Journal of Medicinal Chemistry Article

J. Med. Chem. **XXXX,** *XXX,* 000–000 **A**
DOI: 10.1021/jm900544z

Total Synthesis and Antiproliferative Activity Screening of (±)-Aplicyanins A, B and E and Related Analogues†

Miroslav Šíša,[‡,⊥] Daniel Pla,[‡,§] Marta Altuna,[‡] Andrés Francesch,[∥] Carmen Cuevas,[∥] Fernando Albericio,*[,‡,§,#] and Mercedes Álvarez*[,‡,§,▽]

[‡]*Institute for Research in Biomedicine, Barcelona Science Park—University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain,* [§]*CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Baldiri Reixac 10, E-08028 Barcelona, Spain, and* [∥]*Pharma Mar S.A., Avenida de los Reyes 1, E-28770 Colmenar Viejo, Madrid, Spain.* [⊥]*Current address: Academy of Sciences of the Czech Republic, Palacky University & Institute of Experimental Botany, Šlechtitelu 11, 783 71 Olomouc, Czech Republic.* [#]*Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.* [▽]*Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona, Spain.*

Received April 28, 2009

The first total synthesis of the indole alkaloids (±)-aplicyanins A, B, and E, plus 17 analogues, all in racemic form, is reported. Modifications to the parent compound included changing the number of bromine substituents on the indole, the nature of the substituents on the indole nitrogen (H, Me, or OMe), and/or the oxidation level of the heterocyclic core tetrahydropyrimidine. Each compound was screened against three human tumor cell lines, and 14 of the newly synthesized compounds showed considerable cytotoxicity. The assay results were used to establish structure−activity relationships. These results suggest that the presence of the bromine at position 5 of the indole is critical to activity, as well as the acetyl group on the imine nitrogen does in some compounds.

Introduction

Marine invertebrates such as sponges, tunicates, ascidians, and corals have provided a rich arsenal of new bioactive compounds. The unprecedented structures of these molecules make them excellent synthetic targets, and their potent activity against a broad number of therapeutic indications make these natural products excellent drug lead candidates.[1] A new family of six indole alkaloids, the aplicyanins, was recently isolated from the ascidian *Aplidium cyaneum*.[2] They are cytotoxic to the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A549 (lung carcinoma), and HT-29 (colorectal carcinoma) and also exhibit antimitotic activity.[2] The cellular arrest in mitosis may involve interaction of the drug to either tubulin or in microtubules formation, which usually leads to apoptosis.[3] All aplicyanins contain a 3-(2-amino-1,4,5,6-tetrahydropyrimidin-4-yl)-5-bromoindole nucleus but differ in their respective amino substituents (R^1 = H or Ac), their *N*-indole substituents (R^2 = H or OMe) and in whether or not they contain a second bromine atom at the 6-position of indole (R^3 = H or Br). Some aplicyanins structural traits, namely a six-membered cyclic guanidine (2-amino-1,4,5,6-tetrahydropyrimidin-4-yl) and/or a *N*-methoxyindole, are singular features. This cyclic guanidine is only present in very few natural products, all of which are peptides isolated from extracts of *Streptomyces* sp. (e.g., muraymycins A1−D3[4] and chymostatinols A−C).[5] To the best of our knowledge, aplicyanins are the first marine natural products known to contain this moiety.[6] However, similar compounds sharing a common 3-(pyrimid-4-yl)indole structure are more common and have been isolated from different marine invertebrates and characterized. These include meridianins A−G, from the tunicate *Aplidium meridianum*,[7] the psammopemmins, from an Antarctic marine sponge of the genus *Psammopemma*,[8] and the closely related, but more complex structures, variolins A−D,[9,10] from the Antarctic sponge *Kirkpatrickia varialosa* (Figure 1). Furthermore, the 1-methoxyindole found in some aplicyanins is unprecedented among known marine alkaloids.[11]

Last, given the high cytotoxicity typical of bromoindole derivatives, the presence of a bromoindole in some aplicyanins warrants their investigation as anticancer drugs.[12] Herein is reported the first total synthesis of (±)-aplicyanins A, B, and E and 17 analogues. The analogues differ in the nature of the substituents of the indole nucleus (H and/or Br), of the substituent the indole nitrogen (H, Me, or OMe), and in the oxidation level of the six-member heterocyclic core (2-amino-1,4,5,6-tetrahydropyrimidine or 2-amino-5,6-dihydro-4-pyrimidone). The compounds were screened for cytotoxicity against three human tumor cell lines: A-549, HT-29, and MDA-MB-231. Structure−activity relationships (SAR) were established based on the screening results.

Results and Discussion

Chemistry. Initial attempts to the synthesis of aplicyanins were based on introduction of a three-carbon chain at position 3 of the appropriately substituted indole. The chain had to be adequately functionalized for construction of the 2-amino-1,4,5,6-tetrahydropyrimidine ring. This was tested using two different strategies (Schemes 1 and 2). The

†Dedicated to Professor Peter Stanetty on the occasion of his 65th anniversary.

*Corresponding author: For M.A. and F.A.: phone, +34934037086; fax, +34934037126; E-mail, mercedes.alvarez@irbbarcelona.org.

substituted indoles **1** were either commercially available or prepared by conventional procedures.

Wittig chemistry (Scheme 1) was employed to introduce the chain, starting from the aldehydes **1a**−**d** and the Wittig ylide (**2x**) or phosphonate (**2y**). Addition of guanidine to the β-position of the conjugated double bond and further intramolecular reductive cyclization was the original plan. The *E* stereoisomer was obtained in both cases; however, the stereochemistry of the double bond was irrelevant, as it would ultimately be lost in the subsequent conjugate addition.

Compounds **3a** (71% yield) and **3b** (65% yield) were obtained from **2x** and the aldehydes **1a** or **1b**,[13,14] respectively (Scheme 1). Interestingly, the ethylene acetal was cleaved during silica gel column purification of each compound, providing the corresponding α,β-unsaturated aldehydes in good yield. After different attempts at a tandem Michael cyclization reaction, the product of the reductive guanidylation compound (**4a**) was only obtained in 20% yield.

