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Potential Anticancer Agents—XXXI.* The Relationship of Chemical Structure to Antileukaemic Activity with Analogues of 1-Methyl-3-nitro-1-nitrosoguanidine (NSC–9369)

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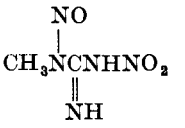
Introduction

The Cancer Chemotherapy National Service Center (CCNSC) of the National Cancer Institute has been testing about one thousand synthetic compounds a month in its search for anticancer agents. Among these compounds, 1-methyl-3-nitro-1-nitrosoguanidine (NSC–9369)† was found to increase the life span



(NSC–9369)

of mice bearing Leukemia L–1210. We have undertaken a study of the relationship of chemical structure to antileukaemic activity with analogues of NSC–9369.

There are several ways in which a team composed of chemists

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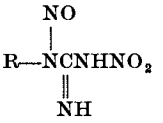
† The NSC accession numbers used in this paper were assigned by the Cancer Chemotherapy National Service Center.

and biologists can approach a structure–activity problem. The most common way is for the chemists to make many random compounds, then wait until the biologists evaluate the compounds, and establish which ones are best. Such an approach can lead to a great deal of wasted time and effort by the team. We prefer to use the 'phase-method' of approach.

With the 'phase-method', a small number of examples of each type of structural change are synthesized and evaluated. In this way, further work on changes in structure which lead to loss of activity can be avoided and, more important, changes which lead to equal or better activity can be followed up in more detail. An added benefit is that if compounds in promising areas of structural change are difficult to synthesize, their synthesis is worthy of the additional effort.

For Phase I evaluation, five classes of compounds were synthesized and evaluated.

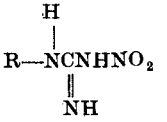
Class 1. Replacement of methyl group by other alkyl groups with and without substituents.



NSC Number	R	Activity[a]
24639	n-C_4H_9—	−
25070	$C_6H_5CH_2$—	−
25073	i-C_4H_9—	±
25959	$ClCH_2CH_2$—	++
25962	$HOCH_2CH_2$—	−
25965	$(CH_3)_2NCH_2CH_2CH_2$—.HNO_3	±

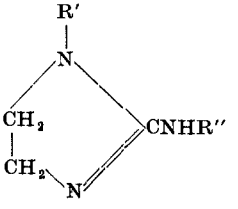
[a] The following definitions are used because of the variation in test data at optimum dosage: (++) is active on all tests, (+) is usually active, (±) is occasionally active, and (−) is inactive; for further detail see testing results.

Class 2.



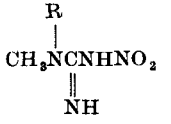
These compounds were synthetic intermediates to class 1. All were inactive.

Class 3. Cyclic analogues.



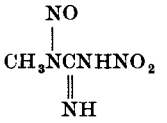
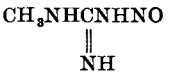
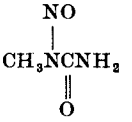
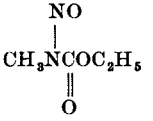
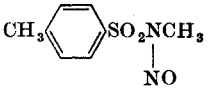
NSC Number	R′	R″	Activity
25961	H	NO_2	–
25973	NO_2	H	–
25958	NO	NO_2	+

Class 4. Replacement of nitroso group by other groups.



NSC Number	R	Activity
25963	CH_3—	–
28104	NO_2—	±
30903	CH_3CO—	–

Class 5. Other diazomethane precursors and miscellaneous compounds.

NSC Number	Compound	Activity
9369		±
30908		–
23909[a]		±
2860[a]		–
5290[a]		–

[a] We wish to thank Dr Howard W. Bond of the CCNSC for this information.

Thus, the first phase, consisting of 26 compounds, led to the following conclusions for Phase II synthesis.

Class 1. Large alkyl or aralkyl groups led to loss of activity. The 2-chloroethyl substituent furnished the most consistently active compound (NSC–25959) obtained in this series; this result suggested that other substituted alkyl groups should be investigated. The vague possibility that an odd number of carbons in the alkyl side chain was necessary for activity suggested that the *n*-propyl and *n*-pentyl compounds be evaluated.

Class 2. All compounds were inactive.

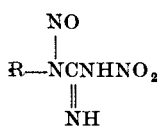
Class 3. The activity of a cyclic nitroso nitroguanidine (NSC–25958) suggested that the next higher cyclic homologue be evaluated in Phase II; unfortunately, the synthesis failed at the nitrosation step.

Class 4. The activity of the dinitroguanidine (NSC–28104), though less than that of the parent NSC–9369, was noteworthy. Apparently, a non-hydrolysable negative group such as nitroso or nitro on the nitrogen bearing the alkyl group is necessary for activity, since the methyl group (NSC–25963) led to loss of activity and the acetyl group (NSC–30903) was hydrolysed too easily. The attempted synthesis of compounds of this class where R was an alkyl- or arylsulphonyl group in Phase II was unsuccessful.

Class 5. Other diazomethane precursors, although toxic, showed no selective effect on tumour over host, except for occasional activity of 1-methyl-1-nitrosourea (NSC–23909).

Based on these attempted correlations, Phase II of the synthetic work was initiated.

Class 1. Replacement of methyl group by other alkyl groups with and without substituents.



NSC Number	R	Activity
33674	n-C_3H_7—	±
34696	$CH_3OCH_2CH_2$—	±
34699	n-C_5H_{11}—	−
35896	$CH_3CO_2CH_2CH_2CH_2$—	−
35906	$ClCH_2CH_2CH_2$—	−
35907	$CH_3CO_2CH_2CH_2$—	−
36885	$BrCH_2CH_2$—	++
38184	$C_6H_5CH_2CH_2$—	−
38191	C_2H_5—	+

From Phase II, it was clear that the best analogues of NSC–9369 made this time were the 2-bromoethyl analogue (NSC–36885) and the 2-chloroethyl analogue (NSC–25959). They appeared to be superior to the originally discovered lead, NSC–9369. The question of whether or not other 2-substituted ethyl analogues are even more effective than NSC–36885 remains to be investigated.

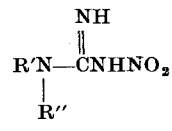
Chemistry

Since McKay[1] has summarized the chemistry of substituted nitroguanidines in an excellent fashion, only reactions not covered by his review will be discussed in any detail here.

A series of 1-substituted-3-nitro-1-nitrosoguanidines (Tables I and II) have been synthesized from the parent 1-substituted-3-nitroguanidines (Tables I and II) by nitrosation using sodium nitrite in 50 per cent nitric acid solution.[2] The 1-substituted-3-nitroguanidines were made by the methods of McKay *et al.*,[2,3] either via reaction of the free amine in water with nitroguanidine at 60° or via reaction of the amine with 1-methyl-3-nitro-1-nitrosoguanidine (I) in ethyl alcohol–water at 0–5°. In general, the latter method proved to give better yields by avoiding the side reactions of hydrolysis to ureas or cyclization of 2-substituted ethyl derivatives to imidazolines. In the case of aryl-substituted derivatives, the second method was the only successful one.

No difficulties were experienced in the nitrosation of 1-alkyl-3-nitroguanidines and 1-aralkyl-3-nitroguanidines using sodium nitrite in 50 per cent nitric acid. Paper chromatography on Whatman No. 1 paper with 1-butanol–acetic acid–water (5 : 2 : 3) as the solvent system gave good separations of the 1-substituted-3-nitroguanidines from the nitroso compounds. The R_f values of the 1-substituted-3-nitroguanidines were always less than those of the corresponding nitroso derivatives. The spots were easily detected by their ultraviolet absorption. Infrared absorption spectra of the nitroso compounds were also very characteristic and different enough from the parent compounds for the success or failure of the nitrosation reaction to be readily ascertained. All of the nitroso derivatives had strong bands near 6·5, 10·0, and 11·0 μ, due to the nitroso group, and also had lost an NH band at 3·10 μ characteristic of the starting material.

