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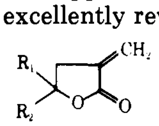
Communications to the Editor

New α-Methylene γ-Lactone Derivatives of Substituted Nucleic Acid Bases as Potential Anticancer Agents

Sir:

It has been recognized[1,2] in the past few years that a number of sesquiterpene lactones and other derivatives obtained from natural sources bearing α-methylene-γ-butyrolactone and related moieties have exhibited interesting biological activity and significant antitumor activity. It soon was characterized that the O=C—C=CH_2 moiety itself, present in vernolepin,[2] elephantopin,[2] etc. is responsible for biological activity. The cytotoxic activity of α-methylene γ-lactones has been attributed to their ability of acting as alkylating agents by a Michael-type reaction with biological cellular nucleophiles such as L-cysteine, glutathione, or thiol-rich or sulfhydryl-containing enzymes such as phosphofructokinase, glycogen synthetase, and DNA polymerase.[3] A large number of possible drug candidates bearing this functionality of the general structure 1 has been synthesized[3-5] with a view to developing effective clinical drugs since naturally found derivatives have therapeutic indices that prelude their clinical use. Several new synthetic approaches to the development of such a moiety are excellently reviewed.[6,7]



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As a part of our anticancer drug development program,[8-10] we were particularly interested in synthesizing suitably substituted nucleic acid bases bearing this moiety. An extensive literature survey revealed that relatively scanty literature references are known except for uracil and thymine α-methylene γ-lactones[3] bearing exocyclic double bonds and adeninyl- and uracilylfuranones in which the double bond in the lactone ring is endocyclic or fully substituted.[11]

Chemistry

For the preparation of purinyl **2a** and **3a** and pyrimidinyl lactone derivatives **4a**, **5a**, **6a**, and **7a**, the efficient route described by Öhler et al.[12] has been employed, which involves treatment of ethyl α-(bromomethyl)acrylate with zinc to form an organozinc intermediate that undergoes a Reformatsky-type reaction with the respective ketones **2–7** (Scheme I). The respective yields of the lactones were fairly good. 6-Chloropurine was alkylated with bromoacetone in the presence of K_2CO_3 to afford the 9-acetonyl derivative **2** in a fairly good yield. It was reported[13] that, when 6-chloropurine was directly alkylated with simple alkyl halides, both 9- and 7-alkyl isomers were obtained, with the 9-derivative as the predominant, if not exclusive, product, and that the relative percentage of the isomers depends on the solvent, nature of the alkyl halide employed, and temperature. However, we could not isolate the isomeric 7-acetonyl derivative and analogous 7-pentanonyl derivative even after extensive column chromatography and purification. It has been found that, under similar reaction conditions as described for the preparation of **2**, a much lower yield of **3** was obtained from 6-chloropurine when it was subjected to reaction with 1-chloro-3-pentanone. This may be attributed to the fact that the latter halide is less reactive than bromoacetone. However, by carrying out the reaction in the presence of the stronger base NaH, **3** was obtained in a comparable yield. For the preparation of **2b** and **2c** from **2a**, a standard literature procedure[14] was adopted. By heating with dilute HCl solution, **2a** gave **2b**, which was also obtained in very low yield during the workup of crude **2a** from **2**. This may be explained by the partial hydrolysis of the labile 6-chloro group during the breaking up of the zinc complex with dilute HCl. By heating equimolar portions of **2a** and thiourea in 1-propanol, compound **2c** was obtained. In an analogous manner, compounds **3b** and **3c** were obtained from **3a**.

Uracil was directly alkylated by 1-chloro-3-pentanone in the presence of K_2CO_3 to furnish **4**, which was converted to **4a**. It has been reported[15] that uracil was alkylated at the N-1 position. It was also reported[15] by Baker et al. that

(1) Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. *J. Med. Chem.* 1971, *14*, 1147 and references cited therein.
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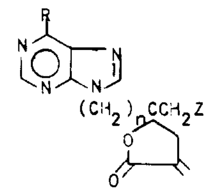
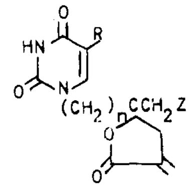
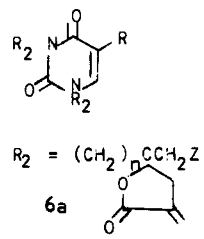
Table I. Physicochemical Data of Lactone Derivatives



