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Potential Anticancer Agents.[1] LXII. The Relationship of Chemical Structure to Antileukemic Activity with Analogs of 1-Methyl-3-nitro-1-nitrosoguanidine (NSC-9369). II

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In a continued investigation of the relationship of structure to antileukemic activity of 1-methyl-3-nitro-1-nitrosoguanidine (NSC-9369), an additional eight nitroso amides bearing an N-(2-substituted ethyl) group were synthesized and evaluated against Leukemia L-1210. The most effective analog was found to be 1-(2-chloroethyl)-1-nitrosourea (NSC-47547).

The first paper[2] on analogs of 1-methyl-3-nitro-1-nitrosoguanidine (I) (NSC-9369)[3] described Phases I and II of the structure-activity study. It was clear that the 2-chloroethyl analog (II) and the 2-bromoethyl analog (III) were superior to the originally discovered lead (I). In this first paper were also posed the following questions: Are other 2-substituted ethyl analogs even more effective than II

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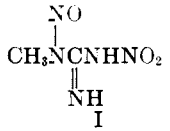
(2) W. A. Skinner, H. F. Gram, M. O. Greene, J. Greenberg and B. R. Baker, *J. Med. Pharm. Chem.*, **2**, 299 (1960).

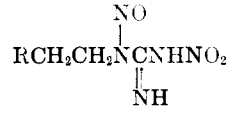
(3) The NSC accession numbers used in this paper were assigned by the Cancer Chemotherapy National Service Center.

or III? Is it possible that the more effective 2-chloroethyl group in II could impart activity to other nitroso amides (IV)?

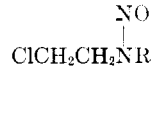
This paper describes Phase III of the structure-activity study designed to shed further light on these questions; thus, selected members of Class 1 and Class 5 compounds[2] were synthesized and evaluated against mouse Leukemia L-1210.



I



II, R = Cl (NSC-25959)
III, R = Br (NSC-36885)



IV

From this Phase III study, it is clear that negatively 2-substituted ethyl analogs of NSC-9369 have enhanced activity, but that the 2-chloroethyl analog (NSC-25959) and the 2-bromoethyl analog (NSC-36885)[2] are still the most effective analogs of the nitroguanidine series. Of even more interest is the high activity of 1-(2-chloroethyl)-1-nitrosourea (NSC-47547), the most effective nitroso amide we have yet found (see Discussion). Additional 1-(2-substituted ethyl)-1-nitrosoureas should be synthesized and evaluated. Since such continued work would overlap into an area being investigated by Dr. John A. Montgomery at Southern Research Institute, no further work is contemplated in this laboratory.

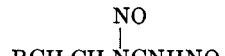
Chemistry

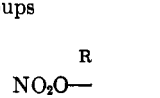
The general chemistry of nitroguanidines has been reviewed by McKay.[4] Additional examples appear in the first paper[2] of this study. In Table I, Compounds V, VIII and X were prepared by reaction of the appropriate amine with 1-methyl-3-nitro-1-nitrosoguanidine at 0–5° according to the method of McKay and Milks.[5] 2-(Methylthio)ethylamine, prepared by methylation of 2-mercaptoethylamine in aqueous base, was converted to X without being isolated. The nitrosoguanidine procedure[5] was unsuccessful for the preparation of XV; the condensation of the appropriate amine with S-methyl-N-nitrothiourea[6] using the method for XII[7] was satisfactory.

(4) A. F. McKay, *Chem. Rev.*, **51**, 301 (1952).
(5) A. F. McKay and J. E. Milks, *J. Am. Chem. Soc.*, **72**, 1616 (1950).
(6) L. Fishbein and J. A. Gallaghan, *ibid.*, **76**, 1877 (1954).
(7) V. Milani, S. Skolnik and R. Evans, *J. Am. Chem. Soc.*, **77**, 2903 (1955).

The remaining two nitroguanidines in Table I were prepared from a preformed nitroguanidine by suitable transformation. Oxidation of 1-(2-methylthioethyl)-3-nitroguanidine (X) with hydrogen peroxide in acetic acid gave an 85% yield of the corresponding sulfone

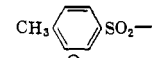
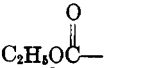
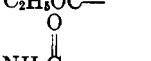
Class 1.—Replacement of methyl group by 2-substituted ethyl groups



NSC No.	R	Activity[a]
40587	NO_2O—	+
40590		±
43420	CH_3S—	−
51451	CH_3SO_2—	+
52394	HOOC—	−
25959	Cl—	++[b]
36885	Br—	++[b]

[a] These definitions are used because of the variation in test data with Leukemia L-1210 at optimum dosage: (++) is active on all tests, (+) is usually active, (±) is occasionally active, and (−) is inactive; for further detail, see testing results. [b] From reference 2.