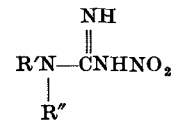
Table I. Known 1-substituted-3-nitroguanidines



No.	NSC No.	R′ Ref.	R″	m.p., °C	Literature m.p. °C	Yield, %	Literature yield, %
II	25071	C_6H_5—[2,6]	H[a]	84–85	84–85	68[d]	53
IV	28106	p-$CH_3OC_6H_4$—[2]	H[a]	152–154	153–154	92[e]	91
V	10386	$C_6H_5CH_2$—[2]	H[c]	184–185	182–183	77	95
VI	25070	$C_6H_5CH_2$—[7]	NO[b]	119–120 (d.)	117–118	59	69
VII	24640	n-C_4H_9—[2]	H[c]	84–85	84–85	68	53
VIII	24639	n-C_4H_9—[2]	NO[b]	121–122 (d.)	121	70	73
IX	25074	i-C_4H_9—[6]	H[c]	122–123	121–122	75	55
X	25960	$ClCH_2CH_2$—[3]	H[a]	116–117	116–117	70	69
XI	25959	$ClCH_2CH_2$—[3]	NO[b]	112–113	114	58	69
XII	25964	$HOCH_2CH_2$—[3]	H[a]	118	118	52[j]	69
XIII	25962	$HOCH_2CH_2$—[3]	NO[b]	105 (d.)	115 (d.)	16[f]	37
XIV	30904	$C_2H_5O_2CCH_2$—[9]	H[a,k]	147–151	150	82	
XV	33675	n-C_3H_7—[6]	H[c]	95–99	98–99	50	30–50
XVI	33674	n-C_3H_7—[7]	NO[b]	118–120 (d.)	118 (d.)	60	73
XVII	33676	C_6H_{11}—[2]	H[a]	196–198	197–198	42[g]	46
XVIII	34701	$Et_2N(CH_2)_3$—[2]	H[a]	134–136	135–136	70[h]	72
XIX	34700	n-C_5H_{11}—[6]	H[c]	91–94	98–99	42	30–50
XX	34697	$CH_3OCH_2CH_2$—[3]	H[a]	120–123	118–120	36	36
XXI	36872	$BrCH_2CH_2$—[3]	H[a]	104–105	102–103	83[i]	83
XXII	—	C_2H_5—[2]	H[c]	146–149	147–148	38	52
XXIII	38191	C_2H_5—[2]	NO[b]	118 (d.)	115	71	77
LIV	29431	—CH_2CH_2—	H	242 (d.)	248 (d.)	82	75

[a] Prepared by reaction of the corresponding amine ($R'NH_2$) with 1-methyl-3-nitro-1-nitrosoguanidine. [b] Prepared by nitrosation of the corresponding 1-R′-3-nitroguanidine with sodium nitrite in 50 per cent nitric acid. [c] Prepared by reaction of the corresponding amine ($R'NH_2$) with nitroguanidine. [d] R_f, 0·86. [e] R_f, 0·81. [f] R_f, 0·52. [g] R_f, 0·88. [h] R_f, 0·55. [i] R_f, 0·78. [j] R_f, 0·43. [k] See Experimental.

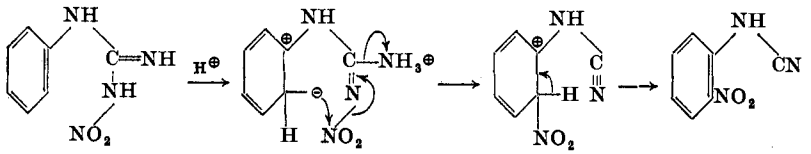
Table II. New 1-substituted-3-nitroguanidines



No.	NSC No.	R′	R″	m.p. °C	R_f	Yield, %	Empirical formula	Calcd.			Found		
								C	H	N	C	H	N
XXIV	25073	*i*-C_4H_9—	NO[b]	82–83 (d.)[g]	—	40	$C_5H_{11}N_5O_3$	31·7	5·82	37·0	31·9	6·01	36·5
XXV	25072	$(CH_3)_2N(CH_2)_3$—	H[d]	105–106[h]	0·39	40	$C_6H_{15}N_5O_2$	38·1	7·99	37·0	38·1	8·07	36·6
XXVI	25965	$(CH_3)_2N(CH_2)_3$— (.HNO_3)	NO[b,c]	132 (d.)	—	55	$C_6H_{15}N_7O_6$	25·6	5·38	34·8	25·6	5·53	34·4
XXVII	25967	*p*-$CH_3C_6H_4SO_2$—	H[c]	180–181	0·86	93	$C_8H_{10}N_4O_4S$	37·2	3·89	21·7	37·4	4·17	21·6
XXVIII	—	$(CH_3)_3N(CH_2)_3$—.I^-	H[c]	141–143[g]	—	81	$C_7H_{18}IN_5O_2$	25·3	5·47	21·1	24·3[e]	5·48	20·6
XXIX	34699	*n*-C_5H_{11}—	NO[b]	124 (d.)	—	55	$C_6H_{13}N_5O_3$	35·5	6·45	34·5	34·8	6·68	34·6
XXX	34696	$CH_3OCH_2CH_2$—	NO[b]	105 (d.)[i]	—	51	$C_4H_9N_5O_4$	25·1	4·74	36·6	25·5	4·54	36·9
XXXI	36874	$CH_3CHOHCH_2$—	H[a]	123–124 (d.)[f,g]	0·67	61	$C_4H_{10}N_4O_3$	29·6	6·21	—	29·6	6·10	—
XXXII	34698	$HO(CH_2)_3$—	H[a]	125–126[g]	0·60	67	$C_4H_{10}N_4O_3$	29·6	6·21	34·5	29·7	6·33	34·6
XXXIII	35897	$CH_3CO_2(CH_2)_3$—	H[c]	129–130[g]	0·76	73	$C_6H_{12}N_4O_4$	35·3	5·92	27·4	35·5	5·92	27·4
XXXIV	35896	$CH_3CO_2(CH_2)_3$—	NO[b]	110–112 (d.)[g]	—	31	$C_6H_{11}N_5O_5$	30·9	4·75	—	31·0	4·90	—
XXXV	35895	$CH_3CO_2CH_2CH_2$—	H[c]	118–120[g]	0·83	89	$C_5H_{10}N_4O_4$	31·6	5·30	29·5	31·5	5·22	29·7
XXXVI	35907	$CH_3CO_2CH_2CH_2$—	NO[b]	93–94 (d.)[g]	—	27	$C_5H_9N_5O_5$	27·4	4·13	—	27·3	4·38	—
XXXVII	35894	$ClCH_2CH_2CH_2$—	H[a]	103–105[g]	—	41	$C_4H_9ClN_4O_2$	26·6	5·02	31·0	26·6	5·15	30·5
XXXVIII	35906	$ClCH_2CH_2CH_2CH_2$—	NO[b]	110–111 (d.)[g]	—	66	$C_4H_8ClN_5O_3$	22·9	3·82	—	23·0	4·17	—
XXXIX	36885	$BrCH_2CH_2$—	NO[c]	101–103 (d.)[j]	0·87	48	$C_3H_6BrN_5O_3$	15·0	2·52	33·3[k]	15·2	2·81	32·6[k]
XL	36876	$C_6H_5CH_2CH_2$—	H[a]	162–164[g]	—	84	$C_9H_{12}N_4O_2$	51·9	5·81	—	52·2	5·93	—
XLI	38184	$C_6H_5CH_2CH_2$—	NO[b]	130–134 (d.)[g]	—	24	$C_9H_{11}N_5O_3$	45·6	4·67	—	45·7	4·75	—

[a] Prepared by reaction of the corresponding amine ($R'NH_2$) with 1-methyl-1-nitro-3-nitrosoguanidine (I).[3] [b] Prepared by nitrosation of the corresponding 1-R′-3-nitroguanidine with sodium nitrite in 50 per cent nitric acid.[3] [c] See Experimental. [d] Prepared by reaction of the corresponding amine ($R'NH_2$) with nitroguanidine.[3] [e] No suitable solvent was found for recrystallization. [f] McKay and Milks[3] have reported 62 per cent yield and m.p. 110–110·5°. [g] Recrystallized from ethanol–water. [h] Recrystallized from absolute ethanol. [i] Recrystallized from 95 per cent ethanol. [j] Recrystallized from methanol–water. [k] Bromine analysis.

It was soon found that 1-aryl-3-nitroguanidines could not be nitrosated using sodium nitrite in 50 per cent nitric acid. When attempts were made to force the nitrosation of 1-nitro-3-phenylguanidine (II) by raising the temperature to 25°, only *o*-nitrocarbanilonitrile (III) could be isolated. Compound III was identified by its melting point of 150° (Elderfield *et al.*,[4] reported m.p. 152°), its elementary analysis, and its infrared absorption spectrum which showed the presence of bands characteristic of nitrile and nitro groups as well as of an *o*-disubstituted benzene. Since there was no indication of the formation of *p*-nitrocarbanilonitrile in this reaction, it is probable that the nitro group on the benzene ring was formed by rearrangement of the nitro group of the nitroguanidine, perhaps by the following mechanism:



(II) (III)

It was felt that the stability of the 1-aryl-3-nitro-1-nitrosoguanidines might be enhanced by the presence of a methoxyl group on the benzene ring: accordingly, 1-(*p*-methoxyphenyl)-3-nitroguanidine (IV) was synthesized and its nitrosation investigated. No 1-nitroso product was obtained using sodium nitrite–50 per cent nitric acid, but sodium nitrite, acetic anhydride, and acetic acid[5] did yield a small amount of an unstable, orange-coloured solid which showed the characteristic infrared absorption bands at 6·5, 10·3, and 11·2 μ of 1-substituted-3-nitro-1-nitrosoguanidines. Unfortunately, the compound was too unstable to be purified further.

In addition to the failure of 1-aromatic substituted-3-nitroguanidines to be nitrosated, McKay[1] also reported that 1-substituted-3-nitroguanidines having the alkyl group attached through a secondary carbon could not be nitrosated. Attempts in this laboratory to nitrosate 1-cyclohexyl-3-nitroguanidine (XVII), using sodium nitrite–50 per cent nitric acid, nitrosyl chloride in

acetic anhydride,[10] or isopentyl nitrite in ethanolic hydrogen chloride, all failed. With nitrosyl chloride in acetic anhydride, some 1-nitroso compound probably formed and decomposed, since the infrared absorption spectrum suggested the product was cyclohexyl acetate.

