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Synthesis of Phidianidines A and B

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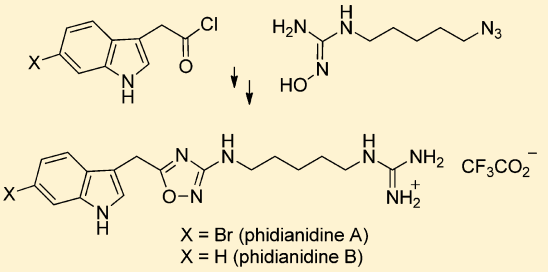
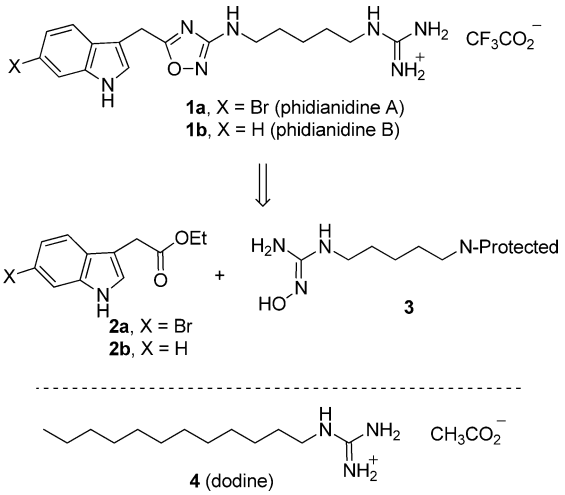
Supporting Information

ABSTRACT: Reaction of a substituted indole-3-acetyl chloride with *N*-5-azidopentyl-*N*′-hydroxyguanidine generated a substituted 3-(5-azidopentylamino)-5-((indol-3-yl)methyl)-1,2,4-oxadiazole. Reduction of the azide with zinc and ammonium formate afforded the amine, which was elaborated to the guanidine, completing short and efficient syntheses of the cytotoxic natural products phidianidines A and B in 19% overall yield by a convergent route that will make analogues readily available for biological evaluation. Initial screening in the NCI 60 cell line at 10^{-5} M indicated that the bromine on the indole is necessary for activity and that the amine precursor to phidianidine A is more potent than phidianidine A.



Guo, Gavagnin, and co-workers recently reported the isolation of the guanidine-containing natural products phidianidines A (**1a**) and B (**1b**) as the trifluoroacetate salts from the shell-less marine opisthobranch mollusk *Phidiana militaris* (see Scheme 1).[1] Despite their relatively simple structure, the phidianidines are of interest because they appear to be the first natural products that contain an 1,2,4-oxadiazole ring.[2] Furthermore, they show significant cytotoxicity at 0.14−5.42 μM concentrations against the highly proliferating C6 rat glioma and HeLa epithelial cervical cancer cell lines and the embryonic 3T3-L1 murine embryonic fibroblast and H9c2 rat embryonic cardiac myoblast cell lines and are less toxic to the less rapidly proliferating CaCo-2 epithelial colorectal adenocarcinoma cell line (35 to 100 μM).[1] The mode of action is not known, but the closely related dodecylguanidinium acetate (**4**, dodine) has been in widespread use since 1956 as a fungicide and may act by membrane disruption.[3] As part of an ongoing program in the synthesis of structurally novel guanidine-containing natural products,[4] we planned to prepare phidianidines A (**1a**) and B (**1b**) by coupling of the appropriate ethyl indole-3-acetate (**2a** or **2b**) with hydroxyguanidine **3** containing a suitably protected nitrogen on the other end of the five-carbon side chain. There is limited precedent for the synthesis of 3-amino-1,2,4-oxadiazoles by coupling of esters with hydroxyguanidines,[5] and nature may use a related route for the biosynthesis of the phidianidines.

