Synthesis and antitumor activity of 2-β-D-ribofuranosyloxazole-4-carboxamide (oxazofurin)

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Variation between duplicate samples was less than 10% (Table II).

Antiviral Activity Determination. The effect of drugs on viral replication was evaluated in plaque-reduction assays in Vero cells according to the procedure of Collins and Bauer.[16] Dose-response lines were drawn by linear-regression technique from which the 50% effective doses (ED_{50}) were calculated (Table II).

Antimycotic and Antibacterial Assays. Antifungal and antibacterial activities were evaluated on recent clinical isolates of *C. albicans*, *Staphylococcus aureus*, *Escherichia coli*, and group D *Streptococcus*. Tests were carried out in Sabouraud-dextrose broth, pH 5.6, in the case of fungi, and nutrient broth containing 5% NaCl, pH 7.2, in the case of bacteria. As for the above assays, compounds were solubilized in DMSO. The initial inoculum of bacteria was 10^3 cells; that of fungi was 10^4 cells. Minimum inhibitory concentrations (MIC) were determined after 18 (bacteria) or 24 h (fungi) of incubation at 37 °C in the presence of different concentrations of the compounds.

Acknowledgment. We are indebted to the Institute Pasteur-Cenci Bolognetti Foundation and to the Regione Autonoma della Sardegna for supporting this research with grants.

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Synthesis and Antitumor Activity of 2-β-D-Ribofuranosyloxazole-4-carboxamide (Oxazofurin)[1]

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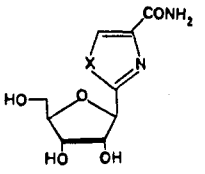
Condensation of 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonyl chloride (**4**) with ethyl 2-amino-2-cyanoacetate (**5**) provided 2-[(3′,4′,6′-tri-*O*-benzoyl-2′,5′-anhydroallonyl)amino]-2-cyanoacetate (**6**). Compound **6** was treated with hydrogen chloride gas to give ethyl 5-amino-2-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate (**8**). Reductive dediazotization of blocked nucleoside **8** provided ethyl 2-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate (**10**), which after deblocking with sodium methoxide and ammonolysis was converted to 2-β-D-ribofuranosyloxazole-4-carboxamide (oxazofurin, **3**), an analogue of the antitumor and antiviral C-nucleoside tiazofurin (**1**). Oxazofurin (**3**) was found to be cytotoxic toward B16 murine melanoma cells in culture but inactive against murine leukemia P388 and L1210.

C-glycosyl nucleosides which are analogues of nicotinamide nucleoside are expected to be converted to the analogues of NAD coenzyme and to inhibit NAD-dependent inosine monophosphate dehydrogenase (IMPD).[2] The inhibition of this enzyme produces the accumulation of IMP and the depletion of guanine nucleotides, which appeared to be linked to DNA synthesis inhibition. So, 2-β-D-ribofuranosylthiazole-4-carboxamide (tiazofurin, **1**)[3]



1 X= S, tiazofurin
2 X= Se, selenazofurin

and 2-β-D-ribofuranosylselenazole-4-carboxamide (selenazofurin, **2**),[4] which are metabolized to analogues of NAD, and thiazole-4-carboxamide adenine dinucleotide (TAD) and selenazole-4-carboxamide adenine dinucleotide (SAD), two strong inhibitors of IMPD, have pronounced antitumor activity in animals and broad-spectrum antiviral activity.[2]

Both tiazofurin and selenazofurin are highly active against Lewis lung carcinoma and P388 and L1210 leukemias in mice. Tiazofurin is also active in vivo against *ara*-C and cytoxan resistant lines of P388 leukemia and Glasgow osteogenic sarcoma. Selenazofurin is about 10 times more active than tiazofurin with a similar spectrum of antitumor activity.

A number of specific modifications of the parent tiazofurin structure have been reported.[5]

Structure-activity relationship studies revealed that the presence of a ring nitrogen adjacent to the carboxamide function and the β-ribofuranosyl moiety as the glycosyl component are the features necessary for biological activity. Substitution of the tiazole ring with isosteric ring systems such as selenazole and 1,2,4-oxadiazole[5] are allowed.

The present report describes the synthesis and biological evaluation of 2-β-D-ribofuranosyloxazole-4-carboxamide (oxazofurin, **3**), an analogue of tiazofurin in which the sulfur atom has been replaced with an oxygen.

