Expedited Articles

Discovery of Novel Antitumor Sulfonamides Targeting G1 Phase of the Cell Cycle

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Described herein is the discovery of a novel series of antitumor sulfonamides targeting G1 phase of the cell cycle. Cell cycle control in G1 phase has attracted considerable attention in recent cancer research, because many of the important proteins involved in G1 progression or G1/S transition have been found to play a crucial role in proliferation, differentiation, transformation, and programmed cell death (apoptosis). We previously reported our first antitumor sulfonamide E7010 as a novel tubulin polymerization inhibitor. Interestingly enough, continuous research on structurally related compounds led us to the finding of another class of antitumor sulfonamides that block cell cycle progression of P388 murine leukemia cells in G1 phase, but not in M phase. Of the compounds examined, *N*-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide (E7070) showed significant antitumor activity against HCT116 human colon carcinoma both in vitro (IC_{50} 0.11 μg/mL in cell proliferation assay) and in vivo (not only growth suppression but also a marked reduction of tumor size in nude mice). Because of its promising efficacy against human tumor xenografts and its unique mode of action, E7070 is currently undergoing phase I clinical trials in European countries.

Introduction

Malignant tumor cells are clearly distinguished from normal cells by their chaotic proliferation due to a serious disorder of the cell cycle regulatory machinery. Cell cycle inhibitors or modulators that halt uncontrollable tumor growth are regarded as highly promising new therapeutic agents against human cancers.[1] We have evaluated many synthetic sulfonamides with respect to the effect on cell cycle progression by flow cytometric analysis following cell growth inhibition assays. This approach produced our original antitumor agent E7010,[2] which was found to cause cell cycle arrest and apoptosis in M phase. E7010 was shown to inhibit microtubule assembly owing to its reversible binding to the colchicine binding site on tubulin[3] and to exhibit good in vivo antitumor activity against various rodent tumors and human tumor xenografts.[4]

A substantial endeavor in our laboratories has been directed to the synthesis of a large number of sulfonamides in order to construct our own compound library for novel chemotherapeutic agents against malignant solid tumors. E7010 is the first optimized drug candidate of the antitumor sulfonamides in this library. In the course of testing many of the sulfonamide collections with a structural relation, we also discovered *N*-(7-indolyl)benzenesulfonamide derivatives which had a distinctly different effect on cell cycle progression of P388 murine leukemia cells than the antimitotic agent E7010.[5] Namely, accumulation of cells in G1 phase was observed in a dose-dependent manner after 24-h drug exposure. Growing interest in these G1-targeting sulfonamides has resulted in the discovery of *N*-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide (E7070)[6] which demonstrated excellent in vivo efficacy against human tumor xenografts, e.g. HCT116 colon carcinoma.

G1 phase of the cell cycle is an important period where various complex signals interact to decide a cell's fate: proliferation, quiescence, differentiation, or apoptosis. It is now well-recognized that malfunctioning of cell cycle control in G1 phase is among the most critical molecular bases for tumorigenesis and tumor progression.[7] Thus, there appears to be a growing possibility that a small molecule targeting the control machinery in G1 phase can be a new type of drug efficacious against refractory clinical cancers. In fact, several "cytostatic" agents that affect G1 progression are currently under clinical investigation: for example, *n*-butyrate derivatives[8] and FR901228,[9] histone deacetylase inhibitors;[10] UCN-01,[11] a PKC-selective protein kinase antagonist; CC1-779,[12] a rapamycin derivative disrupting the FRAP (FKBP−rapamycin binding protein)[13] function; PS-341,[14] a 20S proteasome inhibitor;[15] a geldanamycin derivative[16] targeting the Hsp90 (heat shock protein-90) family of cellular chaperones;[17] TNP-470 (AGM-1470),[18] a fumagillin derivative showing antiangiogenic action through binding to MetAP-2 (methionine aminopeptidase-2).[19] This paper describes the discovery of novel G1-targeting antitumor sulfonamides distinct from mitotic arrest sulfonamides, including the design, synthesis, and structure−activity relationships.

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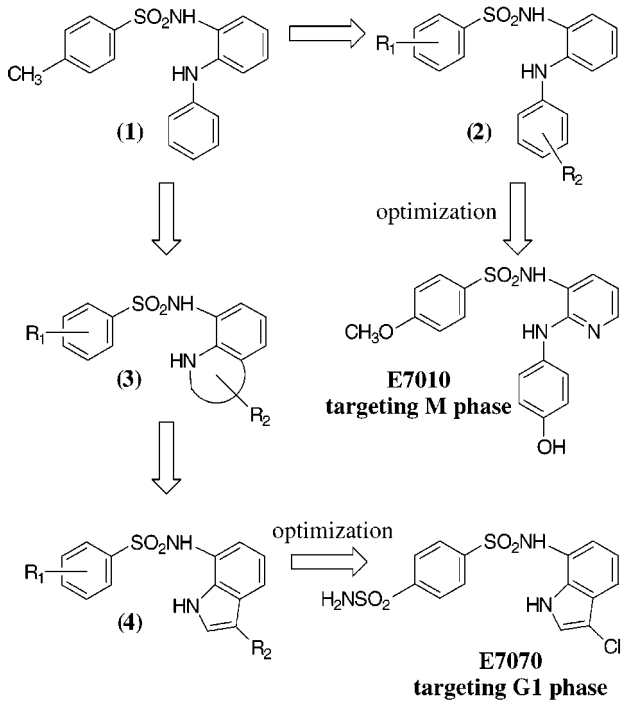
Chart 1. Drug Design



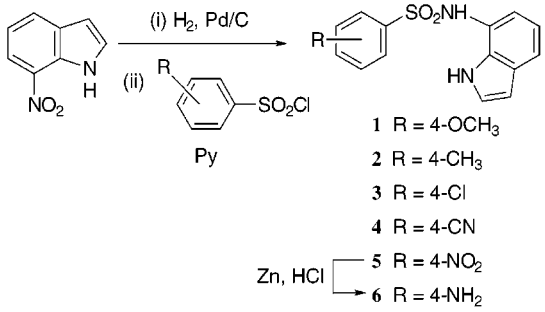
Design and Chemistry

We have focused on making a sulfonamide compound library based upon templates **2**−**4** in Chart 1 ever since compound **1**[2] was found to inhibit cellular growth and mitosis in vitro, but not to be quite potent in vivo. In the design of these templates, the sulfonamide moiety located between two aromatic rings was fixed as a basic motif, and the NH group at the *ortho* position of the sulfonamide was considered a key functionality to afford substantial antiproliferative activity in cell-based assays. The extensive research using template **2** yielded E7010 through the optimization process. Simultaneously, a heterobicyclic molecule was designed as an amine component of the sulfonamide, resulting in the syntheses on templates **3** and **4**. The series of sulfonamides **4** synthesized from 7-indolamines seemed to be of particular interest, because some simple derivatives differed from E7010 with respect to the in vitro antitumor properties such as tumor type selectivity and effect on cell cycle progression. The structure−activity relationship (SAR) study of E7010 and its analogues clarified that the R_1 substituent 4-methoxy was essential for significant antimitotic and antitumor activity.[20] In the present research, not only the 4-methoxy but several other substituents were also introduced onto the benzene ring of template **4** in order to examine the SAR of the new series and to explore the possibility of another potent antitumor sulfonamide. As a result, various *N*-(7-indolyl)benzenesulfonamide derivatives were systematically prepared for biological evaluations.

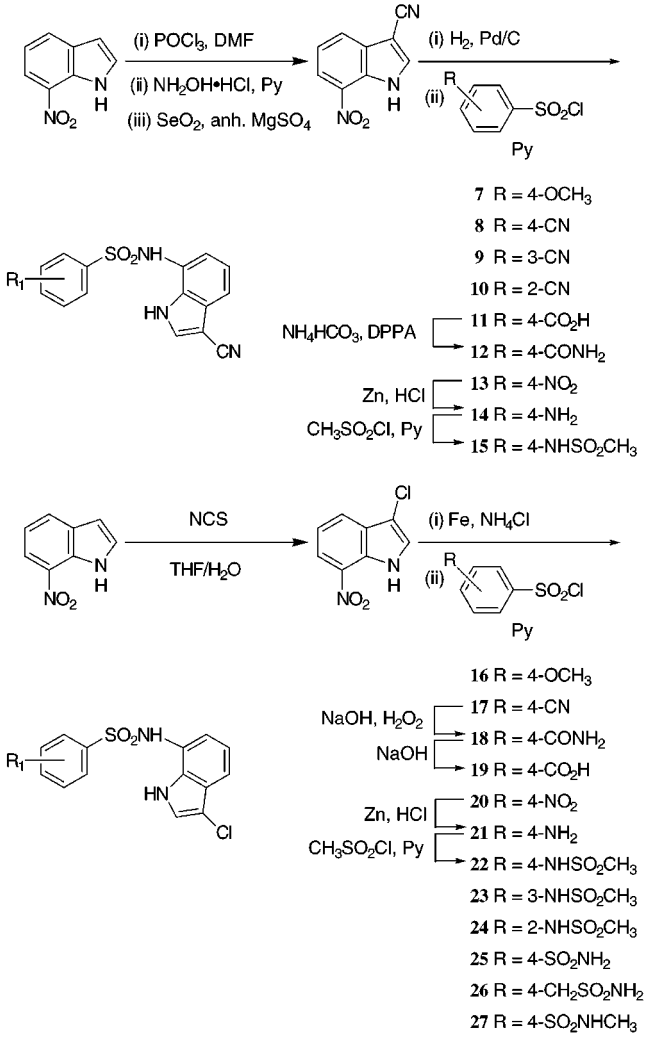
N-(7-Indolyl)benzenesulfonamides **1**−**6** were synthesized according to standard procedures shown in Scheme 1. It is well-known that the indole ring system readily undergoes electrophilic or oxidative reaction particularly at the 3-position. We thus planned to introduce a substituent cyano or chloro into the position to improve the molecular stability under physiological conditions.

