Synthesis and Structure−Activity Relationships of Carbazole Sulfonamides as a Novel Class of Antimitotic Agents Against Solid Tumors

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Two series of carbazole sulfonamides related to Combretastatin A4 (**1**) were synthesized and evaluated for antiproliferative activity. Thirteen of the 26 new sulfonamides exhibited IC_{50} values of <1 μM against CEM leukemia cells. Five compounds were evaluated against a panel of eight human tumor cell lines. 9-Ethyl-*N*-(3,4,5-trimethoxyphenyl)-carbazole-3-sulfonamide (**11a**) showed significant antitumor activity in two human xenograft models (MCF-7 and Bel-7402). Preliminary studies with **11a** showed that the mode of action involves arrest of M-phase cell cycle and induction of apoptosis by increasing expression of p53 and promoting bcl-2 phosphorylation. Unexpectedly, **11a** only weakly inhibits tubulin polymerization, which suggests that the mode of action of **11a** differs from **1** and involves an unidentified target(s). Also, the SAR information gleaned from ring A-substituted analogues varies significantly from that of **1**. Carbazole sulfonamides are a novel promising class of antimitotic agents with clinical development potential.

Introduction

Antimitotic agents arrest the cell cycle at the G_2/M phase and lead to apoptotic cell death and tumor regression.[1,2] Classic antimitotic agents, many of which are tubulin-binding agents, such as taxanes and vinca alkaloids, interfere with microtubule dynamics by targeting tubulin and are widely used to treat human cancers. However, neurotoxicity, difficult synthesis, and development of multidrug resistance, mainly through the expression of P-glycoprotein (Pgp),[a] have limited their clinical use.[3] Therefore, it is important to develop new small molecule tubulin-binding agents or other antimitotic agents with novel modes of action.[4−6]

Combretastatin A4 (**1**), a novel small molecule tubulin-binding agent which was isolated from the South African bush willow *Combretum caffrum* by Pettit et al.,[7] strongly inhibits the polymerization of tubulin by binding to the colchicine binding site (Figure 1).[8] Potent cytotoxicity for **1** has been observed against a wide variety of cell lines, including multidrug resistant lines. However, the low water solubility of this compound has limited its in vivo efficacy. Its water-soluble phosphate prodrug **2** is now in phase II clinical trials.[9] It appears to act by a unique irreversible and selective antivascular effect that disrupts immature endothelial cells' cytoskeleton in vivo.[10]

As a result of the relatively simple chemical structure and unique biological profiles, **1** has stimulated a lot of research in the last two decades (e.g., Figure 1). Structure−activity relationship studies for **1** have shown that the 3,4,5-trimethoxy substituents of the A-ring are essential for good biological activities.[11,12] Therefore, many studies have focused on the design of analogues by altering the linking group and the B-ring of **1** to provide better biological activities.[13,14] We also were interested in exploring the alteration of the linker and the B-ring. In this report, we study the influence of replacing the olefin linker with sulfonamide groups and testing the replacement of the B-ring unit with a carbazole. Furthermore, we examine the influence of various substitutions on the A-ring in this new class of compounds.

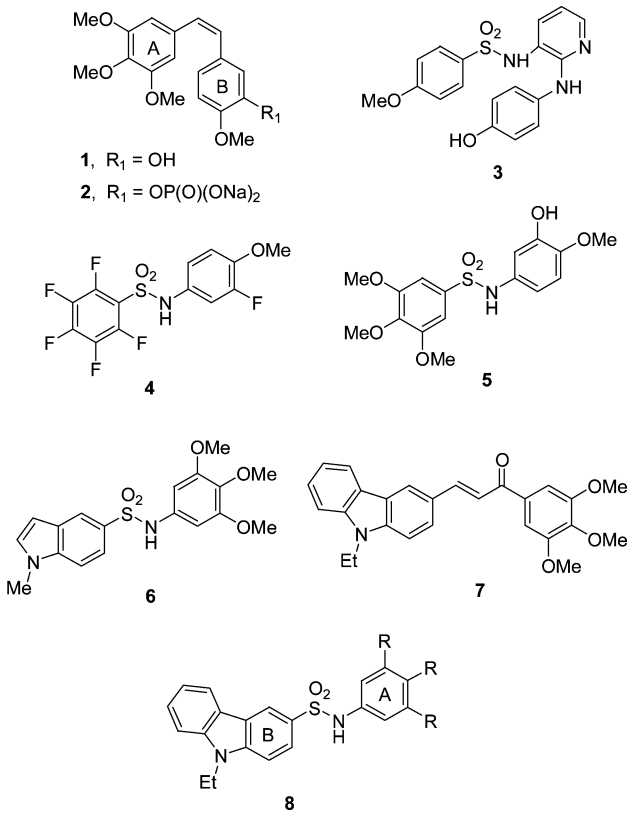

Figure 1. Lead compound and antimitotic agents.

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[a] Abbreviations: Pgp, P-glycoprotein; ip, intraperitoneal; p53, tumor suppressor protein; P-p53, phosphorylated p53; cdc2, cell division cycle 2 kinase; bcl-2, B-cell leukemia/lymphoma 2 protein; bcl-2-p, monophosphorylated bcl-2; bcl-2-2p, biphosphorylated bcl-2; PBS, phosphate buffered saline; PMSF, phenylmethanesulfonyl fluoride; mAb, monoclonal antibody; sc, subcutaneously.

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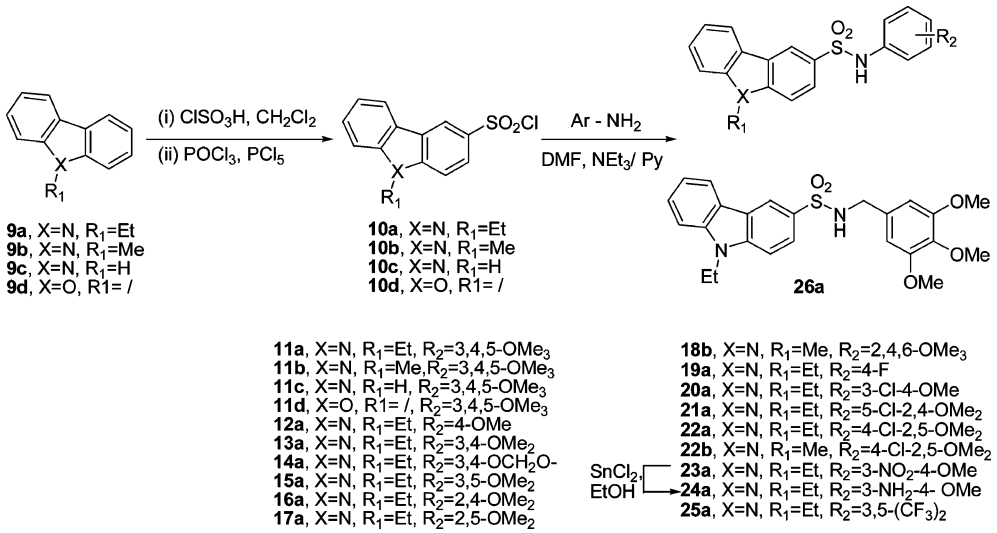
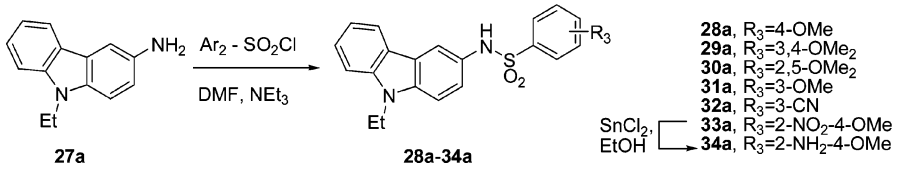
Scheme 1



Scheme 2



In general, sulfonamides constitute a useful class of drugs, displaying a variety of activities including antibacterial, anticarbonic anhydrase, diuretic, hypoglycemic, and antithyroid effectiveness.[15] Since the discovery of **3** (E-7010) in 1992,[16] a number of sulfonamides have been reported to be potent inhibitors of tubulin polymerization and have antiproliferative properties.[15b] In fact, **3** and **4** (T-138067)[17] have entered phase II clinical trials against solid tumors. Novel benzene **5**[18] and indole **6**[19] sulfonamides related to **1** have been claimed by Tularik and Abbott. However, reports of the activity data and SAR information for these compounds, especially in vivo efficacy, is limited. For a related structural class of compounds, **7**, Aventis has shown the utility of substituted carbazoles as tubulin polymerization inhibitors (Figure 1).[20]

In this study, we present the synthesis, in vitro evaluations, SAR analysis, in vivo efficacy, and preliminary mode of action information for the new carbazole sulfonamide compounds based on our conceptional lead compound **8**.

