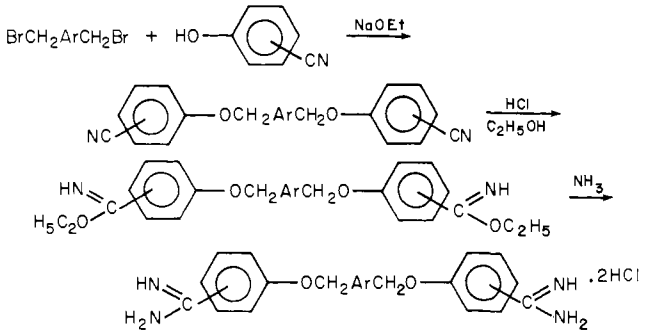
Scheme I



Organic Synthesis. All melting points were determined on a Thomas-Hoover capillary melting point apparatus. Elemental analyses for all compounds were carried out by either Atlantic Microlab, Inc., Atlanta, Ga., or Galbraith Laboratories, Inc., Knoxville, Tenn. Analyses were accepted if the determined values were within ±0.4% of the theoretical values. M & B 4596 [2,7-di(*m*-amidinophenyldiazoamino)-10-ethyl-9-phenylphenanthridinium chloride dihydrochloride] and 2,2'-dibromopropamidine [1,3-bis(4-amidino-2-bromophenoxy)propane diisethionate] were supplied by May & Baker, Ltd., Dagenham, England. The synthesis of 2,2'-dibromopentamidine [1,5-bis(4-amidino-2-bromophenoxy)pentane dihydrochloride] and 2,2'-diiodopentamidine [1,5-bis(4-amidino-2-iodophenoxy)pentane dihydrochloride] has been described earlier.[6] The chemical intermediates, 2-bromo-4-cyanophenol and 4-cyano-2-iodophenol, were prepared by previously described methods.[19] The general procedure for the preparation of target compounds is shown in Scheme I. The dicyano intermediates were prepared according to methods described earlier.[6,7] The diamidino derivatives were synthesized from the corresponding dicyano compounds by procedures described by Ashley et al.[20] and by Berg and Newbery.[19] Purification of the diamidines was accomplished by recrystallization in ethanol, dilute hydrochloric acid, or a mixture of the two solvents. Yields for the conversion of the dicyano intermediates to the diamidines as well as melting points and analyses for the diamidines are given in Table IV. The monocyano intermediate used in the preparation of α-(4-amidinophenoxy)-α'-(4-nitrophenoxy)-*p*-xylene (compound **21**) was prepared in two steps from α,α'-dibromo-*p*-xylene. The two-step ether synthesis was similar to the previously described method for the synthesis of unsymmetrical aliphatic dicyano diether intermediates.[7]

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References and Notes

(1) Kallikrein is a registered trademark assigned to Farbenfabriken Bayer AG, Leverkusen, Federal Republic of Germany.
(2) (a) J. D. Geratz, *Thromb. Diath. Haemorrh.*, **23**, 486 (1970); (b) *ibid.*, **25**, 391 (1971).
(3) J. D. Geratz, *Folia Haematol. (Leipzig)*, **98**, 455 (1972).
(4) J. D. Geratz, *Arch. Int. Pharmacodyn. Ther.*, **194**, 359 (1971).
(5) S. S. Asghar, K. W. Pondman, and R. H. Cormane, *Biochim. Biophys. Acta*, **317**, 539 (1973).
(6) J. D. Geratz, A. C. Whitmore, M. C.-F. Cheng, and C. Piantadosi, *J. Med. Chem.*, **16**, 970 (1973).
(7) J. D. Geratz, M. C.-F. Cheng, and R. R. Tidwell, *J. Med. Chem.*, **18**, 477 (1975).
(8) F. Markwardt, *Haemostasis*, **3**, 202 (1974).
(9) L. J. Loeffler, E.-C. Mar, J. D. Geratz, and L. B. Fox, *J. Med. Chem.*, **18**, 287 (1975).
(10) P. Walsmann, H. Horn, H. Landmann, F. Markwardt, J. Stürzebecher, and G. Wagner, *Pharmazie*, **30**, 386 (1975).
(11) J. H. Milstone, *Proc. Soc. Exp. Biol. Med.*, **103**, 361 (1960).
(12) B. F. Erlanger, N. Kokowsky, and W. Cohen, *Arch. Biochem. Biophys.*, **95**, 271 (1961).
(13) L. Svendsen, B. Blombäck, M. Blombäck, and P. I. Olsson, *Thromb. Res.*, **1**, 267 (1972).
(14) M. Dixon, *Biochem. J.*, **55**, 170 (1953).
(15) L. A. Sternberger, *Br. J. Exp. Pathol.*, **28**, 168 (1947).
(16) S. W. Nye, J. B. Graham, and K. M. Brinkhous, *Am. J. Med. Sci.*, **243**, 279 (1962).
(17) E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry", 2nd ed, Charles C Thomas, Springfield, Ill., 1961, pp 149–153.
(18) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **12**, 408 (1969).
(19) S. S. Berg and G. Newbery, *J. Chem. Soc.*, 642 (1949).
(20) J. N. Ashley, H. J. Barber, A. J. Ewins, G. Newbery, and A. D. H. Self, *J. Chem. Soc.*, 103 (1942).

