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Total syntheses of the bromotyrosine-derived natural products ianthelline, 5-bromoverongamine and JBIR-44

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ABSTRACT

The total syntheses of the bromotyrosine-derived natural products ianthelline, 5-bromoverongamine and JBIR-44 are described and their cytotoxic activity in a cervical cancer (HeLa) cell line and human umbilical vein endothelial cells (HUVECs) are reported.

The *Verongida* order of marine sponges is a rich source of compounds that are characterised by tyrosine metabolites where dibromotyrosine is a signature motif.[1] Such compounds have been found to act as antimicrobials,[2] antifoulants,[3] antifungals[4] and antibacterials with varying degrees of potency.[5] In terms of broad anticancer activity, a large number of these compounds are cytotoxic and antiproliferative.[6] However, some have been identified as having specific antitubulin,[7] antiangiogenic[8] and protein inhibitory[9] activity. For example, ceratamine A (**1**) and B (**2**), isolated from *Pseudoceratina* sp. in 2003,[7a] were found to have antimitotic properties against a breast cancer (MCF-7) cell line (IC_{50} 10 μg/mL) (Fig. 1). The treatment of MCF-7 cells with **1** also resulted in an unusual bundling of cellular microtubules around cell nuclei.[7b] Other related compounds such as the bisulfide mono-bromotyrosine derivatives bisaprasin and psammaplin A act as dual inhibitors of histone deacetylase (HDAC) and DNA methyltransferase in the low nanomolar range,[9a] and aplysamine-6 is one of a few small molecules to be discovered that inhibits, in the low micromolar range, the relatively new cancer target isoprenylcysteine carboxyl methyltransferase (Icmt).[9b] In summary, bromotyrosine-derived natural products are interesting chemical starting points for exploring potential anticancer activities.

The anticancer activity of the three dibromotyrosine compounds ianthelline (**3**), 5-bromoverongamine (**4**) and JBIR-44 (**5**) (Fig. 1) has largely been unexplored owing to the small amount of material obtained from the natural sources.

To enable access to all the three compounds a convenient synthetic strategy involving a common α-oximino acid intermediate **6** was envisaged which would provide sufficient material for preliminary assays (Fig. 2). In previous reports ianthelline (**3**) (isolated from *Ianthella ardis*, 1986) had been found to be moderately active

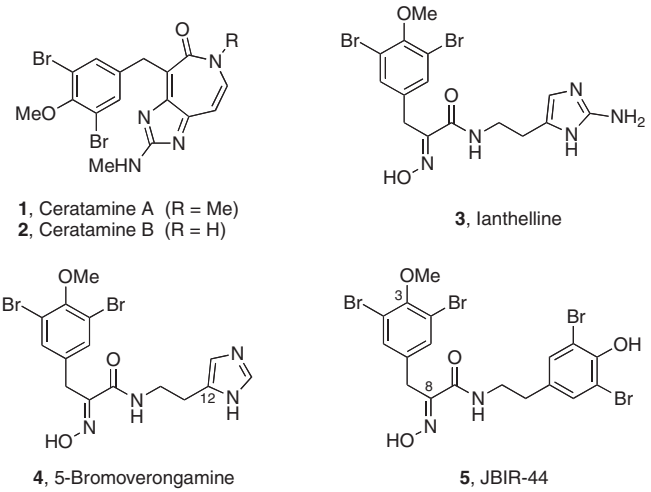

Figure 1. Structures of ceratamine A and B, ianthelline, 5-bromoverongamine and JBIR-44.

