Antitumor Imidazotetrazines. 14.† Synthesis and Antitumor Activity of 6- and 8-Substituted Imidazo[5,1-*d*]-1,2,3,5-tetrazinones and 8-Substituted Pyrazolo[5,1-*d*]-1,2,3,5-tetrazinones

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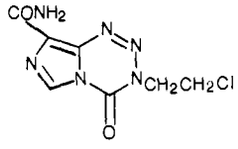
The systematic variation of the potent antitumor agent mitozolomide (1) is extended to cover alteration of substituents at positions 6 and 8 and to change the imidazo[5,1-*d*]-1,2,3,5-tetrazinone (**6**) skeleton to the isomeric pyrazolo-[5,1-*d*]-1,2,3,5-tetrazinone (**17**) skeleton. The series of eight 6-alkyl and 6-aralkyl derivatives of 1 showed optimal antitumor activity when the group was small or linear, but activity diminished as size and branching of this substituent increased. This may reflect altered transport characteristics, or failure of the enlarged derivatives to fit a binding site, or possibly a reduced tendency for the derivatives having bulky groups at position 6 to hydrolytically generate the putatively active triazenes (**21**). Testing of 14 derivatives of 1 differently substituted at position 8 revealed a complex structure–activity relationship, with good antitumor activity obtained for carbamoyl and sulfamoyl groups bearing small substituents. The 8-methylsulfonyl compound had noteworthy activity, but the 8-cyano, 8-nitro, and 8-phenyl derivatives were devoid of useful antitumor activity in these tests. From the limited number of pyrazolotetrazinones (**17**) reported here, it is suggested that the same conclusions as regards activity also hold true for this ring system.

Mitozolomide (1; also known as M&B 39, 565, CCRG 81010, 46241 R.P., NSC 353,451) was first synthesized in 1980 and screened in 1981[1] and was found to exhibit pronounced antitumor effects in a wide range of murine[2] and xenograft[2,3] tumors. Mitozolomide is the first member of a new class of antitumor agent—the azolotetrazinones. The structure of this novel class straddles that of two existing families of antitumor drugs, viz., the nitrosourea family (**2**) and the triazene family, which is exemplified by DTIC (**3**).



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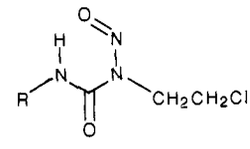

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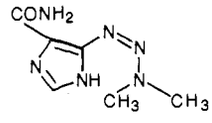
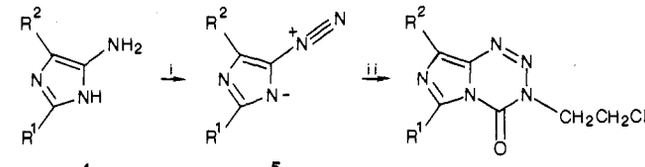


3

The clear advantages of mitozolomide over both the earlier classes of antitumor drugs in terms of antitumor activity,[2,3] oral activity,[2] and pharmacokinetics[4] are currently being tested in clinical trial. In parallel with these developments, we initiated a synthetic program to investigate the structure–activity pattern of this novel class of agent, in which a systematic variation of the key structural features of mitozolomide figured. The variation of activity with change of substitution at the 3-position of the imidazotetrazinone skeleton has already been described,[1,5] and we now report some effects of 6-substitution (compounds **6a–h**) and 8-substitution (compounds **6i–x**) on the antitumor properties of imidazotetrazinones. We also report briefly the antitumor properties of the pyrazolotetrazinone skeleton (compounds **17a–d**).

Scheme I[a]



4 5 6

	R^1	R^2		R^1	R^2
a:	Me	$CONH_2$	**m:**	H	$CON(CH_2C_6H_4OMe)Ph$
b:	Et	$CONH_2$	**n:**	H	$CON(CH_2C_6H_4OMe)CH_2Ph$
c:	*n*-Pr	$CONH_2$	**o:**	H	CONHPh
d:	*i*-Pr	$CONH_2$	**p:**	H	$CONHCH_2Ph$
e:	*n*-Bu	$CONH_2$	**q:**	H	CN
f:	c-Hex	$CONH_2$	**r:**	H	NO_2
g:	$PhCH_2$	$CONH_2$	**s:**	H	SO_2Me
h:	$PhCH_2CH_2$	$CONH_2$	**t:**	H	SO_2NHMe
i:	H	CONHMe	**u:**	H	SO_2NMe_2
j:	H	$CONMe_2$	**v:**	H	$SO_2N(CH_2C_6H_4OMe)H$
k:	H	$CON(CH_2)_4CH_2$	**w:**	H	SO_2NH_2
l:	H	$CON(CH_3)Ph$	**x:**	H	Ph

[a] Reagents: i, HNO_2; ii, $ClCH_2CH_2NCO$.

Chemistry

The general synthesis of azolotetrazinones described earlier[1] was extended to provide the majority of the

† Part 14 of the series "Antitumor Imidazotetrazines". Part 13 is ref 5.
‡ May & Baker Ltd.
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(1) Stevens, M. F. G.; Hickman, J. A.; Stone, R.; Gibson, N. W.; Baig, G. U.; Lunt, E.; Newton, C. G. *J. Med. Chem.* **1984**, *27*, 196.
(2) Hickman, J. A.; Stevens, M. F. G.; Gibson, N. W.; Langdon, S. P.; Fizames, C.; Lavelle, F.; Atassi, G.; Lunt, E.; Tilson, R. M. *Cancer Res.* **1985**, *45*, 3008.
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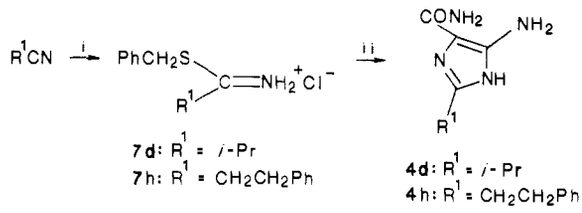
Scheme II[a]

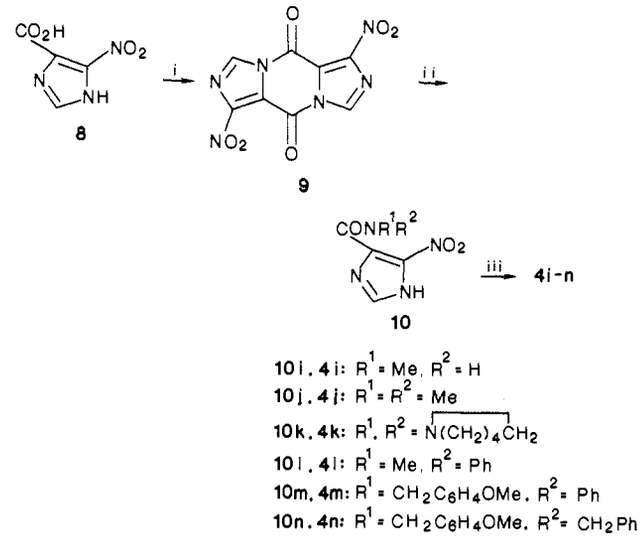


[a] Reagents: i, $PhCH_2SH/HCl$; ii, α-amino-α-cyanoacetamide.

products reported in this paper. Accordingly (Scheme I) 6- or 8-substituted 3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones **6** were prepared from the correspondingly substituted 5-aminoimidazoles **4** by reaction with nitrous acid, yielding 5-diazoimidazoles **5**, which in some cases were isolable and characterized (CAUTION: diazoimidazoles and diazopyrazoles prepared during the course of this work are shock sensitive and should be handled in the dry state with the greatest care). In many cases it proved more convenient not to isolate the intermediate diazo compounds **5** but to extract them directly from the aqueous diazotization mixture into ethyl acetate solution. In either event, dry solutions or suspensions of the diazo compounds **5** were then reacted with 2-chloroethyl isocyanate to yield the desired imidazotetrazinones **6**. Imidazotetrazinones **6** were purified if necessary by crystallization from, or by flash chromatography using, nonnucleophilic solvents (typically mixtures of petrol, ether, methylene chloride, chloroform, acetone, or acetonitrile), since mitozolomide (**1**) has already been shown to be susceptible to nucleophilic degradation.[1] In the preparation of the two 8-carboxamides **6o** and **6p** it was not possible to proceed according to Scheme I directly due to the spontaneous cyclization of the diazo intermediates **5o** and **5p** during preparation. Instead, protected 5-aminoimidazole-4-carboxamides (**4m** and **4n**) were prepared, which were stable during the diazotization process (**5m** and **5n**). Subsequent cycloaddition with 2-chloroethyl isocyanate gave the protected imidazotetrazinones **6m** and **6n**, which were converted to the desired monosubstituted amides **6o** and **6p** by deprotection using trifluoroacetic acid. A similar protection–deprotection (**4v** → **5v** → **6v** → **6w**) operation was necessary to prepare the 8-sulfamoyl derivative **6w** of mitozolomide because of the known instability of 5-aminoimidazole-4-sulfonamide (**4w**).[6]

