Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4745–4751
Bioorganic & Medicinal Chemistry Letters

Discovery of 4-aryl-4*H*-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high-throughput screening assay. 2. Structure–activity relationships of the 7- and 5-, 6-, 8-positions

William Kemnitzer,[a] Shailaja Kasibhatla,[a] Songchun Jiang,[a] Hong Zhang,[a] Jianghong Zhao,[a] Shaojuan Jia,[a] Lifen Xu,[a] Candace Crogan-Grundy,[a] Réal Denis,[b] Nancy Barriault,[b] Louis Vaillancourt,[b] Sylvie Charron,[b] Jennifer Dodd,[b] Giorgio Attardo,[b] Denis Labrecque,[b] Serge Lamothe,[b] Henriette Gourdeau,[b] Ben Tseng,[a] John Drewe[a] and Sui Xiong Cai[a,*]

[a]*Maxim Pharmaceuticals, Inc., 6650 Nancy Ridge Drive, San Diego, CA 92121, USA*
[b]*Shire Biochem Inc., 275 Armand-Frappier Blvd., Laval, Que., Canada H7V 4A7*

Received 8 June 2005; revised 25 July 2005; accepted 25 July 2005
Available online 6 September 2005

Abstract—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 4-aryl-4*H*-chromenes with modifications at the 7- and 5-, 6-, 8-positions. It was found that a small hydrophobic group, such as NMe_2, NH_2, NHEt, and OMe, is preferred at the 7-position. Di-substitution at either the 5,7-positions or the 6,7-positions generally led to a large decrease in potency. Di-substitution at the 7,8-positions, in general, was found to result in potent compounds. 7-NMe_2, 7-NHEt, 7-OMe, and 7,8-di-NH_2 analogs were found to have similar SAR for the 4-aryl group, and several 7-substituted and 7,8-di-substituted analogs were found to have similar potencies as the lead compound MX58151 (**2a**) both as caspase activators and inhibitors of cell proliferation.

Apoptosis, or programmed cell death, is a well-controlled process for the elimination of excessive cells that involves a series of precisely regulated events.[1] During apoptosis, various characteristic changes occur within the cells, including condensation of the nucleoplasm and cytoplasm, chromosomal DNA fragmentation, and the formation of membrane-bound apoptotic bodies, which are rapidly recognized and eliminated by adjacent cells.[2] The mechanism of apoptosis involves a cascade of initiator and effector caspases that are activated sequentially.[3,4] Key effector caspases have been identified including caspase-3, caspase-6, and caspase-7, that cleave multiple protein substrates in cells leading to irreversible cell death.[5,6]

It has been determined that many of the clinically useful cytotoxic agents induce apoptosis in cancer cells.[7,8] The proapoptotic chemotherapeutic agents that target tubulin (taxanes consisting of Taxol and Taxotere, and vinca alkaloids consisting of vincristine, vinblastine, and vinorelbine) are among the most successful and commonly prescribed anticancer therapies. The emergence of drug-resistant tumor cells, as well as 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 that is different from the binding sites of taxanes and vinca alkaloids, represents another potential tubulin target for the development of proapoptotic chemotherapeutic agents. Recently, several tubulin inhibitors were also shown to disrupt the tumor vasculature by targeting endothelial cells, while sparing the normal vasculature.[9,10] Two such vascular targeting agents, combretastatin A-4 phosphate prodrug (CA-4P) and ZD6126 (Chart 1), both destabilize tubulin by binding at the

Keywords: 4-Aryl-4*H*-chromenes; Apoptosis inducers; High-throughput screening; Structure–activity relationships; Cell- and caspase-based.
* Corresponding author. Tel.: +1 858 202 4006; fax: +1 858 202 4000; e-mail: scai@maxim.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.07.066

