Antitumor Imidazotetrazines. 1. Synthesis and Chemistry of 8-Carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one, a Novel Broad-Spectrum Antitumor Agent

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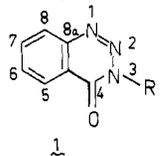
Interaction of 5-diazoimidazole-4-carboxamide and alkyl and aryl isocyanates in the dark affords 8-carbamoyl-3-substituted-imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones. In cold methanol or ethanol, the 3-(2-chloroethyl) derivative **7a** decomposes to afford 2-azahypoxanthine (**14**) and methyl and ethyl *N*-(2-chloroethyl)carbamates, respectively. Compound **7a** has curative activity against L-1210 and P388 leukemia and may act as a prodrug modification of the acyclic triazene 5-[3-(2-chloroethyl)triazen-1-yl]imidazole-4-carboxamide (MCTIC), since it ring opens to form the triazene in aqueous sodium carbonate.

Small molecules bearing NNN linkages in either cyclic (e.g., 1,2,3-triazines[1]) or acyclic (e.g., triazenes[2]) arrangements possess versatile chemical reactivity, which we have long sought to exploit to achieve selective antitumor effects.[3-5] Of the cyclic variants, 3-substituted-1,2,3-benzotriazin-4(3*H*)-ones (**1**) undergo fission at the 1,8a, 2,3,



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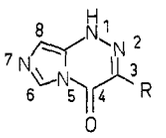

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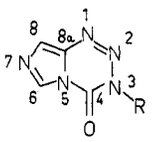

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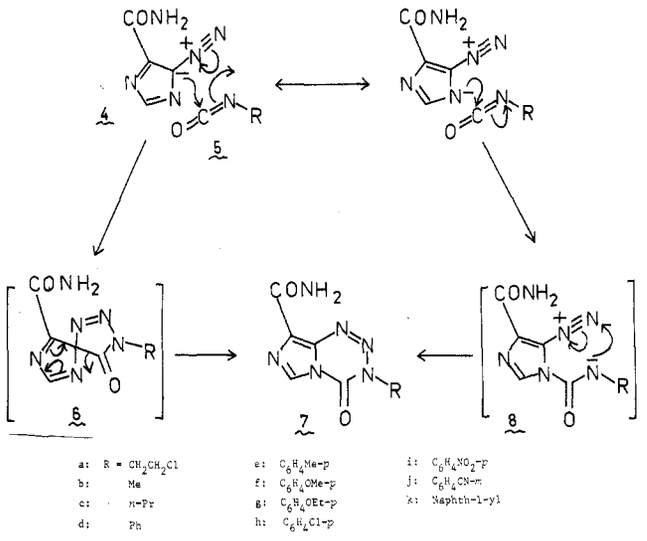
or 3,4 bond, depending on the conditions, and the ionic or radical reactive species thereby generated can be diverted to form a range of products.[1] Ring opening of 3-substituted-imidazo[5,1-c]-1,2,4-triazin-4(3*H*)-ones (**2**) in the presence of hydrazines, on the other hand, proceeds by cleavage of the 4,5 bond only.[6] We were interested in examining the effects elicited by the bridgehead nitrogen atom in 3-substituted-imidazo[5,1-*d*]-1,2,3,5-tetrazin-4-(3*H*)-ones (**3**), which would be hybrids of **1** and **2**, in order to discover whether cleavages of four different bonds (1,8a, 2,3, 3,4, and 4,5) might occur. The prospect that these decompositions might possibly generate a cascade of reactive molecules, some of which, although too unstable for use as pharmaceuticals, are known to possess potent antitumor activity (see later), was felt to warrant investigation.

Chemistry. Ege and Gilbert[7] have described a useful general synthesis of azolotetrazinones based on the interaction of diazoazoles and isocyanates.[7] Accordingly, when 5-diazoimidazole-4-carboxamide (**4**) and 2-chloroethyl isocyanate (**5a**) were stirred in dichloromethane at 25 °C in the dark for 20 days, 8-carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (**7a**; also known as CCRG 81010, M & B 39565, and NSC 353451) was formed in 90% yield. Similarly, interaction of methyl and *n*-propyl isocyanates and a range of aryl isocyanates with **4** in either dichloromethane or ethyl acetate afforded high yields of the appropriate 3-substituted analogues **7b–k** (see Table I).

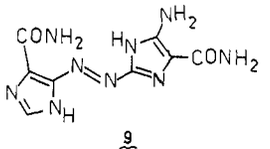
The mechanism of these transformations possibly involves an initial [3 + 2] cycloaddition[8] to form unstable spirobicycles **6**, which rearrange spontaneously by a [1,5] sigmatropic shift to the imidazotetrazinones **7** (Scheme I). Alternatively, the [7 + 2] cycloaddition process favored

Scheme I



by Ege and Gilbert[7] or a two-step ionic mechanism involving initial nucleophilic attack by the imidazole ring nitrogen at the electrophilic carbonyl group of the isocyanate, to yield the dipolar intermediate **8** followed by ring closure, cannot be discounted.