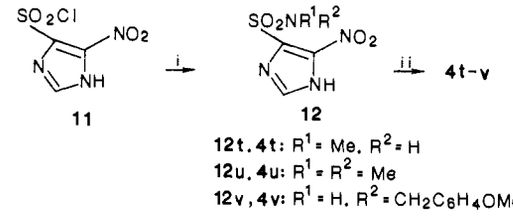
The starting 5-aminoimidazoles **4** were prepared by several methods. Six of the 2-substituted 4-aminoimidazole-5-carboxamides required (**4a–c**, **4e–g**) were prepared as described previously.[7] The remaining two compounds (**4d**, **4h**) were prepared from the appropriate thioimidate salts **7d** and **7h** and α-amino-α-cyanoacetamide by using the procedure described by Rose and Rainsworth[7] (Scheme II). The six 5-aminoimidazole-4-carboxamides substituted on the amide group described here (**4i–n**) were all prepared from 5-nitroimidazole-4-carboxylic acid (**8**)[8] (Scheme III). Thus treatment of **8** with phosphorus pentachloride gave the dimeric lactam **9**.[9] In the presence of primary and secondary amines, **9**

Scheme III[a]



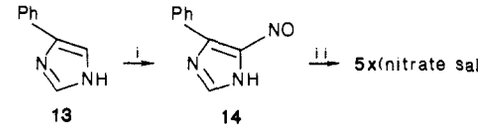
[a] Reagents: i, PCl_5; ii, R^1R^2NH; iii, H_2.

Scheme IV[a]



[a] Reagents: i, R^1R^2NH; ii, H_2.

Scheme V[a]



[a] Reagents: i, HNO_2; ii, NO.

ring opened to give the 5-nitroimidazole-4-carboxamides **10i–n**, which were reduced catalytically to the corresponding 5-aminoimidazole-4-carboxamides **4i–n**. 4-Amino-5-cyanoimidazole (**4q**)[10] and 4-amino-5-nitroimidazole (**4r**) were prepared and converted to their corresponding 4-diazo derivatives **5q**[10] and **5r**[11] as described previously. 4-Amino-5-(methylsulfonyl)imidazole (**4s**)[12] was prepared as described previously. The three sulfonamides **4t–v** were synthesized from 5-nitroimidazole-4-sulfonyl chloride (**11**)[13] by reaction with the appropriate amine, followed by reduction of the nitro sulfonamides **12t–v** with Raney nickel (Scheme IV).

The 8-phenyl analogue of mitozolomide was prepared by an unusual route (Scheme V) in which 4-phenyl-

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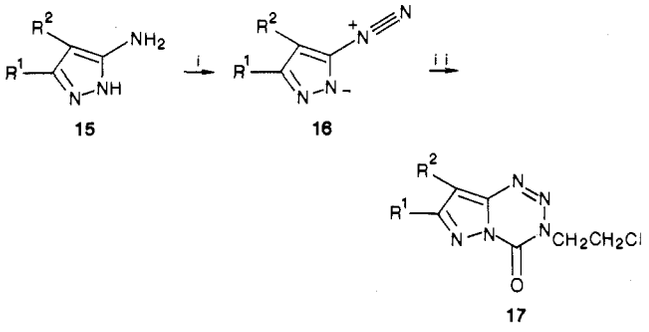
Scheme VI[a]

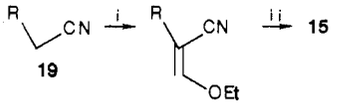


15 16 17

	R^1	R^2
15a, 16a, 17a:	H	$CONH_2$
15b, 16b, 17b:	H	$CONMe_2$
15c, 16c, 17c:	H	NO_2
15d, 16d, 17d:	H	SO_2Me

[a] Reagents: i, HNO_2 or $C_5H_{11}ONO$; ii, $ClCH_2CH_2NCO$.

Scheme VII[a]



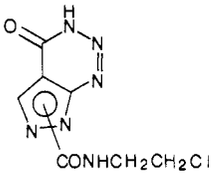
19 20 15

19b, 20b, 15b: R = $CONMe_2$
19d, 20d, 15d: R = SO_2Me

[a] Reagents: i, $(EtO)_3CH$, Ac_2O; ii, NH_2NH_2.

imidazole (**13**) was nitrosated, yielding 4-nitroso-5-phenylimidazole (**14**),[14] which on treatment with nitric oxide in the manner of Effenberger et al.[15] gave the corresponding diazo compound **5x** as its nitrate salt. This salt (**5x**) was converted without isolation to the imidazotetrazinone **6x** by treatment with 1,5-diazabicyclo[4.3.0]-non-5-ene (DBN) (liberating the free diazo compound) followed by cycloaddition with 2-chloroethyl isocyanate.

Pyrazole analogues (**17a–d**) of mitozolomide were prepared from the corresponding 3-aminopyrazoles **15** by diazotization followed by reaction of the intermediate diazopyrazoles **16** with 2-chloroethyl isocyanate in an inert solvent (Scheme VI). Formation of the pyrazolotetrazinones was in general accompanied by greater difficulties in purification than had been observed with the corresponding imidazotetrazinones. Thus, reaction of 3-diazopyrazole-4-carboxamide[16] (**16a**) with 2-chloroethyl isocyanate in ethyl acetate gave the desired product **17a** contaminated with an isomeric carbamoylated pyrazolotriazine (**18**). Separation of the two products **17a** and **18**



18

proved to be difficult and was best achieved by first decomposing **18** in warm Me_2SO followed by flash chromatography of the residue containing **17a**[25] and decomposed

(14) Cusmano, S.; Didonna, G. *Gazz. Chim. Ital.* **1955**, *85*, 208.
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18. Preparation of the corresponding dimethylcarbamoyl derivative **17b** involved preparation of the aminopyrazole **15b**, which has not been reported before. Thus α-cyano-*N,N*-dimethylacetamide[17] (**19b**) was heated with acetic anhydride and triethyl orthoformate, generating the *N,N*-dimethylpropenamide **20b**, which was cyclized with hydrazine to give the aminopyrazole **15b**, isolated as its hydrochloride salt (Scheme VII). Diazotization using amyl nitrite in dry methanol then gave the diazopyrazole **16b**, again isolated as a hydrochloride salt. The free diazo compound **16b** was liberated in situ with DBU and treated with 2-chloroethyl isocyanate in methylene chloride overnight. Chromatography of the reaction products was again necessary to isolate pure pyrazolotetrazinone **17b**.

The corresponding nitro derivative **17c** was prepared via the previously described 3-amino-4-nitropyrazole (**15c**)[18] by diazotization. 3-Diazo-4-nitropyrazole (**16c**) was isolated in 98% yield as its hydrochloride salt. Treatment of this compound (**16c**) with 2-chloroethyl isocyanate gave the desired pyrazolotetrazinone **17c** in low yield after chromatography. Finally, α-(methylsulfonyl)acetonitrile (**19d**) and triethyl orthoformate gave the acrylonitrile **20d**, which was cyclized to 3-amino-4-(methylsulfonyl)pyrazole (**15d**) and subsequently diazotized (**16d**) and reacted with 2-chloroethyl isocyanate, yielding **17d** after chromatography.