The syntheses of *N*-(3-cyano- and 3-chloro-7-indolyl)-benzenesulfonamides **7**−**27** are outlined in Scheme 2.

Scheme 1

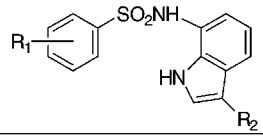


Scheme 2



7-Nitroindole[21] was converted to 3-cyano-7-nitroindole in a good yield by the following sequence of steps: (1) Vilsmeier reaction;[22] (2) condensation with hydroxylamine hydrochloride in the presence of pyridine; (3) dehydration with selenium dioxide and anhydrous magnesium sulfate.[23] The subsequent hydrogenation over 10% palladium on carbon yielded the corresponding amine, which was immediately coupled with several benzenesulfonyl chlorides in pyridine to afford **7**−**11** and **13**. Condensation of **11** with ammonium bicarbonate was achieved by using diphenyl phosphorazidate (DPPA) to afford **12**. Compound **13** was reduced with Zn/HCl to give amine **14**, which was further transformed to **15** through the condensation reaction with methanesulfonyl chloride. A chlorine atom was introduced into the

Table 1. In Vitro Antiproliferative Activity of *N*-(7-Indolyl)benzenesulfonamide Derivatives and E7010



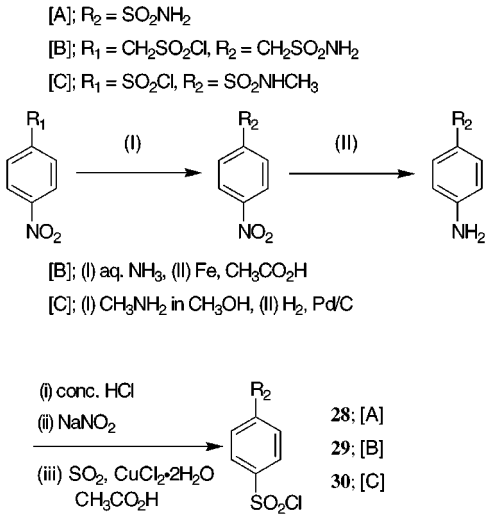
compound	R_1	R_2	mp, °C	formula[a]	IC_{50} (μg/mL)[b] KB	colon 38	P388
1	4-OCH_3	H	161-162	$C_{15}H_{14}N_2O_3S$	0.67	1.5	1.1
2	4-CH_3	H	157-159	$C_{15}H_{14}N_2O_2S$	2.2	2.4	4.3
3	4-Cl	H	163-164.5	$C_{14}H_{11}ClN_2O_2S$	6.0	7.0	6.0
4	4-CN	H	170-170.5	$C_{15}H_{11}N_3O_2S$	8.5	1.8	4.2
6	4-NH_2	H	227-228.5	$C_{14}H_{13}N_3O_2S$	36	6.1	14
7	4-OCH_3	CN	259-261 (dec.)	$C_{16}H_{13}N_3O_3S$	1.4	0.17	0.17
8	4-CN	CN	250.5-252	$C_{16}H_{10}N_4O_2S$	33	0.12	0.53
9	3-CN	CN	214-215	$C_{16}H_{10}N_4O_2S$	8.2	0.09	0.20
10	2-CN	CN	116.5-118.5	$C_{16}H_{10}N_4O_2S$	61	2.8	14
11	4-CO_2H	CN	275-276 (dec.)	$C_{16}H_{11}N_3O_4S$	>100	>100	>100
12	4-$CONH_2$	CN	291.5-293.5 (dec.)	$C_{16}H_{12}N_4O_3S$	15	0.68	2.5
14	4-NH_2	CN	252-255 (dec.)	$C_{15}H_{12}N_4O_2S$	4.4	0.28	0.72
15	4-$NHSO_2CH_3$	CN	258.5-259.5	$C_{16}H_{14}N_4O_4S_2$	15	1.2	3.5
16	4-OCH_3	Cl	170-171	$C_{15}H_{13}ClN_2O_3S$	0.55	0.20	0.76
17	4-CN	Cl	210-211	$C_{15}H_{10}ClN_3O_2S$	11	0.13	1.4
18	4-$CONH_2$	Cl	252.5-253.5 (dec.)	$C_{15}H_{12}ClN_3O_3S$	21	0.19	0.87
19	4-CO_2H	Cl	172-173 (dec.)	$C_{15}H_{11}ClN_2O_4S$	>100	>100	>100
21	4-NH_2	Cl	174.5-176	$C_{14}H_{12}ClN_3O_2S$	10	0.23	0.81
22	4-$NHSO_2CH_3$	Cl	213.5-214 (dec.)	$C_{15}H_{14}ClN_3O_4S_2$	12	0.26	1.6
23	3-$NHSO_2CH_3$	Cl	178-179	$C_{15}H_{14}ClN_3O_4S_2 \cdot 0.5H_2O$	26	1.5	4.8
24	2-$NHSO_2CH_3$	Cl	178-179	$C_{15}H_{14}ClN_3O_4S_2$	18	45	18
25 (E7070)	4-SO_2NH_2	Cl	242-243 (dec.)	$C_{14}H_{12}ClN_3O_4S_2$	4.4	0.10	0.45
26	4-$CH_2SO_2NH_2$	Cl	237-238.5 (dec.)	$C_{15}H_{14}ClN_3O_4S_2$	13	0.12	0.15
27	4-SO_2NHCH_3	Cl	244-246 (dec.)	$C_{15}H_{14}ClN_3O_4S_2$	15	0.24	0.90
E7010					0.29	0.38	0.32

[a] Analyses for C, H, and N were within ±0.4% of the expected values for the formula. [b] Concentration to inhibit cell proliferation by 50% relative to untreated controls after 72 h of continuous drug exposure.

3-position of 7-nitroindole using *N*-chlorosuccinimide (NCS). Reduction of 3-chloro-7-nitroindole with Fe/NH_4Cl gave the corresponding amine hydrochloride, which was then coupled with several benzenesulfonyl chlorides in pyridine to afford **16**, **17**, **20**, and **25**−**27**. The cyano group of **17** was hydrolyzed stepwise under alkaline conditions (NaOH−H_2O_2/DMSO and then NaOH/aqueous EtOH) to provide **18** and **19**, respectively. Nitro compound **20** was converted to amine **21** and further to methanesulfonamide **22** by standard reactions as described above. Similar synthetic transformations gave its regioisomers **23** (the 3-isomer) and **24** (the 2-isomer).

For the synthesis of **25**−**27**, sulfonyl chlorides **28**−**30** were prepared according to general procedures[24] as shown in Scheme 3.

Scheme 3



Pharmacological Results and Discussion

In Vitro Antiproliferative Activity. These new synthetics were initially screened for in vitro antiproliferative activity against KB human nasopharynx carcinoma, colon 38 murine adenocarcinoma, and P388 murine leukemia. After 3-day continuous drug exposure, the drug concentration required for 50% cell growth inhibition (IC_{50}) was determined by the MTT colorimetric assay.[25] All the data are presented in Table 1. Of the compounds listed in the top five rows, **1**−**3** showed almost flat activity against all three cell lines. Although they were less potent than E7010, the pattern of their activities looked similar to that of E7010. On the other hand, **4** and **6** displayed different tumor type selectivity with respect to their higher potency against colon 38 than KB and P388. The subsequent synthetic transformations of these two compounds to the 3-cyanoindole (R_2 = CN) and the 3-chloroindole (R_2 = Cl) derivatives led to a marked enhancement of antiproliferative activ-

ity and selectivity toward colon 38 (see **4** vs **8** and **17**, or **6** vs **14** and **21**).

