piperazine, 110-85-0; 1-(cyanomethyl)-4-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)piperazine, 141529-04-6; 2-hydroxyacetonitrile, 107-16-4; 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5-carbonyl chloride, 14846-34-5; 1-[(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)carbonyl]piperazine, 141529-05-7; (10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)carboxaldehyde, 24391-61-5; 4,6-bis(allylamino)-2-chloro-1,3,5-triazine, 15468-86-7; 1-(2-aminoethyl)-4,4-diethoxypiperidine, 141529-06-8; 1-(cyanomethyl)-4,4-diethoxypiperidine, 141529-07-9; 1-(3-amino-2-hydroxypropyl)-4,4-diethoxypiperidine, 141529-08-0; 1-(3-phthalimido-2-hydroxypropyl)-4,4-diethoxypiperidine, 141529-09-1; 4-(aminocarbonyl)piperidine, 39546-32-2.

Communications to the Editor

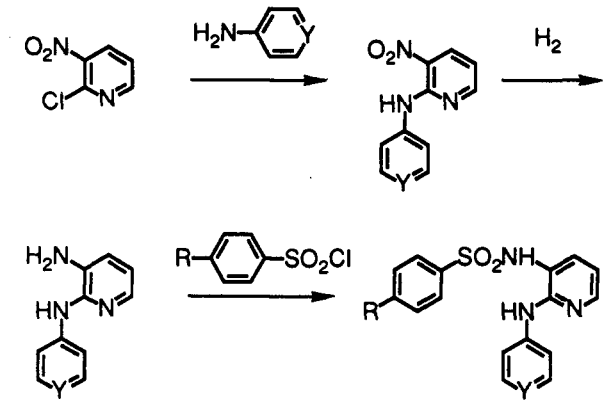
Novel Sulfonamides as Potential, Systemically Active Antitumor Agents

The discovery of new antitumor agents has truly contributed to the treatment of certain types of tumors, i.e. leukemias and lymphomas. Unfortunately, however, chemotherapeutic treatments are far from satisfactory to patients with malignant solid tumors, particularly to those with slowly growing solid tumors. Therefore, we attempted to discover clinically effective drugs against such refractory solid tumors.

It has been repeatedly noted that traditional primary in vivo screening using murine leukemias (P388 and L1210) are unsuitable for the detection of clinically active agents against solid tumors.[1-3] Our main strategy was that structurally novel synthetic compounds with significant antiproliferative activity against solid tumor cells in vitro would be tested for antitumor activity against the slowly growing, murine solid tumors in vivo. We used colon 38 (murine colon adenocarcinoma) in the primary in vivo tests because this model has the following characteristics: (a) colon 38 is a relatively slow growing, murine solid tumor,[4] (b) colon 38 is comparatively resistant to chemotherapy,[5] and (c) colon 38 is subcutaneously (sc) implanted; therefore systemic absorption and delivery of drugs are required in order to inhibit tumor growth. In the case of the traditional murine leukemia models, intraperitoneal (ip) drug treatment after ip tumor implantation has been exclusively used. For the purpose of choosing candidates for in vivo tests, we initially screened new synthetics for in vitro antiproliferative activity against two kinds of solid tumor cells, colon 38 and KB (human carcinoma of the nasopharynx) cells.

In the course of selecting various chemical structures which may be of use in designing novel antitumor agents, we were particularly interested in sulfonamides, because sulfadiazine, an antibacterial sulfonamide, was reported to preferentially accumulate in sc-implanted murine tumors after ip administration.[6] Since it has been well known that sulfonamides have a variety of biological activities such as antibacterial, insulin releasing, carbonic anhydrase inhibitory, and antithyroid,[7] there seemed to be a possibility that new antitumor sulfonamides with mechanisms different from those of known antitumor agents could be discovered. Therefore, we undertook the synthesis and screening of a number of sulfonamides with widely differing chemical structure. Although very little information had appeared in the literature regarding antitumor activity of sulfonamides, chloroquinoxaline sulfonamide (CQS) was reported to have antitumor activity[8] while our work was in progress. The mechanism of action of CQS has not yet been elucidated.[8,9]

Scheme I



2 R = CH_3, Y = C-H
3 R = CH_3O, Y = C-H
4 R = CH_3O, Y = C-OCH_3
5 R = CH_3O, Y = C-OH
6 R = CH_3O, Y = N

Chemistry. The syntheses of the *N*-(2-anilino-3-pyridyl)benzenesulfonamide analogs **2**–**6** are outlined in Scheme I. 2-Anilino-3-pyridinamine derivatives were obtained by the catalytic hydrogenation of 2-anilino-3-nitropyridine derivatives, which were prepared in good yields by heating the mixture of 2-chloro-3-nitropyridine and aniline or its derivatives at ca. 100 °C.[10] Condensation

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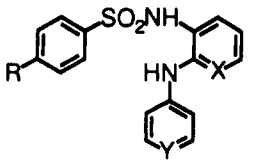
Table I. In Vitro Antiproliferative Activity of Compounds 1–6



compd	R	X	Y	IC_{50} (μg/mL)[a] colon 38	KB
1	CH_3	CH	CH	1.2	0.46
2	CH_3	N	CH	3.2	1.5
3	CH_3O	N	CH	0.45	0.27
4	CH_3O	N	$COCH_3$	3.6	1.9
5	CH_3O	N	COH	0.38	0.29
6	CH_3O	N	N	1.4	1.0

[a] Concentration to inhibit colon 38 and KB cell proliferation by 50% relative to untreated controls after 72 h of continuous drug exposure.

