[Chem. Pharm. Bull. 33(11)5126—5129(1985)]

Antitumor Activity of 5-Substituted 2-Acylamino-1,3,4-thiadiazoles against Transplantable Rodent Tumors

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(Received March 29, 1985)

The antitumor activities of twelve 2-*N*- and/or 5-substituted 2-amino-1,3,4-thiadiazole analogs were tested on Lewis lung carcinoma in mice by intraperitoneal administration. 2-Amino-1,3,4-thiadiazole (ATDA), 2-formamido-1,3,4-thiadiazole and 2-trifluoroacetamido-1,3,4-thiadiazole significantly prolonged the life span of tumor bearing mice. 5-Substituted analogs and 2-urethane and 2-carbamate type compounds were inactive. 2-Formamido-1,3,4-thiadiazole showed more potent activity than the parent compound ATDA against P388, L1210 leukemia, B16 melanoma and Lewis lung carcinoma. The differences in the antitumor activity among ATDA analogs are discussed.

Keywords——antitumor activity; 5-substituted 2-acylamino-1,3,4-thiadiazoles; 2-amino-1,3,4-thiadiazole; 2-formamido-1,3,4-thiadiazole; Lewis lung carcinoma; P388; L1210; B16

Since 2-amino-1,3,4-thiadiazole (ATDA) was first shown to have potent antitumor activity against S91 melanoma, 8110 glioblastoma and 6C3HED lymphosarcoma,[1] many derivatives of ATDA have been tested against several animal tumors, but few compounds with antitumor activity markedly superior to that of the parent compound have been found.[2] On the other hand, Lewis lung carcinoma is commonly used in the screening of antitumor agents, but it is practically resistant to many agents. Consequently, it is important to find compounds effective against this tumor. The present paper describes the antitumor activity of twelve 5-substituted 2-acylamino-1,3,4-thiadiazoles against Lewis lung carcinoma and other experimental tumors, and discusses the differences in activity among these compounds.

Materials and Methods

Materials——The chemical structures and melting points of thiadiazole derivatives used in this study are shown in Table I. Preparation of compounds **1**, **4**, **7**, **10**, **11** and **12** has been reported.[2d,3] 5-Substituted analogs were synthesized by cyclization of the corresponding acylthiosemicarbazides with conc. sulfuric acid, and then the 2-amino group was acylated with formic acid or trifluoroacetic acid. 5-Fluorouracil (5-FU, Kyowa Hakko), 6-mercaptopurine(6-MP, Tokyo Kasei) and cyclophosphamide (EX, Shionogi) were purchased. Compounds **1**, **2**, **4**, **7**, 5-FU and EX were dissolved in physiological saline and other compounds were suspended in 0.5% carboxymethylcellulose (CMC) before use.

Animals and Tumors——Female 6-week-old DBA/2, C57BL and BDF_1 mice were purchased from Shizuoka Laboratory Animal Center, Hamamatsu. P388 and L1210 leukemias were maintained in the peritoneal cavity of DBA/2 mice by weekly transplantation. B16 melanoma and Lewis lung carcinoma (3LL) were maintained by subcutaneous transplantation at 12-day intervals in C57BL mice.

Assay of Antitumor Activity——Female 6-week-old BDF_1 mice were used in all experiments. Mice were intraperitoneally inoculated with leukemia cells (1×10^6 P388 cells/mouse; 1×10^5 L1210 cells/mouse). For solid-type

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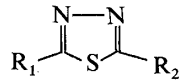
TABLE I. Chemical Structures of 2-Amino-1,3,4-thiadiazole Derivatives



Compound No.	R_1	R_2	mp (°C)
1 (ATDA)	NH_2	H	199—201
2	NH_2	CH_3	227—230.5
3	NH_2	C_6H_5	208.5—210.5
4	NHCHO	H	222—228
5	NHCHO	CH_3	227—230.5
6	NHCHO	C_6H_5	206—212
7	$NHCOCF_3$	H	158—164
8	$NHCOCF_3$	C_2H_5	174—178.5
9	$NHCOCF_3$	$CH_2C_6H_5$	126—130
10	$NHCONH_2$	H	217—219
11	$NHCONHC_6H_5$	H	259—270
12	$NHCO_2C_2H_5$	H	188.5—192

tumors, mice were subcutaneously inoculated in the back with B16 (1×10^6 cells/mouse) or 3LL (5×10^5 or 1×10^6 cells/mouse). The treatment was started 24 h after the tumor cell inoculation. Each compound was intraperitoneally injected once a day for 7 d in the case of leukemias or for 10 d in the case of solid type tumors. Mice that died were autopsied. Sixty days after the tumor cell inoculation, the experiments were terminated, and the survivors were killed and autopsied.

Results and Discussion

The antitumor activities of twelve 2-*N*- and/or 5-substituted 2-amino-1,3,4-thiadiazoles were tested on Lewis lung carcinoma, which is a refractory experimental tumor. The parent compound, ATDA, has been reported to inhibit the *de novo* synthesis of nucleic acids.[4] Thus, two antimetabolites, 5-FU and 6-MP, and EX, which is known to be effective on this tumor, were used as reference compounds. As shown in Table II, though 5-FU and 6-MP were ineffective, three compounds, **1** (ATDA), **4** and **7**, significantly prolonged the life span of 3LL-bearing mice. It has been shown that substitution on the 2-amino group of ATDA led to similarly active or less active compounds,[1,2a,c-e] and 5-substituted analogs except for 5-hydroxy-ATDA were almost inactive compared with the parent compound.[1,4d] This study confirmed that substitution of the 5-position of the thiadiazole ring leads to inactive compounds. On the other hand, the effects of compounds **4** and **7** were equal to that of the parent compound, but compounds **10**, **11** and **12** were much less effective even though they were not substituted at the 5-position. Compounds **4** and **7** possess an amido group at the 2-position, and these groups may be easily hydrolyzed to the amine (ATDA) and the corresponding carboxylic acid. In the case of compounds **10**, **11** and **12**, their carbonyl groups at the 2-position may be inactive because the adjacent groups are electrophilic, and these compounds can hardly release the active compound, ATDA. Compound **7** has been reported to have antitumor activity against P388 leukemia.[2d] The antitumor activity of compound **4** has not previously been described. Therefore, a comparative study on the antitumor activities of compound **1** (ATDA) and compound **4** against several tumors was carried out.

