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# Synthesis and Biological Evaluation of Purpurealidin E-Derived Marine Sponge Metabolites: Aplysamine-2, Aplyzanzine A, and Suberedamines A and B

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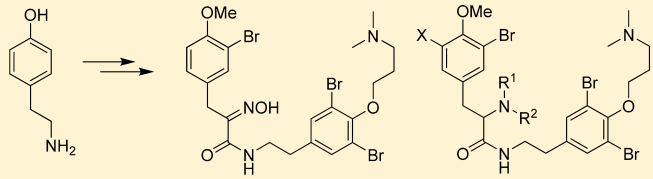
Supporting Information

**ABSTRACT:** Five purpurealidin-derived marine secondary sponge metabolies have been synthesized through the carbodiimide coupling of an appropriate bromotyrosine unit. The structure elucidations have been confirmed through direct comparison with spectroscopic data of isolated natural products. Aplyzanzine A has been shown to be the most active product against a broad bacterial and fungal screen, demonstrating MIC values 2 to 4 times lower than the other metabolites in this study. Compounds **2**, **3**, **4a**, and **5**−**7** exhibit a modest inhibition against slow growing mycobacteria (MIC 25−50 μg/mL), including *Mycobacterium tuberculosis*. *iso*-Anomoian A and suberedamine B showed antitumor activity in the NCI-DTP60 cell line screen at single-digit micromolar concentrations, with *iso*-anomoian A inhibiting 53 cell lines. These molecules present novel scaffolds for further optimization.




Bromotyrosine-derived marine natural products from the order Verongida show unique and diverse biological activities that include antimicrobial, antiviral, antiangiogenic, antifouling, and enzyme-modulatory properties.[1] These structurally diverse secondary metabolites, which accumulate in concentrations of up to 10% sponge dry weight, can act in rapid activated chemical defense mechanisms against a large number of organisms in aqueous environments.[2] Due to substantial and immediate dilution effects once outside the host organism, such compounds display exceptional potency toward predators. These secondary metabolites also display low toxicity toward the host.

One well-researched metabolite, psammaplin A[3] (**1**), and related analogues have been reported to inhibit the detoxification enzyme mycothiol-*S*-conjugate amidase from *Mycobacterium tuberculosis*.[4] This mechanism of inhibition is assumed to involve chelation of zinc in the active site via the oxime moiety.[5] Zinc chelation has also been suggested to be responsible for the cytotoxic activity of **1** through inhibition of histone deacetylase,[6] DNA methyl transferase,[7] and topoisomerase II[8] and/or via peroxisome proliferator-activated receptor gamma[8] and Wnt signal activation.[9]

With the decline in the number of novel antibiotic scaffolds[10] and the ongoing search for more effective anticancer drugs, these brominated metabolites are attractive candidates for further biological evaluation and total synthesis.

Following previous endeavors to synthesize a variety of marine sponge secondary metabolites, purpurealidin E (**2**) was originally isolated (*Psammaplysilla purpurea*, Mandapam Coast), characterized as the *N*-acetyl derivative,[11] and initially synthesized to demonstrate the versatility of acylation using an internally protected coumarin.[12]

Purpurealidin (**2**) was recently synthesized from tyramine, in 39% overall yield after five steps; however, this compound was characterized as the hydrochloride salt of the *N*-acetyl derivative, and no biological evaluation was reported.[13] Other related bromotryosine analogues of **2** have been isolated in milligram quantities and include aplysamine-2 (**3**)[14a] (*Aplysina* sp., New South Wales and *Psammaplysilla purpurea*, Mandapam Coast), anomoian A (**4b**)[14b] (*Anomoianthella popeae*, Great Barrier Reef), aplyzanzine A (**5**)[14c] (*Aplysina* sp., Zanzibar), and suberedamines A (**6**) and B (**7**)[14d] (*Psammaplysilla purea*, Okinawa).

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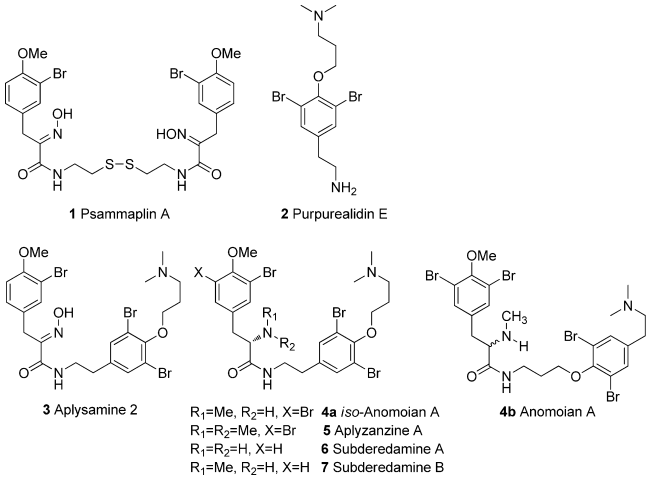


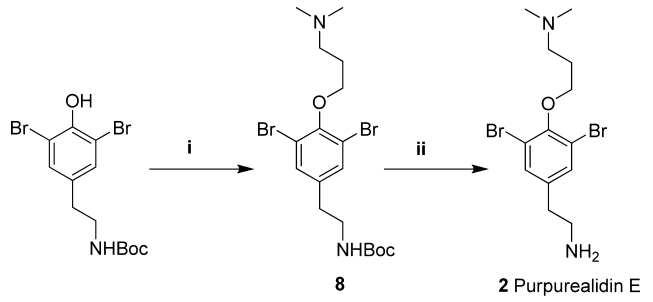

The isolation of purpurealidin E-derived secondary metabolites from marine sponges for further biological evaluation is ecologically destructive (yields below 0.1% dry sponge weight), labor intensive, and costly. Therefore, in order to solve these issues, this paper describes the syntheses of purpurealidin E-derived metabolites and their subsequent biological evaluation. The chosen synthetic routes utilize readily available starting materials and afford the desired compounds on a viable scale via an economical, modular approach.

Also, anomoian A is cited in the literature as both **4a**[1a] and the original isolated structure **4b**,[14b] in which the purpurealidin E-like unit is attached to the bromotyrosine unit via opposing alkylamino chains. The true identity of anomoian A (**4b**) is validated through synthesis of the structural isomer *iso*-anomian A (**4a**).

## RESULTS AND DISCUSSION

Starting from the known *N*-Boc-3,5-dibromo-4-hydroxyphenethylamine,[15] alkylation to the ether **8** followed by Boc-deprotection provided purpurealidin E (**2**) as the free base, in 71% overall yield (Scheme 1, Table 1). The NMR data from isolated

**Scheme 1**[a]




[a]Reagents and conditions: (i) 1-chloro-3-dimethylaminopropane·HCl, $K_2CO_3$, acetone, 75%; (ii) TFA, $CH_2Cl_2$ then $NaHCO_3$, 95%.

**2** showed good correlation data with the exception of $\delta_H$ H-2, $N(CH_3)_2$ and $\delta_C$ C-1, C-2. The addition of DCl or conversion to the triflate salt (Table S1) moves all resonances into the correct ranges, suggesting that purpurealidin E was originally isolated as the protonated form. Tilvi et al. assigned C-4,8 as $CH_2$, which is now corrected to two CH (Table 1).

We have previously synthesized verongamine through amination of the appropriate oximinoester **9** with histamine in MeOH.[16] Using the same conditions and changing the amine to purpurealidin E (**2**) yielded aplysamine-2 (**3**) (87%), giving excellent correlations for the NMR and HRMS data (Scheme 2, Table 2) with those of authentic samples.[11,14a]

Intermediate **11** was readily prepared from known (*S*)-methyl-2-(*N*-Boc-amino)-3-(3,5-dibromo-4-methoxyphenyl)-propanoate (**10b**)[17] under Eschweiler–Clarke conditions.[18] One-pot Boc-deprotection, dimethylation, and hydrolysis gave the desired intermediate in good yield (Scheme 3).

The syntheses of **4a**–**7** were envisaged through carbodiimide coupling of methylated bromotyrosine derivatives **10c**, **12c**, **12d**, and **11** with amine **2**, followed by Boc-protection where necessary (Scheme 5). *N*-Methylation of the known *N*-Boc monobromo and dibromo derivatives **10a**[19] and **10b**,[17] with NaH/MeI in DMF at 0 °C, gave the *N*-alkylated derivatives **12a** (66%) and **12b** (78%) (Scheme 4). Both **12a** and **12b** showed doubling of some signals in both $^1H$ and $^{13}C$ NMR spectra due to the *cisoid*/*transoid* conformations as previously demonstrated with Boc-*N*-Me-Tyr(OMe)-OH due to hindered internal rotation.[20] Of the four compounds demonstrating doubling of peaks (**12a**, **12b**, **12c**, and **12d**), upon high-temperature $^1H/^{13}C$ NMR, only **12a** has peaks that fully coalesce.

Hydrolysis of methyl esters **10b**,[17] **12a**, and **12b** gave good yields of the acids **10c** (89%), **12c** (93%), and **12d** (99%), respectively (Scheme 4), which also demonstrated doubling of $^1H$ and $^{13}C$ NMR signals due to tertiary amide rotamer contributions.

Carbodiimide coupling of acids **10c**, **12c**, **12d**, and **11** with purpurealidin E (**2**) gave **13a** (74%), **13b** (56%), **13c** (72%), and **5** (66%, aplyzanzine A) after purification (Scheme 5). The identity of aplyzanzine A was established through comparison of $^1H$ and $^{13}C$ NMR and HRMS (Table 3) with an authentic sample.[14c]

Kashman et al. reported that aplyzanzine A was obtained as an orange oil with zero optical activity, but the orange oil obtained from the current synthesis displayed an $[\alpha]^{20}_D$ +30, indicating that racemization is unlikely to have occurred from the precursor acid **11** ($[\alpha]^{20}_D$ +70) and peptide aplyzanzine A (**5**).

