Total Synthesis and Anti-tumor Activity Screening of (±)-Aplicyanins A, B and E and Related Analogs

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Title running head. Synthesis and SAR Study of (±)-aplicyanins A, B, and E

Abstract

The first total synthesis of the indole alkaloids (±)-aplicyanins A, B and E, plus seventeen analogs, all in racemic form is reported. Modifications to the parent compound included changing the number of bromine substituents on the indole, the groups 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 fourteen of the newly synthesized compounds showed considerable cytotoxicity. The assay results were used to establish structure-activity relationships. These results suggest that the acetyl group moiety on the imine nitrogen, and the bromine at position 5 of the indole, are both critical to activity.

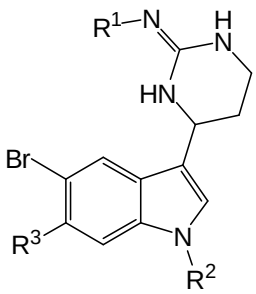
Keywords:

Marine metabolites, indole alkaloids, total synthesis, bromoindole, cytotoxicity

Introduction

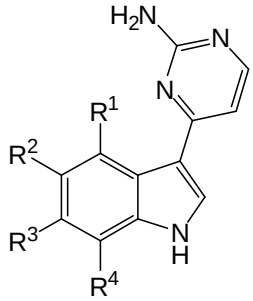
Marine invertebrates such as sponges, tunicates, ascidians and corals have provided a rich arsenal of new bioactive compounds. The unprecedented structures of these molecules make them excellent synthetic targets, and their potent activity against a broad number of therapeutic indications make these natural product 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.[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) and *N*-indole substituents (R^2 = H or OMe), and in whether or not they contain a second bromide at indole position 6 (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 an *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] from the Antarctic sponge *Kirkpatrickia varialosa* (Figure 1). Furthermore, the 1-methoxyindole found in some aplicyanins is unprecedented among known natural compounds.[10]



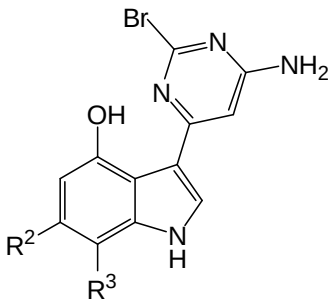
Aplicyanins A-F

A: R^1=R^2=R^3= H
B: R^1= Ac, R^2=R^3= H
C: R^1=R^3= H, R^2= OMe
D: R^1= Ac, R^2= OMe, R^3= H
E: R^1= H, R^2= OMe, R^3= Br
F: R^1= Ac, R^2= OMe, R^3= Br



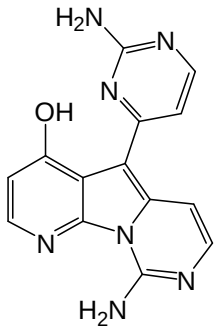
Meridianins A-G

A: R^1= OH, R^2=R^3=R^4= H
B: R^1= OH, R^2=R^4= H, R^3= Br
C: R^1=R^3=R^4= H, R^2= Br
D: R^1=R^2=R^4= H, R^3= Br
E: R^1= OH, R^2=R^3= H, R^4= Br
F: R^1=R^4= H, R^2=R^3= Br
G: R^1=R^2=R^3=R^4= H



Psammopemmins A-C

A: R^1=R^2= H
B: R^1= H, R^2= Br
C: R^1= Br, R^2 = H



Variolin B

Figure 1. Examples of natural bioactive indole alkaloids

Lastly, given the high cytotoxicity typical of bromoindole derivatives, the presence of a bromoindole in some aplicyanins warrants their investigation as anticancer drugs.[11] Herein is reported the first total synthesis of (±)-aplicyanins A, B, and E and seventeen analogs. The analogs differ in the substituents of the indole nucleus (H and/or Br), in the