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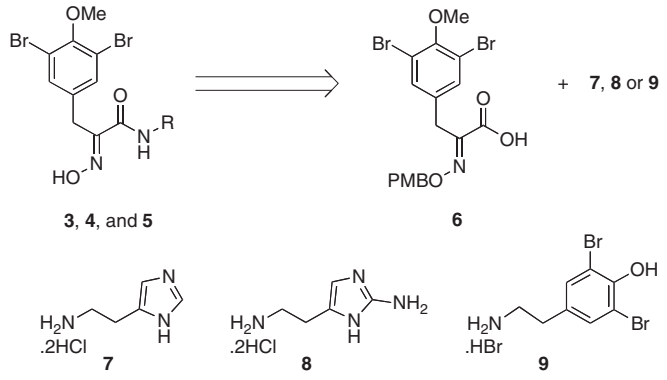
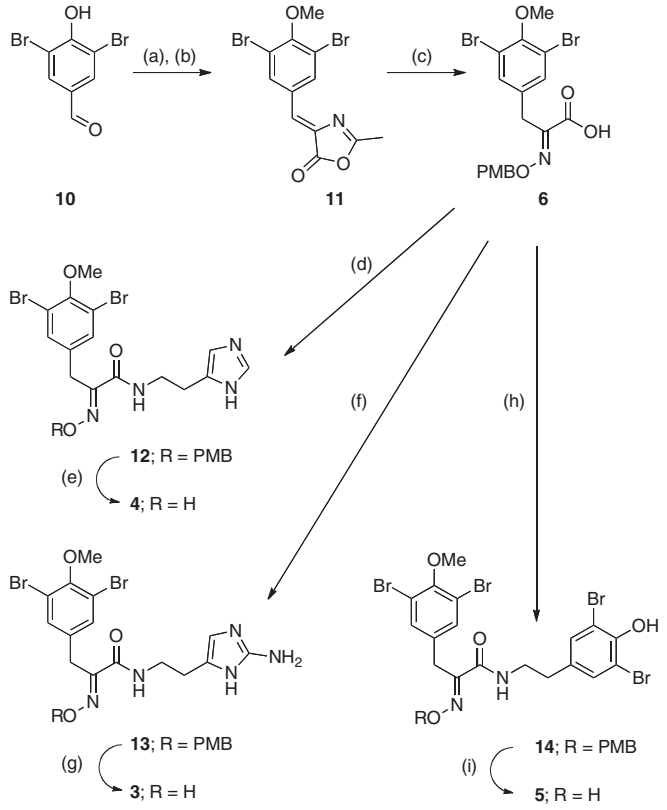


Figure 2. Retrosynthetic analysis for the proposed synthesis of **3**, **4** and **5** involving a common α-oximino acid intermediate **6**.

as an antibacterial and antifungal agent against *Staphylococcus aureus* and *Candida albicans*.[10] 5-Bromoverongamine (**4**) (isolated from *Pseudoceratina* sp., 1998) had been reported to inhibit the settlement of barnacle larvae at 10 mg/mL (EC_{50} 1.03 mg/mL)[11] and was bactericidal towards methicillin-resistant *S. aureus* (MRSA) (MIC 0.0625–0.5 mg L^{-1}).[12] Both **3** and **4** are purported to share a biogenetic relationship to the ceratamines and can be envisioned as open chain cyclisation precursors. JBIR-44 (**5**) (isolated from *Psammaplysilla purpurea*, 2009) was active against a cervical cancer cell line (HeLa, IC_{50} 3.7 μM).[13]

Synthetic routes to α-oximino acids and esters closely related to **6** have been widely discussed in the literature. In addition to the most common approach via an azlactone intermediate,[14] other pathways include cyano ylide couplings[15] and conversion of either amines[16] or α-keto compounds[17] directly to their corresponding oxime. One convenient approach adopts a Horner–Wadsworth–Emmons reaction to couple a functionalised dimethylphosphate to an aldehyde which has been used to good effect in the synthesis of purealidin N[18] and bastadin analogues.[19] Full details of these methods are discussed in a recent review.[1a]



Scheme 1. Synthetic routes to ianthelline (**3**), 5-bromoverongamine (**4**) and JBIR-44 (**5**). Reagents and conditions: (a) MeI, K_2CO_3, acetone, reflux 6 h; (b) *N*-acetylglycine, NaOAc, Ac_2O, 120 °C, 6 h (82% over two steps); (c) $Ba(OH)_2{\cdot}8H_2O$, 1,4-dioxane/H_2O (1:1) 60 °C, 1 h then PMBO–NH_2·HCl, 14 h (54%); (d) DCC, HOBt, **7**, *i*Pr_2EtN, CH_2Cl_2 (81%); (e) $AlCl_3$, anisole, CH_2Cl_2, 5 min, (78%); (f) DCC, HOBt, **8**, *i*Pr_2EtN, CH_2Cl_2/DMF, (41%); (g) $AlCl_3$, anisole, CH_2Cl_2, 5 min (44%); (h) DCC, HOBt, **9**, *i*Pr_2EtN, CH_2Cl_2 (42%); (i) $AlCl_3$, anisole, CH_2Cl_2, 5 min (85%).