The esters **3c**−**e** were obtained in excellent yields by Horner−Wadsworth−Emmons reaction of **1c**−**d**[15] or **1a** and the ethyl phosphonate **2y** (Cs_2CO_3 as a base, dioxane, 70 °C).[16] However, the guanidine chemistry again failed: when ester **3c** was reacted with guanidine in methoxyethanol under microwave irradiation, the only product observed was the acylguanidine **4c**.[17] On the basis of these results, it was decided not to continue with the indolyl acrylates **3d** and **3e**. A survey of the literature reveals that the condensation of α,β-unsaturated esters with guanidine to give tetrahydropyrimidones only involve α,β-unsaturated esters containing an aryl group bearing electron withdrawing substituents at the β-position.[18]

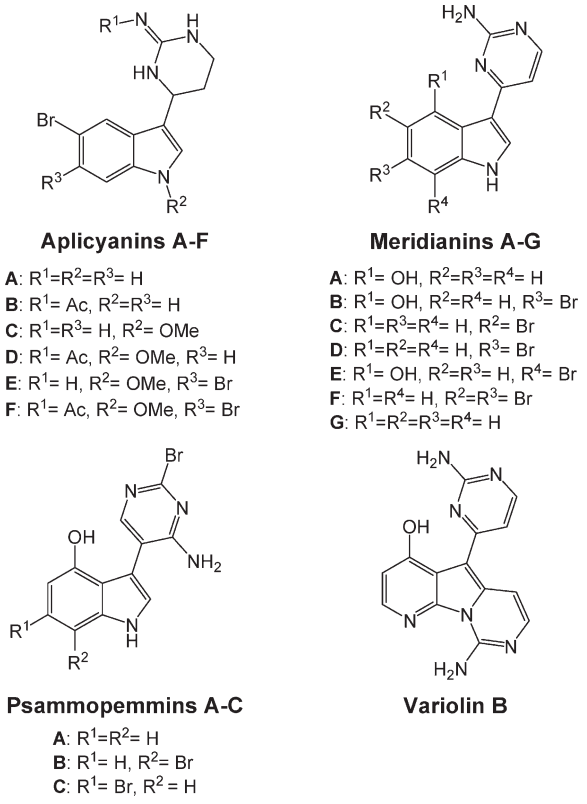
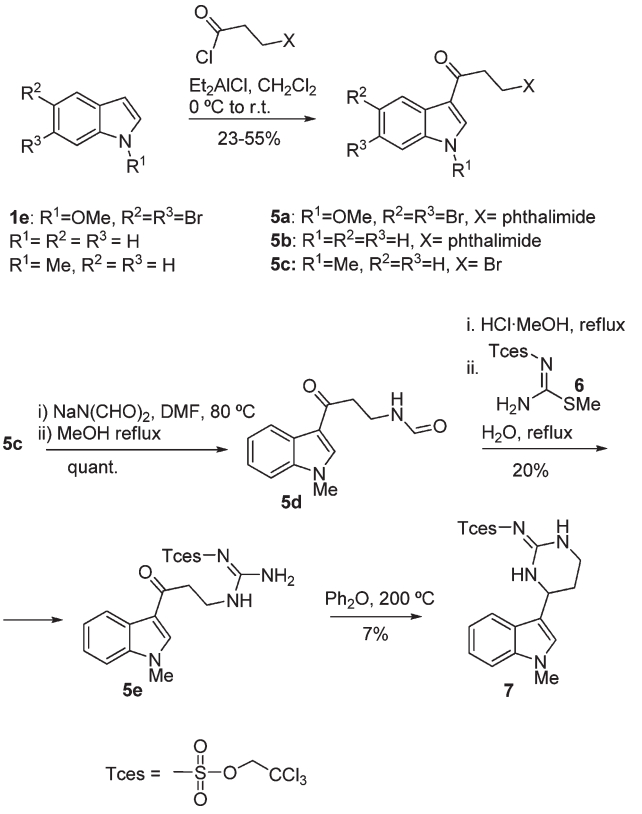
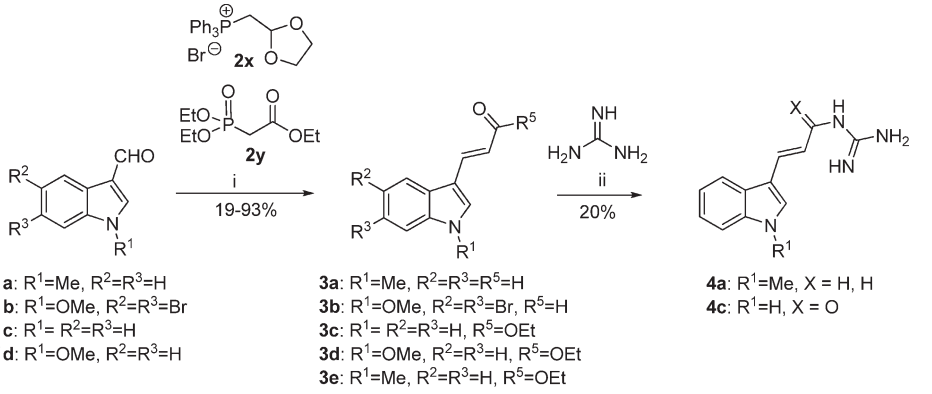


Figure 1. Examples of natural bioactive indole alkaloids.

Scheme 2. Synthetic Strategy by Acylation Route

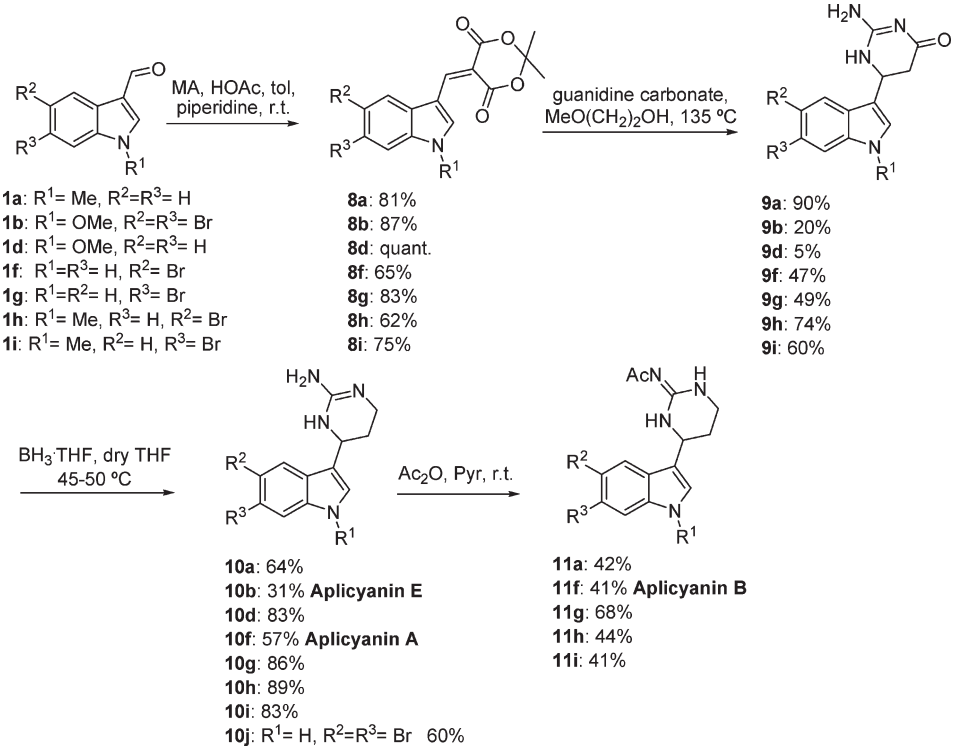


Scheme 1. Synthetic Strategy by Wittig Route[a]



[a] Reagents and conditions: (i) for **3a**-**b**, **2x**, NaOEt, THF-EtOH, rt to reflux; for **3c**−**e**, **2y**, Cs_2CO_3, dioxane-DMSO, 70 °C. (ii) *tert*-BuONa, *tert*-BuOH, reflux, then $NaBH_4$, MeOH, 0 °C to rt.