Further *N*-Boc deprotection of **13a**, **13b**, and **13c** gave the target molecules **6** (suberedamine A, 95%), **7** (suberedamine B, 94%), and **4** (*iso*-anomoian A, 81%) after workup and without the need for further purification. The identities of suberedamines A and B were established through comparison of $^1H$, $^{13}C$, and HRMS data (Tables 4 and 5) with authentic samples.[14d]

The data for compound **4a**, when compared to the $^1H$ and $^{13}C$ assignments of Kernan et al. of the free base of anomoian A (**4b**, Table S2), show good correlation with the bromotyrosine unit and aromatic signals for the tyramine unit. However, on further inspection of the methylene and dimethylamino signals, large discrepancies are observed and further supported by comparison of long-range $^{13}C-^1H$ correlation data (Table S2). Therefore, the structure of anomoian A (**4b**) was confirmed to be the original structure as assigned by Kernan et al., and **4a** is the structural isomer *iso*-anomoian A (**4a**).[21]

**Biological Evaluation.** Compounds **2**–**7** were tested for inhibition against the growth of 10 Gram-negative organisms, eight Gram-positive organisms, and two pathogenic yeasts (Table S3), in accordance with the recommendations of the British Society of Antimicrobial Chemotherapy.[22]

In the concentration range tested purpurealidin E (**2**) showed a lack of activity against any of the species tested (minimum inhibitory concentration, MIC > 128 mg $mL^{-1}$). Compounds **3**–**7** displayed varying degrees of antimicrobial activity, with very little inhibition of the growth of Gram-negative


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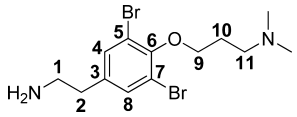
**Table 1. Spectroscopic Data for Purpurealidin E (2)**




| position | $\delta_C$, type | | $\delta_H$, ($J$ in Hz) | |
|---|---|---|---|---|
| | *a* | *b* | *a* | *b* |
| 1 | 40.0, $CH_2$ | 44.0, $CH_2$ | 2.73, t (13.2, 6.6) | 2.86, t (7.3) |
| 2 | 33.6, $CH_2$ | 38.8, $CH_2$ | 3.24, t | 2.70, t (7.3) |
| 3 | 130.3, C | 140.5, C | | |
| 4 | 133.0, $CH_2$ | 134.3, CH | 7.43, s | 7.43, s |
| 5 | 117.3, C | 119.2, C | | |
| 6 | 150.7, C | 152.9, C | | |
| 7 | 117.3, C | 119.2, C | | |
| 8 | 133.0, $CH_2$ | 134.3, CH | 7.43, s | 7.43, s |
| 9 | 69.8, $CH_2$ | 72.8, $CH_2$ | 4.05, t (5.6) | 4.03, t (6.2) |
| 10 | 25.0, $CH_2$ | 29.1, $CH_2$ | 2.23, m | 2.07, m |
| 11 | 55.8, $CH_2$ | 57.6, $CH_2$ | 2.44, t (5.6) | 2.64, t (5.7) |
| $N(CH_3)_2$ | 42.7, $CH_3$ | 45.9, $CH_3$ | 2.90, s | 2.28, s |
| MS | HR-MS (EI +ve) $m/z$ found $(M + H)^+$ calcd for $C_{13}H_{20}Br_2N_2O$ 378.9943, found 378.9768 | HR-MS (EI +ve) $m/z$ found $(M^+)$ calcd for $C_{13}H_{20}{}^{79}Br^{81}BrN_2O$ 379.9922, found 379.9915 | | |

[a]Tilvi, S.; Rodrigues, C.; Naik, C. G.; Parameswaran, P. S.; Wahidhulla, S. *Tetrahedron* **2004**, *60*, 10207−10215 ($^1$H 300 MHz, $CD_3OD$). [b]Current study ($^1$H 300 MHz, $^{13}$C 75 MHz, $CD_3OD$).



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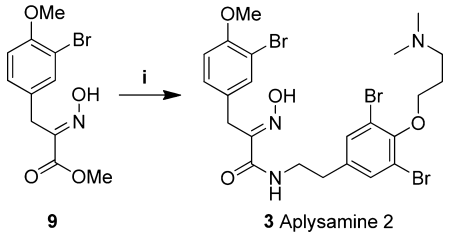
**Scheme 2**[a]




[a]Reagents and conditions: (i) **2**, $Et_3N$, MeOH, reflux, 87%.

bacteria and all eight strains of Gram-positive bacteria showing moderate susceptibility to **3** (MIC 32−64 mg/mL), **4** (MIC 32−128 mg/mL), and **5** (MIC 16−32 mg/mL). Aplyzanzine A (**5**) was the most active compound and exhibited MIC values 2 to 4 times lower than any other compound for seven species of Gram-positive bacteria (most active against *Bacillus subtilis*, *Staphylococcus epidemis*, and *Streptococcus pyogenes*) and demonstrated moderate antifungal activity against the two most common species of pathogenic yeast (*Candida* spp.).

The higher activity of **5** when compared to **4** was notable given their very close similarity in structure. As **2** showed no activity and **5** displayed the highest activity, further investigation of the activity of (*S*)-3-(3-bromo-4-methoxyphenyl)-2-(dimethylamino)propanoic acid (**11**) is warranted.

MICs against mycobacteria were determined using a spot culture growth inhibition assay,[23] which has been previously shown to have identified antimycobacterial activity from both natural products[24,25a] and novel synthetic compounds.[25b] Figure 1 illustrates a representative MIC determination using this assay for the compound aplyzanzine A (**5**), showing an MIC of 25 μg/mL for *M. bovis* BCG and *M. tuberculosis* H37Rv. The marine sponge metabolites **3**−**7** showed moderate activity (MIC 25−50 μg/mL) against both *M. bovis* BCG and *M. tuberculosis* H37Rv. Compound **2** showed no activity up to 50 μg/mL, presumably due to its decreased lipophilicity, which can influence cell penetration.

There is a correlation ($r^2 = 1$) between the MIC on *M. bovis* BCG and the MIC on *M. tuberculosis* H37Rv, an observation that has been reported previously using the spot growth inhibition assay.[25b]

Due to their promising biological activity demonstrated in the initial one-dose screen against the NCI 60 human tumor cell line panel, *iso*-anomian A (**4**) and suberedamine B (**7**) were selected for rescreening by the Biological Evaluation Committee over the ranges given in Table 6.

As a single-digit micromolar $GI_{50}$ (the concentration that causes 50% growth inhibition) is a practical measure of encouraging antitumor activity, the use of total number of cell lines inhibited at single-digit micromolar concentration can also be used as an additional benchmark for overall activity.

*iso*-Anomoian A gave the strongest antitumor activity (53/60 lines) and the broadest spectrum of inhibition at single-digit micromolar concentration, with an average $GI_{50} = 2.17$ μM, and was two and a half times more potent than subederamine B. The results suggest that monomethylation of the tyrosine amine is required for activity and that a second bromine substitution on the aromatic ring decreases activity for the cell lines tested.

In summary, this paper describes the first formal syntheses of the marine sponge secondary metabolites aplysamine-2, suberedamines A and B, aplyzanzine A, purpurealidin E, and the structural isomer *iso*-anomoian A from readily available starting materials. Three of the synthesized compounds, aplysamine-2, *iso*-anomoian A, and aplyzanzine A, were shown to exhibit activity against a range of Gram-positive bacteria, with aplazanzine A being the most active. All natural products, except the parent purpurealidin E, demonstrated activity against *M. bovis* BCG and *M. tuberculosis* H37Rv. *iso*-Anomoian A (**4a**) and suberedamine B (**7**) showed promising results against the NCI 60 cell line cancer screen, and we are currently exploiting the versatility of our synthetic strategy to produce a range of analogues of the compounds reported here in order to obtain SAR data. The results of these further investigations, including the synthesis of anomoian A will be reported in due course.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** All reactions were carried out under an atmosphere of dry argon in oven-dried glassware unless otherwise noted. All solvents were purified according to standard procedures. Diethyl ether and tetrahydrofuran were freshly distilled over sodium wire with a trace of benzophenone. Reaction temperatures were recorded at bath temperatures. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were determined on a Bellingham and Stanley APD410 polarimeter at 20 °C with a path length of 20 cm. Infrared spectra were recorded on a Perkin-Elmer 1600 series Fourier transform spectrophotometer or a Nicolet AVTAR 380 FTIR Smart Endurance; only the principal bands are listed. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian XL-300, a Brüker AVANCE 300 (300 MHz for $^1H$ and 75 MHz for $^{13}C$ spectra), or an AVANCE 500 (500 MHz for $^1H$ and 125 MHz for $^{13}C$ spectra). Coupling constants are given in Hz, and all chemical shifts are relative to the chemical shift of the residual nondeuterated solvent. Low-resolution electrospray mass spectra were obtained on a Brüker Esquire 3000+, and high-resolution spectra on a Brüker APEX II FT or JEOL MStation (JMS-700) mass spectrometer. Elemental analyses were determined by either Atlantic Microlab, Inc. (Norcross, GA, USA) or in-house on an Exeter Analytical CE-440 elemental analyzer. Column chromatography was performed on E. Merck silica gel 60, 230−400 mesh ASTM. Analytical thin-layer chromatography (TLC) was performed on precoated glass plates (Merck Kieselgel 60 $F_{254}$ 0.25 mm), and visualization was effected by UV irradiation and vanillin/$H_2SO_4$ dip followed by charring.

