (1.6)	7.44, s			
8	8.17, d (1.8)	8.35, s					
8-OMe			4.04, s	4.10, s	4.04, s	4.08, s	3.98, s
NH	11.14, s	11.14, s	11.13, s		11.13, s		
COOH	11.98, brs	12.10, brs	N.O.[d]		N.O.		

[a] In DMSO-d_6. [b] In CD_3OD. [c] In DMSO-d_6/H_2O (17:1). [d] N.O. = not observed.

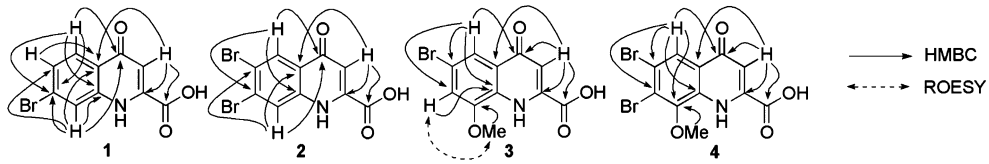


Figure 1. Selected HMBC and ROESY correlations for caelestines A−D (**1**−**4**).

assign the substituents of the benzene ring. The HMBC correlations from one of the aromatic protons at δ_H 8.27 to the carbons at δ_C 176.0 (C-4), 139.6 (C-8a), and 128.1 (C-7) placed this proton at C-5. HMBC correlations from the other aromatic proton at δ_H 8.35 to C-4 ($^4J_{CH}$, weak correlation), C-6, and C-4a located this proton at C-8. Thus only two positions (C-6 and C-7) were left on the benzene ring to place two bromine atoms. The *para* relationship of the two aromatic protons on the benzene ring was further supported by their sharp singlet signals in ^{1}H NMR spectrum, as either an *ortho* or *meta* relationship of these two protons should result in an observable ^{1}H−^{1}H coupling. Thus the structure of caelestine B (**2**) was assigned as 6,7-dibromo-4-oxo-1,4-dihydroquinoline-2-carboxylic acid.

Caelestine C (**3**) was isolated as a pale gum. The (+)-LRESIMS spectrum showed 1:1 ion cluster peaks at *m*/*z* 298/300 $[M + H]^+$ and 320/322 $[M + Na]^+$, indicating the presence of one bromine atom. The molecular formula of $C_{11}H_8BrNO_4$ was determined by (+)-HRESIMS data at *m*/*z* 297.9702 $[M + H]^+$. The ^{1}H NMR spectrum of **3** recorded in DMSO-d_6 (Table 1) showed signals for one exchangeable proton [δ_H 11.13 (s)], two aromatic protons [δ_H 7.74 (d, J = 1.6 Hz) and 7.47 (d, J = 1.6 Hz)], a methoxy group (δ_H 4.04), and a weak and broad olefinic signal at δ_H 6.71. The aforementioned information implied **3** was an analogue of compounds **1** and **2**. Unfortunately, 2D NMR correlations (HSQC and HMBC) arising from the olefinic signal at δ_H 6.71 were very weak in DMSO-d_6, and hence the structure of **3** could not be initially confirmed. This broad signal was possibly due to tautomerization between the 4-oxo-1,4-dihydroquinoline and the 4-hydroxyquinoline moieties, which has been reported for related compounds in various solvents.[13,19] In order to investigate whether a change of deuterated solvents could improve the shape of the H-3 signal, the ^{1}H NMR spectrum of **3** was recorded in CD_3OD. This resulted in all the nonexchangeable protons appearing as sharp signals, suggesting that **3** was present as one preferred tautomeric form in CD_3OD. In a similar manner to caelestines A and B, the HMBC data were critical for the structure assignment of **3**. In CD_3OD the proton at δ_H 6.97 (H-3) showed HMBC correlations to C-2 (δ_C 141.4), C-4 (δ_C 179.7), C-4a (δ_C 128.0), and −*C*OOH (δ_C 164.4), indicating that **3** shared the same ring system as compounds **1** and **2** (Figure 1). The strong $^3J_{CH}$ HMBC correlation from the aromatic proton at δ_H 7.92 to C-4, C-7, and C-8a assigned this proton as H-5. The *meta* coupling constant (J = 1.6 Hz, in DMSO-d_6) between H-5 and the other aromatic proton at δ_H 7.47 required the latter to be placed at C-7. The methoxy group was located at C-8, as the only ROESY correlation observed in **3** was between H-7 and 8-OMe. The existence of the 8-OMe was further supported by the upfield-shifted carbon signals of C-5 ($\Delta\ \delta_C$ >7 ppm) and C-8a ($\Delta\ \delta_C$ >8 ppm) compared with those in compounds **1** and **2**, as the insertion of methoxy group has shielding effects at the *ortho* and *para* positions of a benzene system.[21] Subsequently, a bromine atom was placed at C-6. Hence the structure of caelestine C (**3**) was assigned as 6-bromo-8-methoxy-4-oxo-1,4-dihydroquinoline-2-carboxylic acid.

