Anal. ($C_{24}H_{26}N_8O_8S\cdot3.5CH_3OH$) C, N, S; H: calcd, 5.77; found, 4.60.

2-Amino-1-hydroxy-7-(4-hydroxyanilino)mitosene (17). A mixture of 7 (30 mg, 0.07 mmol)[3] and 5 mL of 0.05 N HCl was stirred at room temperature for 2 h. The resulting purple solution was cooled in an ice bath and brought to pH 8.0 by adding 5% $NaHCO_3$ solution. The mixture was extracted with ethyl acetate (4 × 25 mL) and the combined extract was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by preparative TLC on silica gel (20 × 20 × 0.2 cm) with MeOH–$CHCl_3$ (2:8 v/v) as solvent. The major purple band was scraped off the plate, extracted with MeOH–CH_2Cl_2, filtered, and concentrated to give 10 mg (35% yield) of **17** as a purple solid with mp >250 °C dec; ^1H NMR (Me_2SO-d_6) δ 1.70 (s, 3, 6-CH_3), 3.6–4.2 (m, 3, H-3 + H-2), 4.5 (br s, 1, H-1), 5.2 (s, 2, 10-CH_2), 6.5 (s, 2, $CONH_2$), 6.7–6.9 (br s, 4, phenyl), 8.15 (s, 1 phenolic OH): IR (KBr) 3480, 3400–3420, 3320–3340, 3200, 1700, 1590, 1500 cm^{-1}; UV (MeOH) λ_{max} 254–260, 286–290, 325, 368, 530–550 nm. Anal. ($C_{20}H_{20}N_4O_6\cdot CH_3OH$) C, H, N.

2-Amino-7-(dimethylamidino)-1-hydroxymitosene (18). This compound was prepared by the same procedure as that described for **17**. From 30 mg of **8**[4] was obtained 9 mg (18% yield) of **18** as purple solid with mp >250 °C dec: ^1H NMR (D_2O) δ 1.75 (s, 3, 6-CH_3), 3.08 (s, 6, $N(CH_3)_2$), 3.6–4.2 (m, 3, H-3 + H-2), 4.5 (br s, 1, H-1), 5.06 (s, 2, 10-CH_2), 7.45 (s, 1, NCH=N); ^{13}C NMR (Me_2SO-d_6) δ 11.9 (6-CH_3), 31.48 (NCH_3), 35.82 (NCH_3), 63.01 (C-1), 151.5 (NCH=N); MS (EI, high resolution) calcd for $C_{17}H_{21}N_5O_5$ m/e 375.1543, found m/e 375.1545.

Reduction of 15 by Sodium Dithionite. A solution of 1 mg of **15** in 1 mL of water was deaerated by bubbling N_2 through it for 15 min. A solution of 2 mg of sodium dithionite in 1 mL of water was added and the resulting solution was stirred for 11 min. It turned pale yellow during this time. The nitrogen stream was replaced by oxygen for 5 min, which restored the purple color of the quinone. The reaction mixture was analyzed by TLC on silica gel with two systems, MeOH–chloroform (2:8 v/v) or 2-propanol–1 N NH_4OH (2:8 v/v). Faint spots corresponding to 2,7-diamino-1-hydroxymitosene (**3**) and 6-mercaptopurine ribonucleoside were observed (comparison with authentic samples in parallel spots). There were two more intense spots, neither of which corresponded to 2,7-diaminomitosene. They were not examined further.

Reduction of 14 by Sodium Dithionite. This experiment was carried out in the same way as described for **15**, but on a 5-mg scale. Analysis of the product by TLC using MeOH–chloroform as solvent showed traces of 2,7-diamino-1-hydroxymitosene (**3**) and *ara*-CMP, together with a faster moving unknown spot and a few faint spots near the baseline.

Acknowledgment. This investigation was supported by Grants CA21430 (W.R.) and CA17094 (D. S. Alberts, supporting R.D.), awarded by the National Cancer Institute, DHHS, and by Grant IN 110H (R.D.), awarded by the American Cancer Society. We thank Mr. James Liddil for conducting the MTT assays and Dr. K. H. Schram for mass spectra.

Registry No. **1**, 50-07-7; **2**, 114612-47-4; **3**, 1096-49-7; **6**, 4055-39-4; **7**, 70343-57-6; **8**, 88949-01-3; **9**-5′-UMP, 132019-12-6; **10**-5′-UMP, 132019-14-8; **11**, 132019-13-7; **12**-5′-UMP, 132019-16-0; **13**, 132019-17-1; **14**, 132019-18-2; **15**, 132019-19-3; **16**, 32633-63-9; **17**, 132076-58-5; **18**, 132019-20-6; 5′-*ara*-CMP, 7075-11-8; 5′-uridylic acid, 58-97-9; 5′-cytidylic acid, 63-37-6; 6-mercaptopurine ribofuranoside, 574-25-4; *ara*-cytidine, 147-94-4; 6-mercaptopurine, 50-44-2.

Synthetic Approaches to the Guanosine and Xanthosine Analogues 5-Amino-3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazin-7-one and 3-β-D-Ribofuranosylpyrazolo[3,4-*e*][1,3]oxazine-5,7-dione and Studies of Their Antitumor Potential

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5-Amino-3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazin-7-one has been synthesized via cyclization of the appropriately protected pyrazofurin derivatives and subsequent transformations of the heterocyclic moiety. This guanosine analogue was marginally cytotoxic to L1210 cells in vitro. The xanthosine analogue 3-β-D-ribofuranosylpyrazolo[3,4-*e*]-[1,3]oxazine-5,7-dione was also synthesized, and was found to be highly cytotoxic. It appeared to act as a prodrug of pyrazofurin.

Introduction

Certain purines, purine nucleosides, and purine-like nucleoside antibiotics [e.g., tubercidin (**1**), sangivamycin (**2**), toyocamycin (**3**), formycin (**4**), formycin B (**5**), etc.] have shown significant chemotherapeutic and biological activities.[1-10] Considerable research effort has been devoted to the improvement of the chemotherapeutic and biological activity of these nucleosides by selective chemical

(1) Symposium on Bioorganic Chemistry and Drug Design, Academy of Sciences Latvian SSR, May and June, 1982, Riga, Latvia.

(2) Conference on *Structure–Activity Relationships of Anti-tumor Agents*, Presented lecture on topic entitled, "Antimetabolites" Leewenhorst Conference Centre, 11–13 March 1982, The Netherlands.

(3) New York Academy of Sciences conference on *The Chemistry, Biology and Clinical Uses of Nucleoside Analogs*, 4–6 September 1974, New York, NY.

(4) *Symposium of the Chemistry, Biochemistry and Clinical Aspects of Nucleosides*; Fourteenth National American Chemical Society Medicinal Chemistry Symposium, Durham, NH, June 1974, American Chemical Society: Washington, DC, 1974.