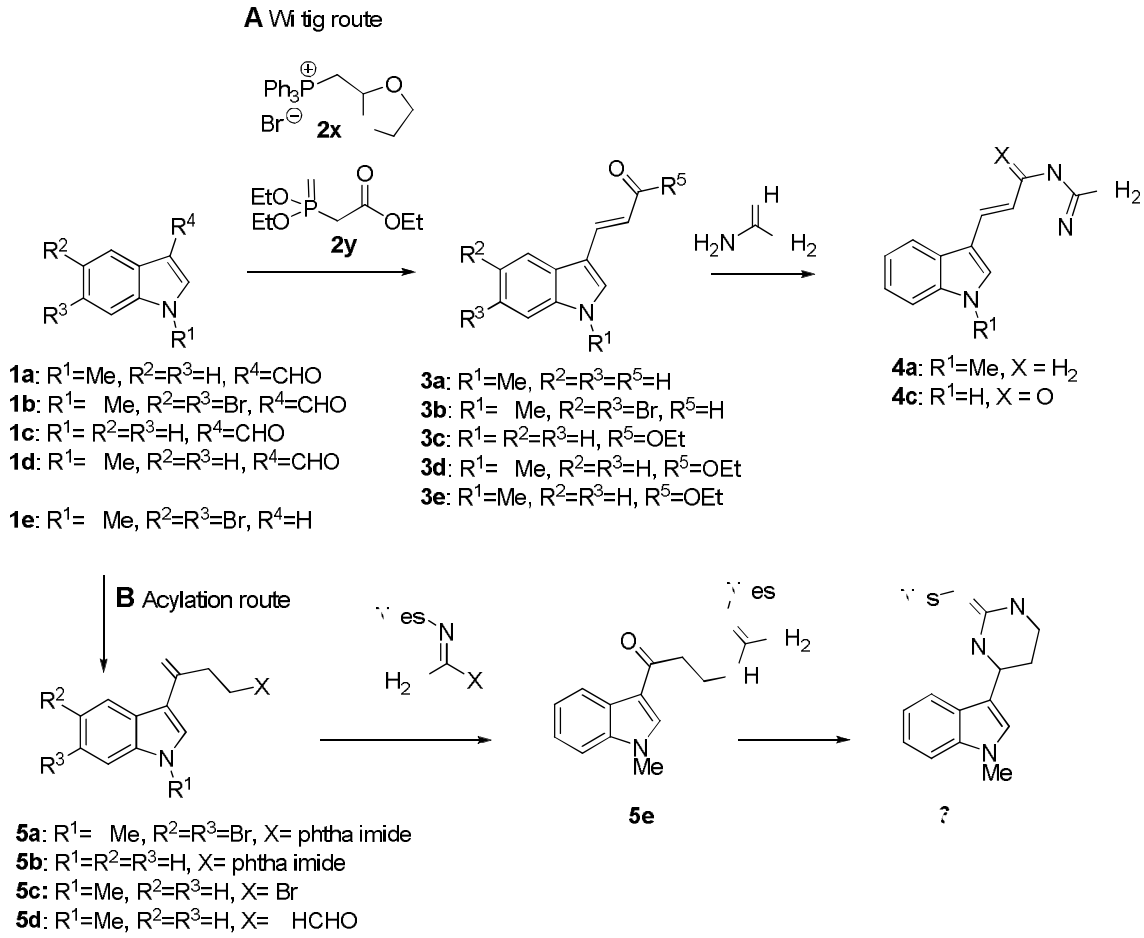
substitution the indole nitrogen (H, Me or OMe), and in the oxidation level of the six members heterocyclic core (2-amino-1,4,5,6-tetrahydropyrimidine or 2-amino-5,6-dihydro-4-pyrimidone). The compounds were screened for cytotoxicity against three human tumor cell lines: A459, HT-29 and MDA-231. Structure 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 (Scheme 1). The substituted indoles **1** were either commercially available or prepared by conventional procedures.

Wittig chemistry (A, 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 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.



Scheme 1. Synthetic routes to the aplicyanins

Compounds **3a**[12] (71% yield) and **3b**[13] (65% yield) were obtained from **2x** and the aldehydes **1a** and **1b**,[14,15] 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**[16] and **1a** and the ethyl phosphonate **2y** (Cs_2CO_3 as base, dioxane, 70 ºC).[17] 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**.[18] Based on these results, it was decided not to continue with the indolyl acrylates **3d** and **3e**. In literature reports on the condensation of α,β-unsaturated esters with guanidines to give tetrahydropyrimidones,[19] all of the reported α,β-unsaturated esters contain an aryl group with electron withdrawing substituents at the β-position.

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 1, B). Acylation of 5,6-dibromo-1-methoxyindole (**1e**)[15] with 3-phthalimidopropionyl chloride[20] using Et_2AlCl in CH_2Cl_2 gave **5a** in only 23% yield. Based on 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.

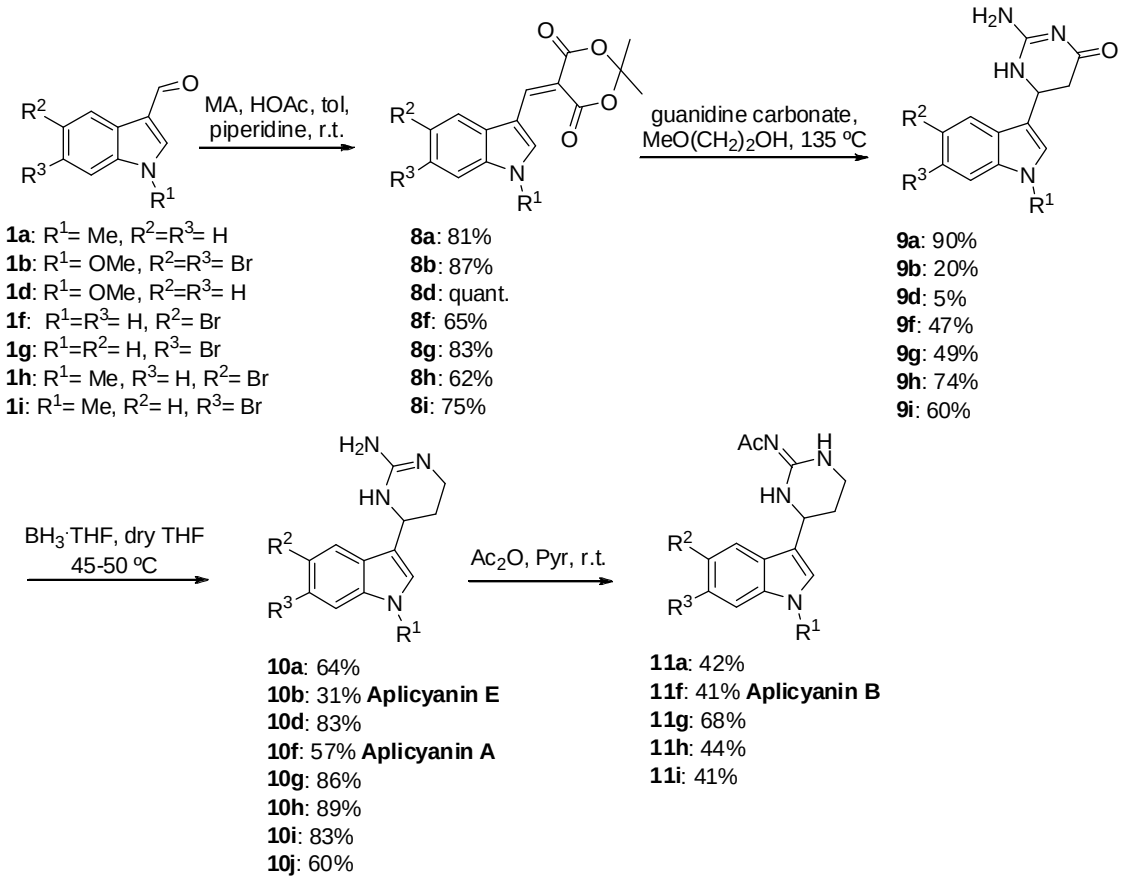
Acylation of *N*-methylindole with 3-bromopropanoyl chloride in the same conditions as above gave the bromoketone **5c** in 30% yield. Reaction of **5c** with the monoacetyl guanidine in DMF produced the loss of hydrobromic acid. The elimination reaction was avoided by using sodium diformylamide, a weaker 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 ºC, 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 2).[25] Thus, the Meldrum acid-indole adducts **8a-i** were prepared in good yields following the procedure described by Jones *et al.*[26] Reaction between adducts **8a-i** and guanidine carbonate in refluxing 2-methoxyethanol[27] gave the 2-aminodihydropyrimidones **9a-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. Firstly, these derivatives are relatively nonreactive, and consequently, require longer times to consume the starting material in the guanidine addition-cyclization reaction. Secondly, they confer instability and low solubility to the 2-amino-dihydropyrimidin-4-ones **9b** and **9d**.

