SYNTHESIS, ANTITUMORIGENIC ACTIVITY, AND ELECTROCHEMICAL PROPERTIES OF URACIL DERIVATIVES OF THE FURAN SERIES*

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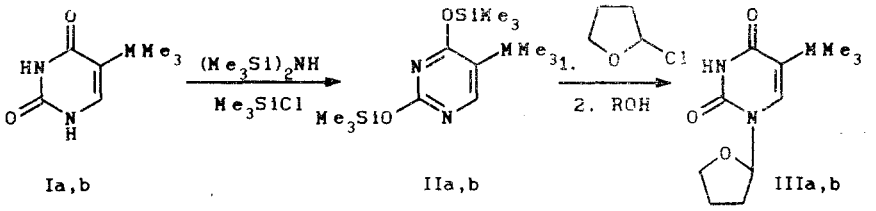
1-(Tetrahydrofuryl) derivatives of 5-timethylgermyl(silyl)uracil and uracil derivatives of 3-(5-nitro-2-furyl)acrylic acid, as well as 1-(γ-triethylgermyl)- and 1-(γ-triethoxysilyl)propylcarbamoyl-5-fluorouracils, were synthesized. Six of the 14 new investigated compounds have high cytotoxic activity in a culture of melanoma B16 cells. 5-Fluorouracil derivatives of 3-(5-nitro-2-furyl)acrylic acid display antitumorigenic activity with respect to lympholeucosis P388 that is comparable to the activity of ftorafur. It was demonstrated by electrochemical studies that the antitumorigenic activity is not determined by the redox properties of the investigated compounds.

The antitumorigenic preparation ftorafur, the active principle of which is 1-(tetrahydro-2-furyl)-5-fluorouracil, is widely known [2]. 1-(Tetrahydrofuryl) derivatives IIIa, b of 5-trimethylgermyl(silyl)uracil, as well as uracil derivatives Va-g and VIa-g of 3-(5-nitro-2-furyl)acrylic acid, were synthesized to study the antitumorigenic properties of other furan derivatives of uracil.

Compounds IIIa, b were synthesized by a method based on the utilization of the silyl method for the synthesis of nucleosides [3] and used to obtain ftorafur [4, 5]. 5-Trimethylgermyl(silyl)uracils Ia, b are silylated with hexamethyldisilazane in the presence of trimethylchlorosilane; the resulting 2,4-bis(trimethylsilyl)-5-trimethylgermyl(silyl)uracils IIa, b are treated with 2-chlorotetrahydrofuran with subsequent isolation of 1-(2-tetrahydrofuryl)-5-trimethylgermyl(silyl)uracils IIIa, b by the action of ethanol on the reaction mixtures:



I-III a M = Ge, b M = Si

Uracil derivatives Va-g and VIf of 3-(5-nitro-2-furyl)acrylic acid were synthesized by the reaction of 2,4-bis(trimethylsilyl)-5-substituted uracils with the chloride of this acid in acetonitrile.

*The preliminary results of this research were presented at the 5th International Symposium on the Chemistry of Furan (Riga) [1].

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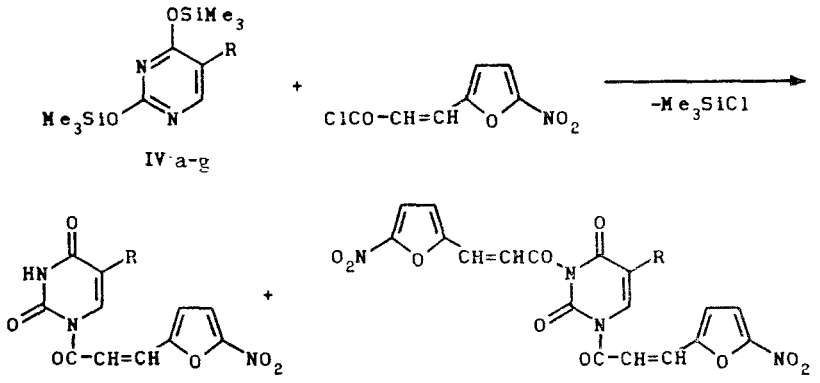
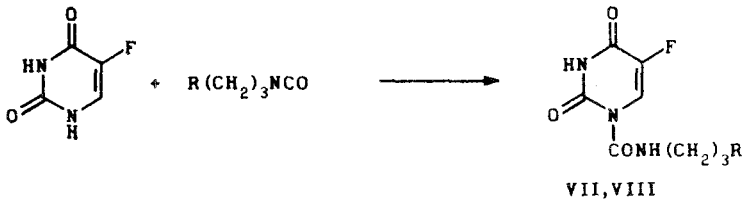


