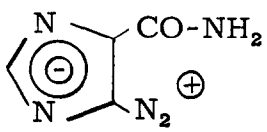
Antitumor activity of triazenoimidazoles*

(*Received* 12 *March* 1962; *accepted* 2 *April* 1962)

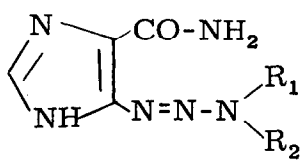
MANY ANALOGUES of the purine and pyrimidine bases of nucleic acids have been shown to inhibit the growth of neoplastic cells *in vitro* and *in vivo*. These analogues are either derivatives of the purine or pyrimidine ring or they are heterocyclic analogues in which the ring system itself has been altered. In contrast, analogues of the imidazoles in the biosynthetic pathway to purine ribonucleotides and nucleic acids have received little attention. We wish to report activity by an imidazole derivative against sarcoma 180, adenocarcinoma 755, and lymphoid leukemia L1210.

An intermediate in the transformation of 5(or 4)-aminoimidazole-4(or 5)-carboxamide (AIC) to 2-azahypoxanthine was isolated and shown to be 5-diazoimidazole-4-carboxamide[1] (I), and brief mention was made of its activity against human epidermoid carcinoma (H. Ep.-2) in tissue culture, Ehrlich ascites carcinoma, and Walker 256 carcinosarcoma. Although 5-diazoimidazole-4-carboxamide can be stored under anhydrous conditions, it cyclizes to 2-azahypoxanthine in solution over a wide range of pH values.[1] This instability in solution limits its potential usefulness as an antitumor agent. It seemed likely that triazene derivatives (II) of I would have antitumor activity for the following reasons: (i) they may serve as latent forms of the active, but unstable, parent compound (I); (ii) they are analogues of AIC; (iii) simpler triazenes, even though they lack a biologically important moiety such as the imidazolecarboxamide portion of II, have shown activity against sarcoma 180 and other tumors.[2] Since triazenes, in general, are acid-sensitive,[3] and the pH of tumor tissue is lower than that of normal tissue,[4] it was thought that dissociation of the triazenoimidazoles to I might occur more readily in tumor tissue than in normal tissue.†

In agreement with the formulation of 5-diazoimidazole-4-carboxamide as an internal diazonium salt (I), it was shown[6] to undergo the



I



II

coupling reaction typical of aromatic diazonium salts. Reaction of I with dimethylamine gave 5(or 4)-(dimethyltriazeno)imidazole-4(or 5)-carboxamide (II, $R_1 = R_2 = CH_3$): explosive decomposition, 250–255°. (Calcd. for $C_6H_{10}N_6O$: C, 39·55; H, 5·53; N, 46·13. Found: C, 39·44; H, 5·50; N, 46·25). The dimethyltriazene is stable in neutral solution in the absence of light.[6]

In repeated tests conducted in accordance with the procedures prescribed by the Cancer Chemotherapy National Service Center,[7] 5(or 4)-(dimethyltriazeno)imidazole-4(or 5)-carboxamide has proved to be active against sarcoma 180 in Swiss mice, adenocarcinoma 755 in BDF_1 mice, and lymphoid leukemia L1210 in BDF_1 mice. Log-probability plots[8] of the host-weight change and the solid-tumor data given in Table 1 clearly indicate that the observed inhibition of S180 and Ca755 is not due to host inanition induced by caloric restriction.[8] At its LD_{10} 5(or 4)-(dimethyltriazeno)imidazole-4(or 5)-carboxamide inhibits the growth of S180 to 32% of controls and of Ca755 to 16% of controls.‡ The life span of L1210-implanted mice is increased 57% at the optimal dose of this compound.

* This work was supported by the Cancer Chemotherapy National Service Center (Contracts SA-43-ph-1740 and SA-43-ph-3784), National Cancer Institute, National Institutes of Health and by the C. F. Kettering Foundation.

† Ross[5] has since discussed the possibility of obtaining selectivity of action against neoplasms through the selective concentration of basic drugs in acidic tumor tissue.

‡ These values were determined from log-probability plots[9] (dosage-response) of the data in Table 1 and from LD_{10} data obtained from nontumor-bearing BDF_1 and Swiss mice.

