# Discovery of 4-Aryl-4*H*-chromenes as a New Series of Apoptosis Inducers Using a Cell- and Caspase-Based High-Throughput Screening Assay. 3. Structure−Activity Relationships of Fused Rings at the 7,8-Positions

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As a continuation of our efforts to discover and develop the apoptosis-inducing 4-aryl-4*H*-chromenes as novel anticancer agents, we explored the SAR of fused rings at the 7,8-positions. It was found that a five-member aromatic ring, such as pyrrolo with nitrogen at either the 7- or 9-position, is preferred. A six-member aromatic ring, such as benzo or pyrido, also led to potent compounds. The SAR of the 4-aryl group was found to be similar for chromenes with a fused ring at the 7,8-positions. These compounds were found to inhibit tubulin polymerization, indicating that cyclization of the 7,8-positions into a ring does not change the mechanism of action. Compound **2h** was identified to be a highly potent apoptosis inducer with an $EC_{50}$ of 5 nM and a highly potent inhibitor of cell proliferation with a $GI_{50}$ of 8 nM in T47D cells.

## Introduction

Apoptosis, or programmed cell death, is the normal cellular process of eliminating unneeded cells that may threaten tissue homeostasis and organ morphogenesis.[1] Apoptosis proceeds with characteristic biochemical and cytological features, including nuclear condensation and DNA fragmentation. Two main pathways of apoptosis have been established.[2] The binding of death activators to receptors such as TNF-α[a] at the cell surface initiates apoptosis through an extrinsic pathway. On the other hand, chemotherapeutic agents, genotoxic stress, and other death stimuli will initiate apoptosis through an intrinsic or mitochondrial pathway. Both pathways involve a cascade of initiator and effector caspases that are activated sequentially. Caspases are a family of cysteine proteolytic enzymes that are normally present inside cells as inactive zymogens.[3] Within the caspase family, caspase-3, -6, and -7 have been identified as the key effector caspases that cleave multiple protein substrates inside cells, leading to irreversible cell death.[4] The acquisition of resistance to apoptosis is one of the hallmarks of cancer.[5] In addition, it has been reported that apoptosis may be involved in the regulation of metastasis.[6]

Apoptosis is known to play a pivotal role in the antitumor efficacy of several clinically useful cytotoxic agents.[7] The proapoptotic chemotherapeutic agents that target tubulin, including paclitaxel and vinblastine, are among the most successful and commonly prescribed anticancer therapies. Paclitaxel is a microtubule-stabilizing agent that binds to the fully formed microtubules and prevents depolymerization of the tubulin subunits. Vinblastine functions by binding to the tubulin monomers and inhibiting their polymerization into microtubules. The emergence of drug-resistant tumor cells, along with dose-limiting neurologic and bone marrow toxicity, however, has limited the use of tubulin targeting agents. 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. Combretastatin A-4 phosphate prodrug **1a** (CA4P) and amide prodrug **1b** (AVE-8062) (Chart 1) both inhibit tubulin polymerization by binding at the colchicine site and are currently in clinical trials.[8] These compounds have additionally been identified as vascular-disrupting agents (VDA) for their ability to disrupt the tumor vasculature by targeting endothelial cells while sparing the normal vasculature.[8,9] Vascular-disrupting agents that induce apoptosis in cancer cells by targeting the clinically validated tubulin/microtubule system offer the attractive possibility of treating all tumor types and improving the effectiveness of conventional clinical chemotherapeutic agents.[10]

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.[11] We have recently reported the discovery of 4-aryl-4*H*-chromenes as a new series of potent apoptosis-inducing agents possessing vascular-disrupting activity.[12] 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 animal models.[13] Starting from our screening hit 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxyphenyl)-4*H*-chromene (**1c**) (Chart 1), we have reported the SAR of the 4-aryl group and the identification of 2-amino-3-cyano-7-dimethylamino-4-(3-bromo-4,5-dimethoxyphenyl)-4*H*-chromene (**1d**) as a lead compound.[14] More recently, we have reported SAR of the 7- and 5-, 6-, 8-positions of 4-aryl-4*H*-chromenes and identified several potent 7-substituted and 7,8-disubstituted analogues, including 2,7,8-triamino-3-cyano-4-(3-bromo-4,5-dimethoxyphenyl)-4*H*-chromene (**1e**).[15] As a continuation of our SAR study, we have explored the synthesis of novel chromenes via cyclization of the 7,8-positions into a five- or six-member ring. Herein, we report the SAR of 7,8-fused-4-

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[a] Abbreviations: TNF-α, tumor necrosis factor-α; VDA, vascular-disrupting agents.

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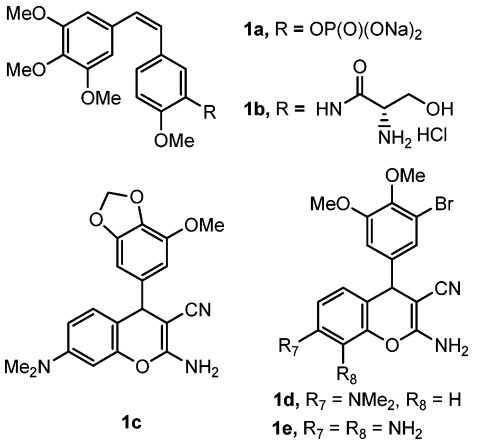
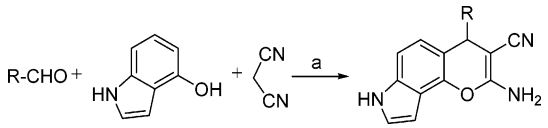
**Chart 1**

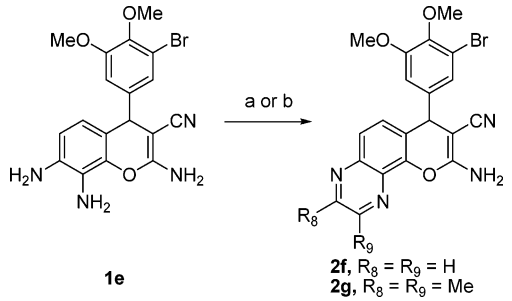



**Scheme 1**[a]




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

**Scheme 2**[a]




[a] Conditions: (a) 1,4-dioxane-2,3-diol, EtOH, room temp, 24 h; (b) butane-2,3-dione, EtOH, reflux, 1 h.

aryl-4*H*-chromenes as inducers of apoptosis and as potential anticancer agents.

