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Original paper

Compounds with potential anti-tumor activity VII. Synthesis and anti-tumor activity of 1-aryl-*N,N'*-di(1,3,4-thiadiazol-2-yl)methylenediamines

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Summary — The synthesis of a series of 1-aryl-*N,N'*-di(1,3,4-thiadiazol-2-yl)methylenediamines is reported. The compounds are obtained by means of reaction between 2-amino-1,3,4-thiadiazole and aromatic aldehydes. The title compounds were evaluated against the leukemia P388 tumor system in mice and some of them were found to be active. 1-(2,6-Dichlorophenyl)-*N,N'*-di(1,3,4-thiadiazol-2-yl)methylenediamine **1**, whose significant activity against P388 leukemia was reported in a previous paper, was tested against other experimental tumors. It exhibits significant activity against L1210 leukemia and M 5076 ascitic sarcoma.

Résumé — **Composés à activité anti-tumorale potentielle VII. Synthèse et activité anti-tumorale d'aryl-1 *N,N'*-di-(thiadiazol-1,3,4 yl-2) méthylènediamines.** *Des nouvelles aryl-1* N,N'*-di(thiadiazol-1,3,4 yl-2) méthylènediamines ont été synthétisées par une réaction de condensation de l'amino-2 thiadiazole-1,3,4 avec des aldéhydes aromatiques. Les composés obtenus ont été testés pour leurs propriétés anti-tumorales dans la leucémie expérimentale P388 sur les souris et certains composés se sont montrés actifs. De plus, la (dichloro-2,6 phényl)-1* N,N'*-di(thiadiazol-1,3,4 yl-2) méthylènediamine dont une activité significative dans la leucémie P388 a été précédemment reportée, s'est avérée active dans la leucémie L1210 et le sarcome M 5076.*

1,3,4-thiadiazole derivatives / *N,N'*-di(1,3,4-thiadiazol-2-yl)methylenediamines / P388 lymphocytic leukemia / anti-tumor activity

Introduction

The anti-tumor activities of 2-amino-1,3,4-thiadiazole (NSC 4728) and many of its derivatives have been reported by numerous investigators [1–8]. For several years, anti-neoplastic clinical trials had been limited due to marked hyperuricemia [9]. Recent studies have shown that concomitant administration of the xanthine oxidase inhibitor, allopurinol, prevents the dose-limiting toxic effect to hyperuricemia [10–12] and have renewed attention in 2-amino-1,3,4-thiadiazole and related compounds [13, 14].

In particular, *N,N'*-di(1,3,4-thiadiazol-2-yl)methylenediamine (NSC 143019) showed a higher and more rapid anti-tumor effect than that of the parent compound (NSC 4738). Consequently, we found it worthwhile to extend our research to derivatives containing a series of substituents at the methylene group, in order to explore the influence of these molecular modifications on anti-leukemic activity.

Recently, the synthesis and anti-leukemic evaluation of a series of 1-arylsubstituted *N,N'*-di(1,3,4-thiadiazol-2-yl)-methylenediamines were reported from our laboratories [15] and many of them were found to be active against murine P388 leukemia. In particular, high anti-leukemic activity (*T/C* 192%) was demonstrated for 1-(2,6-dichlorophenyl)-*N,N'*-di(1,3,4-thiadiazol-2-yl)methylene diamine **1** which was designated a 'selected agent compound' by the National Cancer Institute, Bethesda, MD, U.S.A., for evaluation *versus* human xenograft passaged in athymic mice and other murine tumors. This report outlines the synthesis and the evaluation against P388 leukemia of new *N,N'*-di(1,3,4-thiadiazol-2-yl)methylene diamines **4** and the results of the screening of compound **1** against additional experimental tumors.

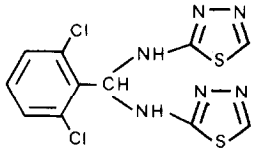

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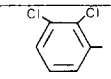
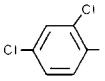
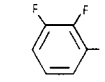
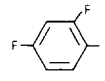
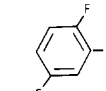
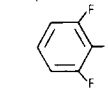
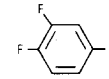
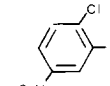
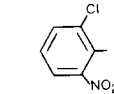
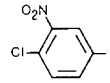
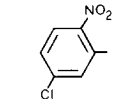
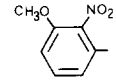
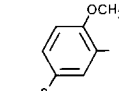
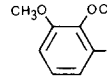
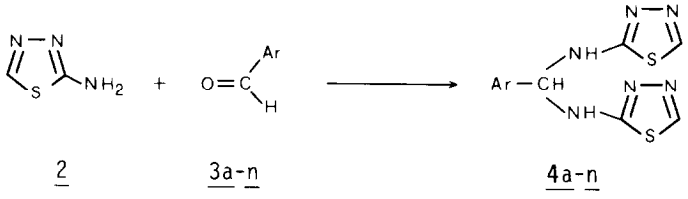
Table I. 1-Aryl-*N,N'*-di(1,3,4-thiadiazol-2-yl)methylenediamines **4a–n**.