Scheme 3. Synthesis of the Aplicyanins and Related Analogues



An alternate strategy was then tried for introducing the bifunctionalized three-carbon chain: acylation of the indole with the acid chloride of *N*-protected *β*-alanine (Scheme 2). Acylation of 5,6-dibromo-1-methoxyindole (**1e**)[19] with 3-phthalimidopropanoyl chloride[20] using Et_2AlCl in CH_2Cl_2 gave **5a** in only 23% yield. On the basis of the low yield and the difficulty of eliminating the phthalimido protecting group by hydrazinolysis, another route was sought.[21] However, using other protecting groups (Alloc or Boc) for the amine of *β*-alanine did not improve the acylation.

Acylation of *N*-methylindole with 3-bromopropanoyl chloride in the same conditions as above gave the bromoketone **5c** in 30% yield. Reaction of **5c** with the monoacetyl guanidine in DMF produced the loss of hydrobromic acid. The elimination reaction was avoided by using sodium diformylamide, a weaker base for the amine introduction.[22] The partially protected aminoketone **5d** was obtained in excellent yield by reacting **5c** and sodium diformylamide in DMF at 80 °C, followed by monodeformylation with MeOH. The Tces-protected guanidine group of **5e** was synthesized by acidic treatment of **5d** to liberate the free amine and its further reaction with Tces-protected methyl carbamimidothioate **6**. Despite having tested several conditions[23] for the intramolecular cyclization of **5e**, only tetrahydropyrimidine **7** was obtained in 7% yield, which underscored the limitations of the latter synthetic approach.[24]

Having deduced that an electron-poor α,*β*-unsaturated ester would be necessary to drive the conjugate addition of guanidine to the double bond, as opposed to reaction with the ester group, as in the formation of compounds **4** (Scheme 1), a new strategy was devised: to decrease the electronic density of the conjugated double bond using a malonic ester derivative such as Meldrum's acid (MA) (Scheme 3).[25] Thus, the Meldrum acid−indole adducts **8a**,**b**,**d**,**f**−**i** were prepared in good yields by following the procedure described by Jones et al.[26] Reaction between adducts **8a**,**b**,**d**,**f**−**i** and guanidine carbonate in refluxing 2-methoxyethanol[27] gave the 2-aminodihydropyrimidones **9a**,**b**,**d**,**f**−**i** in yields that varied according to the indole substituent.[28] As such, *N*-methylindole **9a** was obtained in excellent yield (90%), whereas **9f**−**i**, corresponding to bromine substituents at indole positions 5 or 6, were obtained in lower yields (47−74%), and the *N*-methoxyderivatives **9b** and **9d** were only obtained in 20 and 5% yield, respectively. The poor results for the *N*-methoxy derivatives can be rationalized by two factors. The electron donating character of methoxy in position 1 of indole diminishes the reactivity of compounds **8b** and **8d** toward nucleophilic addition of guanidine. Thus, these derivatives are relatively nonreactive and, consequently, require longer times to consume the starting material in the guanidine addition−cyclization reaction. Second, they confer instability and low solubility to the 2-aminodihydropyrimidin-4-ones **9b** and **9d**.

Reduction of compounds **9a**,**b**,**d**,**f**−**i** with borane−THF[29] afforded the aminotetrahydropyrimidines **10a**,**b**,**d**,**f**−**j** in good yields. The reduction conditions for compounds **9b** and **9d** had to be strictly controlled because longer reaction times or higher temperatures led to the loss of the *N*-methoxy group. Reduction of **9b** produced a 1:2 mixture of **10b** and **10j**. Because the *N*-methoxy group of the indole is acid sensitive, the amino nitrogen in the 2-iminotetrahydropyrimidine ring was acylated, in moderate yields, under basic conditions (Ac_2O, Pyr) to give **11**.

Nearly all of the products were readily purified by column chromatography and obtained in relatively high yields, illustrating the efficiency of our synthetic route. (±)-Aplicyanin A (**10f**) and its acetyl derivative (±)-aplicyanin B (**11f**) were obtained in good overall yield from commercially available 5-bromo-3-formylindole, as were several other derivatives with a bromine at position 6 and/or a methyl group at position 1. However, the most complicated analogue,

Table 1. Cytotoxicity of Compounds **9**, **10**, and **11** to Three Human Tumor Cell Lines (GI_{50} Values Reported in μM)

compd	cell lines		
	MDA-MB-231	A-549	HT-29
9a,**b**,**d**−**h**	na[a]	na	na
9i	2.86	na	na
10a	1.71	2.67	4.29
10b (±)-aplicyanin E	10.9	na	na
10d	na	na	na
10f (±)-aplicyanin A	0.27	0.27	0.11
10g	19.1	na	21.1
10h	25.7	na	13.7
10i	8.79	9.11	4.56
10j	na	na	na
11a	14.4	10.7	7.40
11f (±)-aplicyanin B	0.98	0.51	0.33
11g	5.67	6.86	2.12
11h	0.94	0.43	0.31
11i	8.02	6.30	4.30

[a] na: not active.

(±)-aplicyanin E (**10b**), which contains two bromine atoms at positions 5 and 6 and a methoxy group at position 1, was obtained in just sufficient amount to perform the cytotoxicity assay. The NMR spectral data for (±)-aplicyanins A (**10f**), B (**11f**), and E (**10b**) obtained by total synthesis are in good agreement with the data reported in literature.[2]

Despite the results of (±)-aplicyanin E, the relatively high yields and easy purification of the other compounds are testament to the utility of the herein reported strategy for the synthesis of aplicyanins and their analogues.