Although nitrosation of 1-(3-dimethylaminopropyl)-3-nitroguanidine (XXV) went smoothly with sodium nitrite in 50 per cent nitric acid, attempts to nitrosate 1-(3-diethylaminopropyl)-3-nitroguanidine (XVIII) using either the same method or isopentyl nitrite–hydrochloric acid in ethanol failed, (XVIII) being recovered. Nitration[11] with nitric acid–acetic anhydride also failed. The reason for this unexpected difference in behaviour of the dimethylamino- and diethylamino-propyl compounds is unknown, since study of models does not support steric hindrance as a reasonable explanation.

Since 1-(3-dimethylaminopropyl)-3-nitro-1-nitrosoguanidine (XXVI) could be obtained as the nitrate salt, the synthesis of a quaternary salt of the dimethylamino grouping was attempted. The amine (XXV) was converted to the methiodide (XXVIII) in 81 per cent yield by treatment of (XXV) with methyl iodide. Under nitrosating conditions, the methiodide (XXVIII) was oxidized and a salt of 1-(3-trimethylaminopropyl)-3-nitro-1-nitrosoguanidine could not be isolated. In order to avoid this oxidative sensitivity, the iodide ion was replaced by nitrate, using silver nitrate. However, the nitrosation products from this compound were too water-soluble to be isolated, even by concentration of the solution.

Gagnon *et al.*[9] recently reported the synthesis of ethyl *N*-(nitroamidino)-glycinate (XIV) by dehydration of ethyl *N*-amidinoglycinate nitrate with concentrated sulphuric acid. A more direct route was used here; (XIV) was prepared in 82 per cent yield by reaction of ethyl glycinate with 1-methyl-3-nitro-1-nitrosoguanidine (I). Attempts to nitrosate (XIV) using sodium nitrite–50 per cent nitric acid, nitrosyl chloride in acetic anhydride, or sodium nitrite–acetic anhydride–acetic acid all failed.

When lysine was reacted with 1-methyl-3-nitro-1-nitrosoguanidine (I), a mixture of lysine and another compound resulted which, from its infrared absorption spectrum and paper chromatographic behaviour (R_f, 0·33; R_f of lysine, 0·24), appeared to be the

desired product, N^6-(nitroamidino)-lysine. The product was detected on paper by its ultraviolet absorption and also by its purple colour with ninhydrin reagent. Due to the similarity in solubility of lysine and the product, no solvent separation could be effected. Attempts to separate lysine from the product using Dowex 50 resin pretreated with hydrochloric acid and by elution with various concentrations of ammonium hydroxide solution also failed.

1-(3-Acetoxypropyl)-3-nitroguanidine (XXXIII) and 1-(2-acetoxyethyl)-3-nitroguanidine (XXXV) were prepared by selective acetylation of the corresponding 1-(ω-hydroxyalkyl)-3-nitroguanidines (XXXII and XIII). At the boiling point of acetic anhydride–pyridine, a mixture of mono- and diacetyl derivatives was obtained. When the reaction was carried out at 80° in acetic anhydride–pyridine, only the desired *O*-acetylation products were obtained. That the reaction was selective on the hydroxyl groups was readily shown by the characteristic *O*-acetyl band in their infrared spectra and by subsequent nitrosation to (XXXIV) and (XXXVI).

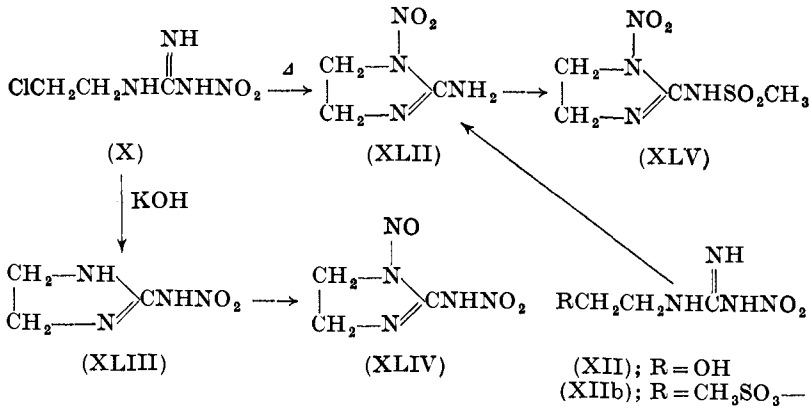
The 1-nitroso derivatives of both 1-(3-hydroxypropyl)-3-nitroguanidine (XXXII) and 1-(2-hydroxypropyl)-3-nitroguanidine (XXXI) were too unstable in the solid state at −10° to warrant characterization.

1,2-Di-(3-nitro-1-guanyl)ethane was prepared in 82 per cent yield by the method of McKay *et al.*,[12] but proved to be too insoluble to be nitrosated in those solvents in which nitrosation could usually be conducted. 1-(*p*-Toluenesulphonyl)-3-nitroguanidine (XXV) also could not be nitrosated.

Some of the 1-(2-substituted-ethyl)-3-nitroguanidines such as (X), (XII), and (XXI) are capable of cyclization to yield either 2-amino-1-nitro-2-imidazoline (XLII) or 2-nitramino-2-imidazoline (XLIII). 1-(2-Chloroethyl)-3-nitroguanidine in the presence of alcoholic potassium hydroxide yields 2-nitramino-2-imidazoline (XLIII).[13] The latter could be nitrosated in nitric acid using sodium nitrite to give 2-nitramino-1-nitroso-2-imidazoline (XLIV) in 72 per cent yield. Refluxing (X) in alcoholic solution without base gave 2-amino-1-nitro-2-imidazoline (XLII),[3] which could not be nitrosated.

Treatment of (XLII) with methanesulphonyl chloride in

pyridine resulted in *N*-(1-nitro-2-imidazolin-2-yl)methanesulphonamide (XLV) in 50 per cent yield. Attempts to mesylate



(XLIII) failed to yield any product, starting material being recovered. Earlier attempts to prepare (XIIb) by mesylation of 1-(2-hydroxyethyl)-3-nitroguanidine (XII) resulted in a product which, from its melting point, chromatographic behaviour (R_f, 0·74), and infrared absorption spectrum, appeared to be an imidazoline that had been *N*-mesylated. The synthesis of the identical material (XLV) by mesylation of (XLII) and the failure of (XLIII) to mesylate proved the structure of the mesylation product of (XII) to be (XLV). Further studies indicated that ring closure of the mesylate (XIIb) to (XLII) was much more rapid than the mesylation of (XII) to (XIIb).

The biological activity shown by (XLIV) prompted the attempted preparation of 1,4,5,6-tetrahydro-2-nitramino-1-nitrosopyrimidine *via* 1,4,5,6-tetrahydro-2-nitraminopyrimidine (XLVI); the latter was synthesized in 19 per cent yield by the method of McKay and Wright.[14] However, under the conditions of nitrosation of (XLIII), i.e., sodium nitrite–50 per cent nitric acid, no nitroso derivative of XLVI could be obtained.

The antitumour activity of 1-(2-chloroethyl)-3-nitro-1-nitrosoguanidine (X) made the synthesis of 1-(2-bromoethyl)-3-nitro-1-nitrosoguanidine (XXXIX) desirable. 1-(2-Bromoethyl)-3-nitroguanidine (XXI) was synthesized in 83 per cent yield from (I) and 2-bromoethylamine. By paper chromatography it was found

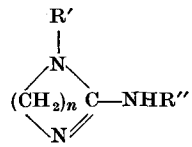
that all of the preparations of (XXI) contained some of the isomeric 2-amino-1-nitro-2-imidazoline (XLII) hydrobromide as a by-product (R_f of XXI, 0·78; R_f of XLII hydrobromide, 0·50). Recrystallization of (XXI) resulted in more of the cyclic compound being produced. It was felt that better purification could be obtained upon nitrosation, since the imidazoline salt should be more water soluble than the desired product (XXXIX). After nitrosation of (XXI) with sodium nitrite in 50 per cent nitric acid, the yellow nitroso compound (XXXIX) separated upon cooling. The paper chromatogram indicated that a mixture of (XXI), the imidazoline (XLII), and (XXXIX) was present. The nitroso compound had an R_f of 0·87.

The use of a 400 per cent molar excess of sodium nitrite in place of the usual 50–100 per cent molar excess, along with increased reaction times, resulted in a purer product (XXXIX), which by paper chromatography was shown to be free of starting material (XXI). When paper chromatograms were run at room temperature (27°), the nitroso compound (XXXIX) decomposed during the chromatographing, the only spot detected corresponding to the imidazoline (XLII). Running the chromatograms at about 3° eliminated this difficulty to a large extent, only trace spots of the imidazoline being present, with the major spot corresponding to the desired (XXXIX). An analytical sample of (XXXIX), m.p. 101–103°, was obtained by recrystallization of the crude nitroso compound at room temperature from methanol by the addition of water followed by cooling.