Chemistry

The syntheses of carbazole sulfonamides **11a**−**c**, **12a**−**17a**, **18b**, **19a**−**24a**, and **22b** are shown in Scheme 1. The preparation of these carbazole sulfonamides was readily achieved by allowing carbazole sulfonyl chlorides and various commercially available substituted anilines to react in the presence of triethylamine (TEA) in dimethylformamide (DMF) solution at room temperature. The carbazole sulfonyl chlorides **10a**−**c** were prepared from commercially available carbazoles **9a**−**c** using the method of Mitsumori et al.[21] Compound **24a** was obtained by reduction of nitro compound **23a** in the presence of $SnCl_2$ in refluxing ethanol.[22] 3,5-Bis-(trifluoromethyl) aniline failed to react with *N*-9-ethylcarbazole-3-sulfonyl chloride **10a** using the TEA/DMF procedure. However, **25a** was obtained by using pyridine both as catalyst and as solvent. The dibenzofuran sulfonamide **11d** was prepared using the TEA/DMF procedure from dibenzofuran **9d**.

The syntheses of *N*-(9-ethylcarbazole-3-yl) benzene sulfonamides **29**−**33a** are shown in Scheme 2. 3-Amino-9-ethyl carbazole was allowed to react with various commercially available sulfonyl chlorides using the TEA/DMF procedure to yield the sulfonamides **29**−**33a**. Compound **34a** was prepared from **33a** using the same method employed for **24a**.

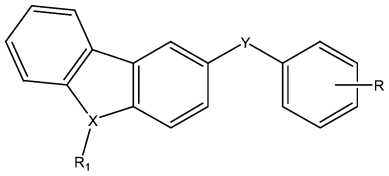
Results and Discussion

1. In Vitro Antiproliferative Activity and SAR. As shown in Table 1, antiproliferative activities of the two series of carbazole sulfonamide compounds were obtained using CEM leukemia cells. CEM leukemia cells were used for initial compound screening because of their known rapid proliferation and high sensitivity to standard anticancer agents. Many compounds exhibited strong activities against human leukemia cells with IC_{50} values of $\leq$1000 to $>$70 nM (**11c**, **15a**, **20a**, **30**−**31a**) or $\leq$70 nM (**11a**,**b**, **16**−**17a**, **18b**, **21a**, **22a**,**b**).

The lead 3,4,5-trimethoxyphenyl-substituted carbazole sulfonamide **11a** has strong cytotoxic activity against human leukemia cells, as reflected by the IC_{50} value of 56 nM. This antiproliferate activity showed that the carbazole tricycle can be used for the B-ring of these sulfonamide compounds related to **1**.[23] The cytotoxicity of the *N*-9 methyl-substituted compound **11b** is similar to that of *N*-9 ethyl compound **11a**. However, **11c**, which has only a hydrogen on *N*-9, showed 10-fold less activity than **11a** and **11b**. Furthermore, when carbazole was replaced by dibenzofuran to yield **11d**, essentially all activity was lost. The results demonstrated that the nitrogen atom of carbazole and the alkylation of *N*-9 are very important for potent activity.

Next, we evaluated the effect of location of the methoxy substitution on the A-ring on cytotoxic activity in the carbazole sulfonamide series. Table 1 shows that the 4-methoxy-substituted compound **12a** is inactive; 3,4-dimethoxy, 3,4-(methylenedioxy), and 3,5-dimethoxy-substituted compounds **13a**−**15a** are more potent than **12a**, however, much less potent

Table 1. Antiproliferative Activity of Carbazole Sulfonamides in CEM Leukemia Cells



compd	X	R_1	Y	R	IC_{50}[a] (nM)
11a	N	Et	SO_2NH	3,4,5-OMe_3	56
11b	N	Me	SO_2NH	3,4,5-OMe_3	46
11c	N	H	SO_2NH	3,4,5-OMe_3	606
11d	O		SO_2NH	3,4,5-OMe_3	>10 000
12a	N	Et	SO_2NH	4-OMe	>10 000
13a	N	Et	SO_2NH	3,4-OMe_2	2436
14a	N	Et	SO_2NH	3,4-OCH_2O-	6252
15a	N	Et	SO_2NH	3,5-OMe_2	974
16a	N	Et	SO_2NH	2,4-OMe_2	57
17a	N	Et	SO_2NH	2,5-OMe_2	61
18b	N	Me	SO_2NH	2,4,6-OMe_3	70
19a	N	Et	SO_2NH	4-F	2171
20a	N	Et	SO_2NH	3-Cl-4-OMe	482
21a	N	Et	SO_2NH	5-Cl-2,4-OMe_2	67
22a	N	Et	SO_2NH	4-Cl-2,5-OMe_2	56
22b	N	Me	SO_2NH	4-Cl-2,5-OMe_2	46
24a	N	Et	SO_2NH	3-NH_2-4-OMe	2200
25a	N	Et	SO_2NH	3,5-$(CF_3)_2$	>10 000
26a	N	Et	SO_2NHCH_2	3,4,5-OMe_3	>10 000
28a[b]	N	Et	$NHSO_2$	4-OMe	>10 000
29a	N	Et	$NHSO_2$	3,4-OMe_2	2651
30a	N	Et	$NHSO_2$	2,5-OMe_2	603
31a	N	Et	$NHSO_2$	3-OMe	789
32a	N	Et	$NHSO_2$	3-CN	1319
33a	N	Et	NH SO_2	2-NO_2-4-OMe	>10 000
34a	N	Et	$NHSO_2$	2-NH_2-4-OMe	>10 000
podophyllotoxin					7.2
1					1.9

[a] IC_{50} = compound concentration required to inhibit CEM leukemia cell proliferation by 50% after 72 h treatment. [b] Commercially available.

than **11a**. Also, the activity of **15a** is greater than that of **13a** and **14a**. From these results, in contrast to **1**, we deduce that methoxy substitution and location on the A-ring plays an important role in affecting cytotoxicity. The effect of substitution involving only 3,4,5-methoxy groups on the sulfonamides is similar to that found for **1**. However, an unexpected difference in SAR information with **1** is noted with other substitutions. 2,4-Dimethoxy, 2,5-dimethoxy, 2,4,6-trimethoxy, 5-chloro-2,4-dimethoxy, and 4-chloro-2,5-dimethoxy-substituted compounds **16a**, **17a**, **18b**, **21a**, **22a**,**b** have similar potent activities as the 3,4,5-trimethoxy-substituted compounds **11a**,**b** and a more than 15-fold stronger activity than the 3,4- or 3,5-dimethoxy-substituted compounds **13a** and **15a**. In contrast, it is well-known that 3,4,5-trimethoxy substituents of the A-ring of **1** are required for potent biological activity.[11,12]

Replacement of the 4-methoxy group in ring A of **12a** by a fluorine atom to yield **19a** enhanced the cytotoxicity. Replacement of one methoxy group of **13a** with a chlorine atom to yield **20a** also enhanced cytotoxicity. The non-3,4,5-trimethoxy analogues **16a**, **17a**, **21a**, and **22a** all exhibit similar potent IC_{50} values. The 3-amino-4-methoxy-substituted analogue **24a** is as active as the 3,4-dimethoxy-substituted compound **13a**, demonstrating that an amino group can replace a methoxy group at the 3-position in this system. Replacement of the 3,5-dimethoxy groups by the strong withdrawing group CF_3 resulted in loss of activity; compare results for **15a** and **25a**. Thus, in contrast to **1**, we note that a number of replacements of the ring A 3,4,5-methoxy groups are tolerated and some enhance the activity.

Table 2. Antiproliferative Activities of **11a**,**b**, **16a**, **17a**, and **22a** in Human Tumor Cell Lines

		IC_{50}[a] (nM)						
cell line	human tumor	**11a**	**11b**	**16a**	**17a**	**22a**	**1**	podophyllo-toxin
CEM	T-cell leukemia	56	46	57	61	56	1.9	7.2
Molt-3	T-cell leukemia	20	19	48	49	67	9.3	14
Bel-7402	hepatoma	201	116	96	219	135	10	9.1
MCF-7[b]	breast cancer	89	70	48	98	67	12	15
DU-145	prostate cancer	603	232	144	365	169	9.3	52
PC-3	prostate cancer	201	116	144	780	90	2.8	10
DND-1	melanoma	89	70	120	195	90	2.5	12
DMS-79	lung cancer	1899	2088	NT[c]	NT	NT	124	23

[a] IC_{50} = compound concentration required to inhibit human tumor cells proliferation by 50% after 72 h treatment. [b] Tularik's compound (**5**; Figure 1) IC_{50} value reported as 150 nM (ref 18). [c] NT = not tested.

These results suggest that the mode of action of these sulfonamides is different from **1** and analogues.

On comparison of the data for the compound **11a** with a $-SO_2NH-$ linkage to that of **26a** with a $-SO_2NHCH_2-$ linkage, it is clear that extension of molecular length by an extra methylene group results in significant loss of activity. Therefore, further modifications with longer linkers were not pursued.