Scheme 1. Retrosynthesis of Phidianidines A (1a) and B (1b)



We chose to use hydroxyguanidine **8** with an azidopentyl side chain because the azide group can be easily elaborated into the guanidine of the phidianidines and a wide variety of analogues (see Scheme 2). Furthermore, 5-azido-1-pentanamine (**6**) is readily available in quantity in 85% yield by selective reduction of diazide **5** by Kim's procedure with triphenylphosphine in a two-phase system.[6a] After monoreduction of the diazide in Et_2O/EtOAc, the azido amine is protonated and partitions into the 5% HCl solution, thereby preventing reduction of the second azide. Reaction of amine **6** with cyanogen bromide[7] in CH_2Cl_2 and aqueous $NaHCO_3$ solution afforded somewhat unstable cyanamide **7**, which was treated with hydroxylamine hydrochloride[8] and K_2CO_3 in EtOH to give the unstable[8d] hydroxyguanidine **8**, which was used immediately for the next step.

Our initial attempt at 3-amino-1,2,4-oxadiazole synthesis using models *N*-butyl-*N*′-hydroxyguanidine and ethyl phenylacetate (3 equiv) with NaOEt (2−3 equiv) in EtOH at reflux for 5 h proceeded in 50−70% yield based on the hydroxyguanidine. However, the yield dropped to 0−15% without both excess ester and NaOEt and the reaction proceeded in only 30% yield with ethyl indole-3-acetate (3

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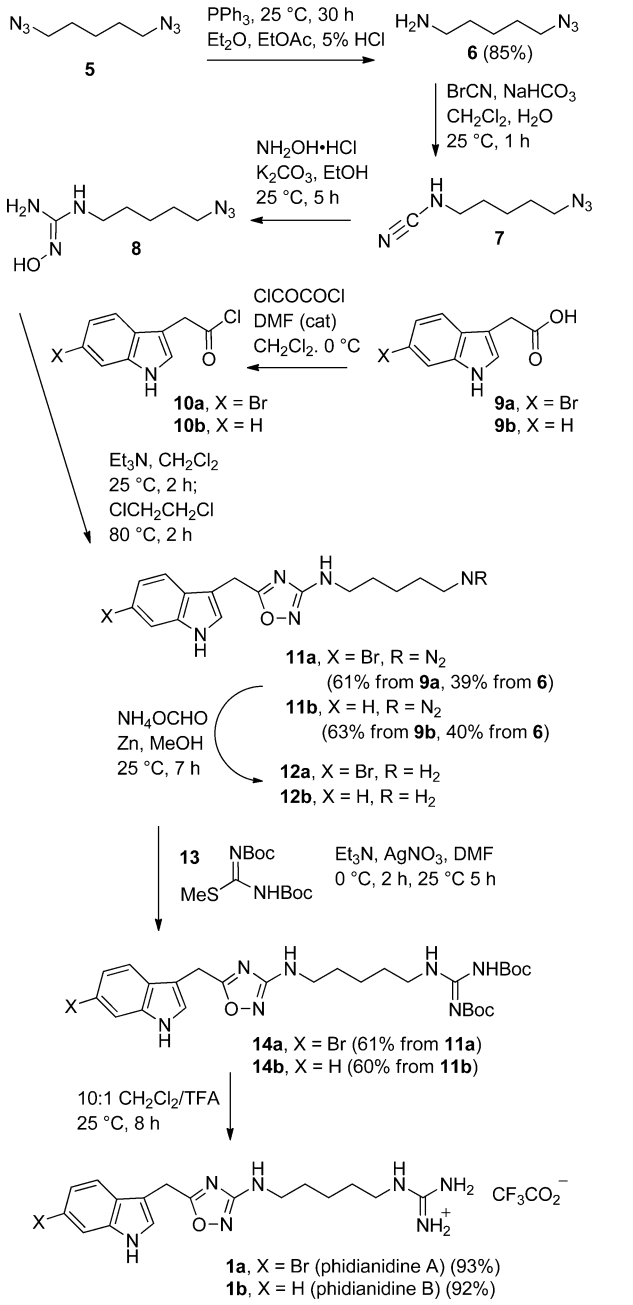
Scheme 2. Synthesis of Phidianidines A (1a) and B (1b)



equiv) and NaOEt (3 equiv). These initial results indicated that an ester is not sufficiently reactive to couple with a hydroxyguanidine unless it is used in large excess. We therefore chose use an acid chloride rather than an ester to form the 3-amino-1,2,4-oxadiazole, a protocol that has been reported in the recent patent literature.[9]