2a-c, 3a -c



4a,5a,7a

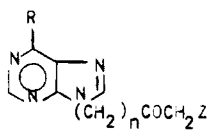


6a

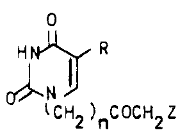
no.	R	*n*	Z	yield, %	mp, °C	crystn solvent[a]	formula
2a	Cl	1	H	61	150–152	C–H	$C_{12}H_{11}ClN_4O_2$
2b	OH	1	H	31[b]	285–288	C–D	$C_{12}H_{12}N_4O_3$
2c	SH	1	H	38[b]	248–252		$C_{12}H_{12}N_4O_2S$
3a	Cl	2	CH_3	64	105–107	E–H	$C_{14}H_{15}ClN_4O_2$
3b	OH	2	CH_3	31[c]	238–240	C–D	$C_{14}H_{16}N_4O_3$
3c	SH	2	CH_3	35[c]	224–228		$C_{14}H_{16}N_4O_2S$
4a	H	2	CH_3	56	147–152	E–H	$C_{13}H_{16}N_2O_4$
5a	F	2	CH_3	59	128–130	C–H	$C_{13}H_{15}FN_2O_4$
6a	F	2	CH_3	54	glassy		$C_{22}H_{27}FN_2O_6$
7a	CH_3	2	CH_3	60	115–118	E–H	$C_{14}H_{18}N_2O_4$

[a] C = $CHCl_3$, H = *n*-hexane, D = Me_2SO, E = EtOAc. [b] Relative yield from **2** based on **2a**. [c] Relative yield from **3** based on **3a**.

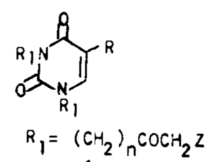
Table II. Physicochemical Data of the Ketones



2,3



4,5,7



6

no.	R	*n*	Z	yield, %	mp, °C	crystn solvent[a]	formula
2	Cl	1	H	61	164–166	C–H	$C_8H_7ClN_4O$
3	Cl	2	CH_3	57	108–110	C–H	$C_{10}H_{11}ClN_4O$
4	H	2	CH_3	52	165–168	B–H	$C_9H_{12}N_2O_3$
5	F	2	CH_3	28	138–140	C–H	$C_9H_{11}FN_2O_3$
6	F	2	CH_3	29	74–75	D	$C_{14}H_{19}FN_2O_4$
7	CH_3	2	CH_3	62	135–136	E	$C_{10}H_{14}N_2O_3$

[a] C = $CHCl_3$, H = *n*-hexane, B = C_6H_6, D = Et_2O, E = EtOAc.

under similar reaction conditions 5-fluorouracil furnished N-3-substituted derivatives. Recently we have shown[16] that, when 5-fluorouracil was directly alkylated with alkylating agents, instead 1-substituted derivatives were obtained. Thus the N-1-substituted derivative **5** along with dialkylated derivative **6** was obtained from 5-fluorouracil. Thymine was alkylated by 1-chloro-3-pentanone in an analogous procedure described for the alkylation of uracil to furnish **7**. The respective ketones were converted to lactones as described earlier. The physicochemical data of the lactones and ketones have been described in Tables I and II, respectively.

^{1}H NMR spectral data are helpful for the identification of the lactones. For the exocyclic methylene group of all the lactones, two apparent triplets (J = 3–4 Hz) or multiplets were observed at δ 5.65 ± 0.15 for the proton syn to the carbonyl group and at δ 6.15 ± 0.12 for the proton anti to the carbonyl group. For most of the lactones the C-4 methylene protons of the lactone group were apparent triplet (J = 2–4 Hz) or multiplets centered at δ 2.85 ± 0.05. IR spectral studies of the lactones showed characteristic bands at 1755 ± 5 and 1255 ± 5 cm^{-1}.