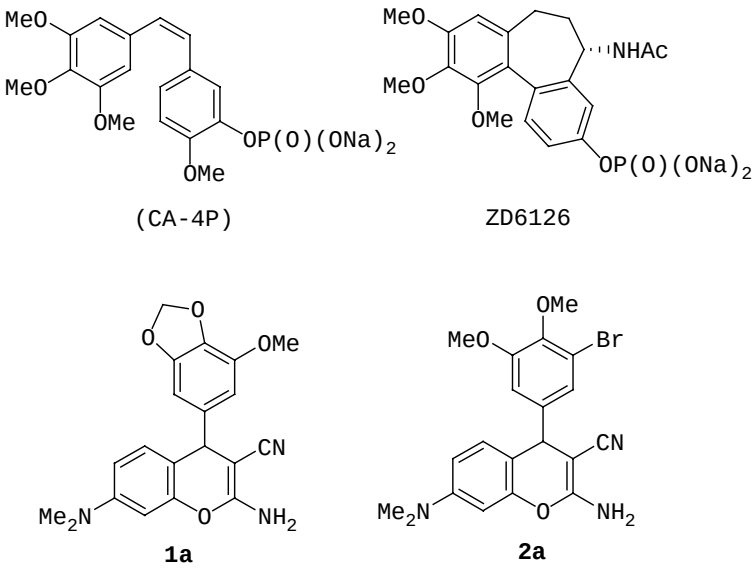
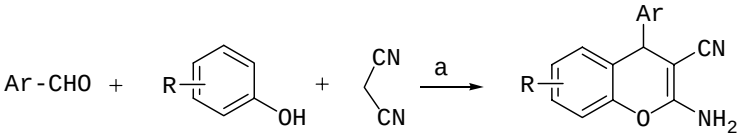


Chart 1.



Scheme 1. Reagents and conditions: (a) EtOH, piperidine, rt.

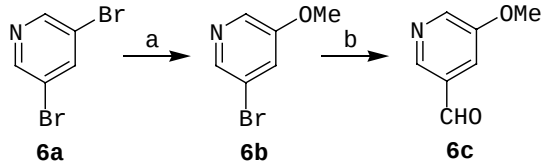
colchicine site and are currently undergoing clinical trials.[11,12] Compounds that induce apoptosis in cancer cells by targeting the clinically validated tubulin/microtubule system, while retaining activity in multi-drug-resistant tumors, have the potential to offer new treatment options in the field of oncology.[13–15]

We have reported the discovery, from our cell- and caspase-based high-throughput screening (HTS) assay, that 4-aryl-4*H*-chromenes are a new series of potent apoptosis inducing agents possessing vascular targeting activity. These compounds were found to be tubulin destabilizers, binding at or close to the binding site of colchicine. They were also active in drug-resistant cancer cell lines including the paclitaxel-resistant, multi-drug-resistant MES-SA/DX5 tumor cells, and were found to be highly active in several anticancer animal models.[16,17] The 4-aryl-4*H*-chromenes, therefore, represent a promising series of novel apoptosis inducers that could be developed into new therapeutic anticancer agents.

We have recently reported the structure–activity relationship (SAR) of the 4-aryl group of 4-aryl-4*H*-chromenes. Starting from our screening hit, 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxyphenyl)-4*H*-chromene (**1a**), by maintaining the 2-amino, 3-cyano, and 7-dimethylamino groups of **1a**, we have identified 2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-7-dimethylamino-4*H*-chromene (**2a**) as a lead compound (Chart 1).[18] As a continuation of our efforts to develop the apoptosis inducing 4-aryl-4*H*-chromenes as potential anticancer therapeutics, we now wish to report the SAR of 4-aryl-4*H*-chromenes (**1**–**5**) with modifications at the 7- and 5-, 6-, 8-positions.

The substituted 4*H*-chromenes were generally prepared by a one-pot reaction of the commercially available substituted aryl aldehydes and phenols with malononitrile in the presence of a base, such as piperidine, in good yield (Scheme 1), according to methods previously described.[18] For compound **3i**, the appropriate starting material, 5-methoxypyridine-3-carboxaldehyde (**6c**), was synthesized in two steps in 16% overall yield. First, 3,5-dibromopyridine (**6a**) was converted to the 5-bromo-3-methoxypyridine (**6b**) by refluxing with NaOMe, and then the aldehyde was formed by treatment of **6b** with *n*-BuLi and DMF (Scheme 2).