Indole-3-acetic acid (**9b**) was reacted with oxalyl chloride and catalytic DMF in CH_2Cl_2 to give acid chloride **10b**, which was treated with 1.5 equiv of freshly prepared **8** and Et_3N in CH_2Cl_2 for 2 h at 25 °C. The solution was concentrated, and a solution of the residue containing the initial coupling product in 1,2-dichloroethane was heated at 80 °C for 2 h to form the 3-amino-1,2,4-oxadiazole giving **11b** in 63% yield from **9b** and 40% yield from **6**. The change of solvents after the initial coupling was necessitated by the high temperature needed for ring closure to form the 1,2,4-oxadiazole and the poor solubility of **8** and **10b** in $ClCH_2CH_2Cl$.

Reduction of the azide group of **11b** was complicated by the sensitivity of the 1,2,4-oxadiazole.[2] Attempted hydrogenation over Pd destroyed the 1,2,4-oxadiazole ring. Reduction with Ph_3P was successful, but removal of the phosphine oxide byproduct was difficult. Eventually we found that reduction of the azide with activated zinc[10] and ammonium formate in MeOH for 7 h at 25 °C proceeded cleanly to give polar amine **12b** that was used without purification.[11a] Coupling of **12b** with Boc-protected *S*-methylisothiourea **13** using Et_3N and $AgNO_3$[4b,12] in DMF for 2 h at 0 °C and 5 h at 25 °C afforded protected guanidine **14b** in 60% yield from **11b**. Deprotection of **14b** by stirring in 10:1 CH_2Cl_2/TFA for 8 h at 25 °C removed the Boc protecting groups providing phidianidine B (**1b**) in 92% yield. The ^1H and ^{13}C NMR spectral data of phidianidine B in both CD_3OD and DMSO-d_6 are identical to those reported for the natural product[1] confirming the presence of the 1,2,4-oxadiazole ring.

Phidianidine A (**1a**) was prepared by an analogous sequence of steps from 6-bromoindole-3-acetic acid (**9a**).[13] Acid chloride **10b** was coupled with hydroxyguanidine **8** to give **11a** in 61% yield from **9a** and 39% yield from **6**. Reduction of the azide of **11a** with zinc and ammonium formate afforded amine **12a** which was coupled with **13** using Et_3N and $AgNO_3$ in DMF to give protected guanidine **14a** in 61% yield from **11a**. Deprotection of **14a** in 10:1 CH_2Cl_2/TFA provided phidianidine A (**1a**) in 93% yield with ^1H and ^{13}C NMR spectral data in both CD_3OD and DMSO-d_6 identical to those reported for the natural product.[1]

Initial screening in the NCI 60 cell line screen at 10^{-5} M showed an average of 33% inhibition of cell growth with phidianidine A (**1a**). The amine precursor **12a** was more potent with an average of 81% inhibition of the 60 cell lines. Phidianidine B (**1b**) and amine **12b** were both much less effective with an average of 5% inhibition of cell growth. These results indicate that the bromine substituent on the indole is important for activity and that the amine of **12a** is more effective than the guanidine of **1a**. Full details for these four compounds with all 60 cell lines are provided in the Supporting Information.

In conclusion, *N*-5-azido-1-pentanamine (**6**) was elaborated to *N*-5-azidopentyl-*N*′-hydroxyguanidine (**8**) in two steps. Reaction of **8** with indole-3-acetyl chloride **10a** or **10b** afforded 3-(5-azidopentylamino)-5-((indol-3-yl)methyl)-1,2,4-oxadiazoles **11a** and **11b** in 61−63% yield. Reduction of the azides with zinc and ammonium formate afforded amines **12a** and **12b**, which were elaborated to the guanidine, completing short and efficient syntheses of the cytotoxic natural products phidianidines A (**1a**) and B (**1b**) in 19% overall yield by a convergent route that will make analogues readily available for biological evaluation.