***N-tert*-Butyl 3,5-Dibromo-4-(3-dimethylaminopropoxy)-phenethylcarbamate (8).** *N-tert*-Butyl 3,5-dibromo-4-hydroxyphenethylamine[15] (5.00 g, 12.65 mmol), *N,N*-dimethyl-3-chloropropylamine hydrochloride (2.40 g, 15.18 mmol), and anhydrous $K_2CO_3$ (4.36 g, 31.60 mmol) were refluxed in dry acetone until no starting material remained by TLC (hexanes/EtOAc/$CH_2Cl_2$). The solvent was removed *in vacuo*, and the resulting residue was suspended in aqueous NaOH (0.1 M, 100 mL) and then extracted with $CHCl_3$ (100 mL). The organic phase was washed with $H_2O$ (100 mL), dried over anhydrous $Na_2SO_4$, and filtered, and finally the solvent was removed *in vacuo* to give **8** as a white solid (4.60 g, 75%): mp 128−129.5 °C; IR (diamond) $\nu_{max}$ 3351 (NH), 1672 (C═O) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.25 (2H, s, H-13, H-17), 4.59 (1H, br s, NH), 3.99 (2H, t, $J$ = 6.3 Hz, 2H, H-18), 3.28 (2H, q, $J$ = 6.9 Hz, H-10), 2.66 (2H, t, $J$ = 6.9 Hz, H-11), 2.56 (2H, t, $J$ = 6.9, Hz, H-20), 2.24 (6H, s, $N(CH_3)_2$), 2.04 (2H, m, H-19) 1.37 (9H, s, $C(CH_3)$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 155.8 (C, $NCO_2C(CH_3)_3$), 151.8 (C, C-15), 137.7 (C, C-12), 132.9 (CH, C-13,17), 118.2 (C, C-14, 17), 79.5 (C, $C(CH_3)_3$), 71.8 ($CH_2$, C-18), 56.4 ($CH_2$,C-20), 45.3 ($CH_3$, $N(CH_3)_2$), 41.5 ($CH_2$, C-10), 35.0 ($CH_2$, C-11), 28.4 ($CH_2$, C-19), 28.1 ($CH_3$, $C(CH_3)_3$); HRCIMS $m/z$ 479.0546 $[M + H]^+$ (calcd for $C_{18}H_{29}{}^{79}Br_2N_2O_3$ 479.0526); anal. calcd for $C_{18}H_{28}Br_2N_2O_3$, C, 45.02; H, 5.88; N, 5.83; found C, 45.11; H, 5.82; N, 5.68.

**Purpurealidin E (2).** TFA (0.34 mL, 3.15 mmol) was added dropwise to *N-tert*-butyl 3,5-dibromo-4-(3-dimethylaminopropoxy)-phenethylcarbamate (**7**, 1.00 g, 2.63 mmol) in $CH_2Cl_2$ (5 mL) and then stirred at room temperature (rt) for 3 h. The solvent was


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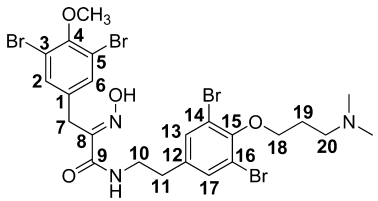
**Table 2. Spectroscopic Data for Aplyamine-2 (3)**




| position | $\delta_C$, type | | | $\delta_H$, ($J$ in Hz) | | |
|---|---|---|---|---|---|---|
| | *a* | *b* | *c* | *a* | *b* | *c* |
| 1 | 113.1, C | 113.2, C | 113.3, C | | | |
| 2 | 134.7, CH | 134.7, CH | 134.9,CH | 7.43, d (2.2) | 7.50, d (1.5) | 7.44, d (2.1) |
| 3 | 130.3, C | 130.2, C | 130.5, C | | | |
| 4 | 155.8, C | 156.0, C | 156.0, C | | | |
| 5 | 112.1, CH | 112.1, CH | 112.3, CH | 6.88, d (8.5) | 6.80, d (7.0) | 6.89, d (8.5) |
| 6 | 131.7, CH | 131.7, CH | 131.9, CH | 7.17, dd (8.5, 2.2) | 7.22, dd (6.0, 1.5) | 7.17, dd (8.5, 2.1) |
| 7 | 28.7, $CH_2$ | 28.7, $CH_2$ | 29.1, $CH_2$ | 3.79, s | 3.65, s | 3.80, s |
| 8 | 152.9, C | 152.9, C | 153.1, C | | | |
| 9 | 165.8, C | 166.0, C | 165.9, C | | | |
| 10 | 41.3, $CH_2$ | 41.2, $CH_2$ | 41.5, $CH_2$ | 3.41, t (7.0) | 3.45, t (7.0) | 3.44, t (7.0) |
| 11 | 35.2, $CH_2$ | 35.1, $CH_2$ | 35.3, $CH_2$ | 2.73, t (7.0) | 2.75, t (6.5) | 2.75, t (7.0) |
| 12 | 140.3, C | 139.8, C | 139.9, C | | | |
| 13 | 134.4, CH | 134.3, CH | 134.5, CH | 7.42, s | 7.35, s | 7.40, s |
| 14 | 118.7, C | 118.8, C | 119.0, C | | | |
| 15 | 152.1, C | 152.5, C | 152.9, C | | | |
| 16 | 118.7, C | 118.8, C | 119.0, C | | | |
| 17 | 134.4, CH | 134.3, CH | 134.5, CH | 7.42, s | 7.35, s | 7.40, s |
| 18 | 71.7, $CH_2$ | 72.1. $CH_2$ | 72.7. $CH_2$ | 4.05, t (5.5) | 4.05, t (6.0) | 4.00, t (6.1) |
| 19 | 26.4, $CH_2$ | 28.1, $CH_2$ | 28.9, $CH_2$ | 2.25, tt (7.6, 5.5) | 2.10, t (6.0) | 2.06, m |
| 20 | 56.9, $CH_2$ | 57.3, $CH_2$ | 57.6, $CH_2$ | 3.46, t (7.6) | 2.82, t (6.5) | 2.66, t (7.7) |
| $OCH_3$ | 56.7, $CH_3$ | 56.7, $CH_3$ | 56.8, $CH_3$ | 3.81, s | 3.84, s | 3.82, s |
| $N(CH_3)_2$ | 43.7, CH3 | 44.8, CH3 | 45.6, CH3 | 2.93, s | 2.45, s | 2.30, s |
| MS | HREI $[M-HCl]^+$ ($C_{23}H_{28}{}^{79}Br_3N_3O_4$) calcd 646.9629, obsd 646.9630 | LRFAB $[M+H]^+$ ($C_{23}H_{28}Br_3N_3O_4$) 654(1), 652(3), 650(3), 648(1) | HRFAB $[M+H]^+$ ($C_{23}H_{29}{}^{79}Br_2{}^{81}BrN_3O_4$) calcd 649.9697, obsd 649.9698 | | | |

[a]Xynas, R.; Capon, R. J. *Aus. J. Chem.* **1989**, *42*, 1427−1433. ($^1$H 400 MHz, $^{13}$C 100 MHz, $CD_3OD$). [b]Rama Rao, M.; Venkatesham, U.; Venkateswarlu, Y. *Ind. J. Chem.* **1999**, *33B*, 1301−1303 ($^1$H 200 MHz, $^{13}$C 50 MHz, $CD_3OD$). [c]Current study ($^1$H 500 MHz, $^{13}$C 125 MHz, $CDCl_3$).



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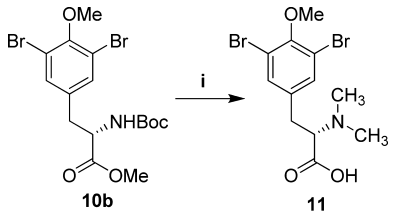
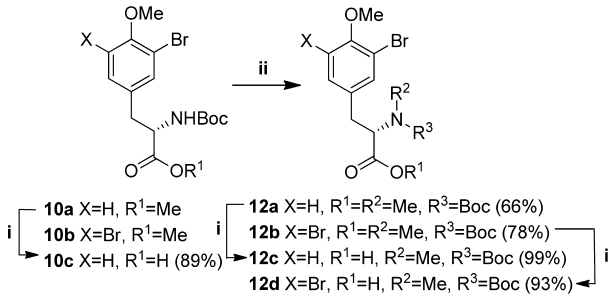
**Scheme 3**[a]



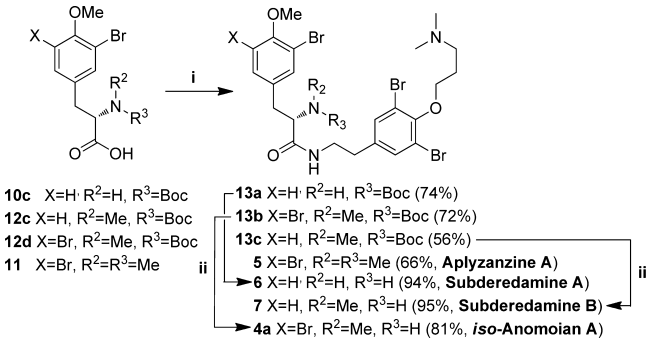

[a]Reagents and conditions: (i) HCHO, $HCO_2H$, reflux, 88%.

**Scheme 4**[a]




[a]Reagents and conditions: (i) LiOH, THF, $H_2O$; (ii) NaH, MeI, DMF.