(5) *First International Round Table on Nucleosides and Their Biological Activities*; 28–30 October 1974, Montpellier, France. *Second International Round Table on the Chemistry and Biology of Nucleosides and Nucleotides*; 172nd National Meeting of the American Chemical Society, Carbohydrate Division, 29 August–3 September 1976, San Francisco, CA. *Third International Round Table on the Chemistry and Biology of Nucleosides and Nucleotides*; Montpellier, France, 4–6 October 1978 (published by INSERM as a monograph in 1979). *Fourth International Round Table on Nucleosides and Their Biological Activities*; Antwerp, Belgium, 3-8 February 1981.

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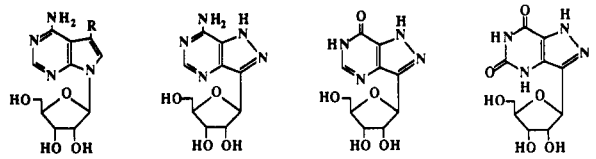
modifications of the heterocyclic moieties.[1-10] These chemical modifications have resulted in definite changes in their chemotherapeutic and biological activity due to perturbations of the conformation and/or the electronic structure of the molecule. One of our modification strategies involves the replacement of a nitrogen atom with an oxygen atom.[11,12] This can produce a significant change in the electron-density distribution of the heterocycle while minimizing the steric change. This eliminates the problem of trying to ascertain if the observed effect is due to the electronic or the steric factor.



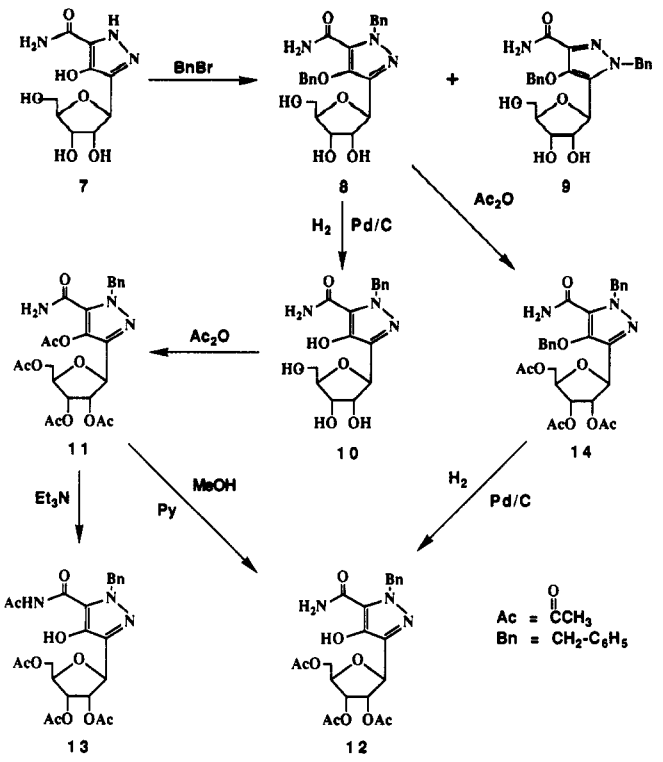
1. R=H
2. R=CONH$_2$
3. R=CN

4 5 6

In our previous studies, we have reported[11,12] the synthesis of several substituted pyrazolo[3,4-*e*][1,3]oxazines and their ribonucleoside derivatives. These compounds could be viewed as analogues of the C-nucleoside antibiotics formycin (**4**), formycin B (**5**), and oxoformycin B (**6**). The present work describes in detail the syntheses and antitumor studies of the guanosine and xanthosine analogues 5-amino-3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazin-7-one (**25**) and 3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazine-5,7-dione (**33**).

This analogue of guanosine can be viewed as a C-nucleoside analogue of the antitumor antibiotic 5-amino-3-β-D-ribofuranosylimidazo[4,5-*d*][1,3]oxazin-7-one (oxanosine).[13] Oxanosine is also an analogue of guanosine and has the single modification of an isosteric substitution of an oxygen atom for the N1 of the purine ring system (while **25** has an oxygen atom in the position analogous to the N3 of purine). It inhibits the growth of HeLa and *ts*/NRK cells and prolongs the life span of L1210 bearing mice.[13,14] The monophosphate derivative has been shown to inhibit IMP dehydrogenase. This inhibition appears to be the primary biochemical mechanism since GMP prevents growth inhibition by oxanosine.[13,14] The potency of oxanosine as an antitumor agent is modest[13] and may be limited in part by cleavage of the glycosidic bond, for example by purine nucleoside phosphorylase. Our new guanosine analogue (**25**) is a C-nucleoside, and consequently would be resistant to cleavage of the glycosidic bond. Therefore, it was of interest to determine whether its antitumor activity would be significantly greater than that of oxanosine.

Scheme I



Chemistry

The naturally occurring[15] C-nucleoside antibiotic pyrazofurin (**7**) has demonstrated significant antiviral activity against a broad spectrum of DNA and RNA viruses,[15-20] as well as limited antitumor activity.[21] The total synthesis of **7** has been achieved via several different routes.[22-25] In our first approach toward the synthesis of the requisite bicyclic nucleosides, the N1 nitrogen atom of pyrazofurin (**7**) was protected with a benzyl group in order to avoid the possible side reactions on the pyrazole nitrogen during the cyclization reaction. The protection of N1 was achieved via dibenzylation and subsequent selective debenzylation at the O4 position. Dibenzylation of pyrazofurin (**7**) with an excess of benzyl bromide gave a mixture of the dibenzylpyrazofurins 1,4-*O*-dibenzylpyrazofurin (**8**) and

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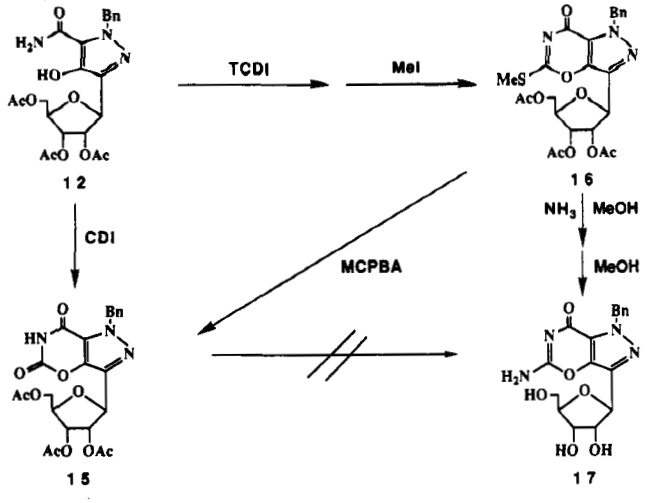
Scheme II



