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Synthesis and Biological Evaluation of *N*-(7-Indolyl)-3-pyridinesulfonamide Derivatives as Potent Antitumor Agents

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Abstract—We herein report the synthesis and antitumor activity of E7070 analogues containing a 3-pyridinesulfonamide moiety. E7070 was selected from our sulfonamide-based compound collections, currently undergoing Phase II clinical trials because of its tolerable toxicity profile and some antitumor responses in the Phase I setting. Of the analogues examined, ER-35745, a 6-amino-3-pyridinesulfonamide derivative, demonstrated significant oral efficacy against the HCT116 human colon carcinoma xenograft in nude mice.

E7070 (**2**), a novel sulfonamide antitumor agent, was discovered from our sulfonamide-focused libraries originating in the lead structure **1** (Fig. 1).[1–3] Because of its promising in vivo efficacy against human tumor xenografts[4] and its unique mechanistic profile,[4–7] E7070 progressed to clinical evaluation. In Phase I studies,[8] the predominant toxicity of E7070 was determined to be myelosuppression such as neutropenia and thrombocytopenia. Some objective responses were observed in patients with advanced cancers. Phase II trials of this compound are currently ongoing in Europe and the United States to assess its clinical efficacy against several solid tumors.

A primary molecular target of E7070 is still unknown, and thus its precise mechanism of antitumor action has yet to be fully understood. However, several reports to date have disclosed that E7070 causes a decrease in the S phase fraction accompanied by the accumulation of cells in the G1 phase and/or the G2 phase of the cell cycle.[2–7] Originally, the compound was found to increase the G1 proportion of P388 murine leukemia cells in time- and dose-dependent manners.[2,4] Thereafter, it was shown that E7070-induced disturbance of the G1/S transition eventually led to G2 arrest in HCT116 human colon cancer cells[6] and A549 human non-small cell lung cancer cells.[7] The study using A549 and its E7070-resistant subline A549/ER further clarified that E7070 inhibited pRb phosphorylation, reduced the protein expression of cyclin A, cyclin B1, CDK2, and CDC2, and repressed CDK2 activity with the induction of p53 and p21 proteins only in the parental A549 cells.[7] All of these findings indicate that E7070 belongs to a new class of cell cycle inhibitors. Given the clinical activity of this interesting small molecule, it appears to be of particular importance to continue the drug discovery research on structurally related compounds. We herein present E7070 analogues

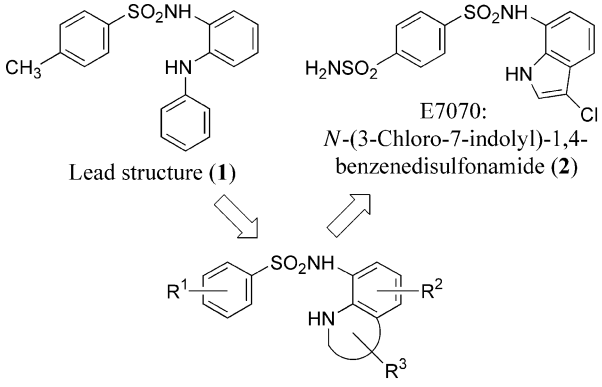

Figure 1. Flow chart for the discovery of E7070.

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containing a 3-pyridinesulfonamide moiety with respect to their antitumor activities against HCT116 cells both in vitro and in vivo.