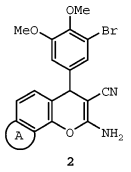
## Results and Discussion

**Chemistry.** Compounds **2a**−**e**,**h**−**j**, **3a**−**h**, **4b**,**d**−**e**, **5a**,**b**, and **6b**,**c** were prepared by a one-pot reaction of the commercially available substituted arylaldehydes and aryl alcohols with malononitrile in good yields according to methods previously described.[14] As an illustration, Scheme 1 depicts the one-pot reaction for the synthesis of pyrrolochromenes. Substituted arylaldehydes for the synthesis of compounds **3i**−**j** and **6d**−**e** are not commercially available and were synthesized according to published procedures.[14,15] For compounds **4a**, **4c**, and **6a** the benzylidene intermediate was synthesized first and then reacted with the appropriate aryl alcohol according to published procedures.[14] Compounds **2f** and **2g** were synthesized in good yields from cyclization of **1e**[15] with 1,4-dioxane-2,3-diol and butane 2,3-dione, respectively (Scheme 2).

**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[16] in human breast cancer cells T47D and human non-small-cell lung cancer cells H1299 (Table 1). By use of the 2-amino, 3-cyano, and the preferred 4-(3-bromo-4,5-dimethoxyphenyl) groups of **1e**, the SAR of chromenes with a fused ring at the 7,8-positions was explored. Compound **2a**, with a benzo ring fused at the 7,8-positions, was only 2-fold less potent than the 7,8-diamino analogue **1e** in T47D cells, suggesting that a ring structure is tolerated at the 7,8-positions. The 2-pyrido (**2b**) and 3-pyrido (**2c**) analogues had activity similar to that of **2a**, suggesting that the aromatic nitrogen at those sites is tolerated. The 5-pyrido (**2d**) analogue was >2-fold more potent than **2a**, suggesting that an aromatic nitrogen at that position might contribute to activity. Replacing the benzo ring in **2a** with a nonaromatic tetrahydrobenzo ring (**2e**) resulted in >2-fold reduction in potency, suggesting that a planar structure is preferred. Interestingly, the pyrazino analogue (**2f**) was >6-fold less potent than **2a** and almost 16-fold less potent than **2d**. The dimethyl substituted pyrazino analogue (**2g**) was >4-fold less potent than **2f**, suggesting a size-limiting pocket around the 8,9-positions.

We next explored chromenes with a five-member ring fused at the 7,8-positions. The pyrrolo analogue (**2h**) is highly potent with an $EC_{50}$ of 5 nM, which is >6-fold more potent than **1e** and almost 15-fold more potent than **2a**, suggesting that a five-member aromatic ring fits well into a pocket at the 7,8-positions. The 8-methylpyrrolo analogue (**2i**) was 6-fold less potent than **2h**, which is similar to the observed decrease in potency from **2f** to **2g**, confirming that there is a size-limiting pocket off the 8-position of the fused ring. The reverse pyrrolo analogue (**2j**) was found to be highly potent with an $EC_{50}$ of 16 nM and was about 3-fold less potent than **2h**.

**Table 1.** SAR of 4-Aryl-2-amino-3-cyano-4*H*-chromenes with a Fused Ring at the 7,8-Positions in the Caspase Activation Assay

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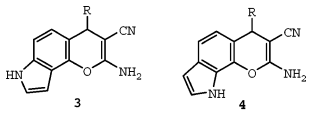
| | | $EC_{50}$ (μM)[a] | |
|---|---|---|---|
| Compound | A | T47D | H1299 |
| **1c** | NA[d] | 0.073 ± 0.006[b] | 0.093 ± 0.012[b] |
| **1d** | NA | 0.019 ± 0.004[b] | 0.043 ± 0.001[b] |
| **1e** | NA | 0.034 ± 0.005[c] | 0.081 ± 0.014[c] |
| **2a** |  | 0.073 ± 0.006 | 0.093 ± 0.012 |
| **2b** |  | 0.065 ± 0.001 | 0.140 ± 0.004 |
| **2c** |  | 0.061 ± 0.003 | 0.150 ± 0.017 |
| **2d** |  | 0.029 ± 0.003 | 0.094 ± 0.012 |
| **2e** |  | 0.190 ± 0.027 | 0.520 ± 0.062 |
| **2f** |  | 0.460 ± 0.018 | 0.860 ± 0.240 |
| **2g** |  | 2.12 ± 0.150 | 2.72 ± 0.031 |
| **2h** |  | 0.005 ± 0.003 | 0.013 ± 0.003 |
| **2i** |  | 0.030 ± 0.004 | 0.057 ± 0.0066 |
| **2j** |  | 0.016 ± 0.001 | 0.053 ± 0.008 |

[a] Data are the mean of three or more experiments and are reported as the mean ± standard error of the mean (SEM). [b] Data from ref 14. [c] Data from ref 15. [d] NA = not applicable.

**Table 2.** SAR of 4-Aryl-2-amino-3-cyanopyrrolo-4*H*-chromenes in the Caspase Activation Assay



3 4

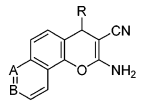
| | | $EC_{50}$ (μM)[a] | |
|---|---|---|---|
| Compound | R | T47D | H1299 |
| **3a** |  | 0.013 ± 0.002 | 0.025 ± 0.001 |
| **3b** |  | 0.030 ± 0.001 | 0.077 ± 0.0126 |
| **3c** |  | 0.030 ± 0.001 | 0.120 ± 0.0206 |
| **3d** |  | 0.052 ± 0.005 | 0.066 ± 0.001 |
| **3e** |  | 0.270 ± 0.017 | 0.430 ± 0.11 |
| **3f** |  | 0.068 ± 0.005 | 0.096 ± 0.0146 |
| **3g** |  | 0.043 ± 0.006 | 0.043 ± 0.007 |
| **3h** |  | 0.110 ± 0.007 | 0.190 ± 0.016 |
| **3i** |  | 0.013 ± 0.002 | 0.065 ± 0.022 |
| **3j** |  | 0.016 ± 0.001 | 0.030 ± 0.004 |
| **4a** |  | 0.035 ± 0.001 | 0.080 ± 0.013 |
| **4b** |  | 0.072 ± 0.005 | 0.180 ± 0.020 |
| **4c** |  | 0.031 ± 0.001 | 0.059 ± 0.016 |
| **4d** |  | 0.088 ± 0.013 | 0.140 ± 0.011 |
| **4e** |  | 0.120 ± 0.008 | 0.130 ± 0.007 |

[a] Data are the mean of three or more experiments and are reported as the mean ± standard error of the mean (SEM).