Chemistry

The synthesis of oxazofurin (**3**) is outlined in Scheme I. Condensation of 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonyl chloride (**4**)[6a,b] with the ethyl 2-amino-2-cyanoacetate (**5**)[7] in anhydrous pyridine at room temperature gave ethyl 2-[(3′,4′,6′-tri-*O*-benzoyl-2′,5′-anhydroallonyl)-

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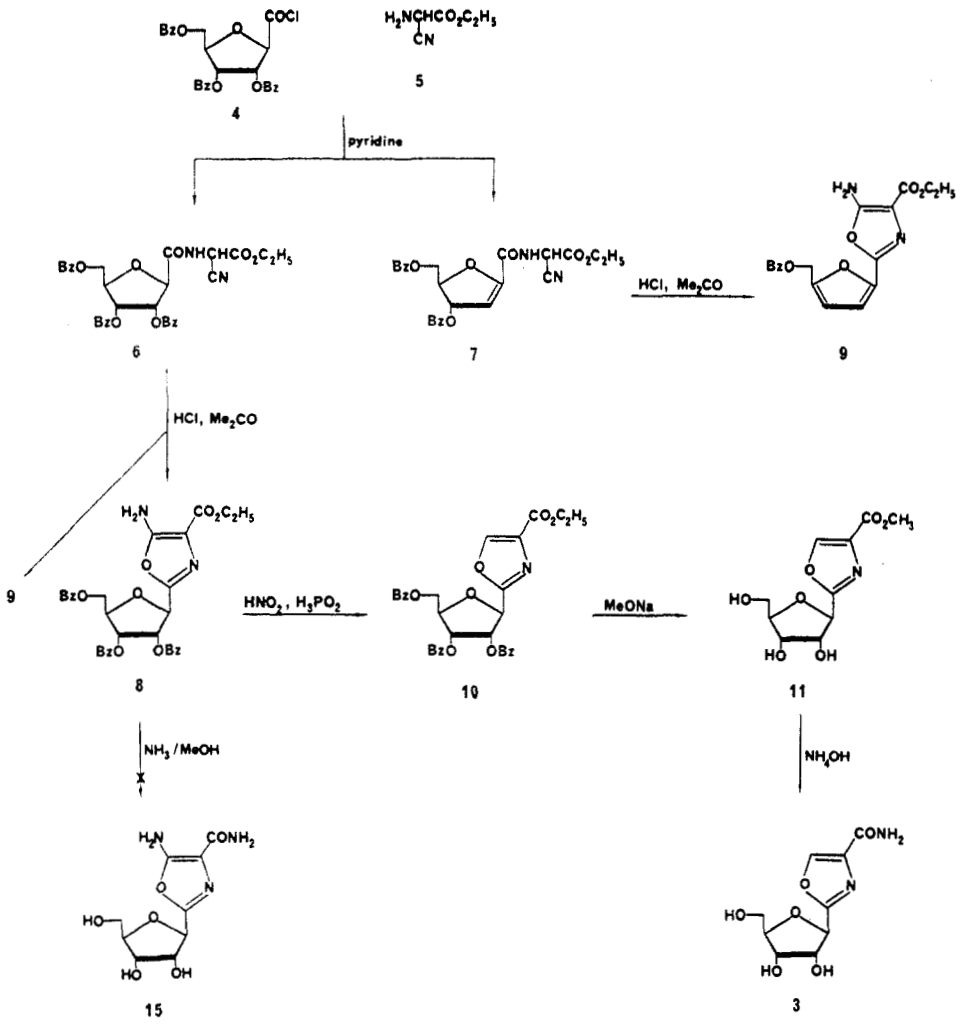
Scheme I



amino]-2-cyanoacetate (**6**) in 21% yield and the (4*S*,-*trans*)-4-(benzoyloxy)-5-[(benzoyloxy)methyl]-*N*-(cyanocarbethoxymethyl)-4,6-dihydrofuran-2-carboxamide (**7**) in 24% yield as mixtures of two diastereomers.

Treatment of **6** with hydrogen chloride gas in anhydrous acetone gave the ethyl 5-amino-2-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate **8** (56%) and the furan derivative ethyl 5-amino-2-[5′-[(benzoyloxy)-methyl]furan-2′-yl]oxazole-4-carboxylate (**9**) in 6% yield.