Biological Results

Compounds **2a–c**, **3a–c**, **4a**, **5a**, **6a**, and **7a** were screened for their in vivo antitumor activity against Ehrlich ascites carcinoma (EAC) in Swiss mice in two doses, viz., 25 and

Table III. In Vitro Cytotoxicity and In Vivo Antitumor Activity

	in vitro ED_{50},[a] µg/mL		in vivo, EAC		
no.	PS cells	KB cells	dose[b]	av days survival	T/C[c]
2a	0.17	1.50	25	24.0/15.0	160
			12	20.0/15.0	133
2b			25	16.5/15.0	110
			12	16.0/15.0	107
2c	>10	>10	25	18.5/15.0	123
			12	16.0/15.0	107
3a	0.25	2.60	25	25.0/15.5	161
			12	19.5/15.5	126
3b			25	17.0/15.5	109
			12	17.5/15.5	113
3c			25	17.5/15.5	113
			12	18.0/15.5	116
4a	0.35	2.80	25	19.5/15.5	126
			12	20.0/15.5	129
5a			25	19.5/15.5	126
			12	23.0/15.5	148
6a			25	21.0/17.0	123
			12	23.5/17.0	138
7a			25	25.5/15.0	170
			12	24.5/15.0	163
5-FU			25	33.0/15.0	220

[a] ED_{50} is the concentration required to reduce the growth rate of PS or KB cells in culture to half of the control rate. $ED_{50} \leq 4$ µg/mL is considered active. [b] Administered to EAC bearing Swiss mice once daily on days 1–7 after inoculation of the animals with tumor cells on day 0; groups of six animals per dose level were used with one control group for every six groups. [c] Testing evaluated by calculating median survival times (MST) of the treated (T) and control (C) groups of mice. T/C ≥ 125 is considered active.

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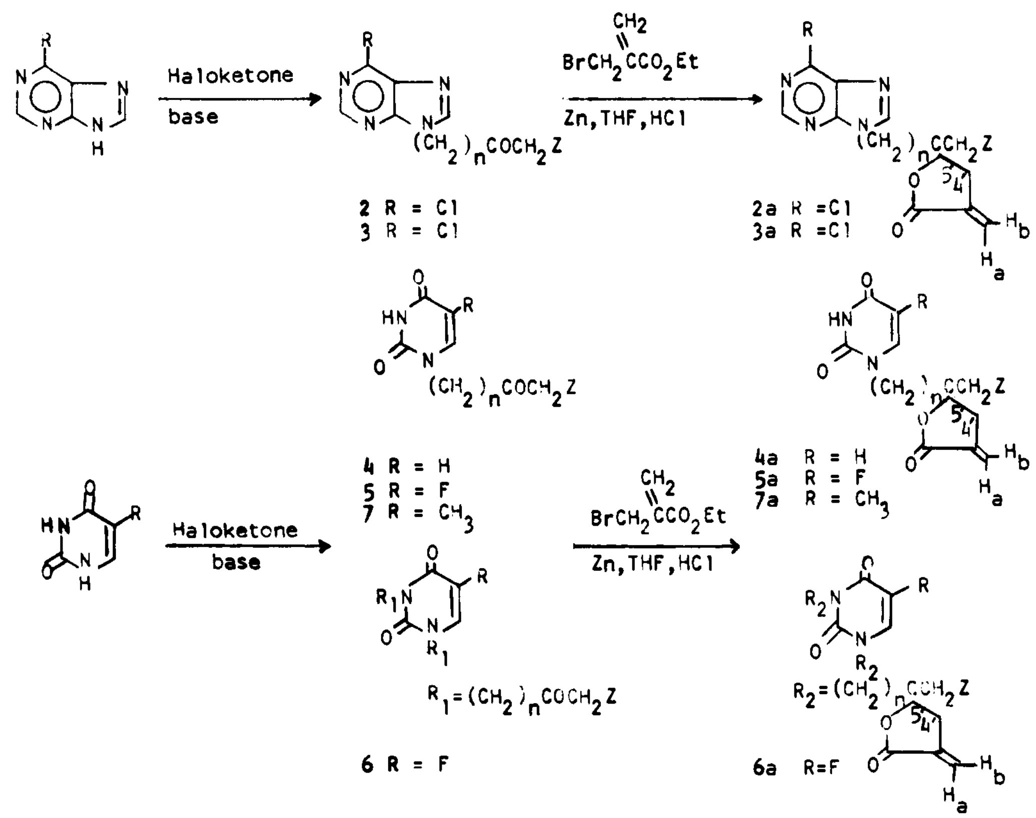
Scheme I



12 mg/kg (Table III). The most interesting compounds showing good anticancer activity are **2a**, **3a**, and **7a** while **4a**, **5a**, and **6a** have shown marginal to moderate activity. The representative ketones **2**, **3**, **5**, and **6** so far tested in vivo against EAC were found to be inactive. Compounds **2c** and **3c** were synthesized with the idea that the 6-mercapto group might exert additional antitumor activity, but when tested, they were found inactive. This finding has also been reflected by the absence of any inhibitory effect of **2c** against P-388 cells and human KB cells in culture.

