# Synthesis and Cytotoxic Evaluation of New Derivatives of the Marine Alkaloid Variolin B

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The marine alkaloid variolin B **2** and eight synthetic derivatives with different substituents at positions C-5 and C-7 have been tested in a panel of sixteen human tumor cell lines to evaluate their cytotoxic potential.

## Introduction

Marine organisms have provided a vast array of structurally diverse and biologically interesting structures.[1] Several new clinical structures with novel modes of action have been identified recently from the intermediate metabolites of marine organisms, leading to great interest in this field.[2] Variolins **1**−**4** (Figure 1) comprise a group of marine heterocyclic substances isolated from the rare, and difficult to access, Antarctic sponge, *Kirkpatrickia variolosa*.[3] This new class of compounds is interesting from both structural and biological points of view. All the variolins have a common tricyclic ring skeleton, which has no precedents in either terrestrial or marine natural products, namely a pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine, with either a heterocyclic or methoxycarbonyl group attached at position C-5 of the core. Variolins can be also considered as guanidine-based alkaloids in which the guanidine moiety is found in the guise of a 2-aminopyrimidine ring.

This type of compounds exhibit a potent cytotoxic activity against P388 murine leukemia cells line, and also being effective against *Herpes simplex* type I.[3] Variolin B **2** is the most active of this family of natural products; oxidation or reduction of the 3-aminopyrimidine ring at position C-5, as in variolin A **1** or *N*-3′-methyl-3′,4′,5′,6′-tetrahydrovariolin B **3**, decreases the activity. This is corroborated by the absence of activity of variolin D **4** in which C-5 carries only a methoxycarbonyl group.

In different human cancer cell lines variolin B **2** and the non natural analogue deoxyvariolin B inhibited colony formation, caused cell cycle perturbations, and induced apoptosis at concentrations ranging from 0.1 to 2 $\mu$M. Although variolins induced an increase in the levels of *p*53 with an increase in *p*21, their cytotoxicities did not appear to be dependent on *p*53 status, as their potency was comparable in cells with wild-type *p*53, or in sublines with inactivated *p*53. Variolin B **2** and deoxyvariolin B prevent the cells from entering S phase, blocking cells in G1, and cause accumulation already evident 4 h after the beginning of treatment. Although intercalation of deoxyvariolin B in DNA has been demonstrated, neither variolin B **2** nor deoxyvariolin B produce detectable breaks in DNA. These results are consistent with the in vitro biochemical assays that also demonstrated that deoxyvariolin B is not a topoisomerase I or II poison. Instead, each of these variolins appears to inhibit cyclin-dependent kinases (CDKs) in the $\mu$M range. CDK1-cyclin B, CDK2-cyclin A, and CDK2/cyclin E complexes were inhibited in a range of concentrations lower than those required to inhibit the activity of CDK4/cyclin D or CDK7/cyclin H complexes. Variolin B **2** and deoxyvariolin B are a new class of CDK inhibitors that activate apoptosis in a *p*53-independent fashion, and thus they may be effective against tumors with *p*53 mutations or deletions.[4]