Biological Results

Compounds **9**, **10**, and **11** were tested against three human tumor cell lines: HT-29 colon, A549 lung, and MDA-MB-231 breast. The cytotoxicities were evaluated for 20 synthesized compounds, and the most significant results are summarized in Table 1. Except for compounds **9a**,**b**,**d**−**h**, the remaining compounds in Table 1 are cytotoxic to MDA-MB-231 breast adenocarcinoma cells: **9i** strongly inhibits growth of these cells at micromolar concentrations, whereas the compounds with the saturated core are more active. The tetrahydropyrimidines **10** are cytotoxic; of these, **10a**, **10f** [(±)-aplicyanin A], and **10i** are the most active against all three cell lines.

The acetylated derivatives **11** also inhibit growth of all three cell lines at micromolar concentration; (±)-aplicyanin B (**11f**) and **11h** are the most active. The comparison of the assay results for *N*-H vs *N*-acetylated compounds shows that in compounds **11g**,**h**,**i**, acetylation increases cytotoxicity, whereas in **11a**,**f**, acetylation decreases activity.

The bromine at position 5 of the indole favor cytotoxic activity in the three cellular lines tested.

In contrast, the substituent of the indole nitrogen gave results of difficult generalization. Among the deacetylated compounds **10**, *N*-methoxy group produced only cytotoxicity of **10b** [(±)-aplicyanin E] among MDA-MB-231; **10f** [(±)-aplicyanin A] (N_{ind}-H) is the most active compound, 100-fold superior to **10h** (N_{ind}-Me). The acetylated compounds **11h** (N_{ind}-Me) and **11f** [(±)-aplicyanin B] (N_{ind}-H) are equally active against the three cell lines.

Conclusions

Herein is reported the total synthesis of the recently discovered marine natural products aplicyanins A, B, and E and 17 analogues. The compounds were screened in cytotoxicity assays against three human tumor cell lines: MDA-MB-231 (breast adenocarcinoma), A549 (lung carcinoma), and HT-29 (colorectal carcinoma).

(±)-Aplicyanin A and its acetyl derivative (±)-aplicyanin B were obtained in good overall yield from commercial 5-bromo-3-formylindole, as were several other derivatives with a bromine substituent at position 6 and/or *N*-methyl substituents. However, the most complicated analogue, (±)-aplicyanin E, which contains two bromine atoms at positions 5 and 6 and a methoxy group at position 1 of the indole, was only obtained in sufficient amount for screening.

Fourteen of the newly synthesized compounds showed considerable cytotoxic activity against three human tumor cell lines. These results suggest that the bromine at position 5 of the indole strongly favors antiproliferative activity, as well as the acetyl group at the imine nitrogen does in some compounds.

(±)-Aplicyanin A results active in the submicromolar range despite the inactivity of the corresponding natural compound. This evidence the activity of the unnatural enantiomer versus the natural one. (±)-Aplicyanin B is as active as its corresponding parent (natural) compound in all three cellular lines, whereas (±)-aplicyanin E maintains the activity only in MDA-MB-231. The decrease of cytotoxycity of the racemic aplicyanin E in front of the natural one indicates again that one enantiomer is more active than the other.

Therefore, these results demonstrate the potential of the aplicyanin structure as a scaffold for anticancer drug discovery and the need of developing enantiomeric synthesis to deepen in structure activity relationships.

Experimental Section

General Data. Melting points (mp) were determined in a Buchi melting point B540. Automatic flash chromatography was done in an Isco Combiflash medium pressure liquid chromatograph with Redisep silica gel columns (47−60 μm). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer and a Gemini 200 MHz spectrometer. HRMS were performed on a Bruker Autoflex high resolution mass spectometer. Microwave-assisted reactions were carried out in a CEM Discover microwave. Reversed phase analytical HPLC was performed on a Waters Alliance separation module 2695 and a Waters 996 PDA detector at 254 nm, using the following columns: a Waters Xterra MS C_{18} column (150 × 4.6 mm, 5 μm) for runs of 15 min, a Waters XBridge C_{18} column (75 mm × 4.6 mm, 2.5 μm) for runs of 8 min. Purification by semipreparative reversed phase HPLC were performed on a Symmetry C18 (5 μm, 30 mm × 100 mm) column, UV detection at 220 nm, with a flow of 10 mL/min, and using H_2O−0.1% TFA/CH_3CN−0.05% TFA as solvent system with a gradient specified for each case. HPLC analytical results to support final compound purity in two solvent systems are summarized in Table 1 of the Supporting Information. Purity determined by this means was superior or equal to 95% for all the compounds.

General Procedure for the Reaction of Formylindoles with 2,3-Dimethyl-1,3-dioxane-4,6-dione (Meldrum Acid). Acetic acid (100 μL, 1.0 mmol) and piperidine (155 μL, 1.1 mmol) were added to a solution of formylindole **1a**,**b**,**d**,**f**−**i** (6.0 mmol) and 2,3-dimethyl-1,3-dioxane-4,6-dione (865.8 mg, 6.0 mmol) in toluene (20 mL). The mixture was stirred at rt overnight. Evaporation afforded a yellow solid, which was recrystallized from ethanol to give pure condensation products.

General Procedure for Preparation of Compounds 9. Meldrum acid adduct **8** (ca. 1.5 mmol) was dissolved in toluene (20 mL).

Guanidine carbonate (1.25 equiv) was then added, and the reaction mixture was stirred at 135 °C until the starting material had been consumed (tracked by HPLC from 2 to 16 h). The crude product was concentrated in vacuo and washed with hexane and CH_2Cl_2. The product was crystallized from ethanol and then washed with hexane, CH_2Cl_2, and cold water to give a slightly yellowish solid. **9b** and **9d** were purified by semipreparative reverse phase HPLC (H_2O−MeCN; gradient 90:10 to 40:60 in 30 min) to afford the pure compound as yellow oil.

General Procedure for the Reduction of Compounds 9. Borane−THF complex (1 M THF solution, 3.0−5.0 mmol) was added to a solution of substrate **9** (1.0 mol) in anhydrous THF (10 mL) under Ar, and the reaction mixture was heated at 45 °C until the starting material has been consumed (tracked by HPLC, 2−15 h). The reaction mixture was then cooled to rt and quenched by stirring with sat. NH_4Cl for 30 min. The organic solution was set aside, and the aqueous phase was extracted with CH_2Cl_2 saturated with NH_3. The organic extracts were combined, dried over $MgSO_4$, and concentrated in vacuo to give the crude materials, which were purified as described below.