Class 5.—Other acylated N-nitrosoethylamines[a]

NSC No.	R	Activity
45628		−
47541		−
47547		++

[a] See Footnote *a*, Class 1.

(XIII). The nitrate ester (VI) was prepared from 1-(2-hydroxyethyl)-3-nitroguanidine with nitric acid in acetic anhydride, according to the procedure described by McKay and Milks.[5]

The 1-nitroso compounds in Table I (VII, IX, XI, XIV, XVI) were prepared by the standard nitrosation procedure in 11 *N* nitric acid with sodium nitrite.[2,5] However, V and XII failed to nitrosate either under these conditions or a variety of others.[2]

Nitrosation of N-(2-chloroethyl)-*p*-toluenesulfonamide (XVII) (Table II) in aqueous acetic acid proceeded smoothly to crystalline XVIII. Similarly, N-(2-chloroethyl)-urea (XXI), prepared from

2-chloroethylamine and potassium cyanate, was nitrosated in dilute sulfuric acid to **XXII**; the yield was poor, but other conditions were even less satisfactory. Ethyl N-(2-chloroethyl)-carbamate (XIX) was readily prepared[8] from 2-chloroethylamine; nitrosation gave XX as an oil that was difficult to purify, since it could neither be crystallized nor distilled. However, the nitrosation conditions successful with the corresponding methyl ester[16] left only a minimum (15%) of starting material, as detected by the loss of NH near 3 μ in its infrared spectrum. Paper chromatograms showed that no other contaminants were present. Since the starting material (XIX) showed toxicity at about 250 mg./kg., the 15% of XIX in XX was not considered deleterious for the testing of XX; the latter was toxic at 2 mg./kg.

All of the compounds in Tables I and II had infrared spectra in agreement with their assigned structures. The nitroso compounds showed *no* absorption near 6.5 μ and sometimes in the 10–12 μ region; in addition, the loss of NH near 3.1 μ by nitrosation was considered significant. These characteristic changes allowed the rapid assay of nitrosation reaction mixtures in order to establish proper reaction conditions.

Experimental[9,10]

1-(2-Methylthioethyl)-3-nitroguanidine (X).—To a solution of potassium hydroxide (11.2 g., 0.20 mole) in 95% ethanol (100 ml.) was added 2-mercaptoethylamine hydrochloride (11.4 g., 0.10 mole). To this stirred suspension was slowly added methyl iodide (23 g., 0.16 mole), the temperature being maintained at 20–25° by cooling. After being stirred for an additional hour at room temperature, the solution was diluted with absolute ethanol (40 ml.), then chilled in an ice-bath. 1-Methyl-3-nitro-1-nitrosoguanidine (7.4 g., 0.05 mole) was added in small portions so that the temperature did not rise above 5°. After the addition was complete, the mixture was stirred for 30 minutes more in the ice-bath, then filtered to remove inorganic material. The filtrate was evaporated to dryness *in vacuo* and the residue triturated thoroughly with water in several portions; yield, 5.3 g. (60%), m.p. 114–115°, that was suitable for nitrosation. For further data, see Table I.

1-(2-Methylsulfonylethyl)-3-nitroguanidine (XIII).—To a solution of 1-(2-

(8) H. M. Curry and J. P. Mason, *J. Am. Chem. Soc.*, **73**, 5043 (1951).

(9) Melting points were taken on a Fisher-Johns block and are uncorrected.

(10) All reactions of 1-methyl-3-nitro-1-nitrosoguanidine with amines should be performed in a good hood, since toxic gases are evolved.[4]

(11) G. K. Hughes and F. Lions, *J. Roy. Soc. N. S. Wales*, **71**, 209 (1938).

(12) A. F. McKay, J. R. G. Bryce and D. E. Rivington, *Can. J. Chem.*, **29**, 382 (1951).