Also, we synthesized *N*-(9-ethylcarbazole-3-yl) benzene sulfonamides **28**−**34a** to test the effect of reversing the orientation of the sulfonamide linkage relative to the A- and B-rings. From the IC_{50} of these compounds, some differences between series are noted. The activity of 4-methoxy and 3,4-dimethoxy-substituted compounds **28a** and **29a** are similar to that of **12a** and **13a** from the original series. However, the 2,5-dimethoxy-substituted compound **30a** in the reversed series is 10-fold less-potent than **17a** in the first series. Interestingly, within the reversed series, the cytotoxicity of the 3-methoxy compound **31a** is comparable to **30a** and more than 3 times greater than that of the 3,4-methoxy compound **29a**. Replacement of the 3-methoxy group of **31a** with the electron-withdrawing 3-cyano group to yield **32a** results in some loss of activity. The introduction of either a nitro or an amino group at the 2-position of compound **28a** to yield **33a** and **34a** did not affect the activity.

2. In Vitro Cancericidal Activity of 11a, 11b, 16a, 17a, and 22a against Several Cancer Cell Lines. The lead carbazole sulfonamide **11a** has potent activity against CEM leukemia cells; consequently, we tested the antiproliferative activity of **11a** against several other human cancer cell lines in vitro. Table 2 contains these results along with comparative data for podophyllotoxin and **1**. The IC_{50} values of **11a** were between 20 and 1899 nM against the cell lines studied (Table 2). The least-sensitive cell line was lung cancer DMS-79 (IC_{50} = 1899 nM). Leukemia cells showed the highest sensitivities (IC_{50} = 20 and 56 nM). The sensitivities of MCF-7 breast cancer cells, melanoma, Bel-7402 hepatoma, and PC-3 prostate cancer cells were also high, with IC_{50} values of less than 201 nM.

With the goal of obtaining more SAR information, we also examined the antiproliferative activity of compounds **11b**, **16a**, **17a**, and **22a** compared to the lead compound **11a** against cancer cell lines. From these results (Table 2), it can be seen that the activity of *N*- methyl carbazole compound **11b** is quite similar to that of **11a**. Compounds **16a**, **17a**, and **22a** have similar activities against leukemia, Bel-7402 hepatocarcinoma, MCF-7 breast cancer cells, and melanoma cell lines. However, for PC-3

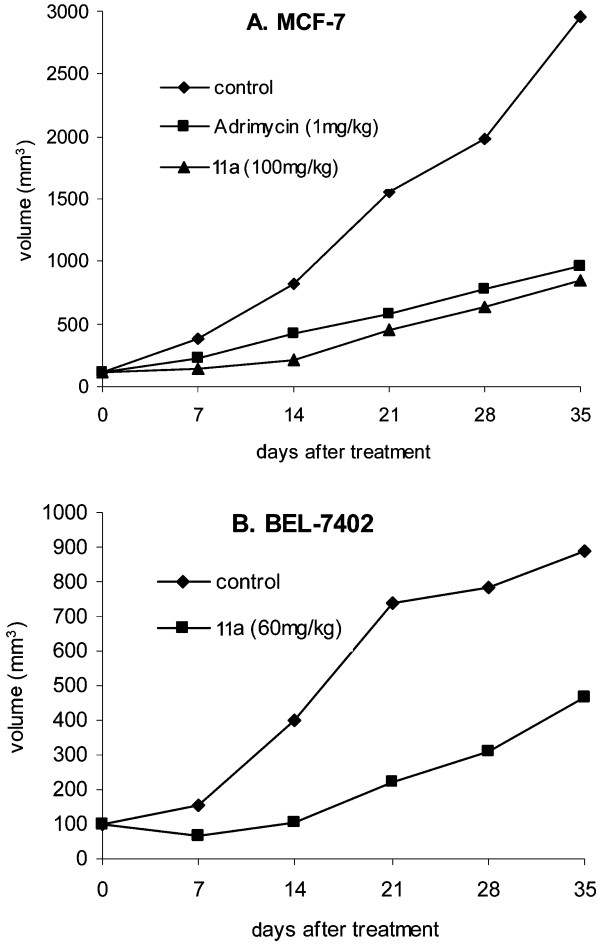


Figure 2. Therapeutic efficacies of **11a** in human breast cancer (MCF-7) and hepatocarcinoma (BEL-7402) in nude mice. In the MCF-7 tumor (A), **11a** was given 100 mg/kg, ip, q2d, with adriamycin (1 mg/kg,ip, q2d) as control. In the BEL-7402 tumor (B), **11a** was given 60 mg/kg, ip, q2d. Each point represents the mean of the tumor volume ($n = 5$).

prostate cancer cells, the 2,5-dimethoxy-substituted compound **17a** is less potent than the other compounds. For the other prostate cancer cell line DU-145, **16a** and **22a** show somewhat higher activity than the other compounds studied. Interestingly, introduction of a 4-chloro atom enhances the antiprostate cancer activity, comparing the results for **17a** and **22a**. Generally, the analogues **16a** and **22a** showed somewhat enhanced activity compared to the lead compound **11a**. These SAR results further demonstrate that 3,4,5- trimethoxy substituents of the A-ring are not indispensable for potent biological activities in this carbazole sulfonamide series. In general, the carbazole analogues are not as active in vitro as the two reference compounds in the cell lines evaluated. However, the activity of **11a** approaches that of the reference compounds in Molt-3 T-cell leukemia and in MCF-7 breast cancer cell lines.

3. Inhibition of Human Breast Cancer and Hepatocarcinoma by 11a in Nude Mice. Because lead compound **11a** has potent antiproliferative activity in human cancer cell lines in vitro, we evaluated the in vivo anticancer efficacy of **11a** in human tumor xenograft models. As shown in Figure 2A, for MCF-7 breast cancer, the average tumor volume of the control group was at 3000 mm^3 on day 35 post-treatment. On treatment with **11a** at 100 mg/kg (ip), the tumor volume was reduced by 75% on the same time course. Compound **11a** was somewhat more effective than adriamycin in this model, which showed a 67% reduction in tumor volume. For human hepatocarcinoma, **11a** given at 60 mg/kg (ip) reduced the tumor volume by 55% (Figure 2B). Overt toxicity to the treated mice at these doses was not noted. The LD_{50} of **11a** was over 500 mg/kg (ip), and additional studies at higher dosage are merited.

Despite the fact that **1** shows strong cytotoxicity in vitro, it shows poor antitumor activities in vivo models because of low water solubility. From the in vivo efficacy of **11a**, it can be deduced that this compound has favorable bioavailability; presumably at least in part due to the presence of the sulfonamide linker.

4. Mode of Action of 11a. Lead compound **11a** has shown potent in vitro and in vivo activities, and the initiation of mode of action studies seemed warranted. Flow cytometric analysis with MCF-7 cells (Figure 3) showed a blockade at the M-phase by **11a** within 24 h of treatment. More than 50% of the cells at the 24 h time point showed dispersed chromosomes characteristic of mitotic arrest (Figure 4) when the concentrations of **11a** ranged between 0.22 and 1.1 μM. Western blot analysis showed remarkable increases of cyclin B1 and cdc2, verifying the accumulation of cells in M-phase after their exposure to **11a** (Figure 5). Leukemia cells treated with **11a** exhibited a similar cell cycle profile and morphological features (data not shown). Also, **11a** induced clear DNA fragmentation and apoptosis in MCF-7 tumor cells (data not shown). Consequently, factors that are related to the apoptotic process were examined. First, p53 levels were assessed because this protein is well-known for its tumor suppressor function. Increased expression of p53 and P-p53 in breast cancer cells treated with **11a** (Figure 5) was observed.[3,24] Bcl-2 expression was also examined because it is an inhibitor of apoptosis. As shown in Figure 5, bcl-2 protein was inactivated by phosphorylation in cells treated with **11a**

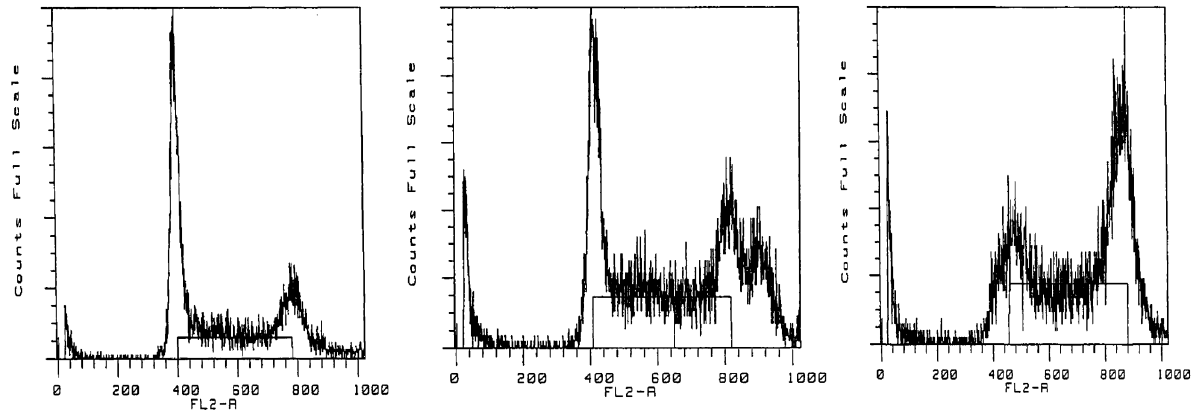


Figure 3. Cell cycle block at the M-phase in MCF-7 cells treated with **11a**. MCF-7 cells treated with **11a** (0.22 μM) were analyzed for cell cycle distribution using flow cytometry. The major block at M-phase appeared between 12 and 24 h post-treatment.