To determine whether a fused ring at the 7,8-positions changes the SAR of the 4-aryl group of the chromene, a group of 7,8-fused pyrrolo and 7,8-fused reverse pyrrolo analogues with different aryls at the 4-position were synthesized (Table 2). The SAR of the 4-aryl groups of 7,8-fused pyrrolochromenes was found to be similar to the reported SAR of 7-substituted and 7,8-disubstituted compounds.[14,15] A 3,4,5-trisubstituted phenyl group, such as 3-bromo-4-hydroxy-5-methoxyphenyl (**3a**) and 3,4,5-trimethoxyphenyl (**3b**), was found to result in potent analogues with potency 2- to 6-fold less than that of **2h**. The 3,5-dimethoxyphenyl analogue (**3c**) and 3-substituted phenyl analogues, including 3-methoxy (**3d**), 3-nitro (**3f**), and 3-cyano (**3g**), all had good activity ranging from 30 to 68 nM. The nonsubstituted phenyl analogue (**3e**) was much less active, confirming the importance of substituents on the phenyl ring, especially at the 3-position. Similar to the reported SAR for 7-$NMe_2$-chromenes,[14] the 3-pyridyl analogue (**3h**) was >2-fold more potent than the phenyl analogue **3e**, and 5-substituted 3-pyridyl analogues **3i** and **3j** were highly potent with potency approaching that of **2h**.

The SAR of the 4-aryl group of reverse pyrrolochromene followed that of the pyrrolochromene. The 3-bromo-4-hydroxy-5-methoxyphenyl analogue (**4a**), 3,4,5-trimethoxyphenyl analogue (**4b**), and 3,5-dimethoyphenyl analogue (**4c**) were 2- to 4-fold less potent than **2j**. The 3-substituted phenyl analogues, including the 3-methoxy (**4d**) and 3-cyano (**4e**), had good activity ranging from 88 to 120 nM.

**Table 3.** SAR of 4-Aryl-2-amino-3-cyanobenzo-4*H*-chromenes and 4-Aryl-2-amino-3-cyanopyrido-4*H*-chromenes in the Caspase Activation Assay



| | | | | $EC_{50}$ (μM)[a] | |
|---|---|---|---|---|---|
| Compound | R | A | B | T47D | H1299 |
| **5a** |  | C | C | 0.027 ± 0.004 | 0.067 ± 0.008 |
| **5b** |  | C | C | 0.064 ± 0.005 | 0.128 ± 0.012 |
| **6a** |  | N | C | 0.160 ± 0.039 | 0.220 ± 0.014 |
| **6b** |  | C | N | 0.120 ± 0.010 | 0.440 ± 0.12 |
| **6c** |  | C | N | 0.064 ± 0.003 | 0.130 ± 0.017 |
| **6d** |  | C | N | 0.120 ± 0.008 | 0.150 ± 0.012 |
| **6e** |  | C | N | 0.230 ± 0.015 | 0.290 ± 0.015 |

[a] Data are the mean of three or more experiments and are reported as the mean ± standard error of the mean (SEM).

The SAR of the 4-aryl group of chromenes with a six-member ring fused at the 7,8-positions was also found to be similar to the reported SAR of 7-substituted and 7,8-disubstituted compounds[14,15] (Table 3). For the analogues with a benzo ring at the 7,8-positions, the 3,4,5-trisubstituted phenyl analogues **5a**,**b** were found to have potencies similar to or slightly more potent than that of **2a**. For the analogues with a pyrido group at the 7,8-positions, the 3,4,5-trisubstituted phenyl analogues **6a**−**c** were found to have potencies similar to or slightly less potent than that of **2b** and **2c**, and the 5-substituted-3-pyridyl analogues **6d** and **6e** were found to be slightly less potent than **2c**.

The activities of these compounds toward the human non-small-cell lung cancer cell line H1299 were roughly parallel to their activity toward T47D cells. In general, H1299 cells were slightly less sensitive (about 2-fold less sensitive as indicated by the $EC_{50}$ value) to the compounds than T47D cells in this assay.

Selected compounds were also tested by the traditional growth inhibition assay to confirm that the active compounds can inhibit tumor cell growth. The growth inhibition assays in T47D and H1299 cells were run in a 96-well microtiter plate as described previously.[16] In brief, T47D and H1299 cells were exposed continuously to test compound for 48 h at 37 °C. CellTiter-Glo reagent (Promega) was added, and the samples were mixed by agitation, incubated at room temperature for 10−15 min, and then read using a luminescent plate reader (Tecan Spectrafluor Plus instrument). The $GI_{50}$ is defined as 50% inhibition of cell proliferation. $GI_{50}$ values, in comparison with their $EC_{50}$ values, are summarized in Table 4.

Compounds **2h** and **2j** were found to be the most potent inhibitors of tumor cell growth among the compounds tested. Compound **2h** has a $GI_{50}$ of 8 and 19 nM, while **2j** has a $GI_{50}$ of 9 and 6 nM, in T47D and H1299 cells, respectively. Compounds **2h** and **2j** are significantly more potent in the growth inhibition assay in T47D cells than the initial lead compound **1d**. Compound **2h** is >11-fold more potent than **1d**, while **2j** is 10-fold more potent than **1d** in the T47D cell line. In general, the compound that is more active in the apoptosis induction assay, as measured by caspase activation, also is more potent in the growth inhibition assay.

**Table 4.** Comparison of Caspase Activation Activity and Inhibition of Cell Proliferation Activity of 4-Aryl-2-amino-3-cyano-4*H*-chromenes

| | T47D | | H1299 | |
|---|---|---|---|---|
| | $EC_{50}$ [a] ($\mu$M) | $GI_{50}$ [b] ($\mu$M) | $EC_{50}$ [a] ($\mu$M) | $GI_{50}$ [b] ($\mu$M) |
| **1d** | 0.019 ± 0.004 | 0.092 ± 0.013 | 0.043 ± 0.001 | 0.016 ± 0.007 |
| **1e** | 0.034 ± 0.005 | 0.15 ± 0.02 | 0.081 ± 0.014 | 0.031 ± 0.001 |
| **2c** | 0.061 ± 0.003 | 0.057 ± 0.003 | 0.150 ± 0.017 | 0.071 ± 0.006 |
| **2f** | 0.46 ± 0.018 | 0.45 ± 0.018 | 0.86 ± 0.24 | 0.55 ± 0.003 |
| **2h** | 0.005 ± 0.003 | 0.008 ± 0.001 | 0.013 ± 0.003 | 0.019 ± 0.009 |
| **2i** | 0.030 ± 0.004 | 0.089 ± 0.026 | 0.057 ± 0.007 | 0.140 ± 0.051 |
| **2j** | 0.016 ± 0.001 | 0.009 ± 0.001 | 0.053 ± 0.008 | 0.006 ± 0.001 |
| **3a** | 0.013 ± 0.002 | 0.057 ± 0.003 | 0.025 ± 0.001 | 0.071 ± 0.006 |
| **3i** | 0.013 ± 0.002 | 0.073 ± 0.005 | 0.065 ± 0.022 | 0.100 ± 0.007 |
| **4a** | 0.035 ± 0.001 | 0.070 ± 0.006 | 0.080 ± 0.013 | 0.070 ± 0.009 |

[a] From Tables 1 and 2. [b] Data are the mean of three experiments and are reported as the mean ± standard error of the mean (SEM).