(13) E. R. Bissell and M. Finger, *J. Org. Chem.*, **24**, 1256 (1959).

methylthioethyl)-3-nitroguanidine (X) (1.00 g., 5.6 mmoles) in acetic acid (25 ml.) and acetic anhydride (25 ml.) cooled in an ice-bath was added dropwise 30% hydrogen peroxide (1.6 ml.). The solution was allowed to stand at room temperature for three days, when no more product had separated. The crystals were collected on a filter and washed with water; yield, 1.00 g. (85%), m.p. 197–200°; this material was suitable for nitrosation. See Table I for additional data.

1-(2-Carboxyethyl)-3-nitroguanidine (XV).—A mixture of β-alanine (3.91 g., 0.044 mole), S-methyl-N-nitrothiourea[7] (5.40 g., 0.40 mole), and water (10 ml.) was heated on a steam-bath under a condenser for 90 minutes; at this time solution was complete. Longer heating (5 hours) led to a complex mixture. The solution was cooled at 0°, the crystalline product (1.7 g.) was collected on a filter and washed with cold water (10 ml.). Recrystallization from water gave 1.1 g. (16%) of product, m.p. 170–172°. See Table I for further data.

The first mother liquor deposited an additional 1.0 g. which was shown by paper chromatography to be about an equal mixture of the thiourea and the product.

1-[2-(2-Nitramino-2-imidazolin-1-yl)-ethyl]-3-nitro-1-nitrosoguanidine (IX) was prepared by the method of McKay, *et al.*,[12] who reported m.p. 176° d., 56% yield, and who modified the general procedure[5] by adding a smaller excess of sodium nitrite to a larger volume of 11 *N* nitric acid solution. However, the usual quantities were found to be advantageous on a small scale. The analytical figures calculated[12] for C and N were in error, and the nitrogen found (42.88) was actually a deficiency. This compound seems difficult to purify and characterize. We found apparently identical infrared spectra and satisfactory analyses for C and H for samples melting in the range 170–190°. Other samples of identical melting points were high in C and H. All were low in N. There was paper chromatographic evidence for VIII in some samples of IX; this may be attributable either to degradation of IX on chromatography or to presence of VIII as a contaminant.

N-(2-Chloroethyl)-*p*-toluenesulfonamide (XVII).—Treatment of 2-aminoethanol with *p*-toluenesulfonyl chloride by the method[17] used for ethylamine afforded 97.6% of N-(2-hydroxyethyl)-*p*-toluenesulfonamide, m.p. 31–35° (lit.[18] 56°). To this intermediate (40.6 g., 0.19 mole) at 35–40°, thionyl chloride (29 ml., 0.35 mole) was added dropwise during 45 minutes while the mixture was slowly heated to complete solution at 65°. Reflux began and was maintained for 2 hours. The mixture solidified on cooling and on recrystallization from methanol afforded 17.7 g., m.p. 99–99.5° (lit.[14,18] 99°,101°). A second crop, 21.2 g., m.p. 96.5–97.5° formed on diluting the filtrate with water (total yield, 87%).

N-(2-Chloroethyl)-N-nitroso-*p*-toluenesulfonamide (XVIII).—A stirred solution of N-(2-chloroethyl)-*p*-toluenesulfonamide (XVII) (10 g., 0.042 mole) in 70 ml. of warm acetic acid was diluted with water (5 ml.) and cooled to 5° in an ice-bath. Solid sodium nitrite (11.6 g., 0.168 mole) was added in small portions over a period of 90 minutes. After being stirred for an additional 20 minutes, the mixture was diluted with ice water (50 ml.). The solid was collected on a filter,

(14) D. H. Peacock and U. C. Dutta, *J. Chem. Soc.*, 1303 (1934).

(15) A. F. Childs, L. J. Goldsworthy, G. F. Harding, S. G. P. Plant and G. A. Weeks, *ibid.*, 2320 (1948).

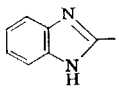
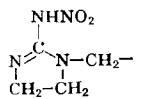
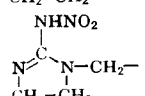
(16) H. Brintzinger and K. Pfannstiel, *Chem. Ber.*, **81**, 378 (1948).

(17) D. H. Hey and T. J. deBoer, *Rec. trav. chim. Pays-Bas*, **73**, 686 (1954).

(18) K. H. Slotta and R. Behnisch, *J. prakt. Chem.*, **135**, 225 (1932).