Biology

Antitumor tests on the compounds described in this paper were performed on two tumor types. The murine L1210 leukemia test was performed at Rhone-Poulenc Santé, Centre de Recherches de Vitry, Paris, France; and the murine TLX5 thymus lymphoma screen was performed at the Cancer Research Campaign Experimental Chemotherapy Group, Aston University, Birmingham, England. The murine L1210 leukemia was originally obtained from the tumor bank, National Cancer Institute, Frederick, MD, and used for anticancer testing in accord with the protocols described by the National Cancer Institute.[19] The TLX5 lymphoma was passaged and used as described previously.[20] Against the L1210 tumor line, all compounds were dosed orally as a suspension in (carboxymethyl)cellulose. In the TLX5 screen, all compounds were dosed with 10% Me_2SO in arachis oil as an injection vehicle. BDF_1 mice were obtained from Charles River Breeding Laboratories and from Iffa Credo Laboratories, and CBA/CA mice were obtained from Bantin and Kingman Ltd., Hull, England.

Discussion

Table VI details the experimental antitumor activity of 22 imidazotetrazinones and four pyrazolotetrazinones, all bearing the 2-chloroethyl group at position 3. Despite the differences between the two tumor models and in the mode of administration, in cases where a compound was screened against both tumor types (10 examples), the same level of activity was observed at very similar dose levels. This direct correspondence in activity between the two screens was observed with mitozolomide and now appears to be a feature of this novel class of agent and permits the following structure–activity conclusions to be drawn from

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Table I. Synthesis and Physical Characteristics of 3-(2-Chloroethyl)azolo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones

no.	general exptl method	rctn solvent	rctn time	rctn temp	yield, %	mp, °C dec	formula	analysis[a]	IR, $\nu_{C=O}$, cm^{-1} (KBr)	NMR chem shifts (Me_2SO), δ
6a	E	EtOAc	5 days	RT	77	170	$C_8H_9ClN_6O_2$	C, H, N	1740, 1685	2.75 (3 H, s, CH_3), 3.95 (2 H, t, J = 6 Hz, CH_2Cl), 4.5 (2 H, t, J = 6 Hz, CH_2N), 7.5 (2 H, br s, $CONH_2$)
6b	E	EtOAc	1 day	RT	60	172–174	$C_9H_{11}ClN_6O_2$	C, H, Cl, N	1740, 1690	1.3 (3 H, t, J = 7 Hz, CH_3), 3.15 (2 H, q, J = 7 Hz, CH_2CH_3), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.55 (2 H, t, J = 6 Hz, CH_2N), 7.65 (2 H, br s, $CONH_2$)
6c	E	EtOAc	1 day	RT	54	145–148	$C_{10}H_{13}ClN_6O_2$	H; C, Cl, N[b]	1745, 1695	0.95 (3 H, t, J = 8 Hz, CH_3), 1.8 (2 H, hex, J = 8 Hz, CH_2CH_3), 3.2 (2 H, t, J = 8 Hz, $CH_2CH_2CH_3$), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 7.7 (2 H, br s, $CONH_2$)
6d	E	EtOAc	5 days	RT	12	188–190	$C_{10}H_{13}ClN_6O_2$	C, H, N	1740, 1690	c
6e	E	EtOAc	1 day	RT	67	165–167	$C_{11}H_{15}ClN_6O_2$	C, H, Cl, N	1740, 1690	0.9 (3 H, t, J = 8 Hz, CH_3), 1.35 (2 H, hex, J = 8 Hz, CH_2CH_3), 1.75 (2 H, qui, J = 8 Hz, $CH_2CH_2CH_3$), 3.15 (2 H, t, J = 8 Hz, $CH_2CH_2CH_2CH_3$), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.55 (2 H, t, J = 6 Hz, CH_2N), 7.65 (2 H, br s, $CONH_2$)
6f	E	EtOAc	1 day	RT	18	245–248	$C_{13}H_{17}ClN_6O_2$	H, Cl, N; C[d]	1740, 1685	1.1–2.2 (10 H, m, c-hex CH_2), 3.55 (1 H, m, CH), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.55 (2 H, t, J = 6 Hz, CH_2N), 7.65 (2 H, br s, $CONH_2$)
6g	E	EtOAc	20 h	RT	20	161–163	$C_{14}H_{13}ClN_6O_2$	C, H, Cl, N	1740, 1685	4.02 (2 H, t, J = 6 Hz, CH_2Cl), 4.59 (2 H, t, J = 6 Hz, CH_2N), 4.62 (2 H, s, $PhCH_2$), 7.2–7.3 (5 H, m, C_6H_5), 7.73 (1 H, br s, $CONH_2$)
6h	E	EtOAc	1 day	RT	19	179–181	$C_{15}H_{15}ClN_6O_2$	C, H, Cl, N	1740, 1680	3.2–3.6 (4 H, m, CH_2CH_2), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 7.28 (5 H, s, Ar H), 7.65 (2 H, br s, $CONH_2$)
6i	F	EtOAc	1 day	RT	44	120–122	$C_8H_9ClN_6O_2$	C, H; N[e]	1745, 1675	2.8 (3 H, d, J = 5 Hz, $NHCH_3$), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 8.35 (1 H, br q, J = 5 Hz, $NHCH_3$), 8.75 (1 H, s, C6-H)
6j	E	EtOAc	1 day	RT	31	114–116	$C_9H_{11}ClN_6O_2$	C, H, N	1735, 1620	3.05 (6 H, s, 2 NCH_3), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 8.85 (1 H, s, C6-H)
6k	E	EtOAc	2 days	RT	56	92–94	$C_{12}H_{15}ClN_6O_2$	H; C, N[f]	1750, 1630	1.6 (6 H, br s, 3′,4′,5′-CH_2), 3.3–3.7 (4 H, m, 2′,6′-CH_2), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 8.7 (1 H, s, C6-H)
6l	F	EtOAc	18 h	RT	15	130–132	$C_{14}H_{13}ClN_6O_2$	C, H, Cl, N	1750, 1640	3.4 (3 H, s, NCH_3), 3.95 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 7.2 (5 H, s, Ar H), 8.65 (1 H, s, C6-H)
6m	F	EtOAc	5 days	RT	44	glass	$C_{21}H_{19}ClN_6O_3$	*g*	1740, 1640	3.6 (3 H, s, OCH_3), 3.9 (2 H, t, J = 6 Hz, CH_2Cl), 4.5 (2 H, t, J = 6 Hz, CH_2N), 5.0 (2 H, s, CH_2 Ar), 6.6–7.2 (9 H, m, Ar H), 8.5 (1 H, s, C6-H)
6n	F	EtOAc	1 day	RT	56	oil	$C_{22}H_{21}ClN_6O_3$	*g*	not recorded	*h*, 3.65 (3 H, s, OCH_3), 3.95 (2 H, t, J = 6 Hz, CH_2Cl), 4.2–4.6 (6 H, m, 3 x CH_2N), 6.6–7.4 (9 H, m, Ar H), 8.5 (s, 1 H, C6-H)
6o	G	TFA	18 h	RT	59	166	$C_{13}H_{11}ClN_6O_2$	C, H, N	1735, 1680	4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 7.0–7.9 (5 H, m, Ar H), 8.9 (1 H, s, C6-H), 10.3 (1 H, br s, NH)
6p	G	TFA	18 h	RT	31	153–155	$C_{14}H_{13}ClN_6O_2$	*g*, *i*	1755, 1660	4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.5 (2 H, d, J = 5 Hz, CH_2NH), 4.6 (2 H, t, J = 6 Hz, CH_2N), 7.3 (5 H, s, Ar H), 8.9 (1 H, s, C6-H), 9.05 (1 H, br t, NH)
6q	E	EtOAc	3 days	RT	69	108–110	$C_7H_5ClN_6O$	C, H; Cl, N[j]	1760	4.05 (2 H, t, J = 6 Hz, CH_2Cl), 4.7 (2 H, t, J = 6 Hz, CH_2N), 9.0 (1 H, s, C6-H)
6r	E	EtOAc	3 days	RT	37	gum	$C_6H_5ClN_6O_3$	C, H, Cl; N[k]	1760	4.15 (2 H, t, J = 6 Hz, CH_2Cl), 4.9 (2 H, t, J = 6 Hz, CH_2N), 8.8 (1 H, s, C6-H)
6s	E	EtOAc	2 days	RT	69	154–155	$C_7H_8ClN_5O_3S$	C, H, Cl, N, S	1750	3.42 (3 H, s, CH_3), 4.06 (2 H, t, J = 6 Hz, CH_2Cl), 4.71 (2 H, t, J = 6 Hz, CH_2N), 9.06 (1 H, s, C6-H)
6t	E	EtOAc	2 days	RT	29	147–148	$C_7H_9ClN_6O_3S$	C, H, N	1745	2.60 (3 H, d, J = 5 Hz, CH_3), 4.00 (2 H, t, J = 6 Hz, CH_2Cl), 4.60 (2 H, t, J = 6 Hz, CH_2N), 7.95 (1 H, q, J = 5 Hz, NH), 8.85 (1 H, s, C6-H)
6u	E	EtOAc	2 days	RT	82	155–156	$C_8H_{11}ClN_6O_3S$	C, H, Cl, N, S	1755	2.8 (6 H, s, 2 CH_3), 3.95 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 8.9 (1 H, s, C6-H)