Compounds **2a**, **3a**, and **4a** were also screened against P-388 (PS) cells and human KB cells in culture at the National Cancer Institute. All of them have displayed significant inhibitory effect against these cell lines. However, they have greater potency against PS cells than KB cells (Table III).

It has been found that both **2a** and **3a** have exhibited comparable biological activity. They do not vary significantly in their effective chain length, being substituted propyl and pentyl derivatives, respectively. To establish the structure–activity relationship of the nucleic acid bases substituted with long-chain ketones that may be converted similarly to α-methylene γ-lactones, further investigation is under active pursuit.

Experimental Section

The melting points were determined in open capillaries on a Thomas-Hoover Unimelt capillary melting point apparatus and were uncorrected. UV spectra in EtOH were recorded on a Hitachi 200-20 spectrophotometer and IR spectra on a Perkin-Elmer 177 grating spectrophotometer in $CHCl_3$ solution or in KBr pellet. ^1H NMR spectra were determined on a Varian EM-390 90MHz spectrophotometer in $CDCl_3$ or Me_2SO-d_6 solution, and chemical shifts are expressed in δ units (ppm) relative to Me_4Si as the internal standard. All spectral data were consistent with the proposed structures. Elemental analyses were performed at the Central Drug Research Institute, Lucknow, India. Satisfactory analytical results indicated by elemental symbols were within ±0.4% of the theoretical values. Silica gel for column chromatography refers to BDH silica gel (60–120 mesh). TLC plates were coated with silica gel G (200 mesh, 0.2 mm) and spots were developed with I_2 vapor. The samples were dried over P_2O_5 in vacuo at 65 °C. Anhydrous Na_2SO_4 was used for drying the solvents. THF was freshly distilled from $LiAlH_4$ under N_2 prior to reaction.

Chemicals. The following halides, namely, bromoacetone and 1-chloro-3-pentanone, were obtained commercially. Ethyl α-(bromomethyl)acrylate[17] was prepared in the laboratory. 6-Chloropurine, uracil, 5-fluorouracil, and thymine were purchased from Sigma Chemical Co., St. Louis, MO.

1-(6-Chloro-9*H*-purin-9-yl)-2-propanone (2). To a stirred mixture of 6-chloropurine (1.54 g, 10 mmol) in DMF (15 mL) and K_2CO_3 (1.4 g, 10 mmol) was added dropwise a solution of bromoacetone (1.50 g, 11 mmol) in DMF (4 mL) under N_2 over a period of 1 h at 5 °C. The resulting mixture was stirred under N_2 for 72 h at room temperature. The reaction mixture was filtered from insoluble inorganic salts, washed with DMF, and DMF spin-evaporated in vacuo. The dark-red oily residue was extracted with $CHCl_3$, washed with brine, and concentrated in vacuo. The crude residue was passed through a silica gel column (25 g) and eluted with solvents of increasing polarity. Elution of the column with C_6H_6–$CHCl_3$ (1:1) afforded the desired product **2** as light creamish solid. The solid was further purified by crystallization from $CHCl_3$–hexane to furnish white crystals of **2**; 1.27 g (61%); mp 164–166 °C; UV 216, 264 nm; IR ($CHCl_3$) 1715 (C=O), 1590 (purine ring) cm^{-1}; TLC, R_f 0.70 ($CHCl_3$–MeOH, 1:1). Anal. ($C_8H_7ClN_4O$) C, H, N.