TABLE I

1-ALKYL-3-NITROGUANIDINES $R'CH_2N(R'')-C(=NH)NHNO_2$

Cpd.	NSC No.	R′	R″	M.p., °C.	R_f[a]	Yield, %	Empirical formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
V	39281		H[b,c,o]	260–261 d.[d]	0.65	46	$C_9H_{10}N_6O_2$	46.2	4.30	35.9	46.2	4.30	36.0
VI	40588	$-CH_2ONO_2$	H[e]	92–93[d,f]	0.83	43							
VII	40587	$-CH_2ONO_2$	NO[g,h]	99–105 d.	0.92	35							
VIII	40589		H[b,i]	195–197[j]	0.59	37							
IX	40590		NO[l]	178 d.	0.75	61	$C_6H_{11}N_9O_5$						
X	39276	CH_3SCH_2-	H[l]	115–116[k]	0.81	60	$C_4H_{10}N_4O_2S$	27.0	5.66	31.5	26.9	5.89	31.6
XI	43420	CH_3SCH_2-	NO[h]	102–103[m]	0.88	52	$C_4H_9N_5O_3S$	23.2	4.38	15.5[n]	23.5	4.65	15.3[n]
XII	48608	F_3C-	H[o]	146–147[m]	0.90	70[p]							
XIII	49798	$CH_3SO_2CH_2-$	H[l]	204–205[q]	0.57	85	$C_4H_{10}N_4O_4S$	22.9	4.80	26.7	23.1	4.76	27.0[r]
XIV	51451	$CH_3SO_2CH_2-$	NO[h]	131–133 d.	0.71	19	$C_4H_9N_5O_6S$	20.1	3.79	29.3	20.3	3.68	29.5[s]
XV	51452	$HOOCCH_2-$	H[l]	170–172	0.65	16	$C_4H_9N_4O_4 \cdot H_2O$	24.7	5.19	28.9	25.1	5.43	29.1
XVI	52394	$HOOCCH_2-$	NO[h]	113–115 d.[m]	0.75	29	$C_4H_7N_5O_5$	23.4	3.44	34.2	23.7	3.58	34.1

[a] Descending on Whatman No. 1 in butanol-acetic acid-water (5/2/3); spots (γ 58) detected by observation under ultraviolet light. [b] Prepared by reaction of the corresponding amine ($R'CH_2NH_2$) with 1-methyl-3-nitro-1-nitrosoguanidine.[2,6] [c] Prepared from 2-(aminomethyl)-benzimidazole dihydrochloride[11] in 30% aqueous ethanol containing two equivalents of potassium hydroxide. [d] Recrystallized from absolute ethanol. [e] Prepared by nitration of 1-(2-hydroxyethyl)-3-nitroguanidine

according to McKay and Milks,[5] who recorded yield of 64% and double m.p. 109–110°, 152–159°. [f] Resolidifies, then remelts at 161°. [g] McKay and Milks[5] record m.p. 105° dec. and yield of 54%. [h] By nitrosation of corresponding guanidine (R″ = H) in 11 *N* nitric acid with aqueous sodium nitrite.[2,5] The procedure for VII was that used for 1-(2-bromoethyl)-3-nitro-1-nitrosoguanidine.[2] [i] Starting material was diethylenetriamine. [j] McKay *et al.*[12] record m.p. 197° and yield of 45%. [k] Recrystallized from ethanol–water. [l] See Experimental. [m] Recrystallized from methanol–water. [n] Sulfur analysis. [o] This compound failed to nitrosate with a variety of experimental conditions. [p] Prepared from S-methyl-N-nitrothiourea[6] and trifluoroethylamine hydrochloride[13] according to Milani *et al.*,[7] who recorded 94% yield and m.p. 147–148°. [q] Recrystallized from 2-methoxyethanol. [r] Calcd.: S, 15.3. Found: S, 15.3. [s] Calcd.: S, 13.4. Found: S, 13.5.

←

dissolved in dichloromethane (100 ml.) and washed with 3 50 ml. portions of ice-cold 0.5 *M* potassium hydroxide. The solution, dried with magnesium sulfate, was evaporated to dryness *in vacuo* (bath 30°) to yield 6.0 g. (59%) of product, m.p. 45–50°. Recrystallization from methanol-water gave pale yellow crystals, m.p. 49–50° (see Table II).