Table I (Continued)

no.	general exptl method	rctn solvent	rctn time	rctn temp	yield, %	mp, °C dec	formula	analysis[a]	IR, $\nu_{C=O}$, cm^{-1} (KBr)	NMR chem shifts (Me_2SO), δ
6v	E	EtOAc	2 days	RT	42	155–156	$C_{14}H_{15}ClN_6O_4S$	C, H, Cl, N, S	1735	3.7 (3 H, s, OCH_3), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.1 (2 H, s, Ar CH_2NH), 4.65 (2 H, t, J = 6 Hz, CH_2N), 6.75 (2 H, d, J = 9 Hz, 3′,5′-benzyl H), 7.10 (2 H, d, J = 9 Hz, 2′,6′-benzyl H), 8.60 (1 H, br s, NH), 8.85 (1 H, s, C6-H)
6w	G	TFA	2 h	RT	72	183	$C_6H_7ClN_6O_3S$	C, H, Cl, N, S	1750	3.95 (2 H, t, J = 6 Hz, CH_2Cl), 4.55 (2 H, t, J = 6 Hz, CH_2N), 7.75 (2 H, br s, NH_2), 8.75 (1 H, s, C6-H)
6x	H	EtOAc	2 days	RT	21	177–178	$C_{12}H_{10}ClN_5O$	C, H, N	1730	4.05 (2 H, t, J = 6 Hz, CH_2Cl), 4.6 (2 H, t, J = 6 Hz, CH_2N), 7.4–7.6 (3 H, m, 3′,4′,5′-aryl H), 8.2–8.4 (2 H, m, 2′,6′-aryl H), 8.9 (1 H, s, C6-H)
17a	M	EtOAc	7 days	RT	20	203–204	$C_7H_7ClN_6O_2$	C, H, Cl, N	1760, 1680	4.05 (2 H, t, J = 6 Hz, CH_2Cl), 4.75 (2 H, t, J = 6 Hz, CH_2N), 7.6 (1 H, br s, NH), 7.75 (1 H, br s, NH), 8.6 (1 H, s, C7-H)
17b	N	CH_2Cl_2	18 h	RT	31	116–118	$C_9H_{11}ClN_6O_2$	C, H, Cl, N	1770, 1750, 1630	3.0 (6 H, s, 2 NCH_3), 4.0 (2 H, t, J = 6 Hz, CH_2Cl), 4.65 (2 H, t, J = 6 Hz, CH_2N), 8.45 (1 H, s, C7-H)
17c	O	CH_2Cl_2	1.5 h	RT	11	168–172	$C_6H_5ClN_6O_3$	*g*, *l*	1760	4.1 (2 H, t, J = 6 Hz, CH_2Cl), 4.85 (2 H, t, J = 6 Hz, CH_2N), 8.75 (1 H, s, C7-H)
17d	P	none	24 h	RT	48	184–185	$C_7H_8ClN_5O_3S$	C, H, N	1760	3.46 (3 H, s, CH_3), 4.10 (2 H, t, J = 6 Hz, CH_2Cl), 4.79 (2 H, t, J = 6 Hz, CH_2N), 8.72 (1 H, s, C7-H)

[a] Compounds 6 and 17 gave satisfactory analyses (± 0.4%) except where indicated. [b] Calcd: C, 42.19; Cl, 12.45; N, 29.52; M, 284.0789. Found: C, 41.1; Cl, 13.5; N, 28.5; $M^{+\cdot}$, 284.0784. [c] NMR not recorded. [d] Calcd: C, 48.07; M, 324.1102. Found: C, 47.06; $M^{+\cdot}$, 324.1082. [e] Calcd: N, 32.7; M, 256.0476. Found: N, 31.9; $M^{+\cdot}$, 256.0484. [f] Calcd: C, 46.4; N, 27.1; M, 310.0945. Found: C, 45.3; N, 26.1; $M^{+\cdot}$, 310.0902. [g] Analysis not recorded. [h] Recorded in acetone-d_6. [i] Mass spectrum (EI), 228 (15), 199 (59), 198 (66), 170 (35), 104 (48), 91 (100) (no molecular ion, m/e 332 seen). [j] Calcd: Cl, 15.8; N, 37.42; M, 224.0213. Found: Cl, 16.4; N, 36.6; $M^{+\cdot}$, 224.0198. [k] Calcd: N, 34.4; M, 244.0112. Found: N, 31.9; $M^{+\cdot}$, 244.0088. [l] Calcd: M, 244.0112. Found: $M^{+\cdot}$, 244.0103.

Table II. Synthesis and Physical Characteristics of 5-Diazoimidazoles

no.	general exptl method	rctn solvent	yield, %	mp, °C vigorous dec	formula	IR, $\nu_{N\equiv N}$, cm^{-1} (KBr)
5a	D	2 M HOAc	74	175	$C_5H_5N_5O$	2070
5b	D	2 M HOAc	72	139	$C_6H_7N_5O$	*b*
5c	D	2 M HOAc	38	*a*	$C_7H_9N_5O$	*b*
5d	D	2 M HOAc	45	120	$C_7H_9N_5O$	2170
5e	D	1 M HCl	33	109–111	$C_8H_{11}N_5O$	*b*
5f	D	2 M HOAc	87	*a*	$C_{10}H_{13}N_5O$	*b*
5g	D	2 M HCl	71	121–122	$C_{11}H_9N_5O$	2180
5h	D	2 M HOAc	98	*a*	$C_{12}H_{11}N_5O$	2150
5j	D	1 M HOAc	84	101–103	$C_6H_7N_5O$	2180
5k	D	1 M HOAc	100	*a*	$C_9H_{11}N_5O$	*b*
5q	D	2 M HOAc	78	90[c]	C_4HN_5	2180
5r	D	1 M H_2SO_4	94	*a*[d]	$C_3HN_5O_2$	*b*
5s	D	2 M HCl	86	128–130	$C_4H_4N_4O_2S$	2125
5t	D	1 M HCl	75	150	$C_4H_5N_5O_2S$	2210
5u	D	2 M HCl	61	109	$C_5H_7N_5O_2S$	2210, 2180
5v	D	2 M HCl	65	144–146	$C_{11}N_{11}N_5O_3S$	2180

[a] Melting point not recorded. [b] IR not recorded. [c] Reference 10 mp 97–98 °C. [d] Reference 11a, mp 40–41 °C.