5′-Methyl-5′-[(6-chloro-9*H*-purin-9-yl)methyl]-2′-oxo-3′-methylenetetrahydrofuran (2a). To a stirred solution of **2** (210 mg, 1 mmol) in dry THF (10 mL) were added activated zinc powder (200–300 mesh, 72 mg, 1.1 mmol) and *p*-hydroquinone (5 mg). A solution of ethyl α-(bromomethyl)acrylate (215 mg, 1.1 mmol) in dry THF (5 mL) was added dropwise for 1/2 h to the above mixture under N_2. The reaction was initiated by warming to 50 °C. After the initial exothermic reaction was over, the resulting mixture was refluxed for 6 h when most of the zinc was consumed. The turbid reaction mixture was cooled and

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carefully decanted from the unreacted zinc. The contents in the flask was washed with a small amount of THF to ensure complete transfer of the material. THF solutions were mixed, and the solvent was evaporated in vacuo. The residue was acidified with ice-cold HCl (10%) and vigorously stirred at room temperature for 15 min. It was thrice extracted with $CHCl_3$, the organic layer washed with brine, and the solvent removed. The residual oil was dissolved in a minimum of $CHCl_3$ when a small amount of solid (20 mg, 9%) remained undissolved. This was characterized to be **2b** by comparison with the authentic sample of **2b** prepared later. The oil was column chromatographed over silica gel (6 g). Elution with C_6H_6–$CHCl_3$ (1:1) resulted in the recovery of unreacted ketone (**2**; 30 mg, 14%). Further elution with C_6H_6–$CHCl_3$ (1:3) afforded a viscous oil (170 mg), which solidified on standing. TLC revealed it to be a mixture of two components with the unreacted ketone present as a trace impurity. The solid was twice crystallized from $CHCl_3$–hexane to furnish **2a** (145 mg, 61%); mp 150–152 °C; UV 216, 265 nm; IR ($CHCl_3$) 1760 (lactone), 1600 (purine ring), 1260 (ester) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (3 H, s, CH_3), 2.90 (2 H, m, CH_2-4′), 4.56 (2 H, s, NCH_2), 5.50 (1 H, d further splitted into *t*, J = 4 Hz, H_b), 6.13 (1 H, d further splitted into t, J = 4 Hz, H_a), 8.30 (1 H, s, H-8), 8.80 (1 H, s, H-2); TLC, R_f 0.68 ($CHCl_3$–MeOH, 1:1). Anal. ($C_{12}H_{11}ClN_4O_2$) C, H, N.

5′-Methyl-5′-[(6-hydroxy-9*H*-purin-9-yl)methyl]-2′-oxo-3′-methylenetetrahydrofuran (2b). A mixture of **2a** (84 mg, 0.3 mmol) and dilute HCl (1.5 mL, 1 N) was heated at 90 °C for 1 h. Next it was evaporated to dryness in vacuo. The gummy residue was triturated with MeOH and the methanol layer decanted. The dried residue was crystallized from $CHCl_3$–Me_2SO to furnish a light creamish powder of **2b** (40 mg, 51%); mp 285–288 °C (wetting at 250 °C); UV 219, 252 nm; IR (KBr) 1760 (lactone), 1600 (purine ring) cm^{-1}. Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

5′-Methyl-5′-[(6-mercapto-9*H*-purin-9-yl)methyl]-2′-oxo-3′-methylenetetrahydrofuran (2c). To a stirred solution of **2a** (168 mg, 0.6 mmol) in dry 1-propanol (6 mL) was added thiourea (52 mg, 0.6 mmol). The mixture was refluxed for 1 h and cooled to 0 °C. A pale yellow solid separated and was collected by filtration and washed once with 1-propanol. The propanol solution was further diluted with water to collect more solid. The solids thus collected were washed with water and dried. It was sufficiently pure for analysis (**2c**; 106 mg, 63%); mp 248–252 °C; UV 219, 318 nm; IR (KBr) 1755 (lactone), 1600 (purine ring), 1260 (ester), 1200 (C=S) cm^{-1}. Anal. ($C_{12}H_{12}N_4O_2S$) C, H, N.

1-(6-Chloro-9*H*-purin-9-yl)-3-pentanone (3). To a stirred suspension of oil-free NaH [from 220 mg (55%) by twice washing with dry hexane] in dry DMF (10 mL) was added under N_2 a solution of 6-chloropurine (780 mg, 5 mmol) in DMF (10 mL) over a period of 1/2 h at 25 °C. The greenish suspension was further stirred for 1 h and cooled to 0 °C. A solution of 1-chloro-3-pentanone (723 mg, 6 mmol) in DMF (5 mL) was added dropwise to the above suspension and stirred overnight at 25 °C. The next day the reaction mixture was heated for 4 h at 80 °C after addition of dry NaI (100 mg). It was cooled and DMF spin-evaporated in vacuo. The residue was diluted with brine and thrice extracted with $CHCl_3$. The organic layer was dried and concentrated to furnish a solid yellow residue. It was applied to a silica gel column (30 g) and eluted with C_6H_6–$CHCl_3$ (1:1) to give **3** (680 mg, 57%); mp 108–110 °C; TLC, R_f 0.69 ($CHCl_3$–MeOH, 1:1). Anal. ($C_{10}H_{11}ClN_4O$) C, H, N.