N-(2-Chloroethyl)urea (XXI).—To a solution of 2-chloroethylamine hydrochloride (1.16 g., 0.01 mole) in water (20 ml.) was added potassium cyanate (0.81 g., 0.01 mole). The solution was evaporated to dryness *in vacuo* (bath 30°). The residue was extracted with hot absolute ethanol and the filtered extract was evaporated to dryness *in vacuo;* yield, 1.11 g. (91%), m.p. 97–104°, that was suitable for nitrosation. Recrystallization from ethanol gave 0.91 g. (75%), m.p. 99–101° (see Table II).

N-(2-Chloroethyl)-N-nitrosourea (XXII).—To a mixture of 96% sulfuric acid (1.5 g.) and water (10 ml.) cooled in an ice-bath was added N-(2-chloroethyl)-urea (XXI) (2.46 g., 0.02 mole). Then a cold solution of sodium nitrite (1.40 g., 0.02 mole) in water (3 ml.) was added dropwise with stirring over a period of 20 minutes, during which time the solid changed from white to a pale yellow. The product was collected on a filter and washed well with water; yield, 0.85 g. (27%), m.p. 75–78° (see Table II).

Biological Activity

Methods.—Assays for activity against three tumors (Sarcoma 180, Adenocarcinoma 755, and lymphoid Leukemia L-1210) were performed according to specifications established by the Cancer Chemotherapy National Service Center.[19] None of the compounds in Tables I and II were active against the sarcoma; therefore, the results are not reported in detail.

(19) *Cancer Chemotherapy Reports*, **1**, 42 (1959), Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md.

TABLE II

CHLOROETHYLAMINE DERIVATIVES $ClCH_2CH_2N(R')R''$

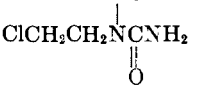
Cpd.	NSC No.	R′	R″	M.p., °C.	Yield, %	Empirical formula	Analyses: Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
XVII	45629		H	99–99.5	85[a,b]							
XVIII	45628		NO[b]	49–50[h]	54	$C_9H_{11}ClN_2O_3S$	41.2	4.23	12.2[c]	41.4	4.34	11.8[c]
XIX	43421		H	Oil[d]	44[e]	$C_5H_{10}ClNO_2$	39.6	6.65	9.24	39.3	6.70	9.37
XX	47541		NO[m]	Oil	100[f]	$C_5H_9ClN_2O_3$[f]	33.2	5.02	19.6[g]	34.1	5.25	20.1[g]
XXI	47538		H[b]	99–101	75[i]	$C_3H_7ClN_2O$	29.5	5.77	22.9	29.2	5.74	22.5[j]
XXII	47547		NO[b]	82–85[k]	27	$C_3H_6ClN_3O_2$	23.8	3.99	27.7	24.0	4.01	27.5[l]

[a] Over-all yield from *p*-toluenesulfonyl chloride; Peacock *et al.*[14] recorded m.p. 99°. [b] See Experimental. [c] Sulfur analysis. [d] B.p. 60–62° (0.5 mm.); $n^{22}D$ 1.4538; Curry and Mason[8] recorded b.p. 76–77° (3 mm.). [e] Prepared from 2-chloroethylamine, liberated from the hydrochloride, according to the procedure of Curry and Mason,[8] modified by the use of an ethereal medium with 1 equivalent of solid potassium bicarbonate as base instead of aqueous sodium hydroxide. [f] Infrared analysis indicated 15% of starting material (XIX) was present[b]; Calcd. with this impurity: C, 34.2; H, 5.27; Cl, 20.2; Childs, *et al.*,[15] reported C, 33.8; H, 5.1. [g] Chlorine analysis. [h] Recrystallized from methanol–water. [i] Recrystallized from ethanol. [j] Calcd. Cl, 29.0. Found: Cl, 28.8. [k] Recrystallized from methanol. [l] Calcd.: Cl, 23.4. Found: Cl, 23.2. [m] Prepared by the method described[16] for the corresponding methyl ester, except that the reaction was maintained at 0–5°, not 15°.