Reduction of compounds **9a-i** with borane-THF[29] afforded the aminotetrahydropyrimidines **10a-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 loss of the *N*-methoxy group. Reduction of **9b** produced a 1:2 mixture of **10b** and **10j**. Since 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**.



Scheme 2. Synthesis of the aplicyanins

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 analog, (±)-aplicyanin E (**10b**), which contains a bromine at positions 5 and 6 and a methoxy group at position 1, was obtained in just enough quantity to perform the cytotoxicity assay. Despite the results of (±)-aplicyanin E, the relatively high yields and easy purification of the other compounds are testament to the utility of the 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. These results suggest that both the acetyl group at the imine nitrogen and the bromine at position 5 of the indole strongly favor activity, and that, in contrast, the substituent on the indole nitrogen is not very influential. Compounds **11h** (N-Me) and **11f** [(±)aplicyanin B] (N-H) are equally active against the three cell lines. Among the de-acetylated compounds, **10f** [(±)aplicyanin A] (N-H) is the most active.

Table 1. Cytotoxicity of compounds **9**, **10** and **11** to three human tumor cell lines (GI_{50} values reported in μM)

Compound	Cell lines		
	MDA-MB-231	A-549	HT-29
9a-h	---	---	---
9i	2.86	---	---
10a	1.71	2.67	4.29
10b (±)-aplicyanin E	10.9	---	---
10d	---	---	---
10f (±)-aplicyanin A	0.27	0.27	0.11
10g	19.1	---	21.1
10h	25.7	---	13.7
10i	8.79	9.11	4.56
10j	---	---	---
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

Conclusions

Herein is reported the total synthesis of the recently discovered marine natural products aplicyanins A, B and E and seventeen analogs. 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 analog, (±)-aplicyanin E, which contains two bromides, 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 both the acetyl group at the imine nitrogen and the bromine at position 5 of the indole strongly favor anti-tumor activity, but that the substituent on the indole nitrogen does not influence activity.

(±)-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. (±)-Aplicyanin A results active in the submicromolar range, despite the inactivity of the corresponding natural compound. These results demonstrate the potential of the aplicyanin structure as a scaffold for anti-cancer drug discovery.

Experimental section

(*E*)-Ethyl 3-(3-indolyl)acrylate (3c).[17] Cs_2CO_3 (6.81 g, 20.7 mmol) was added to a solution of ethyl (diethoxyphosphoryl)acetate (4.81 g, 20.7 mmol) and 3-formylindole (1.06 g, 6.9 mmol) in dioxane-DMSO (99:1, 100 mL). The mixture was stirred at 70

ºC for 43 h. The solvent was removed and the residue was dissolved in EtOAc. The organic solution was washed with sat. NH_4Cl, sat. $NaHCO_3$, and brine. The organic solution was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica-gel chromatography (hexane-EtOAc; 90:10 to 80:20) afforded **3c** (1.39 g, 93%) as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 (t, *J* = 7.2 Hz, 3H, Me); 4.28 (q, *J* = 7.2 Hz, 2H, CH_2); 6.47 (d, *J* = 16.0 Hz, 1H); 7.19-7.30 (m, 2H); 7.38-7.43 (m, 2H); 7.88-7.96 (m, 2H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.5; 60.2; 111.8; 113.1; 113.3; 120.3; 121.4; 123.2; 125.2; 129.0; 137.1; 138.4; 168.4. MS (ESI) 214 (M-1, 100); 215 (M, 7).

(*E*)-Ethyl 3-(1-methoxyindol-3-yl)acrylate (3d). Cs_2CO_3 (3.42 g, 10.5 mmol) was added to a solution of ethyl (diethoxyphosphoryl)acetate (2.39 g, 10.5 mmol) and 1-methoxy-3-formylindole (611 mg, 3.5 mmol) in dioxane-DMSO (99:1, 50 mL). The mixture was stirred at 70 ºC for 43 h. The solvent was removed and the residue was dissolved in EtOAc. The organic solution was washed with sat. NH_4Cl, sat. $NaHCO_3$, and brine. The organic solution was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica-gel chromatography (hexane-EtOAc; 95:10 to 90:10) afforded **3d** (608 mg, 71%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 (t, *J* = 7.2 Hz, 3H, Me); 4.11 (s, 3H, OMe); 4.27 (q, *J* = 7.2 Hz, 2H, CH_2); 6.41 (d, *J* = 16.0 Hz, 1H); 7.20-7.36 (m, 2H); 7.44-7.55 (m, 2H); 7.79-7.91 (m, 2H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.5; 60.1; 66.4; 108.8; 113.9; 120.5; 121.7; 122.2; 123.5; 125.8; 132.8; 137.2; 167.9. MS (ESI) 246 (M+1, 100); 247 (M+2, 30).