IV a-g

V a-g VI a-g

IV—VI a R=H, b R=CH_3, c R=$GeMe_3$, d R=$SiMe_3$, e R=Br, f R=F, g R=NO_2

Both 1-monosubstituted (Va-g) and 1,3-bis(substituted) (VIa-g) derivatives of uracil are formed in the reaction. The formation of the latter is more characteristic for 5-halo-substituted uracils. Considering the antitumorigenic activity of 1-hexylcarbamoyl-5-fluorouracil [6], two germanium- and silicon-containing carbamoyl derivatives of 5-fluorouracil were synthesized. The reaction of 5-fluorouracil with 3-isocyanatopropyltriethylgermane or 3-isocyanatopropyltriethoxysilane in DMF gave, respectively, 1-(γ-triethylgermyl)propylcarbamoyl-5-fluorouracil (VII) and 1-(γ-triethoxysilyl)propylcarbamoyl-5-fluorouracil (VIII):



VII,VIII

VII R = Et_3Ge; VIII R = $(EtO)_3Si$

The antitumorigenic properties of the compounds were studied in a culture of melanoma B16 cells and with respect to two ascitic forms of tumors — lympholeucosis P388 and Lewis lung carcinoma (LLC). Ftorafur was used as a standard preparation (see Table 1).

The cytotoxic activity of the substances was determined in a 4-day test without washing out of the tumor cells. Under such conditions some 1-substituted derivatives of fluoropyrimidines are capable of undergoing partial chemical or enzymatic hydrolysis with the formation of cytotoxic products.

Of the 14 investigated compounds, nine display high/moderate cytotoxic activity; with the exception of ftorafur, all of the fluorine-containing compounds are highly toxic agents with $EC_{50} \leq 3.2$ μg/ml.

The cytotoxic activity of the compounds did not correlated with their toxicity in mice. The maximally tolerable doses of all of the compounds, including the group of highly cytotoxic compounds, range from 100 to 1000 mg/kg.

The antitumorigenic activity of the compounds was studied for two regimens of intraperitoneal introduction in P388 and LLC tumors, which differ substantially with respect to their growth kinetics and sensitivity to antitumorigenic preparations.

Antitumorigenic activity was detected only for fluorine-containing compounds that displayed high cytotoxicity, viz., Vf and VIf. With respect to antitumorigenic activity in lympholeucosis P388 they are comparable to ftorafur — T/C* 125-139%. In contrast to ftorafur, Vf and VIf have a broad spectrum of antitumorigenic action and display activity in the ascitic form of Lewis lung carcinoma.

*T pertains to the test group, and C pertains to the control group.

TABLE 1. Cytotoxicity and Antitumorigenic Activity of 5-Substituted Uracil Derivatives

Compound	EC_{50}, μg/ml	Antitumorigenic activity				Compound	EC_{50}, μg/ml	Antitumorigenic activity			
		P388		LLC				P388		LLC	
		OD/MTD, mg/kg*	T/C, %	Dose, mg/kg	T/C %			OD/MTD, mg/kg*	T/C, %	Dose, mg/kg	T/C, %
Ia	>32	320	100	180	95	Ve	10	1000	103	180	95
Ib	>32	320	100	100	106	Vf	1,0	100	131	32	128
IIIa	>32	320	100	180	114	Vg	10	100	88	32	95
IIIb	>32	100	100	32	105	VIf	1,0	1000	125	320	135
Va	1,0	320	85	180	95	VII	1,0	100	108	56	105
Vb	1,0	320	100	180	95	VIII	1,0	100	108	56	105
Vc	10	320	88	180	95	Ftorafur	10	320	139		
Vd	>32	320	83	100	95						

*OD is the optimal dose, and MTD is the maximally tolerable dose.