According to the NCI anticancer drug screening program "COMPARE analysis",[26] a comparable in vitro antitumor spectrum is to be seen among the drugs sharing the same mode of action.[27] Thus we speculated that these new derivatives might operate, at least in part, by a different biological mechanism than tubulin polymerization inhibition with E7010 and that the R_2 substituents could be in a crucial situation for interacting with a putative cellular target other than tubulin. Conversion of the R_1 substituents 4-amino and 4-cyano into somewhat more polar functional groups generally retained the original antiproliferative profile (see **12**, **15**, **18**, **22**, and **25**−**27**). However, the activity of carboxylic acids **11** and **19** was completely lost. Compounds **7** and **16**, which have a 4-methoxy group as R_1 like E7010, showed less preference for colon 38 even with the R_2 substituents cyano and chloro, respectively, suggesting their potential antimitotic action. Finally, the potency of regioisomers, which differ from each other at the position of R_1, was examined with the substituents cyano and methanesulfonylamino, separately (see **8**−**10** and **22**−**24**). Every location of the cyano group provided the distinctive antiproliferative profile as described above, yet the positions 3 and 4 were more favorable for activity than the position 2. In the case of the methanesulfonylamino group, substitution at the 3- or 4-position gave the expected antiproliferative profile with a rank order of activity of the position 4 > the position 3. Moving the substituent to the 2-position, however, led to a significant decrease in activity. From these results, limitations on the size of the 2-substituent were evident. The 4-position seemed to be most tolerant of the steric bulk of substituents.

Flow Cytometric Analysis. To examine the effect of the new synthetics on cell cycle progression, flow cytometric analysis was performed. Colon 38 was unsuitable for the analysis due to its tight aggregation after trypsinization to collect the adherent cells, so that we employed P388 being less sensitive than colon 38 but still rather responsive to the test compounds. E7010 was used as a control drug of mitotic arrest agents. Figure 1 summarizes the results. As expected, compound **1**, whose pattern of antiproliferative activity was similar to that of E7010, caused G2/M arrest at a concentration of 0.80 μg/mL or more. Likewise, compounds **7** and **16**, with a 4-methoxy group as R_1, exhibited the G2/M arrest phenotype (data not shown). The mitotic arrest with these drugs was also confirmed by an explicit increase in the mitotic index under a microscope. These findings suggest that the 4-methoxy group is a pivotal functionality for binding to tubulin and for disrupting microtubule assembly in the present *N*-(7-indolyl)benzenesulfonamide series as well as in E7010.

By contrast, compounds **8**, **17**, **18**, **22**, and **25** (E7070), showing the aforesaid distinctive antiproliferative profile, were found to cause a decrease in the proportion of the cells in S phase and a clear G1 accumulation in a dose-dependent manner. Despite the cells being extinct using 100 μg/mL of each of the five compounds, no G2/M arrest was observed by drug treatment at 20 μg/mL. Other potent analogues (**9**, **12**, **15**, **23**, **26**, and **27**) with the same kind of antiproliferative profile induced a comparable cellular response (data not shown). Judging from the evident mitotic arrest with 0.16 μg/mL E7010, it is unlikely that tubulin is the primary protein target of these new synthetics; i.e. they should have another putative cellular target regulating cell cycle progression in G1 phase. Compound **21** caused G1 accumulation at 0.80 and 4.0 μg/mL but increased the proportion of the cells in G2/M phase at 20 μg/mL, suggesting that at least around 20 μg/mL of the drug, it may act as a bifunctional molecule to be separated from the other G1-selective compounds.

Inhibition of Tubulin Polymerization. To investigate whether the novel G1-targeting sulfonamides affect microtubule assembly, the inhibition of tubulin polymerization was tested. The following agents were assessed in the experiment: E7010, a positive control, and compounds **18**, **21**, and **25** (E7070), representatives of the G1-targeting sulfonamides. As shown in Figure 2, E7010 demonstrated strong inhibitory potency as reported previously.[3] The IC_{50} value was estimated to be 0.8 μg/mL. On the other hand, **18** and **25** (E7070) showed no significant activity, less than 10% inhibition even at a concentration of 100 μg/mL. Compound **21** was much less potent than E7010 but moderately suppressed microtubule assembly over 33.3 μg/mL, corresponding with the result of flow cytometric analysis. Given a reasonable correlation between the antiproliferative activity and the inhibitory potency against tubulin polymerization in the case of E7010, it can be concluded that **18**, **25** (E7070), and even **21** exhibit their antiproliferative activities via another biological mechanism than the inhibition of tubulin polymerization.

In Vivo Antitumor Activity. The compounds which caused G1 accumulation to P388 cells were then evaluated for in vivo antitumor activity against rodent and human tumors inoculated subcutaneously into mice. The T/C value was calculated as the ratio of tumor weight of the treated group to that of the control group. The NCI activity criteria provide that T/C values of ≤42 are judged moderately active and that T/C values of ≤10 indicate good activity.[28] For the in vivo first screening, we used the murine syngeneic tumor model with colon 38. Following subcutaneous (sc) implantation of the tumor on day 0, intraperitoneal (ip) drug administration was started on day 1 and continued for 4 days. Distinct from a model of ip-ip (ip tumor implantation and ip drug administration), this sc-ip model allows the assessment of systemic absorption and delivery of drug molecules to exhibit in vivo antitumor activity. As shown in Table 2, 3-cyanoindole and 3-chloroindole derivatives with the R_1 substituent 4-cyano, 4-carbamoyl, or 4-methanesulfonylamino were tested to compare their activities. Among the 3-cyanoindole series, only compound **8** demonstrated substantial in vivo efficacy, i.e. good activity at doses of 25 and 50 mg/kg. The results of the 3-chloroindole series provided a pronounced contrast: compounds **18** and **22**, at doses of 25 and 50 mg/kg, displayed moderate and good activity, respectively, whereas compound **17** was effective only at 50 mg/kg. These in vivo antitumor activities did not necessarily correlate with the in vitro antiproliferative activities of the identical compounds, suggesting that the pharmacokinetic properties of the test compounds (i.e. absorption, distribution, metabo-