A maroon pigment, traces of which imparted a coloration to many of the crude imidazotetrazinones, was identified as the imidazolylazoimidazole **9**, which was probably



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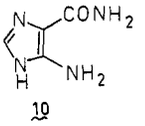

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formed by a coupling interaction[9] between 5-diazoimidazole-4-carboxamide (**4**) and some contaminating 5-aminoimidazole-4-carboxamide (**10**, AIC).

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(1) M. F. G. Stevens, *Prog. Med. Chem.*, **13**, 205 (1976).
(2) K. Vaughan and M. F. G. Stevens, *Chem. Soc. Rev.*, **7**, 377 (1978).
(3) M. F. G. Stevens, A. Gescher, and C. P. Turnbull, *Biochem. Pharmacol.*, **28**, 769 (1977).
(4) J. A. Hickman, *Biochimie*, **60**, 997 (1978).
(5) A. Gescher, J. A. Hickman, R. J. Simmonds, M. F. G. Stevens, and K. Vaughan, *Biochem. Pharmacol.*, **30**, 89 (1981).
(6) G. U. Baig, M. F. G. Stevens, R. Stone, and E. Lunt, *J. Chem. Soc., Perkin Trans. 1*, 1811 (1982).
(7) G. Ege and K. Gilbert, *Tetrahedron Lett.*, 4253 (1979).
(8) A. Padwa and T. Kumagai, *Tetrahedron Lett.*, 1199 (1981).
(9) J. K. Horton and M. F. G. Stevens, *J. Chem. Soc., Perkin Trans. 1*, 1433 (1981).

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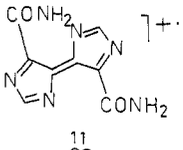
Table I. Synthesis and Physical Characteristics of 8-Carbamoyl-3-substituted-imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones

no.	solvent[g] used in synth	reaction duration, days	yield, %	mp, °C dec	formula[a]	UV λ_{max},[b] nm	IR $\nu_{C=O}$,[c] cm^{-1} [and other absorptions]	NMR chem shifts,[d] δ
7a	A	20	95	164–165[e]	$C_7H_7ClN_6O_2$	325	1748, 1673	4.05 (2 H, t, CH_2CH_2Cl), 4.60 (2 H, t, CH_2CH_2Cl), 7.70 (2 H, br d, NH_2), 8.85 (1 H, s, H-6)
7b	B	20	98	212	$C_6H_6N_6O_2$	327	1750, 1680	3.90 (3 H, s, CH_3), 7.75 (2 H, br s, NH_2), 8.85 (1 H, s, H-6)
7c	B	20	75	167	$C_8H_{10}N_6O_2$	328	1720, 1690	0.95 (3 H, t, CH_3), 1.80 (2 H, m, $CH_2CH_2CH_3$), 4.25 (2 H, t, $CH_2CH_2CH_3$), 7.75 (2 H, br s, NH_2), 8.80 (1 H, s, H-6)
7d	B	4	76	145	$C_{11}H_8N_6O_2$	331	1730, 1695	7.60 (5 H, m, Ph), 7.85 (2 H, br s, NH_2), 8.95 (1 H, s, H-6)
7e	B	4	84	142–144	$C_{12}H_{10}N_6O_2$	335	1735, 1700	2.40 (3 H, s, CH_3), 7.50 (4 H, m, aryl), 7.80 (2 H, br s, NH_2), 8.95 (1 H, s, H-6)
7f	B	4	77	155–160	$C_{12}H_{10}N_6O_3$	337	1730, 1660	3.90 (3 H, s, CH_3), 7.40 (4 H, q, aryl), 7.90 (2 H, br s, NH_2), 9.05 (1 H, s, H-6)
7g	B	4	94	153	$C_{13}H_{12}N_6O_2$	338	1730, 1650	1.35 (3 H, t, CH_3), 4.10 (2 H, q, CH_2), 7.35 (4 H, q, aryl), 7.85 (2 H, br s, NH_2), 8.95 (1 H, s, H-6)
7h	B	4	80	138–141	$C_{11}H_7ClN_6O_2$	334	1730, 1700	7.70 (6 H, br m, Ar and NH_2), 8.95 (1 H, s, H-6)
7i	B	4	89	140–145	$C_{11}H_7N_7O_4$	333	1750, 1680 [1525, 1350 (NO_2)]	*f*
7j	B	4	61	135–138	$C_{12}H_7N_7O_2$	328	1750, 1690 [2210 (C≡N)]	7.45 (4 H, m, aryl), 7.85 (2 H, br s, NH_2), 9.00 (1 H, s, H-6)
7k	B	4	55	144–146	$C_{15}H_{10}N_6O_2$	300	1750, 1680	7.95 (9 H, br, aryl and NH_2), 9.05 (1 H, s, H-6)

[a] All compounds gave satisfactory C, H, and N analyses (±0.4%). [b] In 95% ethanol. [c] As KBr disks. [d] In Me_2SO-d_6. [e] Crystallized from aqueous acetone. [f] Insoluble in Me_2SO-d_6. [g] Solvents used are: A, ethyl acetate at 30 °C in the dark; B, dichloromethane at 25 °C in the dark.