Compd	Ar	Yield[a] (%)	mp (°C)	Formula	IR (cm^{-1})	^1H NMR (DMSO-d_6)[b]
4a		55	155-156	$C_{11}H_8Cl_2N_6S_2$	3368 3187	6.78 (t, 1H, J=6.5, CH); 7.26-7.86 (m, 3H, Ar); 8.72 (s, 2H, CH-5'); 8.78 (d, 2H, J=6.5, NH).
4b		32	118-120	$C_{11}H_8Cl_2N_6S_2$	3315	6.7 (br t, 1H, CH); 7.3-7.96 (m, 3H, Ar); 8.68 (br s, 4H, CH-5' and NH).
4c		49	154-155	$C_{11}H_8F_2N_6S_2$	3320 3280	6.78 (t, 1H, J=6, CH); 7.06-7.76 (m, 3H, Ar); 8.71 (s, 2H, CH-5'); 8.76 (d, 2H, J=6, NH).
4d		58	142-144	$C_{11}H_8F_2N_6S_2$	3340 3190	6.71 (t, 1H, J=6, CH); 6.93-7.88 (m, 3H, Ar); 8.67 (s, 2H, CH-5'); 8.71 (d, 2H, J=6, NH).
4e		92	148-150	$C_{11}H_8F_2N_6S_2$	3200 3115	6.71 (t, 1H, J=6.5, CH); 7.06-7.56 (m, 3H, Ar); 8.68 (s, 2H, CH-5'); 8.75 (d, 2H, J=6.5, NH).
4f		82	170-171	$C_{11}H_8F_2N_6S_2$	3370 3190	6.9 (t, 1H, J=6, CH); 6.96-7.56 (m, 3H, Ar); 8.59 (s, 2H, CH-5'); 8.66 (d, 2H, J=6, NH).
4g		44	155-156	$C_{11}H_8F_2N_6S_2$	3335	6.48 (br t, 1H, CH); 7.25-7.85 (m, 3H, Ar); 8.73 (br s, 4H, CH-5' and NH).
4h		78	175-177	$C_{11}H_8ClN_7O_2S_2$	3315	6.86 (t, 1H, J=6, CH); 7.81 (d, 1H, $J_{3,4}$=8.5, H-3 Ar); 8.25 (dd, 1H, $J_{4,3}$=8.5, $J_{4,6}$=2.5, H-4 Ar); 8.48 (d, 1H, $J_{6,4}$=2.5, H-6 Ar); 8.73 (s, 2H, CH-5'); 8.91 (d, 2H, J=6, NH).
4i		58	161-162	$C_{11}H_8ClN_7O_2S_2$	3240 3160	6.96 (t, 1H, J=6, CH); 7.28-7.95 (m, 3H, Ar); 8.58 (d, 2H, J=6, NH); 8.61 (s, 2H, CH-5').
4j		47	153-155	$C_{11}H_8ClN_7O_2S_2$	3320	6.55 (t, 1H, J=6.5, CH); 7.81 (m, 2H, H-5 and H-6 Ar); 8.22 (d, 1H, $J_{2,6}$=2, H-2 Ar); 8.72 (s, 2H, CH-5'); 8.84 (d, 2H, J=6.5, NH).
4k		48	158-160	$C_{11}H_8ClN_7O_2S_2$	3325 3305	6.96 (br t, 1H, CH); 7.61 (dd, 1H, $J_{4,3}$=8.5, $J_{4,6}$=2, H-4 Ar); 7.71 (d, 1H, $J_{6,4}$=2, H-6 Ar); 8.01 (d, 1H, $J_{3,4}$=8.5, H-3 Ar); 8.65 (br s, 4H, CH-5' and NH).
4l		46	138-140	$C_{12}H_{11}N_7O_3S_2$	3388 3368	3.83 (s, 3H, OCH_3); 6.56 (t, 1H, J=7, CH); 7.03-7.71 (m, 3H, Ar); 8.63 (s, 2H, CH-5'); 8.72 (d, 2H, J=7, NH).
4m		45	150-152	$C_{12}H_{11}BrN_6OS_2$	3345 3188	3.8 (s, 3H, OCH_3); 6.61 (t, 1H, J=6.5, CH); 6.95 (d, 1H, $J_{3,4}$=8, H-3 Ar); 7.43 (dd, 1H, $J_{4,3}$=8, $J_{4,6}$=2, H-4 Ar); 7.51 (d, 1H, $J_{6,4}$=2, H-6 Ar); 8.52 (d, 2H, J=6.5, NH); 8.60 (s, 2H, CH-5').
4n		42	140-141	$C_{13}H_{14}N_6O_2S_2$	3320	3.8 (s, 6H, OCH_3); 6.73 (t, 1H, J=6.5, CH); 7.06 (s, 3H, Ar); 8.55 (d, 2H, J=6.5, NH); 8.65 (s, 2H, CH-5').