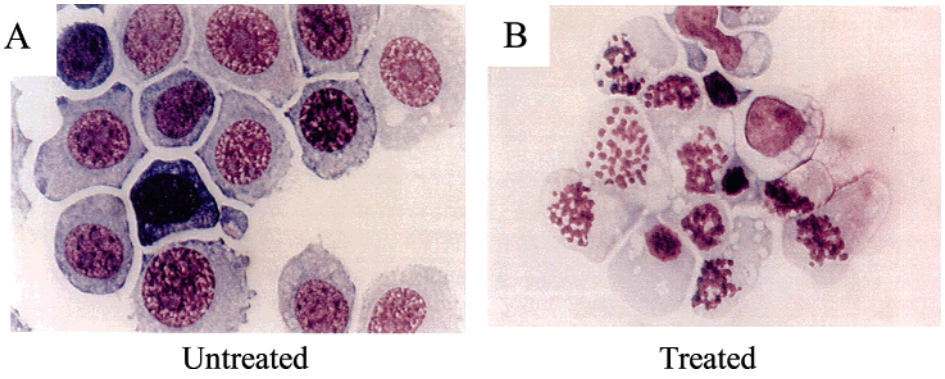


Figure 4. Metaphase arrest by **11a** in MCF-7 Cells. Giemsa staining (400×).

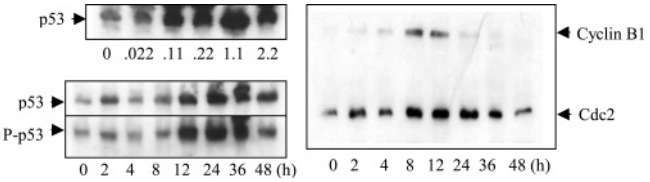


Figure 5. Increased expression of p53, cyclin B1, and cdc2 in MCF-7 cells treated with **11a**. MCF-7 cells were treated at different concentrations (0.022 to 2.2 μM) or at 0.22 μM from 0 to 48 h. Increased p53, P-p53, cyclin B1, and cdc2 expression were noted.

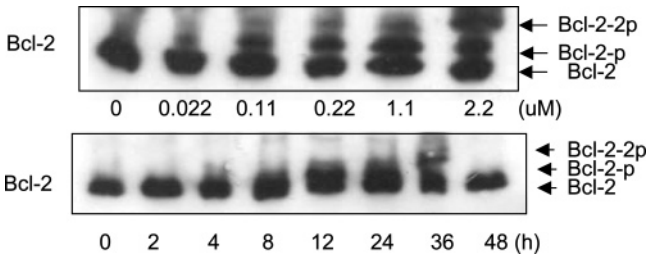


Figure 6. Compound **11a** induced bcl-2 phosphorylation (inactivation) in MCF-7 cells. MCF-7 cells were treated at different concentrations (0.022 to 2.2 μM) or at 0.22 μM from 0 to 48 h. Phosphorylated bcl-2 was noted.

(Figure 6). Increased expression of p53 and bcl-2 phosphorylation processes were dose and time-dependent (Figures 5 and 6). These results strongly suggest that this carbazole sulfonamide has potent cytotoxicity because it can arrest the tumor cell cycle at the M-phase and induce apoptotic cell death by increasing expression of p53 and promoting bcl-2 phosphorylation.

Next, we assessed whether **11a** could directly inhibit microtubule polymerization using purified tubulin in a cell-free system. Surprisingly, lead compound **11a** only weakly affected tubulin polymerization, even at very high concentration (223 μM; Figure 7). This result differs from that of **1** and close analogues, which strongly inhibit tubulin polymerization when their antiproliferate activity is in the submicromolar range. This result suggests the arrest of the cell cycle at the M-phase and the induction of apoptosis by **11a** is unlikely to involve direct action on tubulin. As previously noted, SAR results with these sulfonamides also showed that they act differently from **1** and close analogues. As a result of the size of the carbazole tricycle and different geometry of the sulfonamide linkage,[25] compared to an alkene, it is possible that the sulfonamides are not geometrically suitable for direct tubulin binding and some other cellular targets are likely involved in cell cycle arrest. There are many microtubule-associated proteins (MAPs) that are possible targets, including the dynein and kinesin motor proteins, as well as microtubule regulatory proteins, which can also interact with a microtubule.[1,4] For example, kinesin spindle

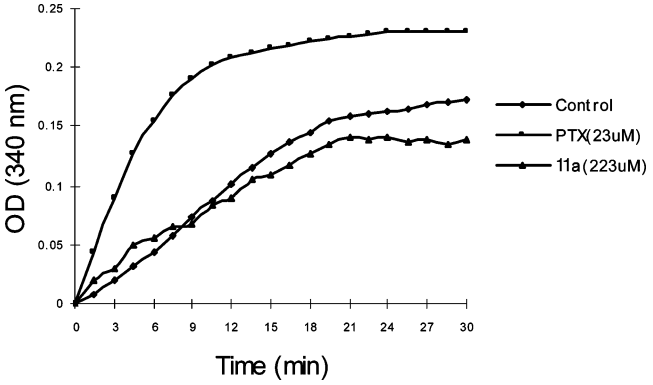


Figure 7. Effect of **11a** on tubulin assembly. Free purified β-tubulin in reaction buffer were incubated with GTP and Mg^{2+} for assembly in the absence or presence of **11a** (223 μM) or paclitaxel (23 μM). Tubulin assembly was determined every 1.5 min by O.D. at 340 nm. Each point represents the mean of two independent experiments.

protein (KSP) inhibitors, which alter microtubule stability, are being developed as a novel potential antimitotic agents for the treatment of cancer.[5] Further studies of the molecular mechanism of action of **11a** is underway.

Conclusions

We have synthesized two series of carbazole sulfonamide compounds structurally related to **1**. New compounds **11a**,**b**, **16a**, **17a**, **18b**, **21a**, and **22a**,**b** exhibit strong activities against human leukemia cells. The SAR information revealed that alkylation of *N*-9 is necessary for potent cytotoxicity. Dibenzofuran replacement of carbazole led to loss of activity. 3,4,5-Trimethoxyphenyl substituents on ring A yield potent activities, however, this array is not essential. 2,4-Dimethoxy, 2,5-dimethoxy, 2,4,6-trimethoxy, 5-chloro-2,4-dimethoxy, and 4-chloro-2,5-dimethoxy ring A-substituted compounds also have similar potent activities. Preliminary mode of action studies demonstrated that the lead compound **11a** arrests tumor cell cycle at M-phase and induces apoptotic cell death by increasing expression of p53 and promoting bcl-2 phosphorylation. However, **11a** only weakly inhibits tubulin polymerization. Lead compound **11a** showed potent antitumor efficacy in vivo models. Lead compounds **11a**, **16a**, and **22a** merit further studies as novel promising antimitotic agents against solid tumors.

Experimental Section

Chemistry. Melting points were determined with a Mel-Temp 3.0 capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian GX400 or Varian Unity Plus 300 spectrometer. Elemental analyses were obtained

from Atlantic Microlab, Inc. (Norcross, GA), and are within ±0.4 of the theoretical values.

9-Ethyl-*N*-(3,4,5-trimethoxyphenyl)-carbazole-3-sulfonamide (11a). To a solution of 3,4,5-trimethoxyaniline (360 mg, 1.97 mmol) in 10 mL DMF at room temperature, 9-ethylcarbazole-3-sulfonyl chloride[21] (600 mg, 2.04 mmol) was added. After stirring for 5 min, TEA (0.30 mL, 2.13 mmol) was added, and the mixture was stirred for an additional 2 h. After adding ice water, the precipitate was filtered, washed with water, dried, and purified by flash chromatography over silica gel, affording the title compound (500 mg, 56%) as a white solid; mp 201−203 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 7.2 Hz, 3H), 3.48 (s, 3H), 3.61 (s, 6H), 4.56 (q, J = 7.2 Hz, 2H), 6.42 (s, 2H), 7.28 (dd, J = 7.2, 7.5 Hz, 1H), 7.53 (dd, J = 7.2, 8.1 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 8.7 Hz, 1H), 7.84 (d, J = 8.7 Hz, 1H), 8.28 (d, J = 8.4 Hz, 1H), 8.65 (s, 1H), 10.02 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 152.9, 141.3, 140.3, 134.2, 133.8, 129.3, 127.0, 124.2, 121.7, 121.5, 120.8, 120.5, 120.0, 109.8, 109.6, 97.4, 60.0, 55.7, 37.3, 13.6. Anal. ($C_{23}H_{24}N_2O_5S\cdot0.4H_2O$) C, H, N.

