Discovery of 4-Aryl-4*H*-chromenes as a New Series of Apoptosis Inducers Using a Cell- and Caspase-Based High Throughput Screening Assay. 4. Structure–Activity Relationships of *N*-Alkyl Substituted Pyrrole Fused at the 7,8-Positions

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In our continuing effort to discover and develop apoptosis inducing 4-aryl-4*H*-chromenes as novel anticancer agents, we explored the structure−activity relationship (SAR) of alkyl substituted pyrrole fused at the 7,8-positions. A methyl group substituted at the nitrogen in the 7-position of the pyrrole ring led to a series of potent apoptosis inducers with potency in the low nanomolar range. These compounds were also found to be low nanomolar or subnanomolar inhibitors of cell growth, and they inhibited tubulin polymerization, indicating that methylation of the 7-position nitrogen does not change the mechanism of action of these chromenes. Compound **2d** was identified as a highly potent apoptosis inducer with an EC_{50} value of 2 nM and a highly potent inhibitor of cell growth with a GI_{50} value of 0.3 nM in T47D cells.

Introduction

Recent advances in our understanding of apoptosis, or programmed cell death, have indicated that excessive or improper inhibition of apoptosis plays a critical role in tumor development and metastasis.[1] As a result, chemotherapeutic agents that target the apoptosis pathways could have broad ramifications for the treatment of cancer. In fact, many commonly used chemotherapeutic drugs induce tumor cell apoptosis.[2] The proapoptotic chemotherapeutic agents that target tubulin, including paclitaxel and vinblastine, are among the most successful and commonly prescribed anticancer therapies. The colchicine binding site located on the monomeric unpolymerized α/β-tubulin represents another potential tubulin target for the development of apoptosis inducing chemotherapeutic agents. Clinical trials are currently in progress for combretastatin A-4 phosphate prodrug **1a** (CA4P) and amide prodrug **1b** (AVE-8062) (Chart 1).[3] Both compounds inhibit tubulin polymerization by binding at the colchicine site. In addition, these compounds have been identified as vascular-disrupting agents (VDA) for their ability to target and disrupt tumor vasculature.[3–5]

We have been interested in the discovery and development of apoptosis inducers as potential anticancer agents and have developed a cell- and caspase-based high throughput screening (HTS) system for the discovery of apoptosis inducers.[6] We have reported the discovery of 4-aryl-4*H*-chromenes as a new series of potent apoptosis inducing agents possessing vascular-disrupting activity.[7] The 4-aryl-4*H*-chromenes inhibit tubulin polymerization and bind at or close to the binding site of colchicine. They are also active in the multidrug resistant MES-SA/DX5 tumor cells and are highly active as single agents and in combination with other anticancer agents in several tumor models.[8] We have previously reported the structure−activity relationship (SAR) of the 4-aryl group of chromene and the identification of 2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-7-dimethylamino-4*H*-chromene (**2a**) (Chart 2) as a lead compound.[9] More recently, we have reported the SAR of a fused ring at the 7,8-positions and identified 2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-pyrrolo[2,3-*h*]chromene (**2b**) as a low nanomolar potent lead compound (Chart 2).[10] Comparison of structure **2a** with structure **2b** suggests that introduction of an alkyl group at the nitrogen of **2b** such as shown in **2d** might increase activity. Herein, we report the discovery and SAR of 7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes as highly potent apoptosis inducers and inhibitors of cell growth.

Chart 1

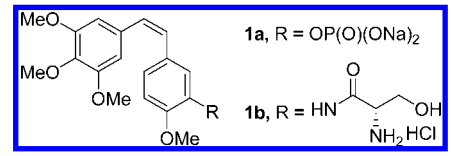
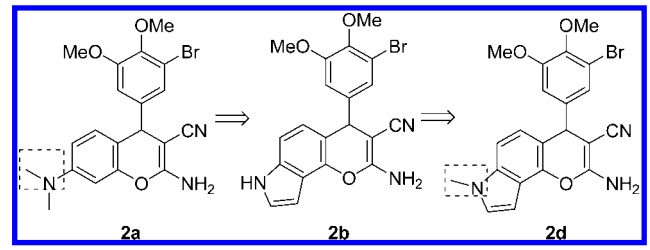


Chart 2



Results and Discussion

Chemistry. The substituted 4-aryl-chromenes **2d**−**2j** and **3a**−**3f** were prepared by a one-pot reaction of substituted aryl aldehyde and aryl alcohol with malononitrile in good yield according to methods previously described (Scheme 1).[9] The intermediate 4-hydroxy-1-methyl-1*H*-indole (**6a**) was synthesized from methylation of 4-benzyloxy-1*H*-indole (**4a**) to give **5a** followed by deprotection, and 7-hydroxy-1-methyl-1*H*-indole