Synthesis and Anticancer Activity of 5-Diethylaminomethyl Derivatives and Nitrogen Mustards of Uracil and 2-Thiouracils

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Several 5-diethylaminomethyl derivatives and nitrogen mustards of uracil and 2-thiouracil have been synthesized and tested for their potential anticancer activity in vitro on KB cells and in vivo on Ehrlich carcinoma. Among the alkylating derivatives tested several showed cytotoxic activity in vitro and compound V [5-[bis(2-chloroethyl)amino]methyl-6-propyluracil hydrochloride] showed both in vitro and in vivo anticancer activity.

Nitrogen mustard I has been independently synthesized by three research teams[1-3] as an alkylating agent related to the clinically used uracil mustard.[4] The structural difference between these two substances is the methylene bridge separating the alkylating functional group of compound I from its uracil moiety. Thus, the enamine conjugation present in the uracil mustard is no longer present in compound I and therefore the alkylation mechanisms displayed by these two substances are quite different. Recently, it has been reported that compound I inhibits the mitotic division in HeLa cells by at least two mechanisms: (i) its incorporation into DNA, and (ii) its

capacity to injure mitotic processes.[5,6] These data prompted us to compare the alkylating activity of compound I with that of uracil mustard itself using mechlorethamine as a reference substance.

Following this two alkyl derivatives of compound I were synthesized and tested for their cytotoxicity; they were the N^1-methyl derivative III, which cannot be transformed into the corresponding nucleotide, and the N^1,N^3-dimethyl derivative IV, which cannot form hydrogen bonds with the uracil-specific carriers or enzymes.

Our investigations were also extended to nitrogen mustard VI, considering the important role played by 2-thiouracil in the pyrimidine metabolism.[7,8] Finally, the diethylaminomethyl derivatives VII–IX have been synthesized in order to compare their activity with that of the analogs bearing the alkylating functional group.

Chemistry. Nitrogen mustards I–IV have been prepared following the successful application of the Mannich reaction to uracil and 6-methyluracil with formaldehyde and β,β'-dichlorodiethylamine as reported in the literature.[3] It should be noted that all these reactions did not take place when ethanol was used as a solvent. The suitable reaction solvents are listed in the Experimental Section.