5′-Ethyl-5′-[2-(6-chloro-9*H*-purin-9-yl)ethyl]-2′-oxo-3′-methylenetetrahydrofuran (3a). Compound **3** (238 mg, 1 mmol) was converted to **3a** as described for the preparation of **2a**. After the identical workup, an insoluble fraction (**3b**, 25 mg, 10%) was obtained. The residual oil was purified by a silica gel column chromatography (6 g). Elution with C_6H_6–$CHCl_3$ (1:1) resulted in the recovery of unreacted **3** (25 mg, 10%). Further elution with C_6H_6–$CHCl_3$ (1:3) furnished **3a** as a colorless solid. It was purified by crystallization from EtOAc–hexane (**3a**; 170 mg, 64%); mp 105–107 °C; ^1H NMR ($CDCl_3$) δ 0.97 (3 H, t, J = 7 Hz, CH_3), 1.80 (2 H, q, J = 7 Hz, CH_2CH_3), 2.30 (2 H, m, CH_2), 2.83 (2 H, t, J = 2 Hz, CH_2-4′), 4.37 (2 H, t, J = 7 Hz, NCH_2), 5.70 (1 H, m, H_b), 6.27 (1 H, m, H_a), 8.17 (1 H, s, H-8), 8.48 (1 H, s, H-2); TLC, R_f 0.66 ($CHCl_3$–MeOH, 1:1). Anal. ($C_{14}H_{15}ClN_4O_2$) C, H, N.

5′-Ethyl-5′-[2-(6-hydroxy-9*H*-purin-9-yl)ethyl]-2′-oxo-3′-methylenetetrahydrofuran (3b). A mixture of **3a** (184 mg, 0.6 mmol) and dilute HCl (3 mL, 1 N) was reacted and worked up as described earlier for the preparation of **2b** to yield a yellowish powder of **3b** (83 mg, 48%); mp 238–240 °C. Anal. ($C_{14}H_{16}N_4O_3$) C, H, N.

5′-Ethyl-5′-[2-(6-mercapto-9*H*-purin-9-yl)ethyl]-2′-oxo-3′-methylenetetrahydrofuran (3c). Compound **3a** (184 mg, 0.6 mmol) was converted to **3c** by an analogous procedure as described for the conversion of **2a** to **2c**. Compound **3c** was obtained as a pale yellow powder (100 mg, 55%); mp 224–228 °C. Anal. ($C_{14}H_{16}N_4O_2S$) C, H, N.

1-Uracil-1-yl-3-pentanone (4). To a stirred solution of uracil (900 mg, 8 mmol) in dry Me_2SO (20 mL) was added anhydrous K_2CO_3 (1.1 g, 8 mmol). After the mixture was stirred at 25 °C for 1/2 h, a solution of 1-chloro-3-pentanone (903 mg, 7.5 mmol) in Me_2SO (8 mL) was added under N_2 over a period of 1 h. Then the reaction mixture was refluxed for 4 h after the addition of anhydrous NaI (100 mg). The insoluble inorganic salts were filtered, and the reaction mixture was spin-evaporated in vacuo. The thick oily residue was diluted with brine (20 mL) and thrice extracted with $CHCl_3$ and worked up in the usual way to furnish a thick oily residue (1.42 g). It was dissolved in $CHCl_3$ (2 mL) and applied to a silica gel column (35 g). Elution with $CHCl_3$–MeOH (19:1) furnished **4** as a white solid. It was further purified by crystallization from C_6H_6–hexane (820 mg, 52%); mp 165–168 °C; UV 214, 260 nm; IR (KBr) 1700 (C=O and uracil ring), 1625 (uracil ring), 1110 (C=O) cm^{-1}; TLC, R_f 0.48 (EtOAc). Anal. ($C_9H_{12}N_2O_3$) C, H, N.