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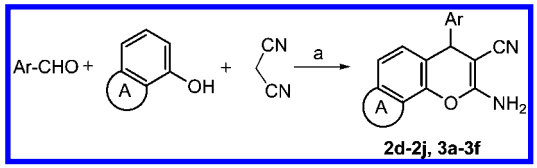
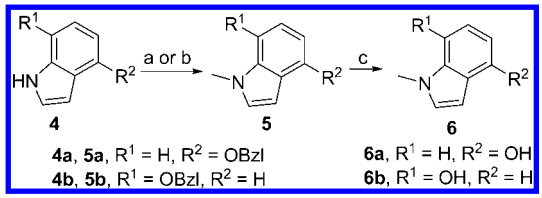
Scheme 1[a]



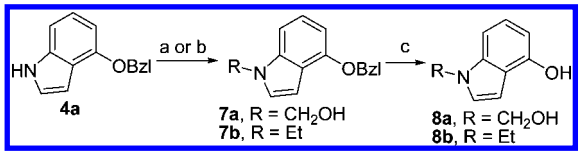
[a] Conditions: (a) EtOH, piperidine, room temperature (rt).

Scheme 2[a]



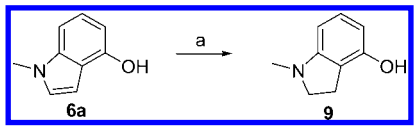
[a] Conditions: (a) NaH, DMF, iodomethane, rt; (b) dimethyl oxalate, KOBu-*t*; (c) 5% Pd/C, H_2(g) at 40 psi, MeOH.

Scheme 3[a]



[a] Conditions: (a) HC(O)H, 2 N NaOH, EtOH, rt; (b) EtBr, NaH, THF; (c) 5% Pd/C, H_2(g) at 50 psi, MeOH.

Scheme 4[a]



[a] Conditions: (a) 5% Pd/C, H_2(g) at 40 psi, MeOH, $HCl_{(conc)}$.

(**6b**) was synthesized similarly from methylation of 7-benzyloxy-1*H*-indole (**4b**) followed by deprotection (Scheme 2). Similarly, the intermediate 4-hydroxy-1-hydroxymethyl-1*H*-indole (**8a**) was prepared from the reaction of **4a** with formaldehyde under basic conditions to give **7a** followed by deprotection, and 1-ethyl-4-hydroxy-1*H*-indole (**8b**) was synthesized from reaction of **4a** with bromoethane in the presence of sodium hydride followed by deprotection (Scheme 3). Hydrogenation of **6a** under acidic conditions produced the saturated intermediate 4-hydroxy-2,3-dihydro-1-methyl-1*H*-indole (**9**) (Scheme 4). 4-Hydroxy-1-methyl-1*H*-benzimidazole, the intermediate for the synthesis of compound **2j**, was prepared from 2-amino-3-nitrophenol in four steps according to reported procedure.[11] Substituted aryl aldehydes for the synthesis of compounds **3e** and **3f** are not commercially available and were synthesized according to published procedures.[9,12]

Structure–Activity Relationship (SAR) Studies. The apoptosis inducing activities of 4-aryl-4*H*-chromenes were measured by our proprietary cell- and caspase-based HTS assay[13] in human breast cancer cells T47D, human colon cancer cells HCT116, and hepatocellular carcinoma cancer cells SNU398. By maintaining the 2-amino, the 3-cyano, and the preferred 4-(3-bromo-4,5-dimethoxyphenyl) groups, the SAR of the alkyl substituted pyrrole ring was explored. Compound **2d**, with a methyl substitution at the 7-position nitrogen, was one of the most potent compounds in this series, having an EC_{50} value of 2 nM in T47D cells, 3 nM in HCT116 cells, and 2 nM in SNU398 cells (Table 1). Compound **2d** was almost 10-fold more potent than **2a** and >2-fold more potent than **2b** in T47D cells. In comparison, compound **2g** from methylation of the 9-position nitrogen of compound **2c** was about 10-fold less active than **2c**, suggesting that there is a space-limiting pocket at the 9-position. Similar to **2g**, the 8-methyl analogue **2h**[10] was 7-fold and 17-fold less active than **2b** and **2d**, respectively, indicating that the position of the methyl group is critical for activity. Replacing the methyl group at the 7-position with a hydroxymethyl group gave the analogue **2e**, which has a potency comparable to that of **2d**, with an EC_{50} value of 3 nM in T47D cells. Moreover, the hydroxy group in **2e** might improve the solubility profile. It also provides a site for the preparation of a phosphate prodrug, similar to what has been successfully done in the case of the combretastatin A-4 phosphate prodrug.[14] In addition, the hydroxy group also provides a site for potential bioconjugation such as attachment of **2e** to a protein. In comparison, the 7-ethyl analogue **2f** was almost 20-fold less active than **2d**, suggesting that the pocket around the 7-position is size limited. The importance of having a methyl substitution at the 7-position was further highlighted by the saturated ring analogue **2i**, which was found to have an EC_{50} value comparable to that of **2d**. In addition, the 7-methylimidazolo[5,4-*h*]chromene analogue **2j** was also highly active, with a potency approaching that of **2d**.