9-Methyl-*N*-(3,4,5-trimethoxyphenyl)-carbazole-3-sulfonamide (11b). The title compound was obtained from 3,4,5-trimethoxyaniline and 9-methylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**.Yield: 65%; white solid, mp 206−208 °C. ^{1}H NMR (DMSO-d_6): δ 3.48 (s, 3H), 3.61 (s, 6H), 3.88 (s, 3H), 6.44 (s, 2H), 7.28 (dd, J = 7.2, 7.8 Hz, 1H), 7.54 (dd, J = 8.1, 7.2 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.73 (d, J = 8.7 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 8.27 (d, J = 7.5 Hz, 1H), 8.65 (s, 1H), 10.02 (s,1H). ^{13}C NMR (DMSO-d_6): δ 152.9, 142.3, 141.4, 134.2, 133.7, 129.1, 126.9, 124.1, 121.5, 121.3, 120.7, 120.3, 120.0, 109.9, 109.7, 97.3, 60.0, 55.7, 29.3. Anal. ($C_{22}H_{22}N_2O_5S\cdot0.25H_2O$) C, H, N.

***N*-(3,4,5-Trimethoxyphenyl)-9*H*-carbazole-3-sulfonamide (11c).** The title compound was obtained from 3,4,5-trimethoxyaniline and 9*H*-carbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 58%; white solid, mp 205−206 °C. ^{1}H NMR (DMSO-d_6): δ 3.47 (s, 3H), 3.60 (s, 6H), 6.41 (s, 2H), 7.22 (dd, J = 7.2, 7.8 Hz, 1H), 7.45 (dd, J = 8.1, 7.2 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 8.7 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 8.22 (d, J = 7.5 Hz, 1H), 8.61 (s, 1H), 9.99 (s, 1H), 11.79 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 152.9, 141.6, 140.5, 134.2, 133.8, 129.1, 126.8, 124.1, 121.9, 121.7, 120.6, 120.4, 119.8, 111.6, 111.3, 97.4, 60.0, 55.7. Anal. ($C_{21}H_{20}N_2O_5S$) C, H, N. High-resolution mass calcd for $C_{21}H_{21}N_2O_5S$, 413.1171; found, 413.1182.

***N*-(3,4,5-Trimethoxyphenyl)-dibenzofuran-2-sulfonamide (11d).** The title compound was obtained from 3,4,5-trimethoxyaniline and dibenzofuran-2-sulfonyl chloride[21] using a procedure similar to compound **11a**. Yield: 84%; white solid, mp 168−170 °C. ^{1}H NMR (DMSO-d_6): δ 3.50 (s, 3H), 3.62 (s, 6H), 6.41 (s, 2H), 7.46 (dd, J = 6.9, 7.5 Hz, 1H), 7.60 (dd, J = 7.5, 7.2 Hz, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.85−7.96 (m, 2H), 8.31 (d, J = 8.1 Hz, 1H), 8.66 (s, 1H), 10.16 (s, 1H). ^{13}C NMR (DMSO- d_6): δ 157.2, 156.2, 153.0, 134.4, 134.1, 133.6, 128.9, 126.3, 123.9, 122.5, 121.9, 121.1, 112.6, 112.0, 98.0, 60.0, 55.7. Anal. ($C_{21}H_{19}NO_6S$) C, H, N.

9-Ethyl-*N*-(4-methoxyphenyl)-carbazole-3-sulfonamide (12a). The title compound was obtained from 4-methoxyaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 71%; brown solid, mp 213-215 °C (lit.[26] mp 215−217 °C). ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 6.9 Hz, 3H), 3.60 (s, 3H), 4.46 (q, J = 6.9 Hz, 2H), 6.74 (d, J = 8.1 Hz, 1H), 7.00 (d, J = 8.1 Hz, 1H), 7.26 (dd, J = 7.2, 7.2 Hz, 1H), 7.52 (dd, J = 7.8, 7.5 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.70−7.78 (m, 2H), 8.22 (d, J = 7.8 Hz, 1H), 8.52 (s, 1H), 9.81 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 156.2, 141.3, 140.3, 130.7, 129.5, 126.9, 124.0, 123.0, 121.7, 121.4, 120.8, 120.0, 119.9, 114.2, 109.8, 109.4, 55.0, 37.3, 13.7.

***N*-(3,4-Dimethoxyphenyl)-9-ethylcarbazole-3-sulfonamide (13a).** The title compound was obtained from 3,4-dimethoxyaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 76%; pale brown solid, mp 175−177 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 7.2 Hz, 3H), 3.59 (s, 6H), 4.46 (q, J = 7.2 Hz, 2H), 6.56 (dd, J = 8.7, 2.1 Hz, 1H), 6.71−6.74 (m, 2H), 7.27 (dd, J = 7.2, 7.5 Hz, 1H), 7.53 (dd, J = 7.8, 7.5 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.78 (d, J = 8.7 Hz, 1H), 8.24 (d, J = 7.8 Hz, 1H), 8.56 (s, 1H), 9.82 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 148.7, 145.7, 141.2, 140.3, 131.1, 129.4, 126.9, 124.1, 121.7, 121.4, 120.8, 120.2, 120.0, 113.0, 112.0, 109.8, 109.4, 106.1, 55.5, 55.3, 37.3, 13.7. Anal. ($C_{22}H_{22}N_2O_4S\cdot0.3H_2O$) C, H, N.

9-Ethyl-*N*-[3,4-(methylenedioxy)phenyl]-carbazole-3-sulfonamide (14a). The title compound was obtained from 3,4-(methylenedioxy)aniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 74%; pale brown solid, mp 182−184 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 6.9 Hz, 3H), 4.46 (q, J = 6.9 Hz, 2H), 5.89 (s, 2H), 6.50 (d, J = 8.4 Hz, 1H), 6.68−6.71 (m, 2H), 7.27 (dd, J = 7.5, 7.2 Hz, 1H), 7.53 (dd, J = 7.8, 7.5 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.72−7.79 (m, 2H), 8.25 (d, J = 7.8 Hz, 1H), 8.55 (s, 1H), 9.89 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 147.3, 144.1, 141.2, 140.3, 132.0, 129.2, 126.9, 124.0, 121.7, 121.5, 120.9, 120.1, 120.0, 114.2, 109.8, 109.5, 108.2, 103.1, 101.2, 37.3, 13.7. Anal. ($C_{21}H_{18}N_2O_4S\cdot0.3H_2O$) C, H, N.

***N*-(3,5-Dimethoxyphenyl)-9-ethylcarbazole-3-sulfonamide (15a).** The title compound was obtained from 3,5-dimethoxyaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 63%; pale brown solid, mp 171−173 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 6.9 Hz, 3H), 3.60 (s, 6H), 4.46 (q, J = 6.9 Hz, 2H), 6.07 (d, J = 2.1 Hz, 1H), 6.32 (d, J = 2.1 Hz, 2H), 7.28 (dd, J = 7.5, 7.2 Hz, 1H), 7.54 (dd, J = 7.8, 7.5 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.77 (d, J = 8.7 Hz, 1H), 7.85 (d, J = 8.7 Hz, 1H), 8.28 (d, J = 7.5 Hz, 1H), 8.66 (s, 1H), 10.20 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 160.6, 141.2, 140.2, 140.0, 129.3, 126.9, 124.0, 121.6, 121.4, 120.8, 120.3, 120.0, 109.7, 109.6, 97.4, 94.8, 55.0, 37.3, 13.6. Anal. ($C_{22}H_{22}N_2O_4S$) C, H, N.

***N*-(2,4-Dimethoxyphenyl)-9-ethylcarbazole-3-sulfonamide (16a).** The title compound was obtained from 2,4-dimethoxyaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 75%; brown solid, mp 185−187 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 7.2 Hz, 3H), 3.43 (s, 3H), 3.60 (s, 3H), 4.78 (q, J = 7.2 Hz, 2H), 6.56 (dd, J = 9.0, 2.7 Hz, 1H), 6.76 (d, J = 9.0 Hz, 1H), 6.86 (d, J = 2.7 Hz, 1H), 7.27 (dd, J = 7.5, 7.2 Hz, 1H), 7.53 (dd, J = 8.4, 7.2 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.84 (dd, J = 8.7, 1.5 Hz, 1H), 8.24 (d, J = 8.1 Hz, 1H), 8.60 (d, J = 1.5 Hz, 1H), 9.32 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 153.4, 145.9, 141.7, 140.8, 130.7, 127.4, 127.3, 124.7, 122.3, 121.8, 121.3, 120.5, 120.4, 113.1, 110.3, 110.0, 109.9, 109.7, 56.6, 55.8, 37.8, 14.1. Anal. ($C_{22}H_{22}N_2O_4S\cdot0.4H_2O$) C, H, N.

***N*-(2,5-Dimethoxyphenyl)-9-ethylcarbazole-3-sulfonamide (17a).** The title compound was obtained from 2,5-dimethoxyaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 70%, brown solid, mp 164−166 °C. ^{1}H NMR(DMSO-d_6): δ 1.30 (t, J = 6.9 Hz, 3H), 3.33 (s, 3H), 3.65 (s, 3H), 4.48 (q, J = 6.9 Hz, 2H), 6.34 (d, J = 2.1 Hz, 1H), 6.41 (dd, J = 9.0, 2.1 Hz, 1H), 7.10 (d, J = 9.0 Hz, 1H), 7.26 (dd, J = 7.5, 7.5 Hz, 1H), 7.52 (dd, J = 7.8, 7.2 Hz, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.68−7.72 (m, 2H), 8.21 (d, J = 7.8 Hz, 1H), 8.45 (s, 1H), 9.07 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 158.5, 154.2, 141.1, 140.2, 130.7, 127.6, 126.7, 124.4, 121.8, 121.2, 120.7, 119.9, 118.3, 109.7, 108.9, 104.5, 98.9, 55.2, 37.3, 13.6. Anal. ($C_{22}H_{22}N_2O_4S$) C, H, N.