Table III. Synthesis and Physical Properties of 5-Aminoimidazoles and Salts

no.	general exptl method	yield, %	mp, °C dec	formula	analysis[b]
4d	A	42.5	gum (HCl)	$C_7H_{12}N_4O{\cdot}HCl$	c
4h	A	26	270–274 (HCl)	$C_{12}H_{14}N_4O{\cdot}HCl$	C, H, N
4i	B	97	184–186[a]	$C_5H_8N_4O$	H; C, N[d]
4j	B	98	179–181	$C_6H_{10}N_4O$	c
4k	B	76	175–177 (HCl)	$C_9H_{14}N_4O{\cdot}HCl$	c
4l	C	88	100 (HCl)	$C_{11}H_{12}N_4O{\cdot}HCl$	c
4m	C	99	gum	$C_{18}H_{18}N_4O_2$	c
4n	C	99	gum (HCl)	$C_{19}H_{20}N_4O_2{\cdot}HCl$	c
4t	C	61	178–180 (HCl)	$C_4H_8N_4O_2S{\cdot}HCl$	C, H, Cl, N
4u	C	78	188–189 (HCl)	$C_5H_{10}N_4O_2S{\cdot}HCl$	C, H, Cl, S; N[e]
4v	C	25	154–155 (HCl)	$C_{11}H_{14}N_4O_3S{\cdot}HCl$	c

[a] Reference 23, mp 200–203 °C. [b] Compounds gave satisfactory analyses (±0.4%) unless otherwise indicated; however, in general 5-aminoimidazoles 4 proved difficult to crystallize to obtain pure and were better used in the preparation of 5-diazoimidazoles directly upon isolation. [c] Analysis not recorded. [d] Calcd: C, 42.9; N, 40.0. Found: C, 43.4; N, 38.9. [e] Calcd: N, 24.7. Found: N, 23.6.

the results (Table VI). Thus, incorporation of an alkyl group at position 6 of the imidazo[5,1-*d*]-1,2,3,5-tetrazinone skeleton is not deleterious to activity when the group is small or linear, but large, bulky groups appear to be distherapeutic, reflecting possibly (1) an increased π value altering transport characteristics, (2) failure of the enlarged

Table IV. Synthesis and Physical Properties of *S*-Benzyl Thioimidate Hydrochlorides

no.	general exptl method	yield, %	mp, °C dec	formula	analysis[b]
7d	I	88	165–166[a]	$C_{11}H_{15}NS\cdot HCl$	C, H, Cl, N
7h	I	87	158–160	$C_{16}H_{17}NS\cdot HCl$	c

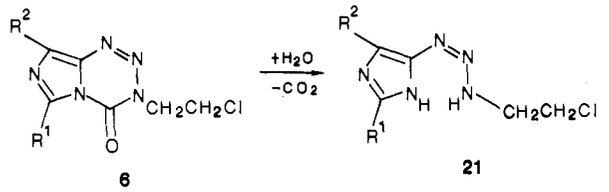
[a] Reference 22, mp 165–168 °C. [b] Compounds gave satisfactory analysis (±0.4%) unless otherwise indicated. [c] Not recorded.

Table V. Synthesis and Physical Characteristics of Nitroimidazoles 9, 10, and 12

no.	general exptl method	yield, %	mp, °C dec	formula	analysis[b]
9	J	100	249–251	$C_8H_2N_6O_6$	c
10i	K	56	278–280[a]	$C_5H_6N_4O_3$	C, H, N
10j	K	32	193–195	$C_6H_8N_4O_3$	C, H, N
10k	K	37	149–150	$C_9H_{12}N_4O_3$	C, H, N
10l	K	33	193	$C_{11}H_{10}N_4O_3$	N; C, H[d]
10m	K	23	212–215	$C_{18}H_{16}N_4O_4$	H, N; C[e]
10n	K	16	167–170	$C_{19}H_{18}N_4O_4$	H, N; C[f]
12t	L	51	260–263	$C_4H_6N_4O_4S$	C, H, N, S
12u	L	63	282–283	$C_5H_8N_4O_4S$	C, H, N, S
12v	L	74	250–251	$C_{11}H_{12}N_4O_5S$	C, H, N

[a] Previously prepared by a different method (ref 24), mp 273 °C. [b] Compounds gave satisfactory analyses (±0.4%) unless otherwise recorded. [c] Mass spectrum, calcd M 278, found *m/z* 278. [d] Calcd: C, 53.66; H, 4.09. Found: C, 52.5; H, 4.95. [e] Calcd: C, 61.4. Found: C, 60.4. [f] Calcd: C, 62.3. Found: C, 61.3.

Scheme VIII



molecule to fit a binding site, or (3) inhibition of hydrolysis at position 4, preventing ring opening of the putative prodrug[1] imidazotetrazinones to the proposed active but unstable triazenes **21** (Scheme VIII). The structure–activity relationship at the 8-position of the imidazo[5,1-*d*]-1,2,3,5-tetrazinone skeleton is more complicated. Thus 8-*N*-methyl-substituted carboxamides are essentially equiactive with mitozolomide, but activity decreases as the bulk of *N*-alkyl substitution increases. The 8-phenyl, 8-cyano, and 8-nitro derivatives show no useful in vivo activity in these tumor models, yet the 8-sulfamoyl and 8-methylsulfonyl compounds are extremely potent antitumor agents with activity at least as good as that of mitozolomide. In particular, the cure rates of the 8-sulfamoyl derivative **6w** against the L1210 leukemia model generally exceeded those generated by mitozolomide when the latter was used as a positive control. It appears that alkyl substitution on 8-sulfonamides has the same effect as that upon 8-amide derivatives.

The pyrazole ring system is represented by four examples (**17a–d**) in this paper, and it appears from the limited number of examples that the conclusions drawn above about structure–activity relationships in the imidazotetrazinones (**6**) hold equally with the pyrazolotetrazinones (**17**). However, the pyrazolotetrazinones appear to be active at higher doses than the imidazotetrazinones, and in addition pyrazolotetrazinones are considerably more difficult to synthesize and obtain pure.

Conclusion

The search for a second-generation analogue of mitozolomide described here and in previous papers in this series has identified to date two types of agent: (i) 8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4-(3*H*)-one,[5] which has interesting antitumor properties that are less pronounced than those of mitozolomide but which are sufficiently different for the compound to be considered complementary to mitozolomide, and (ii) 8-sulfonyl and 8-sulfamoyl derivatives **6t**, **6u**, **6w**, which are extremely potent antitumor agents, particularly against the L1210 leukemia line.

Experimental Section

General experimental details are given in part 1 of this series.[1] General methods A–M are illustrated by specific examples; variations on solvent, temperature, etc. are noted in the corresponding tables.

Method A. Preparation of 2-Substituted 5-Aminoimidazole-4-carboxamides 4d and 4h. A solution of 2-amino-2-cyanoacetamide[21] (3.3 g, 0.033 mol) in ethanol (20 mL) was treated with crude benzenepropanimidothioic acid benzyl ester hydrochloride (**7h**) (9.7 g, 0.033 mol), and the mixture was heated under reflux for 15 min and then cooled. The precipitated solid was recrystallized from methanol, giving **4h**·HCl (2.3 g, 26%) as colorless crystals, mp 270–274 °C. Anal. Calcd for $C_{12}H_{14}N_4O\cdot HCl$: C, 54.0; H, 5.67; N, 21.0. Found: C, 53.8; H, 5.55; N, 21.1.

Method B. Preparation of 5-Aminoimidazole-4-carboxamides 4i–k. A solution of 4-nitro-5-(piperidinocarbonyl)-imidazole (2.68 g, 0.012 mol) in methanol (27 mL) and *N*,*N*-dimethylformamide (27 mL) was treated with platinum oxide (0.27 g) and hydrogenated at room temperature and pressure. When hydrogen uptake was complete, the mixture was filtered and the filtrate evaporated in vacuo, giving crude **4k**.

Compound **4k** was converted to its hydrochloride salt by dissolution in aqueous hydrochloric acid, evaporation to dryness, and trituration with acetone, yielding **4k**·HCl (2.1 g, 76%) as a pale green solid, mp 175–177 °C, pure enough for use in the next stage.

Method C. Preparation of 5-Aminoimidazole-4-carboxamides 4l–n and 5-Aminoimidazole-4-sulfonamides 4t–v. A solution of 5-nitro-*N*-(4-methoxybenzyl)-*N*-phenylimidazole-4-carboxamide (2.1 g, 6.0 mmol) in dry ethanol (200 mL) was hydrogenated at 25 °C (3 atm) pressure with a Raney nickel catalyst. When hydrogen absorption was complete (5 h), the mixture was filtered and evaporated to dryness, yielding 5-amino-*N*-(4-methoxybenzyl)-*N*-phenylimidazolecarboxamide (**4m**) (1.9 g, 99%) as a gum, pure enough for use in the next stage.