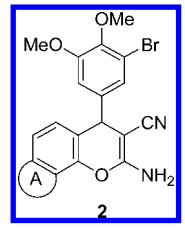
We next explored the SAR of the 4-aryl group of 7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes (Table 2). A 3,4,5-trisubstituted phenyl group, such as the 3,4-methylenedioxo-5-methoxyphenyl analogue (**3a**) led to a >8-fold decrease in potency relative to **2d**. However, the trisubstituted 3,4,5-trimethoxyphenyl analogue (**3b**) was very potent, only 2-fold less potent than **2d**. The 3,5-dimethoxyphenyl analogue (**3c**) and the 3-methoxy analogue (**3d**) were both highly potent analogues, with EC_{50} values only 2–3-fold less potent than those of **2d**. Interestingly, the potency difference between 3,4,5-trisubstituted analogue **2d**, 3,5-dimethoxy analogue **3c**, and 3-methoxy analogue **3d** is relatively small for the 7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes compared to what we observed earlier for 4*H*-pyrrolo[2,3-*h*]chromenes,[10] which had potency differences of 6–10-fold. Similar to the reported SAR for 7-NMe_2, 7,8-disubstituted, and 7,8-fused chromenes,[9,10,12] the 5-substituted 3-pyridyl analogues **3e** and **3f** were also highly potent and about as active as **2d**, with EC_{50} values of 3 nM each in T47D cells.

We also tested four known anti-tubulin apoptosis inducers in our assays, and the results are included in Table 2 for comparison. Vinblastine and vincristine were found to have EC_{50} values of 43 nM against T47D cells, which are >20-fold less active than compound **2d** in the same assay. Colchicine was found to have an EC_{50} value of 9 nM, and Taxol was found to have an EC_{50} value of 36 nM. Overall, these data indicted that **2d** and several of its related analogues are more potent than these reference compounds in our apoptosis activation assay.

The activities of these compounds toward the colon cancer cell line HCT116 and the hepatocellular carcinoma cancer cell line SNU398 were roughly parallel to their activity toward T47D cells. In general, HCT116 cells were slightly less sensitive (about 1.2-fold less sensitive as indicated by the EC_{50} value) to the compounds than T47D cells in this assay. SNU398 cells displayed a tendency to be slightly more sensitive to the compounds than T47D cells in this assay.

Selected compounds were also tested in the standard growth inhibition assay. The growth inhibition assays in T47D, HCT116, and SNU398 cells were run in a 96-well microtiter plate as described previously.[13] In brief, T47D, HCT116, and SNU398 cells were exposed to the test compound for 48 h at 37 °C. CellTiter-Glo reagent (Promega) was added, and the

Table 1. SAR of 2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-chromenes with an Alkyl Substituted Fused Ring at the 7,8-Positions in the Caspase Activation Assay



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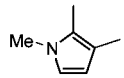
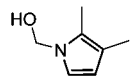
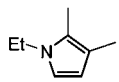
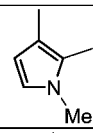
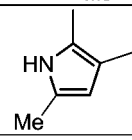
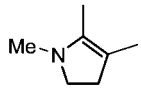
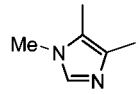
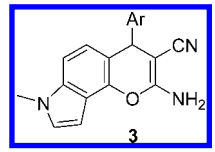
Compound	A	EC_{50} (μM)[a] T47D	HCT116	SNU398
2a	NA	0.019 ± 0.004[b]	0.009 ± 0.0004	0.019 ± 0.0007
2b		0.005 ± 0.0003[c]	0.014 ± 0.002	0.015 ± 0.001
2c		0.016 ± 0.001[c]	0.069 ± 0.003	0.086 ± 0.011
2d		0.002 ± 0.0003	0.003 ± 0.0004	0.002 ± 0.0004
2e		0.003 ± 0.0004	0.003 ± 0.0008	0.002 ± 0.0004
2f		0.039 ± 0.006	0.044 ± 0.008	0.025 ± 0.002
2g		0.15 ± 0.023	0.17 ± 0.028	0.13 ± 0.007
2h		0.030 ± 0.004[c]	0.054 ± 0.005	0.027 ± 0.003
2i		0.002 ± 0.0005	0.004 ± 0.001	0.001 ± 0.0002
2j		0.004 ± 0.0001	0.005 ± 0.0007	0.003 ± 0.0003

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM). [b] Data from ref 9. [c] Data from ref 10. NA, not applied.