9-Methyl-*N*-(2,4,6-trimethoxyphenyl)-carbazole-3-sulfonamide (18b). The title compound was obtained from 2,4,6-trimethoxyaniline and 9-methylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 70%; white solid, mp 209−211°C. ^{1}H NMR (DMSO-d_6): δ 3.29 (s, 6H), 3.71 (s, 3H), 3.93 (s, 3H), 6.09 (s, 2H), 7.26 (dd, J = 7.2, 7.5 Hz, 1H), 7.53 (dd, J = 7.5, 7.8 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.78 (dd, J = 8.7, 1.8 Hz, 1H), 8.22 (d, J = 7.5 Hz,1H), 8.44 (d, J = 1.8 Hz, 1H), 8.52 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 160.3, 158.5, 142.6, 141.9, 133.1, 127.4, 125.2, 122.3, 121.5, 121.0, 120.5, 120.1, 110.3, 109.2, 106.7, 91.4, 55.9, 55.9, 29.8. Anal. ($C_{22}H_{22}N_2O_5S$) C, H, N.

9-Ethyl-*N*-(4-fluorophenyl)-carbazole-3-sulfonamide (19a). The title compound was obtained from 4-fluoroaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 95%; pale brown solid, mp 158−160 °C. ^{1}H NMR (DMSO-d_6): δ 1.24 (t, J = 6.6 Hz, 3H), 4.46 (q, J = 6.6 Hz, 2H), 7.00−7.06 (m, 2H), 7.16−7.26 (m, 2H), 7.24 (d, J = 7.5 Hz, 1H), 7.48 (dd, J = 8.1, 7.2 Hz, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.83 (d, J = 8.7 Hz, 1H), 8.22 (d, J = 7.5 Hz, 1H), 8.62 (s, 1H), 10.20 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 158.9 (d, J = 856.7 Hz), 141.3, 140.3, 134.5 (d, J = 11.4 Hz), 129.2, 127.0, 124.0, 122.4 (d, J = 32.1 Hz), 121.7, 121.6, 120.9, 120.2, 120.0, 115.8 (d, J = 91.8 Hz), 109.7, 109.5, 37.3, 13.6. Anal. ($C_{20}H_{17}FN_2O_2S$) C, H, N.

***N*-(3-Chloro-4-methoxyphenyl)-9-ethylcarbazole-3-sulfonamide (20a).** The title compound was obtained from 3-chloro-4-methoxyaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 72%; brown solid, mp 222−224 °C. ^{1}H NMR (DMSO-d_6): δ 1.33 (t, J = 7.2 Hz, 3H), 3.72 (s, 3H), 4.49 (q, J = 7.2 Hz, 2H), 6.99 (d, J = 9.0 Hz, 1H), 7.05 (dd, J = 8.7, 2.4 Hz, 1H), 7.16 (d, J = 2.4 Hz, 1H), 7.30 (dd, J = 7.5, 7.5 Hz, 1H), 7.56 (dd, J = 7.2, 7.2 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.78−7.79 (m, 2H), 8.28 (d, J = 8.1 Hz, 1H), 8.59 (s, 1H), 10.07 (s, 1H). Anal. ($C_{21}H_{19}ClN_2O_3S$) C, H, N.

***N*-(5-Chloro-2,4-dimethoxyphenyl)-9-ethylcarbazole-3-sulfonamide (21a).** The title compound was obtained from 5-chloro-2,4-dimethoxyaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 76%; brown solid, mp 194−196 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 6.9 Hz, 3H), 3.34 (s, 3H), 3.75 (s, 3H), 4.48 (q, J = 6.9 Hz, 2H), 6.57 (s, 1H), 7.19 (s, 1H), 7.27 (dd, J = 7.5, 7.2 Hz, 1H), 7.52 (dd, J = 7.2, 8.1 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.70−7.75 (m, 2H), 8.23 (d, J = 7.5 Hz, 1H), 8.48 (s, 1H), 9.33 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 153.4, 153.2, 141.2, 140.3, 130.3, 127.4, 126.8, 124.4, 121.8, 121.3, 120.8, 119.9, 118.5, 111.1, 109.8, 109.1, 98.0, 56.3, 55.9, 37.3, 13.7. Anal. ($C_{22}H_{21}ClN_2O_4S$) C, H, N.

***N*-(4-Chloro-2,5-dimethoxyphenyl)-9-ethylcarbazole-3-sulfonamide (22a).** The title compound was obtained from 4-chloro-2,5-dimethoxyaniline and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 56%; brown solid, mp 188−190 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 7.2 Hz, 3H), 3.39 (s, 3H), 3.70 (s, 3H), 4.47 (q, J = 7.5 Hz, 1H), 6.95 (s, 1H), 7.06 (s, 1H), 7.27 (dd, J = 7.8, 7.2 Hz, 1H), 7.53 (dd, J = 8.1, 7.5 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 8.7 Hz, 1H), 7.82 (dd, J = 8.7, 1.5 Hz, 1H), 8.25 (d, J = 7.8 Hz, 1H), 8.60 (d, J = 1.5 Hz, 1H), 9.49 (s, 1H). Anal. ($C_{22}H_{21}ClN_2O_4S$) C, H, N.

***N*-(4-Chloro-2,5-dimethoxyphenyl)-9-methylcarbazole-3-sulfonamide (22b).** The title compound was obtained from 4-chloro-2,5-dimethoxyaniline and 9-methylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 67%; pale white solid, mp 218−220 °C. ^{1}H NMR (DMSO-d_6): δ 3.41 (s, 3H), 3.70 (s, 3H), 3.90 (s, 3H), 6.95 (s, 1H), 7.07 (s, 1H), 7.28 (dd, J = 7.2, 7.8 Hz, 1H), 7.54 (dd, J = 8.4, 7.2 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 8.7 Hz, 1H), 7.83 (d, J = 8.7 Hz, 1H), 8.25 (d, J = 7.8 Hz, 1H), 8.62 (s, 1H), 9.48 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 148.1, 145.7, 142.3, 141.4, 129.8, 126.8, 125.6, 124.3, 121.5, 121.2, 120.6, 120.1, 120.0, 117.0, 113.9, 109.8, 109.3, 108.7, 56.4, 56.3, 29.3. Anal. ($C_{21}H_{19}ClN_2O_4S$) C, H, N.

***N*-(3-Amino-4-methoxyphenyl)-9-ethylcarbazole-3-sulfonamide hydrochloride (24a).** The nitro compound (**23a**) was obtained from 4-methoxy-3-nitroaniline[27] and 9-ethylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a** and used directly in the next step. Yield: 71%; yellow solid, mp 218−220 °C. ^{1}H NMR (DMSO-d_6): δ 1.29 (t, J = 6.9 Hz, 3H), 3.77 (s, 3H), 4.46 (q, J = 6.9 Hz, 2H), 7.21 (d, J = 8.7 Hz, 1H), 7.28 (dd, J = 7.2, 7.8 Hz, 1H), 7.36 (d, J = 9.0 Hz, 1H), 7.53 (dd, J = 7.2, 7.8 Hz, 1H), 7.60 (s, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.52−7.83 (m, 2H), 8.26 (d, J = 7.8 Hz, 1H), 8.60 (s, 1H), 10.31 (s,1H).

To a suspension of the above nitro compound **23a** (180 mg, 0.42 mmol) in 4 mL anhydrous ethanol, $SnCl_2 \cdot 2H_2O$ (520 mg, 2.31 mmol) was added, and the mixture was heated at reflux for 2 h. After cooling to room temperature, 20 mL of water was added and, using 10% aqueous K_2CO_3, the pH of the solution was adjusted to 7−8. The precipitate was filtered and washed with water. The residue was dissolved in 100 mL acetone and filtered. The filtrate was evaporated under reduced pressure, and the precipitate was collected. The precipitate was dissolved in 10 mL anhydrous ethyl acetate and saturated at ice−water bath temperature with anhydrous HCl gas. The solution was then stirred at room temperature for 3 h. The precipitate was filtered to afford the title compound (110 mg, 66%) as a brown solid; mp 223−225 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 7.2 Hz, 3H), 3.52 (s, 3H), 3.71 (s, 3H), 4.46 (q, J = 7.2 Hz, 2H), 6.86 (dd, J = 9.0, 2.1 Hz, 1H), 6.92 (d, J = 9.0 Hz, 1H), 7.13 (d, J = 2.1 Hz, 1H), 7.28 (dd, J = 7.5,7.5 Hz, 1H), 7.53 (dd, J = 7.8, 7.5 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 9.0 Hz, 1H), 7.79 (dd, J = 8.7, 1.5 Hz, 1H), 8.24 (d, J = 7.8 Hz, 1H), 8.59 (d, J = 1.5 Hz, 1H), 10.06 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 147.3, 141.2, 140.2, 131.1, 129.3, 126.9, 125.3, 124.0, 121.7, 121.4, 120.9, 120.1, 120.0, 117.8, 114.1, 112.2, 109.8, 109.5, 55.9, 37.3, 13.7. Anal. ($C_{21}H_{22}ClN_3O_3S \cdot 1.25H_2O$) C, H, N.