2,4-*O*-dibenzylpyrazofurin (**9**) (Scheme I). Chromatographic separation of this isomeric mixture provided the pure isomers **8** and **9** in 65% and 17% yields, respectively. The structural assignment of each isomer was on the basis of the downfield shift of the benzylic protons of **8** relative to the benzylic protons of **9**. This downfield shift was attributed to the anisotropic effect of the adjacent carbonyl group.[11] Selective debenzylation of **8** at the O4 position was effected by the treatment of **8** with hot 6 N HCl or hydrogenolysis over 5% Pd/C to provide 1-benzylpyrazofurin (**10**). The latter method was experimentally preferable since it facilitated the isolation procedure and gave a better yield. A positive ferric chloride test of the product (**10**) indicated that a free 4-hydroxy group had been generated.[11,12] A large bathochromic shift in the UV spectrum of the product (**10**) was also observed in a slightly basic methanol solution. This bathochromic shift was found later to be very characteristic to other pyrazofurin derivatives bearing a free 4-hydroxy group. Compound **10** has been reported[22] in the literature and was synthesized via a cyclization of a C-ribonucleoside derivative. Acetylation of **10** with acetic anhydride in pyridine and careful workup gave the tetraacetyl derivative, 1-benzyl-4,2′,3′,5′-tetra-*O*-acetylpyrazofurin (**11**), in a quantitative yield. Compound **11** was unstable to the liquid–liquid workup procedure and column chromatography on silica. It underwent partial deacetylation to give the desired triacetyl derivative 1-benzyl-2′,3′,5′-tri-*O*-acetylpyrazofurin (**12**) as the major product along with a minor amount of the acetyl migration product (**13**). To avoid this acetyl migration problem, an alternative route to **12** was explored. This was accomplished by successive acetylation of **8** and hydrogenolysis of the intermediate 1,4-*O*-dibenzyl-2′,3′,5′-tri-*O*-acetylpyrazofurin (**14**) over Pd/C. These two reactions proceeded cleanly and gave the appropriately protected pyrazofurin **12** in 94% overall yield.

The treatment of **12** with carbonyldiimidazole (CDI) in THF gave 1-benzyl-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*e*][1,3]oxazine-5,7-dione (**15**) in 68% yield (Scheme II). Attempts to synthesize 5-amino-1-benzyl-3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazin-7-one (**17**) via successive chlorination of **15** and subsequent aminolysis were unsuccessful. Chlorination of **15** with phosphoryl chloride, phosphorus pentachloride, or phenylphosphonic dichloride under various conditions[26] resulted in either no reaction or decomposition. An alternative cyclization of **12** with thiocarbonyldiimidazole (TCDI) followed by methylation with methyl iodide afforded 1-benzyl-5-(methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*e*][1,3]oxazin-7-one (**16**) in 93% yield. The aminolysis of **16** was examined under different reaction conditions. It was found that the C5–O bond was so unstable that only ring-opened products (as indicated by positive ferric chloride tests and UV data) were obtained from these reactions. No recyclization occurred when they were treated with triethylamine or sodium hydride. It was found that the initial ring opened intermediate formed by treating **16** with methanolic ammonia did recyclize when ammonia was removed and the residue was again stirred in methanol. This procedure also provided a concomitant removal of the sugar acetyl groups. After chromatographic purification and recrystallization, the pure 5-amino-1-benzyl-3-β-D-ribofuranosylpyrazolo-[3,4-*e*][1,3]oxazin-7-one (**17**) was obtained in 27% yield. The ^{1}H NMR spectrum (in DMSO-d_6) of **17** showed two overlapping broad singlets at 8.25 and 8.16 (5-NH_2) while a mass spectrum (FAB) gave the protonated parent ion (m/z 375.1299, 6%, MH^+ = 375.1305). A ferric chloride test was negative and the UV spectrum (in MeOH) was similar to that reported for the substituted 5-aminopyrazolo[3,4-*e*][1,3]oxazin-7-one derivatives.[11] A minor product (5%) was isolated and identified as the 5′-*O*-acetyl derivative of **17** by ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. Oxidation of the methylthio group of **16** with *m*-chloroperbenzoic acid (MCPBA)[27] to afford the corresponding sulfone was attempted in order to make it a better leaving group. Surprisingly, the only product obtained was the 5,7-dione derivative **15**. The oxazine ring of compound **17** was very sensitive to acid or base. UV studies revealed that complete decomposition of **17** in methanol required about 2 h at pH 11 or about 1 hour at pH 1. Attempted debenzylation of **17** by hydrogenolysis over 20% Pd/C at 50 psi was unsuccessful. Due to the apparent difference in stability between the oxazine ring and the benzyl protecting group, we found it extremely difficult to deblock the *N*-benzyl group without destroying the oxazine ring. This prompted us to initiate an alternate route.

In our second approach, pyrazofurin (**7**) was treated with benzyl bromide and sodium carbonate to give 4-*O*-benzylpyrazofurin (**18**)[28] in an excellent yield (Scheme III). Silylation of **18** with *tert*-butyldimethylsilyl (TBDMS) chloride afforded 4-*O*-benzyl-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)pyrazofurin (**19**). Although a large excess of the silylating agent was used, the pyrazole nitrogen (N1 or N2) remained unsilylated. We assumed that the *N*-TBDMS group was too unstable and was hydrolyzed upon the liquid–liquid workup.

Hydrogenolysis of **19** over Pd/C gave 2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)pyrazofurin (**20**) in a 98% overall yield from **18**. Cyclization of **20** with TCDI and subsequent methylation with methyl iodide furnished 5-(methylthio)-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrazolo[3,4-*e*][1,3]oxazin-7-one (**21**) in 62% yield. Replacement of the methylthio group with an amino group was attempted by treatment of **21** with ammonia in THF. Under these reaction conditions, only the ring-opened products **22** (44%) and **23** (16%) were isolated along with 35% of the starting material (**21**). The mass