The (chloroethyl)tetrazinone **7a** was isolated from the dichloromethane reaction mixture as a roseate solid, mp 158 °C (effervescence). The IR spectrum (KBr) of the sample showed NH absorption at 3500 and 3240 (broad) and two carbonyl frequencies at 1740 and 1680 cm^{-1}. When crystallized from aqueous acetone or from ethereal 1-methyl-2-pyrrolidinone, the product appeared to change, and the IR spectrum (KBr) showed NH absorption at 3450, 3350, 3230 (broad), and 3120 cm^{-1}, with carbonyl absorptions at 1748 and 1673 cm^{-1}. A cream product (95%) identical with the latter sample was formed directly when 5-diazoimidazole-4-carboxamide and 2-chloroethyl isocyanate were stirred in ethyl acetate at 30 °C for 2 days. The solution IR and ^1H NMR spectra of the two samples were identical, and the discrepancies in the solid-phase IR spectra of uncrystallized and crystallized samples of **7a** are attributed to differing populations of H-bonded modifications of the molecule. A crystallographic analysis of the sample purified from aqueous acetone, mp 164–165 °C, showed the presence of two conformationally distinctive molecules per asymmetric unit: these conformers are rotamers about the C(8)–carboxamide bond, one having the carboxamide group intramolecularly H bonded to N(1), the other not.[10]

The EI-promoted mass spectrum of **7a** showed the expected molecular ion at m/z 242 (244) in low abundance, together with major ions derived from the retro-cycloaddition fragments **4** (m/z 137) and **5a** [m/z 105 (107)]. Similar features were observed with the 3-alkyltetrazinones **7b,c**, but the 3-aryl analogues **7d–k** did not show molecular ions in their spectra: instead abundant radical ions from **4** (m/z 137) and the appropriate aryl isocyanates **5d–k** were observed. The radical ion of low abundance present at m/z 218 in the spectra of all the imidazotetrazinones was attributed to the imidazolylidene–imidazole **11**, a



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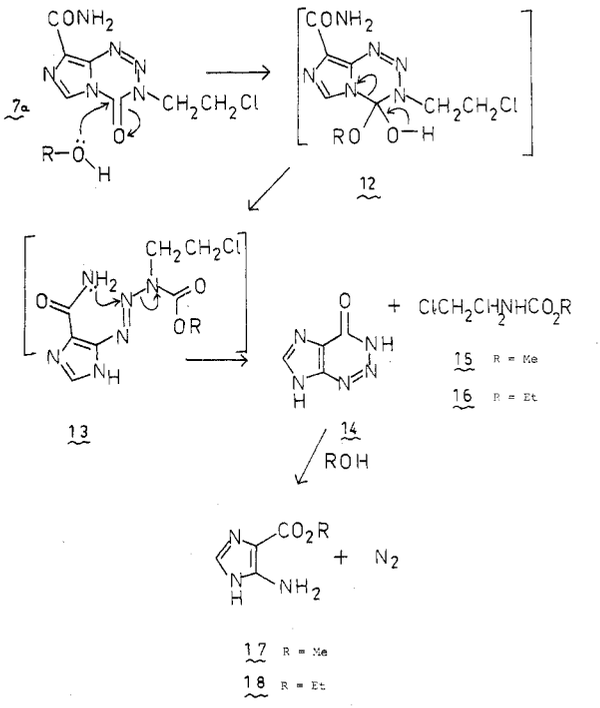
dimer of the carbene generated by nitrogen elimination from the diazoimidazole **4**;[9] this radical ion is also observed in the EI-promoted mass spectra of **4** and 2-azahypoxanthine (**14**).