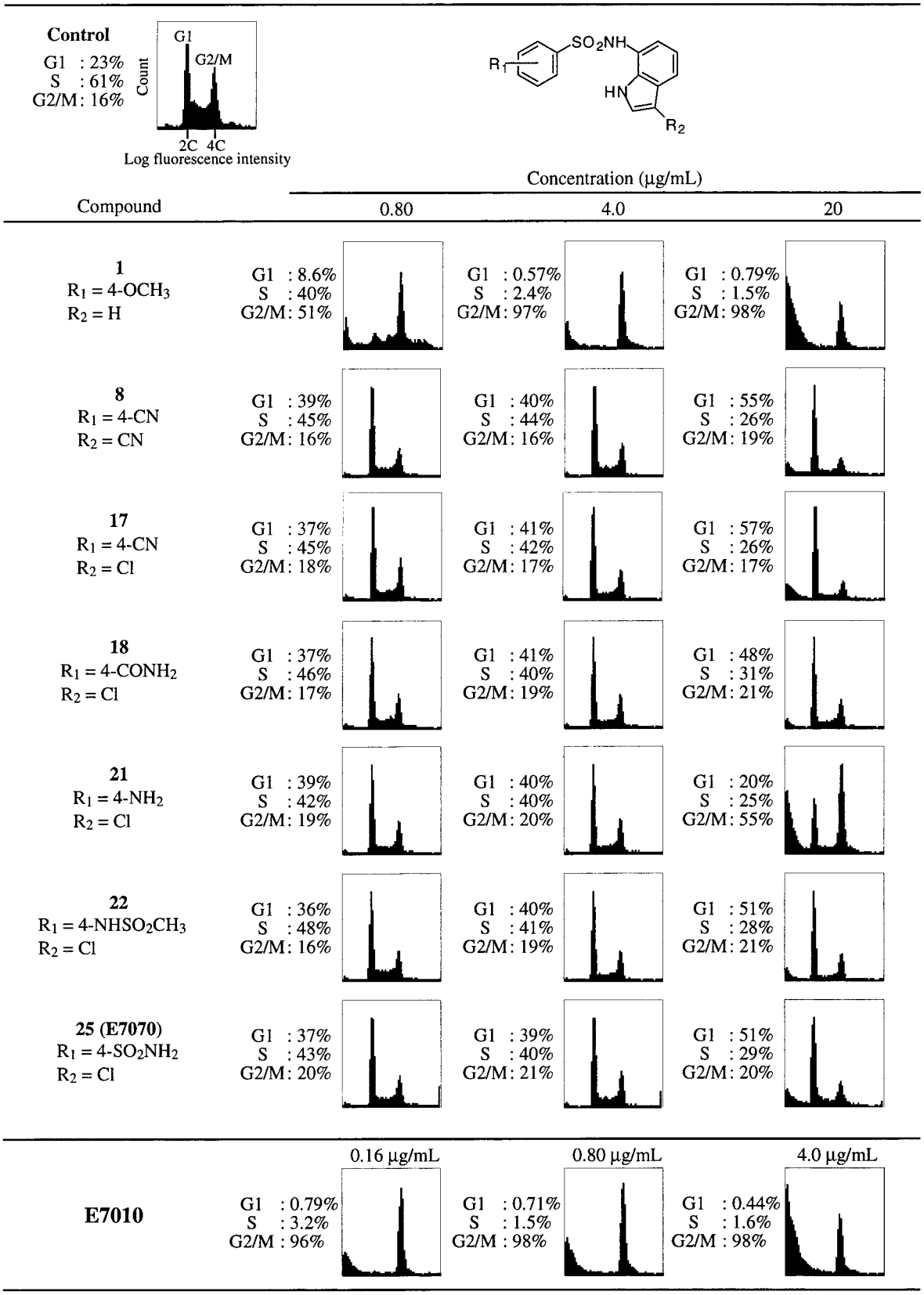


Figure 1. Flow cytometric analysis of several antitumor sulfonamides. Effect of selected compounds on cell cycle progression of P388 murine leukemia cells was examined according to the procedure described in the Experimental Section. The percentage of the cells in each phase was calculated using the ModFit LT software for a flow cytometer (FACSCalibur, Becton-Dickinson).

lism, and excretion) exerted a crucial influence on the in vivo efficacy in an unpredictable manner. The hopeful result of **22** led us to evaluate its regioisomer **23**. The subsequent experiment revealed that **23** was less efficacious against sc colon 38 than **22** as expected by comparing their in vitro activities. However, **23** proved almost equal to **17** and superior to **12** and **15** in the same in vivo screening model despite its in vitro activity

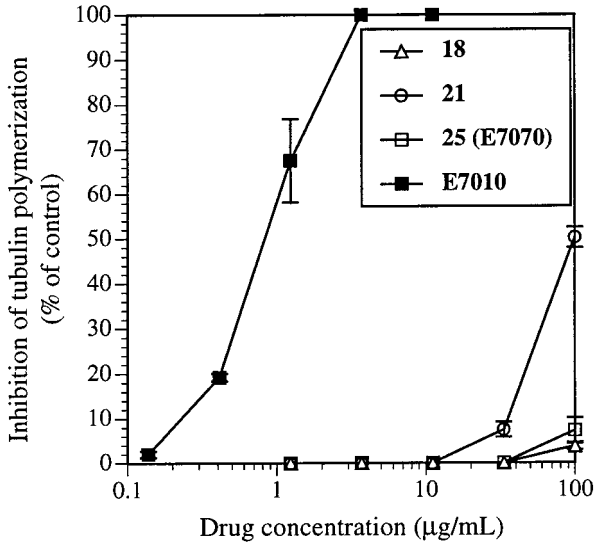


Figure 2. Inhibitory effect of selected antitumor sulfonamides on the polymerization of bovine brain tubulin. Assembly of microtubules was assessed turbidimetrically at 350 nm 30 min after the temperature had been switched from 4 to 37 °C. Polymerization in the absence of any drug gave the control readings. Values are means of four independent experiments ± SEM.

against colon 38 being inferior to those of all three compounds.

Since the data of **22** and **23** indicated a good combination of the sulfonamide moiety in R_1 with the R_2 substituent chloro, compounds **22** and **25**−**27** containing different sulfonamide moieties were screened together with **8**, **9**, and **18** in the human tumor xenograft model[29,30] using HCT116 colon carcinoma. HCT116 was sc implanted into athymic nude mice and allowed to grow to a size of about 100 mm^3, and then daily drug administration was performed for 4 days. The human tumor xenograft is regarded as one of the best test models for predicting drug efficacy in clinic.[31] To obtain good activity in the xenograft model is generally harder than in the syngeneic model, because test drugs are administered after the implanted tumor reaches a steady growth state at an axillary region of nude mice. The relative tumor volume (RTV) was calculated as the ratio of tumor volume on day 9 to that on day 1. The total in vivo antitumor activity was assessed in terms of T/C value and day 9-RTV. The relative body weight (RBW) was calculated as the ratio of body weight on day 5 to that on day 1, indicating the toxicity of each drug treatment to mice. Table 3 reveals that all the compounds diminished the initial tumor size without any significant body weight loss. It was difficult to determine the order of relative merits among active compounds **8**, **18**, and **22** in the previous syngeneic model with colon 38, whereas this xenograft model facilitated the ranking. Especially, **25** (E7070) demonstrated prominent in vivo efficacy: a marked reduction in tumor volume at doses of 25 and 50 mg/kg. It is noteworthy that intravenous (iv) administration of **25** (E7070) produced equal or superior efficacy to that of ip administration, implying the possible use of the clinical administration route. Sulfonamide **27** also exhibited notable in vivo efficacy, but a major metabolite of the compound in rats was found to be exactly **25** (E7070) on HPLC analysis. No correlation between in vitro and in vivo antitumor activity was observed as far as examined.

Further intensive investigations established the prominent antitumor activity of E7070 against several other human tumor xenografts, e.g. T/C = 2% and day 9-RTV = 0.24 with LS174T colon carcinoma, T/C = 11% and day 9-RTV = 0.27 with LX-1 small-cell lung carcinoma, T/C = 34% and day 9-RTV = 0.63 with SW620 colon carcinoma, T/C = 28% and day 9-RTV = 0.46 with HCT15 colon carcinoma, and T/C = 35% and day 9-RTV = 0.93 with PC9 non-small-cell lung carcinoma.[6b] All the data were taken after iv drug administration at 50 mg/kg daily for 4 days. The precise mechanism of action of E7070 still remains to be elucidated, but it seems clearly different from that of any widely used drug in clinic with respect to the G1-targeting fashion and the tumor type selectivity of the drug.[6c] In our experiments using HCT116, E7070 was shown to suppress the activation of CDK2 kinase and cyclin E expression which are required for a transition from G1 to S phase.[7] Because of its promising antitumor activity and its unique mode of action, E7070 is currently undergoing phase I clinical trials in European countries in collaboration with the European Organization for Research and Treatment of Cancer (EORTC).[32]

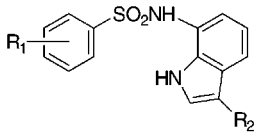
Experimental Section

General. All commercial solvents and reagents were used without further purification. Reactions were generally conducted under nitrogen atmosphere using magnetic stirring. Column chromatography was performed on silica gel (Merck, 230−400 mesh). TLC analyses were done on silica gel plates (Merck, Art. 5715). All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on a Varian UNITY 400 (400 MHz) spectrometer, and chemical shifts are expressed in δ (ppm) units from tetramethylsilane (TMS) as an internal standard. Abbreviations used in NMR analysis are as follows: br = broad, br s = broad singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, m = multiplet, q = quartet, s = singlet, t = triplet, td = triplet of doublets. Mass spectra (FABMS) were determined on a JEOL JMS-HX100 mass spectrometer. Analytical results indicated by element symbols were within ±0.4% of the theoretical values.

***N*-(7-Indolyl)-4-nitrobenzenesulfonamide (5).** A solution of 7-nitroindole[21] (1.83 g, 11.3 mmol) in MeOH (50 mL) was hydrogenated over 10% palladium on carbon (180 mg) under H_2 at 1 atm overnight. After the catalyst was filtered off, the filtrate was evaporated to give almost pure 7-aminoindole. The amine product was immediately dissolved in pyridine (30 mL) and then reacted with 4-nitrobenzenesulfonyl chloride (2.57 g, 11.6 mmol) at room temperature for 5 h. After the reaction mixture was concentrated in vacuo, the resulting residue was suspended in EtOAc and washed successively with 0.2 N aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (eluted with EtOAc−hexane) afforded **5** (3.50 g, 98%) as a yellow solid: mp 188−189 °C (after recrystallization from $CHCl_3$−hexane); ^{1}H NMR (DMSO-d_6) δ 6.42 (1H, dd, J = 2.8, 2.0 Hz), 6.66 (1H, d, J = 7.6 Hz), 6.83 (1H, dd, J = 8.0, 7.6 Hz), 7.31 (1H, dd, J = 3.2, 2.8 Hz), 7.36 (1H, d, J = 8.0 Hz), 7.94−8.02 (2H, m), 8.30−8.38 (2H, m), 10.23 (1H, s), 10.74−10.87 (1H, m); MS (FAB$^+$) *m/e* 317 (M^+). Anal. ($C_{14}H_{11}N_3O_4S$) C, H, N.