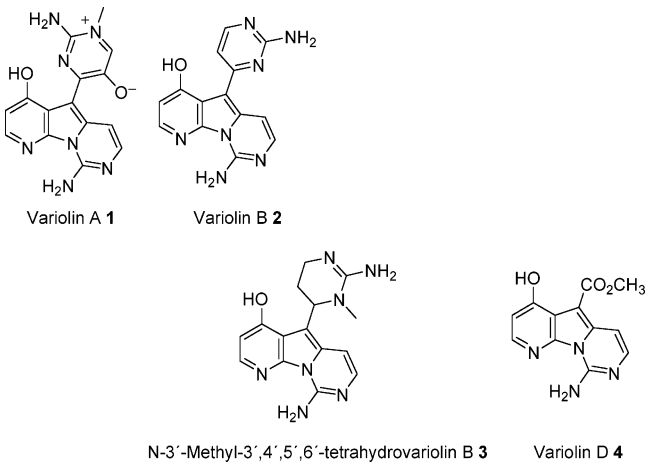


**Figure 1.** Structures of variolins **1**−**4**.

Consequently, its significant bioactivity, interesting structure, and also its low natural occurrence have stimulated different groups of scientific community to elaborate the central tricyclic core of variolins. At this moment, three total syntheses of variolin B **2** have been reported in the literature. The first synthesis of variolin B **2**,[5] starting from 4-chloro-2-methylthiopyrimidine, involved a tandem deoxygenation and cyclization of a triarylmethanol using a combination of triethylsilane and trifluoroacetic acid as the key step. The preparation of variolin B **2** and the non natural deoxyvariolin B from 4-methoxy-7-azaindole has been reported.[6] The method is based on the dihydropyrimido annelation onto the preformed 7-azaindole ring using *N*-tosyldichloromethanimine as cyclization reagent, followed by the installation of the pyrimidine ring with palladium-catalyzed cross-coupling reaction of 2-acetyl-4-(trimethylstannyl)-pyrimidine and an iodopyridopyrrolopyrimidine derivative.

## Results and Discussions

**Chemistry.** Taking into account the key role of the 2-aminopyrimidine ring on the activity of the variolin B, we report here the synthesis and biological evaluation of analogues of variolin B in which this ring either is placed at the C-7 position or is additionally placed at C-7 in the variolin B, giving rise to a complex molecule such as **7** in which two 2-aminopyrimidine rings are attached to the central core of the variolin family.

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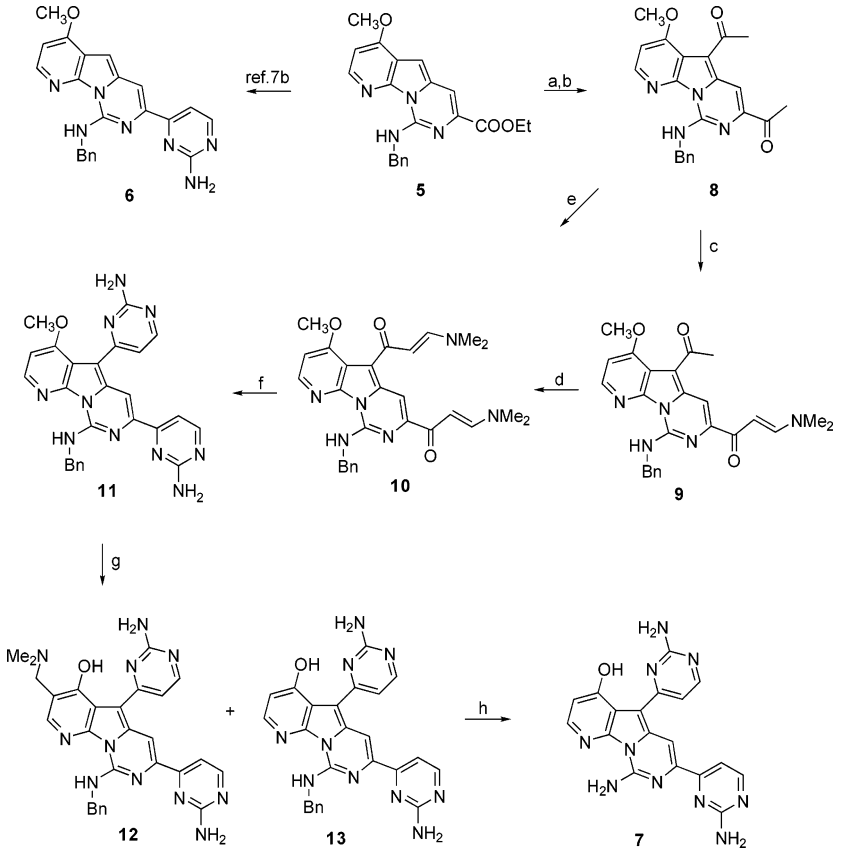
**Scheme 1.** Synthesis of 7-(2-Aminopyrimidin-4-yl)variolin B 7[a]




