[Chem. Pharm. Bull. 30(12)4402—4406(1982)]

Antitumor Activity of 2-Acylamino-1,3,4-thiadiazoles and Related Compounds

Michiko Miyahara,* Masahiro Nakadate, Shoko Sueyoshi, Masayuki Tanno, Makoto Miyahara, and Shozo Kamiya

National Institute of Hygienic Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158, Japan

(Received June 14, 1982)

2-Nitrosoureido-1,3,4-thiadiazoles were effective against both mouse lymphoid leukemia L1210 and rat ascites hepatoma AH13. Some 2-acylamino-1,3,4-thiadiazoles were also effective against L1210. The anti-L1210 action of these 2-acylamino-1,3,4-thiadiazoles was blocked by administration of nicotinamide. Among these compounds, 2-propanoylamino-1,3,4-thiadiazole was the most effective against L1210, with a maximum *T/C* % of 166.

Keywords——2-acylamino-1,3,4-thiadiazole; 2-amino-1,3,4-thiadiazole; nicotinamide antagonist; AH13; L1210; 2-nitrosoureido-1,3,4-thiadiazole; antitumor activity

In 1955 Oleson *et al.*[1] reported antitumor activity of 2-amino-1,3,4-thiadiazoles against S91 melanoma, 8110 glioblastoma and 6C3HED lymphosarcoma on intraperitoneal treatment, and since then, much further work has been done on these compounds.[2] Recently, the ureido derivatives of thiazole, 1,3,4-thiadiazole and related compounds were also shown to have antileukemic activities.[3] The antitumor mechanism has been explained in terms of the inhibition in guanosine monophosphate (GMP) biosynthesis at the step of conversion of inosine monophosphate (IMP) to xanthosine 5′-phosphate.[4]

This paper describes the anitutmor activity of 2-acylamino-1,3,4-thiadiazoles and related compounds, which are expected to decompose *in vivo* to produce the corresponding 2-amino-1,3,4-thiadiazoles, against rat ascites hepatoma AH13 and mouse lymphoid leukemia L1210. The antitumor mechanism is discussed.

Materials and Methods

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a JASCO A-102 spectrophotometer. ^{1}H-Nuclear magnetic resonance (NMR) spectra were measured with a Varian EM 360A spectrometer and ^{13}C-NMR spectra with a JEOL FX-200 spectrometer, with tetramethylsilane as an internal standard and dimethylsulfoxide-d_6 (DMSO-d_6) as the solvent. Mass spectra (MS) were obtained on a JEOL UMS-01SG-2 mass spectrometer.

Materials——1-Methyl-3-(1,3,4-thiadiazol-2-yl)urea (**1**): mp 231°C. IR ν_{max}^{Nujol} cm^{-1}: 3350 (NH), 3050 (NH), 1640 (CO). *Anal.* Calcd for $C_4H_6N_4OS$: C, 30.37; H, 3.82; N, 35.42. Found: C, 30.39; H, 3.90; N, 35.17.

1-Methyl-1-nitroso-3-(1,3,4-thiadiazol-2-yl)urea (**2**): mp 128°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3400 (NH), 1695 (CO), 1485 (NO). *Anal.* Calcd for $C_4H_5N_5O_2S$: C, 25.67; H, 2.69; N, 37.42. Found: C, 25.92; H, 2.81; N, 37.44.

1-(2-Chloroethyl)-3-(1,3,4-thiadiazol-2-yl)urea (**3**): mp 161—162°C. IR ν_{max}^{Nujol} cm^{-1}: 3370 (NH), 3150 (NH), 1680 (CO), 1485 (NO). ^{1}H-NMR (DMSO-d_6) δ (ppm): 11.08 (1H, br, –NHCO–), 9.02 (1H, s, H–C5), 6.85 (1H, t, –N<u>H</u>CH$_2$–), 3.7—3.4 (m, –CH$_2$CH$_2$–). *Anal.* Calcd for $C_5H_7ClN_4OS \cdot 1/4H_2O$: C, 28.44; H, 3.58; N, 26.53. Found: C, 28.53; H, 3.40; N, 26.73.

1-(2-Chloroethyl)-1-nitroso-3-(1,3,4-thiadiazol-2-yl)urea (**4**): mp 123°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3060 (NH), 1700 (CO), 1500 (NO). ^{1}H-NMR (DMSO-d_6) δ (ppm): *ca.* 9.5 (1H, br, –NHCO–), 9.15 (1H, s, H–C5), 4.20 (2H, t, –CH$_2$CH$_2$–), 3.65 (2H, t, –CH$_2$CH$_2$–).

