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A Focused Compound Library of Novel *N*-(7-Indolyl)benzenesulfonamides for the Discovery of Potent Cell Cycle Inhibitors

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Abstract—A series of compounds containing an *N*-(7-indolyl)benzenesulfonamide pharmacophore was synthesized and evaluated as a potential antitumor agent. Cell cycle analysis with P388 murine leukemia cells revealed that there were two different classes of potent cell cycle inhibitors; one disrupted mitosis and the other caused G1 accumulation. Herein described is the SAR summary of the substituent patterns on this pharmacophore template. © 2000 Elsevier Science Ltd. All rights reserved.

In the long history of drug discovery campaigns, it has been repeatedly observed that a series of compounds possessing a common structural feature shows diverse biological activities. The sulfonamides provide a typical example because of their wide variety of pharmacological activities such as antibacterial, insulin-releasing antidiabetic, carbonic anhydrase inhibitory, high-ceiling diuretic, and antithyroid.[1] In addition, more recent papers have reported various sets of novel sulfonamide drugs, e.g., endothelin receptor antagonists,[2] 5-HT_6 receptor antagonists,[3] β_3 adrenergic receptor agonists,[4] thrombin inhibitors,[5] and matrix metalloproteinase inhibitors.[6] Therefore, the sulfonamide moiety appears to be a crucial functionality to interact with many kinds of cellular protein targets. Based upon this consideration, we independently have focused on the construction of a sulfonamide compound library to find new synthetic antitumor agents.

As shown in Scheme 1, a point of departure for our continuous research on antitumor sulfonamides was the finding of compound **A** as a drug lead, which caused substantial growth inhibition and mitotic arrest to cancer cells in vitro.[7] Although the in vivo efficacy of **A** in tumor-bearing mice was only marginal, the optimization process via intensive syntheses on template **B** led to the discovery of E7010 that entered clinical trials as a novel tubulin polymerization inhibitor.[8] E7010 was shown to bind reversibly to the colchicine binding site of β-tubulin.[9] Sulfonamides possessing a heterobicyclic amine component were then designed from lead structure **A**, resulting in another library synthesis on template **C** to aim at the second generation of the antitumor sulfonamide. Here we describe the synthesis of *N*-(7-indolyl)benzenesulfonamide compounds and their in vitro growth inhibition activities against cancer cell lines. The trends in substituent structure–activity relationship (SAR) on this pharmacophore template and cell cycle analysis of selected compounds are also reported.

Chemistry

Illustrated in Scheme 2 is the general synthetic route used to prepare a focused compound library of novel *N*-(7-indolyl)benzenesulfonamides. Commercially available 2-bromonitrobenzenes **D** were converted to 7-bromoindoles **E** utilizing the Bartoli protocol.[10] The conversion of **E** to 7-aminoindoles **F** was achieved by the following sequence of steps: (i) metal-halogen exchange with *n*-butyllithium;[11] (ii) triazene formation with tosyl azide[12] or diphenyl phosphorazidate (DPPA);[13] (iii) reduction with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al).[13] The resulting amines **F** were immediately coupled with various 4-position-substituted benzenesulfonyl

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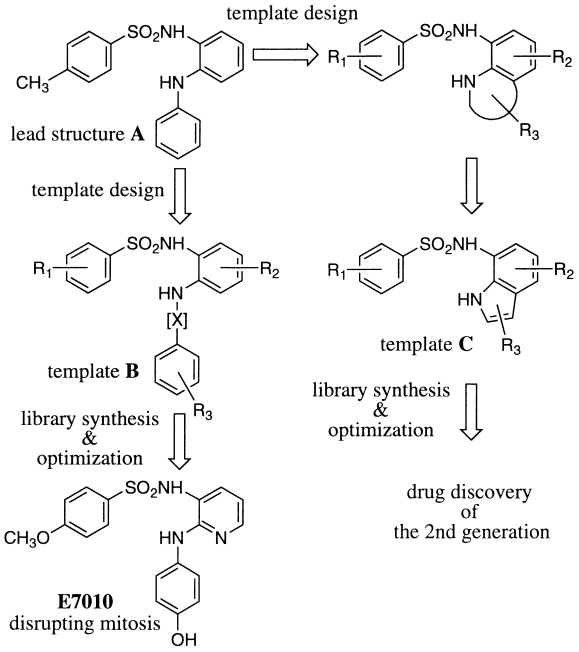