Our experiences in the chemistry of 1,2,3-benzotriazin-4(3*H*)-ones[1] and imidazo[5,1-*c*]-1,2,4-triazin-4(3*H*)-ones[6] led us to expect that the (chloroethyl)tetrazinone **7a** might either revert to the diazoimidazole (**4**) and 2-chloroethyl isocyanate (**5a**) (i.e., cleavage of the 2,3 and 4,5 bonds) or undergo hydrolytic attack at C(4) and subsequent cleavage of the 3,4 and 4,5 bonds to liberate 5-[3-(2-chloroethyl)-triazen-1-yl]imidazole-4-carboxamide (**26**), MCTIC, NSC 157949). Intracellular release of 2-chloroethyl isocyanate, a carbamoylating agent, plays a significant role in modulating the selective toxicity of the nitrosourea BCNU toward the mouse TLX5 lymphoma[11] but not against the L-1210 leukemia.[12] Furthermore, MCTIC is a potent antitumor agent in its own right.[13] We have found evidence confirming the existence of both the aforementioned decomposition routes under different conditions.

Scheme II



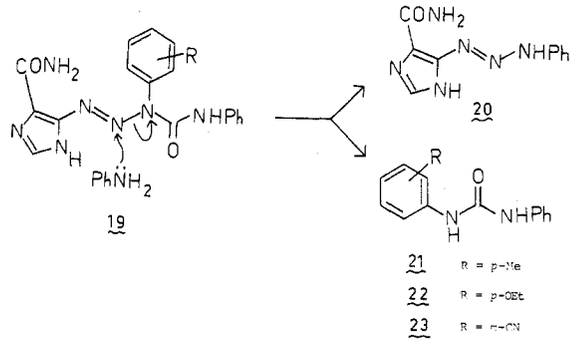
Degradation of the (chloroethyl)tetrazinone **7a** is exquisitely sensitive to the reaction conditions. In a 0.05% w/v solution in methanol at 25 °C, decomposition was very slow; after 35 days, ~60% remained unreacted. TLC and ^1H NMR analysis of the mixture revealed the presence of 2-azahypoxanthine (**14**) and methyl *N*-(2-chloroethyl)-carbamate (**15**). Decomposition of **7a** in boiling methanol was complete in 18 h: the products were **14** (90% of the theoretical yield), **15** (70%), and a trace of an impurity (m/z 141), probably methyl 5-aminoimidazole-4-carboxylate (**17**), derived from further attack on **14** (see below). Degradation of **7a** in boiling absolute ethanol similarly afforded **14**, **16**, and **18**. The rate of decomposition in cold methanol or ethanol was also greatly accelerated by the addition of concentrated aqueous ammonia.

Half-lives of the 3-substitued imidazotetrazinones **7a–k** in 95% ethanol in the dark at 28 °C could be monitored by observing the disappearance of the characteristic band in their UV spectra at 300–340 nm. The 3-alkyl derivatives **7a–c** were, in general, more stable than their 3-aryl counterparts (Table II): electron-withdrawing groups in the aryl substituent had a destabilizing effect. Exposure of solutions to light also decreased the stability of the 3-aryl derivatives, with the exception of the *p*-chlorophenyl analogue **7h**, but had no effect on the half-lives of the 3-alkyl series.

The exact mechanism of these decompositions in cold alcohols and the precise timing of the bond-breaking events is not fully understood. Formally, the product formation from **7a** can be explained by evoking an electrocyclic 6π ring opening, leading to regeneration of 5-diazoimidazole-4-carboxamide and chloroethyl isocyanate, which subsequently yield the observed products. However, it is more likely that degradation is initiated by nucleophilic addition at C(4). Thus, in the case of the 3-(2-chloroethyl) derivative **7a**, attack by the alcohols probably yields the hemiacetals **12** (R = Me or Et), which on cleavage of the 4,5 bond would generate unstable triazenes **13** (R = Me or Et). Intramolecular cyclization with expulsion of the terminal N-moeity would lead to the formation of 2-azahypoxanthine (**14**) and the carbamates **15** or **16** as observed (Scheme II). The esters **17** and **18**, formed as minor

(10) P. R. Lowe and C. H. Schwalbe, to be published.
(11) N. W. Gibson and J. A. Hickman, *Biochem. Pharmacol.*, **31**, 2795 (1982).
(12) K. W. Kohn, *Recent Results Cancer Res.*, **76**, 141 (1981) and references cited therein.
(13) Y. F. Shealy, C. A. O'Dell, and C. A. Krauth, *J. Pharm. Sci.*, **64**, 177 (1975).

Scheme III



byproducts in these degradations, probably arise by alcohol-initiated ring opening of 2-azahypoxanthine (**14**), since partial conversion of **14** to the ethyl ester **18** could be achieved, independently, in boiling ethanol. There are ample precedents for this type of cleavage in 1,2,3-benzotriazin-4(3*H*)-one (**1**, R = H).[14]

The mechanism of the decomposition of representative 3-aryltetrazinones **7e,g,j** in cold acetonitrile containing aniline is similar to that described above, except that the aniline apparently competes successfully with the carboxamide group in attacking N(2) in the acyclic triazenes **19** to afford mixtures of 5-(3-phenyltriazen-1-yl)-imidazole-4-carboxamide (**20**) and the ureas **21–23**, respectively (Scheme III). When **7a** was boiled in acetonitrile alone in the absence of a strongly nucleophilic substrate, it appears that the retro-cycloaddition process does operate, since the IR spectrum of the recovered product shows contaminating bands at 2198 and 1148 cm^{-1} characteristic of the presence of 5-diazoimidazole-4-carboxamide (**4**).