(*E*)-Ethyl 3-(1-methylindol-3-yl)acrylate (3e)[30]. Cs_2CO_3 (1.27 g, 3.8 mmol) was added to a solution of ethyl (diethoxyphosphoryl)acetate (880 mg, 3.8 mmol) and 1-

methyl-3-formylindole (204 mg, 1.3 mmol) in dioxane-DMSO (99:1, 15 mL). The mixture was stirred at 70 ºC for 43 h. The solvent was removed and the residue was dissolved in EtOAc. The organic solution was washed with sat. NH_4Cl, sat. $NaHCO_3$, and brine. The organic solution was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography with (hexane-EtOAc; 90:10 to 85:15) afforded **3e** (234 mg, 80%) as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 (t, *J* = 7.1 Hz, 3H, Me); 3.81 (s, 3H, Me); 4.27 (q, *J* = 7.1 Hz, 2H, CH_2); 6.41 (d, *J* = 16.0 Hz, 1H); 7.21-7.37 (m, 4H); 7.84-7.94 (m, 2H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.5; 33.2; 60.0; 109.9; 112.1; 112.6; 120.6; 121.2; 122.9; 126.0; 133.0; 137.9; 138.0; 168.2. MS (ESI) 230 (M+1, 100); 231 (M+2, 10).

(*E*)-3-(1-Methylindol-3-yl)allylguanidine (4a). *tert*-BuONa (440 mg, 4.4 mmol) was added to a solution of (*E*)-3-(1-methylindol-3-yl)acrylaldehyde (**3a**)[12] (274 mg, 1.5 mmol) and guanidine hydrochloride (708 mg, 7.4 mmol) in *tert*-BuOH (10 mL). The mixture was refluxed for 72 h. The solvent was removed, and the residue was dissolved in MeOH. The solution was then cooled to 0 ºC, and $NaBH_4$ (402 mg, 10.6 mmol) was added in one solid portion. The mixture was stirred for 4 h at r.t. The solvent was removed, and the crude was dissolved in CH_2Cl_2, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (CH_2Cl_2-MeOH; 97:3 to 92:8) afforded **4a** (73 mg, 20%) as a yellow solid. ^{1}H NMR (d_6-DMSO, 400 MHz) δ δ 3.16 (d, *J* = 5.3 Hz, 1H); 3.81 (s, 3H); 6.50 (bs, 3H); 6.57 (d, *J* = 16.0 Hz, 1H); 7.19 (t, *J* = 7.8 Hz, 1H); 7.26 (t, *J* = 8.0 Hz, 1H); 7.51 (d, *J* = 8.0 Hz, 1H); 7.78 (s, 1H); 7.81 (d, *J* = 7.8 Hz, 1H); 7.98 (d, *J* = 16.0 Hz, 1H). ^{13}C NMR (d_6-DMSO, 100 MHz) δ 42.3; 58.1; 120.1; 121.1; 129.1; 131.1; 131.7;

135.1; 140.9; 142.5; 147.3; 176.6; 180.8. MS (ESI) 229 (M+1, 100); 230 (M+2, 14).

5,6-Dibromo-1-methoxy-3-(3-phthalimidopropanoyl)indole (5a). A solution of 3-(3-phthalimidopropanoyl)chloride (139 mg, 0.6 mmol) and 1M Et_2AlCl in hexane (0.6 mL, 0.6 mmol) was stirred in CH_2Cl_2 (10 mL) at 0 ºC under Ar. A solution of 5,6-dibromo-1-methoxyindole (119 mg, 0.4 mmol) in CH_2Cl_2 (5 mL) was then added dropwise after 30 min. The reaction mixture was stirred for 16 h at r.t. and then washed with a sat. $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (hexane-EtOAc; 70:30 to 60:40) afforded **5a** (45.4 mg, 23%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.25 (t, *J* = 7.2 Hz, 2H); 4.14 (s, 3H); 4.15 (t, *J* = 7.2 Hz, 2H); 7.71-7.73 (m, 2H); 7.74 (s, 1H); 7.84-7.86 (m, 2H); 7.92 (s, 1H); 8.69 (s, 1H).

3-(3-Phthalimidopropanoyl)indole (5b). A solution of 3-(3-phtalimidopropanoyl)chloride (471 mg, 1.98 mmol) and 1M Et_2AlCl in hexane (2.0 mL, 2.0 mmol) was stirred in CH_2Cl_2 (10 mL) at 0 ºC under Ar. After 30 min, a solution of indole (155 mg, 1.3 mmol) in CH_2Cl_2 (7 mL) was added dropwise. The reaction mixture was stirred for 2 h at 0 ºC, and then washed with a sat. $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (hexane-EtOAc; 70:30 to 50:50) afforded **5b** (231 mg, 55%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.24 (t, *J* = 7.3 Hz, 2H); 3.94 (t, *J* = 7.3 Hz, 2H); 7.16 (t, *J* = 7.4 Hz, 1H); 7.20 (t, *J* = 7.6 Hz, 1H); 7.45 (d, *J* = 7.4 Hz, 1H); 7.80-7.87 (m, 4H); 8.14 (d, *J* = 7.6 Hz, 1H); 8.27 (s, 1H); 11.95 (bs, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 34.6; 37.9; 112.8; 116.7; 122.0; 122.5; 123.5; 123.7; 125.9; 132.4; 134.8; 135.1; 137.3; 168.4; 193.2.