Scheme 1. Flow chart of drug discovery.

chlorides in the presence of pyridine to afford sulfonamides **G**. The 3-position of the indole moiety of **G** was chlorinated with *N*-chlorosuccinimide (NCS) to give **H**. The addition of a catalytic amount (0.1%) of hydrochloric acid into the THF solution accelerated this substitution reaction.

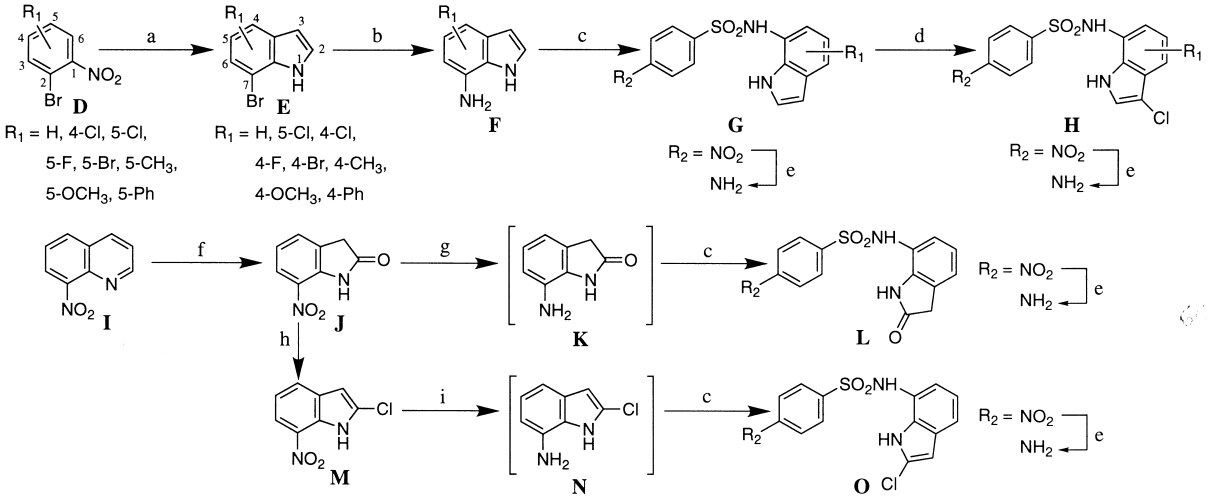
For the preparation of oxindole derivatives **L** and 2-chloroindole derivatives **O**, another indole ring formation protocol was employed, i.e., 8-nitroquinoline **I** was transformed to 7-nitro-oxindole **J** utilizing the procedure reported by Nakashima and Suzuki.[14] The subsequent hydrogenation over 10% palladium on carbon gave 7-amino-oxindole **K**. Compound **J** was separately converted to 2-chloro-7-nitroindole **M** by the treatment of $POCl_3$ under reflux. Reduction of **M** with Fe/NH_4Cl gave 2-chloro-7-aminoindole **N**. Amines **K** and **N** were coupled with sulfonyl chlorides as described for **G** to afford sulfonamides **L** and **O**, respectively. Reduction of the nitro group of **G**, **H**, **L** and **O** with Zn/HCl yielded the corresponding amines.