4-Amino-*N*-(7-indolyl)benzenesulfonamide (6). A solution of **5** (600 mg, 1.89 mmol) in MeOH (10 mL) was hydrogenated over 10% palladium on carbon (60 mg) under H_2 at 1 atm overnight. After the catalyst was filtered off, the

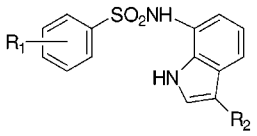
Table 2. In Vivo Antitumor Activity Against Colon 38 Murine Adenocarcinoma



compound	R_1	R_2	dose (mg/kg/day) ip[a], QDx4[b]	deaths/total	T/C[c] (%)
8	4-CN	CN	25	0/6	7
			50	0/6	10
12	4-$CONH_2$	CN	25	1/6	107
			50	0/6	84
15	4-$NHSO_2CH_3$	CN	25	1/6	110
			50	2/6	94
17	4-CN	Cl	25	0/6	98
			50	0/6	26
18	4-$CONH_2$	Cl	25	0/6	15
			50	0/6	14
22	4-$NHSO_2CH_3$	Cl	25	0/6	10
			50	0/6	2
23	3-$NHSO_2CH_3$	Cl	25	0/6	89
			50	0/6	35

[a] Intraperitoneal administration. [b] Daily for 4 days. [c] (Tumor weight of treated mice/tumor weight of control mice) × 100.

Table 3. In Vitro and in Vivo Antitumor Activity Against HCT116 Human Colon Carcinoma



compound	R_1	R_2	IC_{50} (μg/mL)	dose (mg/kg/day) ip, QDx4	deaths/total	Day 5-RBW[a]	Day 9-RTV[b]	T/C (%)
8	4-CN	CN	0.10	25	0/5	1.00	2.08	49
				50	0/5	1.02	0.42	31
				100	0/5	1.04	0.24	23
9	3-CN	CN	0.06	50	0/5	1.06	1.72	44
				100	0/5	1.07	0.86	34
18	4-$CONH_2$	Cl	0.09	25	0/5	1.03	4.90	87
				50	0/5	1.02	1.26	48
				100	0/5	1.03	0.46	20
22	4-$NHSO_2CH_3$	Cl	0.22	25	0/5	0.99	2.32	63
				50	0/5	1.01	0.24	24
				100	0/5	1.02	0.05	3
25 (E7070)	4-SO_2NH_2	Cl	0.10	12.5	0/5	1.00	1.50	45
				25	0/5	0.99	0.43	22
				50	0/5	0.95	0.10	12
				100	5/5	0.78	-	-
				12.5, iv[c]	0/5	1.02	0.81	34
				25, iv[c]	0/5	1.00	0.44	23
				50, iv[c]	0/5	0.93	0.15	8
26	4-$CH_2SO_2NH_2$	Cl	0.15	25	0/5	1.01	0.97	52
				50	0/5	1.01	0.28	29
				100	0/5	0.99	0.17	16
27	4-SO_2NHCH_3	Cl	0.11	50	0/5	1.07	0.44	12
				100	0/5	0.96	0.01	6

[a] (Body weight of mice on day 5)/(body weight of mice on day 1). [b] (Tumor volume on day 9)/(tumor volume on day 1). [c] Intravenous administration.

filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (eluted with EtOAc−hexane) and afforded **6** (494 mg, 91%) as a colorless solid: mp 227−228.5 °C (after recrystallization from EtOAc−hexane); ^1H NMR (DMSO-d_6) δ 5.93 (2H, s), 6.38 (1H, dd, J = 2.8, 1.8 Hz), 6.44−6.51 (2H, m), 6.809 (1H, d, J = 3.2 Hz), 6.812 (1H, d, J

= 5.6 Hz), 7.24 (1H, dd, J = 5.6, 3.2 Hz), 7.29 (1H, dd, J = 2.8, 2.4 Hz), 7.34−7.41 (2H, m), 9.48 (1H, s), 10.66 (1H, s); MS (FAB$^+$) *m/e* 287 (M$^+$). Anal. ($C_{14}H_{13}N_3O_2S$) C, H, N.

Compounds **1**−**4** were synthesized as described for **5** by using the appropriate sulfonyl chlorides.

***N*-(7-Indolyl)-4-methoxybenzenesulfonamide (1).** Obtained as a colorless solid in 95% yield: mp 161−162 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 3.766 and 3.772 (total 3H, s × 2), 6.36−6.44 (1H, m), 6.76 (1H, d, J = 7.7 Hz), 6.82 (1H, td, J = 7.7, 2.2 Hz), 6.97−7.08 (2H, m), 7.26−7.36 (1H, m), 7.28 (1H, d, J = 7.7 Hz), 7.64−7.74 (2H, m), 9.80 (1H, s), 10.72 (1H, br s); MS (FAB$^+$) *m/e* 302 (M$^+$). Anal. ($C_{15}H_{14}N_2O_3S$) C, H, N.

***N*-(7-Indolyl)-4-methylbenzenesulfonamide (2).** Obtained as a colorless solid in 96% yield: mp 157−159 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 6.39 (1H, dd, J = 3.1, 1.8 Hz), 6.75 (1H, dd, J = 7.7, 0.92 Hz), 6.81 (1H, t, J = 7.7 Hz), 7.26−7.32 (4H, m), 7.60−7.66 (2H, m), 9.84 (1H, s), 10.68−10.75 (1H, m); MS (FAB$^+$) *m/e* 286 (M$^+$). Anal. ($C_{15}H_{14}N_2O_2S$) C, H, N.

4-Chloro-*N*-(7-indolyl)benzenesulfonamide (3). Obtained as a colorless solid in 93% yield: mp 163−164.5 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.41 (1H, d, J = 2.1 Hz), 6.70 (1H, d, J = 7.8 Hz), 6.83 (1H, t, J = 7.8 Hz), 7.31 (1H, d, J = 2.1 Hz), 7.33 (1H, d, J = 7.8 Hz), 7.55−7.65 (2H, m), 7.68−7.78 (2H, m), 10.01 (1H, s), 10.75 (1H, s); MS (FAB$^+$) *m/e* 307 (MH$^+$). Anal. ($C_{14}H_{11}ClN_2O_2S$) C, H, N.

4-Cyano-*N*-(7-indolyl)benzenesulfonamide (4). Obtained as a colorless solid in 93% yield: mp 170−170.5 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.41 (1H, dd, J = 2.8, 2.0 Hz), 6.65 (1H, dd, J = 7.5, 0.55 Hz), 6.83 (1H, dd, J = 8.0, 7.5 Hz), 7.31 (1H, t, J = 2.8 Hz), 7.35 (1H, d, J = 8.0 Hz), 7.84−7.91 (2H, m), 7.98−8.04 (2H, m), 10.16 (1H, s), 10.72−10.84 (1H, m); MS (FAB$^+$) *m/e* 297 (M$^+$). Anal. ($C_{15}H_{11}N_3O_2S$) C, H, N.

3-Cyano-7-nitroindole. 7-Nitroindole-3-carboxaldehyde[22] (10.2 g, 53.6 mmol) was dissolved in DMF (150 mL), and then hydroxylamine hydrochloride (3.93 g, 56.6 mmol) and pyridine (4.5 mL, 55.6 mmol) were added to the solution. The obtained mixture was stirred at 80 °C for 2 h, followed by the addition of SeO_2 (6.30 g, 56.8 mmol) and anhydrous $MgSO_4$ (ca. 5 g). After stirring at 80 °C for 2.5 h, the reaction mixture was filtered to remove insolubles and concentrated in vacuo. H_2O was added to the residue to precipitate solid materials. The crude product was collected by filtration, washed with H_2O, and purified by flash chromatography (eluted with EtOAc−hexane) to give the title compound (8.61 g, 86%) as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 7.48 (1H, t, J = 8.1 Hz), 8.17 (1H, d, J = 8.1 Hz), 8.27 (1H, d, J = 8.1 Hz), 8.47 (1H, s), 12.70−13.00 (1H, br); MS (FAB$^+$) *m/e* 188 (MH$^+$).

Compounds **7**−**11** and **13** were prepared by the procedure described for **5**, proceeding from 3-cyano-7-nitroindole instead of 7-nitroindole.

***N*-(3-Cyano-7-indolyl)-4-methoxybenzenesulfonamide (7).** Obtained as a colorless solid in 90% yield: mp 259−261 °C dec (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 3.79 (3H, s), 6.80 (1H, dd, J = 7.6, 2.4 Hz), 7.03 (2H, d, J = 8.8 Hz), 7.07 (1H, dd, J = 8.0, 7.6 Hz), 7.40 (1H, d, J = 8.0 Hz), 7.64 (2H, d, J = 8.8 Hz), 8.20 (1H, dd, J = 2.4, 1.2 Hz), 9.96 (1H, s), 11.82−11.94 (1H, m); MS (FAB$^+$) *m/e* 327 (M$^+$). Anal. ($C_{16}H_{13}N_3O_3S$) C, H, N.