Similar to the hit **1c** and initial lead compound **1d**, compounds **2h** (MX-76747) and **2j** were found to be tubulin inhibitors that bind at or close to the colchicine site of $\beta$-tubulin.[12] Compounds **2h** and **2j** were found to inhibit tubulin polymerization with $IC_{50}$ values of 400 and 900 nM, respectively. In addition, compound **2h** 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,[12] confirming that **2h** and related compounds induce apoptosis similar to other chromenes. Therefore, cyclization of the 7,8-positions into a ring system does not change the mechanism of action of these chromenes.

## Conclusion

In conclusion, we have explored the SAR of the apoptosis-inducing 4-aryl-4*H*-chromenes by fusing the 7,8-positions into a five- or six-member ring. It was found that six-member aromatic rings, including benzo and pyrido, were generally well tolerated while a nonaromatic tetrahydrobenzo ring resulted in significant reduction of apoptotic activity. Interestingly, a pyrazino ring with two heteroatoms also led to a dramatic decrease in apoptotic activity. Significantly, a pyrrolo ring with nitrogen at either the 7- or 9-position was found to result in highly potent analogues. The SAR of the 4-aryl group was found to be similar for chromenes with a fused ring at the 7,8-positions and similar to the previously reported 7-$NMe_2$, 7-OMe, 7-NHEt, and 7,8-disubstituted analogues. The 7,8-fused chromenes were found to be potent in the cell growth inhibition assay and to inhibit tubulin polymerization, similar to chromenes without a fused ring at the 7,8-positions. Several analogues, such as **2h** and **2j**, were identified that have low nanomolar potency in both the caspase assay and the growth inhibition assay, and compound **2h** is significantly more potent than the initial lead compounds **1d** and **1e.** Through SAR studies of the 4-aryl-4*H*-chromenes, an anticancer drug candidate (MX-116407, EPC2407) with potent vascular disrupting activity and in vivo efficacy has been identified[13] and is currently in phase I clinical trial. Additional SAR and in vivo studies of 4-aryl-chromenes will be reported in future publications.

## Experimental Sections

**General Methods and Materials.** Commercial-grade reagents and solvents obtained from Acros, Aldrich, Apin Lancaster, TCI, or VWR were used without further purification unless otherwise indicated. All 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), acetone-$d_6$ ($CD_3COCD_3$, 99.9%) with 1.0% 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 T47D and human small-cell lung H1299 tumor cell lines were purchased from the American Type Culture Collection (Manasas, VA). Tubulin was obtained from Cytoskeleton (Boulder, CO).

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-naphtho[1,2-*b*]pyran (2a).** To a mixture of 5-bromoveratraldehyde (0.100 g, 0.408 mmol), naphthalen-1-ol (0.059 g, 0.41 mmol), and malononitrile (0.027 g, 0.41 mmol) in ethanol (2.0 mL) was added piperidine (0.081 mL, 0.82 mmol). The mixture was stirred for 12 h, and the resulting white solid was collected by filtration, washed with methanol, and dried in vacuo to yield 0.035 g (20%) of **2a** as a white solid: mp 190−192 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 8.23 (d, $J$ = 8.1, 1H), 7.91 (d, $J$ = 8.1, 1H), 7.67−7.59 (m, 3H), 7.23 (br s, 2H), 7.20 (d, $J$ = 8.4, 1H), 7.06 (m, 1H), 6.95 (m, 1H), 4.93 (s, 1H), 3.80 (s, 3H), 3.70 (s, 3H). Anal. ($C_{22}H_{17}BrN_2O_3$) 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 **2a**.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7-dihydro-8-methylpyrano[2,3-*e*]indole (2i).** Compound **2i** was prepared from 5-bromoveratraldehyde, 4-hydroxy-2-methyl-1*H*-indole, and malononitrile and was isolated as a white solid (48%): mp 224−226 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 11.15 (s, 1H), 7.01−6.97 (m, 2H), 6.94 (br s, 2H), 6.86 (d, $J$ = 1.8, 1H), 6.64 (d, $J$ = 8.4, 1H), 6.16 (br s, 1H), 4.77 (s, 1H), 3.79 (s, 3H), 3.70 (s, 3H), 2.38 (s, 3H). Anal. ($C_{21}H_{18}BrN_3O_3$) C, H, N.

**2-Amino-3-cyano-4,7-dihydro-4-(3,4,5-trimethoxyphenyl)-pyrano[2,3-*e*]indole (3b).** Compound **3b** was prepared from 3,4,5-trimethoxybenzaldehyde, 4-hydroxy-1*H*-indole, and malononitrile and was isolated as a white solid (43%): mp 220−222 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 11.29 (s, 1H), 7.35 (t, $J$ = 2.7, 1H), 7.10 (d, $J$ = 8.7, 1H), 6.90 (br s, 2H), 6.76 (d, $J$ = 8.4, 1H), 6.52 (s, 2H), 6.46−6.45 (m, 1H), 4.75 (s, 1H), 3.70 (s, 6H), 3.62 (s, 3H). Anal. ($C_{21}H_{19}N_3O_4$) C, H, N.

**2-Amino-3-cyano-4,7-dihydro-4-(3-pyridyl)pyrano[2,3-*e*]indole (3h).** Compound **3h** was prepared from pyridine-3-carboxaldehyde, 4-hydroxy-1*H*-indole, and malononitrile and was isolated as a yellow solid (11%): mp 226−229 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 11.32 (s, 1H), 8.49−8.41 (m, 2H), 7.53 (d, $J$ = 8.1, 1H), 7.37−7.30 (m, 2H), 7.11 (d, $J$ = 8.4, 1H), 7.01 (br s, 2H), 6.67 (d, $J$ = 8.4, 1H), 6.47 (s, 1H), 4.88 (s, 1H). Anal. ($C_{17}H_{12}N_4O$) C, H, N.

**2-Amino-3-cyano-4,7-dihydro-4-(5-methyl-3-pyridyl)pyrano-[2,3-*e*]indole (3i).** Compound **3i** was prepared from 5-methylpyridine-3-carboxaldehyde, 4-hydroxy-1*H*-indole, and malononitrile and was isolated as a yellow solid (52%): mp 230−233 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 11.32 (br s, 1H), 8.31−8.26 (m, 2H), 7.37 (t, $J$ = 2.9, 1H), 7.32 (br s, 1H), 7.11 (dd, $J$ = 8.4 and 0.6, 1H), 6.98 (br s, 2H), 6.65 (d, $J$ = 8.4, 1H), 6.47 (t, $J$ = 2.1, 1H), 4.82 (s, 1H), 2.23 (s, 3H). Anal. ($C_{18}H_{14}N_4O$) C, H, N.