**Scheme 5**[a]




[a]Reagents and conditions: (i) **2**, EDCI, HOBt, $Et_3N$, $CH_2Cl_2$; (ii) TFA, $CH_2Cl_2$ then $NaHCO_3$.

removed *in vacuo*, and the residue was dissolved in EtOAc (50 mL) and successively washed with saturated $NaHCO_3$ solution (2 × 50 mL) and then brine (50 mL). The organic portion was dried over anhydrous $Na_2SO_4$ and filtered, and finally the solvent removed *in vacuo* to give an orange gum (**2**, 0.75 g, 95%); IR (diamond) $\nu_{max}$ 3369 ($NH_2$), 3283 ($NH_2$) $cm^{-1}$; $^1H$, $^{13}C$ NMR and HRMS see Table 1.

**Aplysamine-2 (3) (refs 11, 14a).** (*E*)-Methyl 3-(3-bromo-4-methoxyphenyl)-2-(hydroxyimino)propanoate[16] (**9** 0.125 g, 0.414 mmol), purpurealidin E (**2**, 0.180 g, 0.473 mmol), and $Et_3N$ (0.2 mL) were refluxed in MeOH (10 mL) for 72 h. The solvent was then removed *in vacuo* to obtain an orange gum. Chromatography ($SiO_2$/hexanes/EtOAc) gave a pale yellow foam (**3**, 0.234 g, 87%); IR (diamond) $\nu_{max}$ 3397 (OH), 1662 (C═O); $^1H$, $^{13}C$ NMR and HRMS see Table 2.

**(*S*)-Methyl 3-(3-Bromo-4-methoxyphenyl)-2-((*N*-*tert*-butoxycarbonyl)(methyl)amino)propanoate (12a).** A solution of (*S*)-methyl 3-(3-bromo-4-methoxyphenyl)-2-((*N*-*tert*-butoxycarbonyl)(amino)-propanoate[19] (5.00 g, 1.33 mmol) and MeI (1.80 mL, 2.94 mmol) in DMF (50 mL) was cooled to 0 °C, NaH (1.40 g, 3.47 mmol) was added in 0.2 g portions until effervescence subsided, and then the mixture was allowed to warm to rt for 15 h. The mixture was quenched with saturated aqueous $NH_4Cl$ (10 mL), and excess DMF was removed *in vacuo* followed by extraction with EtOAc (3 × 100 mL). The organic extracts were combined, washed with $H_2O$ (100 mL), and dried over anhydrous $MgSO_4$, and the solvent was removed *in vacuo* to give a colorless gum (**12a**, 3.50 g, 66%): IR (diamond) $\nu_{max}$ 1740 (ester C═O), 1690 (carbamate C═O) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, 353 K) $\delta$ 7.41 (1H, d, $J$ = 2.0 Hz, H-2), 7.16 (1H, dd, $J$ = 2.0, 8.4 Hz, H-6), 6.86 (1H, d, $J$ = 8.4 Hz, H-5), 4.88 (1H, m, 1H, H-8), 3.89 (3H, s, $ArCOCH_3$), 3.77 (3H, s, $CO_2CH_3$), 3.27 (1H, m, H-7), 3.01 (1H, m, 1H, H-7), 2.76 (3H, s, $NCH_3$), 1.42 (9H, s, $OC(CH_3)_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$, 353K) $\delta$ 171.6 (C, $CO_2CH_3$), 155.8 (C, $NHCO_2C(CH_3)_3$), 154.9 (C, C-4), 133.7 (CH, C-2), 131.3 (C, C-1) 129.1 (CH, C-6), 113.0 (CH, C-5), 79.8 (C, $C(CH_3)_3$), 61.1 (CH, C-8), 56.9 ($CH_3$, $ArOCH_3$), 52.3 ($CH_3$, $CO_2CH_3$), 39.6 ($CH_2$, C-7), 38.9 ($CH_3$, $NCH_3$), 32.4 ($CH_3$, $OC(CH_3)_3$); HRFABMS (NaI/PEG 400/600) $m/z$ 426.0733 $[M + Na]^+$ (calcd for $C_{17}H_{24}{}^{81}BrNaNO_5$, 426.0717).

**(*S*)-Methyl 2-((*N*-*tert*-Butoxycabonyl)(methyl)amino)-3-(3,5-dibromo-4-methoxyphenyl)propanoate (12b).** A solution of (*S*)-methyl 2-((*N*-*tert*-butoxycarbonyl)(amino)-3-(3,5-dibromo-4-methoxyphenyl)propanoate[17] (5.00 g, 1.10 mmol) and MeI (1.5 mL, 2.42 mmol) in DMF (50 mL) was cooled to 0 °C. NaH (1.13 g, 2.86 mmol) was added in portions over 10 min before allowing the reaction mixture to warm to rt over 12 h. The mixture was then quenched with saturated aqueous $NH_4Cl$ (10 mL), before reducing the solvent volume by 2/3 *in vacuo*, and then extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with $H_2O$ (100 mL), dried over anhydrous $MgSO_4$, and filtered; then the solvent was removed *in vacuo* to give a colorless gum (**12b**, 4.10 g, 78%): IR (diamond) $\nu_{max}$ 1741 (ester C═O), 1690 (carbamate C═O) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.27 (2H, s, H-2,6), 4.76 (1H, m, H-8), 3.78 (3H, s, $ArCOCH_3$), 3.67 (s, 3H, $CO_2CH_3$), 3.17 (1H, dd, $J$ = 5.1, 14.4 Hz, H-7), 2.92 (1H, m, H-7), 2.68 (3H, 2*s*, $NCH_3$, rotamers), 1.34 (9H, 2*s*, $C(CH_3)_3$, rotamers); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 171.2 (+ 171.0, C, $CO_2CH_3$), 155.7 (+ 154.8, C, $NHCO_2C(CH_3)_3$), 153.0 (+ 152.8, C, C-4) 136.5 (+ 136.4, C, C-1), 133.5 (+ 133.1, CH, C-2,6), 118.1 (+ 117.9, C, C-3,5), 80.7 (+ 80.6, C, $OC(CH_3)_3$), 60.7 (+ 60.6, $CH_3$, $ArOCH_3$), 59.4 (CH, C-8), 52.5 (+ 52.4, $CH_3$, $CO_2CH_3$), 34.3 (+ 33.8, $CH_2$, C-7), 32.3 (+ 32.0, $CH_3$, $NCH_3$), 28.3 ($CH_3$, $OC(CH_3)_3$); HRFABMS (NaI/PEG 400/600) $m/z$ 503.9812 $[M + Na]^+$ (calcd for $C_{17}H_{23}{}^{79}Br^{81}BrNaNO_5$, 503.9821).

**(*S*)-3-(3-Bromo-4-methoxyphenyl)-2-((*N*-*tert*-butoxycarbonyl)-(methyl)amino)propanoic Acid (12c).** (*S*)-Methyl 2-((*N*-*tert*-butoxycabonyl)(methyl)amino)-3-(3,5-dibromo-4-methoxyphenyl)-propanoate (**12a**, 1.50 g, 3.74 mmol) and LiOH (0.39 g, 9.35 mmol) in THF/$H_2O$ (5 mL/5 mL) were stirred overnight at rt. The solvent was removed *in vacuo*, and the residue acidified with dilute HCl (0.1 M, 10 mL) to pH 1, then extracted with EtOAc (2 × 50 mL). The combined organic portions were washed with $H_2O$ (50 mL) and brine (50 mL), dried over anhydrous $Na_2SO_4$, and filtered, and the solvent was removed *in vacuo* to give a colorless gum (**12c**, 1.44 g, 99%): IR (diamond) $\nu_{max}$ 3220 (OH), 1711(acid C═O), 1691 (carbamate C═O) $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 8.74 (1H, br s, $CO_2H$), 7.33 (1H, 2s, H-2), 7.07 (1H, 2d, $J$ = 8.0 Hz, H-6), 6.76 (1H, d, $J$ = 8.0 Hz, H-5), 4.69 (1H, 2 × dd, $J$ = 5.0, 10.5 Hz, H-8), 3.79 (3H, s, $ArOCH_3$), 3.18 (1H, m, H-7), 3.00 (1H, m, H-7), 2.69 (3H, 2s, $NCH_3$), 1.34 (9H, 2s, $OC(CH_3)_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 175.7 (+ 175.6, C, $CO_2H$), 154.8 (C, $NHCO_2C(CH_3)_3$), 154.7 (C, C-4), 133.7 (+ 133.6, CH, C-2), 131.0 (+ 130.9, C, C-1), 129.1 (+ 129.0, CH, C-6), 112.1 (+ 111.9, C, C-5), 80.9 (C, $OC(CH_3)_3$), 60.9 (+ 61.2, $CH_3$, $ArOCH_3$), 56.3 (CH, C-8), 34.1 (+ 33.6, $CH_2$, C-7) 33.0 (+ 32.4, $CH_3$, $NCH_3$), 28.30 ($CH_3$, $OC(CH_3)_3$); HRFABMS (NaI/PEG 400/600) $m/z$ 410.0590 $[M + Na]^+$ (calcd for $C_{16}H_{22}{}^{79}BrNaNO_5$, 410.0579).