TABLE 2. Polarographic Half-Wave Potentials ($E_{1/2}$) and Limiting Currents (i) for Va-g and VIf in DMF with 0.1 M $(C_4H_9)_4NPF_6$ As the Inert Electrolyte

Compound	$E'_{1/2}$, V	$E''_{1/2}$, V	$E'''_{1/2}$, V	$i'_{1/2}$, μA	$i''_{1/2}$, μA	$i'''_{1/2}$, μA
Va	−0,63	−0,78	−1,11	0,94	0,74	0,61
Vb	−0,63	−0,81	−1,10	0,91	0,81	0,64
Vc	−0,65	−0,80	−1,09	0,57	0,40	0,42
Vd	−0,64	−0,83	−1,12	0,70	0,77	0,47
Ve	−0,56	−0,72	−1,06	0,82	0,96	0,68
Vf	−0,61	−0,76	−1,04	0,60	0,80	0,55
Vg	−0,49	−0,71	−1,03	0,67	0,80	0,55
VIf	−0,57	−0,76	−1,00	1,05	1,03	0,55

It is known that the biological activity of nitrofuran preparations correlates to a certain extent with the potential of the first wave of the electrochemical reduction of these compounds [7]. Since the compounds presented in our research also have biological activity, we carried out a polarographic study of nitrofuryl derivatives Va-g and VIf in DMF to determine the electrochemical-reduction potentials and used cyclic voltammetry to establish the reversibility of the individual steps of the reduction. The mechanism of the electrochemical reduction of the investigated compounds proved to be extremely complex, and the data obtained did not have an unambiguous interpretation and required additional study of model compounds with nitrofuran and uracil structures. In this connection, in the present paper we will present only the preliminary experimental results and some conclusions that follows from their analysis. In the electrochemical process the investigated Va-g and VIf begin to undergo reduction at relatively low potentials (Table 2); this is characteristic for 5-nitrofuran derivatives that contain a substituent with strong electron-acceptor properties, such as a CHO [$E_{1/2} = -0.63$ V relative to a saturated calomel electrode (SCE) [8]] or an NO_2 group ($E_{1/2} = -0.40$ V relative to an SCE [8]). In addition to peaks corresponding to polarographic waves, additional peaks and redox systems appear on the cyclic volt-ampere curves recorded for Va-g and VIf over various ranges of potentials. The existence of reversible redox systems indicates the possibility of the formation of free ion radicals as reaction intermediates. In connection with the high degree of conjugation of the bonds in the molecule, any of the reaction centers present in the molecule, viz., CO, NH, C=C, and NO_2, may be affected vis-à-vis energic favorability. Proceeding from a comparison of the ease of reduction of these reaction centers, it may be assumed that the nitro group in the furan ring is reduced in the initial process, and, in analogy with previously investigated compounds of the 5-nitrofuran series [8], anion radicals of the starting compounds are formed in the first act of the electrochemical reduction of Va-g and VIf. However, the irreversibility of the first step of the reduction of the investigated compounds provides evidence that the products of initial reduction undergo fast chemical transformations. The second wave of the electrochemical reduction of these compounds also proved to be irreversible, and only the third step of the electrochemical reduction is reversible.