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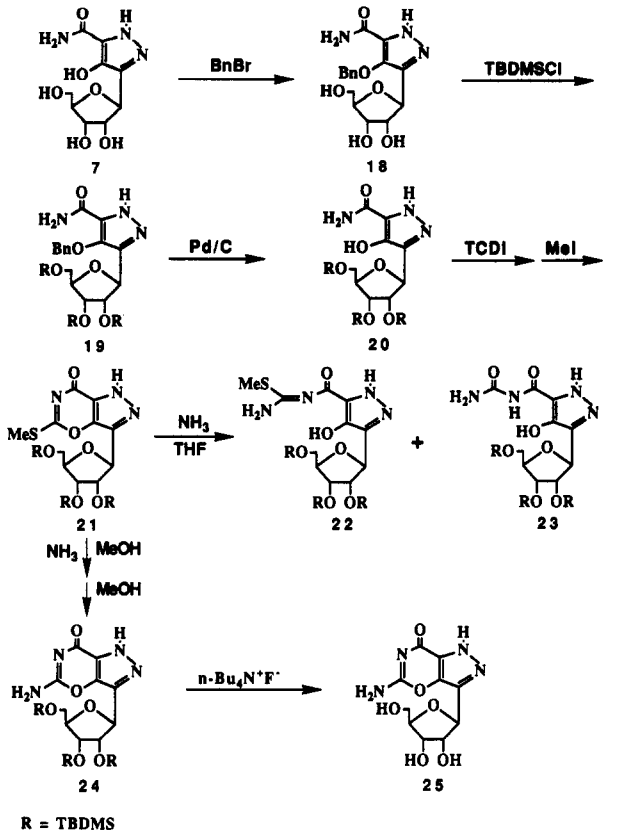
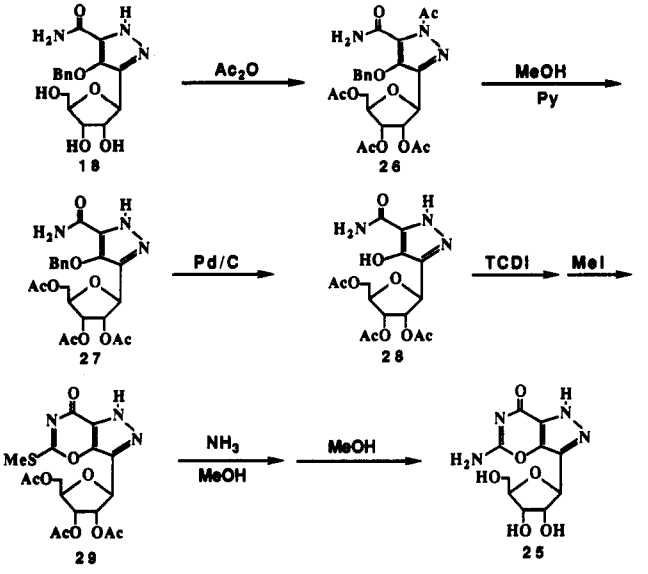
Scheme III



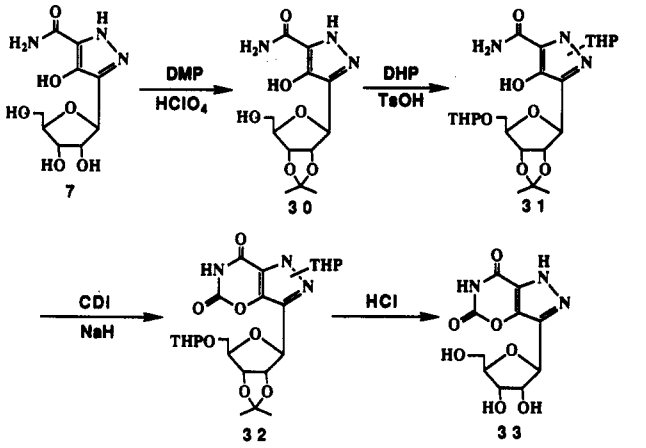
spectra (FAB) of **22** and **23** showed molecular ions of m/z 675 (46, MH^+ = 675) and m/z 645 (7, MH^+ = 645), and both compounds gave a positive ferric chloride test. The ^1H NMR signal of **22** at δ 2.48 (s, 3, MeS) and those of **23** at 8.73, 8.14, 7.44, 5.41 (4 × br s, 4, 5-CONHCONH$_2$, 4-OH) provided additional support for the ring-opened structures **22** and **23**. An attempt to recyclize **22** by treatment with sodium hydride in THF was unsuccessful. An alternative treatment of **21** with methanolic ammonia was followed by the removal of ammonia and stirring the residue again in methanol. This gave 5-amino-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrazolo[3,4-*e*][1,3]-oxazin-7-one (**24**) in 42% yield. Compound **24** gave a negative ferric chloride test, and its spectral properties were similar to those of the 5-amino derivative **17**. Desilylation of **24** was effected with tetra-*n*-butylammonium fluoride. Although TLC indicated the formation of a single product, the isolation and purification of the desired product was difficult and we were unable to obtain a pure sample of 5-amino-3-β-D-ribofuranosylpyrazolo[3,4-*e*]-[1,3]oxazin-7-one (**25**) for analysis.

Although our second approach failed to give the pure compound **25**, it demonstrated that a cyclization of **20** with TCDI and subsequent methylation of the intermediate product was possible without any protection of the pyrazole nitrogen. This prompted us to modify our first approach by leaving the pyrazole nitrogen unprotected. Acetylation of 4-*O*-benzylpyrazofurin (**18**) with acetic anhydride in pyridine afforded the tetraacetyl intermediate **26** (Scheme IV). The subsequent addition of methanol to this reaction mixture resulted in a selective N-deacetylation to give the triacetyl derivative **27** in 97% yield. The purpose of removing this acetyl group was to eliminate the problem of acetyl migration from the N1 position to the carboxamide nitrogen at the cyclization step. Hydrogenolysis of **27** furnished 2′,3′,5′-tri-*O*-acetylpyrazofurin[29a] (**28**) in 96% yield. Successive treatment of

Scheme IV



Scheme V



28 with TCDI and methyl iodide gave 5-(methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*e*][1,3]-oxazin-7-one (**29**) in 41% yield. It is believed that the much lower yield, compared to the cyclization of **12**, was due to side reactions on the unprotected pyrazole nitrogen. Analogous treatment of **29** with methanolic ammonia and subsequent recyclization of the intermediate in methanol yielded the target compound (**25**). Pure **25** was obtained in 20% yield by a successive chromatographic separation on a RP (C-18) silica column followed by recrystallization.

The synthesis of 3-β-D-ribofuranosylpyrazolo[3,4-*e*]-[1,3]oxazine-5,7-dione (**33**)[11] was achieved via a four-step reaction sequence (Scheme V). The 2′,3′-*O*-isopropylidene derivative (**30**) of pyrazofurin has been reported as a precursor to the total synthesis of pyrazofurin.[23] It has also been synthesized from pyrazofurin with *p*-toluenesulfonic acid as a catalyst.[29a] However, this reaction took 3 days to complete, and no yield or analytical data were reported. We employed a modified procedure utilizing perchloric acid as catalyst and this gave the 2′,3′-*O*-isopropylidene derivative (**30**) in 87% yield. Selective protection of the pyrazole nitrogen and the 5′-hydroxy group was effected by treatment of **30** with 3,4-dihydro-2*H*-pyran

(29) (a) Compound **28** was listed in a patent and was prepared by a different method: Gutowski, G. E. U.S. Patent 3960836, 1976; 4053698, 1977. (b) Compound **33** was listed in a patent and was prepared by a different method: Kato, T.; Katagiri, N. U.S. Patent 4656260, 1987; Canadian Patent 1228852, 1987.

(DHP) and *p*-toluenesulfonic acid. After chromatographic separation, the appropriately protected pyrazofurin derivative (**31**) was obtained as a mixture of the N1 and N2 positional isomers and their diastereomers. Cyclization of **31** with CDI furnished the corresponding pyrazolo[3,4-*e*][1,3]oxazine-5,7-dione (**32**) in 80% yield. Complete removal of all the THP groups and the isopropylidene group was achieved by treatment of **32** with HCl gas in dichloromethane. The target compound 3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazine-5,7-dione (**33**)[11,29b] was isolated in 57% yield. Its spectral (^{1}H NMR, MS, UV, and IR) properties were in agreement with its structure.