Biology

The antiproliferative activity of these new synthetics was assessed using KB human nasopharynx carcinoma, colon 38 murine adenocarcinoma, and P388 murine leukemia. After 3-day continuous drug exposure, the concentration required for 50% growth inhibition (IC_{50}) was determined by the MTT colorimetric assay.[15] In order to examine effects of the compounds on cell cycle progression, flow cytometric analysis was carried out using P388 cells. Following 24 h of drug treatment, the cells were fixed in 70% ethanol, treated with 1 mg/mL RNase, stained with 50 μg/mL propidium iodide, and then analyzed for the DNA content with a flow cytometer. The ability of selected compounds to inhibit tubulin polymerization was calculated from the increase in turbidity at 350 nm.[9] Briefly, to the assay buffer solution of bovine brain tubulin (1 mg protein/mL) were added each test drug and GTP (1 mM) at 0 °C. Tubulin polymerization was initiated by warming the reaction mixture from 0 to 37 °C and measured by terbidimetric assay. All the results are shown in Table 1 and Figure 1.

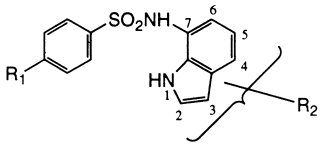
Results and Discussion

In the MTT colorimetric assay, many compounds of the present *N*-(7-indolyl)benzenesulfonamide series displayed significant growth inhibition activity within the concentration range 10^{-6}–10^{-7} M (Table 1). More interestingly, cell cycle analysis with P388 cells revealed that there were two different classes of potent cell cycle inhibitors;

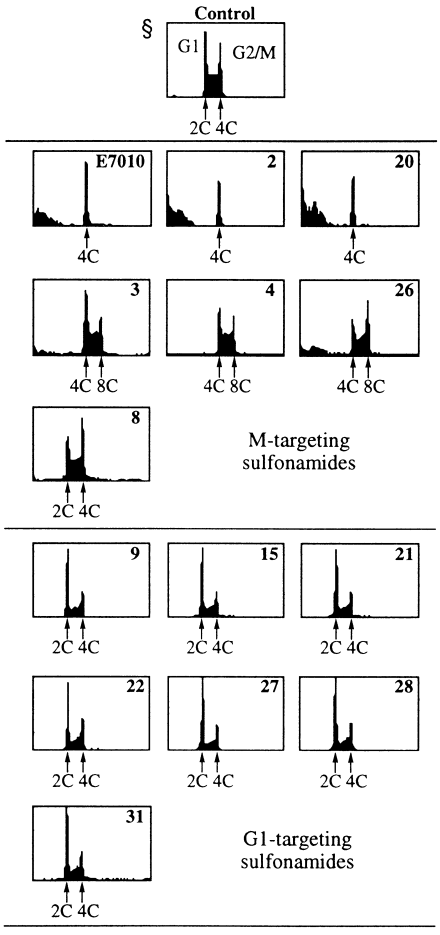


Scheme 2. Synthesis of *N*-(7-indolyl)benzenesulfonamide compounds. Reagents and conditions: (a) $CH_2=CHMgBr$, THF, −40 °C, then aq NH_4Cl; (b) *n*BuLi, THF, −70 °C, then TsN_3 or DPPA, −70 to −40 °C, and finally Red-Al, −40 to 0 °C; (c) 4-R_2-benzenesulfonyl chloride (R_2 = methoxy, chloro, nitro, methyl, or cyano), pyridine, THF or EtOAc; (d) *N*-chlorosuccinimide, aq HCl, THF; (e) Zn, aq HCl, MeOH, reflux; (f) H_2O_2, AcOH, 70 °C; (g) H_2, 10% Pd/charcoal, MeOH, DMF; (h) $POCl_3$, reflux; (i) Fe, NH_4Cl, 2-PrOH, H_2O.