The Mannich bases VII–IX have been obtained by condensing respectively 6-methyluracil, 2-thiouracil, and 6-methyl-2-thiouracil with formaldehyde and diethylamine in ethanol solution according to the procedures of Murakami et al.,[9] Schueler et al.,[10] and Snyder et al.[11]

It should be pointed out that a reaction of 6-methyl-2-thiouracil with diethylamine and formaldehyde in concentrated hydrochloric acid has been described by Monti and Franchi.[12] The structure reported for the isolated product was the same as that we assigned to compound IX but these two substances showed quite different water solubility, uv spectra, and melting points. Since the uv spectra were not identical, we concluded that we were not dealing with two different crystalline forms of a single substance. The structure we assigned to compound IX has been substantiated by nuclear magnetic resonance analysis since it resulted that the proton bound to the carbon atom adjacent to the carbonyl group of 6-methyl-2-thiouracil was substituted by a diethylaminomethyl group. No further evidence in favor of structure IX could be obtained by means of a different synthetic route, starting with the chloromethylation of 6-methyl-2-thiouracil, since an unexpected cyclization occurred during this reaction.[13]

In order to determine the comparative alkylating activities, the method developed by Bardos and co-workers,[14] as modified by Baker and Jordaan,[15] has been applied. According to this procedure, equal volumes of equimolar solutions of a given nitrogen mustard were heated with a large excess of 4-(*p*-nitrobenzyl)pyridine reagent (NBP) for different periods of time. After cooling and the addition of alkali, the intensities of color development were determined spectrophotometrically by measuring the absorbance at a convenient wavelength. The absorbance readings for the different nitrogen mustards were then plotted vs. the corresponding reaction times (Figure 1). The decreases of the alkylating activities produced by hydrolysis were determined by heating equimolar buffered solutions of a given nitrogen mustard for different periods of time. Equal volumes of these solutions were then left to react with NBP for 5 min in order to test the residual alkylating activity, following the previously reported procedure. The pH chosen (4.18) was the most convenient for the kinetic measurements. The resulting absorbance readings were then plotted vs. the hydrolysis time (Figure 2).

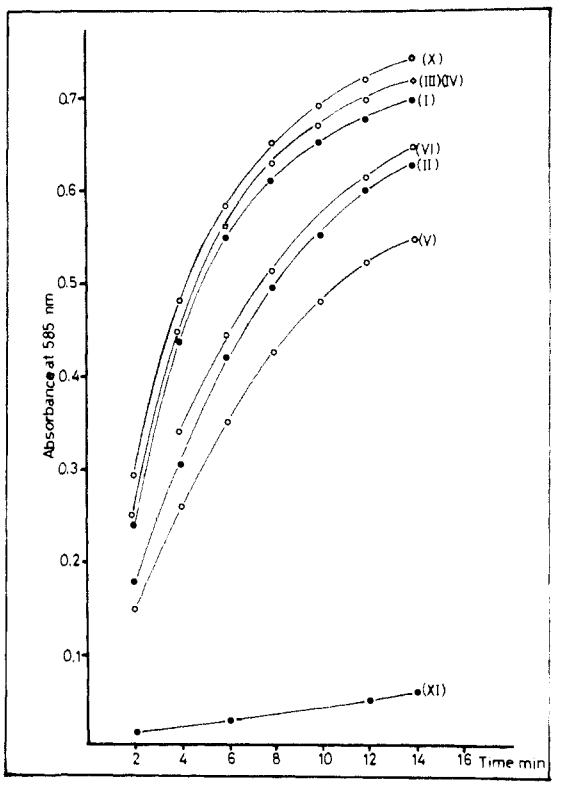


Figure 1. Comparative alkylating activities with NBP at 50° and pH 7.2. Compound X is mechlorethamine and XI is uracil mustard.

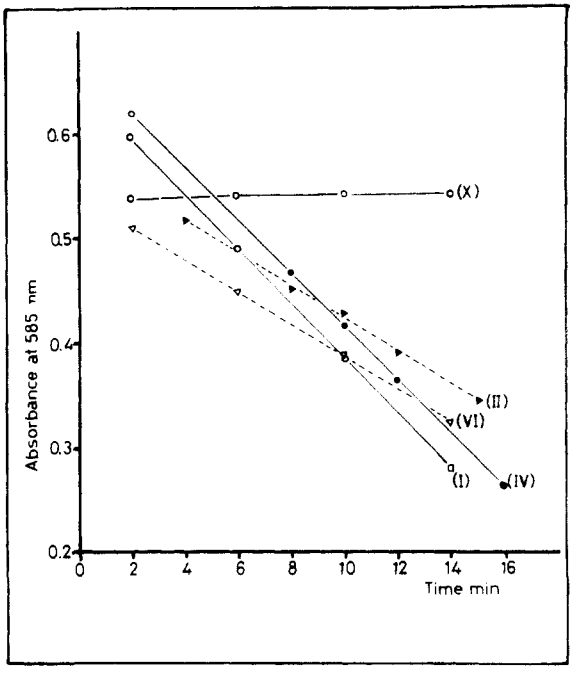
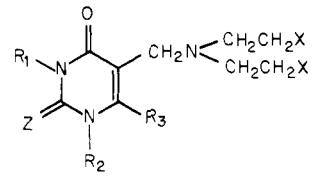