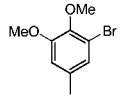
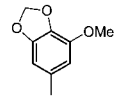
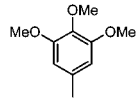
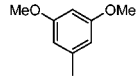
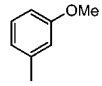
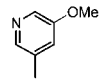
samples were mixed by agitation, incubated at room temperature for 10–15 min, and then read using a luminescent plate reader (Model Spectrafluor Plus Tecan Instrument). The GI_{50} is defined as 50% inhibition of cell growth. GI_{50} values, in comparison with their EC_{50} values, are summarized in Table 3.

Among those tested, compounds **2d** and **2e** were found to be the most potent inhibitors of tumor cell growth in T47D cells. Compound **2d** had a GI_{50} value of 0.3 nM while **2e** had a GI_{50} value of 2 nM in T47D cells. Compounds **2d** and **2e** were significantly more potent in the growth inhibition assay in T47D

Table 2. SAR of 2-Amino-4-aryl-3-cyano-7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes in the Caspase Activation Assay



3

		EC_{50} (μM)[a]		
Compound	Ar	T47D	HCT116	SNU398
2d		0.002 ± 0.0003	0.003 ± 0.0004	0.002 ± 0.0004
3a		0.017 ± 0.0005	0.015 ± 0.0004	0.012 ± 0.001
3b		0.004 ± 0.0001	0.006 ± 0.0007	0.003 ± 0.0003
3c		0.005 ± 0.0009	0.006 ± 0.001	0.003 ± 0.0004
3d		0.006 ± 0.001	0.007± 0.0008	0.005 ± 0.0003
3e		0.003 ± 0.0002	0.003± 0.0005	0.002 ± 0.0001
3f		0.003 ± 0.0006	0.003± 0.0005	0.003 ± 0.0001
Vinblastine	NA[b]	0.043 ± 0.007	0.044 ± 0.007	0.038 ± 0.006
Vincristine	NA[b]	0.043 ± 0.001	0.058 ± 0.012	0.012 ± 0.002
Colchicine	NA[b]	0.009 ± 0.0008	0.015 ± 0.004	0.004 ± 0.0001
Taxol	NA[b]	0.036 ± 0.003	0.024 ± 0.005	0.011 ± 0.002

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM). [b] NA, not applied.

cells than compound **2b**. Compounds **2d** and **2e** were respectively >26-fold and 4-fold more potent than **2b** in T47D cells. Interestingly, compounds **3e** and **2i** were more potent inhibitors of tumor cell growth in SNU398 cells than in T47D cells and

Table 3. Inhibition of Cell Growth Activity of Substituted 2-Amino-4-aryl-3-cyano-4*H*-pyrrolo[2,3-*h*]chromenes

	GI_{50} (μM)[a]		
compound	T47D	HCT116	SNU398
2b	0.008 ± 0.001[b]	0.020 ± 0.004	0.014 ± 0.001
2d	0.0003 ± 0.0001	0.0014 ± 0.0003	0.0002 ± 0.0001
2e	0.002 ± 0.001	0.004 ± 0.001	0.001 ± 0.0003
2i	0.007 ± 0.002	0.004 ± 0.0004	0.001 ± 0.0002
3e	0.008 ± 0.002	0.003 ± 0.001	0.002 ± 0.0002
Vinblastine	0.007 ± 0.002	ND[c]	ND[c]
Colchicine	0.007 ± 0.001	ND[c]	ND[c]

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM). [b] Data from ref 10. [c] ND, not determined.

were comparable to **2e**. We also tested two reference compounds, and the data are included in Table 3. Compound **2d** was found to be >10-fold more potent than the known apoptosis inducers vinblastine and colchicine in the growth inhibition assay in T47D cells.

Similar to compound **2b** (MX-76747), **2d** (MX-126303) was found to be a tubulin inhibitor that binds at or close to the colchicine site of β-tubulin.[7] Compound **2d** was found to inhibit tubulin polymerization with an IC_{50} value of 900 nM. In addition, compound **2d** was found to arrest MCF-7 breast carcinoma cells in the G_2/M phase of the cell cycle followed by apoptosis as measured by cell cycle analysis,[7] confirming that **2d** and related compounds induce apoptosis through similar pathways to those of other chromenes. Therefore, 4-aryl-7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes have the same mechanism of action as our previously reported chromenes.