A portion of this gum was characterized as its picrate. Thus **4m** (0.15 g, 0.47 mmol) was dissolved in dry 1,2-dimethoxyethane and treated with a solution of picric acid (0.25 g, 1.0 mmol) in 1,2-dimethoxyethane, giving 4m picrate dihydrate, mp 207 °C dec. Anal. Calcd for $C_{18}H_{18}N_4O_2\cdot C_6H_3N_3O_7\cdot 2H_2O$: C, 49.1; H, 4.29; N, 16.69. Found: C, 49.1; H, 3.64; N, 16.5.

Method D. Synthesis of 5-Diazoimidazoles 5a–h, 5j, 5k, and 5q–v. A stirred solution of sodium nitrite (0.285 g, 4.1 mmol) in water (4 mL) was cooled in an ice bath and treated dropwise with a solution of 5-amino-*N*-methylimidazole-4-sulfonamide

(21) Smith, L. H., Jr.; Yates, P. *J. Am. Chem. Soc.* **1954**, *76*, 6080. Cook, A. H.; Heilbron, I.; Smith, E. *J. Chem. Soc.* **1949**, 1440.

hydrochloride (0.55 g, 2.6 mmol) in dilute hydrochloric acid (2 M, 2.8 mL). The resulting precipitate was collected and washed with ice-cold water to give **5t** (0.36 g, 75%) as a solid, mp 150 °C (explodes). Anal. Calcd for $C_4H_5N_5O_2S$: C, 25.7; H, 2.69; N, 37.4. Found: C, 25.2; H, 2.47; N, 37.0. A further portion (0.07 g) of product was obtained by extraction of the aqueous liquors at 0 °C with ethyl acetate, drying the extract over magnesium sulphate, and evaporation in vacuo.

Method E. Synthesis of Imidazo[5,1-*d*]tetrazinones 6a–h, 6j, 6k, and 6q–v. A solution of 5-diazo-2-ethylimidazole-4-carboxamide (0.62 g, 3.75 mmol) in dry ethyl acetate (22 mL) was treated with 2-chloroethyl isocyanate (2.4 mL) and the mixture stirred in the dark (24 h). The resulting precipitate was filtered off and washed with ethyl acetate, yielding **6b** (0.59 g, 60%) as pale gray crystals, mp 172–174 °C. Anal. Calcd for $C_9H_{11}ClN_6O_2$: C, 39.9; H, 4.1; Cl, 13.1; N, 31.0. Found: C, 39.8; H, 3.98; Cl, 13.1; N, 30.7.

Method F. Synthesis of Imidazo[5,1-*d*]tetrazinones 6i, 6l, 6m, and 6n. A stirred, ice-cooled solution of sodium nitrite (0.64 g, 9.28 mmol) in water (4.6 mL) was treated dropwise with 4-amino-5-(*N*-methylcarbamoyl)imidazole (1 g, 7.14 mmol) in aqueous acetic acid (1 M, 14.3 mL). The resulting dark red solution was extracted with ethyl acetate (4 × 35 mL), and the combined extracts were dried over magnesium sulfate.

The solution of 4-diazo-5-(*N*-methylcarbamoyl)imidazole was treated immediately with 2-chloroethyl isocyanate (4.3 mL), and the mixture was allowed to stand in the dark at room temperature (1 day). The solution was concentrated and the residue triturated with petroleum ether (bp 40–60 °C) to give an orange gum (4.23 g). The gum was triturated with ethyl acetate (50 mL) and filtered. The filtrate was concentrated to a gum (2.94 g), which was purified by flash chromatography (silica, eluent ethyl acetate/acetonitrile, 4:1), giving **6i** (0.81 g, 44%) as a purple tinged solid, mp 120–122 °C. Anal. Calcd for $C_8H_9ClN_6O_2$: C, 37.4; H, 3.53; N, 32.7. Found: C, 37.3; H, 3.69; N, 31.9.

Method G. Synthesis of Imidazo[5,1-*d*]tetrazinones 6o, 6p, and 6w. A solution of 3-(2-chloroethyl)-8-[*N*-(4-methoxybenzyl)sulfamoyl]imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (0.1 g, 0.25 mmol) was dissolved in trifluoroacetic acid (1.0 mL) and anisole (0.02 mL), and the solution was allowed to stand at room temperature (2 h). The mixture was concentrated and the residue was triturated with ether, giving a yellow solid, which was purified by flash chromatography (silica, eluent petroleum ether/ethyl acetate, 1:1), yielding **6w** (0.05 g, 72%) as a white solid, mp 183 °C dec. Anal. Calcd for $C_6H_7ClN_6O_3S$: C, 25.9; H, 2.53; Cl, 12.72; N, 30.16; S, 11.51. Found: C, 25.9; H, 2.48; Cl, 12.7; N, 30.3; S, 11.4.

Method H. Synthesis of Imidazo[5,1-*d*]tetrazinone (6x). A suspension of 4-nitroso-5-phenylimidazole[14] (1 g, 5.78 mmol) in dry ethyl acetate (50 mL) was treated with a stream of nitric oxide until a solution was obtained. The solution was purged with nitrogen to remove excess nitric oxide.

To this solution of 4-diazo-5-phenylimidazole nitrate was added 1,5-diazabicyclo[4.3.0]non-5-ene (0.7 mL, 5.7 mmol) followed by 2-chloroethyl isocyanate (5 mL) dropwise with ice cooling. The mixture was stirred at room temperature for 48 h. Ether (100 mL) was added, and the supernatant solution was decanted from a dark insoluble residue and concentrated. The concentrate was purified by flash chromatography (silica, eluent methylene chloride). The product was triturated with acetone and dried, giving **6x** (0.35 g, 21%) as colorless plates, mp 177–178 °C. Anal. Calcd for $C_{12}H_{10}ClN_5O$: C, 52.3; H, 3.66; N, 25.4. Found: C, 51.9; H, 3.45; N, 25.4.

Method I. Preparation of Imidothioic Acid Benzyl Ester Hydrochlorides 7d[22] and 7h. A stirred solution of 2-(cyanoethyl)benzene (5 g, 0.038 mol) and benzyl mercaptan (8 g, 0.064 mol) in dry dioxane (90 mL) was treated with hydrogen chloride gas until saturated (3 h). The mixture was allowed to stand at room temperature (5 days) and then treated with ether. The precipitate was collected and washed with ether, giving **7h** (9.7 g, 87%), mp 158–160 °C, pure enough for use in the next stage.

Method J. 1,6-Dinitro-5*H*,10*H*-diimidazo[1,5-*a*:1′,5′-*d*]pyrazine-5,10-dione (9).[9] An intimate mixture of 5-nitroimidazole-4-carboxylic acid[8] (2 g, 0.013 mol) and phosphorus pentachloride (2.67 g, 0.013 mol) was stirred and heated at 120 °C (1 h). The resulting slurry was concentrated at 60 °C (0.1 mm) (30 min) to give **9** (1.9 g, 100%) as a yellow solid, mp 249–251 °C; IR (KBr) 1750 cm^{-1}; MS, m/z 278 (M^+).

Method K. Preparation of 5-Nitroimidazoles 10i–n. A solution of piperidine (6.4 mL, 0.075 mol) in tetrahydrofuran (92 mL) was treated with 1,6-dinitro-5*H*,10*H*-diimidazo[1,5-*a*:1′,5′-*d*]pyrazine-5,10-dione (4.59 g, 0.017 mol) and the mixture stirred at room temperature (1 h). The mixture was concentrated and the residue dissolved in hydrochloric acid (2 M, 92 mL). The solution was extracted with ethyl acetate (3 × 200 mL), and the combined extracts were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (silica, eluent chloroform/methanol, 9:1), giving **10k** (2.72g, 37%) as a yellow solid, mp 149–150 °C. Anal. Calcd for $C_9H_{12}N_4O_3$: C, 48.2; H, 5.39; N, 25.0. Found: C, 48.2; H, 5.33; N, 25.1.