4-Cyano-*N*-(3-cyano-7-indolyl)benzenesulfonamide (8). Obtained as a colorless solid in 91% yield: mp 250.5−252 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.67 (1H, d, J = 7.7 Hz), 7.05 (1H, t, J = 7.9 Hz), 7.44 (1H, d, J = 7.7 Hz), 7.78−7.87 (2H, m), 7.97−8.05 (2H, m), 8.16−8.23 (1H, m), 10.28−10.43 (1H, br), 11.92−12.09 (1H, m); MS (FAB$^+$) *m/e* 322 (M$^+$). Anal. ($C_{16}H_{10}N_4O_2S$) C, H, N.

3-Cyano-*N*-(3-cyano-7-indolyl)benzenesulfonamide (9). Obtained as a colorless solid in 88% yield: mp 214−215 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.71 (1H, d, J = 7.6 Hz), 7.09 (1H, dd, J = 8.0, 7.6 Hz), 7.49 (1H, d, J = 8.0 Hz), 7.74 (1H, dd, J = 8.0, 7.6 Hz), 7.94 (1H, d, J = 8.0 Hz), 8.11−8.14 (2H, m), 8.23 (1H, d, J = 2.8 Hz), 10.30 (1H, br s), 12.05 (1H, br s); MS (FAB$^+$) *m/e* 322 (M$^+$). Anal. ($C_{16}H_{10}N_4O_2S$) C, H, N.

2-Cyano-*N*-(3-cyano-7-indolyl)benzenesulfonamide (10). Obtained as a colorless solid in 35% yield: mp 116.5−118.5 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.65 (1H, d, J = 7.7 Hz), 7.05 (1H, dd, J = 7.9, 7.7 Hz), 7.44 (1H, d, J = 7.9 Hz), 7.81 (1H, ddd, J = 7.5, 7,3, 1.1 Hz), 7.87 (1H, ddd, J = 7.9, 7.5, 1.1 Hz), 7.96 (1H, dd, J = 7.9, 1.1 Hz), 8.03 (1H, dd, J = 7.3, 1.1 Hz), 8.18−8.25 (1H, m), 10.56 (1H, br s), 12.01 (1H, br s); MS (FAB$^+$) *m/e* 322 (M$^+$). Anal. ($C_{16}H_{10}N_4O_2S$) C, H, N.

4-[*N*-(3-Cyano-7-indolyl)sulfamoyl]benzoic Acid (11). Obtained as a colorless solid in 82% yield: mp 275−276 °C dec (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.71 (1H, d, J = 7.6 Hz), 7.06 (1H, dd, J = 8.0, 7.6 Hz), 7.44 (1H, d, J = 8.0 Hz), 7.78−7.84 (2H, m), 8.02−8.04 (2H, m), 8.21 (1H, d, J = 2.7 Hz), 11.96−12.06 (1H, m); MS (FAB$^+$) *m/e* 341 (M$^+$). Anal. ($C_{16}H_{11}N_3O_4S$) C, H, N.

***N*-(3-Cyano-7-indolyl)-4-nitrobenzenesulfonamide (13).** Obtained as a pale-yellow solid in 90% yield: mp 229−232 °C dec (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.70 (1H, d, J = 7.7 Hz), 7.07 (1H, dd, J = 7.9, 7.7 Hz), 7.47 (1H, d, J = 7.9 Hz), 7.91−7.98 (2H, m), 8.22 (1H, d, J = 2.9 Hz), 8.32−8.39 (2H, m), 10.44 (1H, s), 12.05 (1H, s); MS (FAB$^+$) *m/e* 342 (M$^+$). Anal. ($C_{15}H_{10}N_4O_4S$) C, H, N.

4-Carbamoyl-*N*-(3-cyano-7-indolyl)benzenesulfonamide (12). To a mixture of **11** (450 mg, 1.32 mmol), NH_4HCO_3 (209 mg, 2.63 mmol), and Et_3N (370 μL, 2.65 mmol) in DMSO (4.5 mL) was added DPPA (diphenyl phosphorazidate; 340 μL, 1.58 mmol). After stirring at room temperature overnight, the reaction mixture was diluted with EtOAc and washed successively with 0.2 N aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash chromatography (eluted with EtOAc−hexane) afforded **12** (354 mg, 79%) as a colorless solid: mp 291.5−293.5 °C dec (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 6.71 (1H, d, J = 7.9 Hz), 7.04 (1H, t, J = 7.9 Hz), 7.42 (1H, d, J = 7.9 Hz), 7.59 (1H, s), 7.75 (2H, d, J = 8.2 Hz), 7.94 (2H, d, J = 8.2 Hz), 8.12 (1H, s), 8.19 (1H, s), 10.20 (1H, s), 11.96 (1H, s); MS (FAB$^+$) *m/e* 340 (M$^+$). Anal. ($C_{16}H_{12}N_4O_3S$) C, H, N.

4-Amino-*N*-(3-cyano-7-indolyl)benzenesulfonamide (14). To a solution of **13** (3.77 g, 11.0 mmol) in MeOH (100 mL) and concentrated aqueous HCl (5 mL) was added portionwise Zn powder (2.20 g, 33.6 mmol). The mixture was heated under reflux for 30 min and then cooled, neutralized with a large excess of $NaHCO_3$, and filtered to remove insolubles. After the filtrate was concentrated in vacuo, the resulting residue was suspended in EtOAc and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (eluted with EtOAc−hexane) afforded **14** (3.02 g, 88%) as a colorless solid: mp 252−255 °C dec (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 6.05 (2H, s), 6.51−6.58 (2H, m), 6.93 (1H, d, J = 8.0 Hz), 7.13 (1H, dd, J = 8.0, 7.6 Hz), 7.36−7.43 (2H, m), 7.41 (1H, d, J = 7.6 Hz), 8.23 (1H, d, J = 3.2 Hz), 9.73 (1H, s), 11.79−11.91 (1H, m); MS (FAB$^+$) *m/e* 312 (M$^+$). Anal. ($C_{15}H_{12}N_4O_2S$) C, H, N.

***N*-(3-Cyano-7-indolyl)-4-(methanesulfonamido)benzenesulfonamide (15).** To a solution of **14** (625 mg, 2.00 mmol) in pyridine (10 mL) was added dropwise methanesulfonyl chloride (155 μL, 2.00 mmol). After stirring at room temperature overnight, the reaction mixture was concentrated in vacuo. The obtained residue was diluted with EtOAc and washed successively with 0.2 N aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (eluted with EtOH−hexane) afforded **15** (718 mg, 92%) as a colorless solid: mp 258.5−259.5 °C (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 3.09 (3H, s), 6.81 (1H, d, J =

7.8 Hz), 7.08 (1H, dd, J = 8.0, 7.8 Hz), 7.21−7.28 (2H, m), 7.41 (1H, d, J = 8.0 Hz), 7.63−7.70 (2H, m), 8.19 (1H, d, J = 2.4 Hz), 9.90−10.30 (1H, br), 11.81−11.94 (1H, m); MS (FAB$^+$) *m/e* 391 (MH^+). Anal. ($C_{16}H_{14}N_4O_4S_2$) C, H, N.

3-Chloro-7-nitroindole. 7-Nitroindole[21] (15.0 g, 92.5 mmol) was dissolved in THF (120 mL) and 0.5 N aqueous HCl (0.12 mL), and then *N*-chlorosuccinimide (12.6 g, 94.4 mmol) was added portionwise to the solution. The mixture was stirred at room temperature for 5 h, followed by the addition of H_2O (400 mL). The resulting crystalline precipitates were collected by filtration, washed successively with H_2O, MeOH−H_2O (1:1), and isopropyl ether, and finally dried in vacuo to give the title compound (16.9 g, 93%) as a yellow crystalline powder: ^{1}H NMR (DMSO-d_6) δ 7.36 (1H, t, J = 8.0 Hz), 7.73 (1H, s), 8.02 (1H, d, J = 8.0 Hz), 8.21 (1H, d, J = 8.0 Hz), 12.15 (1H, br s); MS (FAB$^+$) *m/e* 197 (MH^+).