**(*S*)-2-((*N*-*tert*-Butoxycarbonyl)(methyl)amino)-3-(3,5-dibromo-4-methoxyphenyl)propanoic Acid (12d).** (*S*)-Methyl 2-(*N*-Boc-(methyl)amino)-3-(3,5-dibromo-4-methoxy-phenyl)propanoate (**12b**, 1.00 g, 2.09 mmol) and LiOH (0.21 g, 5.23 mmol) were stirred overnight at rt in THF/$H_2O$ (5 mL/5 mL). The solvent was removed *in vacuo*, and the residue acidified with dilute HCl (0.1 M, 10 mL) to pH 1, then extracted with EtOAc (2 × 50 mL). The combined organic portions were washed with $H_2O$ (50 mL) and brine (50 mL), dried over anhydrous $Na_2SO_4$, and filtered, and the solvent was removed *in*


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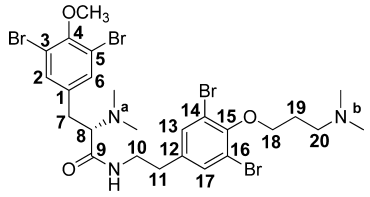
**Table 3. Spectroscopic Data for Aplyzanine A (5)**



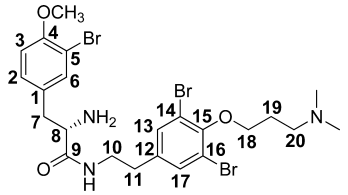

| position | $\delta_C$, type | | $\delta_H$, ($J$ in Hz) | |
|---|---|---|---|---|
| | *a* | *b* | *a* | *b* |
| 1 | 137.7, C | 137.7, C | | |
| 2 | 133.2, CH | 133.4, CH | 7.31, s | 7.34, s |
| 3 | 117.6, C | 117.3, C | | |
| 4 | 152.3, C | 152.6, C | | |
| 5 | 117.6, C | 117.3, C | | |
| 6 | 133.2, CH | 133.4, CH | 7.31, s | 7.34, s |
| 7 | 31.6, $CH_2$ | 33.7, $CH_2$ | 2.71, dd (13.8, 4.5)<br>2.94, dd (13.8, 4.5) | 2.88, m |
| 8 | 69.8, CH | 71.3, CH | 3.14, dd (13.8, 4.5) | 3.00, dd (9.3, 5.1) |
| 9 | 170.8, C | 171.2, C | | |
| 10 | 39.8, $CH_2$ | 39.6, $CH_2$ | 3.29, dt (7.0, 2.8) | 3.25, m |
| 11 | 34.2, $CH_2$ | 33.9, $CH_2$ | 2.54, m<br>2.57, m | 2.60, m |
| 12 | 137.9, C | 138.3, C | | |
| 13 | 132.8, CH | 132.9, CH | 7.23, s | 7.29, s |
| 14 | 117.7, C | 117.6, C | | |
| 15 | 150.9, C | 151.6, C | | |
| 16 | 117.7, C | 117.6, C | | |
| 17 | 132.8, CH | 132.8, CH | 7.23, s | 7.29, s |
| 18 | 69.7, $CH_2$ | 70.3, $CH_2$ | 3.96, t (5.5) | 3.93, t (6.0) |
| 19 | 25.4, $CH_2$ | 27.4, $CH_2$ | 2.18, m | 1.99, m |
| 20 | 55.4, $CH_2$ | 56.1, $CH_2$ | 3.16, m | 3.20, m |
| $OCH_3$ | 60.4, $CH_3$ | 59.7, $CH_3$ | 3.74, s | 3.73, s |
| $N(CH_3)_2$[a] | 41.5, $CH_3$ | 41.1, $CH_3$ | 2.26, s | 2.20, s |
| $N(CH_3)_2$[b] | 42.9, $CH_3$ | 44.0, $CH_3$ | 2.67, s | 2.26, s |
| MS | LRCIMS *m/z* 740 (22), 742 (70), 744, (100), 746 (64) 748 (20) | HRFAB [NaI/PEG600/900/1500] *m/z* 743.9293 $[M+H]^+$ calcd for $C_{24}H_{34}{}^{79}Br_2{}^{81}BrN_3O_3$ 743.9295 | | |

[a]Evan, T.; Rudi, A.; Ilan, M.; Kahman, Y. *J. Nat. Prod.* **2001**, *64*, 226−227 ($^1$H 500 MHz, $^{13}$C 125 MHz, $CDCl_3$ + $CD_3OD$, 10:1). [b]Current study ($^1$H 300 MHz, $^{13}$C 75 MHz, $CD_3OD$).



dx.doi.org/10.1021/np300102z | *J. Nat. Prod.* 2012, 75, 1090−1101

**Table 4. Spectroscopic Data for Suberedamine A (6)**




| position | $\delta_C$, type | | $\delta_H$, ($J$ in Hz) | |
|---|---|---|---|---|
| | *a* | *b* | *a* | *b* |
| 1 | 129.8, C | 127.6, C | | |
| 2 | 136.1, CH | 133.8, CH | 7.47, d (1.9) | 7.43, d (2.0) |
| 3 | 113.6, C | 111.5, C | | |
| 4 | 157.9, C | 155.7, C | | |
| 5 | 114.5, CH | 112.3, C | 7.04 d (8.5) | 7.01, d (8.5) |
| 6 | 131.7, CH | 129.5, CH | 7.20, dd (8.5, 1.9) | 7.17, dd (8.5, 2.0) |
| 7 | 38.3, $CH_2$ | 36.1, $CH_2$ | 3.08, dd (14.0, 7.8) | 3.06 (dd, 14.0, 6.5) |
| | | | 2.98, dd (14.0, 7.3) | 2.95, dd (14.0, 7.5) |
| 8 | 56.5, CH | 71.3, CH | 4.00, t (7.3) | 3.97, t (7.5) |
| 9 | 170.3, C | 168.1, C | | |
| 10 | 42.3, $CH_2$ | 40.1, $CH_2$ | 3.56, m | 3.55, m |
| 11 | 35.8, $CH_2$ | 33.6, $CH_2$ | 3.32, dd (13.2, 6.6) | 3.32, m |
| 12 | 140.9, C | 138.3, C | | |
| 13 | 135.1, CH | 132.9, CH | 7.51, s | 7.47, s |
| 14 | 119.7, C | 117.5, C | | |
| 15 | 153.2, C | 151.0, C | | |
| 16 | 119.7, C | 117.5, C | | |
| 17 | 135.1, CH | 132.9, CH | 7.51, s | 7.47, s |
| 18 | 71.9, $CH_2$ | 69.7, $CH_2$ | 4.13, t (5.6) | 4.10, t (5.5) |
| 19 | 27.2, $CH_2$ | 25.0, $CH_2$ | 2.32, tt (7.9, 5.6) | 2.31, tt (8.0, 5.5) |
| 20 | 57.8, $CH_2$ | 55.6, $CH_2$ | 3.54, t (5.6) | 3.52, m |
| $OCH_3$ | 57.6, $CH_3$ | 55.4, $CH_3$ | 3.91, s | 3.87, s |
| $N(CH_3)_2$ | 44.5, $CH_3$ | 42.3, $CH_3$ | 3.00, s | 2.96, s |
| MS | HRFAB $m/z$ 633.9916 $[M+H]^+$ calcd for $C_{24}H_{31}{}^{79}Br_3{}^{81}BrN_3O_3$ 633.9944 | HRFAB [PEG600] $m/z$ 635.9855 $[M+H]^+$ calcd for $C_{24}H_{33}{}^{79}Br^{81}Br_2\ N_3O_3$ 635.9896 | | |

[a]Tsuda, M.; Sakuma, Y.; Kobayashi, J. *J. Nat. Prod.* **2001**, *64*, 980−982 ($^1$H 500 MHz, $^{13}$C 125 MHz, $CD_3OD$). [b]Current study ($^1$H 500 MHz, $^{13}$C 75 MHz, $CD_3OD$; $\delta_C$ appear to be approximately 2.2 ppm different from "*a*" due to different reference values).



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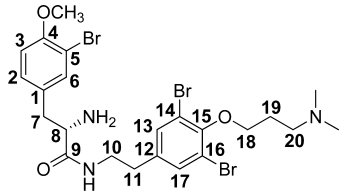
**Table 5. Spectroscopic Data for Suberedamine B (7)**




| position | $\delta_C$, type | | $\delta_H$, ($J$ in Hz) | |
|---|---|---|---|---|
| | *a* | *b* | *a* | *b* |
| 1 | 129.4, C | 127.3, C | | |
| 2 | 136.1, CH | 133.9, CH | 7.44, d (1.1) | 7.42, d (2.0) |
| 3 | 113.6, C | 111.3, C | | |
| 4 | 157.9, C | 155.6, C | | |
| 5 | 114.5, CH | 112.3, C | 7.04 d (8.4) | 7.00, d (8.5) |
| 6 | 131.8, CH | 129.7, CH | 7.18, dd (8.4, 1.1) | 7.18, dd (8.5, 2.0) |
| 7 | 37.4, $CH_2$ | 35.2, $CH_2$ | 3.12, dd (13.6, 5.7) | 3.14 (dd, 13.5, 5.5) |
| | | | 3.05, dd (13.6, 8.6) | 3.05, dd (13.5, 8.5) |
| 8 | 64.9, CH | 62.7, CH | 3.90, m | 3.96, t m |
| 9 | 168.9, C | 166.7, C | | |
| 10 | 42.1, $CH_2$ | 39.9, $CH_2$ | 3.47, m | 3.46, m |
| 11 | 35.8, $CH_2$ | 33.6, $CH_2$ | 3.38, m | 3.37, m |
| 12 | 140.8, C | 138.6, C | | |
| 13 | 135.1, CH | 132.9, CH | 7.47, s | 7.43, s |
| 14 | 119.7, C | 117.5, C | | |
| 15 | 153.2, C | 151.0, C | | |
| 16 | 119.7, C | 117.5, C | | |
| 17 | 135.1, CH | 132.9, CH | 7.47, s | 7.43, s |
| 18 | 71.9, $CH_2$ | 69.8, $CH_2$ | 4.12, t (5.5) | 4.09, t (5.5) |
| 19 | 27.2, $CH_2$ | 25.0, $CH_2$ | 2.32, tt (7.5, 5.5) | 2.31, tt (8.0, 5.5) |
| 20 | 57.8, $CH_2$ | 55.5, $CH_2$ | 3.54, t (7.4) | 3.52, t (8.0) |
| $OCH_3$ | 57.6, $CH_3$ | 55.5, $CH_3$ | 3.90, s | 3.86, s |
| $N(CH_3)_2$[b] | 44.5, $CH_3$ | 42.3, $CH_3$ | 3.00, s | 2.97, s |
| $NCH_3)_2$[a] | 33.3, $CH_3$ | 31.2, $CH_3$ | 2.62, s | 2.59, s |
| MS | HRFAB $m/z$ 648.0092 $[M+H]^+$ calcd for $C_{24}H_{33}{}^{79}Br_3{}^{81}BrN_3O_3$ 648.0072 | HRFAB [PEG600] $m/z$ 652.0035 $[M+H]^+$ calcd for $C_{24}H_{33}{}^{79}Br^{81}Br_2\ N_3O_3$ 652.0035 | | |