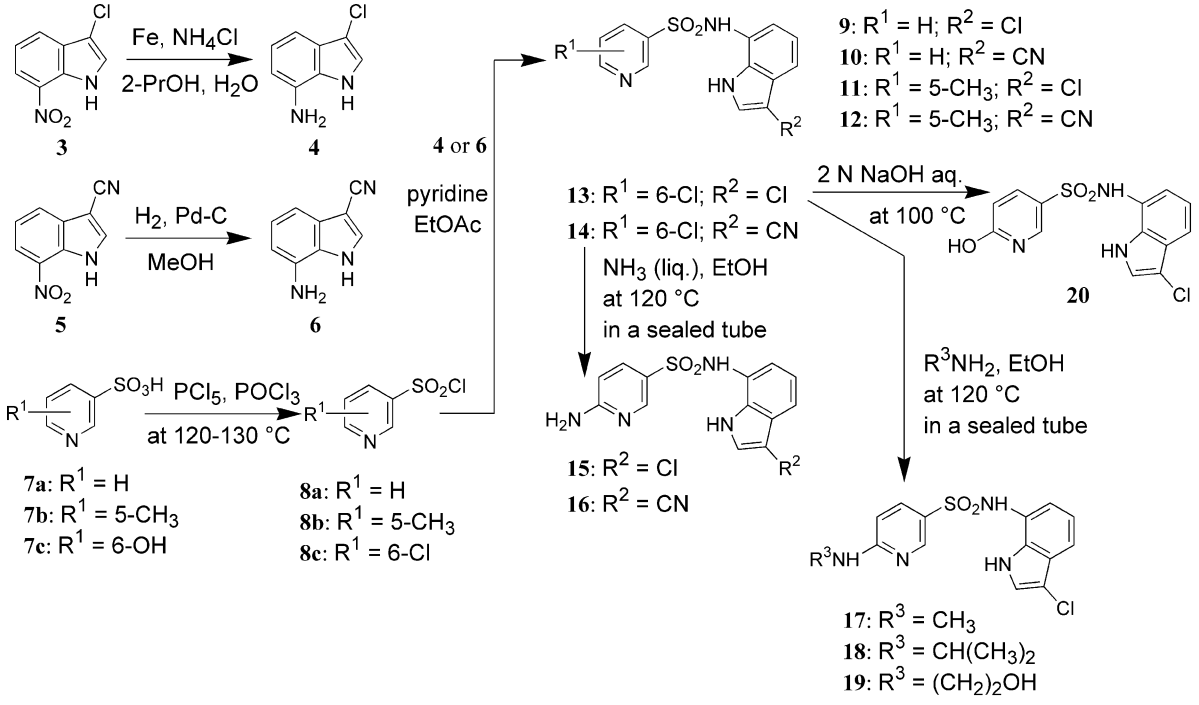
The syntheses of the new analogues are outlined in Scheme 1. Reduction of 3-chloro-7-nitroindole (**3**) and 3-cyano-7-nitroindole (**5**) with Fe/NH_4Cl and H_2/Pd-C gave the corresponding aminoindoles **4** and **6**, respectively.[2] Sulfonic acids **7a**, **7b**,[9] and **7c**[10] were each converted to **8a**, **8b**, and **8c** by using PCl_5 and $POCl_3$ under reflux. These sulfonyl chlorides were coupled with **4** and **6** in the presence of pyridine to afford sulfonamides **9**–**14**. Amination of **13** and **14** with NH_3 in ethanol gave compounds **15** and **16**, respectively. Compound **13** was further transformed to **17**–**20** via the reaction with several amines and aqueous sodium hydroxide.

Compounds **9**–**12** and **15**–**20** were initially screened for in vitro antiproliferative activity against HCT116 human colon cancer cells. Following 72-h continuous drug exposure, the concentration required for 50% cell growth inhibition (IC_{50}) was determined by the MTT colorimetric assay.[11] As shown in Table 1, these new analogues inhibited the proliferation of HCT116 with IC_{50} values ranging from 0.19 to 1.3 μM. The in vitro activities of **9**–**12** were comparable to that of E7070. 3-Chloroindole derivatives (**9**, **11**, and **15**) and the corresponding 3-cyanoindole derivatives (**10**, **12**, and **16**, respectively) were equally active in this in vitro assay.

All these compounds were next evaluated for in vivo antitumor activity on the HCT116 xenograft model. HCT116 cells were implanted subcutaneously into athymic nude mice and allowed to grow to a size of about 100 mm^3. Subsequently, daily drug administration (50 mg/kg/day) was conducted for four days (on days 1–4). We assessed drug efficacy according to the following two criteria: Day 9-RTV (relative tumor volume) and Day 22-T/C(%). The Day 9-RTV value was calculated as the ratio of average tumor volume on day 9 to that on day 1. The RTV value <1.0 indicates that initial tumor volume was reduced by drug treatment. Therefore, it can be used as an index for the assessment of not only tumor growth suppression but also tumor regression. The Day 22-T/C value was calculated on day 22 as the ratio of average tumor weight of the treated group to that of the control group. This is one of the most widely accepted criteria representing drug-induced tumor growth suppression. The T/C value <42 generally indicates in vivo antitumor activity.

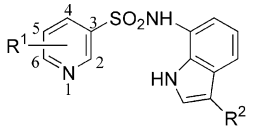
After intraperitoneal (ip) drug administration, compounds **12** and **15**–**18** exhibited potent efficacy based on the T/C criteria. They, except for **16**, also caused a significant decrease in tumor volume on day 9. In the ip treatment, there were no compounds sperior to E7070 in terms of Day 9-RTV and Day 22-T/C values. As far as examined, no correlation between in vitro and in vivo antitumor activities was observed. On this in vivo model, the 3-cyanoindole derivative **12** showed clear efficacy accompanying tumor regression whereas its 3-chloroindole analogue **11** was inactive. By contrast, the efficacy of another 3-cyanoindole derivative **16** was inferior to that of the corresponding 3-chloroindole derivative **15**, particularly with respect to drug effect reducing tumor volume. These observations suggest that the combination of substituents R^1 and R^2 may be critical for pharmacokinetic behavior of this series of compounds.

Of the compounds found to be active in the ip treatment, **12**, **15** (ER-35745), **16**, **18**, and E7070 were further



Scheme 1.