The one-pot reaction does not proceed well when the starting phenol is substituted with electron-withdrawing groups. Thus, compounds **2g** and **2h** could not be synthesized directly via the one-pot reaction on account



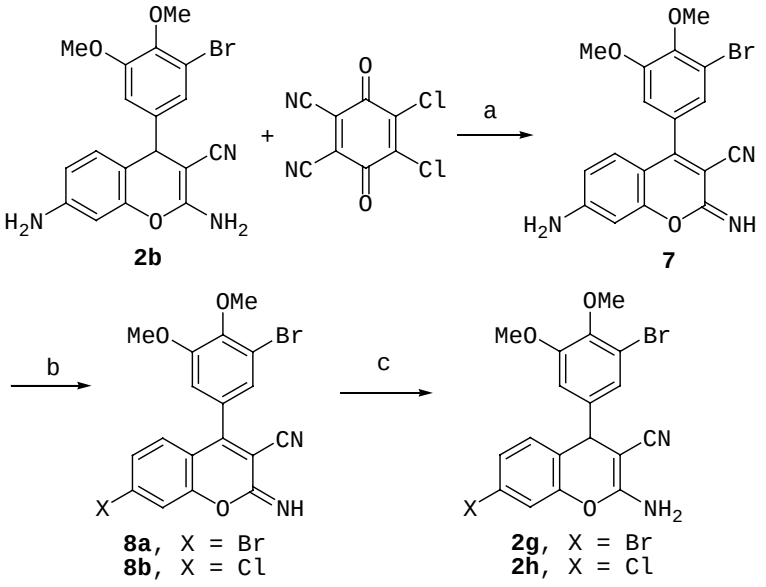
Scheme 2. Reagents and conditions: (a) NaOMe, MeOH, reflux, 3 days; (b) *n*-BuLi, DMF, −78 °C.

of the presence of electron-withdrawing halogens at the meta-position of the phenol ring. We explored the preparation of compounds **2g** and **2h** from the diazotization of the 7-amino group of compound **2b**. To avoid interference with the diazotization process by the 2-amino group, **2b** was oxidized using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone to the imino-2*H*-chromene **7**. Diazotization of **7**, followed by either bromination or chlorination, introduced the desired electron-withdrawing groups at the 7-position (**8a** and **8b**). Reduction of the imino-2*H*-chromenes by sodium borohydride then yielded **2g** and **2h** in overall yields of 14 and 10%, respectively (Scheme 3).

The apoptosis inducing activity of these 4-aryl-4*H*-chromenes was measured by our cell- and caspase-based HTS assay[18] in two cell lines, human breast cancer cells T47D and human non-small cell lung cancer cells H1299, and the results are given in Tables 1 and 2. Starting from our screening hit **1a**, by maintaining the 2-amino, 3-cyano, and 4-(3-methoxy-4,5-methylenedioxyphenyl) groups, we first explored the effects of various substituents at the 7-position (Table 1). The 7-amino analog **1b** was about 16-fold less potent than the 7-dimethylamino analog **1a**, suggesting that a slightly large and more hydrophobic dimethylamino group at the 7-position is important for the apoptosis inducing activity. The 7-ethylamino analog **1c** was about 3-fold more potent than **1b**, supporting the notion that a small and hydrophobic group at the 7-position is important for activity. The 7-diethylamino analog **1d** was about 1.5-fold and 6-fold less potent than **1c** and **1a**, respectively, suggesting that a large group is not preferred at the 7-position. Compounds **1e** and **1f**, with large phenylamino and *N*-morpholino groups at the 7-position, were not active up to 10 μM and are >100-fold less active than **1a**, confirming that a large group is not tolerated at the 7-position. The 7-methoxy analog **1g** had about half the activity of **1a**, confirming that a small and hydrophobic group is preferred at the 7-position. The 7-hydroxy analog **1h** was about 36-fold less active than **1g**, suggesting that a hydrophilic group is not preferred at the 7-position.