[a]Tsuda, M.; Sakuma, Y.; Kobayashi, J. *J. Nat. Prod.* **2001**, *64*, 980−982 ($^1$H 500 MHz, $^{13}$C 125 MHz, $CD_3OD$). [b]Current study ($^1$H 500 MHz, $^{13}$C 75 MHz, $CD_3OD$; $\delta_C$ appear to be approximately 2.2 ppm different from "*a*" due to different reference values).



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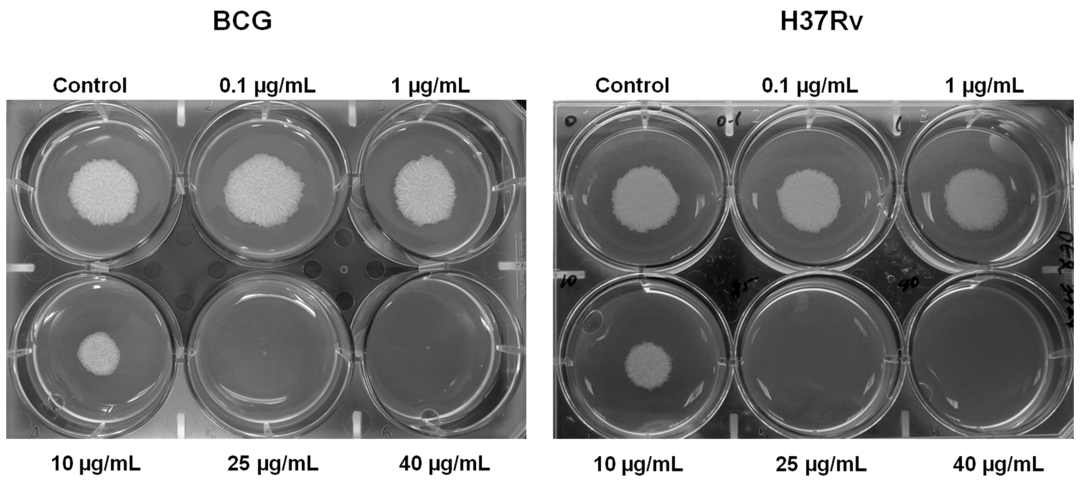


**Figure 1.** Representative assay of the growth of *M. bovis* BCG and *M. tuberculosis* H37Rv on 7H10 agar at 37 °C in the presence of different concentrations of compound **5**. The MIC value of compound **5** is 25 μg/mL for *M. bovis* BCG and *M. tuberculosis* H37Rv. Both mycobacterial species were grown in Middlebrook 7H9 broth medium supplemented with ADC at 37 °C. Cells ($10^3$) were spotted on the center of each well of a six-well plate containing Middlebrook 7H10 agar medium, supplemented with OADC, and different concentrations of **5** (0, 0.1, 1, 10, 25, and 40 μg/mL). Pictures of cultures that grew as spots were taken on the 14th day after inoculation using a digital camera.

**Table 6. In Vitro Antitumor Activities of 4a and 7 against the NCI-DTP 60 Cell Line Screen**[a]

| compound | −log(mean $GI_{50}$)[b] | mean $GI_{50}$ (μM) | $N$[c] | efficacy |
|---|---|---|---|---|
| *iso*-anomoian A (**4a**) (NSC D-752774/1) | 5.32 | 2.17 | 53 | leukemia K562, colon cancer HT29, CNS cancer SNB-75, melanoma M14 and UACC-257, renal cancer UO-31 |
| suberedamine B (**7**) (NSC D-752775/1) | 5.32 | 5.47 | 45 | |

[a]The full 60 cell line data are available free of charge on the Internet at the NCI-DTP site [http://dtp.nci.nih.gov/index.html]. [b]Range given for those compounds selected for a second screening by NCI's Biological Evaluation. [c]The number of cell lines inhibited at single-digit micromolar concentration. [d]Cell lines inhibited in the nanomolar range, with −log $GI_{50}$.

*vacuo* to give a colorless gum (**12d**, 0.91 g, 93%): IR (diamond) $\nu_{max}$ 3210 (OH), 1710 (acid C═O), 1690 (carbamate C═O), $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.20 (1H, br s, 1H, $CO_2H$), 7.28 (2H, s, H-2,6), 4.73 (1H, m, H-8), 3.79 (3H, s, $ArCOCH_3$), 3.20 (2H, dd, $J$ = 4.8, 14.7 Hz, H-7), 3.06 (1H, m, H-7), 2.80 (3H, 2s, $NCH_3$), 1.35 (9H, 2s, $C(CH_3)_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 175.1 (+ 175.1, C, $CO_2H$), 156.1 (+ 155.5, C, $NHCO_2C(CH_3)_3$), 153.2 (+ 153.0, C, C-4), 136.2 (+ 136.1, C, C-1), 135.6 (+ 133.1, CH, C-2,6), 118.1 (+ 118.0, C, C-3,5), 81.2 (+ 81.1, C, $OC(CH_3)_3$), 60.6 (+ 60.1, $CH_3$, $ArOCH_3$), 54.2 (+ 54.1, CH,C-8) 34.0 (+ 33.5, $CH_2$, C-7), 33.1 (+ 32.0, $CH_3$, $NCH_3$), 28.3 ($CH_3$, $OC(CH_3)_3$); HRFABMS (NaI/PEG 400/600) $m/z$ 489.9631 $[M + Na]^+$ (calcd for $C_{16}H_{21}{}^{79}Br^{81}BrNaNO_5$, 489.9665).

**(*S*)-3-(3,5-Dibromo-4-methoxyphenyl)-2-(dimethylamino)-propanoic Acid (11).** (*S*)-Methyl 2-((*N*-*tert*-butoxycarbonyl)amino)-3-(3,5-dibromo-4-methoxyphenyl)propanoate[17] (1.3 g, 2.80 mmol), formaldehyde (37 wt % in $H_2O$, 3.0 mL, 40.30 mmol), and formic acid (2 mL, 53 mmol) were refluxed overnight, and then the solvents removed *in vacuo*. Addition of $H_2O$/EtOAc (50 mL/50 mL) resulted in a colorless precipitate, which was filtered, washed with copious amounts of $CHCl_3$, and then dried *in vacuo* to give a colorless solid (**11**, 0.94 g, 88%): mp 203−205 °C; $[\alpha]^{20}_D$ +70 (*c* 5.0, MeOH); IR (diamond) $\nu_{max}$ 3411 (OH), 1624 (C═O), $cm^{-1}$; $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.56 (2H, s, H-2,6), 3.71 (3H, s, Ar-$OCH_3$), 3.33 (1H, t, $J$ = 7.4 Hz, H-8), 2.85 (2H, dd, $J$ = 7.2, 7.5, 13.8, H-7), 2.27 (6H, s, $N(CH_3)_2$); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) $\delta$ 171.9 (C, $CO_2H$), 152.4 (C, C-4), 139.6 (C, C-1), 134.2 (CH, C-2,6), 117.7 (C, C-3,5), 69.3 (CH, C-8), 61.2 ($CH_3$, $ArOCH_3$), 42.0 ($CH_3$, $N(CH_3)_2$), 33.8 ($CH_2$, C-7); HRFABMS (NBA/PEG 400/600) $m/z$ 381.9482 $[M + H]^+$ (calcd for $C_{12}H_{16}{}^{79}Br^{81}BrNO_3$, 381.9476); anal. calcd for $C_{12}H_{15}Br_2NO_3$, C, 37.82; H, 3.97; N, 3.68; found C, 37.40; H, 3.88; N, 3.57.