General Procedure for the Acylation of 10. Ac_2O (1.5 mL) was added to a solution of compound **10** (0.1 mmol) in pyridine (5 mL), and the resulting mixture was stirred at rt for 15 h. To the mixture were added CH_2Cl_2 (20 mL) and sat. $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were washed with 5% HCl, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude products were purified by semipreparative reverse phase HPLC (H_2O−MeCN; gradient 90:10 to 40:60 in 30 min) to afford yellow oils.[2]

2-Amino-4-(1-methylindol-3-yl)-1,4,5,6-tetrahydropyrimidine (10a). Compound **9a** (50 mg, 0.21 mmol) was reduced and then purified by washing with hexane, CH_2Cl_2, and cold water to obtain **10a** (64%) as a yellow solid; mp (MeCN) 283−285 °C. IR (KBr film) 3259, 2925, 2854, 1663, 1627, 1466, 1382, 1309, 1197, 742. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.15−2.24 (m, 2H), 3.32−3.43 (m, 2H), 3.79 (s, 3H), 4.92 (t, J = 6.4 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 7.14−7.21 (m, 2H), 7.35 (d, J = 7.4 Hz, 1H), 7.55 (d, J = 7.5 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 28.5, 32.9, 38.5, 48.2, 110.9, 114.9, 119.6, 120.5, 123.2, 126.8, 128.1, 139.1, 155.8. MS (ESI-TOF) 229 (M + 1, 100); 231 (M + 3, 27). HRMS m/z calcd for $C_{13}H_{17}N_4$ 229.1453, found 229.1453.

2-Amino-4-(5,6-dibromo-1-methoxyindol-3-yl)-1,4,5,6-tetrahydropyrimidine (10b) and 2-Amino-4-(5,6-dibromoindol-3-yl)-1,4,5,6-tetrahydropyrimidine (10j). Compound **9b** (5 mg, 0.01 mmol) was reduced and then purified by semipreparative reverse phase HPLC (H_2O−MeCN; gradient 90:10 to 40:60 in 30 min) to afford **10b** (31%) and **10j** (60%) as yellow oils.

10b. IR (KBr film) 3522, 2923, 1685, 1560, 1541, 1457, 1204, 1139, 800, 723. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.09−2.27 (m, 2H), 3.32−3.46 (m, 2H), 4.08 (s, 3H), 4.91 (dd, J = 8.1 and 4.4 Hz, 1H), 7.57 (s, 1H), 7.82 (s, 1H), 7.96 (s, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 27.9, 37.9, 47.1, 66.7, 112.0, 114.2, 116.0, 118.9, 123.1, 124.2, 124.4, 133.2, 155.3. MS (ESI-TOF) 401 ($M(Br^{79})_2$ + 1, 50), 403 ($MBr^{79}Br^{81}$ + 1, 100), 405 ($M(Br^{81})_2$ + 1, 54).

10j. ^{1}H NMR (400 MHz, MeOD-d_4): 2.16−2.27 (m, 2H), 3.32−3.47 (m, 2H), 4.08 (s, 3H), 4.92 (dd, J = 7.6 and 5.1 Hz, 1H), 7.31 (s, 1H), 7.73 (s, 1H), 7.91 (s, 1H). ^{13}C NMR (400 MHz, MeOD-d_4) 27.7, 37.9, 47.3, 68.6, 88.4, 101.1, 102.3, 117.1, 123.3, 125.7, 134.9. MS (ESI-TOF) 371 ($M(Br^{79})_2$ + 1, 47), 372 ($M(Br^{79})_2$ + 2, 15), 373 ($MBr^{79}Br^{81}$ + 1, 100), 375 ($M(Br^{81})_2$ + 1, 50). HRMS m/z calcd for $C_{12}H_{13}Br_2N_4$ 370.9501, found 370.9503.

2-Amino-6-(1-methoxyindol-3-yl)-1,4,5,6-tetrahydropyrimidine (10d). Compound **9d** (50 mg, 0.19 mmol) was reduced and then purified by semipreparative reverse phase HPLC (H_2O−MeCN; gradient 90:10 to 40:60 in 30 min) to afford **10d** (83%) as a yellow oil. IR (KBr film) 3425, 2923, 2852, 1678, 1383, 1207, 1139, 801, 724, 523. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.20−2.29 (m, 2H), 3.38−3.49 (m, 2H), 4.09 (s, 3H), 4.96 (dd, J = 6.4 and 6.4 Hz, 1H), 7.10−7.15 (m, 1H), 7.24−7.29 (m, 1H), 7.44−7.48b (m, 2H), 7.61 (d, J = 8.0 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 14.2, 20.6, 38.2, 61.4, 66.3, 109.4, 110.9, 119.7, 121.1, 122.2, 123.9, 151.0, 172.9. MS (ESI-TOF) 245 (M + 1, 100).

2-Amino-4-(5-bromoindol-3-yl)-1,4,5,6-tetrahydropyrimidine (10f). Compound **9f** (200 mg, 0.65 mmol) was reduced and then purified by semipreparative reverse phase HPLC (H_2O−MeCN; gradient 90:10 to 40:60 in 30 min) to afford **10f** (57%) as a yellow oil. IR (KBr film) 3265, 1680, 1630, 1461, 1305, 1203, 1137, 559, 839, 838, 801, 723, 600, 423. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.20−2.27 (m, 2H), 3.34−3.48 (m, 2H), 4.90−4.96 (m, 1H), 7.24 (dd, J = 8.7 and 1.8 Hz, 1H), 7.29−7.35 (m, 2H), 7.74 (d, J = 1.7 Hz, 1H).^{13}C NMR (400 MHz, MeOH-d_4) δ27.7, 37.9, 47.4, 113.0, 113.9, 114.7, 121.3, 124.7, 125.3, 127.5, 136.6, 155.2. MS (ESI-TOF) 293 (MBr^{79} + 1, 91), 294 (MBr^{79} + 2, 10), 295 (MBr^{81} + 1, 100), 296 (MBr^{81} + 2, 9). HRMS m/z calcd for $C_{12}H_{14}BrN_4$ 293.0396, found 293.0399.

2-Amino-4-(6-bromoindol-3-yl)-1,4,5,6-tetrahydropyrimidine (10g). Compound **9g** (100 mg, 0.33 mmol) was reduced and then purified by washing with hexane, CH_2Cl_2, and cold water to obtain **10g** (86%) as a yellow oil. IR (KBr film) 3257, 2942, 1661, 1628, 1455,1333, 1021, 803. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.19−2.27 (m, 2H), 3.35−3.47 (m, 2H), 4.95 (dd, J = 6.3 and 6.3 Hz, 1H), 7.17 (dd, J = 8.5 and 1.7 Hz, 1H), 7.26 (s, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 28.1, 38.2, 47.0, 62.4, 115.4, 115.6, 116.2, 120.6, 123.3, 124.5, 125.0, 139.1. MS (ESI-TOF) 293 (MBr^{79} + 1, 85), 294 (MBr^{79} + 2, 6), 295 (MBr^{81} + 1, 100), 296 (MBr^{81} + 2, 7). HRMS m/z calcd for $C_{12}H_{14}BrN_4$ 293.0396, found 293.0397.