The decomposition of **7a** in aqueous conditions is strikingly pH dependent. The compound is stable in 2 N sulfuric acid and can even be recovered unchanged from hot concentrated sulfuric acid; in phosphate buffer (pH 7.4), the half-life is 98 min at 28 °C (Table II). Preparative-scale degradation of **7a** in 5% aqueous sodium carbonate afforded an unstable solid (50%), which was characterized as MCTIC (**26**), since it was identical with a sample prepared independently by coupling 5-diazoimidazole-4-carboxamide (**4**) with 2-chloroethylamine base in ethyl acetate. At least five other byproducts, including AIC (**10**), were detected (TLC). Preliminary investigation of the decomposition of **7a** in phosphate buffer confirms that AIC (**10**) and 2-chloroethanol (**27**) are the major products: significantly, these compounds have also been identified from the degradation of MCTIC in water or 50% aqueous ethanol.[13]

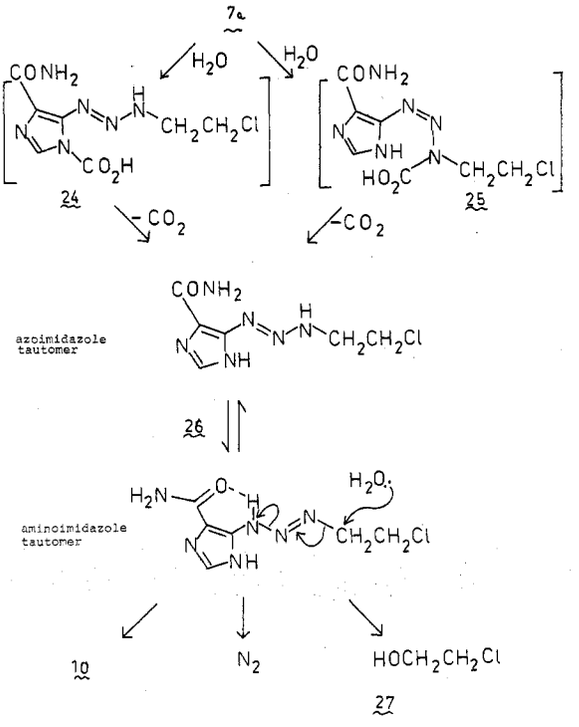
A mechanism that accounts for the salient features of these transformations in aqueous systems is summarized in Scheme IV. Attack by water at C(4) of the imidazotetrazinone **7a**, followed by ring opening, would give unstable carbamic acids **24** or **25**, depending on whether the 3,4 or the 4,5 bond, respectively, was broken initially. Decarboxylation of the carbamic acids would liberate MCTIC (**26**) (as observed in aqueous sodium carbonate), which in turn would alkylate water by an S_N2-type mechanism, ultimately generating AIC, nitrogen, and 2-chloroethanol (**27**). It is possible that intramolecular H bonding controls the chemistry of **26** by stabilizing the aminoimidazole tautomer at the expense of the azoimidazole tautomer, thus activating the electrophilic α-methylene group of MCTIC to attack by nucleophiles.

Table II. Decomposition of 3-Substituted-imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones in Ethanol and Phosphate Buffer

no.	conditions[a]	$t_{1/2}$,[b] min
7a	A	72
	B	70
	C	98
7b	A	495
	B	495
7c	A	155
	B	155
7d	A	23
	B	9
7e	A	40
	B	7.5
7f	A	69
	B	8.5
	C	155
7g	A	78
	B	8
7h	A	6.3
	B	8.2
	C	2.9
7i	A	*c*
7j	A	2.6
7k	A	4.6

[a] A = in 95% ethanol in the dark at 28 °C. B = in 95% ethanol in diffuse laboratory light at 28 °C. C = in phosphate buffer at pH 7.4 in the dark at 28 °C. [b] Decompositions were monitored by measuring the decrease in absorbance of the absorption band in the UV spectrum (see Table I). [c] <1 min.

Scheme IV



The aforementioned chemistry lends weight to the hypothesis that the bicyclic tetrazinone **7a** could be a stable prodrug modification of MCTIC, and this view is supported by a comparison of the biochemical effects of both agents against tumor cells.[15,16] The bicyclic derivative is

(14) J. G. Erickson, *Chem. Heterocycl. Compd.*, **10**, 1 (1956), and references cited therein.