[a] Reagents and conditions: (a) (i) LiOH, THF−$H_2O$ (95%); (ii) MeLi, THF, −15 °C (62%); (b) *N*,*N*-dimethylacetamide, $POCl_3$, 70 °C, (61%); (c) *N*,*N*-dimethylformamide di-*tert*-butylacetal, DMF, 40 °C, 97%; (d) *N*,*N*-dimethylformamide di-*tert*-butylacetal, DMF, 100 °C, 92%; (e) (i) *N*,*N*-dimethylformamide di-*tert*-butylacetal, DMF, 60 °C, (ii) *N*,*N*-dimethylformamide di-*tert*-butylacetal, DMF, 100 °C, 67%; (f) $H_2N(C{=}NH)NH_2{\cdot}HCl$, $K_2CO_3$, 2-methoxyethanol, 110 °C, 79%; (g) NaSMe, DMF, 80 °C, **12** (20%) and **13** (60%); (g) triflic acid, r.t., 63%.

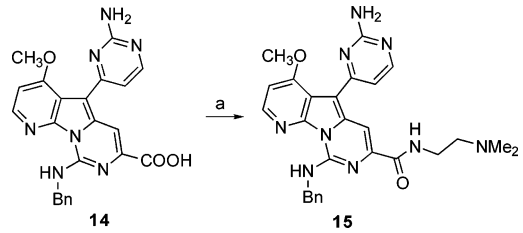
Our strategy[7] to the synthesis of variolin B **2** is based on the key steps: (a) synthesis of appropriately functionalized 2,4-disubstituted-7-azaindole, (b) construction of the central core pyrido[3′,2′:4,5] pyrrolo[1,2-*c*]pyrimidine ring, that typifies this compound, involving a carbodiimide-mediated pyrimido annelation process, and (c) formation of the 2-aminopyrimidine ring at position C-5.

This strategy opens extensive possibility synthetic to prepare new derivatives of this alkaloid. As shown in the Scheme 1, the intermediate **5**, carrying an ethoxycarbonyl group at position C-7 of the core pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine ring, has been used as starting material for the introduction at this position of either different functionalities or of heterocyclic rings. In this context, the synthesis of 7-isovariolin B **6**[7b] involves formation of the 2-aminopyrimidine ring at position C-7 of the tricyclic system, by conversion of the ethoxycarbonyl group into the corresponding 7-acetyl derivative by reaction with methyllithium, followed by the Bredereck protocol[8] to build the aminopyrimidine ring.

Here, we have applied the same strategy to synthesize 7-(2-aminopyrimidin-4-yl)variolin B **7**, bearing two 2-aminopyrimidine rings at positions C-5 and C-7 of the tricyclic core of variolin, starting from 5,7-diacetyl derivative **8** (Scheme 1).