Caelestine D (**4**) was assigned a molecular formula of $C_{11}H_7Br_2NO_4$ on the basis of (−)-HRESIMS data, which had one more bromine atom than **3**. The ^{1}H NMR spectrum of **4** recorded in DMSO-d_6 exhibited only three singlets, which were assigned to an exchangeable proton (δ_H 11.13), an aromatic proton (δ_H 8.15), and a methoxy group (δ_H 4.04). The absence of the olefinic signal (H-3) in DMSO-d_6 implied that **4** also underwent tautomerization. We postulated that **4** might be present as one preferred tautomeric form in a polar protic solvent as shown for **3** in CD_3OD. Unfortunately, **4** was only partially soluble in CD_3OD, which prevented good-quality 2D NMR data from being obtained. Hence one drop of H_2O was added to the DMSO-d_6 solution, which resulted in the appearance of the H-3 signal at δ_H 6.65 in the ^{1}H NMR spectrum. The aforementioned information implied that **4** was an analogue of **3**, with the only structural difference occurring on the benzene ring. Analysis of 2D NMR data recorded in wet DMSO-d_6 allowed the structure of **4** to be determined. The aromatic proton at δ_H 8.11 was assigned as H-5 on the basis of a strong HMBC correlation from this proton to a conjugated ketone at δ_C 176.4 (C-4). As no ROESY correlations were observed between H-5 and the methoxy group, and H-5 had a strong HMBC correlation with the carbon at δ_C 118.9 (C-7) and a weak correlation with the carbon at δ_C 148.8 (C-8), the methoxy group was placed at C-8. This assignment was further confirmed by comparison of the carbon chemical shifts of **4** with **2**. The chemical shifts of C-5, C-7, and C-8a in **4** were shifted upfield by 4.9, 9.2, and 5.8 ppm, respectively, compared to those in **2**, since the presence of a methoxy group at C-8 had shielding effects at the *ortho* and *para* positions.[21] Subsequently, two bromine atoms were placed at C-6 and C-7. Thus the structure of caelestine D (**4**) was assigned as 6,7-dibromo-8-methoxy-4-oxo-1,4-dihydroquinoline-2-carboxylic acid.

Table 3 shows the *in vitro* activity of compounds **1**−**4** against three mammalian cell types. MCF-7 is derived from a breast adenocarcinoma cancer, MM96L is an amelanotic metastatic melanoma cell line, and NFF (neonatal foreskin fibroblasts) is a "normal" primary cell type. The NFF cells are routinely used in toxicity studies to determine compound selectivity for cancer cells. All compounds (**1**−**4**) showed only minor activity at 100 μM. Compounds **1**−**4** were also tested against a panel of ATCC and NCTC type stains of microbes known to be associated with

Table 2. ^{13}C NMR (125 MHz) Data for Caelestines A−D (**1**−**4**)

no.	**1**[a]	**2**[a]	**3**[b]	**4**[c]
2	139.5, C	140.0, C	141.4,[d] C	145.8,[d] C
3	110.5, CH	110.5, CH	111.4, CH	108.6, CH
4	177.2, C	176.0, C	179.7,[d] C	176.4,[d] C
4a	124.5, C	125.9, C	128.0, C	125.9, C
5	127.0, CH	129.3, CH	119.9, CH	124.4, CH
6	127.0, CH	118.9, C	119.3, C	121.9, C
7	126.0, C	128.1, C	116.5, CH	118.9, C
8	121.8, CH	124.6, CH	151.3, C	148.8,[d] C
8a	141.1, C	139.6, C	131.1, C	133.8,[d] C
−*C*OOH	163.4, C	163.2, C	164.4, C	162.8,[d] C
8-OMe			57.7, CH_3	61.7, CH_3

[a] In DMSO-d_6. [b] In CD_3OD. [c] In DMSO-d_6/H_2O (17:1). [d] Signals were assigned from 2D NMR data.