**2-Amino-3-cyano-4,9-dihydro-4-(3,4,5-trimethoxyphenyl)-pyrano[3,2-*g*]indole (4b).** Compound **4b** was prepared from 3,4,5-trimethoxybenzaldehyde, 7-hydroxy-1*H*-indole, and malononitrile and was isolated as a black solid (28%): mp 222−226 °C (dec); $^1$H NMR (acetone-$d_6$) $\delta$ 10.42 (br s, 1H), 7.35−7.34 (m, 1H), 7.27 (d, $J$ = 8.2, 1H), 6.75 (d, $J$ = 8.2, 1H), 6.61 (s, 2H), 6.48−6.47 (m, 1H), 5.97 (br s, 2H), 4.81 (s, 1H), 3.76 (s, 6H), 3.68 (s, 3H). Anal. ($C_{21}H_{19}N_3O_4 \cdot 1.0H_2O$) C, H, N.

**2-Amino-3-cyano-4-(5-methyl-3-pyridyl)-4*H*-pyrano[2,3-*f*]isoquinoline (6e).** Compound **6e** was prepared from 5-methylpyridine-3-carbaldehyde, 5-hydroxyisoquinoline, and malononitrile and was isolated as a white solid (38%): mp 252−256 °C (dec); $^1$H NMR (acetone-$d_6$) $\delta$ 9.27 (d, $J$ = 0.9, 1H), 8.63 (d, $J$ = 5.7, 1H), 8.45 (d, $J$ = 2.1, 1H), 8.33 (d, $J$ = 1.5, 1H), 8.06 (dt, $J$ = 6.3 and 1.2, 1H), 7.83 (dd, $J$ = 8.4 and 0.6, 1H), 7.49 (s, 1H), 7.33 (d, $J$ = 8.4, 1H), 6.60 (br s, 2H), 5.06 (s, 1H), 2.28 (s, 3H). Anal. ($C_{19}H_{14}N_4O$) C, H, N.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,7-dihydropyrano[2,3-*e*]indole (2h).** To a mixture of 5-bromoveratraldehyde (0.100 g, 0.408 mmol), 4-hydroxy-1*H*-indole (0.054 g, 0.41 mmol), and malononitrile (0.027 g, 0.41 mmol) in ethanol (2.0 mL) was added piperidine (0.081 mL, 0.82 mmol). The mixture was stirred for 12 h, and then the solvent was evaporated. The resulting yellow oil was diluted with dichloromethane, and the precipitate was collected by filtration. Recrystallization (EtOAc/hexanes) yielded 0.017 g (10%) of **2h** as a yellow solid: mp 235−237 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 11.33 (br s, 1H), 7.36 (t, $J$ = 2.4, 1H), 7.12 (d, $J$ = 8.4, 1H), 7.00 (br s, 2H), 6.99 (s, 1H), 6.88 (d, $J$ = 1.8, 1H), 6.74 (d, $J$ = 8.7, 1H), 6.46 (br s, 1H), 4.80 (s, 1H), 3.80 (s, 3H), 3.69 (s, 3H). Anal. ($C_{20}H_{16}BrN_3O_3$) C, H, N.

**2-Amino-3-cyano-4-(3,4,5-trimethoxyphenyl)-4*H*-naphtho[1,2-*b*]pyran (5a).** Compound **5a** was prepared from 3,4,5-trimethoxybenzaldehyde, naphthalen-1-ol, and malononitrile by a procedure similar to that described for the preparation of **2h** and was isolated as a white solid (8%): mp 191−193 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.23 (d, $J$ = 8.1, 1H), 7.90 (d, $J$ = 8.4, 1H), 7.66−7.55 (m, 3H), 7.20 (d, $J$ = 8.7, 1H), 7.15 (s, 2H), 6.58 (s, 2H), 4.87 (s, 1H), 3.71 (s, 6H), 3.63 (s, 3H). Anal. ($C_{23}H_{20}N_2O_4\cdot1.5H_2O$) C, H, N.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4,9-dihydropyrano[3,2-*g*]indole (2j).** To 5-bromoveratraldehyde (0.245 g, 1.00 mmol) and malononitrile (0.066 g, 1.0 mmol) in ethanol (4.0 mL) was added piperidine (0.050 mL, 0.50 mmol) and 7-hydroxy-1*H*-indole (0.133 g, 1.00 mmol). The mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was purified by flash column chromatography (elution with EtOAc/hexanes, 1:2) to yield 0.056 g (13%) of **2j** as a white solid: mp 191−192 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.39 (br s, 1H), 7.34−7.25 (m, 2H), 6.91 (d, $J$ = 2.1, 1H), 6.76 (d, $J$ = 2.1, 1H), 6.67 (d, $J$ = 8.1, 1H), 6.56 (d, $J$ = 2.1, 1H), 4.80 (s, 1H), 4.67 (br s, 2H), 3.84 (s, 3H), 3.83 (s, 3H). Anal. ($C_{20}H_{16}BrN_3O_3$) C, H, N.

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

**2-Amino-3-cyano-4,7-dihydro-4-(3-methoxyphenyl)pyrano[2,3-*e*]indole (3d).** Compound **3d** was prepared from 3-methoxybenzaldehyde, 4-hydroxy-1*H*-indole, and malononitrile and was isolated as a white solid (25%): mp 195−198 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.26 (br s, 1H), 7.26−7.18 (m, 2H), 7.09−7.06 (m, 1H), 6.84−6.76 (m, 4H), 6.65−6.63 (m, 1H), 4.80 (s, 1H), 4.65 (br s, 2H), 3.76 (s, 3H). Anal. ($C_{19}H_{15}N_3O_2$) C, H, N.

**2-Amino-3-cyano-4,7-dihydro-4-phenylpyrano[2,3-*e*]indole (3e).** Compound **3e** was prepared from benzaldehyde, 4-hydroxy-1*H*-indole, and malononitrile and was isolated as a yellow solid (42%): mp 209−211 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.30 (br s, 1H), 7.37−7.08 (m, 7H), 6.90 (br s, 2H), 6.67 (d, $J$ = 8.1, 1H), 6.46 (s, 1H), 4.77 (s, 1H). Anal. ($C_{18}H_{13}N_3O$) C, H, N.