Compound **9** was also obtained in a similar way by direct treatment of **7** in 54% yield.

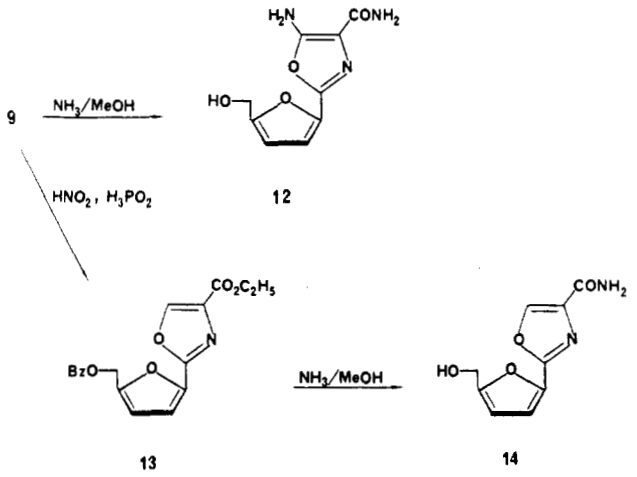
Reductive dediazotization of ethyl 5-amino-2-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate (**8**) with sodium nitrite in aqueous hypophosphorus acid, gave ethyl 2-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate (**10**) in 60% yield. Deblocking with sodium methoxide in methanol of **10** furnished the methyl 2-(β-D-ribofuranosyl)oxazole-4-carboxylate (**11**).

Ammonolysis of **11** in 10% ammonium hydroxide provided 2-β-D-ribofuranosyloxazole-4-carboxamide (oxazofurin, **3**) in 56% yield.

In a similar way, starting from compound **9**, ethyl 2-[5′-[(benzoyloxy)methyl]furan-2′-yl]oxazole-4-carboxylate (**13**) was obtained, which in turn was converted into 2-[5′-(hydroxymethyl)furan-2′-yl]oxazole-4-carboxamide (**14**) by treatment with methanolic ammonia (Scheme II).

We have also attempted to synthesize the 5-amino-2-β-D-ribofuranosyloxazole-4-carboxamide (**15**), an analogue of the bioloically important natural nucleoside 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICR), starting from compound **8**. However, ammonolysis of **8**

Scheme II



with methanolic ammonia or ammonium hydroxide gave only a complex mixture. On the contrary, ammonolysis of **9** with methanolic ammonia at room temperature easily provided 5-amino-2-[5′-(hydroxymethyl)furan-2′-yl]oxazole-4-carboxamide (**12**).

The β-configuration of nucleoside **3** was deduced by the ^{1}H NMR spectrum in Me_2SO-d_6, which shows the 1′-H signal as a doublet at δ 4.67 with a coupling constant of 6.4 Hz, similar to anomers of tiazofurin (**1**) (δ 4.99, $J_{1,2}$ = 5 Hz)[3] and selenazofurin (**9**) (δ 4.88, J = 5 Hz).[4] The β-configuration was confirmed by the proton–proton nuclear Overhauser effects difference experiment. In fact,

Table I. In Vitro Activity (IC_{50} Values) of 3, 12, 14, and Tiazofurin

compd	IC_{50}, μM			
	P388	L1210	B16	HL 60
tiazofurin	16.7	6.2	16.2	6.1
3	*a*	*a*	3015.3	*a*
12	*b*	*b*	*b*	*b*
14	*b*	*b*	*b*	*b*

[a] Inactive until a maximum tested concentration of 4×10^{-3} M. [b] Inactive until a maximum tested concentration of 2.5×10^{-4} M.

selective irradiation of the 1'-H increases by 1% the intensity of the 4'-H signal, while the intensity enhancement of the 3'-H signal is zero; this indicates that 1'-H and 4'-H are located on the same face of the ribosyl ring.[8]

Biological Activity

Oxazofurin 3 and its furan derivatives 14 and 12 have been evaluated in vitro for their ability to inhibit the growth of P388 and L1210 murine leukemia, B16 murine melanoma, and HL 60 human promyelocytic leukemia (Table I).

Activity of compounds 3, 12, 14 against cellular lines was compared with that of tiazofurin.[9] Compounds 12 and 14 have been found to be completely inactive. In our testing system tiazofurin was found to be active on every tested cell line. Oxazofurin 3 was found to be weakly active only against B16 murine melanoma with a 50% inhibitory dose (ID_{50}) value of 3×10^{-3} M.