Table 3. *In Vitro* Cytotoxicity of Caelestines A−D (**1**−**4**)

	mammalian cells [% inhibition at 100 μM (±SD)]		
compound	MCF-7	MM96L	NFF
1	39 (±3)	62 (±11)	57 (±5)
2	49 (±8)	69 (±6)	66 (±2)
3	40 (±5)	54 (±3)	58 (±2)
4	38 (±5)	52 (±6)	68 (±13)

nosocomial infections that included multidrug-resistant *Staphylococcus aureus* (ATCC 43300), methicillin-sensitive *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 19433), *Pseudomonas aeruginosa* (NCTC 10662), and *Candida albicans* (ATCC 10231). At concentrations ranging from 0.1 μM to 10 mM no microbial growth inhibition was observed after 20 h of incubation.

Experimental Section

General Experimental Procedures. UV and IR spectra were recorded on a Jasco V-650 UV/vis spectrophotometer and a Bruker Tensor 27 spectrometer, respectively. NMR spectra were recorded at 30 °C on either a Varian 500 MHz or a 600 MHz Unity INOVA spectrometer. The latter spectrometer was equipped with a triple resonance cold probe. The ^1H and ^{13}C NMR chemical shifts were referenced to the solvent peak for DMSO-d_6 at δ_H 2.49 and δ_C 39.5 and for CD_3OD at δ_H 3.31 and δ_C 49.0. LRESIMS spectra were recorded on a Waters ZQ mass spectrometer. HRESIMS data were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer. A Bioline orbital shaker was used for the large-scale extraction of ascidian material. Alltech Davisil 40−60 μm 60 Å C_{18} bonded silica was used for preadsorption work. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC. A Phenomenex Luna 5 μm C_{18} column (21.2 × 250 mm) was used for semipreparative HPLC separation. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade, and the H_2O was Millipore Milli-Q PF filtered.

Animal Material. The ascidian *Aplidium caelestis* (Monniot 1987) was collected by scuba diving at a depth of 12.9 m from Manta Ray Bommie, North Stradbroke Island, Queensland, Australia, in February 2005. A voucher sample, QMG322279, was lodged at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. The freeze-dried and ground ascidian (20 g) was transferred to a conical flask (1 L), *n*-hexane (250 mL) was added, and the flask was shaken at 200 rpm for 2 h. The *n*-hexane extract was filtered under gravity, then discarded. A $CHCl_3$/MeOH mixture (4:1, 250 mL) was added to the defatted marine material in the conical flask, which was then shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity and set aside. MeOH (250 mL) was added, and the MeOH/ascidian mixture was shaken for a further 2 h at 200 rpm. Following gravity filtration the marine material was extracted with another volume of MeOH (250 mL), while being shaken at 200 rpm for 16 h. All $CHCl_3$/MeOH extracts were combined and dried under reduced pressure to yield a dark brown solid (1.2 g). This solid was redissolved into a $CHCl_3$/MeOH mixture (4:1, 50 mL), then filtered under gravity to remove the salt. The resulting solution was dried to give a desalted residue (0.45 g). This residue was preadsorbed on C_{18}-bonded silica, then packed into a stainless steel cartridge (10 × 30 mm) that was subsequently attached to a C_{18} Luna HPLC column. Isocratic HPLC conditions of 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for the first 10 min; then a linear gradient to MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from time = 0 min. Following lyophilization fractions 29, 31, 33, and 35 yielded pure **1** (1.5 mg, 0.0075% dry wt), **3** (2.1 mg, 0.0105% dry wt), **2** (1.1 mg, 0.0055% dry wt), and **4** (2.3 mg, 0.0115% dry wt), respectively.