5′-Ethyl-5′-(2-uracil-1-ylethyl)-2′-oxo-3′-methylenetetrahydrofuran (4a). Compound **4a** was obtained from **4** (176 mg, 0.9 mmol) by the same procedure described earlier for **2a**. The crude product was purified by column chromatography over silica gel. Elution with 5% MeOH in $CHCl_3$ furnished **4a** as a white powder, which was crystallized from EtOAc–hexane (132 mg, 56%); mp 147–152 °C; ^1H NMR (Me_2SO-d_6) δ 0.80 (3 H, t, J = 6 Hz, CH_3), 1.76 (4 H, m, 2 CH_2), 2.86 (2 H, m, CH_2-4′), 3.80 (2 H, m, NCH_2), 5.63 (1 H, m, H-5), 5.76 (1 H, m, H_b), 6.10 (1 H, m, H_a), 7.73 (1 H, d, J = 7 Hz, H-6), 10.43 (1 H, s, NH); TLC, R_f 0.47 (EtOAc). Anal. ($C_{13}H_{16}N_2O_4$) C, H, N.

1-(5-Fluorouracil-1-yl)-3-pentanone (5) and 1-(5-Fluorouracil-1,3-diyl)-3-pentanone (6). To a stirred suspension of oil-free NaH (191 mg, 8 mmol) [obtained from 344 mg of oily 55% suspension by washing with dry hexane] in DMF (20 mL) was added a solution of 5-fluorouracil (1.04 g, 8 mmol) in DMF (20 mL) under N_2. After 1/2 h a solution of 1-chloro-3-pentanone (964 mg, 8 mmol) in dry DMF (5 mL) was added dropwise over a period of 15 min. Anhydrous NaI (400 mg) was added and the resulting mixture stirred at 70 °C for 3 h. The solvent was spin-evaporated in vacuo and the residue worked up in the usual manner with $CHCl_3$ as the solvent. The resulting residue (1.51 g) was subjected to chromatography over silica gel (40 g). Elution with C_6H_6–$CHCl_3$ (3:1) afforded **6**[8] as a white solid. It was crystallized from Et_2O; mp 74–75 °C; yield 600 mg (29%); UV 224, 272 nm; IR (KBr) 1700–1660 (C=O and 5-FU ring) cm^{-1}; TLC, R_f 0.67 (EtOAc).

Further elution with $CHCl_3$–MeOH (19:1) afforded **5** as a white solid, which was crystallized from $CHCl_3$–hexane (480 mg, 28%); mp 138–140 °C; UV 225, 273 nm; IR (KBr) 1700–1650 (C=O and 5-FU ring), 1130 (C=O) cm^{-1}; TLC, R_f 0.54 (EtOAc). Anal. ($C_9H_{11}FN_2O_3$) C, H, N.

5′-Ethyl-5′-[2-(5-fluorouracil-1-yl)ethyl]-2′-oxo-3′-methylenetetrahydrofuran (5a). Compound **5a** was obtained from **5** (214 mg, 1 mmol) as described for the preparation of **2a** from **2**. The crude oily residue was passed through a silica gel column (10 g). Elution with $CHCl_3$–MeOH (19:1) furnished **5a** as a white solid. This was crystallized from $CHCl_3$–hexane containing a few drops of MeOH to yield 166 mg (59%) of **5a**; mp 128–130 °C; ^1H NMR (Me_2SO-d_6) δ 0.93 (3 H, t, J = 6 Hz, CH_3), 1.63 (2 H, q, J = 6 Hz, CH_2CH_3), 1.96 (2 H, t, J = 6 Hz, NCH_2CH_2), 2.83 (2 H, t, J = 4 Hz, CH_2-4′), 3.76 (2 H, t, J = 6 Hz, NCH_2), 5.73 (1 H, t, J = 4 Hz, H_b), 6.03 (1 H, t, J = 4 Hz, H_a), 8.16 (1 H, d, J = 7 Hz, H-6); TLC, R_f 0.54 (EtOAc). Anal. ($C_{13}H_{15}FN_2O_4$) C, H, N.