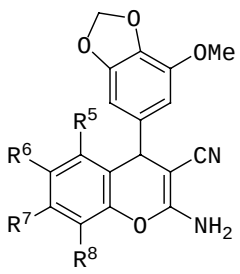
We then explored substitution at the 7-position in combination with substitution at either the 5-, 6-, or 8-position (Table 1). The 5,7-dimethoxy analog **1i** was not active up to 10 μM and is >60-fold less active than 7-methoxy analog **1g**, indicating that a substituent at the 5-position is not tolerated. Interestingly, it has been observed that an ortho-substituent at the 4-phenyl group also resulted in a large reduction of activity.[18] This is probably because a substituent at the 5-position, as well as an ortho-substituent at the 4-phenyl group, causes the 4-aryl group to rotate into an unfavorable position for interaction with its target. The 6,7-dimethoxy analog **1j** was also found to be inactive up to 10 μM and is >60-fold less than the 7-methoxy analog **1g**, indicating that a substituent at the 6-position is not preferred. The 6-methyl-7-ethylamino analog **1k** was about 4-fold less active than the 7-ethylamino analog **1c**, suggesting that a smaller group at the 6-position is more tolerated. Compound **1l**, with the 6- and 7-positions cyclized into a methylenedioxy ring, thus limiting the size of the group at the 6-position, was almost as potent as **1g**, confirming that a smaller group is more tolerated at the 6-position. The 7-amino-8-methyl analog **1m** was almost 4-fold more potent than the 7-amino analog **1b**, suggesting that a substituent at the 8-position might be beneficial for activity.

Based on these results, as well as our earlier results that 2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-3-cyano-7-dimethylamino-4*H*-chromene (**2a**) is a lead compound and 3-bromo-4,5-dimethoxyphenyl is one of the best aryl groups at the 4-position in this series of analogs,[18] we decided to concentrate our exploration efforts on the combination of 3-bromo-4,5-dimethoxyphenyl at the 4-position with different substituents at the 7-position, as well as substituents at both 7- and 8-positions (Table 1). Interestingly, 7-NH_2 analog **2b** was only

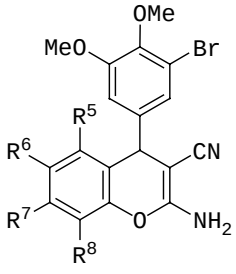


Scheme 3. Reagents and conditions: (a) CH_2Cl_2/rt; (b) ^tBuONO, CH_3CN, $CuBr_2$ or $CuCl_2$, 0°C; (c) $NaBH_4$, THF, rt.

Table 1. SAR of 4-(3-methoxy-4,5-methylenedioxyphenyl)- and 4-(3-bromo-4,5-dimethoxyphenyl)-2-amino-3-cyano-4*H*-chromenes in the caspase activation assay



1



2

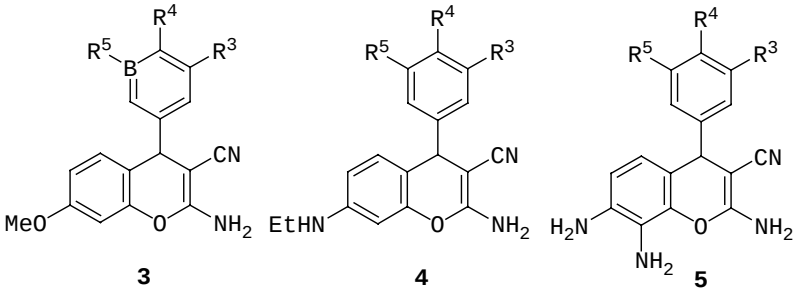
Entry	R^5	R^6	R^7	R^8	EC_{50} (μM)[a]	
					T47D	H1299
1a	H	H	NMe_2	H	0.073 ± 0.006[b]	0.093 ± 0.012[b]
1b	H	H	NH_2	H	1.2 ± 0.03	1.7 ± 0.09
1c	H	H	NHEt	H	0.33 ± 0.06	0.40 ± 0.04
1d	H	H	NEt_2	H	0.48 ± 0.06	0.53 ± 0.01
1e	H	H	NHPh	H	>10	>10
1f	H	H	Mop[c]	H	>10	>10
1g	H	H	OMe	H	0.16 ± 0.02	0.23 ± 0.03
1h	H	H	OH	H	5.8 ± 0.4	5.6 ± 0.4
1i	OMe	H	OMe	H	>10	>10
1j	H	OMe	OMe	H	>10	>10
1k	H	Me	NHEt	H	1.1 ± 0.03	1.4 ± 0.09
1l	H	OCH_2O		H	0.21 ± 0.01	0.27 ± 0.02
1m	H	H	NH_2	Me	0.31 ± 0.01	0.77 ± 0.06
2a	H	H	NMe_2	H	0.019 ± 0.004[b]	0.043 ± 0.001[b]
2b	H	H	NH_2	H	0.033 ± 0.002	0.067 ± 0.005
2c	H	H	NHEt	H	0.014 ± 0.001	0.027 ± 0.002
2d	H	H	OMe	H	0.017 ± 0.001	0.034 ± 0.002
2e	H	H	OEt	H	0.064 ± 0.010	0.092 ± 0.004
2f	H	H	OH	H	0.13 ± 0.01	0.21 ± 0.01
2g	H	H	Br	H	0.14 ± 0.01	0.28 ± 0.02
2h	H	H	Cl	H	0.16 ± 0.02	0.29 ± 0.02
2i	H	H	NH_2	NH_2	0.034 ± 0.005	0.081 ± 0.014
2j	H	H	NH_2	Me	0.026 ± 0.004	0.053 ± 0.007
2k	H	H	Me	Me	0.042 ± 0.002	0.087 ± 0.008
2l	H	H	OH	NH_2	0.061 ± 0.005	0.18 ± 0.034
2m	H	H	OH	OH	1.7 ± 0.18	2.5 ± 0.28