In assays for activity against Adenocarcinoma 755, (C 57 BL/6 JAX 2 × DBA/2 JAX) F_1 hybrid mice were implanted in the axilla with tumor brei. Following implantation the mice were divided, randomly, into groups of ten mice each, each group to be treated with one dosage regimen. An appropriate number of controls, treated only with carboxymethylcellulose solution were used in each series. Treatment was begun 24 hours after tumor implantation and con-

TABLE III

ACTIVITY OF NSC-47547 (XXII) AGAINST ADENOCARCINOMA 755



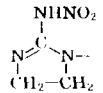
Control No.	Dose (mg./kg.)	Survivors	Change in weight (g.) (test/control)	Tumor weight (mg.) (test/control)	T/C
457	0.9	10/10	0.5/2.0	1084/2125	0.51
467	0.9	4/10	0.1/1.5	430/846	Toxic
474	0.9	10/10	−2.2/1.4	267/1094	0.24
484	0.9	10/10	−1.6/2.4	251/1950	0.12
494	0.9	10/10	−2.2/0.1	96/379	0.25
495	0.9	0/10			Toxic
496	0.9	5/10	−2.4/0.5	154/745	Toxic
501	0.9	10/10	−0.9/2.9	293/1634	0.17
508	0.9	10/10	−1.1/2.2	618/1373	0.45
509	0.9	10/10	−1.9/0.9	174/421	0.40
519	1.8	10/10	−3.2/0.9	170/620	0.27
519	0.9	10/10	−1.3/0.9	236/620	0.38
519	0.45	9/10	0.4/0.9	649/620	1.04
519	0.23	10/10	−0.9/0.9	340/620	0.54
891[a]	1.8	5/10	−5.9/−0.5	00/559	Toxic
891[a]	0.9	10/10	−3.7/−0.5	190/554	0.34
891[a]	0.45	10/10	−3.0/−0.5	256/554	0.46
891[a]	0.23	10/10	−1.8/−0.5	328/554	0.59

[a] Confirmatory Test by Wisconsin Alumni Research Foundation done under contract to the Cancer Chemotherapy National Service Center.

tinued once daily for 10 days. On the eleventh day, the mice were sacrificed and the tumor was excized and weighed. The statistics of evaluation of activity according to the CCNSC are complex. Briefly, a compound is considered active if the average tumor weight of the treated group is less than 50% of the untreated controls. Confirmation of activity is required in at least four of six independent

TABLE IV

ACTIVITY OF 1-ALKYL-3-NITRO-1-NITROSOGUANIDINES AGAINST LEUKEMIA L-1210 $RCH_2CH_2N(NO)C(=NH)NHNO_2$ (Class 1)[2]

No.	NSC No.	R	Series no.	Dose (mg./kg./day)	Survivors on day 5	Weight change (g.) day 0 to day 5 (test/control)	Mean survival time, days (test/control)	T/C
VI	40587	$-ONO_2$	329	30	0/6			Toxic
			341	15	6/6	−0.5/0.1	14.2/8.4	1.69
			341	7.5	6/6	−0.5/0.1	13.0/8.4	1.55
			398	10	6/6	−0.3/−0.3	12.7/9.8	1.29
			398	6.7	6/6	−0.3/−0.3	12.7/9.8	1.29
			398	4.4	6/6	0.6/−0.3	12.5/9.8	1.27
			398	2.8	6/6	0.0/−0.3	12.2/9.8	1.25
			551[a]	30	5/6	−0.2/−0.5	11.4/10.3	1.10
			551[a]	20	5/6	−1.7/−0.5	10.0/10.3	0.97
			551[a]	13	4/6	−0.2/−0.5	10.5/10.3	1.01
			551[a]	9	6/6	−1.9/−0.5	11.0/10.3	1.05
			551[a]	6	6/6	−0.8/−0.5	10.7/10.3	1.03
			1178[b]	30	6/6	−0.1/0.7	12.0/9.6	1.25
			1178[b]	20	6/6	−1.5/0.7	12.8/9.6	1.33
			1178[b]	13	6/6	−0.2/0.7	14.5/9.6	1.51
			1178[b]	9	6/6	0.5/0.7	13.0/9.6	1.35
			1178[b]	6	6/6	−0.4/0.7	13.6/9.6	1.41
			1178[b]	4	6/6	0.2/0.7	16.0/9.6	1.66
			1178[b]	2.7	6/6	0.9/0.7	13.3/9.6	1.38
IX	40590		329	150	6/6	−2.1/0.5	8.5/8.2	1.01
			329	50	6/6	−0.6/0.5	11.7/8.2	1.43
			346	75	6/6	0.6/0.8	9.7/8.3	1.17