4-(Sulfamoylmethyl)benzenesulfonyl Chloride (29). A suspension of 4-nitrophenylmethanesulfonamide[33] (5.00 g, 23.1 mmol) in 90% acetic acid was hydrogenated over 10% palladium on carbon (500 mg) under H_2 at 1 atm overnight. The reaction mixture was filtered to remove the catalyst and the filtrate was concentrated to dryness to give almost pure 4-aminophenylmethanesulfonamide (4.30 g). This amine product was directly added to a mixture comprising H_2O (40 mL) and concentrated aqueous HCl (4.1 mL). The obtained mixture was stirred, followed by the dropwise addition of a saturated aqueous solution of $NaNO_2$ (1.63 g, 23.6 mmol) at 0 °C or below. The reaction mixture was added to an acetic acid solution with SO_2 (prepared by saturating 30 mL of acetic acid with SO_2 and adding 0.97 g of $CuCl_2$ dihydrate to the solution) under cooling with ice and stirring. After stirring at room temperature for 40 min, the whole was poured onto ice−water and then extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give **29** (2.02 g, 32% overall yield) as a pale-yellow solid: ^{1}H NMR (DMSO-d_6) δ 4.26 (2H, s), 7.32 (2H, d, J = 8.4 Hz), 7.59 (2H, d, J = 8.4 Hz); MS (FAB$^+$) *m/e* 270 (MH^+).

Sulfonyl chlorides **28** and **30** were prepared in a manner similar to that for **29**.

4-Sulfamoylbenzenesulfonyl Chloride (28).[24] Prepared from sulfanilamide and obtained as a pale-yellow solid in 66% yield: R_f 0.50 (1:1 EtOAc/hexane); MS (FAB$^+$) *m/e* 256 (MH^+).

4-(*N*-Methylsulfamoyl)benzenesulfonyl Chloride (30). Prepared from 4-nitrobenzenesulfonyl chloride and obtained as a pale-yellow solid in 74% overall yield: R_f 0.55 (1:1 EtOAc/hexane); MS (FAB$^+$) *m/e* 270 (MH^+).

***N*-(3-Chloro-7-indolyl)-1,4-benzenedisulfonamide (25, E7070).** To a solution of 3-chloro-7-nitroindole (3.00 g, 15.3 mmol) in 2-propanol (45 mL) were added Fe powder (2.56 g, 45.8 mmol) and an aqueous solution (9 mL) of NH_4Cl (163 mg, 3.05 mmol). After stirring at 60 °C for 2 h, activated charcoal (ca. 1 g) was added to the reaction mixture. The insoluble materials were filtered off and washed with EtOAc (50 mL), followed by the immediate addition of sulfonyl chloride **28** (4.10 g, 16.0 mmol) and pyridine (3.7 mL, 45.8 mmol). The reaction mixture was stirred at room temperature for 3 h, diluted with EtOAc, and washed successively with 1 N aqueous HCl, H_2O, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (eluted with EtOAc−hexane) afforded **25** (5.30 g, 90%) as a colorless solid: mp 242−243 °C dec (after recrystallization from EtOH−H_2O); ^{1}H NMR (DMSO-d_6) δ 6.75 (1H, d, J = 7.6 Hz), 6.96 (1H, dd, J = 8.0, 7.6 Hz), 7.29 (1H, d, J = 7.6 Hz), 7.50 (1H, d, J = 2.8 Hz), 7.58 (2H, s), 7.90−7.98 (4H, m), 10.23 (1H, s), 11.07−11.17 (1H, m); MS (FAB$^+$) *m/e* 385 (M^+). Anal. ($C_{14}H_{12}ClN_3O_4S_2$) C, H, N.

Compounds **16**, **17**, **20**, **26**, and **27** were synthesized as described for 2**5** by using the appropriate sulfonyl chlorides.

***N*-(3-Chloro-7-indolyl)-4-methoxybenzenesulfonamide (16).** Obtained as a colorless solid in 93% yield: mp 170−171 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 3.78 (3H, s), 6.83 (1H, d, J = 7.6 Hz), 6.95 (1H, dd, J = 8.0, 7.6 Hz), 6.98−7.05 (2H, m), 7.23 (1H, d, J = 8.0 Hz), 7.47 (1H, d, J = 2.7 Hz), 7.62−7.70 (2H, m), 9.84 (1H, s), 10.93−11.03 (1H, m); MS (FAB$^+$) *m/e* 336 (M^+). Anal. ($C_{15}H_{13}ClN_2O_3S$) C, H, N.

***N*-(3-Chloro-7-indolyl)-4-cyanobenzenesulfonamide (17).** Obtained as a colorless solid in 90% yield: mp 210−211 °C (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.71 (1H, dd, J = 7.6, 0.80 Hz), 6.96 (1H, dd, J = 8.0, 7.6 Hz), 7.30 (1H, d, J = 8.0 Hz), 7.48 (1H, dd, J = 2.4, 0.80 Hz), 7.82−7.90 (2H, m), 7.97−8.05 (2H, m), 10.25 (1H, s), 11.04−11.15 (1H, m); MS (FAB$^+$) *m/e* 331 (M^+). Anal. ($C_{15}H_{10}ClN_3O_2S$) C, H, N.

***N*-(3-Chloro-7-indolyl)-4-nitrobenzenesulfonamide (20).** Obtained as a yellow solid in 84% yield: mp 199.5−200.5 °C (after recrystallization from $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 6.72 (1H, d, J = 7.6 Hz), 6.96 (1H, dd, J = 8.0, 7.6 Hz), 7.31 (1H, d, J = 8.0 Hz), 7.47−7.53 (1H, m), 7.92−8.02 (2H, m), 8.30−8.41 (2H, m), 10.33 (1H, s), 11.07−11.22 (1H, m); MS (FAB$^+$) *m/e* 351 (M^+). Anal. ($C_{14}H_{10}ClN_3O_4S$) C, H, N.

***N*-(3-Chloro-7-indolyl)-4-(sulfamoylmethyl)benzenesulfonamide (26).** Obtained as a colorless solid in 85% yield: mp 237−238.5 °C dec (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 4.33 (2H, s), 6.84 (1H, dd, J = 7.7, 0.73 Hz), 6.93 (2H, s), 6.92−6.97 (1H, m), 7.24 (1H, dd, J = 7.9, 0.37 Hz), 7.48 (1H, d, J = 2.7 Hz), 7.48−7.52 (2H, m), 7.75−7.79 (2H, m), 10.08 (1H, br s), 11.04 (1H, br s); MS (FAB$^+$) *m/e* 399 (M^+). Anal. ($C_{15}H_{14}ClN_3O_4S_2$) C, H, N.

***N*-(3-Chloro-7-indolyl)-4-(*N*-methylsulfamoyl)benzenesulfonamide (27).** Obtained as a colorless solid in 92% yield: mp 244−246 °C dec (after recrystallization from acetone−H_2O); ^{1}H NMR (DMSO-d_6) δ 2.39 (3H, d, J = 4.9 Hz), 6.71 (1H, d, J = 7.7 Hz), 6.96 (1H, dd, J = 7.9, 7.7 Hz), 7.30 (1H, d, J = 7.9 Hz), 7.47 (1H, d, J = 2.7 Hz), 7.67 (1H, q, J = 4.9 Hz), 7.86−7.94 (4H, m), 10.19 (1H, s), 11.02−11.10 (1H, m); MS (FAB$^+$) *m/e* 399 (M^+). Anal. ($C_{15}H_{14}ClN_3O_4S_2$) C, H, N.

4-Carbamoyl-*N*-(3-chloro-7-indolyl)benzenesulfonamide (18). To a solution of **17** (1.00 g, 3.01 mmol) in EtOH (9.6 mL) were added aqueous H_2O_2 (30%, 3.6 mL) and 6 N aqueous NaOH (360 μL). The obtained mixture was stirred at 50 °C for 1 h, acidified with 0.2 N aqueous HCl, and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (eluted with EtOAc−hexane) afforded **18** (720 mg, 68%) as a colorless solid: mp 252.5−253.5 °C dec (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 6.76 (1H, d, J = 7.5 Hz), 6.95 (1H, dd, J = 8.1, 7.5 Hz), 7.27 (1H, d, J = 8.1 Hz), 7.49 (1H, d, J = 2.6 Hz), 7.59 (1H, br s), 7.76−7.83 (2H, m), 7.91−7.98 (2H, m), 8.12 (1H, br s), 10.10 (1H, s), 11.01−11.12 (1H, m); MS (FAB$^+$) *m/e* 349 (M^+). Anal. ($C_{15}H_{12}ClN_3O_3S$) C, H, N.

4-[*N*-(3-Chloro-7-indolyl)sulfamoyl]benzoic Acid (19). To a solution of **18** (500 mg, 1.43 mmol) in EtOH (7.2 mL) was added 6 N aqueous NaOH (720 μL). The obtained mixture was stirred under reflux for 2 h and then cooled, acidified with 0.2 N aqueous HCl, and extracted with EtOAc. The organic layer was washed with H_2O, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give **19** (440 mg, 88%) as a colorless solid: mp 172−173 °C dec (after recrystallization from EtOAc−hexane); ^{1}H NMR (DMSO-d_6) δ 6.74 (1H, d, J = 7.7 Hz), 6.95 (1H, dd, J = 8.0, 7.7 Hz), 7.28 (1H, d, J = 8.0 Hz), 7.49 (1H, d, J = 2.7 Hz), 7.84 (2H, d, J = 8.4 Hz), 8.04 (2H, d, J = 8.4 Hz), 10.16 (1H, br s), 11.07 (1H, s), 13.45 (1H, br s); MS (FAB$^+$) *m/e* 350 (M^+). Anal. ($C_{15}H_{11}ClN_2O_4S$) C, H, N.