[a] Data are means of three or more experiments and are reported as mean ± standard error of the mean (SEM).
[b] Data from Ref. 18.
[c] Mop, *N*-morpholino.

slightly less active than **2a**. This result contrasts with the 16-fold decrease in potency that was observed between **1a** and **1b**. In addition, the 7-ethylamino and 7-methoxy analogs **2c** and **2d** were about as potent as **2a**. These results suggest that the 3-bromo-4,5-dimethoxyphenyl at the 4-position reduced the effects of substituents at the 7-position. Analog **2e**, with a slightly larger 7-ethoxy group, was about 4-fold less active than **2d**, confirming that a smaller group at the 7-position is preferred. The 7-hydroxy analog **2f** was >7-fold less active than **2d**, confirming that a hydrophilic group is not preferred at the 7-position. The 7-Br and 7-Cl analogs **2g** and **2h** both were >7-fold less potent than **2d**, indicating that an electron-withdrawing group is less preferred than the electron-donating group at the 7-position. The 7,8-diamino analog **2i** is a potent compound with an EC_{50} value of 34 nM, being only slightly less active than **2a**. The extra amino group at the 8-position is also expected to increase the aqueous solubility of the compound. The 7-amino-8-methyl analog **2j** and 7,8-dimethyl analog **2k** were also highly active with potency around 30–40 nM, confirming that the presence of a substituent at the 8-position is not detrimental to activity. The 7-hydroxy-8-amino analog **2l** was about 2-fold more active than the 7-hydroxy analog **2f**, confirming that a substituent at the 8-position is beneficial for activity. The 7,8-dihydroxy analog **2m** was about 20-fold less active than **2l**, and is the least potent 7,8-disubstituted analog prepared, indicating that the hydrophilic hydroxy group is not tolerated at the 8-position.

To determine whether modification of the 7-position changes the SAR of the 4-aryl group, the 7-OMe, 7-NHEt, and the 7,8-diamino groups were selected to explore different aryls at the 4-position (Table 2). For the analogs with a 7-OMe group, similar to what was

Table 2. SAR of 7-methoxy-, 7-ethylamino-, and 7,8-diamino-4-aryl-2-amino-3-cyano-4*H*-chromenes in the caspase activation assay