Table 1. In vitro and in vivo antitumor activities of E7070 and its analogues



Compd	R^1	R^2	In vitro activity IC_{50} (μM)[a]	Drug administration[b]	In vivo activity		
					Day 9-RTV[c]	Day 22-T/C(%)[d]	Deaths/total
9	H	Cl	0.26	ip	3.4	70	0/5
10	H	CN	0.23	ip	2.6	65	0/5
11	5-CH_3	Cl	0.22	ip	3.7	82	0/5
12	5-CH_3	CN	0.19	ip	0.52	30	0/5
				po	3.4	106	0/5
15 (ER-35745)	6-NH_2	Cl	0.71	ip	0.30	26	0/5
				po	0.19	12	0/5
16	6-NH_2	CN	0.64	ip	1.5	35	0/5
				po	2.3	75	0/5
17	6-$NHCH_3$	Cl	0.86	ip	0.24	38	0/5
18	6-$NHCH(CH_3)_2$	Cl	1.3	ip	0.28	16	0/5
				po	0.90	55	0/5
19	6-$NH(CH_2)_2OH$	Cl	0.65	ip	4.3	89	0/5
20	6-OH	Cl	0.80	ip	3.0	66	0/5
2 (E7070)[e]			0.29	po	0.29	23	0/5
				ip	0.10	12	0/5
				iv	0.15	8	0/5

[a]HCT116 human colon cancer cells were treated with each test compound for 72 h. IC_{50} values were calculated using the least-squares method to afford the means of three independent assay data. Errors were within ±10% of the reported values.
[b]50 mg/kg/day of each test compound was given to nude mice ($n=5$) with HCT116 xenografts following the tumor volume reached about 100 mm^3. Only a vehicle (saline containing 3.5% DMSO and 6.5% Tween 80) was given ip to control mice with the same tumors ($n=10$). Drug administration was performed daily for four days (on days 1–4). Administration routes: ip, intraperitoneal; po, per os; iv, intravenous.
[c]'(Average tumor volume on day 9)/(average tumor volume on day 1)' is designated as Day 9-RTV. Day 9-RTV values <1.0 indicate that drug treatment caused tumor regression. The mean of control Day 9-RTV values was 4.5±1.1.
[d]'(Average tumor weight in treated group on day 22)/(average tumor weight in control group on day 22) × 100' is designated as Day 22-T/C. According to the National Cancer Institute (NCI) activity criteria, Day 22-T/C values <42(%) are judged to be active.
[e]The data for ip and iv injections were reported previously.[2,4]

tested for their oral (po) efficacy. When given orally in a suspension of methyl cellulose, compounds **12**, **16**, and **18** proved to be lacking for in vivo antitumor activity based on the T/C criteria, even though only **18** caused a marginal reduction in tumor volume on day 9. E7070 has been evaluated in preclinical animal models by intravenous (iv) administration that is currently used for clinical trials. In this experiment E7070 was also shown to be quite active via the po route in addition to ip and iv routes reported previously.[2,4] At the same dose level of E7070 (50 mg/kg/day×4), the ip and iv treatments were considered to be more effective than the po treatment. Importantly, ER-35745 demonstrated notable po efficacy that was almost comparable to the efficacy of E7070 administered intravenously. As for the in vivo antitumor activity of ER-35745, the po treatment was superior to the ip treatment, suggesting good pharmacokinetic profiles of this compound given orally. In fact, ER-35745 exhibited rapid adsorption (T_{max} <1 h) and remarkable bioavailability (F ~100) in Sprague–Dawley rats given a po 10 mg/kg dose.

We have recently focused on gene expression analysis using Affymetrix high-density oligonucleotide microarrays (HuGene FL arrays) to identify transcriptional changes closely associated with E7070 efficacy.[12] After 12 h of treatment, 0.8 μM E7070, a pharmacologically relevant and a clinically achievable drug concentration, led to 2-fold or more down-regulation of nine genes in common in three different human cancer cell lines, HCT116-C9 colon carcinoma, MDA-MB-435 breast carcinoma, and MOLT-4 leukemia.[13] These nine genes are cyclin H, DNA polymerase α subunit, mitochondrial $NAD(P)^+$-dependent malic enzyme, medium-chain acyl-CoA dehydrogenase, tropomyosin, calcineurin A1, Rp8 homologue PDCD2, clone 23759, and KIAA0257 (T. Owa, A. Yokoi, and T. Nagasu, unpublished data). It is of particular importance that ER-35745 treatment (at 0.8 μM for 12 h) also repressed more than two-fold the transcript levels of all nine genes in HCT116-C9 cells. This strongly suggests that E7070 and ER-35745 share the same primary mechanism of antitumor action.

Described herein are the synthesis and antitumor activity of E7070 analogues containing a 3-pyridinesulfonamide moiety. We have found the 6-amino-3-pyridinesulfonamide derivative ER-35745 to display significant oral efficacy against the HCT116 human colon carcinoma xenograft in nude mice. ER-7070 and ER-35745 seem to operate by putatively the same mechanism of action, supported by microarray-based gene expression analysis.

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