Method L. Preparation of 5-Nitroimidazoles 12t–v. A water-cooled, stirred, aqueous solution of methylamine (25% w/w, 35 mL) was treated portionwise with 5-nitroimidazole-4-sulfonyl chloride[13] (11) (4.15 g, 0.02 mol). The mixture was stirred at room temperature (15 min) and then concentrated to half-volume. The mixture was acidified (concentrated HCl) and the resulting precipitate recrystallized from water, yielding **12t** (2.07 g, 51%) as pale yellow blades, mp 260–263 °C dec. Anal. Calcd for $C_4H_6N_4O_4S$: C, 23.3; H, 2.93; N, 27.2; S, 15.55. Found: C, 23.1; H, 2.87; N, 27.4; S, 15.4.

Method M. Preparation of 8-Carbamoyl-3-(2-chloroethyl)pyrazolo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (17a). A stirred suspension of 3-diazopyrazole-4-carboxamide[16] (**16a**) (5.9 g, 0.043 mol) in ethyl acetate (150 mL) was treated with 2-chloroethyl isocyanate (24 mL) and stirred at room temperature (7 days) in the dark. The mixture was diluted with ether, giving a mixture of **17a** and **18** (8.36 g) as a solid.

A solution of the mixture (1 g) in Me_2SO (20 mL) was heated at 60 °C (12 h) and then concentrated at 0.1 mmHg. The resulting solid was triturated with methylene chloride and the residue dissolved in boiling acetonitrile. The solution was treated with deactivated silica (3 g containing 20% w/w water) and the suspension evaporated to a powder. The powder was subjected to flash chromatography (silica, eluent ethyl acetate) and the product crystallized from acetonitrile, giving **17a** (0.25 g, 20%) as colorless needles, mp 203–204 °C dec. Anal. Calcd for $C_7H_7ClN_6O_2$: C, 34.65; H, 2.91; Cl, 14.62; N, 34.64. Found: C, 34.8; H, 2.92; Cl, 14.6; N, 34.7.

Method N. Preparation of 17b by the Sequence 19b → 20b → 15b → 16b → 17b. A mixture of α-cyano-*N*,*N*-dimethylacetamide[17] (**19b**) (8.2 g, 0.073 mol), acetic anhydride (21 mL, 0.2 mol), and triethyl orthoformate (21 mL, 0.14 mol) was heated at 160–170 °C, with removal of evolved ethyl acetate (26 mL). Concentration gave a dark oil, which was treated with ethanol and concentrated again. The residue was purified by distillation (bp 160–170 °C (0.5 mm)) and then by flash chromatography (silica, eluent ethyl acetate), affording **20b** (5.3 g, 44%) as an oily solid, used directly in the next stage.

A solution of **20b** (5.3 g, 0.032 mol) in dry ethanol (50 mL) was treated dropwise with hydrazine hydrate (1.58 g, 0.031 mol) and then heated under reflux (6 h). Concentration afforded a residue, which was purified by flash chromatography (silica, eluent chloroform/methanol, 17:3). The product was dissolved in hot 2-propanol (5 mL) and treated with concentrated hydrochloric acid, giving **15b**·HCl (1.4 g, 23%) as colorless crystals, mp 195 °C. Anal. Calcd for $C_6H_{10}N_4O$·HCl:C, 37.8; H, 5.82; Cl, 18.6; N, 29.39. Found: C, 37.8; H, 5.82; Cl, 18.3; N, 29.0.

A suspension of **15b**·HCl (1.4 g, 7.34 mmol) in dry methanol (70 mL) saturated with dry hydrogen chloride was cooled to 0 °C and treated with amyl nitrite (2.55 g, 21.8 mmol) dropwise. The resulting solution was kept at 0 °C (1 h) and then poured into ether, giving the diazopyrazole **16b** as its hydrochloride salt

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(25) Since our paper was submitted, Cheng et al. (*J. Med. Chem.* **1986**, *29*, 1544) have reported on this compound.

Table VI. Antitumor Activity of 3-(2-Chloroethyl)azolo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones

no.	tumor	mouse species	mouse sex	inoculum cells	inoculum route	drug route	day of drug dosing	drug[a] formulation	duration of experiment, days	drug dose, mg/kg	survivors to duration	T/C,[b] %
1 (mitozolomide)	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	40	4/5	>458
										20		167
										10		163
										5		113
	L1210	B6D2F1	♂	10^5	iv	po	3	B	38	40	1/7	>330
										20		217
										10		178
										5		133
6a	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	320		66
										160		80
										80	5/5	>550
										40	2/5	>343
										20		176
	L1210	B6D2F1	♂	10^5	iv	po	3	B	28	160		267
										80		248
										40		180
										10		128
6b	L1210	B6D2F1	♂	10^5	iv	po	3	B	29	40		212
										20		145
										10		105
6c	L1210	B6D2F1	♂	10^5	iv	po	1	B	25	200	2/7	>295
										100		186
										50		151
6d	L1210	B6D2F1	♂	10^5	iv	po	3	B	18	200		137
										100		104
										50		100
6e	L1210	B6D2F1	♀	10^5	iv	po	3	B	25	160		147
										80		113
										40		100
6f	L1210	B6D2F1	♂	10^5	iv	po	1	B	18	200		90
										100		86
										50		94
6g	L1210	B6D2F1	♀	10^5	iv	po	1	B	16	200		127
										100		114
										50		105
6h	L1210	B6D2F1	♀	10^5	iv	po	1	B	16	200		100
										100		100
										50		100
6i	TLX5	CBA/CA	♀	2×10^5	sc	iv	3	A	60	80		72
										40	5/5	>555
										20	1/5	>254
										10		133
										5		104
	L1210	B6D2F1	♂	10^5	ip	po	1	B	39	80		toxic
										40	2/10	>215
										20	10/10	>453
										10	2/10	>192
6j	TLX5	CBA/CA	♀	2×10^5	sc	iv	3	A	60	160		68
										80	3/5	>358
										40	5/5	>508
										20		161
	L1210	B6D2F1	♂	10^5	ip	po	1	B	39	80		toxic
										40	4/10	>235
										20	8/10	>290
										10		197
6k	TLX5	CBA/CA	♀	2×10^5	sc	iv	3	A	60	640		25
										320	1/5	>234
										160		120
										80		102
										40		98
	L1210	B6D2F1	♂	10^5	ip	po	1	B	34	200		171
										100		108
										50		100
6l	L1210	B6D2F1	♀	10^5	iv	po	1	B	16	200		100
										100		100
										50		100
6o	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	320		98
										160		98
										80		100
										40		103
										20		98

Table VI (Continued)

no.	tumor	mouse species	mouse sex	inoculum cells	inoculum route	drug route	day of drug dosing	drug[a] formulation	duration of experiment, days	drug dose, mg/kg	survivors to duration	T/C,[b] %
6p	L1210	B6D2F1	♀	10^5	iv	po	3	B	25	160		183
										80		130
										40		100
6q	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	160		80
										80		104
										40		102
										20		102
										10		96
6r	L1210	B6D2F1	♂	10^5	iv	po	1	B	28	200		98
										100		98
										50		98
6s	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	80		66
										40		66
										20	1/5	>214
	L1210	B6D2F1	♂	10^5	iv	po	3	B	38	40	2/7	>310
										20		257
										10		217
										5		217
6t	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	80		68
										40	1/5	>203
										20	3/5	>375
	L1210	B6D2F1	♂	10^5	iv	po	2	B	45	40		25
										20	6/7	>666
										10		283
6u	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	80		68
										40	3/5	>381
										20	3/5	>385
	L1210	B6D2F1	♂	10^5	iv	po	3	B	45	40	7/7	>750
										20		222
										10		152
6v	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	320		109
										160		123
										80		151
										20		113
6w	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	40		119
										20	5/5	>476
										10		160
										5		121
	L1210	B6D2F1	♂	10^5	iv	po	3	B	38	20	6/7	>605
										10	1/7	>376
										5		207
										2.5		136
6x	L1210	B6D2F1	♀	10^5	iv	po	1	B	16	200		100
										100		100
										50		100
17a	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	160		221
										80		143
										40		116
										20		123
										10		98
	L1210	B6D2F1	♂	10^5	iv	po	3	B	43	200	1/7	363
										100	5/7	>530
										50		186
										25		120
17b	TLX5	CBA/CA	♀	2×10^5	sc	ip	3	A	60	320		48
										160	1/5	>248
										80		131
										40		109
	L1210	B6D2F1	♂	10^5	iv	po	1	B	41	200	10/10	>600
										100		250
										50		144
17c	L1210	B6D2F1	♂	10^5	iv	po	1	B	41	200		101
										100		100
										50		101
17d	L1210	CBA/CA	♂	10^5	iv	po	3	B	30	40	5/7	>447
										20		307
										10		155

[a] A: 10% Me_2SO in arachis oil. B: suspension in solution of 1% (carboxymethyl)cellulose in water. [b] Mean death day treated animals/mean death day control animals.