Therefore, the isosteric replacement of the sulfur atom in tiazofurin with oxygen is detrimental for the antitumor activity. The poor activity of oxazofurin 3 might be due to the lower basicity of the oxazole moiety compared to that of the thiazole moiety of tiazofurin.[10]

Experimental Section

General Procedures. Melting points (uncorrected) were taken on a Büchi apparatus. Elemental analyses were determined on a Carlo Erba Model 1106 analyzer. Infrared spectra (IR in Nujol) were recorded with a Perkin-Elmer Model 297 spectrophotometer. Ultraviolet spectra were recorded with HC 8452A diode-array spectrophotometer driven by an Olivetti M 24. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (Merck); silica gel 60 (Merck) for column chromatography was used. Nuclear magnetic resonance (^1H NMR) spectra were determined at 300 MHz with a Varian VXR-300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O.

Ethyl 2-[(3',4',6'-Tri-*O*-benzoyl-2',5'-anhydroallonyl)-amino]-2-cyanoacetate (6) and (4*S*,*trans*)-4-(Benzoyloxy)-5-[(benzoyloxy)methyl]-*N*-(cyanocarbethoxymethyl)-4,6-dihydrofuran-2-carboxamide (7). To a stirred suspension of 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonyl chloride (4;[6a,b] 11.6 g, 22.79 mmol) in dry pyridine (48 mL) at 0 °C was added slowly ethyl 2-amino-2-cyanoacetate (5;[7] 2.92 g, 22.8 mmol) in dry pyridine (36 mL). The reaction mixture was stirred at room temperature for 13 h under anhydrous conditions and then evaporated to dryness. The residual mixture was treated with EtOAc and filtered; the filtrate was evaporated to dryness and the residue was purified on a silica gel column. Elution with C_6H_6–EtOAc (75:25, v/v) yielded 2.88 g (21%) of a white amorphous solid (6) as a mixture of two diastereomers: IR ν 1730 (C=O); ^1H NMR (Me_2SO-d_6) δ 1.18 (m, 3, CH_3), 4.18 (m, 2, CH_2) 4.62 (m, 2, C_5-CH_2), 4.78 (m, 1, C_4-H), 4.92 (m, 1, C_1-H), 5.61 (m, 1, C_3-H), 5.80 (m, 1, C_2-H), 5.87–5.90 (2 d, 1, CHCN), 7.40–8.00 (m, 15, C_6H_5), 9.48–9.52 (2 d, 1, NH). Anal. ($C_{32}H_{28}N_2O_{10}$) C, H, N.

Further elution of the column with the same mixture of solvents gave 2.9 g (24%) of a clear colorless syrup (7) as a mixture of two diastereomers: ^1H NMR (Me_2SO-d_6) δ 1.18 (m, 3, CH_3), 4.20 (m, 2, CH_2), 4.63 (m, 2, C_5-CH_2), 5.29 (m, 1, C_4-H), 5.85 (2 d, 1, CHCN), 6.12–6.15 (m, 2, C_2-H and C_3-H), 7.39–8.03 (m, 10, C_6H_5), 9.67 (2 d, 1, NH). Anal. ($C_{25}H_{22}N_2O_8$) C, H, N.

Ethyl 5-Amino-2-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate (8) and Ethyl 5-Amino-2-[5'-[(benzoyloxy)methyl]furan-2'-yl]oxazole-4-carboxylate (9). A stirred solution of 6 (2 g, 3.33 mmol) in dry acetone was treated with hydrogen chloride gas for 20 min and stored at 4 °C. After 22 h, the solution was neutralized with $NaHCO_3$ solution and extracted with $CHCl_3$ (2 × 50 mL). The combined $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified on a silica gel column eluting with C_6H_6–EtOAc (70:30, v/v) to yield 0.53 g (26%) of 6 and 1.12 g (56%) of 8 as a white foam: IR ν 1730 (C=O), 3315–3430 (NH_2) cm^{-1}; ^1H NMR δ 1.27 (t, 3, CH_3), 4.21 (d, 2, CH_2), 4.58 (m, 2, C_5-CH_2), 4.78 (m, 1, C_4-H), 5.34 (d, 1, $J_{1,2}$ = 4.3 Hz, C_1-H), 5.85 (t, 1, C_3-H), 6.10 (t, 1, C_2-H), 7.40 (s, 2, NH_2), 7.42–7.92 (m, 15, C_6H_5). Anal. ($C_{32}H_{28}N_2O_{10}$) C, H, N.