**2-Amino-3-cyano-4,7-dihydro-4-(3-nitrophenyl)pyrano[2,3-*e*]indole (3f).** Compound **3f** was prepared from 3-nitrobenzaldehyde, 4-hydroxy-1*H*-indole, and malononitrile and was isolated as a yellow solid (17%): mp 202−204 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.30 (br s, 1H), 8.12−8.08 (m, 1H), 8.05 (t, $J$ = 2.1, 1H), 7.63 (td, $J$ = 4.5 and 2.4, 1H), 7.49 (t, $J$ = 8.1, 1H), 7.25−7.24 (m, 1H), 7.11 (dd, $J$ = 8.4 and 0.9, 1H), 6.69−6.66 (m, 2H), 4.99 (s, 1H), 4.76 (br s, 2H). Anal. ($C_{18}H_{12}N_4O_3$) C, H, N.

**2-Amino-3-cyano-4,7-dihydro-4-(5-methoxy-3-pyridyl)pyrano[2,3-*e*]indole (3j).** Compound **3j** was prepared from 5-methoxypyridine-3-carboxaldehyde, 4-hydroxy-1*H*-indole, and malononitrile and was isolated as a yellow solid (8%): mp 210−214 °C (dec); $^1$H NMR (acetone-$d_6$) $\delta$ 10.44 (br 1H), 8.17 (d, $J$ = 1.8, 1H), 8.15 (d, $J$ = 2.7, 1H), 7.36 (t, $J$ = 2.7, 1H), 7.19−7.16 (m, 2H), 6.76 (d, $J$ = 8.1, 1H), 6.58−6.56 (m, 1H), 6.29 (s, 2H), 4.92 (s, 1H), 3.82 (s, 3H). Anal. ($C_{18}H_{14}N_4O_2\cdot0.3H_2O$) C, H, N.

**2-Amino-3-cyano-4,9-dihydro-4-(3-methoxyphenyl)pyrano[3,2-*g*]indole (4d).** Compound **4d** was prepared from 3-methoxybenzaldehyde, 7-hydroxy-1*H*-indole, and malononitrile and was isolated as a yellow solid (25%): mp 204−206 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.38 (br s, 1H), 7.31−7.19 (m, 3H), 6.84−6.68 (m, 4H), 6.53 (q, $J$ = 2.1, 1H), 4.83 (s, 1H), 4.62 (br s, 2H), 3.76 (s, 3H). Anal. ($C_{19}H_{15}N_3O_2$) C, H, N.

**2-Amino-3-cyano-4-(3-cyanophenyl)-4,9-dihydropyrano[3,2-*g*]indole (4e).** Compound **4e** was prepared from 3-cyanobenzaldehyde, 7-hydroxy-1*H*-indole, and malononitrile and was isolated as a white solid (34%): mp 225−227 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 8.43 (br s, 1H), 7.55−7.51 (m, 2H), 7.47−7.40 (m, 2H), 7.33 (dd, $J$ = 8.1 and 0.9, 1H), 7.28−7.25 (m, 1H), 6.61−6.55 (m, 2H), 4.92 (s, 1H), 4.73 (br s, 2H). Anal. ($C_{19}H_{12}N_4O$) C, H, N.

**2-Amino-3-cyano-4-(3-iodo-4,5-dimethoxyphenyl)-4*H*-naphtho[1,2-*b*]pyran (5b).** Compound **5b** was prepared from 3-iodo-4,5-dimethoxybenzaldehyde, naphthalen-1-ol, and malononitrile and was isolated as a white solid (20%): mp 221−222 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.24 (d, $J$ = 7.5, 1H), 7.91 (d, $J$ = 7.5, 1H), 7.67−7.56 (m , 3H), 7.22 (br s, 2H), 7.19 (d, $J$ = 8.7, 1H), 7.13 (d, $J$ = 1.2, 1H), 7.05 (d, $J$ = 1.5, 1H), 4.90 (s, 1H), 3.78 (s, 3H), 3.67 (s, 3H). Anal. ($C_{22}H_{17}IN_2O_3$) C, H, N.

**2-Amino-3-cyano-4,7-dihydro-4-(3,5-dimethoxyphenyl)pyrano[2,3-*e*]indole (3c).** To a solution of 3,5-dimethoxybenzaldehyde (0.039 g, 0.21 mmol), 4-hydroxy-1*H*-indole (0.028 g, 0.21 mmol), and malononitrile (0.014 g, 0.21 mmol) in ethanol (1.0 mL) was added piperidine (0.041 mL, 0.41 mmol). The resulting solution was stirred for 12 h, the solvent was evaporated, and the residue was purified by flash column chromatography (elution with EtOAc/hexanes, 1:1), yielding a crude yellow solid. It was recrystallized (dichloromethane:hexanes) to yield 0.009 g (12%) of **3c** as a yellow solid: mp 218−220 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 11.30 (s, 1H), 7.35 (t, $J$ = 2.7, 1H), 7.09 (d, $J$ = 9.7, 1H), 6.91 (s, 2H), 6.73 (d, $J$ = 8.4, 1H), 6.45 (s, 1H), 6.34 (s, 3H), 4.70 (s, 1H), 3.69 (s, 6H). Anal. ($C_{20}H_{17}N_3O_3$) C, H, N.

**2-Amino-3-cyano-4-(3-cyanophenyl)-4,7-dihydropyrano[2,3-*e*]indole (3g).** Compound **3g** was prepared from 3-cyanobenzaldehyde, 4-hydroxy-1*H*-indole, and malononitrile by a procedure similar to that described for the preparation of compound **3c** and was isolated as a yellow solid (33%): mp 220−222 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 11.34 (s, 1H), 7.71−7.68 (m, 2H), 7.54−7.52 (m, 2H), 7.37 (t, $J$ = 3.9, 1H), 7.11 (d, $J$ = 8.4, 1H), 7.04 (s, 2H), 6.67 (d, $J$ = 8.7, 1H), 6.47 (s, 1H), 4.93 (s, 1H). Anal. ($C_{19}H_{12}N_4O$) C, H, N.

**2-Amino-3-cyano-4-(3-iodo-4,5-dimethoxyphenyl)-4*H*-pyrano[2,3-*f*]isoquinoline (6c).** To a solution of 3-iodo-4,5-dimethoxybenzaldehyde (0.292 g, 1.00 mmol) and malononitrile (0.066 g, 1.0 mmol) in ethanol (2.0 mL) were added 5-hydroxyisoquinoline (0.145 g, 1.00 mmol) and piperidine (0.050 mL, 0.50 mmol). The mixture was refluxed overnight and then cooled to room temperature. The resulting orange precipitate was collected by filtration, washed with ethanol and hexanes, and then dried in vacuo to yield 0.274 g (57%) of **6c** as a white solid: mp 214−216 °C; $^1$H NMR ($CDCl_3$) $\delta$ 9.22 (d, $J$ = 0.9, 1H), 8.65 (d, $J$ = 6.0, 1H), 7.95 (d, $J$ = 6.3, 1H), 7.68 (d, $J$ = 8.5, 1H), 7.17 (d, $J$ = 8.7, 1H), 7.13 (d, $J$ = 1.8, 1H), 6.77 (d, $J$ = 1.8, 1H), 4.83 (br s, 2H), 4.82 (s, 1H), 3.82 (s, 6H). Anal. ($C_{21}H_{16}IN_3O_3$) C, H, N.