Conclusion

We have explored the SAR of the apoptosis inducing 4-aryl-4*H*-chromenes via introducing an alkyl group on the pyrrole ring fused at the 7,8-positions. A methyl substitution at the nitrogen in the 7-position of the pyrrole ring led to the discovery of compound **2d** with low nanomolar potency. In comparison, a methyl group substituted at either the 8- or 9-positions led to a decrease in potency relative to the nonsubstituted analogues. Significantly, reducing the pyrrole ring to the saturated analogue **2i** maintained high potency and indicates the importance of the *N*-methyl substitution. The 7-hydroxymethyl analogue **2e** is also highly active and has additional possibilities for the preparation of prodrugs or bioconjugation. Interestingly, the 7-ethyl analogue **2f** was about 20-fold less active than **2d**, suggesting a size limited pocket at the 7-position. Further exploration of the SAR of the 4-aryl group of **2d** led to a group of highly potent 7-methyl-4*H*-pyrrolo[2,3-*h*]chromenes. In addition to **2d**, the analogues **3b**−**3f** all had <10 nM potency in the caspase activation assay in T47D, HCT116, and SNU398 cells. These analogues were also found to be highly active in the growth inhibition assay and to inhibit tubulin polymerization, similar to the case of chromenes with a pyrrole fused ring at the 7,8-positions. Compound **2d** is significantly more potent than the previous lead compound **2b**, with an EC_{50} value of 2 nM and a GI_{50} value of 0.3 nM in T47D cells. Additional SAR and in vivo studies of 4-aryl-4*H*-chromenes will be reported in future publications.

Experimental Section

General Methods and Materials. Commercial-grade reagents and solvents obtained from Acros, Aldrich, Chem-Impex, Lancaster, TCI, or VWR were used without further purification, except as indicated. All reaction mixtures were stirred magnetically; moisture-sensitive reactions were performed under argon in oven-dried glassware. Thin-layer chromatography (TLC), usually using ethyl acetate/hexane as the solvent system, was used to monitor reactions. Solvents were removed by rotary evaporation under reduced pressure; where appropriate, the compound was further dried using a vacuum pump. The ^{1}H NMR spectra were recorded at 300 MHz. All samples were prepared as dilute solutions in either deuteriochloroform ($CDCl_3$) with 0.05% v/v tetramethylsilane (TMS) or dimethyl-d_6-sulfoxide (CD_3SOCD_3) with 0.05% v/v TMS. Chemical shifts are reported in parts per million (ppm) downfield from TMS (0.00 ppm), and J coupling constants are reported in hertz. Elemental analyses were performed by Numega Resonance Labs, Inc. (San Diego, CA). Melting points were determined on a glass capillary melting point apparatus. Human breast cancer cells T47D, human colon cancer cells HCT116, and hepatocellular carcinoma cancer cells SNU398 were obtained from the American Type Culture Collection (Manasas, VA). Tubulin was obtained from Cytoskeleton (Boulder, CO).

4-Benzyloxy-1-methyl-1*H*-indole (5a). To a white suspension of sodium hydride (0.089 g, 2.2 mmol) in DMF (4.50 mL) was added 4-benzyloxy-1*H*-indole (**4a**) (0.500 g, 2.2 mmol) in portions over 5 min. The resulting brown mixture was allowed to warm to room temperature over 1 h, and then iodomethane (0.28 mL, 4.5 mmol) was added dropwise. After stirring at room temperature for 5 h, an additional 2 equiv of iodomethane (0.28 mL, 4.5 mmol) was added to the brown mixture. The reaction mixture was stirred for 1 h, quenched with water (10 mL), and then extracted with ethyl acetate (2 × 20 mL). The extracts were dried over Na_2SO_4, filtered through sintered glass, and concentrated to yield 0.607 g of a brown oil residue. The residue was purified by flash column chromatography (elution with EtOAC/hexanes, 1:4) to yield 0.253 g (47%) of **5a** as a brown oil: ^{1}H NMR ($CDCl_3$, δ) 7.52–7.48 (m, 2H), 7.41–7.31 (m, 3H), 7.13–7.03 (m, 2H), 6.98–6.95 (m, 1H), 6.63–6.57 (m, 2H), 5.22 (s, 2H), 3.77 (s, 3H).

4-Hydroxy-1-methyl-1*H*-indole (6a). To a brown solution of **5a** (0.250 g, 1.12 mmol) and methanol (2.25 mL) in a hydrogenation apparatus par shaker flask was added 5% Pd/C (0.119 g) to give a black mixture. The flask was attached to a hydrogenation apparatus, degassed (3×), and then filled with hydrogen gas. After the final degassing, the par shaker flask was filled with hydrogen gas at 40 psi and shaken for 16 h. The reaction product was diluted with methanol (15 mL), filtered through a layer of Celite (2.5 in d × 2 in h), and washed with additional *warm* methanol (75 mL). The organic filtrate was concentrated to yield 0.160 g (97%) of **6a** as black oil: ^{1}H NMR ($CDCl_3$, δ) 7.07 (t, J = 8.2 Hz, 1H), 6.96 (d, J = 2.7 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.54–6.51 (m, 2H), 4.60 (br s, 1H), 3.75 (s, 3H).