Further elution of the column with the same mixture of the solvents gave 0.12 g (6%) of 9 as a solid: mp 171–173 °C; IR ν 1725 (C=O), 3480 (NH_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.27 (t, 3, CH_3), 4.20 (q, 2, CH_2), 5.39 (s, 2, C_5-CH_2), 6.82 (d, 1, $J_{4',3'}$ = 3.4 Hz, H-4 furan ring), 6.93 (d, 1, $J_{3',4'}$ = 3.4 Hz, H-3 furan ring), 7.51–8.0 (m, 5, C_6H_5), 7.53 (s, 2, NH_2). Anal. ($C_{18}H_{16}N_2O_6$) C, H, N.

Ethyl 2-(2',3',5'-Tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate (10). Compound 8 (1 g, 1.66 mmol) in THF (40 mL) was added to a precooled (−20 °C), stirred solution of hypophosphorous acid (50%, 16 mL) containing a few drops of hydrochloric acid. To the above clear solution, a solution of $NaNO_2$ (2.28 g, 33.0 mmol) in water (12 mL) was added slowly (8 min). After 15 min, an additional portion of $NaNO_2$ (0.55 g, 7.9 mmol) dissolved in a small amount of water was slowly added. The stirring was continued for 4 h at −20 °C. The reaction solution was adjusted to pH 6 by careful addition of a saturated solution of sodium bicarbonate. The final reaction mixture was extracted with ethyl acetate (4 × 50 mL) and the organic layer in turn was washed thoroughly with water. The ethyl acetate portion was dried (Na_2SO_4); after evaporation to dryness the crude residue was chromatographed through a silica gel column eluting with C_6H_6–EtOAc (85:15, v/v) to give 580 mg (60%) of 10 as a white foam: ^1H NMR (Me_2SO-d_6) δ 1.3 (t, 3, CH_3), 4.3 (q, 2, CH_2), 4.6 (dd, 1, C_5-CH_2), 4.83 (m, 1, C_4-H), 5.57 (d, 1, $J_{1,2}$ = 4.5 Hz, C_1-H), 5.90 (t, 1, C_2-H), 6.13 (t, 1, C_3-H), 7.42–7.96 (m, 15, C_6H_5), 8.87 (s, 1, C_5-H). Anal. ($C_{32}H_{27}NO_{10}$) C, H, N.

Methyl 2-β-D-Ribofuranosyloxazole-4-carboxylate (11). Compound 10 (0.5 g, 0.85 mmol) was dissolved in anhydrous MeOH (10 mL) containing sodium methoxide (0.61 g, 11.29 mmol) and the mixture was stirred at room temperature overnight. To the reaction solution, 0.35 g of Dowex 50w x8 resin (H^+) (washed with methanol) was added and the suspension was stirred for 1 h. The resin was filtered off, washed well with methanol, and discarded. The filtrate and washings were evaporated under reduced pressure and the residue was purified on a silica gel column using $CHCl_3$–MeOH (90:10, v/v) as eluent. The homogeneous fraction was evaporated to dryness to give 0.1 g (68%) of 11 as white needles: mp 114–115 °C; ^1H NMR (Me_2SO-d_6) δ 3.45 (m, 2, C_5-CH_2), 3.80 (s, 3, CH_3), 3.83 (q, 1, C_4-H), 3.95 (q, 1, C_3-H), 4.25 (q, 1, C_2-H), 4.68 (d, 1, $J_{1,2}$ = 6.1 Hz, C_1-H), 4.78 (m, 1, OH), 5.18 (t, 1, OH), 5.40 (t, 1, OH), 8.88 (s, 1, C_5-H). Anal. ($C_{10}H_{13}NO_7$) C, H, N.