1,1′-Hexamethylene-bis[3-(1,3,4-thiadiazol-2-yl)]urea (**5**): mp 242°C. IR ν_{max}^{Nujol} cm^{-1}: 3340 (NH), 1638 (CO). ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (1H, br, –NHCO–), 8.98 (1H, s, H–C5), 6.59 (1H, t, –N<u>H</u>CH$_2$–), 3.4—3.3 (2H, m, –NHC<u>H</u>$_2$–), 1.8—1.1 (m, –(CH$_2$)$_4$–). *Anal.* Calcd for $C_{12}H_{18}N_8O_2S_2$: C, 38.91; H, 4.90; N, 30.25. Found: C, 39.02; H, 5.05; N, 29.53.

1,1′-Hexamethylene-bis[1-nitroso-3-(1,3,4-thiadiazol-2-yl)]urea (**6**): mp 163°C (dec.). IR ν_{max}^{Nujol} cm^{-1}:

3340 (NH), 1700 (CO), 1490 (NO). ^{1}H-NMR (DMSO-d_6) δ (ppm): 9.07 (1H, s, H-C5), 4.1—3.6 (mul, -N(NO)-CH_2-), 1.6—1.1 (m, -$(CH_2)_4$-).

2-Acetamido-1,3,4-thiadiazole (**7**): mp 268°C. (lit.,[5] 268°C). IR ν_{max}^{Nujol} cm^{-1}: 3150 (NH), 1658 (CO).

2-Benzoylamino-1,3,4-thiadiazole (**8**): mp 206—207°C. (lit.,[5] mp 213—214°C). IR ν_{max}^{Nujol} cm^{-1}: 3110 (NH), 1670 (CO). *Anal.* Calcd for $C_9H_7N_3OS$: C, 52.67; H, 3.44; N, 20.47. Found: C, 52.31; H, 3.45; N, 20.48.

2-(4-Methoxybenzoyl)amino-1,3,4-thiadiazole (**9**): mp 227—228°C. (lit.,[6] mp 234—235°C). IR ν_{max}^{Nujol} cm^{-1}: 3150 (NH), 1656 (CO), 1250 (OCH_3).

2-(4-Nitrobenzoyl)amino-1,3,4-thiadiazole (**10**): mp >280°C. IR ν_{max}^{Nujol} cm^{-1}: 3090 (NH), 1656 (CO). *Anal.* Calcd for $C_9H_6N_4O_3S \cdot 1/4H_2O$: C, 42.44; H, 2.57; N, 21.99. Found: C, 43.66; H, 2.52; N, 21.52.

2-(4-Chlorobenzoyl)amino-1,3,4-thiadiazole (**11**): mp 258—259°C. IR ν_{max}^{Nujol} cm^{-1}: 3140 (NH), 1665 (CO). *Anal.* Calcd for $C_9H_6ClN_3OS$: C, 45.10; H, 2.52; N, 17.53. Found: C, 44.99; H, 2.56; N, 17.55.

2-Propanoylamino-1,3,4-thiadiazole (**12**): mp 232°C. (lit.,[7] 230—232°C). IR ν_{max}^{Nujol} cm^{-1}: 3150 (NH), 1685 (CO). *Anal.* Calcd for $C_5H_7N_3OS$: C, 38.21; H, 4.49; N, 26.73. Found: C, 38.41; H, 4.50; N, 26.63.

2-Butanoylamino-1,3,4-thiadiazole (**13**): mp 174—175°C. IR ν_{max}^{Nujol} cm^{-1}: 3150 (NH), 1680 (CO). *Anal.* Calcd for $C_6H_9N_3OS$: C, 42.09; H, 5.30; N, 24.54. Found: C, 42.10; H, 5.30; N, 24.47.

2-Phenylacetylamino-1,3,4-thiadiazole (**14**): mp 223°C. IR ν_{max}^{Nujol} cm^{-1}: 3100 (NH), 1670 (CO). ^{1}H-NMR (DMSO-d_6) δ (ppm): 12.75 (1H, br, -NH-), 9.17 (1H, s, H-C5), 7.29 (5H, s, -C_6H_5), 3.85 (2H, s, -CH_2-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 169.471 (CO), 158.500 (C-2), 147.238 (C-5), 134.516—126.804 (-C_6H_5), 41.554 (-CH_2-). MS *m/e*: 221 (M^++2), 219 (M^+), 91 (C_7H_7), 85 (C_2HN_2S).

Sulfamethizole (**15**): Japanese Pharmacopeia X.