7-Hydroxy-1-methyl-1*H*-indole (6b). A mixture of 7-benzyloxyindole (**4b**) (0.300 g, 1.34 mmol), dimethyl oxalate (0.317 g, 2.68 mmol), and potassium *tert*-butoxide (0.302 g, 2.68 mmol) in DMF (5.0 mL) was stirred at 110 °C overnight. The solution was poured into a saturated solution of $NaHCO_3$ (20 mL) and extracted with EtOAc. The organic layer was separated, washed with brine, and dried over Na_2SO_4; the solvent was removed by rotary evaporation to give 0.200 g (63%) of 7-benzyloxy-1-methyl-1*H*-indole (**5b**). Compound **5b** was reacted under hydrogenation conditions similar to those of **5a** to produce 0.090 g (46%) of **6b**: ^{1}H NMR ($CDCl_3$, δ) 7.19–7.16 (m, 1H), 6.94 (d, J = 3.0 Hz, 1H), 6.86 (t, J = 7.5 Hz, 1H), 6.48–6.46 (m, 1H), 6.40 (d, J = 3.0 Hz, 1H), 5.05 (s, 1H), 4.07 (s, 3H).

4-Hydroxy-1-hydroxymethyl-1*H*-indole (8a). A solution of **4a** (1.00 g, 4.48 mmol), formaldehyde (2.00 mL, 26.8 mmol), and 2 N NaOH (2.24 mL, 4.48 mmol) in EtOH (10 mL) was stirred at room temperature for 4 h, and the solvent was removed in vacuo. The crude material was purified by flash column chromatography (EtOAc/hexanes, 1:3) to yield 1.13 g of 4-benzyloxy-1-hydroxymethyl-1*H*-indole (**7a**). Compound **7a** was hydrogenated under conditions similar to those of compound **5a** to give 0.580 g (80%) of **8a**: ^{1}H NMR ($CDCl_3$, δ) 7.15–7.07 (m, 3H), 6.60–6.55 (m, 2H), 5.62 (d, J = 7.5 Hz, 2H), 4.93 (s, 1H), 2.37 (t, J = 7.2 Hz, 1H).

1-Ethyl-4-hydroxy-1*H*-indole (8b). To a brown solution of **4a** (1.00 g, 4.48 mmol) in THF (22 mL) at 0 °C was added sodium

hydride (60%, 0.269 g, 11.2 mmol) in portions over 5 min. The resulting suspension was stirred at 0 °C for 5 min, and then bromoethane (0.330 mL, 4.48 mmol) was added dropwise. The reaction mixture was equilibrated to room temperature and then stirred overnight. It was quenched with water (10 mL) and extracted with ethyl acetate (2 × 70 mL), and the organic extracts were then washed with brine, dried over $MgSO_4$, filtered, and concentrated. The brown residue was purified by flash column chromatography (EtOAC/hexanes, 1:20) to yield 0.585 g (52%) of 4-benzyloxy-1-ethyl-1*H*-indole (**7b**) as a white solid. Compound **7b** was hydrogenated under conditions similar to those of compound **5a** to yield **8b** (100%) as a black oil: ^{1}H NMR ($CDCl_3$, δ) 7.08–7.02 (m, 2H), 6.94 (d, J = 8.3 Hz, 1H), 6.54–6.48 (m, 2H), 4.70 (br s, 1H), 4.13 (q, J = 7.3 Hz, 2H), 1.43 (t, J = 7.4 Hz, 3H).

4-Hydroxy-2,3-dihydro-1-methyl-1*H*-indole (9). Compound **6a** (0.410 g, 2.79 mmol) was hydrogenated with 5% Pd/C in methanol (20 mL) and 0.28 mL of concentrated aqueous HCl under H_2 (40 psi) to yield 0.120 g (29%) of **9**: ^{1}H NMR ($CDCl_3$, δ) 6.98 (t, J = 8.5 Hz, 1H), 6.19–6.12 (m, 2H), 4.51 (s, 1H), 3.34 (t, J = 8.4 Hz, 2H), 2.90 (t, J = 8.7 Hz, 2H), 2.75 (s, 3H).

2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7-dihydro-7-methyl-pyrano[2,3-*e*]indole (2d). To a yellow solution of 5-bromoveratraldehyde (2.50 g, 10.2 mmol), ethanol (34.0 mL), malononitrile (0.673 g, 10.2 mmol), and **6a** (1.50 g, 10.2 mmol) was added piperidine (0.500 mL, 5.10 mmol), and the solution was stirred at room temperature for 12 h. The resulting precipitate was collected by filtration, washed with ethanol (50 mL), and dried in vacuo to yield 3.00 g (66%) of **2d** as a yellow solid: mp 207–209 °C (dec); ^{1}H NMR ($CDCl_3$, δ) 7.07–7.02 (m, 2H), 6.91 (d, J = 2.1 Hz, 1H), 6.77–6.75 (m, 2H), 6.57 (dd, J = 0.9 Hz, 1H), 4.78 (s, 1H), 4.70 (br s, 2H), 3.82 (s, 6H), 3.78 (s, 3H). Anal. ($C_{21}H_{18}BrN_3O_3$) C, H, N.