Figure 2. Hydrolytic loss of the alkylating power at 50° and pH 4.18. Compound X is mechlorethamine.

The results concerning the comparative alkylating activities of the studied compound (Figure 1) clearly indicated that, under the chosen experimental conditions, all the nitrogen mustards reported in Table I reacted similarly to mechlorethamine but differently from uracil mustard. The small difference in reactivity observed between compounds II and V probably depended on steric hin-

Table I. List of Compounds Tested



Code no.	R_1	R_2	R_3	Z	X	Formula	Yield, %	Mp, °C	Analyses
I	H	H	H	O	Cl	$C_9H_{13}Cl_2N_3O_2 \cdot HCl$	*a*	228	C, H, N
II	H	H	CH_3	O	Cl	$C_{10}H_{15}Cl_2N_3O_2 \cdot HCl$	*b*	238 dec[c]	C, H, N
III	H	CH_3	H	O	Cl	$C_{10}H_{15}Cl_2N_3O_2 \cdot HCl$	52	220-221	C, H, N
IV	CH_3	CH_3	H	O	Cl	$C_{11}H_{17}Cl_2N_3O_2 \cdot HCl$	60	162-163	C, H, N
V	H	H	$CH_2CH_2CH_3$	O	Cl	$C_{12}H_{19}Cl_2N_3O_2 \cdot HCl$	18.7	188-190 dec	C, H, N
VI	H	H	CH_3	S	Cl	$C_{10}H_{15}Cl_2N_3OS \cdot HCl$	85	238-240	C, H, N
VII	H	H	CH_3	O	H	$C_{10}H_{17}N_3O_2$	*d*	Dec 270[e]	C, H, N
VIII	H	H	H	S	H	$C_9H_{15}N_3OS$	23.5	196	C, H, N
IX	H	H	CH_3	S	H	$C_{10}H_{17}N_3OS$	22	170-171	C, H, N

[a] See ref 1-3. [b] See ref 3. [c] Lit.[3] 204-205° dec. [d] See ref 9. [e] Lit.[9] dec >300°.

drance effects operating during the alkylation process. In the case of the uracil mustard, the interaction with the alkylating functional group determined a reactivity very similar to that found by Bardos and co-workers for aromatic nitrogen mustards.[14]

The hydrolytic reaction between mechlorethamine and the nitrogen mustards tested showed no similarity (Figure 2). At pH 4.18, this substance was very stable while the remaining nitrogen mustards (I, II, IV, and VI) were rapidly hydrolyzed.

The differences observed between mechlorethamine and compound I might be due to their different pK_a values. For mechlorethamine[16] at 0° the pK_a was 6.45 ± 0.2 while at 5° for compound I the pK_a was 5.21 ± 0.03.

Our attempts to determine a simultaneous hydrolytic mechanism peculiar for nitrogen mustards I–IV were unsuccessful. Ultraviolet spectrophotometry limited the choice of experimental conditions, while the poor solubility of these substances did not allow a satisfactory NMR study.

Experimental Section

Melting points were determined in open-glass capillary tubes using a Buchi apparatus and were not corrected. Infrared spectra (as Nujol mulls) were obtained on a Perkin-Elmer Model 225 spectrophotometer. Ultraviolet spectra were obtained on a Hitachi Perkin-Elmer Model 124 spectrophotometer and NMR spectra on a JEOL C60 HL spectrometer, using Me_4Si as internal standard in Me_2SO-d_6 solution and DSS in D_2O. Eastman chromatogram 6060 sheets were used for thin-layer chromatography and they were developed with ligroine–methyl alcohol–ethyl acetate (2:5:2).