Table III indicates that both thiadiazoles are more effective than 6-MP against P388, B16 and 3LL than 6-MP, whereas 6-MP is more effective than the thiadiazoles against L1210. The antitumor activity of compound **4** against all tumors appeared to be higher than that of ATDA, while the toxicities of both compounds seemed to be similar (when 200 mg/kg of each

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TABLE II. Antitumor Activities of 2-Amino-1,3,4-thiadiazole Derivatives against Lewis Lung Carcinoma

Compound No.	Dose (mg/kg)	Survival days (mean ± S.D.)	%ILS[a]	60-day survivors[b]
1 (ATDA)	100	37.2 ± 4.8[c]	61	1
	10	27.0 ± 0.9	17	0
2	100	25.2 ± 1.6	9	0
3	100	23.3 ± 3.5	−1	0
4	100	38.3 ± 4.0[c]	66	1
	10	24.5 ± 1.3	6	0
5	100	26.3 ± 1.2	12	0
6	100	24.2 ± 3.3	3	0
7	100	37.0 ± 4.5[c]	60	1
	10	24.7 ± 3.1	7	0
8	100	24.5 ± 1.6	4	0
9	100	24.0 ± 3.4	2	0
10	100	25.6 ± 4.0	9	0
11	100	22.8 ± 3.0	−3	0
12	100	25.1 ± 3.0	7	0
6-MP	50	28.2 ± 2.1	20	0
5-FU	20	22.3 ± 0.7	−3	0
EX	40	26.2 ± 2.1	13	1
0.5% CMC		23.5 ± 1.2	—	0
Non-treated		23.1 ± 1.5	—	0

Treatment with each compound was started 24 h after the subcutaneous implantation of tumor cells (1×10^6) and the indicated dose was administered once a day for 10 d. Experiments were done with 6 mice per group. *a*) $[(T-C)/C] \times 100$; T, mean survival days of treated mice; C, mean survival days of control mice. *b*) Macroscopic tumor findings were negative. *c*) Significantly different from the control group ($p < 0.05$).

TABLE III. Antitumor Activities of 2-Amino-1,3,4-thiadiazole (**1**) and 2-Formamido-1,3,4-thiadiazole (**4**)

Compound No.	Dose (mg/kg)	P388 %ILS[a]	L1210 %ILS	B16 %ILS	3LL %ILS	3LL 60-day survivors[b]
1	200	Toxic	Toxic	Toxic	Toxic	
	100	95	43	38	33	5
	50	57	35	19	10	0
4	200	Toxic	Toxic	Toxic	Toxic	
	100	138	67	75	96	5
	50	82	53	54	34	0
	25	45	28	55	28	0
6-MP	50	32	NT	1	8	0
	30	NT	98	NT	NT	NT

Mice were inoculated with tumor cells (1×10^6 P388 cells, 1×10^5 L1210 cells, 1×10^6 B16 cells, or 5×10^5 3LL cells/mouse) on day 0 and were intraperitoneally administered once a day from day 1 to day 7 in the case of ascites-type tumors (P388 and L1210) or from day 1 to day 10 in the case of solid-type tumors (B16 and 3LL). Experiments with ascites-type tumors and solid-type tumors were done with 6 and 10 mice per group, respectively. The mean survival times of control mice bearing P388, L1210, B16 and 3LL were 9.8, 7.4, 25.3 and 27.4 d, respectively. *a*, *b*) Described in the footnotes to Table II. NT: Not tested.

compound was intraperitoneally injected once a day for 7 d, 10 out of 32 mice given ATDA and 14 out of 32 mice given compound **4** died at an early stage in the treatment period, but 100 mg/kg of these compounds did not cause any toxic symptoms in mice).

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Many investigators have studied the antitumor activity and the mechanism of action of ATDA.[4] ATDA inhibits inosinic acid (IMP) dehydrogenase by competing with nicotine adenine dinucleotide (NAD^+), depresses the *de novo* synthesis of xanthylic acid (XMP) from IMP and thus inhibits the synthesis of nucleic acids in the cells. It is also known that 6-MP inhibits the conversion of IMP to XMP by competition with IMP for IMP dehydrogenase.[5] In spite of the similarity of the sites of action of ATDA and 6-MP, a difference in the antitumor spectrum was observed in this study. This may be due to differences in the affinities of these compounds for tumor cells or in the extent of NAD^+ dependency of the biochemical reactions in tumor cells. Recently, it has been reported that 2,2′-(methylenediimino)bis-1,3,4-thiadiazole was converted *in vivo* to two ATDA molecules and one formaldehyde molecule and was more effective than the parent compound, but formaldehyde did not potentiate the effect of ATDA.[6] Thus, the difference in the activities of ATDA and compound **4** may not be due to formaldehyde released from compound **4** showing antitumor activity itself or potentiating the effect of ATDA, but may arise because the membrane permeability of compound **4** is higher than that of ATDA (amides are generally more lipophilic than the corresponding amines).

In conclusion, the results of this study indicate that ATDA and its analogs are effective against several tumors, including 3LL, and raise the possibility that a simple modification such as formylation of the amino group of an antitumor agent might enhance the activity.

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