2-β-D-Ribofuranosyloxazole-4-carboxamide (Oxazofurin, 3). Treatment of compound 11 (0.13 g, 0.5 mmol) with 10% ammonium hydroxide (6 mL) for 6.5 h at room temperature and evaporation to dryness afforded a product which was purified on a silica gel column using $CHCl_3$–MeOH (70:30, v/v) as eluent. The homogeneous fraction was evaporated to dryness to give 69 mg (56%) of 3 as white needles: mp 132–134 °C; UV (EtOH) λ_{max} 214 nm (ε 13 100); ^1H NMR (Me_2SO-d_6) δ 3.46 (m, 2, C_5-CH_2),

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3.84 (dd, 1, C_4-H), 3.97 (dd, 1, C_3-H), 4.28 (dd, 1, C_2-H), 4.67 (d, 1, $J_{1,2}$ = 6.4 Hz, C_1-H), 4.80 (t, 1, C_5-CH_2), 5.12 and 5.35 (2 d, 2, $C_{2',3'}$-OH), 7.5 and 7.68 (2 s, 2, $CONH_2$), 8.58 (s, 1, C_5-H). Anal. ($C_9H_{12}N_2O_6$) C, H, N.

5-Amino-2-[5'-(hydroxymethyl)furan-2'-yl]oxazole-4-carboxamide (12). A solution of **9** (100 mg, 0.28 mmol) in MeOH/NH_3 (10 mL, saturated at 0 °C) was stirred at room temperature for 53 h in a pressure bottle. The solvent was evaporated to dryness and the residue was purified on a silica gel column using $CHCl_3$-MeOH-NH_4OH (80:19:1 v/v) as eluent. The homogeneous fractions were pooled and evaporated to dryness, and the residue was crystallized from methanol to give 25 mg (40%) of **12**; mp 132-135 °C; IR ν 1605 and 1688 (C=O), 3318 (NH_2), cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.43 (d, CH_2OH), 5.37 (t, 1, OH), 6.41 (d, 1, $J_{4',3'}$ = 3.0 Hz, H-4 furan ring), 7.02 (d, 1, $J_{3',4'}$ = 3.0 Hz, H-3 furan ring), 7.30 and 7.70 (2 br s, 4, NH_2, $CONH_2$). Anal. ($C_9H_9N_3O_4$) C, H, N.

Ethyl 2-[5'-[(Benzoyloxy)methyl]furan-2'-yl]oxazole-4-carboxylate (13). Compound **9** (0.5 g, 1.4 mmol) in THF (18 mL) was added to a precooled (-20 °C) stirred solution of hypophosphorous acid (50%, 14 mL) containing a few drops of hydrochloric acid. To the above yellow solution, a solution of $NaNO_2$ (250 mg, 3.62 mmol) in water (3 mL) was added slowly (8 min). The stirring was continued for 3.5 h at -20 °C. The reaction mixture was adjusted to pH 6 by careful addition of a saturated solution of sodium bicarbonate and extracted with ethyl acetate (4 × 30 mL). The organic layer in turn was washed thoroughly with water, and the ethyl acetate portion was dried (Na_2SO_4); after evaporation to dryness, the crude residue was chromatographed on a silica gel column eluting with C_6H_6-EtOAc (80:20, v/v). The homogeneous solid that was obtained after evaporation of the solvent was crystallized from ethyl acetate-petroleum ether to give 180 mg (37%) of **13** as a white solid: mp 107-109 °C; ^1H NMR (Me_2SO-d_6) δ 1.30 (t, 3, CH_3), 4.32 (q, 2, CH_2), 5.46 (s, 2, CH_2), 6.90 (d, 1, $J_{4',3'}$ = 3.1 Hz, H-4 furan ring), 7.31 (d, 1, $J_{3',4'}$ = 3.1 Hz, H-3 furan ring), 7.50-8.0 (m, 5, C_6H_5), 8.93 (s, 1 C_5-H). Anal. ($C_{18}H_{15}NO_6$) C, H, N.

2-[5'-(Hydroxymethyl)furan-2'-yl]oxazole-4-carboxamide (14). Treatments of compound **13** (150 mg, 0.43 mmol) with MeOH-NH_3 (40 mL, saturated at 0 °C) for 5 days at room temperature and evaporation to dryness yielded a product which was purified on a silica gel column using $CHCl_3$-MeOH (90:10, v/v) as eluent. The homogeneous solid was crystallized from EtOH to yield 40 mg (36%) of **14**: mp 193-195 °C; IR ν 1610 and 1660 (C=O), 3290 and 3340 (NH_2) cm^{-1}; UV (EtOH) λ_{max} 206 (ϵ 11700), 282 nm (ϵ 19200); ^1H NMR (Me_2SO-d_6) δ 4.48 (d, 2, CH_2), 5.47 (t, 1, OH), 6.56 (d, 1, $J_{4',3'}$ = 3.4 Hz, H-4 furan ring), 7.14 (d, 1, $J_{3',4'}$ = 3.4 Hz, H-3 furan ring), 7.57 and 7.70 (br 2 s, 2, NH_2), 8.63 (s, 1, H-5 oxazole ring). Anal. ($C_9H_8N_2O_4$) C, H, N.