Table 1. In vitro antiproliferative activity against cancer cell lines[a] and effect on cell cycle progression[b]



Compound	R_1	R_2	Cancer cell line, IC_{50} (μg/mL) KB	Colon 38	P388	Cell cycle analysis[c]
1	OCH_3	H	0.67	1.5	1.1	G2/M arrest and 4C→8C*
2	OCH_3	2-Cl	0.12	0.17	0.11	G2/M arrest
3	OCH_3	3-Cl	0.55	0.20	0.76	G2/M arrest and 4C→8C*
4	OCH_3	4-Cl	0.92	0.65	1.1	G2/M arrest and 4C→8C*
5	OCH_3	5-Cl	0.21	0.64	0.27	G2/M arrest
6	OCH_3	2-OH(oxindole)	0.10	0.22	0.11	G2/M arrest
7	Cl	H	6.0	7.0	6.0	No effect
8	Cl	2-Cl	4.0	10	7.4	G2/M increase
9	Cl	3-Cl	8.8	0.28	4.3	G1 increase
10	Cl	4-Cl	9.7	0.66	4.6	G1 increase
11	Cl	5-Cl	9.1	8.5	9.9	No effect
12	Cl	2-OH(oxindole)	1.1	4.5	2.2	G2/M arrest and 4C→8C*
13	NO_2	H	15	7.2	8.2	No effect
14	NO_2	2-Cl	18	19	21	No effect
15	NO_2	3-Cl	14	0.34	2.3	G1 increase
16	NO_2	4-Cl	9.1	0.67	3.3	G1 increase
17	NO_2	5-Cl	12	13	13	No effect
18	NO_2	2-OH(oxindole)	>100	62	>100	No effect
19	CH_3	2-Cl	0.11	0.18	0.14	G2/M arrest
20	CH_3	2-OH (oxindole)	0.093	0.17	0.14	G2/M arrest
21	CN	3-Cl	11	0.13	1.4	G1 increase
22	CN	4-Cl	15	0.22	1.8	G1 increase
23	CN	3,4-diCl	13	0.12	0.59	G1 increase
24	NH_2	H	36	6.1	14	No effect
25	NH_2	2-Cl	1.4	2.9	2.2	G2/M arrest and 4C→8C*
26	NH_2	2-OH (oxindole)	0.86	1.8	1.2	G2/M arrest and 4C→8C*
27	NH_2	3-Cl	10	0.23	0.81	G1 increase
28	NH_2	3,4-diCl	14	0.10	0.49	G1 increase
29	NH_2	3-Cl, 4-F	14	0.11	0.51	G1 increase
30	NH_2	3-Cl, 4-Br	13	0.14	0.64	G1 increase
31	NH_2	3-Cl, 4-CH_3	16	0.10	0.37	G1 increase
32	NH_2	3-Cl, 4-OCH_3	20	2.3	5.5	No effect
33	NH_2	3-Cl, 4-Ph	15	19	19	No effect
E7010			0.29	0.38	0.32	G2/M arrest



§The horizontal axis of diagrams represents a relative DNA content based on log fluorescence intensity. The level of the vertical direction corresponds to the number of cells.

[a]Compounds were tested at concentrations of 1.0×10^{2}, 33, 11, 3.7, 1.2, 4.1×10^{-1}, 1.4×10^{-1}, 4.6×10^{-2}, and 1.5×10^{-2} μg/mL.
[b]P388 cells were treated with test compounds at 4.0 μg/mL for 24 h.
[c]*This means that drug-treated cells underwent an extra DNA replication without the completion of mitosis.

one arrested mitosis like E7010 and the other caused a cellular accumulation in G1 phase (Table 1). The mitotic arrest observed here was shown to result from the inhibition of tubulin polymerization (Fig. 1), whereas the precise mechanism of the G1 increase is currently under investigation. With respect to in vitro cell proliferation, three cancer cell lines, KB, colon 38, and P388, were almost equally suppressed by the M (mitosis)-targeting sulfonamides. In contrast, the G1-targeting sulfonamides displayed a distinctly different antitumor spectrum, i.e., preferential potency towards colon 38 rather than KB and P388, reflecting the presence of another putative cellular target than tubulin.