In order to determine whether a nitro group in the 1-position of 1-substituted-3-nitroguanidines would give antitumour activity as well as the nitroso group, 1-methyl-1,3-dinitroguanidine (XLVII) and 1-butyl-1,3-dinitroguanidine (XLVIII) were synthesized. Nitration was readily accomplished by using nitric acid–acetic anhydride[11] mixtures.

Substitution of another negative grouping in place of the 1-nitroso group was accomplished by acetylation of 1-methyl-3-nitroguanidine with acetic anhydride to *N*-methyl-*N*-(nitroamidino)acetamide (XLIX) in 40 per cent yield. Attempts to prepare *N*-methyl-*N*-(nitroamidino)methanesulphonamide or *N*-methyl-*N*-(nitroamidino)-*p*-toluenesulphonamide from 1-methyl-3-nitroguanidine resulted in recovery of the starting material.

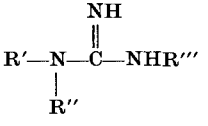
Table III. Imidazolines



No.	NSC No.	n	R′	R″	R_f	m.p. °C	Literature m.p. °C	Yield, %	Literature yield, %
XLII[d]	25973	2	NO_2	H	0·50	191–192	188–189	70	70
XLIII[a]	25961	2	H	NO_2	—	221–222	220–221 (d.)	56	65
XLIV[e]	25958	2	NO	NO_2	—	139–141	141–142	59	88
XLV[b]	38948	2	NO_2	CH_3SO_2—	0·65	167–169		50	
XLVI[a]	38182	3	H	NO_2	—	251–252 (d.)	251–252 (d.)	19	55
LII[c]	36875	2	$HOCH_2CH_2$—	NO_2	0·66	130–132	131–132	48	42
LIII[b]	38183	2	$ClCH_2CH_2$	NO_2	0·84	142–144		52	

[a] Prepared according to the method of McKay and Wright.[14] [b] See Experimental. [c] Prepared according to the method of McKay, Bryce and Rivington.[12] [d] Prepared according to the method of McKay and Milks;[3] this compound is a hydrochloride salt. [e] Prepared according to the method of McKay, Park and Viron.[15]

Table IV. Miscellaneous 1-alkyl-1,3-disubstituted-guanidines

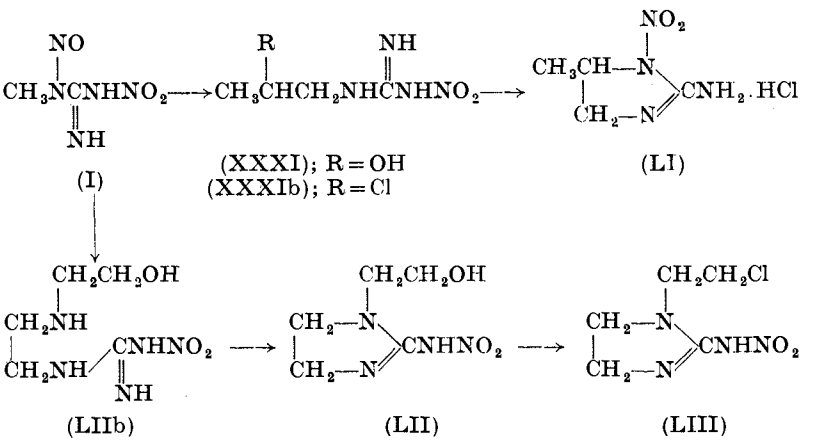


No.	NSC No.	R′	R″	R‴	m.p. °C	Literature m.p. °C	Yield, %	Literature yield, %
XLVII	28104[a]	CH_3—	NO_2	NO_2	81–82	80–82	51	52
XLVIII	38192[a]	n-C_4H_9—	NO_2	NO_2	69–70	71–72	17	24
XLIX	30903[c]	CH_3—	CH_3CO—	NO_2	121–123		40	
L	30908[e]	CH_3—	H	NO[b]	91 (d.)	95 (d.)	64	22
LV	25963[d]	CH_3—	CH_3	NO_2	200	194–195	56	59

[a] Prepared according to the method of Meen and Wright.[11] [b] R_f, 0·54. [c] See Experimental. [d] Prepared according to the method of McKay and Wright.[8] [e] Prepared according to the method of Davis and Rosenquist[16] except that, after completion of the reaction, the solution was concentrated *in vacuo* at room temperature.

Variation of the 3-nitro group of 1-methyl-3-nitro-1-nitrosoguanidine was accomplished by reduction of 1-methyl-3-nitroguanidine to 1-methyl-3-nitrosoguanidine in 64 per cent yield using zinc and ammonium chloride as the reducing agent.[16] The use of paper chromatography made the following of this and other reductions relatively easy, since good separations were obtained. The reduction of 1-methyl-1,3-dinitroguanidine (XLVII) resulted in a mixture of 1-methyl-3-nitroguanidine (R_f, 0·62) and 1-methyl-3-nitrosoguanidine (L) (R_f, 0·54); none of the hoped for 1-methyl-1,3-dinitrosoguanidine was present. Reduction of 1-methyl-3-nitro-1-nitrosoguanidine (I) with zinc and ammonium chloride gave 1-methyl-3-nitroguanidine as the major product. Attempts to prepare 1-methyl-1,3-dinitrosoguanidine by nitrosation of 1-methyl-3-nitrosoguanidine (L) using sodium nitrite–50 per cent nitric acid resulted only in the decomposition of (L).

The ease of cyclization of 2-substituted ethyl or propyl derivatives of 1-substituted-3-nitroguanidines was also illustrated by the following experimental results. Attempts to prepare 1-(2-chloropropyl)-3-nitroguanidine (XXXIb) either *via* reaction of 2-chloropropylamine with 1-methyl-3-nitro-1-nitrosoguanidine (I) or *via* chlorination of 1-(2-hydroxypropyl)-3-nitroguanidine (XXXI) with thionyl chloride resulted in a high-melting product,



m.p. 190–198° (d.), which analyzed correctly for the isomeric 2-amino-5-methyl-1-nitro-2-imidazoline hydrochloride (LI). This

could be distinguished from the desired open chain compound (XXXIb) by its infrared absorption spectrum and paper chromatographic behaviour, which were very much like those of 2-amino-1-nitro-2-imidazoline (XLII) hydrochloride.

The reaction of (I) with 2-(2-aminoethylamino)ethanol also resulted in spontaneous cyclization of the intermediate (LIIb) to yield 2-(2-nitramino-1-imidazolinyl)-1-ethanol (LII) in 48 per cent yield.[12] Treatment of (LII) with thionyl chloride resulted in a 52 per cent yield of 1-(2-chloroethyl)-2-nitramino-2-imidazoline (LIII). The latter compound was of interest since 2-nitramino-1-nitroso-2-imidazoline had shown some activity and (LIII) can be considered as having an alkylating group in the 1-position of the imidazoline ring in the place of the nitroso group.

Experimental*†

Rearrangement of 1-phenyl-3-nitroguanidine (II) to o-nitrocarbanilonitrile (III). A solution of 1-phenyl-3-nitroguanidine (26·6 g, 0·145 mole) in concentrated nitric acid (52 ml) was diluted with water (52 ml). To this stirred solution, cooled with an ice bath, was added dropwise a solution of sodium nitrite (22·0 g, 0·32 mole) in water (36 ml), the temperature being maintained between 30–35°. After 30 min the mixture was filtered and the yellow crystals were washed with water. The solid was then washed with ether until the washings were free of colour. The cream-coloured solid (15·7 g, 60 per cent) was identified with 1-phenyl-3-nitroguanidine (II) by paper chromatography and infrared spectrum.

The ether layer was separated from the filtrate and the aqueous layer extracted with ether (100 ml). The combined ether extracts were concentrated *in vacuo* to a yellow-brown solid (10 g), m.p. 115–130° (d.). This solid was recrystallized from methanol to yield *o*-nitrocarbanilonitrile (2·6 g, 11 per cent), m.p. 152–155° (sublimes about 130°); $\lambda_{max\,(\mu)}^{Nujol}$ 3·15 (NH), 4·45 (C≡N), 6·62

* Paper chromatograms were run by the descending technique on Whatman No. 1 paper using the solvent system 1-butanol–acetic acid–water (5 : 2 : 3). Spots as low as 5γ could be detected by their ultraviolet absorption. Melting points were taken on a Fisher–Johns block and are uncorrected.

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

and 7·46 (NO_2), 13·55 (*o*-disubstituted benzene). A similar preparation, m.p. 148–149°, was analyzed.

Anal. Calcd. for $C_7H_5N_3O_2$: C, 51·5; H, 3·09; N, 25·8. Found: C, 51·1; H, 3·22; N, 25·8.