5′-Ethyl-5′-[2-(5-fluorouracil-1,3-diyl)ethyl]-2′-oxo-3′-methylenetetrahydrofuran (6a). A mixture of **6** (300 mg, 1 mmol) in THF (8 mL), activated zinc powder (157 mg, 2.4 mmol), *p*-hydroquinone (10 mg), and ethyl α-(bromomethyl)acrylate (465 mg, 2.4 mmol) in THF (8 mL) was reacted and worked up as before. Applying the crude residue on a silica gel column (15 g) and elution with $CHCl_3$–MeOH (49:1) furnished **6a** as a light yellow oil, which on charcoalization in MeOH gave a colorless glassy product; yield 235 mg (54%); ^{1}H NMR (Me_2SO-d_6) δ 0.83 (6 H, t, J = 6 Hz, 2 CH_3), 1.40–2.16 (8 H, m, 2 CH_2CH_3 and 2 NCH_2CH_2), 2.46 (2 H, ill-defined t, J = 2 Hz, CH_2-4′), 2.80 (2 H, ill-defined t, J = 2 Hz, CH_2-4′), 3.36–3.93 (4 H, m, 2 NCH_2), 5.70 (2 H, apparently s, two olefinic H), 6.03 (2 H, apparently s, two olefinic H), 8.13 (1 H, d, J = 6 Hz, H-6); TLC, R_f 0.64 (EtOAc). Anal. ($C_{22}H_{27}FN_2O_6$) C, H, N.

1-Thymin-1-yl-3-pentanone (7). Thymine (504 mg, 4 mmol) in Me_2SO (15 mL) was alkylated by 1-chloro-3-pentanone (482 mg, 4 mmol) in the presence of K_2CO_3 (552 mg, 4 mmol) in a manner analogus for the preparation of 4. The pure compound was isolated by column chromatography over silica gel as usual. Elution with 2% MeOH in $CHCl_3$ furnished **7** as a white solid. It was crystallized from EtOAc (520 mg, 62%); mp 135–136 °C; UV 218, 268 nm; IR (KBr) 1700, 1610 (thymine ring), 1115 (C=O) cm^{-1}; TLC R_f 0.45 (EtOAc). Anal. ($C_{10}H_{14}N_2O_3$) C, H, N.

5′-Ethyl-5′-(2-thymin-1-ylethyl)-2′-oxo-3′-methylenetetrahydrofuran (7a). Compound 7 (210 mg, 1 mmol) was converted to **7a** as usual. The crude compound was passed through a silica gel column (13 g). Elution with $CHCl_3$–EtOAc (1:1) furnished the crude lactone. This on further crystallization from EtOAc–hexane gave pure **7a** (170 mg, 60%); mp 115–118 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.90 (3 H, t, J = 6 Hz, CH_3), 1.80 (3 H, s, CH_3-5), 1.83 (2 H, q, J = 6 Hz, CH_2CH_3), 2.50 (2 H, t, J = 3 Hz, NCH_2CH_2), 2.90 (2 H, t, J = 3 Hz, CH_2-4′), 3.87 (2 H, t, J = 7 Hz, NCH_2), 5.80 (1 H, t, J = 3 Hz, H_b), 6.13 (1 H, t, J = 3 Hz, H_a), 7.40 (1 H, s, H-6), 11.03 (1 H, br s, NH); TLC, R_f 0.44 (EtOAc). Anal. ($C_{14}H_{18}N_2O_4$) C, H, N.

In Vivo Tumor Screen. Swiss mice bearing Ehrlich ascites carcinoma (EAC) were obtained from the Karolinska Institute, Sweden, and maintained in the same strain in this Center by serial transplantation. The EAC cells were aspirated under aseptic condition from an adult Swiss mouse bearing tumor for 10–11 days. The tumor cells were diluted with normal saline and counted under a microscope. Swiss male mice weighing 18–20 g were chosen for the experiments and inoculated intraperitoneally (ip) with 0.2 mL of diluted solution containing 1×10^6 EAC cells on day 0. Groups of six animals per dose level were used with one control group for every six groups. Drug treatment by the ip route was started 24 h later and continued daily for 7 days. The test solutions of different doses of compounds were prepared by homogenization in normal saline containing 2% Tween-80 and applied at a dose volume not to exceed 0.01 mL/g of body weight. The control group received equal volume of saline containing 2% Tween-80. The number of deaths was counted daily during the test. The body weight of the animals were recorded on day 0 and 5. The testing was evaluated by calculating median survival times (MST) of the treated (T) and control (C) groups and expressed as T/C.

The compounds were screened for in vitro cytotoxicity against murine lymphocytic leukemia P-388 cells and human carcinoma of the nasopharynx (KB) cells at the National Cancer Institute, according to the standard protocol.[18]

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