The pK_a value of compound I has been determined by adding different volumes of 0.1 M NaOH (from 0.1 to 0.4 ml) to 20 ml of 0.0025 M water solutions of compound I cooled at 5°. The pH was measured with a pH meter 4d radiometer. The resulting pK_a value was 5.21 ± 0.03.

For the synthesis of compounds III–V, the procedure reported here for compound IV has been followed. Compound III precipitated after cooling, while compound V was recovered evaporating the solvent under reduced pressure. Both were recrystallized from absolute ethanol.

5-[Bis(2-chloroethyl)amino]methyl-1,3-dimethyluracil Hydrochloride (IV). Paraformaldehyde (0.4 g, 4.4 mmol), 1.75 g (8.5 mmol) of 1,3-dimethyluracil, and 2.25 g (12.6 mmol) of β,β'-dichlorodiethylamine hydrochloride in 10 ml of glacial acetic acid were refluxed 3 h at 100° under stirring. The solvent was removed under reduced pressure and the residue washed twice with ether and recrystallized from ethyl acetate.

5-[Bis(2-chloroethyl)amino]methyl-6-methyl-2-thiouracil (VI). Paraformaldehyde (0.8 g, 8.9 mmol), 3.25 g (23 mmol) of 6-methyl-2-thiouracil, and 4.5 g (25 mmol) of β,β'-dichlorodiethylamine in 45 ml of dimethylformamide were heated at 85–90° for 1 h. The precipitate formed after cooling was recrystallized from methyl alcohol.

5-(Diethylamino)methyl-6-methyluracil (VII). Paraformaldehyde (3.3 g, 36 mmol), 12.6 g (65 mmol) of 6-methyluracil, and 7.3 g (0.1 mol) of diethylamine in 500 ml of ethanol were refluxed 3 h. Paraformaldehyde (0.5 g, 5.5 mmol) and 1.0 g (14 mmol) of diethylamine were added and the reaction mixture was refluxed for an additional 5 h, cooled, and filtered. The filtrate was evaporated under reduced pressure and the residue suspended in boiling acetone (60 ml) and filtered. The residue (2.9 g) was recrystallized from acetone.

5-(Diethylamino)methyl-2-thiouracil (VIII). 2-Thiouracil (1.28 g, 0.01 mmol), 0.73 g (0.01 mmol) of diethylamine, 0.9 ml of formalin, and 0.1 ml of glacial acetic acid in 25 ml of ethanol were refluxed 2 h. Diethylamine (0.2 ml, 2.7 mmol) and 0.2 ml of formalin were added and the reaction mixture was refluxed for 3 h and then filtered. The solvent was evaporated and the residue recrystallized from ethanol.

5-(Diethylamino)methyl-6-methyl-2-thiouracil (IX). 6-Methyl-2-thiouracil (3.5 g, 25 mmol), 13 g (0.18 mmol) of diethylamine, 5 ml of formalin, and 0.5 ml of glacial acetic acid in 50 ml of ethanol were refluxed 3 h. The solvent was evaporated under reduced pressure and the residue triturated twice in ether (total volume 200 ml). The resulting yellowish powder was suspended under stirring in 100 ml of methanol and rapidly filtered.

The specimens were allowed to stand for 15–20 h and then the precipitate was collected and washed with methanol–ether on the filter and recrystallized from cold water. After two recrystallizations large crystals were obtained (1 g, 22% yield): mp 170–171° (determined in a Kofler apparatus); uv (MeOH) 274, 285 nm (sh); ir 3125 (NH), 1630, 1495, 1460 cm^{-1} (C=O); NMR (D_2O) 1.40 (6 H, t, CH_3), 2.43 (3 H, s, CH_3), 3.32 (4 H, m, CH_2).