TABLE 1. INHIBITION OF TUMOR GROWTH BY 5(OR 4)-(DIMETHYLTRIAZENO)IMIDAZOLE-4(OR 5)-CARBOXAMIDE

Tumor	Dosage (mg/kg/day)	Mortality	Avg. host wt. change, T/C* (g)	Avg. tumor wt.		No. of expts.
				T/C (mg)	% of control	
S180	200	1/6	−5·6/−1·5	232/981	23	1
	100	1/37	−1·5/+0·9	419/1307	32	6
	50	0/6	−2·4/−2·2	481/1101	43	1
	25	1/6	−2·5/−2·2	751/1101	68	1
Ca755	200	4/10	−4·3/+2·8	17/1888	1	1
	125	5/50	−2·7/+2·2	159/1163	14	5
	100	1/10	−1·6/+2·8	427/1888	22	1
	50	1/10	+0·3/+3·1	394/1305	30	1
	25	0/10	+1·5/+3·1	485/1305	37	1
	12	0/10	+1·2/+3·1	660/1305	50	1
				Avg. survival time		
				T/C (days)	% Increase over control	
L1210	180	0/6	−1·8/+0·8	13·8/9·7	42	1
	125	0/12	−1·0/+1·1	12·3/8·4	47	2
	120	1/18	−1·5/+0·7	13·8/8·8	57	3
	80	0/6	−1·2/+0·8	14·3/9·7	47	1
	53	0/6	+0·0/+0·8	12·3/9·7	26	1

* T/C = treated/control.

Preliminary evaluation of other 5(or 4)-(substituted-triazeno)-imidazole-4(or 5)-carboxamides (II) indicates that some of them inhibit the growth of one or more of the transplantable mouse tumors. Further studies on the synthesis and antitumor effects of these and related compounds are in progress.

Acknowledgement—The authors express their appreciation to Dr. F. M. Schabel, Jr., and to Dr. H. E. Skipper for their encouragement in this work.

Kettering-Meyer Laboratory,
Southern Research Institute,
Birmingham, Ala., U.S.A.

Y. FULMER SHEALY
JOHN A. MONTGOMERY
W. RUSSELL LASTER, JR.

REFERENCES

1. Y. F. SHEALY, R. F. STRUCK, L. B. HOLUM and J. A. MONTGOMERY, *J. org. Chem.* **26**, 2396 (1961).
2. D. A. CLARKE, R. K. BARCLAY, C. C. STOCK and C. S. RONDESTVEDT, JR., *Proc. Soc. exp. Biol., N.Y.* **90**, 484 (1955); C. P. DAGG, D. A. KARNOFSKY, C. C. STOCK, C. R. LACON and J. RODDY, *Ibid.* p. 489; J. H. BURCHENAL, M. K. DAGG, M. BEYER and C. C. STOCK, *Ibid.* **91**, 398 (1956).
3. K. H. SAUNDERS, *The Aromatic Diazo Compounds and Their Technical Applications*. Edward Arnold, London (1949).
4. J. P. GREENSTEIN, *Biochemistry of Cancer* 2nd ed., pp. 450–453. Academic Press, New York (1954); H. MILLET, *J. biol. Chem.* **78**, 281 (1928).
5. W. C. J. ROSS, *Biochem. Pharmacol.* **8**, 235 (1961).
6. Y. F. SHEALY, C. A. KRAUTH and J. A. MONTGOMERY, *J. org. Chem.* In press.

7. J. LEITER, A. R. BOURKE, S. A. SCHEPARTZ and I. WODINSKY, *Cancer Chemotherapy Screening Data VIII, Cancer Res.* **20** (No. 10, part 2), 734 (1960); Cancer Chemotherapy National Service Center Specifications for Screening Chemical Agents and Natural Products Against Animal Tumors, *Cancer Chemotherapy Reports*, No. 1, pp. 42–64 (1959).
8. W. R. LASTER, JR., F. M. SCHABEL, JR., H. E. SKIPPER, W. S. WILCOX and J. R. THOMSON, *Cancer Res.* **21**, 895 (1961).
9. F. M. SCHABEL, JR., J. A. MONTGOMERY, H. E. SKIPPER, W. R. LASTER, JR. and J. R. THOMSON, *Cancer Res.* **21**, 690 (1961).