2-Amino-4-(5-bromo-1-methylindol-3-yl)-1,4,5,6-tetrahydropyrimidine (10h). Compound **9h** (200 mg, 0.62 mmol) was reduced and then purified by washing with hexane, CH_2Cl_2, and cold water to obtain **10h** (89%) as a white solid; mp (MeCN) 314−316 °C. The samples for bioassays were crystallized from MeOH. IR (KBr film) 3209, 3053, 2969, 2879, 1665, 1621, 1476, 1422, 1323, 1124, 1090, 792, 808, 619, 595. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.19−2.26 (m, 2H), 3.36−3.49 (m, 2H), 3.79 (s, 3H), 4.93 (dd, J = 7.5 and 5.2 Hz, 1H), 7.26 (s, 1H), 7.29−7.37 (m, 2H), 7.74 (d, J = 1.3 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 28.6, 33.4,38.8, 48.1, 77.0, 109.6, 113.0, 114.1, 122.4, 123.1, 126.2, 129.9, 154.7. MS (ESI-TOF) 307 (MBr^{79} + 1, 100), 308 (MBr^{79} + 2, 15), 309 (MBr^{81} + 1, 90), 310 (MBr^{81} + 2, 12). HRMS m/z calcd for $C_{13}H_{16}BrN_4$ 307.0553, found 307.0552.

2-Amino-4-(6-bromo-1-methylindol-3-yl)-1,4,5,6-tetrahydropyrimidine (10i). Compound **9i** (200 mg, 0.62 mmol) was reduced and then purified by washing with hexane, CH_2Cl_2, and cold water to obtain **10i** (83%) as a yellow oil. The samples for bioassays were further purified by HPLC (C_{18} column). IR (KBr film) 3175, 3099, 3062, 2924, 1660, 1624, 1548, 1476, 1321, 1134, 797. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.19−2.26 (m, 2H), 3.33−3.47 (m, 2H), 3.77 (s, 3H), 4.95 (dd, J = 6.3 and 6.3 Hz, 1H), 7.19−7.23 (m, 2H), 7.51 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 1.6 MHz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 28.3, 33.0, 38.4, 47.9, 111.2, 114.0, 115.3, 116.7, 121.1, 123.7, 125.7, 129.1, 155.7. MS (ESI-TOF) 307 (MBr^{79} + 1, 100), 308 (MBr^{79} + 2, 12), 309 (MBr^{81} + 1, 85), 310 (MBr^{81} + 2, 10). HRMS m/z calcd for $C_{13}H_{16}BrN_4$ 307.0553, found 307.0555.

2-(Acetylamino)-4-(1-methylindol-3-yl)-1*H*-3,4,5,6-tetrahydropyrimidine (11a). Compound **10a** (20 mg, 0.09 mmol) was converted into **11a** (42%) as a colorless oil. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.19 (s, 3H), 2.29−2.36 (m, 2H), 3.45−3.63 (m, 2H), 3.79 (s, 3H), 5.13 (t, J = 6.0 Hz, 1H), 7.07−7.12 (m, 1H), 7.20−7.25 (m, 2H), 7.40 (d, J = 8.3 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 24.1, 26.9, 38.6, 83.8, 87.6, 110.9, 113.7, 119.4, 120.7, 123.3, 124.2, 128.3, 138.7, 195.0. MS (ESI-TOF) 271 (M + 1, 100). HRMS m/z calcd for $C_{15}H_{19}N_4O$ 271.1553, found 271.1557.

2-(Acetylamino)-4-(5-bromoindol-3-yl)-1*H*-3,4,5,6-tetrahydropyrimidine (11f). Compound **10f** (200 mg, 0.37 mmol) was converted into **11f** (41%) as a yellow oil. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.20 (s, 3H), 2.29−2.35 (m, 2H), 3.46−3.63 (m, 2H), 5.11 (t, J = 6.1 Hz, 1H), 7.26 (dd, J = 8.7 and 1.8 Hz, 1H), 7.32−7.36 (m, 2H), 7.78 (d, J = 1.6 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 24.1, 26.9, 38.6, 48.2, 86.2, 113.7, 114.6, 121.7, 125.5, 126.1, 127.8, 129.7, 137.2, 191.5. MS (ESI-TOF) 335 (MBr^{79} + 1, 100), 336 (MBr^{79} + 2, 12), 337 (MBr^{81} + 1, 93), 338 (MBr^{81} + 2, 10). HRMS m/z calcd for $C_{14}H_{16}BrN_4O$ 335.0502, found 335.0505.

2-(Acetylamino)-4-(6-bromoindol-3-yl)-1*H*-3,4,5,6-tetrahydropyrimidine (11g). Compound **10g** (50 mg, 0.09 mmol) was converted into **11g** (68%) as a yellow oil. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.20 (s, 3H), 2.32 (q, J = 5.9 and 5.8 Hz, 2H), 3.45−3.61 (m, 2H), 5.13 (t, J = 6.0 Hz, 1H), 7.19 (dd, J = 8.5 and 1.6 Hz, 1H), 7.31 (s, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.58 (d, J = 1.3 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 24.2, 27.0, 38.5, 48.3, 87.4, 115.0, 115.8, 116.7, 120.7, 123.8, 125.0, 139.4, 152.3, 174.0. MS (ESI-TOF) 335 (MBr^{79} + 1, 100), 336 (MBr^{79} + 2, 10), 337 (MBr^{81} + 1, 90), 338 (MBr^{81} + 2, 9). HRMS m/z calcd for $C_{14}H_{16}BrN_4O$ 335.0502, found 335.0501.

2-(Acetylamino)-4-(5-bromo-1-methylindol-3-yl)-1,2,3,4-tetrahydropyrimidine (11h). Compound **10h** (50 mg, 0.16 mmol) was converted into **11h** (44%) as a yellow oil. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.17 (s, 3H), 2.23−2.30 (m, 2H), 3.42−3.58 (m, 2H), 3.76 (s, 3H), 5.07 (dd, J = 6.7 and 5.3 Hz, 1H), 7.26 (s, 1H), 7.27−7.34 (m, 2H), 7.75 (d, J = 1.4 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 24.2, 27.0, 33.2, 38.5, 48.1, 112.8, 113.7, 114.1, 122.1, 126.2, 128.3, 129.9, 137.8, 180.7. MS (ESI-TOF) 348 (MBr^{79} + 1, 100), 350 (MBr^{81} + 1, 90).