The hydrochloride was obtained by suspending the base in anhydrous ether saturated with hydrogen chloride and recrystallization from acetone–methanol (1:1): mp 208–210°; ir 2570, 1645 and 1455 cm^{-1} (C=O); NMR (D_2O) 1.34 (6 H, t, CH_3), 2.27 (3 H, s, CH_3), 3.21 (4 H, m, CH_2), 4.10 (2 H, s, CH_2); the NMR spectrum was coincident with that of a D_2O solution of the base after addition of a few drops of DCI.

The product, reported by Monti and Franchi,[13] has been prepared and, after several recrystallizations from boiling water, it was found to be homogeneous (TLC): ir 1710, 1650 cm^{-1}; uv (MeOH) 273 and a small shoulder at about 295 nm; the uv spectrum was different from that of an analytical sample of IX.

Determination of Comparative Alkylating Activities. The procedure reported by Bardos and co-workers,[14] as modified by Baker and Jordaan,[15] has been followed. According to this procedure, a 0.2 mM 2-methoxyethanol solution of the substance to be tested was prepared and stored at once for 30 min at 5 °C. Aliquots (1 ml) were pipetted into a series of 10-ml flasks and 1 ml of a 5% w/v solution of 4-(*p*-nitrobenzyl)pyridine (NBP) in 2-methoxyethanol and 1 ml of 0.05 M potassium phthalate buffer (pH 4.18) were added. Blanks were identical but did not

Table II. Anticancer Activity of Compounds Tested

Code no.	EORTC	In vitro: Dose, μg/ml	In vitro: Effect on KB cells,[a] growth index %[b] 16 h	In vitro: Effect on KB cells,[a] growth index %[b] 72 h	In vivo: Dose, mg/kg × 6 days	In vivo: Effect on Ehrlich ascites carcinoma, tumor-free[c] total
I	625	1	119	100	0.5	7/10
		10	60	66	1.0	9/10
		100	1†	−93‡	2.5	9/10
II	627	1	67	32‡	0.5	8/10
		10	63†	9‡	1.0	8/10
		100	−77‡	−91‡	2.5	0/10
III	634	1	69	4‡	0.5	0/10
		10	84	−36‡	1.0	8/10
		100	65	−61‡	2.5	0/10
IV	633	1	58	99	0.5	1/10
		10	27	59‡	1.0	8/10
		100	30	−43‡	2.5	0/10
V	1626	1	110	42†	0.5	10/10
		10	−27‡	−7‡	1.0	9/10
		100	−24‡	−32‡	2.5	9/10
VI	631	1	49	59	0.5	5/10
		10	22	12‡	1.0	8/10
		100	−80‡	−34‡	2.5	3/10

[a] See ref 17 and 18. [b] Growth index % is the percentage of the growth of controls, when negative it represents the percentage of the initial population, independently from the growth of controls. Statistical analysis compares the actual mean with the mean of controls by Student's t test (†, $p < 0.05$; ‡ $p < 0.01$). [c] Tumor-free survivors have been evaluated 80 days after implantation, ip, of 10^5 Ehrlich ascites carcinoma cells [mean survival time of controls 20 (19–22) days].

contain the alkylating agent dissolved in methoxyethanol. The flasks were maintained at 50 °C, removed at timed intervals, and placed on ice for 10 min. Subsequently 0.7 ml of 0.1 M KOH in 80% ethanol was added and the content was immediately diluted to 10 ml with 2-methoxyethanol, while the flasks were being shaken. The absorption was measured at 585 nm; the pH of the reaction mixtures was constant at 7.2.

In order to determine the hydrolytic losses of the alkylating capacity, a 0.2 mM 2-methoxyethanol solution of the alkylating agent was prepared and stored immediately at 5 °C for 30 min. Aliquots of 1 ml were pipetted into a series of 10-ml flasks, and 1 ml of potassium phthalate buffer (pH 4.18) was added. Blanks were prepared in the same manner, without the alkylating agent. The flasks were maintained at 50 °C and removed at timed intervals and placed on ice for 10 min; then 1 ml of the NBP solution was promptly added and the flasks were maintained at 50 °C for 5 min. After cooling for 10 min on ice, the procedure was repeated and the absorptions were measured at 585 nm.