**(*S*)-3-(3-Bromo-4-methoxyphenyl)-2-((*N*-*tert*-butoxycarbonyl)-amino)propanoic Acid (10c).** (*S*)-Methyl 3-(3-bromo-4-methoxyphenyl)-2-((*N*-*tert*-butoxycarbonyl)amino)propanoate[19] (2.00 g, 5.15 mmol) and NaOH (0.515 g, 0.012 mmol) were dissolved in THF/$H_2O$ (5 mL/5 mL) and stirred overnight at rt. The solvent was removed *in vacuo*, and dilute HCl (10 mL) was added to adjust the pH to 1, before extraction with EtOAc (2 × 50 mL). The combined organic portions were washed with dilute HCl (0.1 M, 25 mL), $H_2O$ (50 mL), and brine (50 mL), dried over anhydrous $Na_2SO_4$, and filtered, and finally the solvent was removed *in vacuo* to give a white solid (**10c**, 1.70 g, 89%): mp 167−169 °C; IR (diamond) $\nu_{max}$ 3332 (NH), 1709 (acid C═O), 1693 (carbamate C═O) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 10.0 (1H, br s, $CO_2H$), 7.29 (1H, d, $J$ = 2.1 Hz, H-2), 7.04 (1H, dd, $J$ = 2.1, 8.4 Hz, H-6), 6.76 (1H, d, $J$ = 8.4 Hz, H-5), 4.96 (1H, br s, H-8), 3.79 (3H, s, $ArOCH_3$), 3.08 (2H, m, H-7), 1.35 (9H, 2s, $OC(CH_3)_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 176.0 (C, $CO_2H$), 155.4, (C, C-4), 155.0 (C, $NHCO_2C(CH_3)_3$), 134.2 (CH, C-2), 129.6 (C, C-1), 129.4 (CH, C-6), 112.1 (CH, C-5), 111.7 (C, C-3), 80.5 (C, $OC(CH_3)_3$), 56.3 ($CH_3$, $ArOCH_3$), 54.3 (CH, C-8), 36.7 ($CH_2$, C-7), 28.3 ($CH_3$, $OC(CH_3)_3$); HRFABMS (NaI/PEG 400/600) $m/z$ 396.0424 $[M + Na]^+$ (calcd for $C_{15}H_{20}{}^{79}Br$-$NaNO_5$, 396.0423); anal. calcd for $C_{15}H_{20}BrNO_5$, C, 48.14; H, 5.39; N, 3.74; found C, 48.19; H, 5.37; N, 3.75.

**Aplyzanzine A (5).** A mixture of the acid **11** (0.186 g, 49 mmol), purpurealidin E (**2**, 0.188 g, 49 mmol), DCC (0.11 g, 54 mmol), HOBt (0.075 g, 49 mmol), and DIEA (0.3 mL, 147 mmol) in dry DMF (10 mL) was stirred at rt until no starting material remained by TLC (neutral $Al_2O_3$, EtOAc/MeOH, 9:1). Water (25 mL) was added and extracted with EtOAc (3 × 25 mL), the solution was washed with brine (25 mL) and dried over anhydrous $Na_2SO_4$, and the solvent was removed *in vacuo* to give a pale yellow gum. Column chromatography (neutral $Al_2O_3$, EtOAc/MeOH, 9:1) yielded a pale orange oil (**5**, 0.245 g, 66%): $[\alpha]^{20}_D$ +30 (*c* 5.0, MeOH); IR (diamond) $\nu_{max}$ 1648 (C═O) $cm^{-1}$; $^1H$, $^{13}C$ NMR and HRMS see Table 3.

**(*N*-*tert*-Butoxycarbonyl)suberedamine A (13a).** A mixture of acid (**10c**, 0.20 g, 5.00 mmol), purpurealidin E (**2**, 0.18 g, 5.00 mmol), EDCI (0.14 g, 7.00 mmol), HOBt (0.070 g, 5.00 mmol), and $Et_3N$ (0.60 mL, 15.00 mmol) in dry $CH_2Cl_2$ (10 mL) was stirred at rt until no starting material remained by TLC (neutral $Al_2O_3$, EtOAc/MeOH, 9:1). The reaction mixture was diluted with $H_2O$ (25 mL), extracted with EtOAc (3 × 25 mL), washed with brine (25 mL), and dried


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over anhydrous $Na_2SO_4$, and the solvent removed *in vacuo* to give a pale yellow gum. Column chromatography (neutral $Al_2O_3$, EtOAc/dry MeOH, 9:1) yielded a colorless gum (**13a**, 0.28 g, 74%): IR (diamond) $\nu_{max}$ 3332 (N−H), 1666 (C═O) $cm^{-1}$; $^1H$ NMR (300 MHz, $CD_3OD$) $\delta$ 7.32 (2H, s, H-13,17), 7.30 (1H, d, $J$ = 2.1 Hz, H-2), 7.04 (1H, dd, $J$ = 2.1, 8.4 Hz, H-6), 6.83 (1H, d, $J$ = 8.4 Hz, H-5), 4.10 (1H, q, $J$ = 6.0 Hz, H-8), 3.93 (2H, t, $J$ = 6.0 Hz, H-18), 3.72 (3H, s, Ar-OC$H_3$), 3.30 (2H, m, H-10), 3.24 (2H, m, H-10), 2.87 (2H, m, H-7) 2.64 (2H, m, H-20), 2.24 (6H, s, N(C$H_3$)$_2$), 1.97 (2H, m, H-19) 1.25 (9H, s, C(C$H_3$)$_3$); $^{13}C$ NMR (75 MHz, $CD_3OD$) $\delta$ 172.6 (C, CONH), 156.0 (C, $NHCO_2C(CH_3)_3$), 155.9 (C, C-4), 151.58 (C, C-15), 138.3 (C, C-12), 133.7 (CH, C-2), 132.9 (CH, C-13, 17), 131.0 (C, C-1), 129.2 (CH, C-6), 117.6 (C, C-14, 16), 112.2 (CH, C-5), 111.1 (C, C-3), 79.6 (C, OC$(CH_3)_3$), 71.3 ($CH_2$, C-18), 56.0 ($CH_2$, C-20), 55.6 ($CH_3$, O$CH_3$), 43.9 ($CH_3$, N(C$H_3$)$_2$), 40.0 (CH, C-8), 37.2 ($CH_2$, C-7), 33.8 ($CH_2$, C-11), 27.4 ($CH_3$, OC(C$H_3$)$_3$, 27.3 ($CH_2$, C-19); HRFABMS (PEG 400/600) $m/z$ 736.0439 $[M + H]^+$ (calcd for $C_{28}H_{39}{}^{79}Br_2{}^{81}BrN_3O_5$, 736.0421).

**Suberedamine A (6).** (*N-tert*-Butoxycarbonyl)suberedamine A (**13a**, 0.12 g, 16.00 mmol) and TFA (0.03 mL, 36.00 mmol) were stirred for 3 h in dry $CH_2Cl_2$ (5 mL). The solvent was then removed *in vacuo* to give a colorless gum. The gum was suspended in $CHCl_3$ (10 mL), washed with saturated $NaHCO_3$ (2 × 5 mL) and saturated NaCl (10 mL), dried over anhydrous $Na_2SO_4$, and filtered, and finally the solvent removed *in vacuo* to give a colorless solid (**6**, 0.095 g, 94%): mp 65−68 °C (lit. mp 64−67 °C[14d]); $[\alpha]^{20}_D$ +19.5 (*c* 1.0, MeOH), lit. $[\alpha]^{25}_D$ +21;[14d] IR (diamond) $\nu_{max}$ 2934, 2475, 1642, 1461 $cm^{-1}$; $^1H$, $^{13}C$ NMR and HRMS see Table 4.

**(*N-tert*-Butoxycarbonyl)suberedamine B (13b).** A mixture of the acid **12c** (1.00 g, 2.69 mmol), purpurealidin E (**2**, 1.02 g, 2.69 mmol), EDCI (0.77 g, 4.03 mmol), HOBt (0.36 g, 2.69 mmol), and $Et_3N$ (1.20 mL, 8.06 mmol) in dry THF (10 mL) was stirred at rt until no starting material remained by TLC ($Al_2O_3$, EtOAc/MeOH, 9:1). Water (25 mL) was added, and the mixture extracted with EtOAc (3 × 25 mL). The combined organic portions were washed with brine (25 mL), dried over anhydrous $Na_2SO_4$, and filtered, and the solvent was removed *in vacuo* to give a pale yellow gum. This was purified by column chromatography (neutral $Al_2O_3$ EtOAc/MeOH, 9:1) to yield a colorless gum (**13b**, 1.42 g, 72%): IR (diamond) $\nu_{max}$ 3329 (N−H), 1670 (C═O) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.31 (1H, d, $J$ = 1.8 Hz, H-2), 7.23 (2H, s, H-13,17), 7.06 (1H, br s, 1H, H-6), 6.76 (1H, d, $J$ = 8.4 Hz, H-5), 6.36 (1H, br s, N*H*), 4.64 (1H, br s, 1H, H-8), 4.01 (2H, t, $J$ = 5.4 Hz, H-18), 3.78 (3H, s, ArOC$H_3$), 3.38 (2H, br s, H-10), 3.26 (2H, m, H-20), 3.20 (1H, m, H-7), 2.80 (1H, m, H-7), 2.73 (6H, s, N(C$H_3$)$_2$), 2.63 (3H, s, N-C$H_3$), 2.56 (2H, m, H-11), 2.36 (2H, m, H-19), 1.31 (9H, br s, OC(C$H_3$)$_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.5 (C, CONH), 154.6 (C, $NHCO_2C(CH_3)_3$), 151.0 (C, C-4), 138.2 (C, C-15), 133.7 (CH, C-2), 133.0 (CH, C-13, 17), 131.2 (C, C-1), 129.1 (CH, C-6), 118.0 (C, C-14, 16), 112.0 (CH, C-5), 111.5 (C, C-3), 80.8 (C, OC$(CH_3)_3$), 69.92 ($CH_2$, C-18), 60.4 (CH, C-8), 56.3 ($CH_3$, O$CH_3$), 55.9 ($CH_2$, C-20), 43.4 ($CH_3$, N-(C$H_3$)$_2$), 40.3 ($CH_2$, C-10), 34.5 ($CH_2$, C-7), 33.0 ($CH_2$, C-11), 31.1 ($CH_3$, N-C$H_3$), 28.3 ($CH_3$, OC(C$H_3$)$_3$), 25.7 ($CH_2$, C-19); HRFABMS (NaI/PEG 600) $m/z$ 750.0567 $[M + H]^+$ (calcd for $C_{29}H_{41}{}^{79}Br_2{}^{81}BrN_3O_5$, 750.0578).