2-(Acetylamino)-4-(6-bromo-1-methylindol-3-yl)-1*H*-3,4,5,6-tetrahydropyrimidine (11i). Compound **10i** (50 mg, 0.52 mmol) was converted into **11i** (41%) as a yellow oil. ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.19 (s, 3H), 2.25−2.32 (m, 2H), 3.43−3.60 (m, 2H), 3.77 (s, 3H), 5.11 (dd, J = 5.9 and 5.9 Hz, 1H), 7.19−7.22 (m, 1H), 7.25 (s, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.61 (d, J = 1.1 Hz, 1H). ^{13}C NMR (400 MHz, MeOH-d_4) δ 24.1, 26.9, 33.1, 38.4, 48.1, 114.1, 114.3, 116.9, 120.9, 123.8, 125.5, 129.3, 139.8, 152.2, 173.9. MS (ESI-TOF) 348 (MBr^{79} + 1, 100), 350 (MBr^{81} + 1, 95).

Cytotoxicity Assay. Established human-derived cell lines used in this study were purchased from American Type Culture Collection (ATCC): A-549, human lung carcinoma, HT-29, human colorectal adenocarcinoma, and MDA-MB-231, human breast adenocarcinoma. All cell lines were maintained in DMEM supplemented with 10% FBS and 100 units/mL penicillin and streptomycin at 37 °C and 5% CO_2.

Triplicate cultures were incubated for 72 h in the presence or absence of test compounds **9a**−**i**, **10a**−**j**, **11a**−**i**. A colorimetric assay using sulforhodamine B (SRB) was adapted for a quantitative measurement of cell growth and viability, following a previously described method.[30] Cells were plated in 96-well microtiter plates at a density of 5×10^3/well and incubated for 24 h. One plate from each different cell line was fixed, stained, and used for T_z reference (see next paragraph). The cells were then treated with vehicle alone (control) or the test compounds at the concentrations indicated. The treated cells were incubated for additional 72 h and then assayed for cytotoxicity via colorimetric analysis.

The cells were washed twice with PBS, fixed for 15 min in 1% glutaraldehyde solution, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at rt. The cells were then rinsed several times in 1% acetic acid solution and air-dried. SRB was then extracted in 10 mM trizma base solution, and the absorbance at 490 nm was then measured. Cell survival is expressed as percentage of control cell growth.

Dose−response curves were obtained by using the NCI algorithm:[31] T_z = number of control cells at time t_0, C = number of control cells at time t, and T = number of treated cells at time t.

If $T_z < T < C$ (growth inhibition), then the result is $100 \times ([T - T_z]/[C - T_z])$.

If $T < T_z$ (net cell death), then the result is $100 \times ([T - T_z]/T_z)$.

After dose-curve generation, the following parameter is calculated by interpolation: GI_{50}, concentration that causes 50% growth inhibition.

Acknowledgment. This study was partially supported by CICYT (grant BQU 2006-03794), Generalitat de Catalunya, and the Barcelona Science Park.

Supporting Information Available: General data, experimental procedures, and characterization of compounds **3a**−**e**, **4a**, **5a**−**e**, **7**, **8a**,**b**,**d**,**f**−**i**, **9a**,**b**,**d**,**f**−**i**. ^{1}H and ^{13}C NMR spectra and HPLC chromatograms of compounds **9**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Recent reviews on marine natural products: (a) Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G; Northcote, P. T.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2009**, *26*, 170–244. (b) Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G; Northcote, P. T.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2008**, *25*, 35–94. (c) Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G; Northcote, P. T.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2007**, *24*, 31–86. (d) Blunt, J. W.; Copp, B. R.; Munro, M. H. G; Northcote, P. T.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2006**, *23*, 26–78. (e) Blunt, J. W.; Copp, B. R.; Munro, M. H. G; Northcote, P. T.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2005**, *22*, 15–61.

(2) (a) Reyes, F.; Fernández, R.; Rodríguez, A.; Francesch, A.; Taboada, S.; Ávila, C.; Cuevas, C. Aplicyanins A−F, new cytotoxic bromoindole derivatives from the marine tunicate *Aplidium cyaneum*. *Tetrahedron* **2008**, *64*, 5119–5123. (b) Reyes, F.; Francesch, A.; Cuevas, C.; Altuna, M.; Pla, D.; Álvarez, M.; Albericio F. Indole derivatives as antitumor compounds. WO 2007/054748 A1, **2007**.

(3) Jordan, M. A.; Wilson, L. Microtubules as a target for anticancer drugs. *Nat. Rev. Cancer* **2004**, *4*, 253–265.

(4) (a) McDonald, L. A.; Barbieri, L. R.; Carter, G. T.; Kruppa, G.; Feng, X.; Lotvin, J. A.; Siegel, M. M. FTMS structure elucidation of natural products: application to muraymycin antibiotics using ESI multi-CHEF SORI-CID FTMS(n), the top-down/bottom-up approach, and HPLC ESI capillary-skimmer CID FTMS. *Anal. Chem.* **2003**, *75*, 2730–2739. (b) McDonald, L. A.; Barbieri, L. R.; Carter, G. T.; Lenoy, E.; Lotvin, J.; Petersen, P. J.; Siegel, M. M.; Singh, G.; Williamson, R. T. Structures of the muraymycins, novel peptidoglycan biosynthesis inhibitors. *J. Am. Chem. Soc.* **2002**, *124*, 10260–10261.

(5) Hamano, K.; Tanzawa, K.; Takahashi, M.; Enokida, R.; Okazaki, H.; Kinoshita, T.; Takamatsu, Y. Chymostatinols manufacture with Streptomyces for treatment of osteoporosis. Jpn. Kokai Tokkyo Koho JP08003188 A 19960109, **1996**.

(6) Two aminoimidazolinyl (five-membered cyclic guanidine) compounds have been isolated from marine sponges. (a) Sun, H. H.; Sakemi, S. A brominated (aminoimidazolinyl)indole from the sponge *Discodermia polydiscus*. *J. Org. Chem.* **1991**, *56*, 4307–4308. (b) Cohen, J.; Paul, G. K.; Gunasekera, S. P.; Longley, R. E.; Pomponi, S. A. 6-Hydroxydiscodermindole, a new discodermindole from the marine sponge Discodermia polydiscus. *Pharm. Biol.* **2004**, *42*, 59–61.

(7) Hernández Franco, L.; Bal de Joffe, E.; Puricelli, L.; Tatian, M.; Seldes, A. M.; Palermo, J. A. Indole alkaloids from the tunicate *Aplidium meridianum*. *J. Nat. Prod.* **1998**, *61*, 1130–1132.

(8) Butler, M. S.; Capon, R. J.; Lu, C. C. Psammopemmins (A-C), novel brominated 4-hydroxyindole alkaloids from an Antarctic sponge, *Psammopemma* sp. *Aust. J. Chem.* **1992**, *45*, 1871–1877.