Antitumor Evaluation. The following cell lines were used: P388 murine lymphocytic leukemia, L1210 murine lymphocytic leukemia, B16 murine melanoma, and HL-60 human promyelocytic leukemia. Cell lines, maintained in vitro in exponential growth, were cultured in RPMI-1640 supplemented with antibiotics (penicillin 100 units/mL, streptomycin 100 μg/mL, gentamicin 50 μg/mL), g/mL), 3 mM glutamine, 10 mM HEPES buffer, and 15% (for P388 and L1210 cell lines) heat-inactivated new born calf serum or 10% (for B16 cell line) or 15% (for HL-60 cell line) heat-inactivated fetal calf serum. In order to determine cell growth inhibition, an antimetabolic assay was performed. Tiazofurin (1)[9] and compound **3** were solubilized in water and then in culture medium. Compounds **12** and **14** were solubilized in DMSO, and then water and culture medium were added; the final concentration of DMSO (not more than 0.5%) had no cytotoxic effect in our testing system. Various concentrations of each compound were placed, in quadruplicate, in flat-bottomed microculture wells with tumor cell suspensions for 48 h at 37 °C. Cells were placed in aliquots of 0.2 mL at the following concentrations: P388, 10^5 cell/well; L1210, 5 × 10^4 cell/well; B16, 3 × 10^3 cell/well; HL 60, 10^5 cell/well. Antiproliferative activity was determined by adding to the cultured cells [^{125}I]-5-iodo-2'-deoxyuridine together with 5-fluoro-2'-deoxyuridine, for an additional 18 h. Harvesting was performed by a multiple suction filtration apparatus on a fiber-glass filter. Immediately before harvesting, B16 cells were treated with trypsin 0.05% plus EDTA 0.02%. The filter paper was washed six times with 0.85% NaCl solution and the paper disks containing the aspirates cells were read in a γ-scintillation counter. At each dose level of compounds tested, cell-growth inhibition was expressed as a percentage of inhibition of radioisotope incorporation in the treated cultures with respect to control cultures. A dose resulting in 50% inhibition of radioisotope incorporation (ID_{50}) was determined; ID_{50} mean of at least three experiments was reported.

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8-Substituted 5-[(Aminoalkyl)amino]-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-ones as Potential Antineoplastic Agents. Synthesis and Biological Activity

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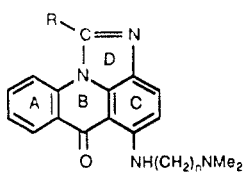
A series of 8-substituted 5-[(aminoalkyl)amino]-6*H*-*v*-triazolo[4,5,1-*de*]acridin-6-ones (**2**), structurally related to the imidazoacridinones (**1**), was synthesized and tested for cytotoxic and antineoplastic activity. Preliminary biological results indicated that the 8-OH derivatives possess the highest antitumor activity. No relationship has been found between the nature of the C-8 substituent and antitumor activity.

Among the antineoplastic compounds, a growing interest has been observed in recent years in the development of synthetic DNA-interacting agents. They have as a common general structural feature a tri- or tetracyclic chromophore bearing one or two side chains containing an (aminoalkyl)amino residue. Anthracenediones (ametantrone, mitoxantrone),[1] anthrapyrazoles,[2] pyrazoloacridines,[3] and acridine-4-carboxamides[4] belong, among others, to this broad class of compounds. We recently described a further example of active compounds in this class, i.e. the imidazoacridinones (**1**).[5]



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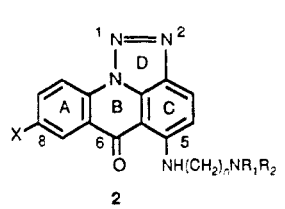

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The results obtained so far indicate that the presence of an (aminoalkyl)amino side chain is crucial for the bio-

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