^{1}H NMR studies showed that the N1-unprotected pyrazofurin derivatives and pyrazolo[3,4-*e*][1,3]oxazines exist in tautomeric forms in DMSO-d_6 based on the fact that the ^{13}C proton signals for the heterocyclic carbons were broadened or depleted. In contrast, only one set of proton signals were observed in $CDCl_3$. However, the resolution was usually poor, and signals were broadened due to tautomerism.

Antitumor Results and Discussion

The capacity of the target compounds to inhibit growth of tumor cells in vitro was evaluated with use of L1210 murine leukemic cells. The xanthosine analogue **33** was highly cytotoxic with an IC_{50} of 0.2 μM. The guanosine analogue **25** was much less cytotoxic with IC_{50} = 100 μM. To obtain a preliminary indication of the biochemical mechanism of action and route of metabolic activation of **33**, the ability of various natural nucleosides to protect L1210 cells from its growth-inhibitory effect was tested. Total protection from growth inhibition by **33** at concentrations up to 10 μM, the highest tested, was provided by 1 mM uridine or by 100 μM cytidine. In contrast, 10 μM adenosine, 10 μM thymidine, or 50 μM hypoxanthine did not significantly affect the growth inhibition by **33**. These results strongly suggested that **33** exerted its growth-inhibitory effect by specifically inhibiting the biosynthesis of pyrimidine nucleotides. Uridine can be metabolized to UMP via the reutilization pathways for pyrimidine nucleosides, and cytidine is converted to uridine in the tissue culture medium,[30] presumably by cytidine deaminase in the horse serum it contains. In either case an exogenous source of pyrimidine nucleotides has been provided, enabling the cells to grow normally without synthesizing pyrimidine nucleotides endogenously.

These results with **33** are very similar to those reported previously for pyrazofurin (**7**) in several cell lines.[31] In the present studies with L1210 cells, the IC_{50} for pyrazofurin was the same as for **33**, 0.2 μM. The results on protection by natural nucleosides and bases also were identical with those obtained for **33**: Complete protection from the growth-inhibitory effect of pyrazofurin up to 10 μM, the highest concentration tested, by 1 mM uridine or 100 μM cytidine. No protection by 10 μM adenosine, 10 μM thymidine, or 50 μM hypoxanthine. These results are as would be expected since pyrazofurin is known to block the endogenous synthesis of pyrimidine nucleotides due to the inhibition of orotidylate decarboxylase by pyrazofurin monophosphate.[32]

In addition, the pyrazolo[3,4-*e*][1,3]oxazine ring system was observed in chemical studies to be unstable, suggesting the possibility that **33** might exert its cytotoxic action after a conversion to pyrazofurin nucleotides. This conversion might occur at the nucleoside level, spontaneously in the cell cultures, providing pyrazofurin which would then enter the cells. To further investigate this possibility, the UV spectrum of **33** was monitored with time of incubation in phosphate-buffered saline at 38 °C, the same ionic strength, pH, and temperature as for cell cultures. The UV spectra of pyrazofurin and of **33** are shown in Figure 1A. The shift of the absorbance maximum for the solution of **33** from 240 (λ_{max} of **33**) to 305 nm (λ_{max} of pyrazofurin) is shown by the spectra taken hourly (Figure 1B). The final spectrum after 24 h is also shown in Figure 1A. Using the extinction coefficients of **33** and pyrazofurin, it was calculated that approximately 65% of the **33** was converted to pyrazofurin in 24 h. In conclusion, the inhibition of L1210 cell growth observed in cultures treated with **33** appeared to be primarily due to its conversion to pyrazofurin.

The activation of pyrazofurin to its monophosphate is catalyzed by adenosine kinase.[32] Therefore, it would be expected that 5-iodotubercidin (ITu), a potent inhibitor of adenosine kinase,[33] would at least partially protect the cells from the growth-inhibitory effect of pyrazofurin, as

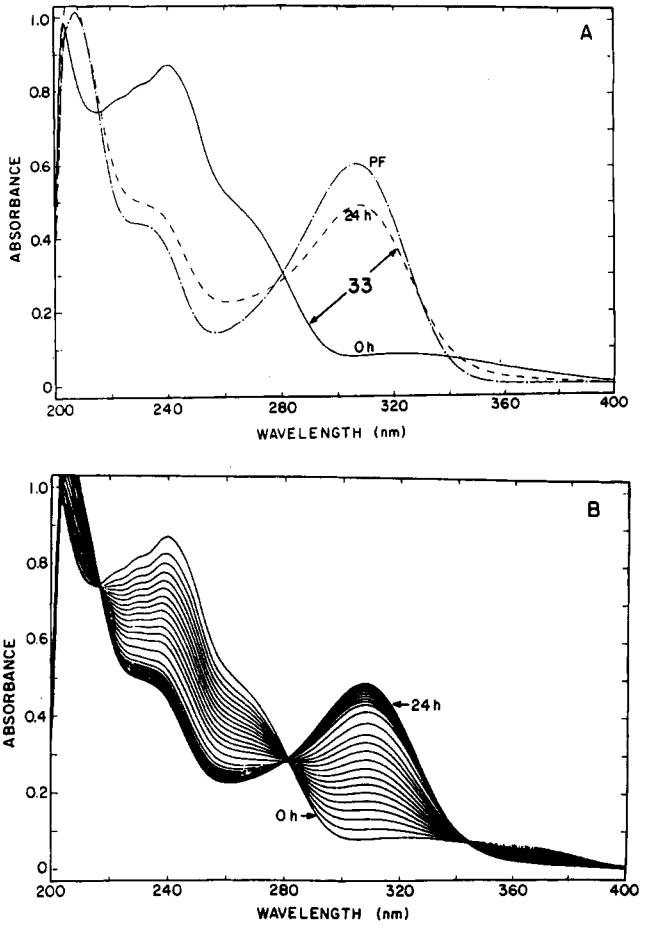


Figure 1. Spontaneous conversion of the xanthosine analogue **33** to pyrazofurin (PF). A solution of **33** in phosphate-buffered saline (0.025 M KH_2PO_4, 0.1 M NaCl, pH 7.6) was incubated at 38 °C and the UV spectrum was obtained at zero time and at hourly intervals for 24 h. A, UV spectra of 95 μM **33**, freshly prepared (—) and after 24 h at 38 °C (- - -). The spectrum of 79 μM PF (stable under these conditions) is shown for comparison (—·—). B, spectra recorded hourly during incubation of 95 μM **33** as described above.