[a]Yield of pure, isolated product.
[b]δ in ppm downfield from TMS; *J* values are in Hz.

Chemistry

The 1-aryl-*N*,*N*′-di(1,3,4-thiadiazol-2-yl)methylenediamines **(4a–n)** were obtained in good yields by condensation of 2-amino-1,3,4-thiadiazole **2** with an equimolecular amount of the appropriate disubstituted aromatic aldehyde **(3a–n)** as shown in Scheme 1. The structure of the obtained compounds was ascertained by means of IR and ^{1}H NMR spectral data which are reported in Table I and supported by satisfactory elemental analysis. In the ^{1}H NMR spectra the benzylic hydrogen couples with the NH protons. The NH signals disappeared and the ArCH signal became a singlet upon addition of deuterium oxide.



2 3a-n 4a-n

Scheme 1.

Pharmacological results and Discussion

Table II indicates the results of the evaluation of the title compounds *versus* P388 lymphocytic leukemia in mice. Only compounds **4b**, **4e**, **4f** and **4j** increased the median survival time of mice with a *T*/*C*% maximum of 131, 174, 160 and 150, respectively. A Topliss analysis [16] of antineoplastic activities of **4a–n** did not reveal π or σ dependencies and no unfavorable steric effect was noted.

Also the stability of compounds of our series was examined under simulated physiological conditions (phosphate buffer, pH 7.4, at 37°C) and no difference was observed between active and inactive compounds. All derivatives are stable until 40°C but they are totally hydrolized at higher temperature.

Table III reports the screening of **1** against various murine tumors and *versus* human xenograft implanted in mice. Apart from being a good P388 anti-leukemic (*T*/*C* 192%), as previously reported, **1** is particularly active against lymphocytic leukemia L1210 (*T*/*C* 223%), more than the parent 2-amino-1,3,4-thiadiazole [13], and against ascitic sarcoma M 5076 (*T*/*C* 195%).

Table II. P388 murine lymphocytic leukemia anti-tumor activity.

Compd.	Dose (mg/kg)	*T*/*C*%	Compd.	Dose (mg/kg)	*T*/*C*%
4a	240	96	**4h**	240	119
	120	100		120	104
	60	89		60	101
4b	400	131	**4i**	240	118
	200	114		120	106
	100	109		60	96
4c	200	93	**4j**	240	150
	100	93		120	137
	50	99		60	109
4d	240	107	**4k**	240	126
	120	96		120	106
	60	105		60	107
4e	240	174	**4l**	240	101
	120	126		120	98
	60	119		60	98
4f	240	160	**4m**	240	90
	120	109		120	92
	60	108		60	87
4g	240	100	**4n**	240	100
	120	101		120	91
	60	101		60	87

Table III. Evaluation of compound **1** against various tumors in mice.

Tumor system	Sex	Dose (mg/kg)	Toxicity day survivors	b.wt. change[a] (g)	*T/C* (%)
P388	F	400	6/6	−0.8	192
lymphocytic		200	6/6	−1.0	174
leukemia		100	6/6	−0.4	148
	M	400	6/6	−2.4	186
		200	6/6	−1.1	162
		100	6/6	0.8	127
		50	6/6	0.4	124
L1210	M	400	5/5	0.9	223
lymphocytic		240	6/6	0.7	171
leukemia		144	5/5	0.5	151
		100	6/6	−1.0	134
		50	6/6	−0.4	131
M 5076	F	800	10/10	−2.0	195
ascitic		400	10/10	−0.8	164
sarcoma		200	10/10	−0.2	137
		100	10/10	0.6	125
		50	10/10	−0.6	131
	M	800	9/10	−2.0	157
		400	10/10	0.2	136
		200	10/10	−0.9	136
		100	10/10	−0.1	142
		50	10/10	−0.2	122
LOX	F	200	6/6	−2.9	126
amelanotic		100	6/6	−2.1	106
melanoma		50	6/6	−2.1	106
Human mammary	F	400	4/4	−1.7	44
carcinoma MX-1		200	6/6	−1.7	73
xenograft		100	6/6	−1.5	94

[a]Body weight change difference (g) computed between toxicity day and day 1.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded in hexachlorobutadiene on a Perkin–Elmer model 257 spectrophotometer. ^{1}H NMR spectra were measured with a Bruker WP 80 SY spectrometer in DMSO-d_6 using tetramethylsilane as the internal standard: chemical shifts are expressed in δ (ppm) and coupling constants (*J*) in Hz.

Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} Merck 5 × 10 (0.025 mm) plates using $CHCl_3/CH_3OH$ (8:2) as the eluant. Elemental analyses (C, H, N, S) were obtained on a C. Erba model 1106 elemental analyzer and were within ±0.4% of the theoretical values.

General procedure for the synthesis of compounds ***4a–n***

To 5.05 g (0.05 mol) of 2-amino-1,3,4-thiadiazole **2** was added the equimolecular amount of the appropriate aldehyde (**3a–n**). The reaction mixture, in the absence of solvent, was vigorously stirred at 160°C for a few minutes to obtain a complete fusion. After cooling, the crude product obtained was purified by washing repeatedly with ethanol and diethyl ether. All attempts to recrystallize the compounds failed because decomposition occurred upon heating. However, final products were shown to be pure by TLC.

Pharmacology

All the biological data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Can-

cer Treatment, National Cancer Institute, Bethesda, MD, U.S.A., according to their protocols [17].

P388 lymphocytic leukemia
The tumor inoculum consisted of 10^6 ascites cells, injected i.p. into CD_2F_1 mice on day 0. The compounds **4a–n** (Table II) were administered i.p. in saline with Tween 80 or in hydroxypropylcellulose daily for 5 days starting on day 1. Activity was expressed as $T/C \times 100$, T being the median survival time of treated animals and C being the median survival time of control animals. A T/C value $\geq 127\%$ is considered necessary to demonstrate moderate activity. A T/C value $\geq 175\%$ is considered significant activity. A T/C value $<85\%$ indicates a toxic test.

Compound **1** was also tested against other tumors (Table III) as reported below.

L1210 lymphocytic leukemia
L1210 lymphocytic leukemia (0.1 ml of diluted ascitic fluid containing 10^5 cells) was injected i.p. into male CD_2F_1 mice on day 0; compound **1**, as a suspension in saline with Tween 80, was injected on day 1 and the treatment continued daily for a total of 5 injections. The final evaluation day was day 30 and toxicity was evaluated on day 5. The parameter measured was median survival time: an initial $T/C \geq 125\%$ is considered necessary to demonstrate moderate activity. A reproducible $T/C \geq 150\%$ is considered significant activity. A $T/C < 86\%$ indicates toxicity.

M 5076 ascitic sarcoma
M 5076 ascitic sarcoma, 10^6 cells of ascitic fluid diluted up to 0.1 ml, was injected i.p. into $B_6C_3F_2$ mice (male or female) on day 0; compound **1**, as a suspension in saline with Tween 80, was injected i.p. every 4th day for a total of 4 injections beginning on day 1. The final day of testing was day 60 and toxicity was evaluated on day 14. The parameter measured was median survival time; a T/C value $\geq 125\%$ is considered necessary to demonstrate activity. A reproducible $T/C \geq 150\%$ is considered significant activity. A T/C value $<86\%$ indicates toxicity.

LOX amelanotic melanoma
0.1 ml of diluted ascitic fluid containing 10^6 cells was injected i.p. into athymic Ncr-nu female mice; a suspension of compound **1** in saline with Tween 80 was injected i.p. one day after tumor implantation and the treatment was repeated every 4th day for a total of 3 injections. Final evaluation day was day 60 and toxicity was evaluated on day 10. The parameter measured was median survival time: an initial T/C value of $\geq 140\%$ demonstrates activity and $T/C < 86\%$ indicates toxicity.

Human mammary carcinoma MX-1 xenograft
A fragment of MX-1 human mammary carcinoma having an average diameter between 9 and 12 OMUs (ocular micrometer unit) was implanted under the subrenal capsule of athymic female mice. The i.p. treatment with compound **1**, suspended in saline with Tween 80, started 1 day after tumor implantation and was repeated every 4th day for a total of 3 injections. Day 11 was the final evaluation day and was also the toxicity evaluation day. The parameter measured was mean tumor weight change for test and control groups; an initial $T/C < 20\%$ is considered necessary to demonstrate activity.

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