TABLE 3. Hyperfine Structure (hfs) Constants of the EPR Spectra of the Ion Radicals Formed in the Electrochemical Generation (ECG) of Va-g and VIf in DMF

Compound	ECG potential, V	hfs constants, Oe				
		$a_N^{NO_2}$	a_H^4	a_H^3	a_H^α	a_H^β
Va	−1,1	5,6	3,6	1,0	—	3,6
	−1,4	7,0	4,3	1,2	0,8	2,6
Vb	−1,1	5,7	3,7	1,2	—	3,7
	−2,2	6,9	4,3	1,2	0,9	2,7
Vc	−1,1	hfs not decoded				
	−1,2	7,5	4,4	1,2	0,9	2,7
Vd	−1,1	6,0	3,8	1,1	—	3,8
	−1,4	7,0	4,3	1,2	0,8	2,7
Ve	−1,1	hfs not decoded				
	−2,4	7,1	4,3	1,2	0,8	2,6
Vf	−1,1	5,2	3,7	1,1	0,5	3,7
	−2,0	7,1	4,4	1,2	0,9	2,7
Vg	−1,1*	4,4	2,9	0,7	...	2,9
	−2,1	6,9	4,3	1,2	0,8	2,7
VIf	−1,5	5,3	3,6	1,0	0,5	3,6
	−2,6	7,0	4,3	1,2	0,8	2,7

*The EPR spectrum contained additional lines from another radical with a 5-nitrofurylvinylene structure.

We carried out the electrochemical generation of the radicals in the resonator of the EPR spectrometer [9] for Va-g and VIf and obtained the hyperfine structure (hfs) constants of the corresponding EPR spectra (Table 2). Two types of free-radical particles were primarily detected; however, the potentials at which the radicals develop and the electrochemical characteristics constitute evidence that neither of them is a primary anion radical of the starting compounds. The first radicals were recorded at the potential of the third polarographic wave. It follows from a comparison with the previously studied EPR spectra of the anion radicals of 5-nitro-2-furylacrolein derivatives [10] that in our case, although the starting molecules did undergo electrochemical transformations, the free radicals are anion radicals of 5-nitrofuran derivatives with retention of the vinylene grouping. Consequently, the first steps of the electrochemical reduction affect the substituent in the 2 position of the 5-nitrofuran ring, i.e., the uracil fragment of the molecule rather than the nitro group itself in the furan ring. For all of the investigated compounds at more negative potentials of electrochemical generation we recorded EPR spectra of another free radical. Analysis of the hfs provides evidence that, with respect to its structure, this radical also corresponds to a substituted nitrofuran with retention of the vinylene grouping in the 2 position of the 5-nitrofuran ring (Table 2). From the data on the increase in the hfs constants due to the presence of the nitrogen atom of a nitro group and a proton in the 4 position of the furan ring it may be asserted that in this radical, as compared with the preceding radical, the electrophilic properties of the substituent in the 2 position are decreased, i.e., subsequent reduction of the uracil ring rather than of the nitro group occurred. The distribution of the density of the unpaired electron in the first radical is extremely sensitive to a change in the electron-acceptor properties of substituent R in the uracil fragment, whereas in the case of the second radical it is virtually independent of substituent R. On the basis of this it may be assumed that in the secondary radicals either the degree of conjugation between substituent R and the nitrofuran ring decreased or bond cleavage occurred.

Since the initial reduction of Va-g and VIf does not involve the reduction of the nitro group in the furan ring, the biological activity of these compounds is probably not determined by their redox properties, and Vf and VIf, which displayed antitumorigenic activity, therefore do not differ from the remaining compounds with respect to their electrochemical properties.

EXPERIMENTAL

The PMR and ^{13}C and ^{29}Si NMR spectra of solutions of the compounds in d_6-DMSO were recorded with a WH-90 spectrometer. The progress of the reactions and the purity of the products were monitored by TLC on Silufol UV-254 plates in acetone—chloroform systems (4:5, 1:4, or 1:9) with detection by UV light. The electrochemical studies were carried out with a PAR-170 polarograph in anhydrous DMF with 100 mmole·liter^{-1} $(C_4H_9)_4NPF_6$ as the inert electrolyte. The free

radicals were generated under steady-state conditions of electrochemical reduction on a platinum electrode in a cell located in the resonator of a Carl Zeiss ER-9 spectrometer (Jena) by the method in [9].