**Suberedamine B (7).** TFA (0.09 mL, 12.26 mmol) was added dropwise to (*N-tert*-butoxycarbonyl)suberedamine B (**13b**, 0.45 g, 6.13 mmol) in dry $CH_2Cl_2$ (5 mL) at 0 °C. The reaction mixture was stirred for 12 h; then the solvent was removed *in vacuo* to give a colorless gum. The gum was suspended in $CHCl_3$ (10 mL), washed with saturated $NaHCO_3$ solution (2 × 5 mL), then brine (10 mL), dried over anhydrous $Na_2SO_4$, and filtered, and the solvent was removed *in vacuo* to give a colorless gum (**7**, 0.36 g, 95%): $[\alpha]^{20}_D$ +15.5 (*c* 1.0, MeOH), lit. $[\alpha]^{25}_D$ +16.0;[14d] IR (diamond) $\nu_{max}$ 1666 (C═O) $cm^{-1}$; $^1H$, $^{13}C$ NMR and HRMS see Table 5.

**(*N-tert*-Butoxycarbonyl)-*iso*-anomoian A (13c).** A mixture of the acid (**12d**, 0.80 g, 1.77 mmol), purpurealidin E (**2**, 0.67 g, 1.77 mmol), EDCI (0.51 g, 2.66 mmol), HOBt (0.24 g, 1.77 mmol), and $Et_3N$ (0.80 mL, 5.32 mmol) in dry THF (10 mL) was stirred at rt until no starting material remained by TLC ($Al_2O_3$, EtOAc/MeOH, 9:1). The reaction mixture was diluted with water (25 mL) and extracted with EtOAc (3 × 25 mL). The combined organic portions were washed with brine (25 mL), dried over anhydrous $Na_2SO_4$, and filtered, and the solvent was removed *in vacuo* to give a pale yellow gum. Purification by column chromatography (neutral $Al_2O_3$ EtOAc/MeOH, 9:1) gave a colorless gum (**13c**, 0.81 g, 56%); IR (diamond) $\nu_{max}$ 1670 (C═O) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.27 (2H, s, H-13,17), 7.23 (2H, s, H-2,6), 4.67 (1H, br s, H-8), 3.98 (2H, t, $J$ = 6.3 Hz, H-18), 3.77 (3H, s, 3H, OC$H_3$), 3.55 (2H, m, H-10), 3.35 (2H, m, H-20), 3.12 (1H, m, H-7), 2.81 (1H, m, H-7), 2.62 (3H, s, N-C$H_3$), 2.55 (2H, m, H-11), 2.23 (6H, s, N(C$H_3$)$_2$), 2.03 (2H, tt, $J$ = 6.3, 9.9 Hz, H-19), 1.39 (9H, s, C(C$H_3$)$_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.1 (*C*-9), 152.8 (C, $NHCO_2C(CH_3)_3$), 152.1 (C, C-4), 138.2 (C, C-15), 137.3 (C, C-12), 136.5 (C, C-1), 133.1 (CH, C-2, 6), 132.8 (CH, C-13, 17), 118.1 (C, C-3, 5), 118.0 (C, C-14, 16), 81.1 (C, OC$(CH_3)_3$), 71.9 ($CH_2$, C-18), 60.6 ($CH_3$, O$CH_3$), 59.5 (CH, C-8), 56.4 ($CH_2$, C-20), 45.4 ($CH_3$, N(C$H_3$)$_2$), 40.5 ($CH_2$, C-10), 34.5 ($CH_2$, C-7), 32.9 ($CH_2$, C-11), 31.0 ($CH_3$, NC$H_3$), 29.72 ($CH_3$, OC(C$H_3$)$_3$), 28.24 ($CH_2$, *C*-19); HRFABMS (NaI/PEG 600/900/1500) $m/z$ 829.9689 $[M + H]^+$ (calcd for $C_{29}H_{40}{}^{79}Br_2{}^{81}Br_2N_3O_5$, 829.9664).

***iso*-Anomoian A (4).** TFA (0.80 mL, 11.08 mmol) was added dropwise to (*N-tert*-butoxycarbonyl)-*iso*-anomoian A (**13c**, 0.45 g, 5.54 mmol) in dry $CH_2Cl_2$ (5 mL) at 0 °C. The mixture was stirred for 12 h; then the solvent was removed *in vacuo* to give a colorless gum. The gum was suspended in $CHCl_3$ (10 mL), washed with a saturated $NaHCO_3$ solution (2 × 5 mL) and saturated NaCl (10 mL), dried over anhydrous $Na_2SO_4$, and filtered, and the solvent was removed *in vacuo* to give a colorless gum (**4**, 0.32 g, 81%): $[\alpha]^{20}_D$ +4.5 (*c* 1.0, MeOH), lit. $[\alpha]^{25}_D$ +5.1;[14b] IR (diamond) $\nu_{max}$ 1669 (C═O) $cm^{-1}$; $^1H$, $^{13}C$ NMR and HRMS see Table S2.

**Determination of Antibacterial and Antifungal Activities.** For each putative antimicrobial, determination of the antimicrobial susceptibility was performed in strict accordance with the recommendations of the British Society for Antimicrobial Chemotherapy. Isosensitest agar (Oxoid) was prepared according to the manufacturer's instructions and sterilized by autoclaving at 116 °C for 20 min. This was then cooled to 50 °C in a water bath. A 10 mg sample of each antimicrobial was dissolved in 1 mL of DMSO. A 256 μL aliquot was added to 19.744 mL of molten Isosensitest agar at 50 °C and mixed well. This was poured into a Petri dish to produce a culture plate containing a final concentration of 128 μg/mL. Smaller volumes of solution (in the range 128−2 μL) were also incorporated into agar plates in a similar fashion to produce a final concentration range of 128−1 μg/mL. A set of control plates was prepared containing DMSO at an identical concentration range without antimicrobial.

A panel of 20 microorganisms was obtained from the National Collection of Type Cultures (NCTC), Colindale, UK, the National Collection of Pathogenic Fungi (NCPF), Colindale, UK, and the American Type Culture Collection (ATCC), Manassas, VA, USA. The panel included a range of pathogenic species and comprised 10 Gram-negative bacteria, eight Gram-positive bacteria, and two pathogenic yeasts (Table S1). Each strain was cultured onto Columbia agar (Oxoid) and incubated overnight at 37 °C. Colonies were then suspended in sterile distilled water (SDW) to produce a suspension of 1.5 × $10^8$ colony forming units (cfu)/mL using a densitometer. This suspension was then diluted 1/15 in SDW, and 1 μL was inoculated onto all test media using a semiautomated multipoint inoculator (final inoculum: 10 000 cfu per spot). All media were incubated at 37 °C for 24 h and examined for the presence of growth. The minimum inhibitory concentration of each compound was recorded as the lowest concentration to completely inhibit visible growth. All tests were repeated on a separate occasion to ensure reproducibility. All of the test strains were able to grow on the test medium at all concentrations of solvent, thus allowing interpretation of MICs.

**Determination of Antimycobacterial Activity.** *M. bovis* BCG was grown in 100 mL roller bottles in a 37 °C incubator, with rolling at 2 rpm in Middlebrook 7H9 medium supplemented with 10% (v/v) albumin-dextrose-catalase (ADC; BD) and 0.05% Tween 80 until the

midexponential phase ($1OD_{600}$). *M. tuberculosis* H37Rv was grown as a standing culture in 30 mL unbreakable universals in a 37 °C incubator, containing Middlebrook 7H9 medium supplemented with 10% (v/v) ADC and 0.05% Tween-80 until the midexponential phase (McFarland standard 1).

For the quality control of the mycobacterial cultures, *M. bovis* BCG was stained with a modified Ziehl–Neelsen staining protocol using a Tb-color kit (Bund Deutscher Hebammen Laboratory) according to the manufacturer's procedure, and *M. tuberculosis* H37Rv was grown on a blood agar plate in order to detect contamination.

For the spot-culture inhibition assay, mycobacterial cultures were serially diluted up to $10^3$ cfu/mL. Then, 10 μL of that culture was spotted in 5 mL of Middlebrook 7H10 agar medium, supplemented with 10% (v/v) oleic acid-albumin-dextrose-catalase (OADC; BD) in a six-well plate containing various concentrations of compounds, and incubated at 37 °C for 2 weeks. A well containing DMSO (5 μL) instead of compound was used as a negative control and isoniazid as a positive control. The MIC was determined as the lowest concentration at which there is no growth of mycobacteria in the well.

***In Vitro* Antitumor Assay.** Tests of toxicity on NCI60, a set of 60 human cancer cell lines derived from nine tissue types, were performed at the Developmental Therapeutics Program (DTP) of the U.S. National Cancer Institute. The cytotoxicity of orthotopolin riboside was evaluated by measuring total cell protein using the sulforhodamine B method according to the standard DTP protocol (http://dtp.nci.nih.gov/docs/compare/compare_methodology.html) at both time 0 and after 48 h. $GI_{50}$ values (concentration of a drug inducing 50% reduction of growth) were estimated from the dose–response curves.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

$^1H$, $^{13}C$, and 2D NMR spectra (COSY, HMBC, and HMQC) of compounds **2**–**8**, **10c**, **11**, **12a**–**d**, **13a**–**c** associated with this article are available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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