(9) (a) Perry, N. B.; Ettouati, L.; Litaudon, M.; Blunt, J. W.; Munro, M. H. G. Alkaloids from the antarctic sponge *Kirkpatrickia varialosa*. Part 1: Variolin B, a new antitumor and antiviral compound. *Tetrahedron* **1994**, *50*, 3987–3992. (b) Trimurtulu, G.; Faulkner, D. J.; Perry, N. B.; Ettouati, L.; Litaudon, M.; Blunt, J. W.; Munro, M. H. G.; Jameson, G. B. Alkaloids from the antarctic sponge Kirkpatrickia varialosa. Part 2: Variolin A and N(3′)-methyl tetrahydrovariolin B. *Tetrahedron* **1994**, *50*, 3993–4000.

(10) Walker, S. R.; Carter, E. J.; Huff, B. C.; Morris, J. C. Variolins and Related Alkaloids. *Chem. Rev.* **2009**, *109*, 3080–3098.

(11) The only known naturally occurring example is a 3,3-disubstituted-1-methoxyoxindole, found in alkaloids isolated from *Gelsemium*

elegans: Xu, Y.-K.; Yang, S.-P.; Liao, S.-G.; Zhang, H.; Lin, L.-P.; Ding, J.; Yue, J.-M. Alkaloids from *Gelsemium elegans*. *J. Nat. Prod.* **2006**, *69*, 1347–1350.

(12) (a) Gribble, G. W. Naturally occurring organohalogen compounds—a survey. *J. Nat. Prod.* **1992**, *55*, 1353–1395. (b) Davidson, B. S. Ascidians: producers of amino acid-derived metabolites. *Chem. Rev.* **1993**, *93*, 1771–1791. (c) Tasdemir, D.; Bugni, T. S.; Mangalindan, G. C.; Concepcion, G. P.; Harper, M. K.; Ireland, C. M. Cytotoxic bromoindole derivatives and terpenes from the Philippine marine sponge Smenospongia sp. *Z. Naturforsch.* **2002**, *57c*, 914–922.

(13) For a general review in Vilsmeier formilation of indoles, see: Black, D. S. Applications of iminium cation chemistry to activated indoles. In *In Advances in Nitrogen Heterocycles*; Moody C. J., Ed; JAI Press Inc.: Stamford, CT, 1998; Vol. 3, pp 85−116.

(14) 5,6-Dibromo-1-methoxyindole-3-carbaldehyde (**1b**) was obtained in 50% yield from 5,6-dibromo-1-methoxyindole (**1e**, see ref 19) by Vilsmeier reaction, following the methodology described for the preparation of 1-methoxyindole-3-carbaldehyde (see ref 15). **1b**: ^{1}H NMR (400 MHz, $CDCl_3$) 4.16 (s, 3H), 7.76 (s, 1H), 7.85 (s, 1H), 8.59 (s, 1H), 9.89 (s, 1H).

(15) Compound **1d** was obtained by Vilsmeier formylation of 1-methoxyindole. Hanley, A. B.; Parsley, K. R.; Lewis, J. A.; Fenwick, G. R. Chemistry of indole glucosinolates: intermediacy of indol-3-ylmethyl isothiocyanates in the enzymic hydrolysis of indole glucosinolates. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2273–2276.

(16) Mouloungui, Z.; Murengezi, I.; Delmas, M.; Gaset, A. Cesium carbonate in a weakly hydrated solid−liquid heterogeneous medium: a new reagent for anionic activation synthesis. *Synth. Commun.* **1988**, *18*, 1241–1245.

(17) Transformation of ethyl ester into the guanidine-amide was observed by ^{1}NMR and MS. **4b**: ^{1}H NMR (400 MHz, D_2O) 6.05 (d, J = 7.8 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 8.1 Hz, 1H), 7.45 (s, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H). MS (ESI) 229 (M+1, 100).

(18) Zee-Cheng, K.-Y.; Robins, R. K.; Cheng, C. C. Pyrimidines. III. 5,6-Dihydropyrimidines. *J. Org. Chem.* **1961**, *26*, 1877–1884.

(19) 5,6-Dibromo-1-methoxyindole (**1e**) was obtained in 20% yield from 2,3-dibromo-6-nitrotoluene following the procedure described for the preparation of 1-methoxyindole by Somei, M.; Shoda, T. The chemistry of indoles. XVII. A facile route to 1-acetoxy- and 1-methoxyindoles. *Heterocycles*, **1981**, *16*, 1523−1525. **1e**: ^{1}H NMR (400 MHz, $CDCl_3$) 4.08 (s, 3H), 6.28 (d, J = 3.5 Hz, 1H), 7.26 (d, J = 3.5 Hz, 1H), 7.73 (s, 1H), 7.85 (s, 1H). MS (CI) 302 (MBr^{79}, 40); 304 (MBr^{81}, 100).

(20) 3-Phthalimidopropanoyl chloride was obtained from β-alanine by N-protection with phthalic anhydride followed by reaction with oxalyl chloride.

(21) **5b** was prepared in the same conditions as **5a** starting from indole to test several experimental conditions for removing the phthalimide protecting group. None of treatments of **5a** with hydrazine in different reaction solvent, temperature, or time afforded the amine.

(22) Yinglin, H.; Hongwen, H. A convenient synthesis of primary amines using sodium diformylamide as a modified Gabriel reagent. *Synthesis* **1990**, 122–124.

(23) Other cyclization conditions tested were: formic acid in refluxing dioxane; formic acid; formic acid in refluxing 1,2-dichloroethane; and (i) 2 equiv $Ti(OiPr)_4$ in EtOH at rt followed by (ii) $NaBH_4$ in MeOH at rt.

(24) Compound **7** was only obtained in 7% yield, using $Ti(OiPr)_4$ in MeOH, followed by reduction with $NaBH_4$ at rt overnight.

(25) Ostras, K. S.; Gorobets, N. Y.; Desenko, S. M.; Musatov, V. I. An easy access to 2-amino-5,6-dihydro-3*H*-pyrimidin-4-one building blocks: the reaction under conventional and microwave conditions. *Mol. Diversity* **2006**, *10*, 483–489.

(26) Benzies, D. W. M.; Martínez-Fresneda, P.; Jones, R. A.; McNab, H. Flash-vacuum pyrolysis of 5-(indol-2- and -3-ylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-diones. *J. Chem. Soc., Perkin Trans 1* **1986**, 1651–1654.

(27) Microwave-assisted cyclization did not provide any improvement in yield.

(28) Protected guanidines as acetyl- or Boc-guanidines did not improve the reaction yield.

(29) Agami, C.; Dechoux, L.; Melaimi, M. Chemoselective reduction of pyrimidines. An access to enantiopure tetrahydropyrimidinones. *Tetrahedron Lett.* **2001**, *42*, 8629–8631.

(30) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Waren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

(31) Boyd, M. R.; Paull, K. D. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res.* **1995**, *34*, 91–109.