EXPERIMENTAL SECTION

General Experimental Methods. Reactions were conducted in flame- or oven-dried glassware under a nitrogen atmosphere and were stirred magnetically. The phrase "concentrated" refers to removal of solvents by means of a rotary-evaporator attached to a diaphragm pump (15−60 Torr) followed by removal of residual solvents at <1 Torr with an vacuum pump. Flash chromatography was performed on silica gel 60 (230−400 mesh). Analytical thin-layer chromatography (TLC) was performed using silica gel 60 F-254 precoated glass plates (0.25 mm). TLC plates were analyzed by short-wave UV illumination or by dipping in CAM stain (40 g of ammonium molybdate, 1.6 g of

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ceric ammonium molybdate, 80 mL of concentrated sulfuric acid and 720 mL of water) and heating on a hot plate, or by spraying with permanganate solution (5 g $KMnO_4$ in 495 mL water). THF and ether were dried and purified by distillation from sodium/benzophenone. Et_3N, pyridine, acetonitrile, and benzene were distilled from CaH_2. ^1H and ^{13}C NMR spectra were obtained on a 400 MHz spectrometer in $CDCl_3$ with tetramethylsilane as internal standard unless specifically indicated. Chemical shifts are reported in δ (ppm downfield from tetramethylsilane). Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), and br (broad). IR spectra were acquired on an FT-IR spectrometer and are reported in wave numbers (cm^{-1}). High-resolution mass spectra were obtained using electrospray ionization (ESI) analyzed by quadrupole time-of-flight (QTof).

5-Azido-1-pentanamine (5).[6] To a solution of **5**[6,14] (1.1 g, 7.1 mmol) in 5 mL of Et_2O were added 5 mL of EtOAc and 9 mL of 5% HCl aqueous solution successively. To the resulting mixture at 0 °C was added PPh_3 (1.9 g, 7.2 mmol, 1.0 equiv) in small portions over 1 h. The mixture was stirred at room temperature for 30 h. HCl solution (1 M, 10 mL) was added, the layers were separated, and the organic layer was discarded. The aqueous layer was washed with CH_2Cl_2 (20 mL × 2), which was discarded, and neutralized with 6 M NaOH until the pH reached 12. The basic aqueous layer was saturated with NaCl and extracted with CH_2Cl_2 (20 mL × 4). The combined organic layers were dried over Na_2SO_4 and concentrated to give 780 mg (85% from **5**) of **6** as a colorless oil: ^1H NMR 3.28 (t, 2, J = 6.8), 2.73 (t, 2, J = 6.8), 2.12 (br, 2, NH_2), 1.67−1.58 (m, 2), 1.55−1.45 (m, 2), 1.48−1.38 (m, 2); ^{13}C NMR 51.3, 41.7, 32.7, 28.6, 24.0; IR (neat) 3318 (br), 2933, 2862, 2089, 1558, 1469, 1390, 1300, 1259. The spectral data are identical to those previously reported.[6]

***N*-(5-Azidopentyl)-5-[(1*H*-indol-3-yl)methyl]-1,2,4-oxadiazol-3-amine (11b).** To a suspension of **9b** (350 mg, 2.0 mmol) in 10 mL of CH_2Cl_2 at 0 °C were added DMF (2 drops) and oxalyl chloride (0.51 mL, 5.9 mmol, 3.0 equiv) successively. The mixture was stirred at 0 °C for 1.5 h and concentrated to give 450 mg of crude 1*H*-indole-3-acetyl chloride (**10b**), which was used for the synthesis of **11b** without further purification.

To a solution of **6** (400 mg, 3.1 mmol) in 6 mL of CH_2Cl_2 were added $NaHCO_3$ (1.6 g, 19 mmol, 6.0 equiv) and 7 mL of H_2O successively. To the resulting mixture was slowly added a solution of BrCN (394 mg, 3.7 mmol, 1.2 equiv) in 2 mL of CH_2Cl_2 at 0 °C. The mixture was stirred at 0 °C for 0.5 h and at room temperature for 1 h. The reaction was quenched by addition of water (10 mL). The mixture was extracted with CH_2Cl_2 (15 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated to give 460 mg (96% from **6**) of >90% pure **7** as a somewhat unstable pale yellow oil that can be stored for a few days at 0 °C: ^1H NMR 3.73 (br, 1, NH), 3.31 (t, 2, J = 6.8), 3.10 (dt, 2, J = 6.8, 6.8), 1.71−1.59 (m, 4), 1.52−1.42 (m, 2); ^{13}C NMR 116.0, 51.1, 46.0, 29.1, 28.3, 23.4; IR (neat) 3211 (br), 2937, 2865, 2215, 2090, 1454, 1351, 1246, 1159.