The following compounds were prepared from the corresponding aryl aldehyde, aryl alcohol, and malononitrile with piperidine in ethanol by a procedure similar to that described for the preparation of compound **2d**.

2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7-dihydro-7-(hydroxylmethyl)pyrano[2,3-*e*]indole (2e). Compound **2e** was prepared from 5-bromoveratraldehyde, malononitrile, and **8a** and was isolated as a white solid (42%): mp 196–198 °C; ^{1}H NMR ($CDCl_3$, δ) 7.22–7.18 (m, 2H), 6.90 (d, J = 1.8 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.75 (d, J = 1.8 Hz, 1H), 6.64 (d, J = 3.3 Hz, 1H), 5.61 (s, 2H), 4.77 (s, 1H), 4.70 (br s, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 2.47 (br s, 1H). Anal. ($C_{21}H_{18}BrN_3O_4$) C, H, N.

2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7-dihydro-7-ethylpyrano[2,3-*e*]indole (2f). Compound **2f** was prepared from 5-bromoveratraldehyde, malononitrile, and **8b** and was isolated as a white solid (67%): mp 156–158 °C; ^{1}H NMR ($CDCl_3$, δ) 7.13 (d, J = 3.0 Hz, 1H), 7.06 (d, J = 8.5 Hz, 1H), 6.92 (d, J = 1.9 Hz, 1H), 6.76 (d, J = 1.9 Hz, 1H), 6.74 (d, J = 8.8 Hz, 1H), 6.58 (d, J = 3.0 Hz, 1H), 4.77 (s, 1H), 4.69 (br s, 2H), 4.14 (q, J = 7.4 Hz, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 1.46 (t, J = 7.4 Hz, 3H). Anal. ($C_{22}H_{20}BrN_3O_3$) C, H, N.

2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,9-dihydro-9-methylpyrano[3,2-*g*]indole (2g). Compound **2g** was prepared from 5-bromoveratraldehyde, malononitrile, and **6b** and was isolated as a white solid (52%): mp 206–207 °C (dec); ^{1}H NMR ($CDCl_3$, δ) 7.27 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 3.3 Hz, 1H), 6.90 (d, J = 1.8 Hz, 1H), 6.77 (d, J = 2.4 Hz, 1H), 6.61 (d, J = 8.1 Hz, 1H), 6.42 (d, J = 3.0 Hz, 1H), 4.79 (s, 1H), 4.62 (br s, 2H), 4.09 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H). Anal. ($C_{21}H_{18}BrN_3O_3$) C, H, N.

2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7,8,9-tetrahydro-7-methylpyrano[2,3-*e*]indole (2i). Compound **2i** was prepared from 5-bromoveratraldehyde, malononitrile, and **9** and was isolated as a white solid (26%): mp 199–201 °C; ^{1}H NMR ($CDCl_3$, δ) 6.88 (s, 1H), 6.72 (s, 1H), 6.67 (d, J = 8.4 Hz, 1H), 6.20 (d, J = 8.4 Hz, 1H), 4.60–4.57 (m, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 3.40 (t, J = 8.1 Hz, 2H), 2.98 (t, J = 8.7 Hz, 2H), 2.74 (s, 3H). Anal. ($C_{21}H_{20}BrN_3O_3$) C, H, N.

2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7-dihydro-7-methylpyrano[2,3-*e*]benzimidazole (2j). Compound **2j** was prepared from 5-bromoveratraldehyde, malononitrile, and 4-hydroxy-1-methyl-1*H*-benzimidazole and was isolated as a light brown solid (48%): mp 243–245 °C (dec); ^{1}H NMR ($CDCl_3$, δ) 7.88 (s, 1H), 7.11 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 1.8 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 6.72 (d, J = 2.1 Hz, 1H), 4.84 (br s, 2H), 4.80 (s, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H). Anal. ($C_{20}H_{17}BrN_4O_3$) C, H, N.