3-(3-Bromopropanoyl)-1-methylindole (5c). A solution of 3-bromopropionyl chloride (1.54 mL, 15.2 mmol) and 1M Et_2AlCl in hexane (11.4 mL, 11.4 mmol) was stirred in CH_2Cl_2 (10 mL) at 0 ºC under Ar. After 30 min, a solution of 1-methylindole (1.0 g, 7.6 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The mixture was stirred for 4 h at r.t. and then washed with sat. $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (hexane-EtOAc; 70:30 to 60:40) afforded **5c** (602 mg, 30%) as a red oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.43 (t, *J* = 7.0 Hz, 2H); 3.80 (t, *J* = 7.0 Hz, 2H); 3.86 (s, 3H); 7.31-7.36 (m, 3H); 7.74 (s, 1H) and 8.36-8.38 (m, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 27.2; 33.8; 42.7; 109.9; 116.2; 122.8; 123.1; 123.8; 126.4; 135.8; 137.7; 191.7. MS (CI) 267 (MBr^{81}, 100); 265 (MBr^{79}, 92).

3-(3-Formamidopropanoyl)-1-methylindole (5d). To a stirring solution of **5c** (98 mg, 0.37 mmol) in dry DMF (5 mL) was added sodium diformylamide (105 mg, 1.1 mmol) in one portion. The mixture was stirred at 80 ºC overnight, and then concentrated *in vacuo* and the crude mixture was dissolved in MeOH (10 mL) and refluxed for 2 h. The mixture was concentrated, and then dissolved in CH_2Cl_2, washed with water, dried over $MgSO_4$ and concentrated again to give **5d** in quantitative yield as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.14 (t, *J* = 5.5 Hz, 2H); 3.75 (dt, *J* = 5.5, 5.5 Hz, 2H); 3.87 (s, 3H); 6.46 (bs, 1H); 7.35 (m, 3H); 7.76 (s, 1H); 8.14 (s, 1H); 8.33 (m, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 33.7; 33.9; 38.8; 110.0; 120.1; 122.5; 123.1; 123.8; 135.9; 161.4.

***N*-(2,2,2-Trichloroethoxysulfonyl)-*N'*-(3-(1-methylindol-3-yl)-3-oxopropyl)guanidine (5e).** A solution of **5d** (1.56 g, 6.78 mol) in MeOH (1.25 M

HCl) was refluxed with for 2 h. The solvent was evaporated off and the crude was dissolved in CH_2Cl_2, washed with an aqueous solution of 50% NaOH, dried, and concentrated *in vacuo* to give 3-(3-aminopropanoyl)-1-methylindole in quantitative yield as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.00 (t, *J* = 6.2 Hz, 2H); 3.13 (t, *J* = 6.2 Hz, 2H); 3.84 (s, 3H); 7.32 (m, 3H); 7.74 (s, 1H); 8.38 (m, 1H).

A solution of 3-(3-aminopropanoyl)-1-methylindole (1.37 g, 6.78 mmol) and protected isothiourea **6** (2.25 g, 7.46 mmol) in H_2O (50 mL) was refluxed overnight, and then cooled to r.t.. The crude mixture were diluted with 20 mL of H_2O, extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel chromatography (hexane-EtOAc; 60:40 to 50:50) afforded **5e** (632 mg, 20%) as a yellow solid. m.p. 172-174 ºC. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.22 (bs, 2H); 3.70 (m, 2H)); 3.81 (s, 3H); 4.56 (bs, 2H); 6.39 (bs, 1H); 7.32 (m, 3H); 7.79 (s, 1H) and 8.24 (bs, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 29.9; 33.8; 37.2; 78.3; 94.4; 116.0; 122.3; 123.3; 123.9; 126.2; 137.0; 137.7; 158.3. MS (CI) 911 (2M, 25); 457 (M+2, 100); 403 (20); 227 (20); 130 (55); 124 (95). HRMS m/z calcd. for $C_{15}H_{17}Cl_3N_4O_4S$ $(M+H)^+$ 455.0109, found 455.0119.

2,2,2-Trichloroethyl 6-(1-methylindol-3-yl)-tetrahydropyrimidin-2(1*H*)-ylidenesulfamate (7). A solution of **5e** (251 mg, 0.55 mmol) in diphenylether (8 mL) and two drops of formic acid was stirred at 200 ºC overnight. The reaction was mixture was cooled to r.t. and run through a small column of silica gel: firstly, with hexane, to remove all the diphenylether, and then, with EtOAc, to elute the crude product. Purification by semipreparative reverse phase HPLC (H_2O-MeCN; gradient 60:40 to 50:50 in 30 min) afforded **7** (10 mg, 7%) as a yellow solid. mp. 104-106 ºC.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.25 (m, 2H); 3.78 (s, 3H); 4.60 (s, 2H); 5.00 (m, 1H); 6.74 (bs, 1H); 6.99 (bs, 1H); 7.02 (s, 1H); 7.15 (t, *J* = 7.0, 1H); 7.29 (t, *J* = 8.0 Hz, 1H); 7.34 (d, *J* = 7.0 Hz, 1H); 7.54 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 27.2; 32.9; 37.9; 47.6; 77.9; 94.5; 109.9; 113.7; 118.4; 119.8; 122.5; 124.9; 126.6; 137.5; 154.6.

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 r.t. overnight. Evaporation afforded a yellow solid which was recrystallized from ethanol to give pure condensation products.