To a solution of crude **7** (460 mg, 3.0 mmol) in 10 mL of dry EtOH were added $NH_2OH{\cdot}HCl$ (252 mg, 3.6 mmol, 1.2 equiv) and K_2CO_3 (1.24 g, 9.0 mmol, 3.0 equiv) successively. The reaction mixture was stirred at room temperature for 5 h, diluted with EtOAc (20 mL), and filtered through Celite. The filtrate was dried over Na_2SO_4 and concentrated to give 502 mg of crude **8** as an unstable pale yellow sticky oil, which decomposed on storage at 0 °C overnight and should be used immediately in the next step: ^1H NMR (DMSO-d_6) 9.50 (br, 1, NH or OH), 7.49 (br, 1, NH or OH), 7.32 (br, 2, NH or OH), 3.33 (t, 2, J = 7.2), 3.14−3.06 (m, 2), 1.60−1.42 (m, 4), 1.38−1.26 (m, 2).

To a solution of freshly prepared crude **8** (502 mg, from 400 mg (3.1 mmol) of **6**) and NEt_3 (0.83 mL, 6.0 mmol) in 10 mL of CH_2Cl_2 at 0 °C was added a solution of crude **10b** (450 mg, from 350 mg (2.0 mmol) of **9b**) in 4 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 2 h and concentrated. To the residue was added 15 mL of $ClCH_2CH_2Cl$. The mixture was heated at 80 °C for 2 h and concentrated. To the residue was added 15 mL of saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (15 mL × 4). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash chromatography of the residue on silica gel (6:1 CH_2Cl_2/EtOAc) gave 410 mg (63% from **9b**, 40% from **6**) of **11b** as a beige solid: mp 58−59 °C; ^1H NMR 8.16 (br, 1, NH) 7.63 (br d, 1, J = 8.0), 7.37 (br d, 1, J = 8.0), 7.22 (br dd, 1, J = 8.0, 8.0), 7.20 (br s, 1), 7.15 (br dd, 1, J = 8.0, 8.0), 4.29 (br, 1, NH), 4.22 (s, 2), 3.25 (t, 2, J = 6.8), 3.23 (dt, 2, J = 6.8, 6.8), 1.68−1.56 (m, 4), 1.49−1.37 (m, 2); ^{13}C NMR 177.3, 168.6, 136.1, 126.7, 123.0, 122.5, 119.9, 118.7, 111.3, 108.1, 51.2, 43.1, 28.9, 28.5, 23.9, 23.4; IR (neat) 3306 (br), 2092, 1703, 1661, 1594, 1456, 1339, 1247, 740; HRMS (ESI) calcd for $C_{16}H_{20}N_7O$ (MH^+) 326.1729, found 326.1727.

***N*-(5-Azidopentyl)-5-[(6-bromo-1*H*-indol-3-yl)methyl]-1,2,4-oxadiazol-3-amine (11a).** To a suspension of **9a** (254 mg, 1.0 mmol) in 10 mL of CH_2Cl_2 at 0 °C were added DMF (2 drops) and oxalyl chloride (0.26 mL, 3.0 mmol, 3.0 equiv) successively. The mixture was stirred at 0 °C for 1.5 h and concentrated to give 320 mg of crude **10a**, which was used for the synthesis of **11a** without further purification.