1-(3-Dimethylaminopropyl)-3-nitro-1-nitrosoguanidine nitrate (*XXVI*). 1-(3-Dimethylaminopropyl)-3-nitroguanidine (XXV) (7·56 g, 0·04 mole) was dissolved in concentrated nitric acid (10 ml) and diluted with water (5 ml). The solution was stirred and cooled to 2°. Then a solution of sodium nitrite (4·2 g, 0·06 mole) in water (7 ml) was added slowly keeping the temperature below 5°. After being stirred for one hour, the reaction mixture was allowed to stand at −5° to 0° for one additional hour during which time the yellow product crystallized. It was collected on a filter and washed well with ether; yield, 6·15 g (55 per cent), m.p. 132·5° (d.); $\lambda_{max\,(\mu)}^{Nujol}$ 2·95, 3·05 (NH), 6·15, 6·51 (C=N, NH), 7·61 (NO_2), 7·32, 12·17 (NO_3^-), 10·26, 10·84, 11·54 (N—NO).

See Table II for analytical data obtained from a similar preparation after the product had been washed thoroughly with absolute ethanol.

Attempts to prepare the corresponding 1-(3-diethylaminopropyl)-3-nitro-1-nitrosoguanidine were unsuccessful, since the product was too soluble. Concentration of the nitric acid solution *in vacuo* with little or no heating gave inorganic salts as the only isolable material.

(3-Guanidinopropyl)trimethylammonium iodide (*XXVIII*). A solution of 1-(3-dimethylaminopropyl)-3-nitroguanidine (XXV) (1·89 g, 0·01 mole) was refluxed in methanol (5 ml) and methyl iodide (2·13 g, 0·015 mole) for 0·5 h. The solution was concentrated *in vacuo* to a colourless syrup which was crystallized from methanol. The white solid was collected on a filter and washed with acetone; yield, 2·7 g (81 per cent), m.p. 141–143°; $\lambda_{max\,(\mu)}^{Nujol}$ 2·95, 3·05 (NH), 6·05 (NH, C=N), 6·50, 7·90 (NO_2), 7·23 (CH_3).*

Ethyl N-(*nitroamidino*)*glycinate* (*XIV*). A cold solution of potassium hydroxide (11·2 g, 0·20 mole) in ethanol (300 ml) was added to ethyl glycinate hydrochloride (27·9 g, 0·20 mole). To the stirred suspension at 15° 1-methyl-3-nitro-1-nitrosoguanidine

* See Table II for analytical data.

(I) (14·7 g, 0·10 mole) was added in portions over a period of about 30 min. The mixture was then heated rapidly to 60°, filtered, and the insoluble potassium chloride washed with hot absolute alcohol (50 ml). The combined filtrate and washings were chilled at 0° until crystallization was complete. The product was collected on a filter and washed with cold ethanol; yield, 15·7 g (82 per cent), m.p. 147–151° (lit.[6] 150°); $\lambda_{max\,(\mu)}^{Nujol}$ 3·10, 3·25 (NH), 5·78 (ester C=O), 6·14, 6·22 (NH, C=N), 6·43 (NO_2), 8·05 (ester C—O—C).

Attempts to prepare *N*-(nitroamidino)glycine from sodium glycinate and 1-methyl-3-nitro-1-nitrosoguanidine failed.

1-(2-Acetoxyethyl)-3-nitroguanidine (*XXXV*). A mixture of 1-(2-hydroxyethyl)-3-nitroguanidine (13·5 g, 0·091 mole), pyridine (50 ml) and acetic anhydride (10·2 g, 0·1 mole) was heated on a steam bath at about 80° for 2 h, solution occurring in 5 min. Concentration *in vacuo* gave a white solid which was recrystallized from 95 per cent ethanol; yield, 15·3 g (89 per cent), m.p. 114–117°. The melting point was raised to 118–120° after two additional crystallizations from 95 per cent ethanol; $\lambda_{max\,(\mu)}^{Nujol}$ 5·85 (ester C=O), 8·05 (ester C—O—C), 6·05, 6·22 (NH, C=N), 6·45 (NO_2).*

1-(3-Acetoxypropyl)-3-nitroguanidine (*XXXIII*). By acetylation of 1-(3-hydroxypropyl)-3-nitroguanidine (XXXII) (16·2 g, 0·1 mole) in pyridine (50 ml) and acetic anhydride (15·3 g, 0·15 mole), as described above for (XXV), 14·9 g (73 per cent) of product was obtained, m.p. 128–132°. Recrystallization from 95 per cent ethanol altered the melting point to 129–130°; $\lambda_{max\,(\mu)}^{Nujol}$ 5·75 (ester C=O), 6·01, 6·25 (NH, C=N), 6·42 (NO_2), 8·01 (ester C—O—C).*

3-Nitro-1-(p-*toluenesulphonyl)guanidine* (*XXVII*). The potassium salt of 3-nitro-1-(*p*-toluenesulphonyl)guanidine was prepared according to the method of Henry[17] in quantitative yield; m.p. 188–189°.

3-Nitro-1-(p-*toluenesulphonyl)guanidine* was obtained in 93 per cent yield, m.p. 184·5–185·5°, by neutralization of a solution of the potassium salt dissolved in 10 times its weight of warm water. The product was recrystallized from 95 per cent ethanol to give colourless crystals, m.p. 180–180·5°; $\lambda_{max\,(\mu)}^{Nujol}$ 6·15 (NH_2, C=N),

* See Table II for analytical data.

6·55 (NO_2, aryl), 7·52, 8·61 (—SO_2N—), 12·30 (*p*-disubstituted benzene).*

N-(*1-Nitro-2-imidazolin-2-yl*)*methanesulphonamide* (*XLV*). (a) A suspension of 1-(2-hydroxyethyl)-3-nitroguanidine (XII) (1·48 g, 0·01 mole) in pyridine (5 ml) was stirred in an ice bath and methanesulphonyl chloride (1·75 g, 0·015 mole) in pyridine (2 ml) was added dropwise. Solution was complete in several minutes but stirring was continued for 2 h. The reaction mixture was poured into ice-water (20 ml) and extracted three times with 15-ml portions of ethyl acetate. The dried organic layer was concentrated *in vacuo* to a yellow syrup (1·4 g) which crystallized rapidly when rubbed. The material was recrystallized from methanol; yield, 0·3 g, m.p. 155–160°; $\lambda_{max\,(\mu)}^{Nujol}$ 2·95 (NH), 6·04 (NH, NO_2), 7·45, 8·65 (—SO_2N—), 7·95 (NO_2). The compound travelled as one major spot (R_f, 0·65). The starting material had an R_f of 0·43. Two recrystallizations of the solid gave a sample with m.p. 167–171°.

Anal. Calcd. for $C_4H_8N_4O_4S$: C, 23·1; H, 3·87; N, 26·9. Found: C, 22·9; H, 4·24; N, 26·4.

(b) Methanesulphonyl chloride (4·15 g, 0·036 mole) was added to a suspension of 2-amino-1-nitro-2-imidazoline (XLII) (3·0 g, 0·018 mole) in pyridine (30 ml) cooled in an ice bath. After being stirred for 2 h in the ice bath, the mixture was poured into 25 g of ice and water. The product was collected on a filter and washed with water; yield, 1·9 g (50 per cent), m.p. 167–169°. A mixture with preparation (a) gave no depression in m.p. Both preparations gave identical infrared absorption spectra and paper chromatograms.

1-(2-Bromoethyl)-3-nitro-1-nitrosoguanidine (*XXXIX*). Sodium nitrite (5·24 g, 0·076 mole) in water (5 ml) was added dropwise, over a period of 20 min, to a solution of 1-(2-bromoethyl)-3-nitroguanidine (XXI) (3·98 g, 0·019 mole) in concentrated nitric acid (8·4 ml) and water (3·6 ml) cooled to −5°. The solution was stirred for 1 h at 0°, the yellow-coloured product filtered and washed with cold water (5 ml); yield, 2·24 g (48 per cent), m.p. 85–88° (d.); $\lambda_{max\,(\mu)}^{Nujol}$ 2·95, 3·05 (NH), 6·15 (C=N), 6·49 (N=C), 8·0 (NO_2), 11·16, 11·6 (N=O). The crude sample was recrystallized from methanol by adding water at room temperature until

* See Table II for analytical data.

cloudy, and cooling; m.p. 92–94° and after thorough drying, m.p. 101–103°. When the crude compound was run on Whatman No. 1 paper in 1-butanol–acetic acid–water (5 : 2 : 3) at room temperature only one spot, R_f, 0·52, corresponding to 2-amino-1-nitro-2-imidazoline (XLII) hydrobromide, was detected. Running the same chromatogram at 3° gave (XXXIX), R_f, 0·87, as a major spot and the imidazoline (XLII) as a trace spot. Similar results were obtained with purified material.*

N-*methyl*-N-(*nitroamidino*)*acetamide* (*XLIX*). A solution of 1-methyl-3-nitroguanidine (3·0 g, 0·024 mole) in acetic anhydride (30 ml) was refluxed for 2 h. After concentration *in vacuo*, the residue was crystallized from water (20 ml) to give colourless crystals (1·5 g, 40 per cent), m.p. 103–118°, that had an infrared spectrum essentially identical to that of the analytical sample. Two recrystallizations (0·3 g) from water (5 ml) gave an analytical sample, m.p. 120·5–123°; $\lambda_{max\,(\mu)}^{Nujol}$ 3·00, 3·12 (NH), 5·08 (C=O of acetyl attached to a negative nitrogen).