The results of elementary analysis for C, H, and N were in agreement with the calculated values.

5-Trimethylsilyluracil (Ib) was synthesized by the method described in [1] and had mp 308-309°C [mp 305°C (from water) [11]].

1-(Tetrahydro-2-furyl)-5-trimethylsilyluracil (IIIb). A 1.3-g (12 mmole) sample of 2-chlorotetrahydrofuran in 5 ml of acetonitrile was added slowly with stirring at 0-5°C to a solution of 3.2 g (0.01 mole) of 2,4,5-tris(trimethylsilyl)uracil in 15 ml of dry acetonitrile, after which the mixture was stirred for 2 h at 15-20°C and for 30 min at 50-60°C. It was then cooled and evaporated, and the oily residue was treated with ethanol and ether. Two recrystallizations from 50% aqueous ethanol gave 1.4 g (55%) of a substance with mp 162-164°C. PMR spectrum: 0.21 (s, $SiMe_3$), 2.05 [m, $C(CH_2)_2C$], 4.01 (m, OCH_2), 5.96 (m, OCH), 7.21 (s, C=CH), 11.14 ppm (s, NH). ^{13}C NMR spectrum: −0.70 ($SiMe_3$); 25.13, 32.64 [$C(CH_2)C$]; 70.43 (OCH_2); 87.85 (OCH); 109.78 (Si—C=); 145.14 (=CH); 151.83, 166.85 ppm (C=O). ^{29}Si NMR spectrum: −5.5 ppm.

5-Trimethylgermyluracil (Ia) was obtained by the method in [12] and had mp 245-247°C.

1-(Tetrahydro-2-furyl)-5-trimethylgermyluracil (IIIa). A 1.2-g (5 mmole) sample of 5-trimethylgermyluracil was heated with a mixture of 1.5 ml of hexamethyldisilazane and 0.15 ml of trimethylchlorosilane at 140-150°C for 4 h. The resulting 2,4-bis(trimethylsilyl)-5-trimethylgermyluracil was dissolved in 10 ml of dry methylene chloride, a solution of 0.58 g (6 mmole) of 2-chlorotetrahydrofuran in 5 ml of methylene chloride was added with stirring at −10°C to −15°C, and the mixture was stirred at 15-20°C for 2 h. Compound IIIa [1.2 g (41%)] was isolated in the same way as IIIb in the form of a colorless crystalline powder with mp 155-157°C. PMR spectrum: 0.34 (s, $GeMe_3$), 1.92-2.22 [m, $C(CH_2)_2C$], 3.80-4.04 (m, OCH_2), 5.81 (m, OCH), 7.01 (s, C=CH), 7.2 ppm (s, NH).

Silylation of the Uracil Derivatives. A 100-mmole sample of the corresponding uracil derivative was heated with a mixture of 30 ml of hexamethyldisilazane and 3 ml of trimethylchlorosilane at 140-150°C for 4-5 h, after which the excess amounts of the silylating agents were removed by distillation, and the residue was fractionally distilled in vacuo. This procedure gave IVa [bp 90°C (1 mm)], IVb [bp 92°C (1 mm)], IVc [110-112°C (2-3 mm)], IVd [105-109°C (1-2 mm)], IVe [bp 125-130°C (1-2 mm)], IVf [112°C (12-13 mm)], and IVg [bp 125-130°C (1-2 mm)].