			346	50	6/6	0.8/0.8	10.2/8.3	1.23
			346	33	5/6	0.4/0.8	9.0/8.3	1.08
			346	22	6/6	0.2/0.8	10.7/8.3	1.29
			1301[b]	125	4/6	−0.7/1.2	11.0/9.2	1.14
			1301[b]	62	6/6	0.5/1.2	10.5/9.2	1.14
			1301[b]	31	6/6	1.6/1.2	10.3/9.2	1.11
			1301[b]	15	6/6	0.7/1.2	10.2/9.2	1.10
XI	43420	CH_3S—	406	200	5/6	−0.3/−0.7	9.0/9.2	0.97
			406	70	6/6	−0.8/−0.7	10.5/9.2	1.14
			406	20	6/6	−0.3/−0.7	9.3/9.2	1.01
			438	70	5/6	−3.2/0.5	8.0/9.0	0.88
XIV	51451	CH_3SO_2—	518	90	6/6	−1.8/0.2	12.0/8.8	1.36
			528	90	6/6	1.0/1.1	10.3/9.6	1.07
			538	90	6/6	−2.0/1.1	11.6/9.1	1.27
			538	60	6/6	−0.6/1.1	15.3/9.1	1.68
			538	45	6/6	−1.6/1.1	14.0/9.1	1.53
			552	135	6/6	−2.2/1.9	11.8/9.9	1.19
			552	90	6/6	−1.8/1.9	12.1/9.9	1.22
			552	60	6/6	−1.3/1.9	13.1/9.9	1.32
			552	45	6/6	−0.5/1.9	12.1/9.9	1.22
			552	30	6/6	−0.5/1.9	13.3/9.9	1.34
			552	20	6/6	0.4/1.9	11.6/9.9	1.17
			552	8.8	6/6	1.6/1.9	12.0/9.9	1.21
XVI	52394	—COOH	529	90	6/6	−2.0/0.4	11.2/9.9	1.13
			574	80	6/6	−0.5/2.0	10.1/7.0	1.44
			574	53	6/6	−0.7/2.0	10.1/7.0	1.44
			574	35	5/6	1.8/2.0	8.8/7.0	1.25
			574	23	5/6	−0.6/2.0	10.2/7.0	1.45

[a] Confirmatory test by Microbiological Associates. [b] Confirmatory test by Southern Research Institute.

TABLE V

ACTIVITY OF N-(2-CHLOROETHYL)-N-NITROSO AMIDES AGAINST LEUKEMIA L-1210 $ClCH_2CH_2\underset{\displaystyle NO}{\underset{|}{N}}R$

No.	NSC No.	R	Series no.	Dose (mg./kg./day)	Survivors on day 5	Weight change (g.) day 0 to day 5 (test/control)	Mean survival time, days (test/control)	T/C
XVIII	45682	CH_3-C$_6$H$_4$-SO_2-	467	45	6/6	−1.4/0.8	10.0/9.2	1.0
			574	45	6/6	−1.1/2.0	6.6/7.0	0.94
			574	30	6/6	0.1/2.0	6.3/7.0	0.90
			574	20	6/6	0.0/2.0	7.0/7.0	1.00
			574	13	6/6	1.0/2.0	7.0/7.0	1.00
		O ‖	467	2	4/6	−2.3/0.8	6.5/9.2	Toxic
			480	1	6/6	−0.4/0.0	12.3/10.5	1.17
XX	47541	C_2H_5OC—	574	2.0	6/6	1.0/2.0	8.1/7.0	1.15
			574	1.3	6/6	0.4/2.0	8.1/7.0	1.15
			574	0.8	6/6	0.1/2.0	8.1/7.0	1.15
			574	0.5	6/6	1.1/2.0	8.3/7.0	1.18
		O ‖	469	0.9	6/6	−1.4/0.4	15.6/11.1	1.40
			486	0.9	6/6	−0.8/0.6	10.3/10.3	1.00
XXII	47547	NH_2C—	519	0.9	6/6	−1.1/−0.1	23.0/9.2	2.50
			519	0.6	6/6	−1.6/−0.1	15.3/9.2	1.66
			519	0.4	6/6	−0.2/−0.1	12.6/9.2	1.36
			519	0.2	6/6	−0.2/−0.1	11.5/9.2	1.25
			535	2.0	6/6	−2.0/0.3	23.5/9.6	2.44
			535	1.3	6/6	−2.9/0.3	25.3/9.6	2.63
			535	0.9	6/6	−1.0/0.3	19.6/9.6	2.04
			535	0.6	6/6	−2.2/0.3	19.3/9.6	2.01