To this end, the intermediate **5** by initial hydrolysis followed by sequential treatment with methyllithium and the Vilsmeier acylation with *N*,*N*-dimethylacetamide in the presence of $POCl_3$ provided the 5,7-diacetylated product **8** in 61% yield. The preferential reactivity of the acetyl group at C-7 position with respect to the same group located at C-5 position is corroborated by the selective preparation of enaminone **9**. We have observed different reactivity of the acetyl group at positions C-5 and C-7 of pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine core in the reaction with *N*,*N*-dimethylformamide di-*tert*-butylacetal, and it was possible to prepare selectively the monoenaminone **9**, only by control of reactions conditions. When compound **8** reacts with *N*,*N*-dimethylformamide di-*tert*-butylacetal at 40 °C for 24 h, the monoenaminone **9** was obtained in 95% yield. The second condensation was achieved by heating of **9** at 100 °C for 12 h to give the dienaminone **10** in 92% yield. The direct formation of the 5,7-dienaminone **10** from the diacetyl derivative **8** was carried out with *N*,*N*-dimethylformamide di-*tert*-butylacetal in DMF at 60 °C for 18 h followed by addition of a slight excesss of the reagent and then heating at 100 °C for 12 h. Under these conditions, compound **10** was isolated in 67% yield. The simultaneous formation of the two 2-aminopyrimidine rings was realized in one step in 79% yield, by treatment of **10** with guanidine hydrochloride in 2-methoxyethanol in the presence of anhydrous potassium carbonate to afford **11**. The last steps were the *O*-methyl and *N*-benzyl deprotection. To remove the *O*-methyl group, compound **11** was treated with an excess of sodium methanethiolate in dry DMF; however, the unexpected compound **12**, bearing a *N*,*N*-dimethylaminomethyl group at position C-3, was isolated in 20% yield along with the expected compound **13** in 60% yield. Probably, the formation of compound **12** involves formation of the intermediate *N*,*N*-dimethyl(methylene)ammonium followed by amino alkylation into the ortho position. Finally, the *N*-benzyl protecting group was removed from **13** by treatment with triflic acid at room

**Scheme 2.** Synthesis of Amide **15**[a]




[a] Reagents and conditions: (a) *N*,*N*-dimethyletylendiamine, EDC·HCl, THF, $CH_2Cl_2$, 0 °C then r.t., 70%.

temperature to give 9-amino-5,7-bis(2-aminopyrimidin-4-yl)-pyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidin-4-ol **7** in 63% yield (Scheme 1).

Finally, the amide **15** was prepared in 70% yield from the acid **14**,[7b] by reaction with *N*,*N*-dimethylethylendiamine in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) (Scheme 2).

All the compounds prepared here, **7**−**13** and **15**, as well as those previously reported, **2**, **6**, **16**, and **17**, were isolated as solids and purified by column chromatography to give the target compounds in an analytically pure state (see Experimental Section).

**Biological Results.** A panel of sixteen human tumor cell lines was used to evaluate the cytotoxic potential of the variolin B derivatives (Figure 2): prostate carcinoma tumor cells (DU-145 and LN-CaP), SKOV-3 ovary adenocarcinoma, ovarian cells sensitive (IGROV) or resistant (IGROV-ET) to ET-743, SK-BR-3 breast adenocarcinoma, MEL-28 malignant melanoma, H-MEC-1 endothelium cells, A-549 lung carcinoma NSCL, K-562 chronic myelogenous leukemia, PANC-1 pancreatic epitheloid carcinoma, HT-29 colon carcinoma cells, LoVo lymph node metathesis cells and the corresponding LoVo-Dox cells resistant to doxorubicin, and cervix epitheloid carcinoma (HeLa) or resistant (HeLa-Apl) to aplidine.

A conventional colorimetric assay was set up to estimate $GI_{50}$ values, i.e., the drug concentration which causes 50% cell growth inhibition after 72 h continuous exposure to the test molecules. Variolin B **2** is included in the test for comparison. The results obtained are shown in Table 1, and several general observations can be made.

a. As in the case of variolin D **4**, the biological importance of the substituent at C-5 in variolins is corroborated by the loss of activity of some of the new variolin B derivatives tested with a 3-aminopyrimidine at position C-7 (**16**, **17**, and 7-isovariolin B **6**) but not at C-5.

b. For the rest of derivatives tested, the combination of substituents at C-5 and C-7 decreases the activity with respect to variolin B **2**.

c. Only compounds **12** and **15**, which contain an amino alkyl chain ((dimethylamino)methyl at C-3 and 3-aminopyrimidine at position C-7 and 2-(dimethylamino)ethyl carboxamide at C-7, respectively) in addition to a 3-aminopyrimidine at position C-5, have a micromolar activity.

d. Derivatives **12** and **15** are scaffolds of new types for further preparation of new antitumor compounds.