1,3-Bis[3-(5-nitro-2-furyl)acrylyl]-5-fluorouracil (VIf, $C_{18}H_9FN_4O_{10}$). A solution of 5.4 g (20 mmole) of 2,4-bis(trimethylsilyl)-5-fluorouracil and 8.0 g (40 mmole) of 3-(5-nitro-2-furyl)acrylic acid chloride in 30 ml of dry CH_3CN was maintained at 15-20°C for 24 h, after which the precipitate was removed by filtration, washed with CH_3CN, and dried to give 8.1 g (90%) of Vf with mp 212-214°C (dec.) and R_f 0.89 [acetone—chloroform (1:4)]. PMR spectrum: 7.16, 8.00 (d, CH=CH); 7.35, 7.75 (d, CH=CH, furyl); 7.44, 7.49 (d, CH=CH, furyl); 7.58, 7.74 (d, CH=CH); 8.61 ppm (s, CH, uracil).

1-[3-(5-Nitro-2-furyl)acrylyl]uracil (Va, $C_{11}H_7N_3O_6$). A 4.0-g (20 mmole) sample of 3-(5-nitro-2-furyl)acrylic acid chloride in 20 ml of CH_3CN was added slowly at 20-25°C to a solution of 5.0 g (20 mmole) of 2,4-bis(trimethylsilyl)uracil in 10 ml of dry CH_3CN, during which a precipitate formed. The mixture was then stirred for 2 h, after which the precipitate was removed by filtration, washed with CH_3CN, and dried to give 5.0 g (91%) of Va with mp 256-258°C (dec., from 1,4-dioxane) and R_f [acetone—chloroform (1:4)]. PMR spectrum: 5.82, 8.04 (d, CH=CH, uracil); 7.26, 7.37 (d, CH=CH, furyl); 7.57 (s, CH=CH); 11.62 ppm (s, NH).

1-[3-(5-Nitro-2-furyl)acrylyl]-5-methyluracil (Vb, $C_{12}H_9N_3O_6$) was obtained in the same way as Va from 5.4 g (20 mmole) of 2,4-bis(trimethylsilyl)-5-methyluracil and 4.0 g (20 mmole) of 3-(5-nitro-2-furyl)acrylic acid chloride. Workup gave 3.2 g (55%) of a product with mp 230-232°C (dec., from 1,4-dioxane) and R_f 0.78 [acetone—chloroform (1:4)]. PMR spectrum: 1.85 (s, CH_3); 7.29, 7.76 (d, CH=CH, furyl); 7.61 (s, CH=CH); 7.99 (q, CH, uracil); 11.72 (s, NH).

1-[3-(5-Nitro-2-furyl)acrylyl]-5-trimethylgermyluracil (Vc, $C_{14}H_{15}GeN_3O_6$). A mixture of 1.1 g (5 mmole) of 5-trimethylgermyluracil, 1.5 ml of hexamethyldisilazane, and 0.15 ml of trimethylchlorosilane was heated at 140-150°C for 4 h, after which it was cooled and treated with 10 ml of dry CH_2Cl_2, and 1.0 g (5 mmole) of 3-(5-nitro-2-furyl)acrylic acid chloride in 5 ml of CH_2Cl_2 was added at 0°C to −5°C. The mixture was maintained at 20-25°C for 24 h, and the resulting precipitate (0.2 g) was removed by filtration. Evaporation of the solvent and two recrystallizations of the residue from acetone—water (2:1) gave 0.8 g (41%) of Vc with mp 200-202°C (dec.) and R_f 0.91 [acetone—chloroform (1:4)]. PMR spectrum: 0.32 (s, $GeMe_3$); 7.26, 7.69 (d, CH=CH, furyl); 7.55 (s, CH=CH); 7.71 (s, CH, uracil); 11.49 ppm (s, NH).