(30) Wotring, L. L.; Borysko, K. Z. Unpublished observations.
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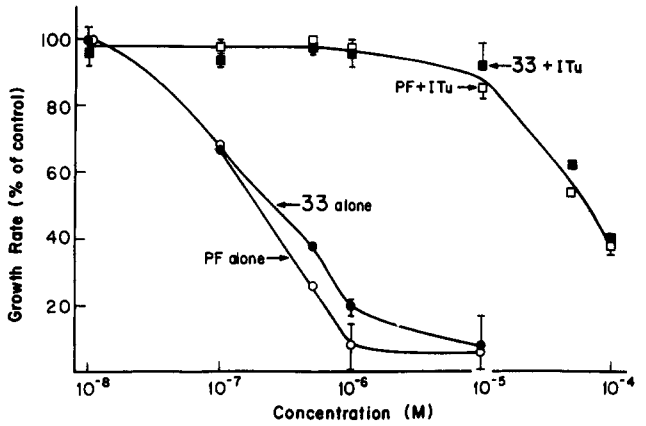

Figure 2. Protection of L1210 cells from the growth inhibitory effects of **33** and pyrazofurin (PF) by 5-iodotubercidin (ITu). L1210 cells were treated continuously with the indicated compounds, which were added to the cultures simultaneously. Growth rate was determined as described in the Experimental Section for **33** alone (●), **33** + ITu (0.1 μM) (■), PF alone (○), and PF + ITu (0.1 μM) (□). The values are averages of two to three independent determinations in separate experiments, except for 50 and 100 μM **33** + ITu, which are from a single experiment. Bars indicate range. Where no bar is shown the range is within the size of the point, except for the two single experiments mentioned above.

demonstrated previously for several cytotoxic adenosine analogues.[34,35] If as proposed above, the cytotoxicity of **33** is primarily due to its conversion to pyrazofurin in the cell culture medium, ITu would also be expected to protect cells from growth inhibition by **33**. As shown in Figure 2, the addition of 0.1 μM ITu to L1210 cell cultures simultaneously with **33** or pyrazofurin caused an increase of the IC_{50} for both compounds from 0.2 μM to 70 μM. These results support the conclusion that adenosine kinase is required for the activation of **33** as well as pyrazofurin, and are consistent with the hypothesis that **33** is converted to pyrazofurin, in the cell culture medium and/or inside the cells, and then activated to pyrazofurin monophosphate by adenosine kinase.

In conclusion, it appears that due to the instability of the pyrazolo[3,4-*e*][1,3]oxazine ring system, the highly cytotoxic xanthosine analogue **33** acts primarily as a prodrug form of the previously reported antitumor nucleoside pyrazofurin.[21] The guanosine analogue **25** was much less cytotoxic, indicating that either the ring system was more stable with the 5-amino than with the 5-one substituent, or if it was not, that breakdown of **25** did not yield pyrazofurin. As for the comparison of **25** with oxanosine, it appeared that the two compounds have similar cytotoxic potency. Recalculation of the IC_{50} from the data of Yagisawa et al.[13] on the same basis as the current study gave a value of 20 μM for inhibition of L1210 cell growth by oxanosine, slightly lower than the value of 100 μM obtained here for **25**.

Experimental Section

Chemistry. General Procedures. Melting points (mp) were taken on a Thomas-Hoover Unimelt apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WM-360 spectrometer operating in FT mode. The chemical shift values were reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. Mass spectra (MS) were determined by the Mass Spectrometry Laboratory of the Chemistry Department, University of Michigan. High-resolution MS measurements were obtained on a VG 70-250-S MS spectrometer using a direct probe for sample introduction. Nominal MS spectra were obtained on a Finnigan 4021 instrument. Ultraviolet (UV) absorption spectra were recorded with a Hewlett-Packard 8450A diode array spectrophotometer and infrared (IR) spectra were recorded on a Perkin-Elmer 281 spectrometer. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Chemical reactions and column chromatographic separations were followed by thin-layer chromatography (TLC) on silica gel precoated glass plates (layer thickness 0.2 mm) purchased from Analtech, Inc. The TLC plates were observed under UV light (254 nm). Evaporations were effected with a Buchler flash evaporator equipped with a Dewar "dry ice" condenser under water aspirator or mechanical oil pump vacuum at 40 °C or cooler.

1-Benzyl-4-(benzyloxy)-3-β-D-ribofuranosylpyrazole-5-carboxamide (1,4-*O*-Dibenzylpyrazofurin) (8) and 1-Benzyl-4-(benzyloxy)-5-β-D-ribofuranosylpyrazole-3-carboxamide (2,4-*O*-Dibenzylpyrazofurin) (9). A solution of benzyl bromide (2.38 mL, 20 mmol) in 50 mL of CH_3CN was added to a solution of pyrazofurin (7, 2.59 g, 10 mmol) and Na_2CO_3 (2.12 g, 20 mmol) in 50 mL of H_2O. The reaction mixture was stirred at room temperature for 6 days with fresh benzyl bromide/CH_3CN (2.38 mL/25 mL) and Na_2CO_3/H_2O (2.12 g/25 mL) being added every 2 days. The reaction mixture was then extracted with $CHCl_3$ (200 mL × 2), and the $CHCl_3$ solution was washed with saturated NaCl solution (200 mL × 2), dried (Na_2SO_4), and evaporated. The resulting residue was chromatographed on a silica column (3.8 × 26 cm, eluted successively with 1%, 3%, and 5% MeOH/$CHCl_3$ v/v). Evaporation of the appropriate fractions gave 2.86 g (65%) of **8** as a white solid: MS (EI) *m/z* 439.1734 (1, M^+ = 439.1743); UV (MeOH)[36] λ max 253 nm, (MeOH, pH 1) λ max 253 nm, (MeOH, pH 11) λ max 253 nm; ^1H NMR (DMSO-d_6) δ 7.72, 7.15 (2 × s, 2, NH_2), 7.50–7.00 (m, 10, 2 × Ph), 5.64 (m, 2, NCH_2Ph), 5.08 (2 d, 2, OCH_2Ph); ^{13}C NMR (DMSO-d_6)[37] δ 159.72 ($CONH_2$), 137.74, 136.27, 128.35, 127.31, 127.06 (Ph), 85.03 (C4'), 77.35 (OCH_2Ph), (NCH_2Ph).

Further elution with 7% MeOH/$CHCl_3$ v/v and evaporation of the appropriate fractions gave 0.73 g (17%) of **9** as a white foam: MS (EI) *m/z* 439.1747 (3, M^+ = 439.1743); UV (MeOH)[36] λ max ~250 nm (sh), (MeOH, pH 1) λ max ~250 nm (sh), (MeOH, pH 11) λ max ~250 nm (sh); ^1H NMR (DMSO-d_6) δ 7.60–7.10 (m, 12, NH_2 and 2 × Ph), 5.44 (2 × d, 2, NCH_2Ph), 5.05 (2 × d, 2, OCH_2Ph).