1-Methyl-3-(5-mercapto-1,3,4-thiadiazole-2-yl)urea (**16**): mp 243—244°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3350 (NH), 3080 (NH), 1650 (CO). *Anal.* Calcd for $C_4H_6N_4OS_2$: C, 25.31; H, 3.26; N, 29.12. Found: C, 25.25; H, 3.18; N, 29.45.

2-Acetamido-5-benzylthio-1,3,4-thiadiazole (**17**): mp 167—169°C. Purchased from Aldrich Chem. Co.

2-Azido-5-methyl-1,3,4-thiadiazole (**18**): mp 89.5—90.5°C. IR ν_{max}^{Nujol} cm^{-1}: 2130 (N_3). *Anal.* Calcd for $C_3H_3N_5S$: C, 25.52; H, 2.14; N, 49.62. Found: C, 25.45; H, 2.02; N, 49.52.

1,3-Bis(5-methyl-1,3,4-thiazol-2-yl)triazene (**19**): mp 243—244°C (dec.). *Anal.* Calcd for $C_6H_7N_7S_2$: C, 29.87; H, 2.92; N, 40.64. Found: C, 29.70; H, 2.93; N, 39.33.

2-Amino-1,3,4-thiadiazole (**20**): mp 193°C (lit.,[8] mp 191—192°C).

Hydrolysis of 2-Benzoylamino-1,3,4-thiadiazole in Hydrochloric Acid——2-Benzoylamino-1,3,4-thiadiazole (140 mg) was heated in 20 ml of 20% hydrochloric acid at 90—100°C for 4 h. The reaction mixture was cooled and neutralized to pH 7 with 20% sodium hydroxide solution, and the precipitate which formed was filtered off and recrystallized from ether. The product was found to be identical with authentic benzoic acid by infrared (IR) spectroscopy (mp 119°C, Merck Index, mp 122.4°C). Yield, 36 mg (43%). The filtered solution was evaporated to dryness. The residue was dissolved in hot acetone and the filtered acetone solution was evaporated to dryness to yield 2-Amino-1,3,4-thiadiazole, whose IR spectrum was indistinguishable from that of an authentic sample (mp 187—189°C, lit.,[8] mp 191—192°C). Yield, 33 mg (48%).

Methods——AH13 Test System: Details were given in our previous paper.[9] Criteria for antitumor activity are as follows. −; 0/6 survival rate at the 60th day. ±; 1/6 survival rate at the 60th day, or 0/6 suvival rate but with survival time increased to more than twice that of the control. +; 2/6—3/6 survival rate at the 60th day. ++; 4/6—6/6 survival rate at the 60th day.

L1210 Test System: L1210 cells, provided by the Cancer Chemotherapy Center, Tokyo, were propagated by intraperitoneal inoculation in DBA/2 mice, and CDF_1 mice were used as test animals. These mice were supplied by Shizuoka Agricultural Co. Association for Laboratory Animals. The test compounds were intraperitoneally administered after intraperitoneal inoculation of 10^5 L1210 cells per mouse on the following schedule; 1) Days 2 and 6 (3 mice/group) 2) Days 1—10 (except on the sixth day after inoculation, 6 mice/group) 3) Day 1 only (10 mice/group). When combination treatment with nicotinamide and a test compound was employed, nicotinamide was given 30 minutes before the 1,3,4-thiadiazole treatment. Antitumor activity was evaluated in terms of *T/C* % (*T*=mean survival time of treated animals, *C*=mean survival time of control animals). A *T/C* % value of 120—124 was considered to indicate slight effectiveness and one of 125 or more indicated effective antitumor activity against L1210.

Results and Discussion

The effects of compounds **1**—**20** on the lifespan of mice bearing L1210 are shown in Table I. Compounds **2**, **4**, **9**, **10**, **11**, **12**, **13**, **17** and **18** were effective with maximum *T/C* % values of 171, 188, 123, 136, 134, 166, 134, 129 and 124, respectively, on the days 2 and 6 treatment. The reference compound **20** showed a maximum *T/C* % of 138.

Among the tested compounds shown in Table I, only the nitrosoureido derivatives,

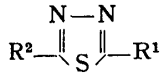
compounds **2** and **6**, were effective against AH13 (± and +, respectively). The other tested compounds were all ineffective against AH13.

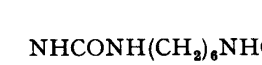
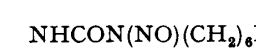
Thus, 2-acylamino-1,3,4-thiadiazoles were effective only against L1210 and the two 1,3,4-thiadiazoles containing a nitrosoureido group were effective against not only L1210 but also AH13. The antitumor mechanism of the latter compounds may therefore be different from that of the former compounds. Among the former compounds, 2-propanoylamino-1,3,4-thiadiazole (**12**) was the most effective (T/C %: 166). The anti-L1210 activities of the two 2-nitrosoureido-1,3,4-thiadiazoles were less than those of typical cancer chemotherapy agents, such as 1,3-bis(2-chloroethyl)-1-nitrosourea and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. Moreover, no synergistic effect due to having two antitumor chemical functional moieties, 2-amino-1,3,4-thiadiazole and alkyl-nitrosoureido, could be observed.