Table II. In Vitro Antitumor Activity of Compounds 2–6 against Colon 38[a]

compd	dose (mg/kg/day)	deaths/ total	T/C[b] (%)
2	100	0/6	20
	200	0/6	19
	400	0/6	1
3	50	0/6	27
	100	0/6	2
	200	0/6	1
4	50	0/6	30
	100	0/6	1
	200	2/6	0
5	12.5	0/6	59
	25	0/6	32
	50	0/6	3
	100	0/6	2
6	12.5	0/6	41
	25	0/6	35
	50	0/6	14

[a] The tumor was introduced by sc implantation of a 75-mg tumor fragment into the axillary region of female BDF_1 mice on day 0. Compounds 2–6 were administered orally on days 1–8. The tumors were excised and weighed on day 21. [b] (Tumor weight of treated mice/tumor weight of control mice) × 100.

of *p*-methyl or *p*-methoxybenzenesulfonyl chloride with 2-anilino-3-pyridinamine derivatives in pyridine at room temperature gave compounds 2–6 in good yields. The structures of these compounds were established by spectroscopic and analytical data.

Biological Activity and Discussion. A number of sulfanilamides, i.e., sulfadiazine, sulfapyridine, sulfamerazine, sulfamethazine, sulfisomidine, sulfadimethoxine, sulfathiazole, sulfamethoxazole, sulfamethizole, and sulfisoxazole, were tested for in vitro antiproliferative activity against colon 38 and KB cells. None of the sulfanilamides showed inhibitory activity against these cells (IC_{50} > 100 μg/mL).

Of the compounds tested, 1 was the first discovered sulfonamide with substantial in vitro antiproliferative activity (Table I). Although 1 showed only marginal in vivo antitumor activity against colon 38, further modification of the structure of 1 led to the discovery of analog 2 with good in vivo activity (Table II). Compound 2 showed antitumor activity against colon 38 in a dose-dependent manner after oral (po) administration on days 1–8. Replacement of the methyl group of 2 by a methoxy group (analog 3) increased both in vitro and in vivo activity. Substitution with a hydroxy group in position 4 of the anilino group of 3 (analog 5) did not affect in vitro activity but increased in vivo activity. No correlation between in vitro and in vivo antitumor activity was observed upon modification of the anilino group of 3 (3 vs 4–6). It is noteworthy that 2, 3, and 5 have a broad range of effective doses. Compound 5 has been selected for further evaluation. Compound 5 was found to inhibit mitosis of P388 cells at cytotoxic concentrations (IC_{50} = 0.32 μg/mL) as shown in Figure 1. Its excellent activity and wide range of effective doses in the colon 38 model and its structural novelty, unrelated to known antineoplasms, suggest that 5 may be a hopeful new antitumor agent for human solid tumors. Reports on the structure–activity relationships of this series of compounds, further data on the efficacy of 5 (E7010) in this and other tumor models, and the precise mechanism of action will be forthcoming.

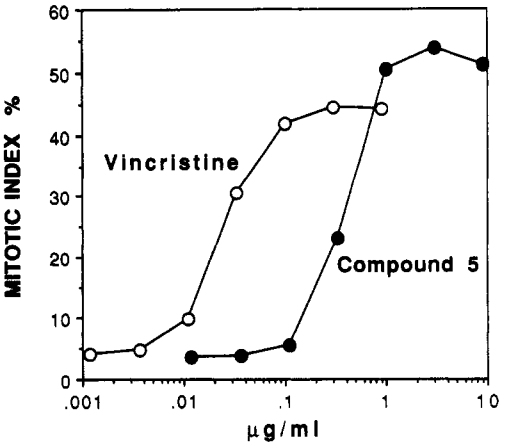


Figure 1. Effects of compound 5 and vincristine on the mitotic index of P388 cells in vitro. P388 cells were cultured with the drugs for 12 h. The cells were treated with 75 mM KCl, fixed with MeOH–AcOH, and stained with crystal violet. The mitotic index was determined by counting at least 300 cells (control 4.5%). Similar results were obtained in a second experiment.

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Isopropyl and Phenyl Esters of 3β-(4-Substituted phenyl)tropan-2β-carboxylic Acids. Potent and Selective Compounds for the Dopamine Transporter

Since (−)-cocaine (1) is a popular drug of abuse, much effort has been devoted to elucidating its neurochemical mode of action. Ritz et al.[1] correlated the potencies of cocaine and cocaine analogs in self-administration studies with their potencies in inhibiting [^{3}H]mazindol binding to the dopamine (DA) transporter in rat striatum and con-

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