To a solution of freshly prepared crude **8** (see preparation of **11b** for procedure) (254 mg, from 200 mg (1.6 mmol) of **6**) and NEt_3 (0.42 mL, 3.0 mmol) in 5 mL of CH_2Cl_2 at 0 °C was added a solution of crude **10a** (320 mg, from 254 mg (1.0 mmol) of **9a**) in 2 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 2 h and concentrated. To the residue was added 8 mL of $ClCH_2CH_2Cl$. The mixture was heated at 80 °C for 2 h and concentrated. To the residue was added 15 mL of saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (15 mL × 4). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash chromatography of the residue on silica gel (6:1 CH_2Cl_2/EtOAc) gave 246 mg (61% from **9a**, 39% from **6**) of **11a** as a beige solid: mp 86−87 °C; ^1H NMR 8.21 (br, 1, NH) 7.51 (br s, 1), 7.47 (br d, 1, J = 8.4), 7.24 (br d, 1, J = 8.4), 7.16 (br s, 1), 4.29 (br, 1, NH), 4.18 (s, 2), 3.26 (t, 2, J = 6.8), 3.23 (dt, 2, J = 6.8, 6.8), 1.68−1.56 (m, 4), 1.49−1.37 (m, 2); ^{13}C NMR 177.0, 168.6, 136.9, 125.6, 123.7, 123.2, 120.0, 116.1, 114.2, 108.3, 51.2, 43.1, 28.9, 28.5, 23.9, 23.3; IR (neat) 3296 (br), 2092, 1594, 1453, 1330, 1236, 893, 803, 735; HRMS (ESI) calcd for $C_{16}H_{19}BrN_7O$ (MH^+) 404.0834, found 404.0833.

***N*-[5-[[Bis[[(1,1-dimethylethoxy)carbonyl]amino]-methylene]amino]pentyl]-5-[(1*H*-indol-3-yl)methyl]-1,2,4-oxadiazol-3-amine (14b).** To a solution of **11b** (410 mg, 1.26 mmol) in 8 mL of MeOH were added ammonium formate (397 mg, 6.30 mmol, 5.0 equiv) and activated zinc[10] (328 mg, 5.04 mmol, 4.0 equiv) successively. The mixture was stirred at room temperature for 7 h. The reaction was quenched by addition of 1 M NaOH (15 mL). The mixture was extracted with CH_2Cl_2 (15 mL × 4). The combined organic layers were dried over Na_2SO_4 and concentrated to give 390 mg of crude **12b**, which was used in the next step without purification: ^1H NMR 8.14 (br, 1, NH), 7.63 (br d, 1, J = 8.0), 7.38 (br d, 1, J = 8.0), 7.22 (br dd, 1, J = 8.0, 8.0), 7.22 (br s, 1), 7.15 (br dd, 1, J = 8.0, 8.0), 4.26 (br, 1, NH), 4.22 (s, 2), 3.23 (dt, 2, J = 6.8, 6.8), 2.68 (t, 2, J = 6.8), 1.68−1.54 (m, 2), 1.52−1.32 (m, 4).

To a solution of crude **12b** (390 mg) in 8 mL of DMF were added 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea (**13**) (561 mg, 1.89 mmol) and NEt_3 (1.40 mL, 10.1 mmol) successively. To the resulting mixture at 0 °C was added $AgNO_3$ (429 mg, 2.52 mmol) in small portions. The reaction was stirred at 0 °C for 2 h and at room temperature for 5 h. The reaction was quenched by addition of EtOAc (50 mL) and filtered through Celite. The filtrate was washed with saturated $NaHCO_3$ (50 mL × 4), dried over Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (2:3 EtOAc/hexanes) gave 417 mg (61% from **11b**) of **14b** as a pale yellow sticky oil: ^1H NMR 11.49 (br s, 1, NH), 8.30 (br, 2, NH), 7.62 (br d, 1, J = 8.0), 7.37 (br d, 1, J = 8.0), 7.21 (br dd, 1, J = 8.0, 8.0), 7.20 (br s, 1), 7.14 (br dd, 1, J = 8.0, 8.0), 4.33 (br, 1, NH), 4.22 (s, 2), 3.37 (dt, 2, J = 6.8, 6.8), 3.20 (dt, 2, J = 6.8, 6.8), 1.64−1.54 (m, 4), 1.50 (s, 9), 1.49 (s, 9), 1.42−1.32 (m, 2); ^{13}C NMR 177.2, 168.7, 163.6, 156.1, 153.3, 136.1, 126.7, 123.0, 122.3, 119.8, 118.7, 111.3, 108.0, 83.1, 79.3, 43.1, 40.7, 29.0, 28.6, 28.3 (3 C), 28.0 (3 C), 24.0, 23.4; IR (neat) 3324 (br), 1719, 1597, 1414, 1366, 1325, 1129, 1053, 912, 731; HRMS (ESI) calcd for $C_{27}H_{40}N_7O_5$ (MH^+) 542.3091, found 542.3085.