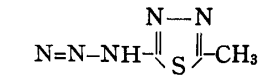
As shown in Table II, the effect of serial administration of compound **12** on the survival time of mice bearing L1210 under the days 1—10 treatment (max. T/C %: 157 at total dose of 1215 mg/kg) was similar to that of the days 2 and 6 treatment (max. T/C %: 166 at total dose of 800 mg/kg). On the other hand, the effect of the reference compound **20** depended on the schedule, and administration on days 1—10 was the most effective (Tables I, II and III).

The antileukemic action of compounds **12** and **20** in mice bearing L1210 was reversed by administration of nicotinamide, as shown in Table III. Single administration of nicotinamide

Table I. Antitumor Effects of the 2-Acylamino-1,3,4-thiadiazoles and Related Compounds on the Survival Time of Mice bearing L1210 and Rats bearing AH13



Compd. No.*	R^1	R^2	L1210 Dose[a] (mg/kg)	L1210 T/C %	AH13 Evaluation[b]
1	$NHCONHCH_3$	H	25	100	
			50	109	
			100	113	−
			200	66	
2	$NHCON(NO)CH_3$	H	25	125	
			50	141	±
			100	171	
3	$NHCONHCH_2CH_2Cl$	H	100	100	
			200	113	−
			400	100	
4	$NHCON(NO)CH_2CH_2Cl$	H	50	146	
			100	188	−
			200	toxic	
5		H	100	104	
			200	100	−
			400	96	
6		H	50	109	
			100	109	+
			200	116	
7	$NHCOCH_3$	H	100	98	
			200	102	−
			400	109	
8	$NHCOC_6H_5$	H	100	112	
			200	115	−
			400	119	
9	$NHCOC_6H_4OCH_3$	H	100	115	
			200	115	−
			400	123	
10	$NHCOC_6H_4NO_2$	H	100	136	
			200	123	−
			400	127	

Compd. No.	R^1	R^2	L1210 Dose[a] (mg/kg)	L1210 T/C %	AH13 Evaluation[b]
11	$NHCOC_6H_4Cl$	H	100	121	
			200	121	–
			400	134	
12	$NHCOCH_2CH_3$	H	100	116	
			200	129	–
			400	166	
13	$NHCOCH_2CH_2CH_3$	H	100	116	
			200	134	–
			400	134	
14	$NHCOCH_2C_6H_5$	H	100	105	
			200	95	–
			400	113	
15	$NHSO_2C_6H_4NH_2$	CH_3	100	101	
			200	105	–
			400	95	
16	$NHCONHCH_3$	SH	100	113	
			200	100	–
			400	95	
17	$NHCOCH_3$	$C_6H_5CH_2S$	100	129	
			200	124	–
			400	110	
18	N_3	CH_3	100	114	
			200	114	–
			400	124	
19		CH_3	100	114	
			200	114	–
			400	100	
20	NH_2	H	100	125	
			200	134	–
			400	138	

a) All compounds were administered intraperitoneally on days 2 and 6 after inoculation.
b) All compounds were administered intraperitoneally on days 3—7 after inoculation. Criteria are described in "Materials and Methods."

TABLE II. Effects of Serial Administration of 1,3,4-Thiadiazoles on the Survival Time of Mice bearing L1210 (Days 1—5 and 7—10)

Compd. No.	T/C % at doses (mg/kg) of				
	27	40	60	90	135
12	105	96	103	128	157
20	153	165	151	132	111

on day 1 had no effect against L1210. However, when combination treatment was employed, the nicotinamide treatment depressed the antitumor effect of compounds **12** and **20**. Consequently, these 1,3,4-thiadiazoles, except for the 2-nitrosoureido-1,3,4-thiadiazoles, are nicotinamide antagonists which inhibit purine nucleotide biosynthesis. However, the antitumor effect of 2-nitrosoureido-1,3,4-thiadiazole, compound **2**, was not affected by administration of nicotinamide (Table III). The antitumor effect of compounds **2**, **4** and **6** may therefore be mainly due to alkylation.