2-Amino-3-cyano-4-(3,4-methylenedioxo-5-methoxyphenyl)-4,7-dihydro-7-methylpyrano[2,3-*e*]indole (3a). Compound **3a** was prepared from myristicin aldehyde, malononitrile, and **6a** and was isolated as a white solid (55%): mp 186–188 °C (dec); ^{1}H NMR ($CDCl_3$, δ) 7.05–7.01 (m, 2H), 6.79 (d, J = 8.4 Hz, 1H), 6.55 (d, J = 3.0 Hz, 1H), 6.46 (s, 1H), 6.33 (d, J = 1.2 Hz, 1H), 5.92–5.90 (m, 2H), 4.75 (s, 1H), 4.64 (br s, 2H), 3.88 (s, 3H), 3.77 (s, 3H). Anal. ($C_{21}H_{17}N_3O_4$) C, H, N.

2-Amino-3-cyano-4-(3,4,5-trimethoxyphenyl)-4,7-dihydro-7-methylpyrano[2,3-*e*]indole (3b). Compound **3b** was prepared from 3,4,5-trimethoxybenzaldehyde, malononitrile, and **6a** and was isolated as a white solid (66%): mp 182–184 °C; ^{1}H NMR ($CDCl_3$, δ) 7.06–7.02 (m, 2H), 6.80 (d, J = 8.1 Hz, 1H), 6.57 (d, J = 2.4 Hz, 1H), 6.42 (s, 2H), 4.79 (s, 1H), 4.66 (s, 2H), 3.81 (s, 3H), 3.79 (s, 6H), 3.78 (s, 3H). Anal. ($C_{22}H_{21}N_3O_4$) C, H, N.

2-Amino-3-cyano-4-(3,5-dimethoxyphenyl)-4,7-dihydro-7-methylpyrano[2,3-*e*]indole (3c). Compound **3c** was prepared from 3,5-dimethoxybenzaldehyde, malononitrile, and **6a** and was isolated as a white solid (60%): mp 196–198 °C; ^{1}H NMR ($CDCl_3$, δ) 7.04–7.00 (m, 2H), 6.81 (d, J = 8.4 Hz, 1H), 6.55 (d, J = 3.0 Hz, 1H), 6.37–6.31 (m, 3H), 4.77 (s, 1H), 4.64 (br s, 2H), 3.77 (s, 3H), 3.74 (bs, 6H). Anal. ($C_{21}H_{19}N_3O_3$) C, H, N.

2-Amino-3-cyano-4-(3-methoxyphenyl)-4,7-dihydro-7-methylpyrano[2,3-*e*]indole (3d). Compound **3d** was prepared from 3-methoxybenzaldehyde, malononitrile, and **6a** and was isolated as a white solid (69%): mp 189–192 °C; ^{1}H NMR ($CDCl_3$, δ) 7.23–7.18 (m, 1H), 7.05–7.00 (m, 2H), 6.83–6.74 (m, 4H), 6.56 (d, J = 3.0 Hz, 1H), 4.81 (s, 1H), 4.64 (br s, 2H), 3.763 (s, 3H), 3.757 (s, 3H). Anal. ($C_{20}H_{17}N_3O_2$) C, H, N.

2-Amino-3-cyano-4-(5-methylpyridin-3-yl)-4,7-dihydro-7-methylpyrano[2,3-*e*]indole (3e). Compound **3e** was prepared from 5-methylpyridine-3-carbaldehyde, malononitrile, and **6a** and was isolated as a brown solid (73%): mp 222–226 °C (dec); ^{1}H NMR ($CDCl_3$, δ) 8.34 (d, J = 2.1 Hz, 1H), 8.31 (m, 1H), 7.29 (s, 1H), 7.06 (d, J = 3.0 Hz, 1H), 7.02 (m, 1H), 6.71 (d, J = 8.4 Hz, 1H), 6.57 (m, 1H), 4.85 (s, 1H), 4.77 (br s, 2H), 3.77 (s, 3H), 2.26 (s, 3H). Anal. ($C_{19}H_{16}N_4O$) C, H, N.

2-Amino-3-cyano-4-(5-methoxypyridin-3-yl)-4,7-dihydro-7-methylpyrano[2,3-*e*]indole (3f). Compound **3f** was prepared from 5-methoxypyridine-3-carbaldehyde, malononitrile, and **6a** and was isolated as a white solid (12%): mp 212–216 °C (dec); ^{1}H NMR ($CDCl_3$, δ) 8.19–8.15 (m, 2H), 7.07–6.99 (m, 3H), 6.73 (d, J = 8.4 Hz, 1H), 6.57 (d, J = 3.0 Hz, 1H), 4.89 (s, 1H), 4.75 (br s, 2H), 3.81 (s, 3H), 3.78 (m, 3H). Anal. ($C_{19}H_{16}N_4O_2$) C, H, N.

Supporting Information Available: Caspase activation assay (EC_{50}), cell growth inhibition assays (GI_{50}), the tubulin inhibition assay, and a table of elemental analysis data for the targeted compounds **2d**−**2g**, **2i**−**2j**, and **3a**−**3f**. This material is available free of charge via the Internet at http://pubs.acs.org.

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