## Conclusions

We reported here the synthesis and biological evaluation of eight new variolin B derivatives as potential cytotoxic agents. Variolin B **2** and derivatives have been tested in a panel of sixteen human tumor cell lines to evaluate their cytotoxic potential. Some of the derivatives tested have similar activity than the natural compound variolin B **2**.

## Experimental Section

**General Methods.** All reactions were carried under $N_2$, and solvents were dried by standard procedures. Column chromatography purifications were performed using silica gel (60 Å c.c. 70−200 $\mu$m, SDS) as the stationary phase. All melting points were determined on a Kofler hot-plate melting point apparatus and are uncorrected. IR spectra were determined as Nujol emulsions or films on a Nicolet Impact 400 spectrophotometer and were expressed in $cm^{-1}$. NMR spectra were recorded on a Bruker AC200 (200 MHz), a Varian Unity 300 (300 MHz) or a Bruker Avance 400 (400 MHz) using $CDCl_3$ or DMSO-$d_6$. Chemical shifts were reported in ppm ($\delta$ scale) relative to $Me_4Si$ as an internal standard, and all *J* values were in Hz. Assignments were made by DEPT or two-dimensional NMR experiments. The EI mass spectra were recorded on a Fisons AUTOSPEC 500 VG spectrometer.

**1-[5,7-Bis(acetyl-9-(*N*-benzylamino)-4-methoxypyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine (8).** To a solution of $POCl_3$ (0.53 mL, 5.72 mmol) in anhydrous $CHCl_3$ (5 mL) was added *N*,*N*-dimethylacetamide (0.59 mL, 6.36 mmol) at 0 °C under $N_2$. The mixture was stirred at room temperature for 25 min. Then, a solution of monoacetyl derivative (0.11 g, 0.32 mmol) in $CHCl_3$ (6 mL) was added, and the reaction mixture was heated at 70 °C for 30 h. After cooling, the mixture was poured into a saturated aqueous solution of NaOAc (100 mL) and then extracted with $CH_2Cl_2$ (5 × 30 mL). The combined organic layers were washed with $H_2O$ (2 × 10 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column using EtOAc:$CH_2Cl_2$ (1:9) as eluent to give **8** (0.075 g, 61% yield); $R_f$: 0.37 ($SiO_2$, EtOAc:$CH_2Cl_2$, 1:9); mp 225−227 °C (yellow prisms from $CH_2Cl_2$:$Et_2O$). Anal. ($C_{22}H_{20}N_4O_3$) C, H, N.

**(*E*)-1-[5-Acetyl-9-(*N*-benzylamino)-7-[(*N*,*N*-dimethylamino)-2-propenon-1-yl]-4-methoxypyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine (9).** A mixture of the diacetyl derivative **8** (0.105 g, 0.27 mmol), *N*,*N*-dimethylformamide di-*tert*-butylacetal (0.27 g, 1.35 mmol), and anhydrous DMF (15 mL) was stirred at 40 °C for 24 h under $N_2$. After cooling, the solution was poured into a saturated aqueous solution of NaCl (20 mL) and then extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to dryness under reduced pressure. The residue was chromatographed on a silica gel column using $CH_2Cl_2$:MeOH (9:1) as eluent to give **9** (0.114 g, 95% yield); mp 216−218 °C (from $CH_2Cl_2$:$Et_2O$). Anal. ($C_{25}H_{25}N_5O_3$) C, H, N.

**(*E*)-9-(*N*-Benzylamino)-5,7-bis[3-(*N*,*N*-dimethylamino)-2-propenon-1-yl]-4-methoxypyrido [3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine (10). Method A:** A mixture of the diacetyl derivative **8** (0.1 g, 0.25 mmol), *N*,*N*-dimethylformamide di-*tert*-butylacetal (0.26 g, 1.29 mmol), and anhydrous DMF (15 mL) was stirred at 60 °C for 18 h under $N_2$. Then *N*,*N*-dimethylformamide di-*tert*-butylacetal (0.52 g, 2.50 mmol) was added and the reaction mixture was stirred at 100 °C for 12 h. After cooling, the solution was poured into a saturated aqueous solution of NaCl (20 mL) and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to dryness under reduced pressure. The residue was chromatographed on a silica gel column using $CH_2Cl_2$:MeOH (9:1) as eluent to give **10** (0.086 g, 67% yield); mp 115−116 °C (from $CH_2Cl_2$:$Et_2O$).