2,2-Dimethyl-5-[(1-methylindol-3-yl)methylene]-1,3-dioxane-4,6-dione (8a). Starting from **1a** (1.05 mg, 6.60 mmol) and following the general procedure, **8a** (81%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.77 (s, 6H); 3.97 (s, 3H); 7.37-7.45 (m, 3H); 7.93-7.99 (m, 1H); 8.9 (s, 1H); 9.39 (s, 1H). MS (CI) 286 (M+1, 100).

2,2-Dimethyl-5-[(5,6-dibromo-1-methoxyindol-3-yl)methylene]-1,3-dioxane-4,6-dione (8b). Starting from **1b**[16] (200 mg, 0.60 mmol) and following the general procedure, **8b** (87%) was obtained which was in the following steps used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74 (s, 6H); 4.38 (s, 3H); 8.10 (s, 1H); 8.48 (s, 1H); 8.67 (s, 1H); 9.46 (s, 1H). MS (ESI-TOF) 460 (M+1, 100).

2,2-Dimethyl-5-[(1-methoxyindol-3-yl)methylene]-1,3-dioxane-4,6-dione (8d). Starting from **1d** [31] (627 mg, 3.58 mmol) and following the general procedure, **8d** (quantitative yield) was obtained. ^{1}H NMR (400 MHz, d_6-acetone) δ 1.78 (s, 6H);

4.26 (s, 3H); 7.37-7.45 (m, 2H); 7.52-7.57 (m, 1H); 7.93-8.00 (m, 1H); 8.88 (s, 1H); 9.55 (s, 1H). MS (ESI-TOF) 625 (2M+Na, 100).

2,2-Dimethyl-5-[(5-bromoindol-3-yl)methylene]-1,3-dioxane-4,6-dione (8f). Starting from **1f** (500 mg, 2.23 mmol) and following the general procedure, **8f** (65%) was obtained. ^{1}H NMR (400 MHz, d_6-acetone) δ 1.74 (s, 6H); 7.48 (dd, *J* = 8.6 and 2.0 Hz, 1H); 7.62 (d, *J* = 8.6 Hz, 1H); 8.18 (d, *J* = 2.0 Hz, 1H); 8.77 (s, 1H); 9.44 (s, 1H). MS (ESI-TOF) 723 (2M+Na, 100).

2,2-Dimethyl-5-[(6-bromoindol-3-yl)methylene]-1,3-dioxane-4,6-dione (8g). Starting from **1g** (500 mg, 2.23 mmol) and following the general procedure, **8g** (650 mg, 83%) was obtained. ^{1}H NMR (400 MHz, d_6-acetone) δ 1.74 (s, 6H); 7.50 (dd, *J* = 8.4 and 2.0 Hz, 1H); 7.85 (d, *J* = 2.0 Hz, 1H); 7.96 (d, *J* = 8.4 Hz, 1H); 8.79 (s, 1H); 9.44 (s, 1H). MS (ESI-TOF) 237 (MBr^{79}+1, 100); 239 (MBr^{81}+1, 93); 723 (2M+Na, 100).

2,2-Dimethyl-5-[(5-bromo-1-methylindol-3-yl)methylene]-1,3-dioxane-4,6-dione (8h). Starting from **1h**[32] (1.06 g, 4.46 mmol) and following the general procedure, **8h** (62%) was obtained. ^{1}H NMR (400 MHz, d_6-acetone) δ 1.73 (s, 6H); 4.11 (s, 3H); 7.53 (dd, *J* = 8.4 and 1.6 Hz, 1H); 7.63 (d, *J* = 8.4 Hz, 1H); 8.16 (d, *J* = 1.6 Hz, 1H); 8.70 (s, 1H); 9.30 (s, 1H). MS (ESI-TOF) 308 (M+1, 100).

2,2-Dimethyl-5-[(6-bromo-1-methylindol-3-yl)methylene]-1,3-dioxane-4,6-dione (8i). Starting from **1i**[32] (1.05 g, 4.41 mmol) and following the general procedure, **8i** (75%) was obtained. ^{1}H NMR (400 MHz, d_6-acetone) δ 1.73 (s, 6H); 4.11 (s, 3H); 7.52 (dd, *J* = 8.4 and 1.6 Hz, 1H); 7.87 (d, *J* = 1.6 Hz, 1H); 7.93 (d, *J* = 8.4 Hz, 1H); 8.71 (s, 1H); 9.29 (s, 1H). MS (ESI-TOF) 308 (M+1, 100).

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 eq.) was then added and the reaction mixture was stirred at 135 ºC until the starting material had been consumed (tracked by HPLC from 2-16 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 two yellow oils.

2-Amino-6-(1-methylindol-3-yl)-1*H*-5,6-dihydropyrimidin-4-one (9a). Compound **8a** (1.05 g, 1.43 mmol) was converted into **9a** (90%). IR (KBr film): 3418, 2977, 1717, 1657, 1552, 1509, 1477, 1427, 1411, 1385, 1357, 1326, 1304, 1250, 1174, 1156, 1141, 1072, 1013, 795, 746, 714. ^{1}H NMR (400 MHz, d_4-MeOH) δ: 2.70-2.90 (m, 2H), 3.78 (s, 3H); 4.94 (dd, *J* = 5.9 and 9.2 Hz, 1H); 7.09 (t, *J* = 7.5 Hz, 1H); 7.16-7.25 (m, 2H); 7.37 (d, *J* = 8.1 Hz, 1H,); 7.63 (d, *J* = 8.0 Hz, 1H,). ^{13}C NMR (400 MHz, d_4-MeOH) δ: 29.7, 32.6, 46.5, 47.0, 68.2, 110.4, 119.5, 120.1, 122.8, 126.8, 127.5, 131.1, 138.7. MS (ESI-TOF) 243 (M+1, 100); 265 (M+Na, 82). HRMS *m/z* calcd. for $C_{13}H_{14}N_4NaO$ 265.1060, found 265.1058.