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***N*-[5-[[Bis[[(1,1-dimethylethoxy)carbonyl]amino]-methylene]amino]pentyl]-5-[(6-bromo-1*H*-indol-3-yl)methyl]-1,2,4-oxadiazol-3-amine (14a).** To a solution of **11a** (246 mg, 0.61 mmol) in 5 mL of MeOH were added ammonium formate (192 mg, 3.05 mmol, 5.0 equiv) and activated zinc[10] (159 mg, 2.44 mmol, 4.0 equiv) successively. The mixture was stirred at room temperature for 7 h. The reaction was quenched by addition of 1 M NaOH (15 mL). The mixture was extracted with CH_2Cl_2 (15 mL × 4). The combined organic layers were dried over Na_2SO_4 and concentrated to give 228 mg of crude **12a**, which was used in the next step without purification: ^{1}H NMR 8.15 (br, 1, NH) 7.53 (br s, 1), 7.48 (br d, 1, J = 8.4), 7.24 (br d, 1, J = 8.4), 7.19 (br s, 1), 4.29 (br, 1, NH), 4.18 (s, 2), 3.23 (dt, 2, J = 6.8, 6.8), 2.68 (t, 2, J = 6.8), 1.68−1.54 (m, 2), 1.52−1.32 (m, 4).

To a solution of crude **12a** (228 mg) in 4 mL of DMF were added 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea (**13**) (267 mg, 0.92 mmol) and NEt_3 (0.70 mL, 5.06 mmol) successively. To the resulting mixture at 0 °C was added $AgNO_3$ (207 mg, 1.22 mmol) in small portions. The reaction was stirred at 0 °C for 2 h and at room temperature for 5 h. The reaction was quenched by addition of EtOAc (30 mL) and filtered through Celite. The filtrate was washed with saturated $NaHCO_3$ (30 mL × 4), dried over Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (2:3 EtOAc/hexanes) gave 226 mg (60% from **11a**) of **14a** as a pale yellow sticky oil: ^{1}H NMR 11.50 (br s, 1, NH), 8.70 (br, 1, NH), 8.31 (br, 1, NH), 7.52 (br s, 1), 7.47 (br d, 1, J = 8.0), 7.23 (br d, 1, J = 8.0), 7.16 (br s, 1), 4.47 (br, 1, NH), 4.18 (s, 2), 3.32 (dt, 2, J = 6.8, 6.8), 3.16 (dt, 2, J = 6.8, 6.8), 1.58−1.46 (m, 4), 1.49 (s, 18), 1.34−1.24 (m, 2); ^{13}C NMR 176.9, 168.7, 163.5, 156.1, 153.3, 136.9, 125.7, 123.7, 123.1, 120.0, 115.9, 114.3, 108.2, 83.1, 79.4, 43.1, 40.7, 29.0, 28.6, 28.3 (3 C), 28.0 (3 C), 24.0, 23.3; IR (neat) 3322 (br), 1718, 1598, 1413, 1366, 1330, 1131, 1052, 910, 803, 730; HRMS (ESI) calcd for $C_{27}H_{39}BrN_7O_5$ (MH^+) 620.2196, found 620.2187.