Caelestine A (1): pale gum; UV (MeOH) λ_{max} (log ε) 356 (3.21), 340 (3.32), 251 (3.63), 223 (3.87) nm; IR ν_{max} (KBr) 1696 (br), 1623, 1589, 1561, 1509, 1456, 1402, 1295 (br), 1243, 1205, 1141, 1065, 826 cm^{-1}; ^1H (600 MHz) and ^{13}C (125 MHz) NMR, see Tables 1 and 2, respectively; (+)-LRESIMS *m*/*z* (35 eV) (rel int) 268 $[C_{10}H_6{}^{79}BrNO_3 + H]^+$ (92), 270 $[C_{10}H_6{}^{81}BrNO_3 + H]^+$ (100); (+)-HRESIMS *m*/*z* 267.9597 (calcd for $C_{10}H_7{}^{79}BrNO_3$, 267.9604).

Caelestine B (2): pale gum; UV (MeOH) λ_{max} (log ε) 350 (3.53), 263 (4.00), 224 (4.12) nm; IR ν_{max} (KBr) 1721 (br), 1628, 1587, 1494, 1439, 1403, 1205, 1137, 801 cm^{-1}; ^1H (600 MHz) and ^{13}C (125 MHz) NMR, see Tables 1 and 2, respectively; (+)-LRESIMS *m*/*z* (35 eV) (rel int) 346 $[C_{10}H_5{}^{79}Br_2NO_3 + H]^+$ (42), 348 $[C_{10}H_5{}^{79}Br^{81}BrNO_3 + H]^+$ (100), 350 $[C_{10}H_5{}^{81}Br_2NO_3 + H]^+$ (42); (−)-HRESIMS *m*/*z* 343.8547 (calcd for $C_{10}H_4{}^{79}Br_2NO_3$, 343.8563).

Caelestine C (3): pale gum; UV (MeOH) λ_{max} (log ε) 347 (3.25), 244 (3.80), 227 (3.77) nm; IR ν_{max} (KBr) 1679 (br), 1514, 1439, 1203, 1138, 1064, 801 cm^{-1}; ^1H (600 MHz) and ^{13}C (125 MHz) NMR, see Tables 1 and 2, respectively; (+)-LRESIMS *m*/*z* (35 eV) (rel int) 298 $[C_{11}H_8{}^{79}BrNO_4 + H]^+$ (95), 300 $[C_{11}H_8{}^{81}BrNO_4 + H]^+$ (100), 320 $[C_{11}H_8{}^{79}BrNO_4 + Na]^+$ (35), 322 $[C_{11}H_8{}^{81}BrNO_4 + Na]^+$ (38); (+)-HRESIMS *m*/*z* 297.9702 (calcd for $C_{11}H_9{}^{79}BrNO_4$, 297.9709).

Caelestine D (4): pale gum; UV (MeOH) λ_{max} (log ε) 355 (3.48), 340 (3.47), 255 (3.93), 225 (4.01) nm; IR ν_{max} (KBr) 1623, 1582, 1549, 1503, 1452, 1417, 1392, 1196, 1039, 932, 805 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR, see Tables 1 and 2, respectively; (+)-LRESIMS *m*/*z* (35 eV) (rel int) 376 $[C_{11}H_7{}^{79}Br_2NO_4 + H]^+$ (45), 378 $[C_{11}H_7{}^{79}Br^{81}BrNO_4 + H]^+$ (100); 380 $[C_{11}H_7{}^{81}Br_2NO_4 + H]^+$ (48); (−)-HRESIMS *m*/*z* 373.8662 (calcd for $C_{11}H_6{}^{79}Br_2NO_4$, 373.8669).

Mammalian Cell Assays. The experimental details of the mammalian cell assays have been described in a previous publication.[22] Doxorubicin was used as a positive control for all assays and gave 99% inhibition of MCF-7 and MM96L and 95% inhibition of NFF at 1 μM.

Antimicrobial Assays. Antimicrobial activities were evaluated using the CLSI broth microdilution assay[23] with each compound screened in a decimal dilution series commencing from 0.1 μM to 10 mM.

Acknowledgment. We thank H. T. Vu from Griffith University for acquiring the HRESIMS measurements. We would also like to thank J. N. A. Hooper from the Queensland Museum for ascidian collection and identification. M. Boddington and R. Graham from the University of Southern Queensland are acknowledged for technical assistance with the antimicrobial assays.