Anal. Calcd. for $C_4H_8N_4O_3$: C, 30·0; H, 5·03; N, 35·0. Found: C, 30·0; H, 4·96; N, 34·9.

2-Amino-5-methyl-1-nitro-2-imidazoline hydrochloride (*LI*). Thionyl chloride (0·6 ml, 8 millimoles) was added to a suspension of 1-(2-hydroxypropyl)-3-nitroguanidine (XXXII) (0·81 g, 5 millimoles) in chloroform (6 ml). The mixture was refluxed for 1 h, causing the deposition of an opaque oil which crystallized. The mixture was concentrated *in vacuo* to dryness, triturated in ethanol, and the white solid collected on a filter; yield, 0·41 g (22 per cent), m.p. 190–198° (d.), R_f, 0·57. Two recrystallizations from 95 per cent ethanol did not change the melting point; $\lambda_{max\,(\mu)}^{Nujol}$ 3·14, 6·57 (NH), 5·81 (C=NH), 6·30 (NO_2).

Anal. Calcd. for $C_4H_9ClN_4O_2$: C, 26·6; H, 5·02; Cl, 19·6; N, 31·0. Found: C, 26·7; H, 5·44; Cl, 19·5; N, 31·0.

Reaction of 2-chloropropylamine with 1-methyl-3-nitro-1-nitrosoguanidine in water, in the usual manner, gave a solution which showed only one spot on paper chromatography. The R_f of 0·54 showed this to be the cyclic compound (LI), since the open chain 1-(2-chloropropyl)-3-nitroguanidine would travel faster on paper in the solvent system used.

1-(2-Chloroethyl)-2-nitramino-2-imidazoline (*LIII*). Thionyl

* See Table II for analytical data.

chloride (6 ml, 0·08 mole) was added to a suspension of 2-nitramino-2-imidazoline-1-ethanol (LII) in chloroform (90 ml). The reaction mixture was refluxed for one hour; solution took place during the first 5 min and was followed by separation of an oil which solidified upon cooling. The mixture was concentrated *in vacuo* and the solid residue recrystallized from methanol; yield, 5·0 g (52 per cent), m.p. 141–144°. A portion, recrystallized again from methanol for analysis, had m.p. 142–144°; $\lambda_{max\,(\mu)}^{Nujol}$ 3·01 (NH), 6·40, 7·70–7·90 (NO_2).

Anal. Calcd. for $C_5H_9ClN_4O_2$: C, 31·2; H, 4·70; Cl, 18·4; N, 29·1. Found: C, 31·2; H, 5·30; Cl, 18·6; N, 28·8.

Biological Activity

Methods

Assays for activity against three tumours (Sarcoma 180, Adenocarcinoma 755, and lymphoid Leukaemia L1210) were performed according to specifications established by the Cancer Chemotherapy National Service Center.[18] Only the results obtained with Leukaemia L1210 will be reported in detail, since none of the compounds exhibited any significant activity against the sarcoma or adenocarcinoma.

In assays for activity against Leukaemia L1210, ascites form, (C57Bl/6 Jax × DBA/2 Jax)F1 hybrid mice were used. Animals of a single sex were used for any one experiment. Tests of nitroguanidines were included in series in which 25 assays were run, each drug assay being done with 6 animals, each series having 30 untreated controls. All mice were implanted intraperitoneally with 100,000 ascitic cells contained in 0·1 ml of sterile Locke's solution. Treatment was begun 24 h after tumour 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 or Adenocarcinoma 755 tumours. No dose higher than 500 mg/kg of body weight was used.

Mean survival time was calculated for treated and control groups. Five days were subtracted from mean survival of treated (T) and untreated (C) groups. On the basis of experience,[18] a T/C value of 1·71 is considered possibly significant. Compounds which produced such a value of T/C were retested at several

dilutions bracketing those found 'active'. For the purpose of calculating mean survival time, mice failing to survive the fifth day were not counted. If two or more mice failed to survive the fifth day, the compound was considered to be toxic at this dosage. If, in calculating mean survival as described, the T/C value was 0·67 or less, the dosage regimen was considered toxic.

Results

None of the compounds discussed in this paper had any demonstrable, reproducible activity against Sarcoma 180 or Adenocarcinoma 755 in mice.

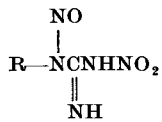
The results of tests with Leukaemia L1210 are given in Tables V–IX. Only nine compounds produced increases in survival time which are considered significant. All but one, (XLVII), are 1-alkyl-3-nitro-1-nitrosoguanidines; (XLVII) is a 1,3-dinitroguanidine. Of the nine 'active' compounds, only four had significant activity in more than one test: 1-ethyl- (XXIII), 1-(2-chloroethyl)- (XI), 1-(2-bromoethyl)- (XXXIX), 3-nitro-1-nitrosoguanidine and the nitrosoimidazoline (XLIV). Of the 16 nitrosoguanidines, seven were 'active' in one test, but only three had confirmable activity. Only one of the imidazoline compounds was 'active' (XLIV), and only one of the compounds in Table VIII was 'active' (XLVII).

In general, the 1-nitroso derivatives of 1-alkyl-3-nitroguanidines were more toxic than their *des*-nitroso analogues. However, among nitroso compounds there was little apparent correlation between toxicity and antitumour activity. Even the 'active' compounds inhibited the tumour over a narrow range.

It is difficult to compare the relative activities of the compounds tested in different series at different times. Nevertheless, 1-(2-chloroethyl)-3-nitro-1-nitrosoguanidine (XI) and the bromoethyl analogue (XXXIX) appear to be the most active compounds. Even though the chloroethyl analogue (XI) appears to be better than the bromoethyl analogue (XXXIX), it would be necessary to test these concurrently in the same test series.

The highest mean survival time of any treated group was 12·7 days, compared to untreated controls which normally die in 8 to 11 days. No compound increased survival time more than about 50 per cent.

Table V. Antileukaemic activity of 1-alkyl-3-nitro-1-nitrosoguanidines (Class 1)



No.	NSC No.	R	Dose mg/kg/day	Series No.	Change in weight (g) day 0 to day 5 (treated/control)	Survivors on day 5	Mean survival time, days (treated/control)	*T/C*
VI	25070	$C_6H_5CH_2$—	125	208	0·0/0·7	6/6	7·8/9·0	0·70
			125	210	−1·4/−1·5	6/6	11·2/11·7	0·93
			80	224	0·5/0·0	6/6	7·8/8·1	0·90
			40	224	0·2/0·0	6/6	7·2/8·1	0·71
			20	202	0·3/0·0	6/6	9·5/8·3	1·36
VIII	24639	*n*-C_4H_9—	40	208	0·5/0·7	6/6	8·8/9·0	0·95
			20	202	−0·2/0·0	6/6	7·2/8·3	0·67
			10	195	−0·2/−0·4	6/6	8·2/8·7	0·86
			10	210	−0·9/1·5	5/6	11·2/11·7	0·93
XI	25959	$ClCH_2CH_2$—	20	191	−0·3/0·6	6/6	12·5/7·6	*2·88*
			20	201	−0·4/0·4	6/6	12·0/7·5	*2·80*
			40	202	−0·2/0·0	6/6	8·3/8·3	1·00
			80	208	−1·1/0·7	6/6	10·2/9·0	1·30
			320	214		0/6		
			160	214		0/6		
			80	214	−1·4/0·6	4/6	6·8/7·1	0·86
			60	214	−1·2/0·6	6/6	7·3/7·1	1·10
			40	214	−1·5/0·6	6/6	7·7/7·1	1·29
			20	214	−0·8/0·6	6/6	11·3/7·1	*3·00*
			10	214	0·7/0·6	6/6	10·3/7·1	*2·52*

XIII	25962	$HOCH_2CH_2$—	20	191	0·0/0·6	6/6	8·8/7·6	1·46
			40	202	0·9/0·0	6/6	8·5/8·3	1·06
			80	208	−1·3/0·7	6/6	11·7/9·0	1·68
			160	218	−0·2/0·8	1/6	6·0/7·6	0·38
			80	224	−0·5/0·0	6/6	8·7/8·1	1·19
			40	224	−0·8/0·0	6/6	8·7/8·1	1·19
			20	224	0·6/0·0	6/6	8·8/8·1	1·23
			10	224	0·5/0·0	6/6	7·8/8·1	0·90
			5	224	−0·4/0·0	5/6	9·4/8·1	1·42
XVI	33674	n-C_3H_7—	200	239	−0·3/0·4	6/6	9·2/7·8	1·50
			150	250	−2·5/−0·4	3/6	12·7/9·4	*1·75*
			100	250	−1·2/−0·4	6/6	11·7/9·4	1·52
			66	250	−0·9/−0·4	5/6	10·2/9·4	1·18
			44	250	−0·3/−0·4	6/6	11·0/9·4	1·36
XXIII	38191	CH_3CH_2—	70	292	−2·3/0·3	6/6	14·2/9·1	*2·24*
			105	308	−2·3/0·7	5/6	11·2/8·7	*1·81*
			70	308	−2·5/0·7	6/6	11·3/8·7	1·70
			47	308	0·3/0·7	6/6	9·3/8·7	1·16
			31	308	−0·5/0·7	6/6	11·5/8·7	*1·76*
XXIV	25073	i-C_4H_9—	400	206	−4·4/0·5	2/6	8·7/6·6	*2·31*
			200	212	−1·8/0·8	2/6	8·0/7·4	1·25
			100	220	−0·2/0·0	6/6	8·2/8·6	0·89
XXVI	25965	$(CH_3)_2N(CH_2)_3$—HNO_3	20	201	0·0/0·4	6/6	10·0/7·5	*2·00*
			40	202	1·5/0·0	6/6	11·2/8·3	*1·88*
			20	208	0·6/0·7	6/6	11·5/9·0	1·63
			160	228		0/6		
			80	228	−0·9/0·6	4/6	6·3/9·1	0·32
			40	228	−1·3/0·6	6/6	7·2/9·1	0·54
			20	228	−1·2/0·6	6/6	8·7/9·1	0·90
			10	228	−0·5/0·6	6/6	11·2/9·1	1·51
			5	228	0·8/0·6	6/6	10·0/9·1	1·22