**Method B:** A mixture of the 5-acetyl-7-enaminone **9** (0.11 g, 0.25 mmol), *N*,*N*-dimethylformamide di-*tert*-butylacetal (0.5 g, 2.5 mmol), and anhydrous DMF (10 mL), was stirred at 100 °C for 12 h under $N_2$. After cooling, the solution was poured into a saturated aqueous solution of NaCl (20 mL) and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to dryness under reduced pressure. The residue was chromatographed on a silica gel column using $CH_2Cl_2$:MeOH (9:1) as eluent to give **10** (0.114 g, 92% yield). Anal. ($C_{28}H_{30}N_6O_3$) C, H, N.

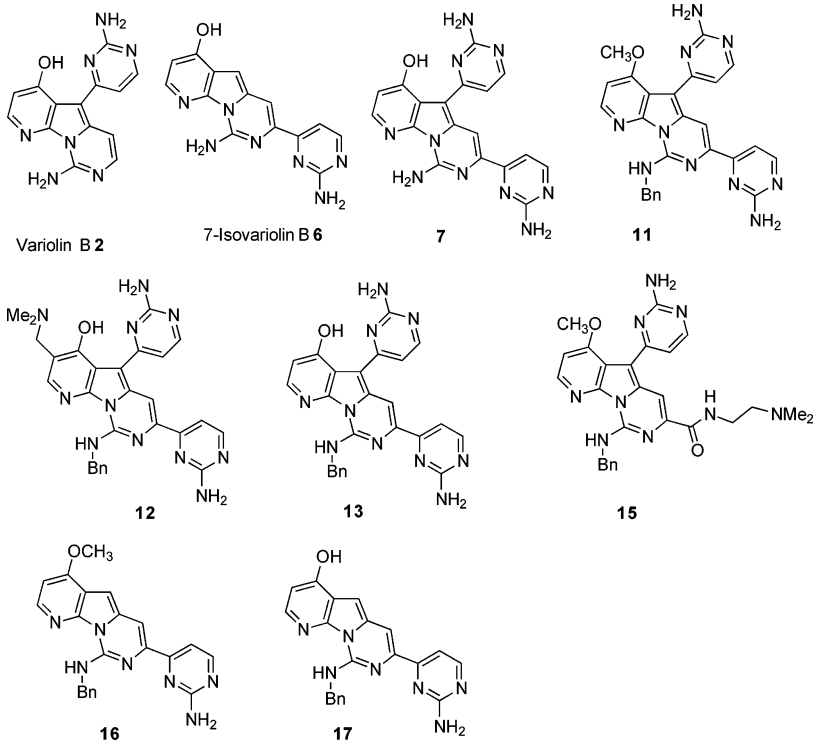



**Figure 2.** Structures of variolin B derivatives tested in vitro cytotoxicity.

**Table 1.** Data of in Vitro Cytotoxicity ($GI_{50}$, $\mu$M) of the Compounds **2**, **6**, **7**, **11**, **12**, **13**, **15**, **16**, and **17**[a]