Supporting Information Available: NMR data for caelestines A−D (**1**−**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bolognese, A.; Liberatore, R.; Riente, G.; Scherillo, G. *J. Heterocycl. Chem.* **1988**, *25*, 1247–1250.
(2) Schildknecht, H. *Z. Naturforsch., B* **1969**, *24*, 38–47.
(3) Wang, S. F.; Braekman, J. C.; Daloze, D.; Pasteels, J.; Soetens, P.; Handjieva, N. V.; Kalushkov, P. *Experientia* **1996**, *52*, 628–630.
(4) Gonzalez, A.; Schroeder, F.; Meinwald, J.; Eisner, T. *J. Nat. Prod.* **1999**, *62*, 378–380.
(5) Neuenhaus, W.; Budzikiewicz, H.; Korth, H.; Pulverer, G. *Z. Naturforsch. B: Chem. Sci.* **1980**, *35*, 1569–1571.
(6) Breidingmack, S.; Zeeck, A. *J. Antibiot.* **1987**, *40*, 953–960.
(7) Nagata, H.; Ochiai, K.; Aotani, Y.; Ando, K.; Yoshida, M.; Takahashi, I.; Tamaoki, T. *J. Antibiot.* **1997**, *50*, 537–542.
(8) Starratt, A. N.; Caveney, S. *Phytochemistry* **1996**, *42*, 1477–1478.
(9) Mitaku, S.; Skaltsounis, A. L.; Tillequin, F.; Koch, M.; Pusset, J.; Sevenet, T. *Nat. Prod. Res.* **1995**, *7*, 219–225.

(10) Al-Khalil, S.; Alkofahi, A.; El-Eisawi, D.; Al-Shibib, A. *J. Nat. Prod.* **1998**, *61*, 262–263.
(11) Lepkoovsky, S. R. E.; Haagen-Smit, A. J. *J. Biol. Chem.* **1943**, *149*, 195–201.
(12) Lewis, R. J., Sr. In *Sax's Dangerous Properties of Industrial Materials*, 10th ed.; John Wiley and Sons: New York, 2000; Vols. 1−3.
(13) Molinski, T. F.; Faulkner, D. J. *Tetrahedron Lett.* **1988**, *29*, 2137–2138.
(14) Desilva, E. D.; Miao, S. C.; Andersen, R. J.; Schultz, L. W.; Clardy, J. *Tetrahedron Lett.* **1992**, *33*, 2917–2920.
(15) Pearce, A. N.; Appleton, D. R.; Babcock, R. C.; Copp, B. R. *Tetrahedron Lett.* **2003**, *44*, 3897–3899.
(16) McKay, M. J.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **2005**, *68*, 1776–1778.
(17) Davis, R. A.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **2002**, *65*, 454–457.
(18) Davis, R. A.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 1405–1409.
(19) Yin, S.; Cullinane, C.; Carroll, A. R.; Quinn, R. J.; Davis, R. A. *Tetrahedron Lett.* **2010**, *51*, 3403–3405.
(20) Leeson, P. D.; Baker, R.; Carling, R. W.; Curtis, N. R.; Moore, K. W.; Williams, B. J.; Foster, A. C.; Donald, A. E.; Kemp, J. A.; Marshall, G. R. *J. Med. Chem.* **1991**, *34*, 1243–1252.
(21) Pretsch, E.; Bühlmann, P.; Affolter, C. In *Structure Determination of Organic Compounds*; Springer-Verlag: Berlin, 2000; p 97.
(22) Davis, R. A.; Carroll, A. R.; Andrews, K. T.; Boyle, G. M.; Tran, T. L.; Healy, P. C.; Kalaitzis, J. A.; Shivas, R. G. *Org. Biomol. Chem.* **2010**, *8*, 1785–1790.
(23) Wikler, M. A.; Low, D. E.; Cockerill, F. R.; Sheehan, D. J.; Craig, W. A.; Tenover, F. C.; Dudley, M. N. In *Methods for Dilution. Antimicrobial susceptibility tests for bacteria that grow aerobically*; Approved standard, 7th ed.; CLSI (formerly NCCLS), **2006**; Vol. 26, pp M7−A7.

NP100329W