Biological Results and Discussion. The potential anticancer activity of the above-mentioned alkylating compounds has been tested in vitro on the KB cell line, derived from human carcinoma of the pharynx, and in vivo on Ehrlich carcinoma transplanted in mice.

The in vitro test has been carried out according to the standards used for EORTC screening,[17] the only change being that, instead of evaluating the effect after a 48-h treatment, it was evaluated at 16 and 72 h in an attempt to separate the acute cytotoxicity from delayed activities on growth and survival.

In the in vivo test, CD_1 mice, carrying Ehrlich carcinoma in the ascites form (inoculum 10^5 cells ip), were treated daily for 6 days; treatment began on the day of tumor implantation.

The three nonalkylating compounds VII–IX are deprived of any cytotoxic and anticancer activity. Compounds II–VI (Table II) are instead more effective than the reference compounds I after 72-h treatment in the in vitro test. However, among these substances, only compound V appears to be at least as active as compound I on Ehrlich carcinoma in vivo.

Compound V (1 μg/ml) inhibits the growth of KB cells after a 72-h treatment and shows acute cytotoxicity when raised to 10 μg/ml.

It should also be noted that III is never cytotoxic in the acute (16 h) in vitro experiment, whereas after a 72-h treatment it inhibits the grown of KB cells in a concentration of 1 μg/ml and becomes cytotoxic at 10 μg/ml.

This tendency to exhibit a delayed effect can also be observed for compound IV at a concentration of 100 μg/ml.

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References and Notes

(1) L. O. Ross, W. W. Lee, M. G. M. Schelstraete, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 3021 (1961).
(2) J. Frakás and F. Sorm, *Collect. Czech. Chem. Commun.*, **26**, 893 (1961).
(3) R. C. Elderfield and J. R. Wood, *J. Org. Chem.*, **26**, 3042 (1961).
(4) G. P. Ellis and G. B. West, Ed., "Progress in Medicinal Chemistry", Vol. 8, Part I, Butterworth, London, 1971, p 101.
(5) T. Margot, F. Fakan, and Z. Muchnova, *Neoplasma*, **16**, 249 (1969).
(6) J. Slotova and Z. Karpfel, *Biol. Plant*, 11, 49 (1969).
(7) R. H. Lindsay, C. J. Romine, and M. Y. Wong, *Arch. Biochem. Biophys.*, **126**, 812 (1968).
(8) P. T. Cardeilhac, *Proc. Soc. Exp. Biol. Med.*, **125**, 692 (1967).
(9) M. Murakami, Y. Kawashima, N. Ito, and K. Yano, German Offen. 1,802,922 (1969); *Chem. Abstr.*, 71, 61417d (1969).
(10) H. Schueler, H. Grabhoefer, and H. Ulrich, German Offen. 1,163,142 (1964); *Chem. Abstr.*, **60**, 14518e (1964).
(11) H. R. Snyder, H. M. Foster, and G. A. Nussberger, *J. Am. Chem. Soc.*, **76**, 2441 (1954).
(12) L. Monti and G. Franchi, *Gazz. Chim. Ital.*, **79**, 447 (1949).
(13) M. Calligaris, S. Fabrissin, M. de Nardo, and C. Nisi, *J. Org. Chem.*, **36**, 602 (1971).
(14) T. J. Bardos, N. Datta-Gupta, P. Hebborn, and D. J. Triggle, *J. Med. Chem.*, **8**, 167 (1965).
(15) B. R. Baker and J. H.. Jordaan, *J. Heterocycl. Chem.*, **2**, 21 (1965).
(16) B. Cohen, E. R. Van Artsdalen, and J. Harris, *J. Am. Chem. Soc.*, **70**, 282 (1948).
(17) EORTC Screening Group, *Eur. J. Cancer*, 8, 185 (1972).
(18) L. Morasca and A. Leonardi, *Rev. Fr. Etud. Clin. Biol.*, **10**, 759 (1965).