| cell line | 2 | 6 | 7 | 11 | 12 | 13 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|
| DU-145 | 0.89 | 34.1 | 26.9 | 21.0 | 4.74 | 21.7 | 2.38 | 25.2 | 26.1 |
| LN-caP | 0.05 | 5.28 | 26.9 | 18.6 | 9.13 | 21.7 | 2.37 | 16.0 | 12.9 |
| SKOV-3 | 1.21 | n.d. | n.d. | n.d. | n.d. | 21.7 | 0.72 | 25.2 | 19.0 |
| IGROV | 1.14 | 27.9 | 26.9 | 21.0 | 4.03 | 21.7 | 2.80 | 15.1 | 4.46 |
| IGROV-ET | 1.28 | 31.9 | 26.9 | 21.0 | 3.56 | 9.90 | 2.05 | 25.2 | 12.9 |
| SK-BR-3 | 0.85 | 31.9 | 14.8 | 8.24 | 2.79 | 21.7 | 2.13 | 25.2 | 13.1 |
| MEL-28 | 1.20 | 3.41 | 26.9 | 21.0 | 4.12 | 21.7 | 4.42 | 25.2 | 24.4 |
| H-MEC-1 | 0.27 | n.d. | n.d. | n.d. | n.d. | 21.7 | 0.46 | 15.0 | 6.31 |
| A-549 | 0.98 | 34.1 | 26.9 | 21.0 | 18.7 | 21.7 | 3.38 | 25.2 | 14.1 |
| K-562 | 1.55 | 9.24 | 26.9 | 21.0 | 4.78 | 21.7 | 3.36 | 25.2 | 26.1 |
| PANC-1 | 1.68 | 21.8 | 26.9 | 19.9 | 17.7 | 21.7 | 3.97 | 25.2 | 26.1 |
| HT-29 | 2.85 | 34.1 | 26.9 | 21.0 | 18.7 | 21.7 | 3.44 | 25.2 | 25.1 |
| LOVO | 0.80 | 34.1 | 26.9 | 21.0 | 13.6 | 21.7 | 3.62 | 14.4 | 8.74 |
| LOVO-DOX | 1.02 | 34.1 | 26.9 | 21.0 | 3.05 | 21.7 | 3.46 | 11.0 | 6.76 |
| HELA | n.d. | 6.14 | 26.9 | 3.03 | 4.52 | n.d. | n.d. | n.d. | n.d. |
| HELA-APL | n.d. | 8.86 | 26.9 | 3.68 | 3.88 | n.d. | n.d. | n.d. | n.d. |

[a] n.d. = not determined.

**9-(*N*-Benzylamino)-5,7-bis(2-aminopyrimidin-4-yl)-4-methoxypyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine (11).** A mixture of the 5,7-dienaminone **10** (0.141 mg, 0.28 mmol), guanidine hydrochloride (0.27 g, 2.83 mmol), anhydrous $K_2CO_3$ (0.43 g, 3.08 mmol), and dry 2-methoxyethanol (15 mL) was heated at 100 °C for 40 h. After cooling, the mixture was poured into a saturated aqueous solution of $NH_4Cl$ (30 mL) and extracted with $CH_2Cl_2$ (4 × 25 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to dryness under reduced pressure. The residue was chromatographed on a desactive silica gel column using first $CH_2Cl_2$ and then $CH_2Cl_2$:MeOH (9:1) as eluent to give **11** (0.11 mg 79%); mp 269−271 °C (orange prisms from $CH_2Cl_2$:$Et_2O$). Anal. ($C_{26}H_{22}N_{10}O$) C, H, N.

**9-(*N*-Benzylamino)-5,7-bis(2-aminopyrimidin-4-yl)-4-hydroxipyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine (13).** To a solution of compound 5,7-dipyrimidine **11** (0.11 mg, 0.224 mmol) in anhydrous DMF (12 mL) was added sodium methanethiolate (0.157 mg, 2.24 mmol) under $N_2$. The reaction mixture was stirred at 80 °C for 24 h. After cooling, the mixture was poured into a saturated aqueous solution of NaCl (50 mL) and extracted with EtOAc:dioxane (2:1, 5 × 40 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel column using $CH_2Cl_2$:MeOH (9:1) as eluent to give **13** (0.064 mg, 60% yield); $R_f$: 0.27 ($SiO_2$, $CH_2Cl_2$:MeOH, 9:1); mp: >300 °C (red prisms from $CH_2Cl_2$). Anal. ($C_{25}H_{20}N_{10}O$) C, H, N.