(15) C. Horgan, M. F. G. Stevens, and M. J. Tisdale, *Br. J. Cancer*, **48**, 132 (1982).

(16) C. Horgan, M. J. Tisdale, E. Erban, M. D'Incalci, and S. Pepe, *Br. J. Cancer*, **48**, 139 (1983).

Table III. Antitumor Activity of 8-Carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (7a) against L-1210[a] and P388[b] Leukemia in Mice

tumor	schedule	dose, mg kg^{-1} day^{-1}	mortality at day 5	median survival time, days (T/C)	T/C, %	survivors[c]
L-1210	day 1 only	80[d]	0/5	14.1/8.1	177	0/5
		40	0/5	>60/8.1	>740	5/5
		20	0/5	16/8.1[e]	198	1/5
		10	0/5	10.6/8.1	131	0/5
		5	0/5	9.0/8.1	111	0/5
L-1210	days 1-5	20	0/5	>60/10.4	>577	5/5
		10	0/5	>60/10.4	>577	5/5
		7.5	0/5	17.0/10.4	163	0/5
		5	0/5	14.4/10.4	139	0/5
		2.5	0/5	11.6/10.4	112	0/5
L-1210[f]	days 1-9	50[d]	0/6	11.3/8.2	137	0/6
		25	0/6	>61.1/8.2	>745	6/6
		12.5	0/6	>61.1/8.2	>745	6/6
		6.25	0/6	15.1/8.2	184	0/6
		3.12	0/6	12.1/8.2	147	0/6
P388	day 1 only	80[d]	0/5	7.4/10.0	74	0/5
		40	0/5	>60/10.0	>600	5/5
		20	0/5	>60/10.0	>600	5/5
		10	0/5	16.4/10.0	164	0/5
		5	0/5	13.2/10.0	132	0/5
P388	days 1-5	20	0/5	>60/10.8	>555	5/5
		10	0/5	>60/10.8	>555	5/5
		7.5	0/5	>60/10.8	>555	5/5
		5	0/5	30/10.8[e]	277	3/5
		2.5	0/5	16/10.8	148	0/5
P388[f]	days 1-5	50[d]	0/6	15/9.8	153	0/6
		25	0/6	38/9.8[e]	387	4/6
		12.5	0/6	27/9.8[e]	275	1/6
		6.25	1/6	17.8/9.8	180	0/6

[a] 10^5 L-1210 cells implanted intraperitoneally in BDF_1 mice on day 0. Animals treated on day 1 by the intraperitoneal route with drug dissolved in 10% Me_2SO in arachis oil (0.1 mL) and thereafter as indicated in the schedule. [b] 10^6 P388 cells implanted intraperitoneally in BDF_1 mice on day 0. Treatment as in footnote *a*. [c] At day 60. [d] Toxic at this dose. [e] Only dead mice were included in the calculation of the median survival time when long-term survivors were involved in the test experiment. [f] Results were obtained by Dr. G. Atassi, Institut Jules Bordet, Brussels.

a more attractive clinical candidate[17] than either MCTIC or its 1-aryl-3-(2-chloroethyl)triazene counterparts,[18] which are unstable moeities likely to engender insurmountable formulation problems.

Antitumor Activity. In standard antitumor tests against mouse L-1210 leukemia on day 1, days 1–5, and days 1–9 drug administration schedules, **7a** exhibited pronounced antitumor effects, with 60-day cures being elicited in all schedules (Table III). The antitumor activity and toxicity of **7a** in the day 1 and days 1–9 schedules closely parallel that reported for MCTIC against L-1210 leukemia.[13] Compound **7a** also showed pronounced activity against P388 leukemia (Table III), with 100% cures being produced at more than one dose level in day 1 and days 1–5 schedules. The drug has activity rated (++) on the NCI activity scale against B16 melanoma, colon 38 tumor, Lewis lung carcinoma, and the LX-1 lung tumor xenograft.[19] Full antitumor results on **7a** and its analogues against a wide spectrum of tumors, cross-resistance studies, and results of experiments designed to unravel the biologically relevant chemistry and mode of action of this interesting new moiety will be published in future parts of this series.

Experimental Section

Melting points were determined on an Electrothermal instrument and are uncorrected. NMR spectra were recorded on a Varian EM 360 or CFT 20 instrument; solvents are indicated in the text. IR spectra were recorded on a Pye Unicam SP 1000, SP 3200, or SP 4000 instrument as potassium chloride disks, unless otherwise indicated. UV spectra were run on a Pye Unicam SP 8000; for the half-life determinations, a Unicam SP 8005 program controller operating in the repeat scan mode was employed. Mass spectra were determined on a V.G. Micromass 12 operating at 70 eV with a source temperature in the range of 150–300 °C. Elemental microanalyses were performed on a Carlo Erba 1106 instrument.