Entry	B	R^3	R^4	R^5	EC_{50} (μM)[a]	
					T47D	H1299
3a	C	OMe	OMe	OMe	0.026 ± 0.002	0.057 ± 0.003
3b	C	OMe	H	OMe	0.015 ± 0.001	0.032 ± 0.004
3c	C	OMe	H	H	0.052 ± 0.004	0.089 ± 0.012
3d	C	Br	H	H	0.052 ± 0.004	0.095 ± 0.014
3e	C	Cl	H	H	0.080 ± 0.012	0.16 ± 0.02
3f	C	NO_2	H	H	0.089 ± 0.012	0.17 ± 0.01
3g	C	H	H	H	0.36 ± 0.06	0.52 ± 0.01
3h	N	H	H	H	0.17 ± 0.02	0.29 ± 0.03
3i	N	OMe	H	H	0.047 ± 0.009	0.10 ± 0.01
4a	C	OMe	OMe	OMe	0.049 ± 0.006	0.11 ± 0.01
4b	C	OMe	H	OMe	0.055 ± 0.010	0.12 ± 0.02
4c	C	OMe	H	H	0.11 ± 0.01	0.19 ± 0.02
4d	C	Cl	H	H	0.12 ± 0.01	0.19 ± 0.03
4e	C	NO_2	H	H	0.11 ± 0.01	0.17 ± 0.02
5a	C	Cl	OMe	OMe	0.024 ± 0.001	0.058 ± 0.004
5b	C	I	OMe	OMe	0.049 ± 0.006	0.10 ± 0.01
5c	C	Br	OH	OMe	0.023 ± 0.003	0.062 ± 0.006
5d	C	OMe	H	OMe	0.092 ± 0.007	0.19 ± 0.01
5e	C	CN	H	H	0.39 ± 0.06	0.46 ± 0.04
5f	C	Br	H	H	0.15 ± 0.01	0.23 ± 0.06
5g	C	NO_2	H	H	0.39 ± 0.05	0.42 ± 0.05

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

observed for analogs with a 7-NMe_2 group,[18] the 3,4,5-trimethoxyphenyl analog **3a** was only slightly less active than **2d**, the analog with the potent 3-bromo-4,5-dimethoxyphenyl group at the 4-position. The 3,5-dimethoxyphenyl analog **3b** and 3-substituted-phenyl analogs **3c**–**f** all had good activity ranging from 15 to 89 nM. The non-substituted phenyl analog **3g** was much less active, confirming the importance of a substituent at the phenyl ring, especially at the 3-position. The 3-pyridyl analog **3h** was slightly more potent that the phenyl analog **3g** and the 5-methoxy-3-pyridyl analog **3i** was about as potent as the 3,4,5-trisubstituted-phenyl analog **3a**, confirming our previous observation that a 5-substituted-3-pyridyl has potency similar to that of a 3,4,5-trisubstituted-phenyl at the 4-position.[18]

With an NHEt group at the 7-position, the 3,4,5-trimethoxyphenyl analog **4a** and the 3,5-dimethoxyphenyl analog **4b** were slightly less potent than **2e**, the one with the potent 4-(3-bromo-4,5-dimethoxyphenyl) group. Analogs **4c**–**e**, with a 3-substituted-phenyl group at the 4-position, all had good activity with EC_{50} values of around 100 nM, slightly less potent than the 3,4,5-trisubstituted and 3,5-disubstituted analogs.

In the 7,8-diamino series, the 3-chloro-4,5-dimethoxyphenyl analog **5a** and the 3-iodo-4,5-dimethoxyphenyl analog **5b** were about as potent as **2i**, as expected from our SAR studies in the 7-NMe_2 series that the 3-bromo group can be replaced by a chloro or iodo group.[18] Analog **5c** was about as potent as **2i**, indicating that 3-bromo-4-hydroxy-5-methoxyphenyl group is a good replacement for the 3-bromo-4,5-dimethoxyphenyl group, similar to what was reported previously.[18] Moreover, the hydroxy group in **5c** might improve the solubility profile, as well as provide a useful handle for the preparation of a phosphate prodrug, similar to what has been successfully done in the case of combretastatin A-4 phosphate prodrug.[11] The 3,5-dimethoxyphenyl analog **5d** also had good activity. As expected, the 3-substituted analogs **5e**–**g** were less active than the 3,4,5-trisubstituted analogs.