Table V—*continued*

No.	NSC No.	R	Dose mg/kg/day	Series No.	Change in weight (g) day 0 to day 5 (treated/control)	Survivors on day 5	Mean survival time, days (treated/control	*T/C*
XXIX	34699	n-C_5H_{11}—	200	245	0·7/0·1	5/6	7·8/7·4	1·17
			75	245	0·0/0·1	6/6	6·7/7·4	0·71
XXX	34696	$CH_3OCH_2CH_2$—	50	245	−1·9/0·1	6/6	9·2/7·4	*1·75*
			20	245	−1·0/0·1	6/6	8·2/7·4	1·33
			75	254	−0·1/0·1	4/6	8·0/8·6	0·83
			50	254	0·9/0·1	4/6	10·8/8·6	1·61
			33	254	0·1/0·1	4/6	6·5/8·6	0·42
			22	254	−0·3/0·1	4/6	9·3/8·6	1·19
XXXIV	35896	$CH_3CO_2(CH_2)_3$—	250	257		0/6		
			100	257	−2·8/0·1	4/6	7·8/10·2	0·54
			20	257	0·2/0·1	6/6	12·2/10·2	1·48
XXXVI	35907	$CH_3CO_2(CH_2)_2$—	250	257		0/6		
			100	257		0/6		
			20	257	−0·8/0·1	6/6	11·8/10·2	1·31
XXXVIII	35906	$ClCH_2CH_2CH_2$—	250	257		0/6		
			100	257	−0·7/0·1	4/6	13·0/10·2	1·54
			20	257	−1·2/0·1	6/6	12·8/10·2	1·50
XXXIX	36885	$BrCH_2CH_2$—	125	269	−2·0/0·9	5/6	11·8/8·1	*2·19*
			60	269	−1·0/0·9	6/6	12·7/8·1	*2·48*
			183	280	−1·4/1·7	5/6	11·2/8·2	*1·94*
			125	280	−1·9/1·7	6/6	12·3/8·2	*2·28*
			83	280	0·2/1·7	6/6	12·3/8·2	*2·28*
			56	280	−0·2/1·7	6/6	10·7/8·2	*1·78*
XLI	38184	$C_6H_5CH_2CH_2$—	140	292	−1·0/0·3	3/6	10·7/9·1	1·39
			70	306	0·9/1·4	6/6	8·0/8·3	0·91

Table VI. Antileukaemic activity of 1-alkyl-3-nitroguanidines (Class 2)

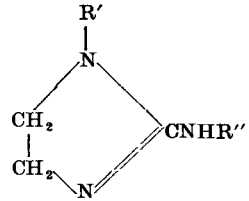
$$R\text{—}NHC(=NH)NHNO_2$$

No.	NSC No.	R	Dose mg/kg/day	Series No.	Change in weight (g) day 0 to day 5 (treated/control)	Survivors on day 5	Mean survival time, days (treated/control)	T/C
II	25071	C_6H_5—	450	202	−0·6/0·0	1/6	6·0/8·3	0·30
			225	208	0·0/0·7	6/6	7·2/9·0	0·55
			110	218	1·4/0·8	5/6	6·8/7·6	0·69
IV	28106	p-$CH_3OC_6H_4$—	500	196	−1·0/0·2	5/6	11·8/11·2	1·10
VII	24640	n-C_4H_9—	500	195		0/6		
			100	201	0·1/0·6	6/6	7·0/7·5	0·80
IX	25074	i-C_4H_9—	400	202	0·0/0·0	1/6	9·0/8·3	1·21
			200	208	0·2/0·7	6/6	8·3/9·0	0·83
X	25960	$ClCH_2CH_2$—	500	191	−2·2/0·6	2/6	6·5/7·6	0·58
			200	216	−0·8/0·1	5/6	7·6/7·4	1·08
			200	218	0·1/0·8	6/6	7·2/7·6	0·85
XII	25964	$HOCH_2CH_2$—	500	191	0·0/0·6	6/6	6·5/7·6	0·58
			250	202	0·0/0·0	4/6	7·0/8·3	0·61
XIV	30904	$C_2H_5O_2CCH_2$—	500	210	−1·8/−1·5	6/6	11·7/11·7	1·00
			250	210	2·3/−1·5	6/6	11·1/11·7	0·91
XV	33675	n-C_3H_7—	450	246	0·3/0·1	6/6	7·0/7·7	0·74

Table VI—*continued*

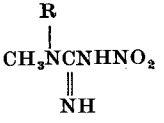
No.	NSC No.	R	Dose mg/kg/day	Series No.	Change in weight (g) day 0 to day 5 (treated/control)	Survivors on day 5	Mean survival time days (treated/control)	*T/C*
XVII	33676	C_6H_{11}—	400	246		0/6		
			200	252	−0·1/0·4	5/6	6·4/8·4	0·41
XVIII	34701	$Et_2N(CH_2)_3$—	400	261	−0·2/0·3	5/6	7·0/7·4	0·83
XIX	34700	*n*-C_5H_{11}—	350	261	0·3/0·3	6/6	6·2/7·4	0·50
XX	34697	$CH_3OCH_2CH_2$—	450	261	0·3/0·3	6/6	7·7/7·4	1·13
XXI	36872	$BrCH_2CH_2$—	225	299	−1·0/1·4	6/6	9·3/9·0	1·08
			110	312	−0·1/0·9	6/6	7·3/8·2	0·72
XXV	25072	$(CH_3)_2N(CH_2)_3$—	400	202	−0·6/0·0	6/6	7·7/8·3	0·82
XXXI	36874	$CH_3CHOHCH_2$—	400	281	−1·0/0·7	6/6	8·5/7·8	1·25
XXXII	34698	$HOCH_2CH_2CH_2$—	450	261	0·4/0·3	5/6	6·0/7·4	0·42
XXXIII	35897	$CH_3CO_2(CH_2)_3$—	450	273	−3·5/0·4	6/6	7·0/8·5	0·57
XXXV	35895	$CH_3CO_2CH_2CH_2$—	450	273	0·1/0·4	6/6	8·0/8·5	0·86
XXXVII	35894	$ClCH_2CH_2CH_2$—	90	281	−1·1/0·7	6/6	8·2/7·8	1·14
XL	36876	$C_6H_5CH_2CH_2$—	400	281	−0·8/0·7	5/6	6·0/7·8	0·36
			200	300	0·6/1·3	6/6	12·0/9·6	1·52
			100	312	3·5/0·9	6/6	6·5/8·2	0·47
LIV	29431	—CH_2CH_2—	500	205	−1·4/−0·4	5/6	9·6/7·9	1·59
			500	208	0·4/0·7	6/6	7·5/9·0	0·63
			250	218	0·9/0·8	5/6	6·8/7·6	0·69

Table VII. Antileukaemic activity of cyclic nitroguanidines (Class 3)



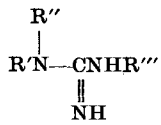
No.	NSC No.	R′	R″	Dose mg/kg/day	Series No.	Change in weight (g) day 0 to day 5 (treated/control)	Survivors on day 5	Mean survival time, days (treated/control)	*T/C*
XLII	25973	NO_2	H	125	198	−2·2/0·4	1/6	7·0/8·1	0·65
				62·5	204	−0·3/−0·2	6/6	8·7/8·5	1·06
XLIII	25961	H	NO_2	500	191	−1·9/0·6	6/6	7·3/7·6	0·88
XLIV	25958	NO	NO_2	20	191	0·7/0·6	6/6	8·5/7·6	1·35
				40	202	−0·2/0·0	6/6	10·8/8·3	*1·76*
				80	208	0·0/0·7	6/6	10·7/9·0	1·43
				160	218	0·1/0·8	6/6	7·5/7·6	0·96
				40	220	−0·4/0·0	6/6	11·2/8·6	*1·72*
				320	234	−3·1/1·0	2/6	8·0/7·8	1·07
				160	234	−0·9/1·0	5/6	9·0/7·8	1·43
				80	234	0·4/1·0	6/6	10·2/7·8	*1·86*
				40	234	−0·1/1·0	6/6	9·8/7·8	*1·71*
				20	234	0·8/1·0	6/6	8·7/7·8	1·32
				10	234	−0·1/1·0	6/6	8·3/7·8	1·18
LII	36875	$HOCH_2CH_2$	NO_2	450	281	0·7/0·7	4/6	6·5/7·8	0·54
				225	299	0·0/1·4	6/6	9·5/9·0	1·13
LIII	38183	$ClCH_2CH_2$	NO_2	450	309		0/6		
				225	316	−0·7/−1·2	5/6	8·0/8·7	0·81