2-Amino-6-(5,6-dibromo-1-methoxyindol-3-yl)- 1*H*-5,6-dihydropyrimidin-4-one (9b). Compound **8b** (240 mg, 0.52 mmol) was converted into **9b** (20%). IR (KBr film) 2924, 1665, 1459, 1205, 801, 724. ^{1}H NMR (400 MHz, d_4-MeOH) δ 3.01 (dd, *J* = 16.8 and 5.0 Hz, 1H); 3.21 (dd, *J* = 16.8 and 10.0 Hz, 1H); 4.11 (s, 3H); 5.26 (dd, *J* = 10.0 and 5.0 MHz, 1H); 7.68 (s, 1H); 7.88 (s, 1H); 8.12 (s, 1H). ^{13}C NMR (400 MHz,

d_4-MeOH) δ 37.0, 46.4, 66.9, 108.7, 114.4, 116.6, 119.4, 123.3, 124.5, 124.9, 133.2, 168.4, 198.4. MS (ESI-TOF) 415 ($M(Br^{79})_2$+1, 48); 416 ($M(Br^{79})_2$+2, 8); 417 ($MBr^{79}Br^{81}$+1, 100); 419 ($M(Br^{81})_2$+2, 54). HRMS *m/z* calcd. for $C_{13}H_{13}Br_2N_4O_2$ 414.9400, found 414.9401.

2-Amino-6-(1-methoxyindol-3-yl)-1*H*-5,6-dihydropyrimidin-4-one (9d). Compound **8d** (1.08 g, 3.58 mmol) was converted into **9d** (5%). IR (KBr film) 3315, 2933, 1611, 1542, 1473, 1373, 1320, 1303, 1232, 1147, 1094, 1030, 945, 750, 738, 703, 575, 548, 508, 425. ^{1}H NMR (400 MHz, d_4-MeOH) δ 2.68-2.82 (m, 2H); 4.47 (s, 1H); 5.01 (dd, *J* = 8.8 and 6.4 Hz, 1H) δ 7.06-7.11 (m, 1H); 7.19-7.24 (m, 1H); 7.39-7.43 (m, 2H); 7.62 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (400 MHz, d_4-MeOH) δ 38.1, 47.3, 66.5, 83.2, 84.6, 100.4, 109.6, 120.2, 121.3, 122.1, 124.0, 162.5, 188.8. MS (ESI-TOF) 259 (M+1, 90); 260 (M+2, 100). HRMS *m/z* calcd. for $C_{13}H_{15}N_4O_2$ 259.1190, found 259.1190.

2-Amino-6-(5-bromoindol-3-yl)-1*H*-5,6-dihydropyrimidin-4-one (9f). Compound **8f** (1.35 g, 3.84 mmol) was converted into **9f** (47%). IR (KBr film) 3261, 1631, 1567, 1506, 1434, 1363. ^{1}H NMR (400 MHz, d_4-MeOH) δ 2.71-2.87 (m, 1H); 5.03 (dd, *J* = 8.4 and 6.2 Hz, 1H); 7.22-7.34 (m, 3H); 7.80 (d, *J* = 1.6 Hz, 1H). ^{13}C NMR (400 MHz, d_4-MeOH) δ 46.9, 54.6, 121.0, 123.3, 124.2, 130.7, 133.4, 133.8, 136.7, 144.7, 171.3, 186.5. MS (ESI-TOF) 307 (MBr^{79}+1, 98); 308 (MBr^{79}+2, 17); 309 (MBr^{81}+1, 100); 310 (MBr^{81}+2, 15). HRMS *m/z* calcd. for $C_{12}H_{12}BrN_4O$ 307.0189, found 307.0188.

2-Amino-6-(6-bromoindol-3-yl)-1*H*-5,6-dihydropyrimidin-4-one (9g). Compound **8g** (50 mg, 0.14 mmol) was converted into **9g** (49%). IR (KBr film) 3462,

1721, 1624, 1500, 1452, 1422, 1348, 1231, 1145, 1110, 803, 533. ^{1}H NMR (400 MHz, d_4-MeOH) δ 2.68-2.82 (m, 2H); 4.99 (dd, *J* = 8.6 and 6.0 Hz, 1H); 7.17-7.30 (m, 3H); 7.76 (d, *J* = 1.7 Hz, 1H). ^{13}C NMR (400 MHz, d_4-MeOH) δ: 47.1, 54.8, 123.7, 123.8, 124.6, 130.2, 131.1, 133.3, 133.8, 146.9, 171.4, 186.4. MS (ESI-TOF) 307 (MBr^{79}+1, 98); 308 (MBr^{79}+2, 15); 309 (MBr^{81}+1, 100); 310 (MBr^{81}+2, 15). HRMS *m/z* calcd. for $C_{12}H_{12}BrN_4O$ 307.0189, found 307.0188.

2-Amino-6-(5-bromo-1-methylindol-3-yl)-1*H*-5,6-dihydropyrimidin-4-one (9h). Compound **8h** (994 mg, 2.73 mmol) was converted into **9h** (74%). IR (KBr film) 3353, 3267, 3031, 2977, 1662, 1632, 1543, 1498, 1428, 1446, 1386, 1324, 1269, 1148, 1060, 797, 677, 516. ^{1}H NMR (400 MHz, d_6-DMSO) δ 3.73 (s, 3H); 4.88 (dd, *J* = 7.2 and 7.2 Hz, 1H); 7.25-7.29 (m, 2H); 7.41 (d, *J* = 8.8, 1H); 7.82 (d, *J* = 2.0, 1H). MS (ESI-TOF) 321 (MBr^{79}+1, 100); 322 (MBr^{79}+2, 18); 323 (MBr^{81}+1, 90); 324 (MBr^{81}+2, 16). HRMS *m/z* calcd. for $C_{13}H_{14}BrN_4O$ 321.0346, found 321.0348.