***N*-[3,5-Di-(trifluoromethyl)phenyl]-9-ethylcarbazole-3-sulfonamide (25a).** To a solution of 3,5-bis-(trifluoromethyl)aniline (0.31 mL, 2.0 mmol) in 5 mL pyridine at room temperature, 9-ethylcarbazole-3-sulfonyl chloride[21] (600 mg, 2.05 mmol) was added. After stirring for 1.5 h, the pyridine was removed under reduced pressure. The residue was purified by flash chromatography over silica gel to afford the title compound (852 mg, 88%) as a white solid; mp 152−153 °C. ^{1}H NMR (DMSO-d_6): δ 1.32 (t, J = 7.2 Hz, 3H), 4.49 (q, J = 7.2 Hz, 2H), 7.32 (dd, J = 7.2, 7.8 Hz, 1H), 7.57 (dd, J = 7.8, 7.2 Hz, 1H), 7.70−7.74 (m, 4H), 7.84 (d, J = 8.7 Hz, 1H), 7.88 (d, J = 8.7 Hz, 1H), 8.32 (d, J = 7.5 Hz, 1H), 8.74 (s, 1H), 11.14 (s,1H). ^{13}C NMR (DMSO-d_6): δ 142.0, 140.9, 140.8, 131.6 (q, J = 132.0 Hz), 128.6, 127.5, 124.2, 123.3 (q, J = 1084.0 Hz), 122.2, 122.1, 121.3, 120.8, 120.6, 118.7, 116.6, 110.4, 110.3, 37.8, 14.0. Anal. ($C_{22}H_{16}F_6N_2O_2S$) C, H, N.

9-Ethyl-*N*-(3,4,5-trimethoxybenzyl)-carbazole-3-sulfonamide (26a). The title compound was obtained from 3,4,5-trimethoxybenzylamine and 9-methylcarbazole-3-sulfonyl chloride using a procedure similar to compound **11a**. Yield: 81%; white solid, mp 187−189 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 6.9 Hz, 3H), 3.33 (s, 3H), 3.57 (s, 6H), 3.96 (d, J = 6.3 Hz, 2H), 4.47 (q, J = 6.9 Hz, 2H), 6.43 (s, 2H), 7.27 (dd, J = 7.2, 7.8 Hz, 1H), 7.52 (t, J = 7.5, 7.2 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.83 (dd, J = 8.7, 2.1 Hz, 1H), 7.95 (t, J = 6.3 Hz, 1H), 8.24 (d, J = 8.1 Hz, 1H), 8.54 (d, J = 1.5 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 152.5, 141.0, 140.3, 136.1, 133.1, 130.9, 126.8, 124.0, 121.9, 121.5, 120.8, 119.9, 119.8, 109.6, 109.3, 104.7, 59.5, 55.5, 46.6, 37.2, 13.6. Anal. ($C_{24}H_{26}N_2O_5S$) C, H, N.

3,4-Dimethoxy-*N*-(9-ethylcarbazole-3-yl)-benzene sulfonamide (29a). The title compound was obtained from 3,4-dimethoxybenzenesulfonyl chloride and 3-amino-9-ethylcarbazole using a procedure similar to compound **11a**. Yield: 77%; pale brown solid, mp 168−169 °C. ^{1}H NMR (DMSO-d_6): δ 1.24 (t, J = 6.6 Hz, 3H), 3.68 (s, 3H), 3.73 (s, 3H), 4.35 (q, J = 6.6 Hz, 2H), 6.98 (d, J = 8.7 Hz, 1H), 7.11−7.23 (m, 4H), 7.43 (dd, J = 7.8, 7.2 Hz, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.55 (d, J = 8.1 Hz, 1H), 7.83 (s, 1H), 8.03 (d, J = 7.5 Hz, 1H), 9.85 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 151.9, 148.4, 139.9, 137.1, 131.1, 129.1, 126.0, 122.1, 121.7, 121.5, 120.6, 120.3, 118.7, 114.5, 110.9, 109.5, 109.3, 109.2, 55.6, 36.9, 13.6. Anal. ($C_{22}H_{22}N_2O_4S \cdot 0.25H_2O$) C, H, N.

2,5-Dimethoxy-*N*-(9-ethylcarbazole-3-yl)-benzene sulfonamide (30a). The title compound was obtained from 2,5-dimethoxybenzenesulfonyl chloride and 3-amino-9-ethylcarbazole using a procedure similar to compound **11a**. Yield: 84%; off-white solid, mp 237−239 °C. ^{1}H NMR (DMSO-d_6): δ 1.25 (t, J = 6.6 Hz, 3H), 3.62 (s, 3H), 3.91 (s, 3H), 4.35 (q, J = 6.6 Hz, 2H), 7.07−7.17 (m, 5H), 7.39−7.45 (m, 2H), 7.54 (d, J = 8.1 Hz, 1H), 7.80 (s, 1H), 8.01 (d, J = 7.8 Hz, 1H), 9.71 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 152.0, 150.3, 139.9, 137.0, 128.8, 127.1, 125.9, 122.0, 121.6, 121.1, 120.1, 119.5, 118.7, 115.1, 113.9, 113.9, 109.2, 109.1, 56.3, 55.6, 36.9, 13.6. Anal. ($C_{22}H_{22}N_2O_4S \cdot 0.2H_2O$) C, H, N.

***N*-(9-Ethylcarbazole-3-yl)-3-methoxybenzenesulfonamide (31a).** The title compound was obtained from 3-methoxybenzenesulfonyl

chloride and 3-amino-9-ethylcarbazole using a procedure similar to compound **11a**. Yield: 71%; pale brown solid, mp 176−178 °C. ^{1}H NMR (DMSO-d_6): δ 1.25 (t, J = 7.2 Hz, 3H), 3.70 (s, 3H), 4.35 (q, J = 7.2 Hz, 2H), 7.10−7.18 (m, 3H), 7.27−7.29 (m, 2H), 7.37−7.48 (m, 3H), 7.55 (d, J = 8.1 Hz, 1H), 7.82 (d, J = 1.5 Hz, 1H), 8.03 (d, J = 7.5 Hz, 1H), 10.02 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 159.2, 140.8, 139.9, 137.2, 130.3, 128.7, 126.1, 122.1, 121.6, 121.5, 120.3, 118.9, 118.8, 118.4, 114.6, 111.7, 109.4, 109.2, 55.5, 37.0, 13.7. Anal. ($C_{21}H_{20}N_2O_3S$) C, H, N.

3-Cyano-*N*-(9-ethylcarbazole-3-yl)-benzenesulfonamide (32a). The title compound was obtained from 3-cyanobenzenesulfonyl chloride and 3-amino-9-ethylcarbazole using a procedure similar to compound **11a**. Yield: 71%; white solid, mp 198−200 °C. ^{1}H NMR (DMSO-d_6): δ 1.25 (t, J = 7.2 Hz, 3H), 4.36 (q, J = 7.2 Hz, 2H), 7.06 (dd, J = 8.7, 1.8 Hz, 1H), 7.16 (dd, J = 7.2, 7.5 Hz, 1H), 7.44 (dd, J = 7.5, 7.8 Hz, 1H), 7.48 (d, J = 8.7 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.71 (dd, J = 8.1, 7.8 Hz, 1H), 7.82 (d, J = 1.8 Hz, 1H), 7.94 (d, J = 8.1 Hz, 1H), 8.05−8.09 (m, 3H), 10.20 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 140.8, 140.0, 137.5, 136.3, 131.2, 130.6, 130.2, 127.9, 126.2, 122.3, 121.9, 121.6, 120.4, 118.8, 117.4, 115.4, 112.3, 109.5, 109.3, 37.0, 13.6. Anal. ($C_{21}H_{17}N_3O_2S\cdot0.2H_2O$) C, H, N.

***N*-(9-Ethylcarbazole-3-yl)-4-methoxy-2-nitrobenzenesulfonamide (33a).** The title compound was obtained from 4-methoxy-2-nitrobenzenesulfonyl chloride and 3-amino-9-ethylcarbazole using a procedure similar to compound **11a**. Yield: 80%; yellow solid, mp 79−81 °C. ^{1}H NMR (DMSO-d_6): δ 1.26 (t, J = 6.9 Hz, 3H), 3.81 (s, 3H), 4.37 (q, J = 6.9 Hz, 2H), 7.15−7.19 (m, 2H), 7.24 (dd, J = 9.0, 2.4 Hz, 1H), 7.44 (dd, J = 8.4, 7.2 Hz, 1H), 7.49−7.52 (m, 2H), 7.57 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 9.0 Hz, 1H), 7.86 (s, 1H), 8.05 (d, J = 8.1 Hz, 1H), 10.25 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 162.7, 156.0, 149.5, 140.0, 137.4, 131.9, 127.8, 126.1, 122.7, 122.2, 121.7, 120.4, 118.8, 117.1, 114.8, 109.8, 109.5, 109.3, 56.6, 37.0, 13.7. Anal. ($C_{21}H_{19}N_3O_5S\cdot0.2H_2O$) C, H, N.