**9-(*N*-Benzylamino)-5,7-bis(2-aminopyrimidin-4-yl)-3-[(*N*,*N*-dimethylamino)methyl]-4-hydroxi-pyrido[3′,2′:4,5] pyrrolo[1,2-*c*]pyrimidine (12).** (0.024 mg, 20% yield); $R_f$: 0.48 ($SiO_2$, $CH_2Cl_2$:MeOH, 9:1); mp >300 °C (red needles from dioxane:$Et_2O$). Anal. ($C_{28}H_{27}N_{11}O$) C, H, N.

**9-Amino-5,7-bis(2-aminopyrimidin-4-yl)-4-hydroxi-pyrido-[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine (7).** A solution of compound **13** (57 mg, 0.12 mmol) in triflic acid (3 mL) was stirred at room temperature for 16 h. After cooling at 0 °C, MeOH (5 mL) was added and the resulting mixture was stirred at that temperature for 5 min. Afterward, a solution of 30% ammonium hydroxide was added until basic pH. The solvent was removed off at room temperature under reduced pressure. To residue was added a saturated aqueous solution of NaCl (10 mL) and extracted with EtOAc (3 × 10 mL). The residue was chromatographed on a silica amine ($SiO_2$−$NH_2$) using $CH_2Cl_2$:MeOH (9:1) as eluent to afford **7** (30 mg, 65% yield); mp > 300 °C (red prisms from $CH_2Cl_2$:MeOH).). Anal. ($C_{18}H_{14}N_{10}O$) C, H, N.

**5-(2-Aminopyrimidin-4-yl)-9-(*N*-benzylamino)-*N*-[2-(*N*,*N*-dimethylamino)ethyl]-4-methoxypyrido[3′,2′:4,5]pyrrolo[1,2-*c*]pyrimidine-7-carboxamide (15).** To a solution of acid **14** (30 mg, 0.068 mmol) and *N*,*N*-dimethyletylendiamine (10 $\mu$L, 0.092 mmol) in anhydrous THF (4 mL) at 0 °C under $N_2$ was added a solution of *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (14.3 mg, 0.075 mmol) in anhydrous $CH_2Cl_2$ (2 mL). The mixture was stirred at room temperature for 24 h. The solvent was concentrated to dryness under reduced pressure. The residue was chromatographed on a silica gel column using $CH_2Cl_2$:MeOH (9:1) as eluent to give **15** (24 mg, 70% yield); mp: 170−171 °C ($CH_2Cl_2$:$Et_2O$).). Anal. ($C_{27}H_{29}N_9O_2$) C, H, N.

**Cell Growth Inhibition Assay: Screening.** A colorimetric assay using sulforhodamine B (SRB) has been adapted for a quantitative measurement of cell growth and viability, following a previously described method.[10] Cells were seeded in 96-well microtiter plates, at 5 × $10^3$ cells per well in aliquots of 195 $\mu$L of RPMI medium, and they are allowed to attach to the plate surface by growing in drug free medium for 18 h. Afterward, samples are added in aliquots

of 5 $\mu$L (dissolved in DMSO:$H_2O$, 3:7). After 72 h exposure, the antitumor effect is measured by the SRB methodology: cells are fixed by adding 50 $\mu$L of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. Plates are washed with deionized $H_2O$ and dried; 100 $\mu$L of SRB solution (0.4wt %/vol in 1% acetic acid) is added to each microtiter well and incubated for 10 min at room temperature. Unbound SRB is removed by washing with 1% acetic acid. Plates are air-dried and bound stain is solubilized with Tris buffer. Optical densities are read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analysis are generated automatically by LIMS implementation. Using control OD values ($C$), test OD values ($T$), and time zero OD values ($T_0$), the drug concentration that causes 50% growth inhibition ($GI_{50}$ value) was calculated from the equation: $100 \times [(T - T_0)/C - T_0)] = 50$.

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**Supporting Information Available:** Analytical and spectroscopic data of compounds **7**−**13** and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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