**2-Amino-4-(3-bromo-4-hydroxy-5-methoxyphenyl)-3-cyano-4,7-dihydropyrano[2,3-*e*]indole (3a).** To a solution of 4-acetoxy-3-bromo-5-methoxybenzaldehyde (1.02 g, 3.74 mmol), malononitrile (0.247 g, 3.74 mmol), and 4-hydroxy-1*H*-indole (0.497 g, 3.74 mmol) in ethanol (10.0 mL) was added piperidine (0.19 mL, 1.9 mmol). The mixture was refluxed for 4 h and cooled to room temperature, and then the solvent was evaporated to yield the crude product. It was purified by flash column chromatography (elution

with EtOAc/hexanes, 1:1), yielding 0.10 g (7%) of **3a** as a yellow solid: mp 238−242 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.27 (br s, 1H), 7.31−7.27 (m, 1H), 7.12−7.10 (m, 1H), 6.91 (d, $J$ = 2.1, 1H), 6.75−6.70 (m, 2H), 6.65 (s, 1H), 5.82 (s, 1H), 4.76 (s, 1H), 4.68 (br s, 2H), 3.86 (s, 3H). Anal. ($C_{19}H_{14}BrN_3O_3 \cdot 0.2H_2O$) C, H, N.

**2-Amino-3-cyano-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrano[2,3-*f*]isoquinoline (6b).** Compound **6b** was prepared from 3,4,5-trimethoxybenzaldehyde, 5-hydroxyisoquinoline, and malononitrile by a procedure similar to that described for the preparation of **3a** and was isolated as a white solid (4%): mp 215−219 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 9.22 (s, 1H), 8.65 (d, $J$ = 5.8, 1H), 7.95 (d, $J$ = 6.6, 1H), 7.67 (d, $J$ = 8.8, 1H), 7.21 (d, $J$ = 8.5, 1H), 6.42 (s, 2H), 4.85 (s, 1H), 4.82 (br s, 2H), 3.83 (s, 3H), 3.80 (s, 6H). Anal. ($C_{22}H_{19}N_3O_4 \cdot 0.4H_2O$) C, H, N.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-pyrano[2,3-*f*]quinoline (2b).** To a solution of 5-bromoveratraldehyde (0.245 g, 1.00 mmol) and malononitrile (0.066 g, 1.0 mmol) in ethanol (2.0 mL) was added piperidine (0.10 mL, 1.0 mmol). The mixture was stirred at room temperature for 15 min, then refluxed for 1 h, and cooled to room temperature. To the mixture were added piperidine (0.10 mL, 1.0 mmol) and 5-hydroxyquinoline (0.145 g, 1.00 mmol). The mixture was refluxed for 1 h, and the solvent was evaporated. The residue was purified by flash column chromatography (elution with EtOAc/hexanes, 1:2), yielding a white solid. It was recrystallized by EtOH to yield 0.050 g (11%) of **2b** as a white solid: mp 235−237 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 8.95 (s, 1H), 8.50 (d, $J$ = 9.0, 1H), 7.82 (d, $J$ = 9.0, 1H), 7.50 (br s, 1H), 7.30 (s, 1H), 6.92 (s, 1H), 6.76 (s, 1H), 4.82 (br s, 3H), 3.84 (s, 6H). Anal. ($C_{21}H_{16}BrN_3O_3$) C, H, N.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-pyrano[2,3-*f*]isoquinoline (2c).** A mixture of 5-bromoveratraldehyde (0.245 g, 1.00 mmol) and malononitrile (0.066 g, 1.0 mmol) in ethanol (2.0 mL) and piperidine (0.10 mL, 1.0 mmol) was stirred at room temperature for 15 min, then refluxed for 1 h, and cooled to room temperature. To the mixture was added piperidine (0.10 mL, 1.0 mmol) and 5-hydroxyisoquinoline (0.145 g, 1.00 mmol). The mixture was refluxed for 1 h, and the resulting white solid was collected by filtration, washed with ethanol and ether, and then dried in vacuo to yield 0.070 g (16%) of **2c** as a white solid: mp 218−220 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 9.23 (s, 1H), 8.66 (d, $J$ = 6.0, 1H), 7.95 (d, $J$ = 5.7, 1H), 7.69 (d, $J$ = 8.4, 1H), 7.18 (d, $J$ = 8.7, 1H), 6.92 (d, $J$ = 1.8, 1H), 6.75 (d, $J$ = 1.8, 1H), 4.85 (m, 3H), 3.84 (s, 6H). Anal. ($C_{21}H_{16}BrN_3O_3$) C, H, N.

The following compounds were prepared by a procedure similar to that described for the preparation of compound **2c**.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-pyrano[3,2-*h*]quinoline (2d).** Compound **2d** was prepared from 5-bromoveratraldehyde, malononitrile, and 8-hydroxyquinoline and was isolated as a white solid (41%): mp 126−128 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 9.00 (dd, $J$ = 4.3 and 1.5, 1H), 8.16 (dd, $J$ = 8.4 and 1.5, 1H), 7.55−7.50 (m, 2H), 7.15 (d, $J$ = 8.5, 1H), 6.98 (dd, $J$ = 1.9 and 0.8, 1H), 6.76 (d, $J$ = 2.2, 1H), 5.11 (br s, 2H), 4.85 (s, 1H), 3.83 (s, 6H). Anal. ($C_{21}H_{16}BrN_3O_3 \cdot 1.5H_2O$) C, H, N.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-7,8,9,10-tetrahydro-4*H*-naphtho[1,2-*b*]pyran (2e).** Compound **2e** was prepared from 5-bromoveratraldehyde, malononitrile, and 5,6,7,8-tetrahydro-1-hydroxynaphthalene and was isolated as a white solid (10%): mp 200−202 °C; $^1$H NMR ($CDCl_3$) $\delta$ 6.89 (d, $J$ = 1.9, 1H), 6.80−6.72 (m, 3H), 4.64−4.63 (m, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 2.73−2.72 (m, 4H), 1.81−1.79 (m, 4H). Anal. ($C_{22}H_{21}BrN_2O_3 \cdot 0.2H_2O$) C, H, N.