2-Amino-*N*-(9-ethylcarbazole-3-yl)-4-methoxybenzenesulfonamide (34a). The title compound was obtained from the above compound **33a** using a procedure similar to compound **24a**. Yield: 74%; pale brown foam. ^{1}H NMR (DMSO-d_6): δ 1.25 (t, J = 6.9 Hz, 3H), 3.62 (s, 3H), 4.35 (q, J = 6.9 Hz, 2H), 5.99 (s, 2H), 6.06 (d, J = 9.0 Hz, 1H), 6.22 (s, 1H), 7.12 (d, J = 9.0 Hz, 1H), 7.15 (dd, J = 7.5, 7.2 Hz, 1H), 7.32 (d, J = 9.0 Hz, 1H), 7.43 (dd, J = 8.1, 7.2 Hz, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.55 (d, J = 8.1 Hz, 1H), 7.78 (s, 1H), 8.00 (d, J = 8.1 Hz, 1H), 9.81 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 163.3, 148.3, 139.9, 136.9, 131.6, 129.2, 126.0, 122.1, 121.7, 120.8, 120.2, 118.7, 113.4, 111.8, 109.3, 109.2, 102.7, 99.5, 55.0, 37.0, 13.7. Anal. ($C_{21}H_{21}N_3O_3S$) C, H, N.

Biological Methods

Tumor Cell Lines. The human cell lines CEM (leukemia), MCF-7 (breast cancer), DU-145 (prostate cancer), PC-3 (prostate cancer), and DMS-79 (lung cancer) were from the American Tissue Culture Collection (ATCC, Rocksville, MD). Molt-3 (human leukemia) and DND-1 (human melanoma) cell lines were from Dr. T. Ohnuma (Mount Sinai School of Medicine, New York, NY). Bel-7402 cell line originated from Chinese human hepatocarcinoma patients were provided by the Cancer Institute of Chinese Academy of Medical Sciences (Beijing, China). Cells were cultivated in RPMI-1640 supplemented with 10% heat inactivated fetal calf serum. The media were treated with penicillin (150 ug/mL) and streptomycin (150 ug/mL). Cells (cultured in 5% CO_2 at 37 °C) in exponential growth were used in all experiments.

Cancericidal Activity in Vitro. Cells were distributed into 24-well plates (Falcon, Oxnard, CA) with 5×10^4 cells and a total volume of 250 μL per well, followed by treatment with the study compound at concentrations between 0 and 10 μM at 37 °C for 72 h. The IC_{50} values were determined in duplicates, and these values were defined as drug concentrations killing 50% cells in comparison with untreated controls and calculated by nonlinear regression analysis.

Morphology. Cell sample slides were made on a Cytospin centrifuge (Shandon Southern Products, Ltd., England) at 700 rpm for 10 min. The slides were air-dried, fixed in methanol for 5 min, stained with Giemsa (Harleco, NJ) at room temperature for 15 min, and preserved with resin. Morphological features were observed by light microscopy (BH-2 OLYMPUS, Japan). Cells in M-phase arrest were identified if spreading of the chromosomes was seen in the cytoplasm.

Cell Cycle Analysis. Tumor cells were treated with **11a** for indicated periods of time and harvested after a digestion with a mixture of 0.25% trypsin/0.02% EDTA. The cells (1.5×10^6) were washed twice with PBS, followed by the treatment with RNase (Sigma, St. Louis, MO) at a final concentration of 0.5 μg/μL at 37 °C for 30 min. Cells were then fixed in 4 mL of ice-cold 70% ethanol at 4 °C for at least 12 h. After washing in PBS, cells were stained with propidium iodide at a final concentration of 0.1 μg/μL at 4 °C for 60 min. At least 10^4 cell events per sample were analyzed in an EPICS flow cytometer (Beckman Coulter, San Jose, CA) using EPICS software (version 2.0).

DNA Fragmentation. DNA from untreated or **11a** treated cells was extracted. Briefly, after washing with PBS, cells were lysed with 500 μL of lysis buffer (0.05 M Tris-HCl, pH 7.5; 0.15 M NaCl; 0.005 M EDTA, pH 8.0 and 1% sodium dodecyl sulfate) containing 100 μg/mL proteinase K at 56 °C for 2 h. The resulting products were extracted with phenol and then a mixture of chloroform/isoamyl alcohol (24:1, v/v), precipitated at 4 °C overnight with absolute ethanol, followed by centrifugation (12000 g, PM180R, ALC, Ltd., Italy) at 4 °C for 20 min. The ethanol was removed, and the dry pellet of nucleic acid was resuspended in 10 mM Tris-HCl/1 mM EDTA solution. The DNA samples were then treated with RNase (Sigma, St. Louis, MO) at a final concentration of 0.1 μg/μL at 37 °C for 20 min. DNA content was determined spectrophotometrically by measuring absorption at 260 nm (A_{260}). All samples had $A_{260}/A_{280} \geq 2$. The DNA sample (5 ug) was analyzed in 1.5% horizontal agarose gel prepared with 1% ethidium bromide. Electrophoresis was performed at 75 V for 2 h, and the DNA migration was visualized under UV light.

Microtubule Polymerization Assays. The HTS-tubulin polymerization assay kit (Cytoskeleton, Inc., Denver, CO) was used to measure microtubule polymerization, following the protocol provided by the vendor. Study compounds were added into each well of the 96-well plate placed on ice and using G-PEM buffer as diluent (80 mM PIPES, pH 6.9, 1 mM EGTA, 5% glycerol, 1 mM GTP). Each well contained G-PEM buffer, study compound at the concentration indicated, and β-tubulin at a concentration of 1 mg/mL. The plate was shaken for 20 s and warmed to 37 °C, and the absorbance was read at 340 nm every 1.5 min for 30 min.

Western Blot Assay. Cells were harvested, aliquoted and then lysed in lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.02% NaN_3, 1% NP-40, 1 mM PMSF, 1 μg/mL aprotinin) on ice for 1 h, followed by centrifugation (12 000 g) at 4 °C for 20 min. Supernatants were collected and the protein concentration was determined by the Bradford method. The protein sample (90 μg/20 μL/test) was subjected to electrophoresis in a 10% or 15% SDS-polyacrylamide gel (10% gel for proteins with molecular mass >50 KD; 15% gel for proteins with molecular mass <50 KD) and then transferred to a nitrocellulose membrane with an electrotransfer system (Bio-Rad Laboratories, Munich, Germany). The membrane was blocked with 5% nonfat milk in TBST buffer (20 mM Tris-HCl, 137 mM NaCl, 0.05% Tween-20) at 4 °C overnight. Expression of bcl-2, p53, cdc-2, and cyclin B1 were probed with anti-bcl-2 monoclonal antibody (mAb, CALBIOCHEM, San Diego, CA), anti-p53 mAb (Santa Cruz Biotechnology, Inc.), anti-cdc-2 mAb and anti-cyclin B1 mAb (Oncogene Research Products, Cambridge, MA), respectively. After washing in TBST four times, the membrane was reacted with goat anti-mouse horseradish peroxidase (HRP). After washing in TBST and then in Tris-buffered saline (twice for each buffer and 10 min for each wash), the signals were detected by an enhanced chemiluminescence (ECL) system (Santa Cruz Biotechnology, Inc.) and preserved by exposure to X-film.

Human Tumor Models and Treatment. Nude mice (female, 5−6 weeks of age and 15−20 g in weight) were supplied by

Experimental Animal Center, Chinese Academy of Medical Sciences (Beijing, China) and used for human hepatocarcinoma and breast cancer (Bel-7402 and MCF-7) xenografts.

For the human hepatocarcinoma, nude mice received subcutaneous injection of BEL-7402 (5×10^6/mouse) on their backs. For the human breast cancer model, female nude mice received subcutaneous injection of estrogen (0.25 mg/mouse) 24 h before the tumor was implanted. MCF-7 (8×10^6/mouse) cells were then implanted (sc) on the backs of the mice. When the average tumor volume was in 90−120 mm^3 range, the tumor bearing mice were randomly divided into solvent control and treatment cages with five mice per group, followed by **11a** ip administration to the treatment group. The dose of **11a** was 100 mg/kg for MCF-7 tumor and 60 mg/kg for Bel-7402 tumor. The treatment was continued for 5−6 weeks q2d (every other day). The known drug control was adriamycin (1 mg/kg, ip, q2d). The therapeutic response to **11a** treatment was determined by measuring tumor volume. Two perpendicular tumor diameters, width and length, were obtained with calipers, followed by calculations using the formula, tumor volume = length × width^2 × 0.52. [28]

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Supporting Information Available: Element analysis data of new carbazole sulfonamide compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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