2-Amino-6-(6-bromo-1-methylindol-3-yl)-1*H*-5,6-dihydropyrimidin-4-one (9i). Compound **8i** (1.14 g, 3.13 mmol) was converted into **9i** (60%). IR (KBr film) 3322, 3094, 1636, 1609, 1544, 1494, 1407, 1323, 1294, 1255, 1230, 1134, 1134, 836, 804, 675, 590. ^{1}H NMR (400 MHz, d_6-DMSO) δ 3.73 (s, 3H); 4.88 (dd, *J* = 7.6 and 7.6 Hz, 1H); 7.16 (d, *J* = 8.4 Hz, 1H); 7.58 (d, *J* = 8.8, 1H); 7.69 (s, 1H). ^{13}C NMR (400 MHz, d_6-DMSO) δ 41.9, 46.9, 108.1, 122.2, 123.8, 130.1, 131.0, 133.9, 137.1, 147.1, 157.1, 186.3. MS (ESI-TOF) 321 (MBr^{79}+1, 100); 322 (MBr^{79}+2, 15); 323 (MBr^{81}+1, 92); 324 (MBr^{81}+2, 15). HRMS *m/z* calcd. for $C_{13}H_{14}BrN_4O$ 321.0346, found 321.0352.

General procedure for the reduction of compounds 9. Borane-THF complex (1M

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 ºC until the starting material has been consumed (tracked by HPLC, 2–15 h). The reaction mixture was then cooled to r.t. 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.

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 solid. IR (KBr film) 3259, 2925, 2854, 1663, 1627, 1466, 1382, 1309, 1197, 742. ^{1}H NMR (400 MHz, d_4-MeOH) 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, d_4-MeOH) 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-tetrahydropyrimidin-4-one (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, d_4-MeOH) 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, d_4-MeOH) 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}NMR (400 MHz, d_4-MeOD): 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, d_4-MeOD) 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, d_4-MeOH) 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, d_4-MeOH) 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, d_4-MeOH) 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, d_4-MeOH) δ 27.7, 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 semisolid. IR (KBr film) 3257, 2942, 1661, 1628, 1455, 1333, 1021, 803. ^{1}H NMR (400 MHz, d_4-MeOH) 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, d_4-MeOH) 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 semisolid. 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, d_4-MeOH) 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, d_4-MeOH) 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 semisolid. 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, d_4-MeOH) 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, d_4-MeOH) 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.

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 r.t. 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-(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%). ^{1}H NMR (400 MHz, d_4-MeOH) 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, d_4-MeOH) 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%). ^{1}H NMR (400 MHz, d_4-MeOH) 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, d_4-MeOH) 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%). ^{1}H NMR (400 MHz, d_4-MeOH) 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, d_4-MeOH) 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%). ^{1}H NMR (400 MHz, d_4-MeOH) 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, d_4-MeOH) 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%). ^{1}H NMR (400 MHz, d_4-MeOH) 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, d_4-MeOH) 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 ATCC (American Type Culture Collection): A-549, human lung carcinoma, 0HT-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.[33] 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 Tz 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 r.t. 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:[34] Tz = 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 $Tz < T < C$ (growth inhibition), then the result is $100 \times ([T - Tz]/[C - Tz])$.

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

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

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

Supporting Information Available: General data, ^{1}H- and ^{13}C-NMR spectra, and HPLC chromatograms of compounds **9-11**. This information is available free of charge via the Internet at http://pubs.acs.org.

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^{1}H NMR (400 MHz, $CDCl_3$) 4.15 (s, 3H), 6.63 (dd, *J* = 15.9 and 7.7 Hz, 1H); 7.50 (d, *J* = 15.9 Hz); 7.64 (s, 1H), 7.77 (s, 1H), 8.12 (s, 1H), 9.61 (d, *J* = 7.7 Hz, 1H).

14. 5,6-Dibromo-1-methoxy-indole-3-carbaldehyde (**1b**) was obtained in 50% yield from 5,6-dibromo-1-methoxyindole by Vilsmeier reaction, following the methodology described for the preparation of 1-methoxyindole-3-carbaldehyde in Hanley, A. B.; Parsley, K. R.; Lewis, J. A.; Fenwick, G. R. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2273-2276. **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. 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-methoxy-indole by Somei, M.; Shoda, T. *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).

16. **1b** and **1d** were obtained by Vilsmeir formylation of 5,6-dibromo-1- methoxy-indole and 1-methoxy-indole, respectively.

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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.

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

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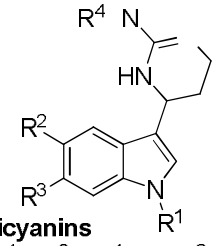
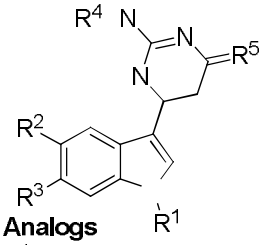
TOC Graphic



Ap icyanins
A: $R^1 = R^3 = R^4 = H$; $R^2 = Br$
B: $R^1 = R^3 = H$; $R^2 = Br$; $R^4 = Ac$
E: $R^1 = OMe$; $R^2 = R^3 = Br$; $R^4 =$



Analogs
$R^1 = H$, Me, OMe
$R^2 = R^3 = H$, Br
$R^4 = H$, Ac; $R^5 = H_2$, O