As discussed above, access to all the three natural products **3**, **4** and **5** was envisaged by elaborating the α-oximino acid derivative **6** (Fig. 2). Amide coupling of **6** with the amine salts **7**, **8** or **9** followed by the removal of the *O-para* methoxybenzyl (PMB) group would furnish the desired bromotyrosine derivatives.

The α-oximino acid intermediate **6** was accessed in three steps from the commercially available 3,5-dibromo-4-hydroxybenzaldehyde **10** (Scheme 1). Methylation of the phenol group of **10** with methyl iodide under refluxing conditions[20] and conversion of the product, after isolation, to the corresponding azlactone **11** was achieved by the treatment with *N*-acetylglycine and sodium acetate in acetic anhydride (Erlenmeyer conditions).[21] These two steps proceeded in an overall yield of 82% and the geometry of **11** was confirmed by X-ray crystallographic analysis to be the thermodynamically favoured *Z*-isomer.[22] The one-pot saponification of the azlactone **11** with barium hydroxide octahydrate and subsequent condensation with *O-para* methoxybenzyl hydroxylamine[23] afforded the oxime acid intermediate **6** on gram-scale in 54% yield. The oxime acid **6** was then coupled with the appropriate amine (**7**, **8**[23] or **9**[24]) using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt) to furnish the *O*-PMB-protected oximes **12**, **13** and **14**, respectively. Removal of the PMB group with $AlCl_3$ and anisole[14b] proceeded smoothly to furnish the bromotyrosine-derived products ianthelline (**3**), 5-bromoverongamine (**4**) and JBIR-44 (**5**) in 78%, 44% and 85% yield, respectively.

The ^1H and ^{13}C NMR spectra for synthetic ianthelline (**3**) and JBIR-44 (**5**) matched those of the natural materials. However, in the case of synthetic **5** the original assignments of C-8 (δ_C 153.8) and C-3 (δ_C 152.0) should be reversed on the basis of HMBC spectroscopy (see Supplementary data).[25] In addition, whilst the ^1H NMR spectrum of synthetic 5-bromoverongamine (**4**) agreed with that of the natural material, the assignment of the C-12 quaternary centre (δ_C 134.0) in the natural material did not correlate with the shift of C-12 (δ_C 135.9) in the synthetic material.[26] However, X-ray crystallographic analysis confirmed that the structure of synthetic **4** matched with that proposed for the natural material (Fig. 3). Furthermore, this data also confirmed the *E*-geometry of the oxime and the tautomeric form of the imidazole.[27]

In the MTS assays[28] that were conducted (Table 1), JBIR-44 (**5**) was found to be cytotoxic against HeLa cells (IC_{50} 14 μM). This was within the range reported for the natural material against the same cell line (IC_{50} 3.7 μM). Ianthelline (**3**) was moderately

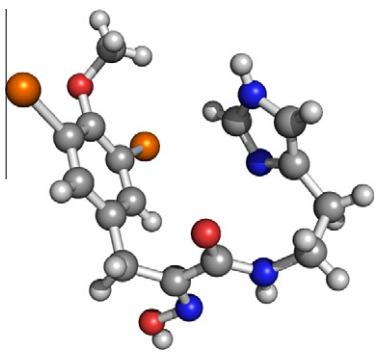


Figure 3. X-ray structure of 5-bromoverongamine (**4**).

Table 1
Cytotoxic activities of natural products **3**, **4**, and **5** against HeLa and HUVEC cell lines

Compound	Cell line [IC_{50} (μM)]	
	HeLa	HUVEC
Ianthelline (**3**)	35	74
5-Bromoverongamine (**4**)	>50	36
JBIR-44 (**5**)	14	24

cytotoxic (IC_{50} 35 μM) and 5-bromoverongamine (**4**) was the least active (IC_{50} >50 μM). Since the related dibromotyrosine derivative aeroplysinin-1 has been reported to inhibit the proliferation of the bovine arterial endothelial cells (IC_{50} ~2 μM),[29] it was also of interest to investigate the activity against endothelial cells. To this end, an xCELLigence assay using human umbilical vein endothelial cells (HUVECs) was conducted and all the three compounds (Table 1) were found to be cytotoxic to HUVECs in the micromolar range (IC_{50} 24–74 μM).