4-Amino-*N*-(3-chloro-7-indolyl)benzenesulfonamide (21). Compound **20** was treated according to the same procedure described in the preparation of **14** to afford **21** as a colorless solid in 95% yield: mp 174.5−176 °C (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 5.97 (2H, br s), 6.48 (2H, d, J = 8.8 Hz), 6.88 (1H, d, J = 7.6 Hz), 6.95 (1H, dd, J = 8.0, 7.6 Hz), 7.19 (1H, d, J = 8.0 Hz), 7.36 (2H, d, J = 8.8 Hz), 7.46 (1H, d, J = 2.4 Hz), 9.56 (1H, s),

10.86−10.98 (1H, m); MS (FAB$^+$) *m/e* 321 (M^+). Anal. ($C_{14}H_{12}ClN_3O_2S$) C, H, N.

***N*-(3-Chloro-7-indolyl)-4-(methanesulfonamido)benzenesulfonamide (22).** Compound **21** was treated according to the same procedure described for the preparation of **15** to afford **22** as a colorless solid in 90% yield: mp 213.5−214 °C dec (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 3.08 (3H, s), 6.83 (1H, d, J = 7.7 Hz), 6.96 (1H, dd, J = 7.9, 7.7 Hz), 7.23 (2H, d, J = 8.8 Hz), 7.24 (1H, d, J = 7.9 Hz), 7.47 (1H, d, J = 2.7 Hz), 7.68 (2H, d, J = 8.8 Hz), 9.92 (1H, s), 10.38 (1H, s), 10.99 (1H, s); MS (FAB$^+$) *m/e* 399 (M^+). Anal. ($C_{15}H_{14}ClN_3O_4S_2$) C, H, N.

Compounds **23** and **24** were prepared analogously, proceeding from 3- and 2-nitrobenzenesulfonyl chlorides, respectively.

***N*-(3-Chloro-7-indolyl)-3-(methanesulfonamido)benzenesulfonamide (23).** Obtained as a colorless solid in 82% overall yield: mp 178−179 °C (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 2.90 (3H, s), 6.80 (1H, d, J = 7.6 Hz), 6.96 (1H, dd, J = 8.0, 7.6 Hz), 7.26 (1H, d, J = 8.0 Hz), 7.35 (1H, ddd, J = 7.6, 2.0, 1.6 Hz), 7.42 (1H, ddd, J = 8.0, 2.0, 1.6 Hz), 7.47 (1H, dd, J = 8.0, 7.6 Hz), 7.48 (1H, d, J = 2.8 Hz), 7.59 (1H, t, J = 1.6 Hz), 10.07 (1H, s), 10.96−11.03 (1H, m); MS (FAB$^+$) *m/e* 399 (M^+). Anal. ($C_{15}H_{14}ClN_3O_4S_2 \cdot 0.5H_2O$) C, H, N.

***N*-(3-Chloro-7-indolyl)-2-(methanesulfonamido)benzenesulfonamide (24).** Obtained as a colorless solid in 77% overall yield: mp 178−179 °C (after recrystallization from EtOH−hexane); ^{1}H NMR (DMSO-d_6) δ 3.07 (3H, s), 6.63 (1H, d, J = 7.5 Hz), 6.94 (1H, dd, J = 7.9, 7.5 Hz), 7.17−7.24 (1H, m), 7.32 (1H, d, J = 7.9 Hz), 7.52 (1H, d, J = 2.6 Hz), 7.59−7.70 (3H, m), 8.81 (1H, s), 10.37 (1H, s), 11.16 (1H, s); MS (FAB$^+$) *m/e* 399 (M^+). Anal. ($C_{15}H_{14}ClN_3O_4S_2$) C, H, N.

Cancer Cell Lines. Colon 38 murine adenocarcinoma and P388 murine leukemia were supplied by the Cancer Chemotherapy Center, Japan Foundation for Cancer Research, Tokyo, Japan. KB human nasopharynx carcinoma and HCT116 human colon carcinoma were purchased from the American Type Culture Collection, Rockville, MD.

In Vitro Antiproliferative Activity. All cell lines were maintained in RPMI1640 medium supplemented with 10% fetal bovine serum, penicillin (100 units/mL), streptomycin (100 μg/mL), mercaptoethanol (5×10^{-2} mM), and sodium pyruvate (1 mM). Exponentially growing cells were seeded in 96-well microtiter plates at 2.5×10^3 cells/well (100 μL) for KB and colon 38 or at 1.25×10^3 cells/well (100 μL) for P388 and then incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. A test compound was dissolved in DMSO at a concentration of 20 mg/mL and further diluted with RPMI1640 medium containing 10% fetal bovine serum to prepare 3-fold serial dilutions with the maximum concentration being 200 μg/mL. The obtained dilutions were each poured into the well of the above-described culture plate in an amount of 100 μL. The culture was then continued at 37 °C under the 5% CO_2 atmosphere for 3 days. Thereafter, a solution of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide][25] having a concentration of 3.3 mg/mL in phosphate-buffered saline (PBS) was added to each well in an amount of 50 μL. The resulting mixtures were further incubated for 1−2 h. The supernatant was removed from each well by suction. Formed formazan was dissolved in 100 μL of DMSO. The absorbance was measured by using a microplate reader at a test wavelength of 540 nm and a reference wavelength of 660 nm to be taken as an index of the number of viable cells. The IC_{50} value (the drug concentration required for 50% cell growth inhibition) was determined by the least-squares method.

Flow Cytometric Analysis. Exponentially growing P388 cells were seeded at 5×10^5 cells/mL in 24-well pates. In a similar way to the growth inhibition assay, a test compound was prepared with the medium at graded concentrations and added to the cell plates. The incubation was then continued at 37 °C under 5% CO_2 atmosphere for 24 h. Thereafter, the cells were harvested by centrifugation, resuspended in 1 mL of ice-cold 70% ethanol, and fixed at 4 °C for more than 1 h. They were further washed with PBS and treated with 0.25 mL of 1 mg/mL RNaseA at 37 °C for 20 min. After the cells were washed with PBS twice, 0.25 mL of 50 μg/mL propidium iodide was added to the treated cells. The analysis of the DNA content was performed by quantitation of red fluorescence in a flow cytometer (FACSCalibur, Becton-Dickinson).

Inhibition of Tubulin Polymerization. Test compounds dissolved in DMSO at graded concentrations were added to the RB buffer solution of microtubule protein (1 mg protein/mL) from bovine brain. After 30 min of incubation at 4 °C, the mixture was heated to 37 °C with 1 mM GTP to start the polymerization reaction. Inhibitory activity of the drugs was determined from the increase in turbidity at 350 nm resulting from tubulin polymerization.

In Vivo Antitumor Activity. (a) Murine Syngeneic Tumor Model with Colon 38. About 75 mg of colon 38 was transplanted sc into the flank of BDF_1 mice (7−8 weeks old, female) on day 0. According to the predetermined doses (as indicated in Table 1), a test compound was suspended in saline containing 3.5% DMSO and 6.5% Tween 80 so that the injection volume would be 0.1 mL/10 g of body weight. The obtained suspensions were administered ip to the mice of the test groups (n = 6) once a day for 4 days (days 1−4). On the other hand, only the vehicle was administered ip to the mice of the control group (n = 10). The tumors were extirpated and weighed on day 21. Antitumor activity was determined by comparing the mean tumor weight of each test group (T) with that of the control group (C) and expressed as a T/C percentage.

(b) Human Tumor Xenograft Model with HCT116. 5×10^6 cells of HCT116 were inoculated sc into the flank of BALB/c·nu/nu mice (7 weeks old, female). On the seventh day after inoculation (day 1), the mice with an estimated tumor volume of about 100 mm^3 were divided into the control group (n = 10) and the test groups (n = 5). The treatments were performed in the same manner as the colon 38 model. Each tumor was measured on days 5, 9, and 15 with a sliding caliper. The tumor volume (TV) was calculated by the following formula: TV (mm^3) = $(a \times b \times b)/2$, where a represents the length and b represents the width (mm). On day 22, the tumors were extirpated from all mice and weighed for determining a T/C percentage. The relative tumor volume (RTV) on day 9 was also calculated as an index of in vivo antitumor activity by comparing the mean tumor volume on day 9 with that on day 1 in each group. RTV values on day 9 < 1.0 indicate initial tumor volume was reduced with drugs.

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