(*continued on page 13*)

TABLE V—*Compound XXII continued from p. 12*

Series no.	Dose (mg./kg./day)	Survivors on day 5	Weight change (g.) day 0 to day 5 (test/control)	Mean survival time, days (test/control)	T/C
553	6.7	6/6	−5.4/1.4	7.6/7.7	0.98
553	4.5	5/6	−3.2/1.4	11.8/7.7	1.53
553	3.0	6/6	−3.2/1.4	14.8/7.7	1.92
553	2.0	6/6	−2.3/1.4	21.5/7.7	2.74
553	1.3	6/6	−2.2/1.4	15.8/7.7	2.05
646[a]	0.9	6/6	−1.6/−1.1	20.3/11.2	1.81
646[a]	0.6	6/6	−1.4/−1.1	19.2/11.2	1.71
646[a]	0.4	6/6	−0.8/−1.1	17.0/11.2	1.51
646[a]	0.2	6/6	−1.1/−1.1	15.5/11.2	1.38

[a] Confirmatory Test by Microbiological Associates.

assays. A regimen is considered toxic if less than 7 of 10 mice survived treatment.

In assays for activity against Leukemia L-1210, ascites form, (C 57 Bl/6 JAX × DBA/2 Jax) F_1 hybrid mice were used. All mice were implanted intraperitoneally with 10^5 ascites cells. Each drug regimen was tested in six animals, an appropriate number of placebo controls being included in each series. Treatment was begun 24 hours after tumor implantation and continued once daily until death of the animals. Dosages were chosen on the basis of previous experience with treating mice bearing Sarcoma 180 and Adenocarcinoma 755 tumors. Mean survival time was calculated for treated (T) and control (C) groups. A compound is considered active if the value of T/C exceeded 1.25 in an appropriate number of independent tests. A compound is considered toxic if the value of T/C $\leqq$ 0.85 or if two or more mice failed to survive the fifth day. It should be noted that in the previous paper in this series[2] T/C $\cong$ T-5/C-5; these previous results can be converted readily if so desired.

Due to their instability, all of the nitroso compounds were stored in a freezer and freshly prepared ice-cold suspensions were used for injection.

Results.—None of the compounds in Table I and II had any significant, reproducibile activity against Sarcoma 180.

The des-nitroso compounds had no activity against any of the tumors and were relatively non-toxic. Detailed data, therefore, are not presented.

Only XXII (NSC-47547) showed activity against Adenocarcinoma 755 and the results with it alone are shown in Table III. The activity

not only was reproducible in this laboratory but was confirmed in one other laboratory.

Results with Leukemia L-1210 are presented in Tables IV and V. Compound X exhibited minimal activity in at least one dose in each of two tests, but its activity could not be reproduced in another laboratory. Compounds XI, XVIII and XX were inactive. Compounds XIV and XVI showed slight but significant activity, not as yet independently confirmed. Compound XXII was the most active compound tested in this or in the previous series of experiments[2]; its activity has been confirmed in at least one other laboratory.

Discussion

The most significant biological observations in this paper concern compound XXII (NAS 47547). It is the most active compound against Leukemia L-1210 tested in this and the previous series.[2] No other nitroso-compound increased survival beyond 15.7 days; in general, mice treated with "active" compounds survived about 12 days. In contrast some groups of mice treated with chloroethylnitrosourea (XXII) survived over 20 days. Furthermore, chloroethylnitrosourea (XXII) is the only nitroso compound tested which had even minimal inhibitory activity against a solid tumor, Adenocarcinoma 755, though it, too, was inactive against Sarcoma 180. It is obvious that the nitroguanidine portion of the alkylnitrosoguanidines can be replaced by another hydrolyzable group, the urea moiety. It is also obvious that the most active compounds in the nitrosourea and the nitrosoguanidine series have a negative substituent on the 2-position of the ethyl radical, particularly bromo or chloro. However, not all hydrolyzable groups, even with N-(1-chloroethyl) substituents, can be substituted and still retain antitumor activity; the ethylcarbamate (XX) and the *p*-tolylsulfonamide (XVIII) were inactive.

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