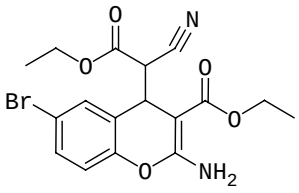
The activities of these compounds toward the human non-small cell lung cancer cell line H1299 were roughly parallel to their activity toward T47D cells. In general, H1299 cells were slightly less sensitive (about 2-fold less sensitive as measured by the EC_{50} value) to the compounds than T47D cells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

Selected compounds were also tested by the traditional inhibition of cell proliferation (GI_{50}) assay to confirm that the active compounds can inhibit tumor cell growth. The growth inhibition assays in T47D and

Table 3. Comparison of caspase activation activity and inhibition of cell proliferation activity of 4-aryl-2-amino-3-cyano-4*H*-chromenes

Entry	T47D			H1299		
	EC_{50}[a] (μM)	GI_{50}[b] (μM)	GI_{50}/EC_{50}	EC_{50}[a] (μM)	GI_{50}[b] (μM)	GI_{50}/EC_{50}
1b	1.2 ± 0.03	2.4 ± 0.52	2.1	1.7 ± 0.08	1.2 ± 0.17	0.74
1g	0.16 ± 0.02	0.26 ± 0.06	1.6	0.23 ± 0.025	0.24 ± 0.036	1.0
1h	5.8 ± 0.35	6.08 ± 0.99	1.0	5.63 ± 0.38	5.50 ± 0.76	0.98
1i	>10	>10	1	>10	>10	1
1j	>10	>10	1	>10	>10	1
1m	0.31 ± 0.01	0.49 ± 0.08	1.6	0.77 ± 0.06	0.86 ± 0.16	1.1
2a	0.019 ± 0.004	0.092 ± 0.014	4.8	0.043 ± 0.001	0.029 ± 0.010	0.67
2b	0.033 ± 0.002	0.077 ± 0.015	2.3	0.067 ± 0.005	0.076 ± 0.013	1.1
2d	0.017 ± 0.001	0.008 ± 0.002	0.47	0.034 ± 0.002	0.009 ± 0.003	0.26
2f	0.13 ± 0.01	0.80 ± 0.19	6.1	0.21 ± 0.01	0.19 ± 0.01	0.90
2h	0.16 ± 0.02	0.064 ± 0.014	0.4	0.29 ± 0.02	0.055 ± 0.002	0.19
2i	0.034 ± 0.005	0.15 ± 0.02	4.4	0.081 ± 0.014	0.031 ± 0.001	0.38
2l	0.061 ± 0.005	0.12 ± 0.06	2.0	0.18 ± 0.03	0.11 ± 0.047	0.61
3i	0.047 ± 0.009	0.065 ± 0.009	1.4	0.10 ± 0.01	0.072 ± 0.007	0.72

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



HA14-1

Chart 2.

H1299 cells were run in a 96-well microtiter plate, as described previously.[18] The GI_{50}, along with the EC_{50} data and the ratio of GI_{50}/EC_{50}, are given in Table 3. Results show that **2a**, **2b**, **2d**, **2h**, and **3i** are the most potent inhibitors of tumor cell growth among the compounds tested. Compound **2d** had a GI_{50} value of 0.008 and 0.009 μM in T47D and H1299 cells, respectively. The GI_{50}/EC_{50} ratio for most of these compounds was >1 or close to 1 both with T47D and H1299 cells, with a few exceptions. For example, the GI_{50}/EC_{50} ratio for compounds **2d** and **2h** was less than 0.5 for both cell lines, indicating that both compounds are more potent in the growth inhibition assay than in the caspase assay. Table 3 also shows that in general, the compound that is more active in the apoptosis induction assay, as measured by caspase activation, is also more potent in the growth inhibition assay.

We have reported that compound **2a** and related compounds are tubulin inhibitors that bind at or are close to the colchicine site of β-tubulin.[18] Compounds **2b** and **2i** were tested in the tubulin polymerization assay. Both compounds were found to inhibit tubulin polymerization with IC_{50} values less than 500 nM, similar to **2a**. Therefore, modification at the 7- and 8-positions does not change the mechanism of action of these compounds, that is induction of apoptosis through inhibition of tubulin polymerization. Interestingly, a somewhat structurally related 4-substituted chromene HA14-1 (Chart 2) has been reported to bind to Bcl-2 protein and induce apoptosis in HL-60 cells.[19] However, HA14-1 is not active up to 10 μM in our apoptosis induction assay in several cell lines including T47D and DLD-1.