(0.92 g, 63%) as colorless crystals, mp 150 °C (explodes); IR (KBr) 2280 cm^{-1}. Anal. Calcd for $C_6H_7N_5O$·HCl: C, 35.74; H, 4.00; N, 34.74. Found: C, 35.3; H, 3.79; N, 34.3.

A suspension of the hydrochloride of diazopyrazole **16b** (0.92 g, 4.56 mmol) in methylene chloride (50 mL) was treated consecutively with 2-chloroethyl isocyanate (2.5 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.7 g, 4.60 mmol), and the mixture was stirred at room temperature (18 h). The mixture was concentrated and the residue triturated with petroleum ether. The insoluble residue was purified by flash chromatography (eluent ethyl acetate) and the obtained product crystallized from ethyl acetate, affording the pyrazolo[5,1-*d*]tetrazinone **17b** (0.38 g, 31%) as colorless crystals, mp 116–118 °C dec. Anal. Calcd for $C_9H_{11}ClN_6O_2$: C, 39.93; H, 4.1; Cl, 13.1; N, 31.05. Found: C, 39.7; H, 3.96; Cl, 13.1; N, 30.9.

Method O. Preparation of 17c by the Sequence 15c → 16c → 17c. A stirred saturated solution of hydrogen chloride in methanol (56 mL) was treated with **15c**[18] (2.0 g, 0.016 mol) and cooled to 0 °C. Amyl nitrite (7.2 g, 0.062 mol) was added dropwise. The mixture was stirred at 2 °C (1 h) and the resulting suspension was poured into ether (200 mL), giving **16c**·HCl (2.5 g, 92%) as a white solid, mp 113 °C dec (lit.[18] mp 104–105 °C). Anal. Calcd for $C_3HN_5O_2$·HCl: C, 20.53; H, 1.15; Cl, 20.2; N, 39.9. Found: C, 20.5; H, 0.91; Cl, 19.0; N, 40.8.

A suspension of 3-diazo-4-nitropyrazole (**16c**) hydrochloride salt (2.45 g, 0.014 mol) in methylene chloride (50 mL) was treated with 2-chloroethyl isocyanate (8.9 g) and then with 1,8-diazabicyclo[5.4.0]undec-7-ene (2.13 g, 0.014 mol) dropwise. The resulting solution was stirred at room temperature in the dark (1.5 h), concentrated, and triturated with petroleum ether (bp 60–80 °C). The residue was purified by flash chromatography (silica, eluent ethyl acetate/petroleum ether (bp 40–60 °C) 1:2), giving **17c** (0.384 g, 11%) as a pale yellow solid, mp 168–172 °C dec. Anal. Calcd for $C_6H_5ClN_6O_3$: C, 29.46; H, 2.06; Cl, 14.5; N, 34.4. Found: C, 30.4; H, 2.31; Cl, 15.6; N, 32.4.

Method P. Preparation of 17d by the Sequence 19d → 20d → 15d → 16d → 17d. A solution of (methylsulfonyl)acetonitrile (10.5 g, 0.089 mol) in triethyl orthoformate (21 mL, 0.14 mol) and acetic anhydride (21 mL, 0.2 mol) was heated at 160 °C with removal of evolved ethyl acetate (35 mL). The dark oily residue was dissolved in methanol (60 mL) and the solution was concentrated and distilled, giving **20d** (11.98 g, 78%) as a pale yellow oil, bp 165–168 °C (1.0 mm).

A solution of the 3-ethoxy-2-(methylsulfonyl)propenenitrile (**20d**) (3.49 g, 0.02 mol) in ethanol (20 mL) was treated with hydrazine hydrate (1 g, 0.02 mol) and heated under reflux (6 h). Concentration and purification of the residue by flash chromatography (silica, eluent $CHCl_3$/MeOH, 85:15) gave **15d** as a pink oil (1.37 g). This oil was dissolved in ethyl acetate (10 mL) and treated with ethereal HCl, yielding **15d**·HCl (0.97 g, 24%) as a colorless solid, mp 201–203 °C dec. Anal. Calcd for $C_4H_7N_3O_2S$·HCl: C, 24.3; H, 4.08; Cl, 17.9; N, 21.3; S, 16.2. Found: C, 24.4; H, 4.09; Cl, 17.9; N, 21.5; S, 16.6.

A stirred solution of sodium nitrite (0.46 g, 6.67 mmol) in water (3.5 mL) was treated dropwise with a solution of 5-amino-4-(methylsulfonyl)pyrazole hydrochloride (**15d**·HCl) (1.0 g, 5.06 mmol) in hydrochloric acid (1 M, 12.2 mL) at 0 °C. The solution was adjusted to pH 7 (sodium hydrogen carbonate) and extracted with ethyl acetate (3 × 50 mL). The extracts were dried ($MgSO_4$) and evaporated (30 °C (10 mm), then 30 °C (0.1 mm)), giving **16d** (0.68 g, 78%) as a yellow solid, mp 117–119 °C dec. Anal. Calcd for $C_4H_4N_4O_2S$: C, 27.9; H, 2.34; N, 32.5. Found: C, 27.8; H, 2.32; N, 31.9.

A solution of 5-diazo-4-(methylsulfonyl)pyrazole (1.3 g, 7.55 mmol) in 2-chloroethyl isocyanate (16 mL) was kept at room temperature (24 h). The solution was concentrated and the residue purified by flash chromatography (silica, eluent ethyl acetate/toluene, 4:6). The product was triturated with petroleum ether, giving **17d** (1 g, 48%) as a colorless solid, mp 184–185 °C dec. Anal. Calcd for $C_7H_8ClN_5O_3S$: C, 30.3; H, 2.90; N, 25.2. Found: C, 29.9; H, 2.81; N, 25.1.

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NMR Spectroscopic Studies of Intermediary Metabolites of Cyclophosphamide. 2. Direct Observation, Characterization, and Reactivity Studies of Iminocyclophosphamide and Related Species

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4-Hydroxy-5,5-dimethylcyclophosphamide (**6**) was synthesized as a stable (to fragmentation) analogue of 4-hydroxycyclophosphamide (**1**). In anhydrous Me_2SO-d_6 (≤0.03 mol % water), *cis*- and *trans*-**6** were observed by multinuclear NMR spectroscopy to equilibrate with α,α-dimethylaldophosphamide (**7**) and 5,5-dimethyliminocyclophosphamide (**8**). Identification of **8** was based on ^1H, ^{13}C, and ^{31}P chemical shifts, selective INEPT and two-dimensional NMR correlation experiments, and temperature-dependent equilibria data. The interconversion of *cis*-/*trans*-**6** and -**7** was also observed in lutidine buffer; **8** was not detected under the aqueous conditions. In Me_2SO-d_6, hydroxy metabolite **1** underwent dehydration to give iminocyclophosphamide (**5**), as evidenced by chemical shift data and a selective INEPT experiment. Concentrations of *cis*-/*trans*-**1**, aldophosphamide (**2**), and **5** were found to be temperature-dependent with higher temperatures favoring **2** and **5** in a reversible manner, thus indicating that **1**/**2**/**5** were interconverting. The addition of small amounts of water to Me_2SO-d_6 solutions of imine **5** resulted in the immediate disappearance of its NMR signals. The role of imine **5** in the conversion of **1** to C-4 substituted analogues of **1** was elucidated for the formation of 4-cyanocyclophosphamide (**3a**) from **1** and sodium cyanide in lutidine buffer.

There is considerable evidence that the unique therapeutic efficacy of the anticancer drug cyclophosphamide is related to its intermediary metabolites, 4-hydroxycyclophosphamide (**1**) and aldophosphamide (**2**) (Scheme I).[1-6] Mechanistic bases for the oncostatic selectivity of

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