Table VIII. Antileukemic activity of 1-substituted-1-methyl-3-nitroguanidines (Class 4)



No.	NSC No	R	Dose mg/kg/day	Series No.	Change in weight (g) day 0 to day 5 (treated/control)	Survivors on day 5	Mean survival time, days (treated/control)	*T/C*
XLVII	28104	NO_2	20	196	−0·1/0·2	6/6	10·7/11·2	0·92
			50	208	−0·7/0·7	6/6	10·0/9·0	1·25
			100	214	−1·1/0·6	6/6	8·8/7·1	*1·81*
			200	221	−3·0/−0·5	4/6	6·8/8·8	0·47
			100	222	−0·5/0·8	6/6	9·5/7·1	*2·14*
			200	232	−1·0/0·9	6/6	8·2/8·8	0·84
			150	232	−1·1/0·9	6/6	9·8/8·8	1·26
			100	232	−1·4/0·9	6/6	10·3/8·8	1·39
			50	232	0·8/0·9	6/6	10·8/8·8	1·53
			25	232	0·7/0·9	6/6	10·2/8·8	1·37
XLIX	30903	CH_3CO	250	210	0·6/−1·5	6/6	12·2/11·7	1·07
LV	25963	CH_3	500	191	−1·4/0·6	6/6	8·3/7·6	1·27

Table IX. Miscellaneous



No.	NSC No.	R′	R″	R‴	Dose mg/kg/day	Series No.	Change in weight (g) day 0 to day 5 (treated/control)	Survivors on day 5	Mean survival time, days (treated/control)	*T/C*
XXVII	25967	*p*-$CH_3C_6H_4SO_2$—	H	NO_2	400	202	0·2/0·0	3/6	7·7/8·3	0·82
					200	191	−0·1/0·6	6/6	7·5/7·6	0·96
XLVIII	38192	*n*-C_4H_9—	NO_2	NO_2	180	292	−3·0/0·3	6/6	10·3/9·1	1·29
L	30908	CH_3—	H	NO	500	210		0/6		
					250	210	−3·5/−1·5	4/6	7·0/11·7	0·30

Discussion

Before entering upon any discussion of the results and their significance for cancer chemotherapy, it is best to examine the limitations of the test system. Inherent in the test for activity against Leukaemia L1210 are all the variables one would expect from the interaction of a three-factor system (host, tumour, and therapeutic agent). These interactions become especially significant in a test which measures survival time because survival is often a compromise between tumour inhibition and drug toxicity. This compromise becomes especially critical with therapeutic agents which are effective over a narrow range close to the toxic dose. It is aggravated even more in the instance of a tumour, such as Leukaemia L1210, to the containing of which the host contributes little. Under these conditions small variations in the lethal properties of the tumour or the therapeutic agent are magnified. For these reasons, if for no other, there is, as seen in the tables, considerable variation in results from trial to trial. If to this one adds normal expected fluctuation in human errors, the reliability of results is again diminished. These conclusions have, therefore, to be tentative.

Some idea of the fluctuations obtained in these tests with such compounds can be seen from the fact that the parent nitrosoguanidine, 1-methyl-3-nitro-1-nitrosoguanidine (NSC–9369), used as a standard treatment for comparison in many tests, produced variable results. In some 21 tests NSC–9369 was used at a level of 10 mg/kg. In only 16 of these tests could NSC–9369 be considered significantly effective by the criteria used in this paper. The mean T/C was 2·11, with a range of 0·98 to 2·94.

With these limitations on interpretation of the data in mind, there are some conclusions which seem to be true. None of the *des*-nitroso compounds were selectively active against the leukaemia with the exception of one compound (1-methyl-1,3-dinitroguanidine) in one test. The nitroso group could not be replaced by hydrogen, methyl, or acetyl.

Concerning the second substituent on the 1-nitrogen, significant activity in any test was limited to 7 of 16 compounds. Only three of these had activity confirmed in subsequent tests. All three of these were ethyl or halogenoethyl derivatives. Activity

appeared to be limited to compounds with one or two carbon atoms. Any longer carbon chain reduced or limited activity.

In this series there were six compounds with a substituted ethyl group and one with a substituted methyl group. The hydroxy-, methoxy-, acetoxy-, and phenyl-(2-substituted-1-ethyl)-3-nitro-1-nitrosoguanidines were inactive or inconsistently active. Only the bromoethyl and chloroethyl derivatives were reproducibly active. In fact, taking the data at face value, these two compounds were the most active in the series. In considering mechanism of action and structure–activity relationships, it should be noted that other substituents as bulky as the halogens did not contribute to activity and that the 3-chloropropyl derivative was inactive.

The seemingly simplest explanation of the mechanism of action of these compounds (such of them as are active) is that they are degraded to diazoalkanes, which act as alkylating agents. To explain the rather restricted number of active compounds in the series, one would have to postulate that the inactive compounds did not, under physiological conditions, yield a diazoalkane at a sufficiently high rate. Or, if they did, the diazoalkane product did not preferentially inhibit the tumour. One could adduce limited cell penetration or increased toxicity to normal tissues as explanation for the inactivity of the therapeutically inactive diazoalkanes. This would mean that diazoethane and its halogenated derivatives were uniquely selective in their toxicity to the tumour. That this may be true can be deduced from the fact that there appeared to be little correlation between toxicity and antitumour activity in this extended series of compounds. On the other hand, the toxicity data should be taken cautiously because no serious attempt was made to define the toxicity of any of the compounds.

The ' diazoalkane ' hypothesis, in addition to its basic assumptions, leaves unexplained the high activity of the relatively stable imidazoline (XLIV). Its degradation in the mouse is unknown. Although chemically the imidazoline (XLIV) is a logical analogue of 1-methyl-3-nitro-1-nitrosoguanidine (NSC–9369), its mechanism of action may be entirely different, since it cannot be converted to a diazoalkane.

It is not possible to state for certain whether the nitrocarbamoyl portion of 1-methyl-3-nitro-1-nitrosoguanidine (I) can or

cannot be replaced by other hydrolysable groups (see Class 5 table) and still maintain activity. In this regard, one should note that 1-methyl-1-nitrosourea (NSC–23909) had occasional activity and that a series of (2-chloroethyl)nitrosocarbamates have been reported[19] to have toxicological properties similar to nitrogen mustards. It is possible that other diazomethane precursors in Class 5 have borderline activity which could be justifiably missed in one test. In order to check this important point, *N*-(2-chloroethyl)-*N*-nitroso-*p*-toluenesulphonamide, ethyl (2-chloroethyl)nitrosocarbamate,[19] and 1-(2-chloroethyl)-1-nitrosourea will be synthesized and evaluated against L1210, since superior and more consistent activity was obtained by replacement of the methyl group of 1-methyl-3-nitro-1-nitrosoguanidine (I) with a 2-bromoethyl (XXXIX) or a 2-chloroethyl (XI) group.

Since peak activity was obtained with 1-(2-chloroethyl)-3-nitro-1-nitrosoguanidine (XI) and the corresponding 2-bromoethyl analogue (XXXIX), it could be of value to synthesize and investigate the activity of other 2-substituted-ethyl analogues, particularly with negative substituents, since the 2-hydroxy-, 2-acetoxy-, and 2-methoxyethyl derivatives were not significantly active.

Finally, it should be pointed out that not even the active compounds in the present series inhibited Sarcoma 180 or Adenocarcinoma 755 significantly. The specific activity of these compounds against leukaemia might be attributable to peculiarities in the biochemistry and physiology of this tumour. On the other hand, we have evidence that the nitrosoguanidines are active against several ascites tumours but not against the corresponding solid tumour. It seems more likely that the nitrosoguanidines inhibit Leukaemia L1210, not because it is a lymphosarcoma, but because it is an ascites tumour.

Summary. In order to investigate the relationship of chemical structure to the antileukaemic activity of 1-methyl-3-nitro-1-nitrosoguanidine (NSC–9369), a series of 52 analogues were synthesized and evaluated against Leukaemia L1210. Of these, 1-(2-chloroethyl)-3-nitro-1-nitrosoguanidine (NSC–25959) and 1-(2-bromoethyl)-3-nitro-1-nitrosoguanidine (NSC–36885) showed the highest and most consistent activity, being better than the parent NSC–9369. 1-Ethyl-3-nitro-1-nitrosoguanidine (NSC–38191) and 2-nitramino-1-nitroso-2-imidazoline (NSC–25958) showed consistent, but lower, degrees of activity.

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