In conclusion, we have explored the SAR of the apoptosis inducing 4-aryl-4*H*-chromenes as potential anticancer agents via modifications at the 7- and 5-, 6-, 8-positions. It was found that small hydrophobic groups, such as NMe_2, OMe, or NHEt, are preferred at the 7-position, while larger groups, such as NHPh, result in significant reduction of apoptotic activity. Interestingly, disubstitution at the 7,8-positions, such as 7,8-diamino or 7,8-dimethyl, results in potent compounds. Disubstitution at either the 5,7-positions or the 6,7-positions generally results in a drastic decrease in activity, with the exception of the cyclic 6,7-methylenedioxy analog that retains good potency. The 7-NMe_2, 7-NHEt, 7-OMe, and 7,8-di-NH_2 analogs were found to have similar SAR for the 4-aryl group. Several 7-substituted and 7,8-di-substituted analogs, such as **2b**–**d**, **2i**–**l**, **3a**–**b**, and **5a**–**c**, were found to be as potent as the lead compound **2a**. In addition, some of these compounds offer better potential for aqueous solubility, particularly **2i** and **5a**–**c**. Compound **5c** also provides a handle for the preparation of prodrugs.

References and notes

1. Henson, P. M.; Bratton, D. L.; Fadok, V. A. *Curr. Biol.* **2001**, *11*, R795.
2. Steller, H. *Science* **1995**, *267*, 1445.
3. Kasibhatla, S.; Tseng, B. *Mol. Cancer Ther.* **2003**, *2*, 573.
4. Green, D. R.; Reed, J. C. *Science* **1998**, *281*, 1309.
5. Nicholson, D. W. *Cell Death Differ.* **1999**, *6*, 1028.
6. Stennicke, H. R.; Ryan, C. A.; Salvesen, G. S. *Trends Biochem. Sci.* **2002**, *27*, 94.
7. Herr, I.; Debatin, K. M. *Blood* **2001**, *98*, 2603.
8. Rich, T.; Allen, R. L.; Wyllie, A. H. *Nature* **2000**, *407*, 777.
9. Chaplin, D. J.; Dougherty, G. J. *Br. J. Cancer* **1999**, *80*, 57.
10. Denekamp, J. *Eur. J. Clin. Invest.* **1999**, *29*, 733.
11. Kanthou, C.; Tozer, G. M. *Blood* **2002**, *99*, 2060.

12. Thorpe, P. E.; Chaplin, D. J.; Blakey, D. C. *Cancer Res.* **2003**, *63*, 1144.
13. Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. *Med. Res. Rev.* **1998**, *18*, 259.
14. Li, Q.; Sham, H.; Rosenburg, S. *Annu. Rev. Med. Chem.* **1999**, *34*, 139.
15. Haggarty, S. J.; Mayer, T. U.; Miyamoto, D. T., et al. *Chem. Biol.* **2000**, *7*, 275.
16. Kasibhatla, S.; Gourdeau, H.; Meerovitch, K.; Drewe, J.; Reddy, S.; Qiu, L.; Zhang, H.; Bergeron, F.; Bouffard, D.; Yang, Q.; Herich, J.; Lamothe, S.; Cai, S. X.; Tseng, B. *Mol. Cancer Ther.* **2004**, *3*, 1365.
17. Gourdeau, H.; Leblond, L.; Hamelin, B.; Desputeau, C.; Dong, K.; Kianicka, I.; Custeau, D.; Bourdeau, C.; Geerts, L.; Cai, S. X.; Drewe, J.; Labrecque, D.; Kasibhatla, S.; Tseng, B. *Mol. Cancer Ther.* **2004**, *3*, 1375.
18. Kemnitzer, W.; Kasibhatla, S.; Jiang, S.; Zhang, H.; Wang, Y.; Zhao, J.; Jia, S.; Herich, J.; Labreque, D.; Storer, R.; Meerovitch, K.; Bouffard, D.; Rej, R.; Denis, R.; Blais, C.; Lamothe, S.; Attardo, G.; Gourdeau, H.; Tseng, B.; Drewe, J.; Cai, S. X. *J. Med. Chem.* **2004**, *47*, 6299.
19. Wang, J. L.; Liu, D.; Zhang, Z. J.; Shan, S.; Han, X.; Srinivasula, S. M.; Croce, C. M.; Alnemri, E. S.; Huang, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7124.