Hydrolysis of 2-benzoylamino-1,3,4-thiadiazole in 20% hydrochloric acid gave 2-amino-1,3,4-thiadiazole and benzoic acid in 48% and 43% yields, respectively. From this experiment, it can be presumed that 2-acylamino-1,3,4-thiadiazoles (compounds **7**—**15** and **17**) will be hydrolyzed *in vivo*, perhaps enzymatically, to produce the corresponding 2-amino-1,3,4-

Table III. Reversal Effects of Nicotinamide on Antileukemic Action of 1,3,4-Thiadiazoles in Mice with L1210

Treatment day 1, *i.p.*	Dose (mg/kg)	*T/C* %
Control	500	100
Nicotinamide	500	103
Compound **12**	500	147
Nicotinamide+Compound **12**	500+500	103
Compound **20**	500	129
Nicotinamide+Compound **21**	500+500	107
Compound **2**	150	152
Nicotinamide+Compound **2**	500+150	179

All compounds were administered intraperitoneally on the 1st day after inoculation. When combination treatment was employed, nicotinamide was given 30 min prior to the 1,3,4-thiadiazoles.

thiadiazole, and the 2-amino-1,3,4-thiadiazole may act as a coenzyme analog to inhibit IMP dehydrogenase in purine biosynthesis.[2a] Some azides[10] and triazenes[11] also give the amino compound in acidic or alkaline solution. In spite of the possibility of the formation of amino-thiadiazole, however, a triazene derivative (compound **19**) was ineffective.

Compound **12** was as effective against L1210 as other inhibitors of purine biosynthesis,[12] including 6-mercaptopurine, 6-thioinosine and 6-thioguanine, so further studies with other animals and tumors may be worthwhile to determine its antitumor spectrum.

Acknowledgement We are grateful to Dr. Shigeru Tsukagoshi, Cancer Chemotherapy Center, Tokyo, for providing animals bearing tumors and for helpful advice. We are also grateful to Dr. Kenzo Kanohda of this institute for mass spectral measurement.

References

1) J.J. Oleson, A. Sloboda, W.P. Troy, S.L. Halliday, M.J. Landes, R.B. Angier, J. Semb, K. Cyr and J.H. Williams, *J. Am. Chem. Soc.*, **77**, 6713 (1955).
2) *a*) M.M. Ciotti, S.R. Humphreys, J.M. Venditti, N.O. Kaplan and A. Goldin, *Cancer Res.*, **20**, 1195 (1960); H.F. Oettfen, J.A. Reppert, V. Coley and J.H. Burchenal, *Cancer Res.*, **20**, 1597 (1960); S.R. Humphreys, J.M. Venditti, C.J. Ciotti, I. Kline, A. Goldin and N.O. Kaplan, *Cancer*, **22**, 483 (1962); T. Matsumoto, K. Ootsu and Y. Okada, *Cancer Chemother. Rep.*, **58**, 331 (1974); K. Takatori, K. Imai, S. Nakano, T. Hasegawa and S. Asano, *Yakugaku Zasshi*, **96**, 471 (1976).
3) R.K.Y. Zee-Cheng and C.C. Cheng, *J. Med. Chem.*, **22**, 28 (1979).
4) K. Tsukamoto, M. Suno, K. Igarashi, Y. Kozai and Y. Sugino, *Cancer Res.*, **35**, 2631 (1975); J.A. Nelson, L.M. Rose and L.L. Bennett, Jr., *Cancer Res.*, **36**, 1375 (1976); J.A. Nelson, L.M. Rose and Bennett, Jr., *Cancer Res.*, **37**, 182 (1977).
5) G. Werber, F. Buccheri and M. Gentile, *J. Heterocycl. Chem.*, **14**, 1263 (1977).
6) A.M. Granr, S.V. Krees, A.B. Mauger, W.J. Rzeszotarski, and F.W. Wolff, *J. Med. Chem.*, **15**, 1082 (1972).
7) F. Ueda, T. Ueda and S. Toyoshima, *Yakugaku Zasshi*, **79**, 920 (1959).
8) M. Kanaoka, *Yakugaku Zasshi*, **75**, 1149 (1955).
9) Mi. Miyahara, M. Miyahara, M. Nakadate, I. Suzuki and S. Odashima, *Gann*, **69**, 187 (1978).
10) K. Clusius and H. Hurzeler, *Helv. Chim. Acta*, **37**, 383 (1954); K. Clusius and M. Vecchi, *Ann. Chem.*, **607**, 16 (1957).
11) R.J. LeBlanc and K. Vaughan, *Can. J. Chem.*, **50**, 2544 (1972).
12) M. Fukui, M. Inaba, S. Tsukagoshi, and Y. Sakurai, *Cancer. Res.*, **42**, 1098 (1982).