***N*-[5-[(Aminoiminomethyl)amino]pentyl]-5-[(1*H*-indol-3-yl)-methyl]-1,2,4-oxadiazol-3-amine Trifluoroacetic Acid Salt (Phidianidine B, 1b).** Compound **14b** (417 mg, 0.77 mmol) was taken up in 20 mL of 1:10 TFA/CH_2Cl_2, and the resulting solution was stirred at room temperature for 8 h. The mixture was diluted with 25 mL of MeOH and concentrated to give 326 mg (93%) of >95% pure **1b** as a pale yellow oil: ^{1}H NMR (CD_3OD) 7.52 (br d, 1, J = 8.0), 7.35 (br d, 1, J = 8.0), 7.21 (br s, 1), 7.11 (br dd, 1, J = 8.0, 8.0), 7.01 (br dd, 1, J = 8.0, 8.0), 4.22 (s, 2), 3.15 (t, 2, J = 6.8), 3.14 (t, 2, J = 6.8), 1.68−1.54 (m, 4), 1.46−1.36 (m, 2); (DMSO-d_6) 11.04 (br s, NH), 7.55 (br t, 1, J = 5.6, NH), 7.51 (br d, 1, J = 8.0), 7.37 (br d, 1, J = 8.0), 7.31 (br d, 1, J = 2.2), 7.09 (br dd, 1, J = 8.0, 8.0), 6.99 (br dd, 1, J = 8.0, 8.0), 6.72 (br t, 1, J = 5.6, NH), 4.20 (s, 2), 3.06 (dt, 2, J = 5.6, 5.6), 3.01 (dt, 2, J = 5.6, 5.6), 1.56−1.40 (m, 4), 1.35−1.25 (m, 2); ^{13}C NMR (CD_3OD, referenced to the residual solvent peaks centered at δ 49.15) 179.5, 170.2, 158.8, 138.2, 128.3, 124.8, 122.9, 120.2, 119.4, 112.5, 108.3, 43.9, 42.5, 29.9, 29.7, 25.0, 24.2; (DMSO-d_6, referenced to the residual solvent peaks centered at δ 39.51) 176.9, 168.5, 156.7, 136.2, 126.7, 124.2, 121.2, 118.7, 118.4, 111.5, 106.9, 42.3, 40.7, 28.1 (2 C), 23.4, 22.7; IR 3303 (br), 1671, 1599, 1201, 1137; HRMS (ESI) calcd for $C_{17}H_{24}N_7O$ (M^+) 342.2042, found 342.2047. In both solvents, the indole carbons near the nitrogen are doubled in various ratios due to the presence of both NH and ND forms of the indole.[15]

***N*-[5-[(Aminoiminomethyl)amino]pentyl]-5-[(6-bromo-1*H*-indol-3-yl)methyl]-1,2,4-oxadiazol-3-amine Trifluoroacetic Acid Salt (Phidianidine A, 1a).** Compound **14a** (100 mg, 0.16 mmol) was taken up in 5 mL of 1:10 TFA/CH_2Cl_2, and the resulting solution was stirred at room temperature for 8 h. The mixture was diluted with 5 mL of MeOH and concentrated to give 79 mg (92%) of >95% pure **1a** as a pale yellow oil: ^{1}H NMR (CD_3OD) 7.52 (br d, 1, J = 1.7), 7.45 (br d, 1, J = 8.0), 7.23 (br s, 1), 7.13 (br dd, 1, J = 8.0, 1.7), 4.20 (s, 2), 3.15 (br t, 4, J = 7.2), 1.66−1.54 (m, 6), 1.46−1.36 (m, 2); (DMSO-d_6) 11.18 (br, s, NH), 7.56 (br d, 1, J = 1.7), 7.49 (br s, NH), 7.47 (br d, 1, J = 8.4), 7.35 (br d, 1, J = 2.5), 7.14 (br dd, 1, J = 8.4, 1.7), 6.73 (br t, 1, J = 6.0, NH), 4.20 (s, 2), 3.06 (dt, 2, J = 6.0, 6.0), 3.00 (dt, 2, J = 6.0, 6.0), 1.56−1.42 (m, 4), 1.34−1.22 (m, 2); ^{13}C NMR (CD_3OD, referenced to the residual solvent peaks centered at δ 49.15) 179.1, 170.3, 158.8, 139.1, 127.3, 125.9, 123.4, 121.0, 116.3, 115.5, 108.8, 43.9, 42.5, 29.9, 29.7, 25.1, 24.1; (DMSO-d_6, referenced to the residual solvent peaks centered at δ 39.51) 176.7, 168.5, 156.7, 137.0, 125.8, 125.3, 121.6, 120.2, 114.2, 114.0, 107.4, 42.3, 40.7, 28.1 (2 C), 23.3, 22.5; IR 3280 (br), 1670, 1601, 1184, 1137; HRMS (ESI) calcd for $C_{17}H_{23}BrN_7O$ (M^+) 420.1147, found 420.1135. In both solvents, the indole carbons near the nitrogen are doubled in various ratios due to the presence of both NH and ND forms of the indole.[15]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Results from the NCI 60 cell screens of phidianidine A (**1a**), phidianidine B (**1b**), amine **12a**, and amine **12b**, tables of spectral data, and copies of ^{1}H and ^{13}C NMR spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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