**2-Amino-4-(3-bromo-4-hydroxy-5-methoxyphenyl)-3-cyano-4,9-dihydropyrano[3,2-*g*]indole (4a).** To 2-(3-bromo-4-hydroxy-5-methoxybenzylidene)malononitrile (0.279 g, 1.00 mmol) and 7-hydroxy-1*H*-indole (0.133 g, 1.00 mmol) in ethanol (5.0 mL) was added piperidine (0.050 mL, 0.50 mmol). The mixture was refluxed for 2 h and then cooled to room temperature. The solvent was evaporated, and the residue was diluted with ethyl acetate (50 mL), washed with brine (10 mL), dried over $Na_2SO_4$, and concentrated in vacuo to yield a crude product. It was purified by flash column chromatography (elution with EtOAc/hexanes, 1:1), yielding 0.011 g (3%) of **4a** as a yellow solid: mp 197−201 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 8.39 (br s, 1H), 7.31 (d, $J$ = 8.7, 1H), 7.26−7.25 (m, 1H), 6.90 (d, $J$ = 2.1, 1H), 6.71−6.65 (m, 2H), 6.54−6.52 (m, 1H), 5.84 (s, 1H), 4.78 (s, 1H), 4.66 (br s, 2H), 3.87 (s, 3H). Anal. ($C_{19}H_{14}BrN_3O_3 \cdot 0.2H_2O$) C, H, N.

The following compounds were prepared by a procedure similar to that described for the preparation of compound **4a**.

**2-Amino-3-cyano-4,9-dihydro-4-(3,5-dimethoxyphenyl)pyrano-[3,2-*g*]indole (4c).** Compound **4c** was prepared from 7-hydroxy-1*H*-indole and 2-(3,5-dimethoxybenzylidene)malononitrile and was isolated as a white solid (26%): mp 185−186 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.39 (br s, 1H), 7.30 (d, $J$ = 8.1, 1H), 7.23−7.21 (m, 1H), 6.71 (d, $J$ = 8.4, 1H), 6.54−6.52 (m, 1H), 6.38 (d, $J$ = 2.1, 2H), 6.34−6.32 (m, 1H), 4.78 (s, 1H), 4.63 (br s, 2H), 3.75 (s, 6H). Anal. ($C_{20}H_{17}N_3O_3$) C, H, N.

**2-Amino-3-cyano-4-(3,4,5-trimethoxyphenyl)-4*H*-pyrano[2,3-*f*]quinoline (6a).** Compound **6a** was prepared from 2-(3,4,5-trimethoxybenzylidene)malononitrile and 5-hydroxyquinoline and was isolated as a white solid (14%): mp 229−230 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 8.96 (m, 1H), 8.52 (d, $J$ = 8.7, 1H), 7.82 (d, $J$ = 9.0, 1H), 7.52 (m, 1H), 7.33 (d, $J$ = 8.7, 1H), 6.43 (s, 2H), 4.83 (s, 3H), 3.83 (s, 3H), 3.80 (s, 6H). Anal. ($C_{22}H_{19}N_3O_4$) C, H, N.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-pyrano[2,3-*f*]quinoxaline (2f).** To a mixture of 2,7,8-triamino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-chromene (0.300 g, 0.719 mmol) and ethanol (3.6 mL) was added glyoxal (0.095 mL, 40% in $H_2O$, 0.79 mmol). The mixture was stirred at room temperature overnight. The resulting precipitate was collected and washed with ethanol and hexanes and then dried in vacuo to yield 0.20 g (62%) of **2f** as a brown solid: mp 157−159 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 8.94 (s, 2H), 7.85 (d, $J$ = 9.1, 1H), 7.39 (d, $J$ = 9.1, 1H), 6.97 (d, $J$ = 1.9, 1H), 6.77 (d, $J$ = 1.6, 1H), 5.02 (br s, 2H), 4.88 (s, 1H), 3.84 (s, 6H). Anal. ($C_{20}H_{15}BrN_4O_3 \cdot 1.0H_2O$) C, H, N.

**2-Amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-8,9-dimethyl-4*H*-pyrano[2,3-*f*]quinoxaline (2g).** To a mixture of 2,7,8-triamino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-chromene (0.050 g, 0.12 mmol) and ethanol (0.60 mL) was added butane-2,3-dione (0.010 mL, 0.12 mmol). The mixture was refluxed for 1 h and then cooled to room temperature. The resulting precipitate was collected and washed with ethanol and then dried in vacuo to yield 0.051 g (91%) of **2g** as a brown solid: mp 245−247 °C (dec); $^1$H NMR ($CDCl_3$) $\delta$ 7.70 (d, $J$ = 8.7, 1H), 7.25−7.24 (m, 1H), 6.95 (d, $J$ = 2.1, 1H), 6.73 (d, $J$ = 2.1, 1H), 4.98 (br s, 2H), 4.83 (s, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 2.80 (s, 3H), 2.75 (s, 3H). Anal. ($C_{22}H_{19}BrN_4O_3 \cdot 0.5H_2O$) C, H, N.

**2-Amino-4-(5-bromo-3-pyridyl)-3-cyano-4*H*-pyrano[2,3-*f*]isoquinoline (6d).** To 5-bromopyridine-3-carbaldehyde (0.093 g, 0.50 mmol) and malononitrile (0.033 g, 0.50 mmol) in ethanol (5.0 mL) at 0 °C was added piperidine (0.100 mL, 1.00 mmol). The mixture became a yellow solution upon stirring overnight, and then 5-hydroxyisoquinoline was added (0.074 mL, 0.51 mmol). The resulting mixture was heated at 80 °C for 6 h, and the resulting white solid was collected by filtration, washed with ether, and then dried in vacuo to yield 0.031 g (16%) of **6d** as a white solid: mp 238−240 °C (dec); $^1$H NMR (acetone-$d_6$) $\delta$ 9.29 (d, $J$ = 2.5, 1H), 8.64−8.63 (m, 2H), 8.60 (d, $J$ = 2.5, 1H), 8.07 (d, $J$ = 5.8, 1H), 7.95 (t, $J$ = 2.2, 1H), 7.86 (d, $J$ = 8.5, 1H), 7.38 (d, $J$ = 8.5, 1H), 6.71 (br s, 2H), 5.18 (s, 1H). Anal. ($C_{18}H_{11}BrN_4O$) C, H, N.

**Supporting Information Available:** Details of the caspase activation assay ($EC_{50}$), cell growth inhibition assays ($GI_{50}$), and tubulin inhibition assay and table of elemental analysis data for the targeted compounds **2a**−**j**, **3a**−**j**, **4a**−**e**, **5a**,**b**, and **6a**−**e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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