8-Carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (7a). A suspension of 5-diazoimidazole-4-carboxamide (0.3 g)[9] in anhydrous dichloromethane (10 mL) was stirred with 2-chloroethyl isocyanate (1.0 mL) for 20 days in the dark at 25 °C. Addition of ether (30 mL) to the mixture gave a roseate precipitate (90%) of **7a**, which was collected and washed with more ether. The product had mp 158 °C (effervescence); IR ν_{max} (KBr) 3500, 3240, 1740, 1680, 1660 cm^{-1}; UV λ_{max} (EtOH) 325 nm. Anal. Calcd for $C_7H_7ClN_6O_2$: C, 34.7; H, 2.9, N, 34.8. Found: C, 35.1; H, 2.7; N, 34.8. Crystallization from aqueous acetone afforded cream microprisms, mp 164–165 °C (effervescence). Anal. ($C_7H_7ClN_6O_2$) C, H, N. The physical characteristics of the specimen, mp 164–165 °C, are recorded in Table I.

When 5-diazoimidazole-4-carboxamide and 2-chloroethyl isocyanate were stirred in ethyl acetate at 30 °C in the dark for 2 days, the product (95%) was the cream form of **7a**, mp 164–165 °C (effervescence).

Yields, physical characteristics, and spectroscopic features of other 8-carbamoyl-3-substituted-imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-ones **7b–k** formed from 5-diazoimidazole-4-carboxamide and isocyanates in either dichloromethane or ethyl acetate are recorded in Table I.

Degradation of 8-Carbamoyl-3-(2-chloroethyl)imidazo-[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (7a) in Organic Solvents. A. Methanol. A suspension of **7a** (2.46 g) in methanol (123 mL) was boiled (18 h) and evaporated under vacuum to yield a solid

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residue, which was triturated with ether. The tan solid (1.51 g) melted slowly at 150 °C (dec) and exploded when dropped into a preheated probe at 200 °C. Analysis of the solid by ^{1}H NMR, ^{13}C NMR, IR, MS, and TLC revealed the presence of **14** (~90%): ^{1}H NMR (Me_2SO-d_6) δ 8.45 (1 H, s, H-6), 12.0 (2 H, br s, 2 NH exchangeable with D_2O); ^{13}C NMR δ 119.6, 143.0, 152.0, 152.3. The ^{13}C NMR spectrum of the mixture showed the presence of additional impurities absorbing at δ 42.4, 43.2, 50.5. 51.3, and 135.0; the IR spectrum (KBr) showed a broad band at 1690 cm^{-1}, and the MS was essentially that of **14** (m/z 218, 110, 109, 83), with an impurity at m/z 141, which is probably the ester **17**.[20]

Evaporation of the ethereal solution gave a colorless oil (1.0 g): bp 120 °C (15 mmHg); ^{1}H NMR (Me_2SO-d_6) δ 3.5–3.7 (4 H, m, CH_2CH_2), 3.75 (3 H, s, CH_3), 5.6 (1 H, br s, NH); IR ν_{max} (film) 3340 (NH), 1710 (CO) cm^{-1}. Anal. Calcd for $C_4H_8ClNO_2$: C, 34.9; H, 5.9; N, 10.2; Cl, 25.8. Found: C, 36.0; H, 6.1; N, 10.5; Cl, 25.3. The oil was identical with a sample of methyl *N*-(2-chloroethyl)carbamate (**15**) prepared by reacting methanol with 2-chloroethyl isocyanate.

When compound **7a** (6.0 g) was suspended in methanol (480 mL) and treated with concentrated aqueous ammonia (120 mL), a clear solution was obtained. The mixture was stirred at 25 °C for 10 min, and solvent was removed under vacuum (temperature <40 °C). The solid residue was triturated with ether, collected, and dried at 100 °C (15 mmHg). The product, the azapurinone **14** (3.4 g, 100%), melted at >150 °C (dec) and was identical (IR) with an authentic sample. The ethereal filtrate yielded methyl *N*-(2-chloroethyl)carbamate (**15**; 1.75 g, 51%) as a colorless oil. Anal. Calcd for $C_4H_8ClNO_2$: C, 34.9; H, 5.9; N, 10.2; Cl, 25.8. Found: C, 34.5; H, 5.8; N, 10.5; Cl, 25.1.