In conclusion, the total syntheses of the bromotyrosine-derived products ianthelline (**3**), 5-bromoverongamine (**4**) and JBIR-44 (**5**), obtained in five steps from the commercially available aldehyde **10**, have been reported. Ianthelline and JBIR-44 exhibit anticancer activity in HeLa cells and all the three compounds were cytotoxic towards HUVECs. In addition, this short synthetic route will allow for rapid development of analogues to explore structure–activity relationships.

Acknowledgements

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Supplementary data

Supplementary data (synthesis procedures, spectral data, ^{13}C NMR for natural and synthetic **4**, HMBC spectra for synthetic **5**, biological assay protocols) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.07.016.

References and notes

1. (a) Hentschel, F.; Lindel, T. *Synthesis* **2010**, 181–204; (b) Fusetani, N.; Masuda, Y.; Nakao, Y.; Matsunaga, S.; van Soest, R. W. M. *Tetrahedron* **2001**, *57*, 7507–7511; (c) Benharref, A.; Païs, M.; Debitus, C. *J. Nat. Prod.* **1996**, *59*, 177–180; (d) Aiello, A.; Fattorusso, E.; Menna, M.; Pansini, M. *Biochem. Syst. Ecol.* **1995**, *23*, 377–381; (e) Albrizio, S.; Ciminiello, P.; Fattorusso, E.; Magno, S.; Pansini, M. *Tetrahedron* **1994**, *50*, 783–788; (f) Kernan, M. R.; Cambie, R. C.; Bergquist, P. R. *J. Nat. Prod.* **1990**, *53*, 615–622.
2. Pick, N.; Rawat, M.; Arad, D.; Lang, J.; Fan, J.; Kende, A. S.; Av-Gay, Y. *J. Med. Microbiol.* **2006**, *55*, 407–415.
3. (a) Schoenfeld, R. C.; Ganem, B. *Tetrahedron Lett.* **1998**, *39*, 4147–4150; (b) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1996**, *52*, 181–186; (c) Ortlepp, S.; Sjögren, M.; Dahlström, M.; Weber, H.; Ebel, R.; Edrada, R.; Thoms, C.; Schupp, P.; Bohlin, L.; Proksch, P. *Marine Biotech.* **2007**, *9*, 776–785.
4. Jang, J. –H.; van Soest, R. W. M.; Fusetani, N.; Matsunaga, S. *J. Org. Chem.* **2007**, *72*, 1211–1217.
5. Takada, N.; Watanabe, R.; Suenaga, K.; Yamada, K.; Ueda, K.; Kita, M.; Uemura, D. *Tetrahedron Lett.* **2001**, *42*, 5265–5267.
6. (a) Hernández–Guerrero, C. J.; Zubía, E.; Ortega, M. J.; Luis Carballo, J. *Bioorg. Med. Chem.* **2007**, *15*, 5275–5282; (b) Shinde, P. B.; Lee, Y. M.; Dang, H. T.; Hong, J.; Lee, C.-O.; Jung, J. H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6414–6418.
7. (a) Manzo, E.; van Soest, R.; Matainaho, L.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2003**, *5*, 4591–4594; (b) Karjala, G.; Chan, Q.; Manzo, E.; Andersen, R. J.; Roberge, M. *Cancer Res.* **2005**, *65*, 3040–3043; (c) Coleman, R. S.; Campbell, E. L.; Carper, D. J. *Org. Lett.* **2009**, *11*, 2133–2136.
8. Córdoba, R.; Salvador Tormo, N.; Medarde, A. F.; Plumet, J. *Bioorg. Med. Chem.* **2007**, *15*, 5300–5315.
9. (a) Piña, I. C.; Gautschi, J. T.; Wang, G. Y.; Sanders, M. L.; Schmitz, F. J.; France, D.; Cornell–Kennon, S.; Sambucetti, L. C.; Remiszewski, S. W.; Perez, L. B.; Bair, K. W.; Crews, P. *J. Org. Chem.* **2003**, *68*, 3866–3873; (b) Buchanan, M. S.; Carroll, A. R.; Fechner, G. A.; Boyle, A.; Simpson, M.; Addepalli, R.; Avery, V. M.; Hooper, J. N. A.; Cheung, T. H. C.; Quinn, R. J. *J. Nat. Prod.* **2008**, *71*, 1066–1067.