1-Benzyl-4-hydroxy-3-β-D-ribofuranosylpyrazole-5-carboxamide (1-Benzylpyrazofurin) (10). A mixture of 1,4-*O*-dibenzylpyrazofurin (**8**, 1.38 g, 3.14 mmol) and 0.15 g of 5% Pd/C in 50 mL of EtOH was hydrogenated at room temperature, under 50 psi of hydrogen for 3 h. This mixture was then filtered, and filtrate was evaporated and coevaporated with EtOH and toluene to give 1.07 g (98%) of **10** as a white solid: MS (EI) *m/z* 349.1282 (6, M^+ = 349.1274); UV (MeOH)[36] λ max 231 and 271 nm, (MeOH, pH 1) max 231 and 271 nm, (MeOH, pH 11) λ max 236 nm (sh) and 313 nm; ^1H NMR (DMSO-d_6) δ 10.0–8.0 (very br s, 1, 4-OH), 7.53, 7.09 (2 × br s, 2, NH_2), 7.40–7.00 (m, 5, Ph), 5.63 (2 × d, 2, CH_2Ph).

1-Benzyl-4-acetoxy-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazole-5-carboxamide (1-Benzyl-4,2',3',5'-tetra-*O*-acetylpyrazofurin) (11). Acetic anhydride (0.8 mL) was added to a solution of **10** (0.34 g, 0.97 mmol) in 5 mL of pyridine. The reaction mixture was stirred at room temperature for 5 h, and volatile materials were evaporated. The residue was coevaporated with toluene (3×), $CHCl_3$ (3×), and then ether. The resulting foam was dried in vacuo to give 0.5 g (100%) of **11**: MS (EI) *m/z* 517.1703 (21, M^+ = 517.1696); UV (MeOH, neutral or pH 2)[36] apparent absorption between 220–270 nm with no peaks, (MeOH, pH 11) λ max 254 nm (sh) and 332 nm (the large bathochromic shift was due to the apparent facile cleavage of the

(34) Wotring, L. L.; Townsend, L. B. *Cancer Res.* **1979**, *39*, 3018.
(35) Wotring, L. L.; Crabtree, G. W.; Edwards, N. L.; Parks, R. E., Jr.; Townsend, L. B. *Cancer Treat. Rep.* **1986**, *70*, 491.

(36) Acidic MeOH solution was prepared by mixing 0.9 mL of MeOH with 0.1 mL of HCl/KCl buffer solution (pH 1). Basic MeOH solution was prepared by mixing 0.9 mL of MeOH with 0.1 mL of NaOH/NaCl/glycine buffer solution (pH 11).
(37) Assignments of signals to the base carbons were tentative.

4-*O*-acetyl group under basic conditions); ^{1}H NMR (DMSO-d_6) δ 7.75, 7.50 (2 × br s, 2, NH_2), 7.40–7.10 (m, 5, Ph), 5.59 (s, 2, CH_2Ph).

1-Benzyl-4-(benzyloxy)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazole-5-carboxamide (1,4-*O*-Dibenzyl-2′,3′,5′-tri-*O*-acetylpyrazofurin) (14) and 1-Benzyl-4-hydroxy-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazole-5-carboxamide (1-Benzyl-2′,3′,5′-tri-*O*-acetylpyrazofurin) (12). Acetic anhydride (5 mL) was added to a solution of **8** (2.12 g, 4.82 mmol) in 30 mL of pyridine. The reaction mixture was stirred at room temperature for 3.5 h. Volatile materials were evaporated, and the residue was coevaporated with toluene (3×) and EtOH (3×) to give **14** as a semisolid: MS (EI) *m/z* 565.2056 (1, MH^+ = 565.2060); UV (MeOH)[36] λ max ~252 nm (sh), (MeOH, pH 1) λ max ~252 nm (sh), (MeOH, pH 11) λ max ~252 nm (sh); ^{1}H NMR (DMSO-d_6) δ 7.75, 7.22 (2 × br s, 2, NH_2), 7.50–7.00 (m, 10, 2 × Ph), 5.60 (s, 2, NCH_2Ph), 5.07 (s, 2, OCH_2Ph).

EtOH (100 mL) and 0.20 g of 20% Pd/C were added to the above product **14**. The reaction mixture was hydrogenated at room temperature, under 50 psi of hydrogen overnight. The solid materials were removed by filtration and the filtrate was evaporated to give 2.15 g of **12** (94% from **8**) as a white foam: MS (EI) *m/z* 475.1581 (3, MH^+ = 475.1591); UV (MeOH)[36] λ max 231 and 267 nm, (MeOH, pH 1) λ max 231 and 267 nm, (MeOH, pH 11) λ max 236 nm (sh) and 314 nm; ^{1}H NMR (DMSO-d_6) δ 9.96 (s, 1, 4-OH), 7.60, 7.20 (2 × br s, NH_2), 7.30–7.00 (m, 5, Ph), 5.65 (s, 2, CH_2Ph), 2.07, 2.02, 1.95 (3 × s, 9, 3 × Ac).

1-Benzyl-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*e*][1,3]oxazine-5,7-dione (15). To a solution of 0.340 g (0.715 mmol) of **12** in 10 mL of dry THF was added 0.086 g of NaH (60% in oil). After the bubbling had ceased, CDI (0.232 g, 1.43 mmol) was added and the reaction mixture was stirred at 60 °C for 1 h. Volatile materials were evaporated, and the residue was partitioned between EtOAc/NH_4Cl solution (50 mL/50 mL). The EtOAc solution was washed with NH_4Cl solution (50 mL), dried (Na_2SO_4), evaporated, and coevaporated with toluene (2×) and MeOH (2×) to give a white foam. This foam was purified on a silica column (2 × 25 cm) using 1% and 3% MeOH/$CHCl_3$ v/v as eluants. Evaporation of the appropriate fractions gave 0.243 g (68%) of **15** as a white foam: MS (FAB) *m/z* 502 (15, MH^+ = 502); UV (MeOH)[36] λ max 236 and 264 nm, (MeOH, pH 1) λ max 236 and 264 nm, (MeOH, pH 11) λ max 244 nm; ^{1}H NMR (DMSO-d_6) δ 12.15 (br s, 1, 6-NH), 7.30 (m, 5, Ph), 5.58 (s, 2, CH_2Ph).

1-Benzyl-5-(methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*e*][1,3]oxazin-7-one (16). To a solution of **12** (1.273 g, 2.677 mmol) in 30 mL of dry THF was added 0.161 g of NaH (60% in oil). After the bubbling had ceased, TCDI (0.621 g, 3.484 mmol) was added and the reaction mixture was stirred at room temperature for 4.5 h. Methyl iodide (0.5 mL, 8.032 mmol) was added to the mixture and stirring was continued at room temperature overnight. Volatile materials were evaporated, and the residue was suspended in 100 mL of $CHCl_3$. The suspension was filtered and the filtrate was washed with H_2O (70 mL × 3), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (2 × 25 cm) using $CHCl_3$ as the eluant. Evaporation of the appropriate fractions gave 1.320 g (93%) of **16** as a yellowish foam: MS (EI) *m/z* 531.1298 (4, MH^+ = 531.1312); UV (MeOH)[36] λ max 236 and 265 nm, (MeOH, pH 1) λ max 236 and 265 nm, (MeOH, pH 11) λ max 236 nm and 265 nm; ^{1}H NMR (DMSO-d_6) δ 7.30 (m, 5, Ph), 5.66 (s, 2, CH_2Ph), 2.60 (s, 3, MeS), 2.09, 2.01, 1.98 (3 × s, 9, 3 × Ac).