1-[3-(5-Nitro-2-furyl)acrylyl]-5-trimethylsilyluracil (Vd, $C_{14}H_{15}N_3O_6Si$). A 0.5-g (2.5 mmole) sample of 3-(5-nitro-2-furyl)acrylic acid chloride in 10 ml of $CHCl_3$ was added at 0-5°C to a solution of 0.8 g (2.5 mmole) of 2,4,5

-tris(trimethylsilyl)uracil in 10 ml of dry $CHCl_3$, after which the mixture was stirred at 20-25°C for 3 h and filtered. The product was isolated in the same way as Vc to give 0.6 g (69%) of Vd with mp 227-229°C (dec.) and R_f [acetone—chloroform (1:9)]. PMR spectrum: 0.19 (s, $SiMe_3$); 7.32, 7.74 (d, CH=CH, furyl); 7.57 (s, CH=CH); 7.79 (s, uracil CH); 11.30 ppm (s, NH).

1-[3-(5-Nitro-2-furyl)acrylyl]-5-bromouracil (Ve, $C_{11}H_6BrN_3O_6$). A 2.0-g (10 mmole) sample of 3-(5-nitro-2-furyl)acrylic acid chloride in 20 ml of CH_3CN was added at 20-25°C to a solution of 3.3 g (10 mmole) of 2,4-bis(trimethylsilyl)-5-bromouracil in 10 ml of dry CH_3CN, and the mixture was maintained at this temperature for 4 h. The precipitate was removed by filtration and recrystallized from 1,4-dioxane to give 1.9 g (53%) of Ve with mp 234-236°C (dec.) and R_f 0.69 [acetone—chloroform (1:4)]. PMR spectrum: 7.28, 7.78 (d, CH=CH, furyl); 7.60, 7.64 (d, CH=CH); 8.37 (s, uracil CH); 12.24 ppm (s, NH).

1-[3-(5-Nitro-2-furyl)acrylyl]-5-fluorouracil (Vf, $C_{11}H_6FN_3O_6$) was obtained in the same way as Ve from 6.7 g (25 mmole) of 2,4-bis(trimethylsilyl)-5-fluorouracil and 5.0 g (25 mmole) of 3-(5-nitro-2-furyl)acrylic acid chloride and had mp 209-211°C (dec.) and R_f 0.75 [acetone—chloroform (1:4)]. PMR spectrum: 7.30, 7.82 (d, CH=CH, furyl); 7.69 (s, CH=CH); 8.28 (d, uracil CH); 12.10 ppm (s, NH). The yield was 2.5 g (34%).

1-[3-(5-Nitro-2-furyl)acrylyl]-5-nitrouracil (Vg, $C_{11}H_6N_4O_8$) was obtained in the same way as Ve from 1.5 g (5 mmole) of 2,4-bis(trimethylsilyl)-5-nitrouracil and had mp 128-130°C (from 1,4-dioxane) and R_f [acetone—chloroform (1:4)]. PMR spectrum: 7.04, 7.73 (d, CH=CH); 7.38, 7.73 (d, CH=CH, furyl); 9.23 ppm (s, uracil CH). The yield was 0.9 g (56%).

1-(γ-Triethylgermyl)propylcarbamoyl-5-fluorouracil (VII, $C_{14}H_{24}FGeN_3$). A solution of 1.3 g (10 mmole) of 5-fluorouracil and 3.0 g (12 mmole) of 3-isocyanatopropyltriethylgermane in 20 ml of DMF was heated for 4 h at 110-120°C, after which the solvent was removed by distillation. Chloroform (20 ml) was added to the residue, and the resulting precipitate of unchanged 5-fluorouracil (1.0 g) was removed by filtration and washed with chloroform. The solvent was removed by distillation, petroleum ether was added to the oily residue, and the precipitated VII was removed by filtration, washed, and air dried to give a product with mp 127-128°C and R_f 0.85 [acetone—chloroform (4:5)]. PMR spectrum: 0.61, 0.65 (t, $GeCH_2$); 0.90 (q, CH_3); 1.48 (m, CCH_2C); 3.19 (m, NCH_2); 8.32 (d, CH); 9.11 (t, $NHCH_2$); 12.20 ppm (s, NH). The yield was 0.7 g (19%).