10. Litaudon, M.; Guyot, M. *Tetrahedron Lett.* **1986**, *27*, 4455–4456.
11. Thirionet, I.; Daloze, D.; Braekman, J. C.; Willemsen, P. *Nat. Prod. Lett.* **1998**, *12*, 209–214.
12. Kottakota, S. K.; Harburn, J. J.; O'Shaughnessy, A.; Gray, M.; Konstanidis, P.; Yakubu, D. E. 13th International Electronic Conference on Synthetic Organic Chemistry (ESOC-13), 1–30 November, 2009.
13. Fujiwara, T.; Hwang, J. H.; Kanamoto, A.; Nagai, H.; Takagi, M.; Shinya, K. *J. Antibiot.* **2009**, *62*, 393–395.
14. (a) Nishiyama, S.; Yamamura, S. *Bull. Chem. Soc. Jpn.* **1985**, 3453–3456; (b) Zhu, G.; Yang, F.; Balachandran, R.; Höök, P.; Vallee, R. B.; Curran, D. P.; Day, B. W. *J. Med. Chem.* **2006**, *49*, 2063–2076.
15. Wasserman, H. H.; Wang, J. *J. Org. Chem.* **1998**, *63*, 5581–5586.
16. García, J.; Pereira, R.; de Lera, A. R. *Tetrahedron Lett.* **2009**, *50*, 5028–5030.
17. Godert, A. M.; Angelino, N.; Woloszynska-Read, A.; Morey, S. R.; James, S. R.; Karpf, A. R.; Sufrin, J. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3330–3333.
18. Boehlow, N. T. R.; Harburn, J.; Spilling, C. D. *J. Org. Chem.* **2001**, *66*, 3111–3118.
19. Bailey, K. L.; Molinski, T. F. *Tetrahedron Lett.* **2002**, *43*, 9657–9661.
20. Okamoto, K. J.; Clardy, J. *Tetrahedron Lett.* **1987**, *28*, 4969–4972.
21. Erlenmeyer, E. *Justus Liebigs Ann. Chem.* **1893**, *275*, 1–8.
22. *Crystal data for* **11**: $C_{12}H_9Br_2NO_3$, M = 375.02, monoclinic, space group $P2(1)/c$, a = 11.5810(3) Å, b = 14.3132(3) Å, c = 7.6379(2) Å, V = 1261.35(8) Å^3, Z = 4, D_c = 1.975 Mg m^{-3}, T = 180(2) K. CCDC 778410.
23. Prepared following the procedure described in Yang, F. Ph.D. Thesis, University of Pittsburgh, 2006.
24. Prepared following the procedure described in Ullah, N.; Arafeh, K. M. *Tetrahedron Lett.* **2009**, *50*, 158–160.
25. For the natural sample, the authors report a strong *m*-coupling between aromatic protons H-1 and H-5 (δ_H 7.52) and the aromatic carbon (δ_C 152.0) and also observe the long range coupling of methylene protons H–7 (δ_H 3.87) to C-8 (δ_C 153.8). However, in the synthetic sample, we observe a strong *m*-coupling between aromatic protons H-1 and H-5 (δ_H 7.52) and the signal at (δ_C 153.8) which we have assigned to C-3. There is also a long range coupling of methylene protons H-7 (δ_H 3.87) to the signal at (δ_C 152.0) which we have assigned to C-8. The HMBC spectra for the natural sample are not available for direct comparison.
26. The original ^{13}C NMR spectrum for the natural material was kindly provided by Professor Jean Claude Braekman for comparison and is included in the Supplementary data.
27. *Crystal data for* **4**: $C_{15}H_{16}Br_2N_4O_3$, M = 460.14, monoclinic, space group $P2(1)/n$, a = 8.9668(2) Å, b = 21.2760(3) Å, c = 9.3347(2) Å, V = 1752.45(6) Å^3, Z = 4, D_c = 1.744 Mg m^{-3}, T = 180(2) K. CCDC 777660.
28. The (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) (MTS) assay gives a colourimetric measure of the number of viable cells present in vitro.
29. Rodríguez-Nieto, S.; González-Iriarte, M.; Carmona, R.; Muñoz-Chápuli, R.; Medina, M. A.; Quesada, A. R. *FASEB J.* **2002**, *16*, 261–263.