The SAR summary is described with particular focus on the substituent patterns of both two aromatic rings, benzene and indole. The phenyl ring substituent (R_1, see Table 1) was introduced into the 4-position. Electron-donating groups OCH_3 and CH_3 were found crucial for the mitotic arrest phenotype, corresponding with the SAR of E7010 series compounds.[7–9] Substitution with an electron-withdrawing group NO_2 or CN abolished the antimitotic property; instead it resulted in the G1 increase phenotype in combination with the 3- and/or 4-Cl substitution of the indole moiety. In case of the compounds possessing Cl or NH_2 on the phenyl ring, both phenotypes were observed depending on the substituent pattern of the indole.

The Cl substituent on the indole (R_2, see Table 1) was varied with respect to its position. Oxindole derivatives **6**, **12**, **18**, **20** and **26** were also prepared for the SAR study. Substitution at the 2-position proved to particularly promote mitotic arrest ability (see **1** versus **2** or **6**; **7** versus **8** or **12**; **24** versus **25** or **26**) and to enhance antiproliferative activity in most cases. Note that compounds **2** and **6** exhibited a comparable potency to that of E7010 in the MTT assay and the tubulin polymerization test

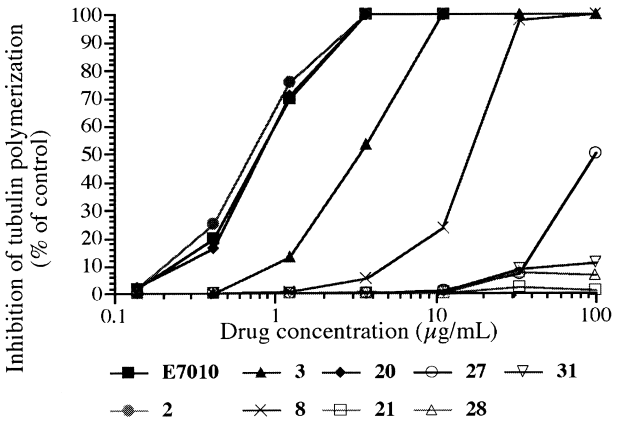


Figure 1. Tubulin polymerization assay. Tubulin polymerization in the absence of any drug gave the control readings. Plotted values are the means of four independent experiments.

(Table 1 and Fig. 1). Additionally, the 5-Cl substitution also produced a somewhat good effect on antimitotic and antiproliferative activity in case of the R_1 substituent OCH_3 (see **1** versus **5**). For the G1 increase phenotype, substitution at the 3- and/or 4-position of the indole was essential. Especially the 3-Cl substitution seemed to have predominant effect, judging from the data that the 3-Cl compounds were about two times more potent against colon 38 than the 4-Cl ones on the IC_{50} value basis (see **9** versus **10**; **15** versus **16**; **21** versus **22**). The tubulin polymerization test disclosed that compound **27**, causing G1 accumulation at 4.0 μg/mL, moderately inhibited microtubule assembly over 33.3 μg/mL. However, the incidental antimitotic action was significantly diminished in 3,4-di-substituted compounds **28** and **31**. Steric limitations of the 4-substituent were evident by seeing antiproliferative profiles of compounds **32** and **33**.

As for selected antimitotic compounds, E7010, **2**, **3**, **8** and **20**, there was a reasonable correlation among antiproliferative activity, mitotic arrest ability at 4.0 μg/mL, and inhibitory activity against tubulin polymerization, with a rank order of E7010 ≈ **2** ≈ **20** > **3** > **8**. Considering chemical structures of the new antimitotic sulfonamides, they are likely to bind reversibly to β-tubulin in the same manner as E7010. In this aspect, our compounds are clearly distinguished from another antimitotic sulfonamide T138067 that contains the pentafluorobenzenesulfonamide moiety and has been reported to modify β-tubulin covalently at the Cys-239 residue.[16] On the other hand, intensive research on the G1-targeting sulfonamides led us to the discovery of *N*-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide (E7070), which has progressed to Ph I clinical trials in Europe because of its promising in vivo efficacy against human tumor xenografts and its unique mode of action.[17] Further data on the SAR of E7070-related compounds will be forthcoming.

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