1-(γ-Triethoxysilyl)propylcarbamoyl-5-fluorouracil (VIII, $C_{14}H_{24}FN_3O_6Si$). A solution of 2.6 g (20 mmole) of 5-fluorouracil and 4.9 g (20 mmole) of 3-isocyanatopropyltriethoxysilane in 25 ml of dry DMF was heated at 90-100°C for 2 h, after which the solvent was removed by distillation. Chloroform (30 ml) was added to the oily residue, and unchanged 5-fluorouracil (1.7 g) precipitated. The chloroform was evaporated, petroleum ether was added to the residue, and the resulting precipitate was removed by filtration and air dried to give 2.8 g (74%) of VIII with mp 102-104°C and R_f 0.70 [acetone—chloroform (1:4)]. PMR spectrum: 0.52 (t, $SiCH_2$), 1.14 (t, CH_3), 1.58 (m, CCH_2C), 3.30 (m, NCH_2), 3.71 (q, OCH_2), 8.33 (d, CH), 9.12 (t, $NHCH_2$), 12.25 ppm (s, NH).

Preparation of the melanoma B16 cell cultures was carried out at 37°C in an atmosphere of 10% CO_2 in the Dulbeko complete medium (DCM) containing 25 mM HEPES buffer, 10% fetal serum, and a standard mixture of streptomycin, penicillin, and Fungizone (Gibso). The first 10 passages of B16 cells obtained from the tumorous material of the melanoma strain were used in the cytotoxic test [13].

The cytotoxic testing was carried out in 96-cell flat-bottomed plates (Linbro) in 300 μl of the DCM. A total of $5 \cdot 10^3$ to $10 \cdot 10^3$ B16 cells were sown in the plate cells, and the next day the compounds were introduced in tenfold dilutions over the concentration range 0.32-32 μg/ml. After 4 days, the number of cells in the plate cells was determined with respect to the DNA by a microfluorimetric method by means of the Choest N 33342 dye (Calbiochem).

The B16 cells were fixed in 70% ethanol and lysized in 0.5 M NaOH at 55°C for 1 h. The cell lysate was neutralized with 0.6 M KH_2PO_4 containing 2 M NaCl in the presence of the Choest dye in a final concentration of 0.1 μg/ml. The excitation and emission wavelengths of 360 and 460 nm, respectively, were used in the fluorimetry.

The cytotoxicity of the compounds was evaluated from the effectiveness of the concentration (in micrograms per milliliter) that suppresses the cell growth by 50% (EC_{50}). The calculation and analysis with respect to the static (0.251 log) and functional (1.0 log) ranks of cytotoxicity on a logarithmic scale of the concentration were carried out by the method of linear regression with an Apple IIe computer. The functional thresholds of high and moderate cytotoxicity were 3.2 and 32 μg/ml, respectively.

The antitumorigenic activity of the compounds was studied with respect to lympholeucosis P388 and Lewis lung carcinoma (LLC) in mice.

Lympholeucosis P388 was implanted intraperitoneally in C57BL/6×DBA/2 male mice (with masses of 18-20 g) in the amount of 10^6 cells per mouse. The compounds were introduced intraperitoneally on the second and ninth days at doses that increased by 0.5 log on the logarithmic scale until toxicity developed.

Lewis lung carcinoma was implanted intraperitoneally in C57BL/6×DBA/2 male hybrids (with masses of 18-22 g) in the amount of 10^6 cells. The compounds were introduced on the first to third and eighth to tenth days at doses amounting to 0.32-0.56 of the optimal doses in the case of active substances or of the maximally tolerable doses in the absence of activity, as determined with respect to lympholeucosis P388.

The antitumorigenic activity was evaluated from the life span of the mice in test groups (T) as compared with control groups (C) and was expressed in percent (T/C, %). The number of mice in the test groups was three for P388 and five for LLC, which made it possible to determine the reliable effect ($P < 0.05$) of the toxicity and antitumorigenic activity at 80% and 120% levels, respectively. The functional thresholds of moderate and high antitumorigenic activity were 150% and 200%, respectively.

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