B. Absolute Ethanol. Compound **7a** (2.06 g) was boiled in ethanol for 6 h, and the residue that formed when the solution was evaporated was triturated with ether to give a pink solid (1.26 g): mp >150 °C dec; ^{1}H NMR analysis of the mixture showed it to contain **14** and **18** in the ratio 3.5:1. The ester **18** was prepared independently from **14** in refluxing ethanol (18 h), after which time ~60% conversion was achieved. The ^{1}H NMR spectrum of **18** showed absorptions at δ 1.35 (3 H, t, J = 6 Hz, CH_3), 4.3 (2 H, q, J = 6 Hz, CH_2), and 7.35 (1 H, s, H-2), identical with the spectrum of an authentic sample.[21]

Evaporation of the ethereal phase of the original reaction mixture gave an oil (1.07 g, 83%), which was identical with an authentic sample of ethyl *N*-(2-chloroethyl)carbamate: IR ν_{max} (film) 3370 (NH), 1710 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (3 H, t, J = 6 Hz, CH_3), 3.5–3.7 (4 H, m, CH_2CH_2), 4.15 (2 H, q, J = 6 Hz, CH_2), 5.40 (1 H, br s, NH exchangeable with D_2O). Anal. ($C_5H_{10}ClNO_2$) C, H, N.

C. 95% Ethanol. A solution of **7a** in ethanol (concentration 0.001%) was maintained in a foil-covered quartz cuvette or a cuvette exposed to diffuse laboratory light. The change in absorbance of the long-wavelength absorption band at 325 nm, against time, was monitored in a Unicam SP 8000 spectrometer, with temperature control (28 °C) achieved by an external water bath. The half-life of **7a** and its analogues **7b–k** under these conditions are recorded in Table II.

D. Acetonitrile. A sample of crude **7a** (2.0 g) formed from the interaction of 5-diazoimidazole-4-carboxamide and 2-chloroethyl isocyanate in dichloromethane was refluxed in acetonitrile (2 h). The hot solution was filtered to remove a maroon solid (~5 mg), which was identical (UV and IR) with a sample of 5-amino-2-[(4-carbamoylimidazol-5-yl)azo]imidazole-4-carboxamide (**9**) prepared by coupling 5-diazoimidazole-4-carboxamide and 5-aminoimidazole-4-carboxamide.[9] Evaporation of the acetonitrile solution under vacuum gave a cream solid (1.95 g): IR (KBr) 3450, 3350, 3230, 3120, 2198 (diazo), 1748, 1673, 1148 cm^{-1}.

5-(3-Phenyltriazen-1-yl)imidazole-4-carboxamide (20). A mixture of 5-diazoimidazole-4-carboxamide (0.2 g) and aniline (0.2 g) was stirred in 95% ethanol (3.0 mL) for 12 h at 25 °C in the dark. The yellow precipitate of the triazenylimidazole (72%) was washed with ether: mp 159–160 °C dec; UV λ_{max} (EtOH) 372 nm; IR ν_{max} (KBr) 3400–3200 (bonded NH), 1650, 1600 cm^{-1}; mass spectrum, m/z 230 (M$^+$, 20%), 202 (15), 185 (15), 169 (20), 168 (13), 167 (10), 126 (33), 109 (45), 104 (40), 93 (66), 77 (100). Anal. ($C_{10}H_{10}N_6O$) C, H, N.

Solutions of the imidazotetrazinones **7e,j,k** (0.1 g) in acetonitrile (5 mL) were stirred in the dark at 25 °C for 12 h with aniline (0.1 g). TLC examination (0.25-mm silica plates, with chloroform/methanol as the developing solvent) revealed in each case four spots. Two of the spots cochromatographed with reference samples of the starting materials and one with 5-(3-phenyltriazen-1-yl)imidazole-4-carboxamide, prepared as above. The fourth spot cochromatographed with specimens of *N*-*p*-tolyl- (**21**), *N*-(*p*-ethoxyphenyl)- (**22**), and *N*-(*m*-cyanophenyl)-*N'*-phenylureas (**23**), prepared independently by decomposing *p*-tolyl-, *p*-ethoxyphenyl-, and *m*-cyanophenyl isocyanates, respectively, in aniline.

5-[3-(2-Chloroethyl)triazen-1-yl]imidazole-4-carboxamide (26). A mixture of **7a** (2.0 g) and 5% aqueous sodium carbonate (40 mL) was stirred at 25 °C for 4 h. A buff solid (0.96 g) was collected and washed rapidly with ice-cold water. The product, mp 123 °C (**explodes!**), was identical [IR (KBr) 3490, 3280, 3080, 2950, 2820, 2640, 1640, 1590 cm^{-1}] with a sample prepared independently by coupling 5-diazoimidazole-4-carboxamide with 2-chloroethylamine in ethyl acetate at 25 °C in the dark.[13]

The red aqueous filtrate was neutralized to pH 7 with 2 N hydrochloric acid and evaporated under vacuum to yield a pink residue. TLC examination of the residue showed the presence of five products. Preparative-scale TLC separation of the major products gave a sample with δ 5.35 (2 H, br s, NH_2), 6.50 (2 H, br s, $CONH_2$), 6.95 (1 H, s, H-2), which cochromatographed with an authentic sample of 5-aminoimidazole-4-carboxamide (**10**).

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