5-Amino-1-benzyl-3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazin-7-one (17). A mixture of **16** (2.95 g, 5.55 mmol) in 110 mL of NH_3/MeOH was stirred at room temperature for 5 h. Volatile materials were removed by evaporation and coevaporation with MeOH. The residue was stirred in 20 mL of MeOH at room temperature overnight. The resulting suspension was allowed to stand at 5 °C for 1 day and then filtered. The solid product was absorbed on a small amount of silica and chromatographed on a silica column (2 × 15 cm, eluted successively with 5%, 10%, 15%, 20% MeOH/$CHCl_3$ v/v). Evaporation of the appropriate fractions and recrystallization from MeOH gave 564 mg (27%) of **17** as white crystalline needles: mp 208–211 °C dec; MS (FAB) *m/z* 375.1299 (100, MH^+ = 375.1305); UV (MeOH)[36] λ max 248 nm, (MeOH, pH 1) λ max 248 nm, (MeOH, pH 11) λ max 248 nm; ^{1}H NMR (DMSO-d_6) δ 8.25, 8.16 (2 overlapping br s, 2, NH_2), 7.30 (m, 5, Ph), 5.60 (m, 2, PhCH_2). Anal. ($C_{17}H_{18}N_4O_6$) C, H, N.

A minor product was isolated from the column and was purified by recrystallization from MeOH. This compound (121 mg, 5%, as white crystals) was characterized as the 5′-*O*-acetyl derivative of **17**: mp 215–216 °C; MS (FAB) *m/z* 417.1407 (100%, MH^+ = 417.1410); UV (MeOH)[36] λ max 248 nm, (MeOH, pH 1) λ max 248 nm, (MeOH, pH 11) λ max 248 nm; ^{1}H NMR (DMSO-d_6) δ 8.26, 8.14 (2 overlapping br s, 2, NH_2), 7.30 (m, 5, Ph), 5.60 (s, 2, PhCH_2), 1.96 (s, 3, 5-OAc). Anal. ($C_{19}H_{20}N_4O_7$) C, H, N.

4-(Benzyloxy)-3(5)-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrazole-5(3)-carboxamide (4-*O*-Benzyl-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)pyrazofurin) (19) and 4-Hydroxy-3(5)-(2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)pyrazofurin) (20). Imidazole (8.170 g, 120 mmol) and TBDMSCl (9.044 g, 60 mmol) were added to a solution of 3.493 g (10 mmol) of **18**[28] in 50 mL of DMF. The reaction mixture was stirred at room temperature for 20 h. Volatile materials were evaporated and coevaporated with toluene (3×). The residue was partitioned between hexane/H_2O (100 mL/100 mL). The H_2O layer was extracted with hexane (100 mL × 2). The combined hexane layer was washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and evaporated. The resulting foam (**19**) was dried in vacuo over MeOH for 10 h: UV (MeOH)[36] λ max ~250 nm (sh), (MeOH, pH 1) λ max ~250 nm (sh), (MeOH, pH 11) λ max ~250 nm (sh); MS (CI) *m/z* 692.3920 (100, MH^+ = 692.3946); ^{1}H NMR ($CDCl_3$) δ 7.38 (m, 5, Ph), 6.78, 5.49 (2 × br s, 2, NH_2), 5.15 (2 × d, 2, PhCH_2), 4.88 (s, 1, 1′-H), 0.94, 0.87, 0.84 (3 × s, 27, 3 × *t*-Bu), 0.16, 0.04, 0.02, 0.01, –0.01 (5 × s, 18, 6 × Me).

The above foam was dissolved in 100 mL of EtOH. The solution was hydrogenated at room temperature, under 50 psi of hydrogen for 3 h with 0.30 g of 20% Pd/C as catalyst. The reaction mixture was filtered and the solid was washed with MeOH. The filtrate and washings were evaporated to give 5.922 g (98%) of **20** as a white foam: MS (FAB) *m/z* 602 (5, MH^+ = 602); UV (MeOH)[36] λ max 225 and 266 nm, (MeOH, pH 1) λ max 225 and 266 nm, (MeOH, pH 11) λ max 236 nm (sh) and 314 nm; ^{1}H NMR ($CDCl_3$) δ 11.15 [br s, 1, 1(or 2)-NH], 7.76 (br s, 1, 4-OH), 6.68, 5.77 (2 × br s, 2, NH_2), 0.95, 0.88, 0.87, (3 × 27, 3 × *t*-Bu), 0.17, 0.16, 0.05, 0.04, 0.02, 0.01 (6 × s, 18, 6 × Me).

5-(Methylthio)-3-[2,3,5-tris-*O*-(*tert*-butyldimethysilyl)-β-D-ribofuranosyl]pyrazolo[3,4-*e*][1,3]oxazin-7-one (21). NaH (0.115 g, 60% in oil) was added to a solution of 2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)pyrazofurin (**20**, 1.575 g, 2.616 mmol) in 25 mL of THF at room temperature. After the bubbling had ceased, TCDI (0.559 g, 3.139 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Methyl iodide (10% by volume in THF, 1.95 mL, 3.134 mmol) was then added and stirring was continued at room temperature overnight. Volatile materials were removed by evaporation, and the residue was partitioned between H_2O/$CHCl_3$ (100 mL/100 mL). The $CHCl_3$ layer was washed with H_2O (100 mL × 2), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (3 × 15 cm, eluted with $CHCl_3$, 1%, 2%, MeOH/$CHCl_3$ v/v). Evaporation of the appropriate fractions gave 1.073 g (62%) of **21** as a yellowish foam: MS (FAB) *m/z* 658 (73, MH^+ = 658); UV (MeOH)[36] λ max 236 and 261 nm, (MeOH, pH 1) λ max 236 and 261 nm, (MeOH, pH 11) λ max 239 and 272 nm; ^{1}H NMR ($CDCl_3$) δ 5.09 (d, 1, 1′-H, $J_{1'-2'}$ = 4.0 Hz), 2.62 (s, 3, MeS), 0.90, 0.89, 0.81 (3 × s, 27, 3 × *t*-Bu), 0.12, 0.11, 0.04, 0.03, 0.00, –0.10 (6 × s, 18, 6 × Me).

5-Amino-3-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrazolo[3,4-*e*][1,3]oxazin-7-one (24). A mixture of **21** (0.141 g, 0.214 mmol) in 5 mL of NH_3/MeOH was stirred at room temperature for 2 h. The NH_3/MeOH was removed by evaporation and coevaporation with MeOH. The residue was stirred in 10 mL of MeOH at 65 °C for 8 h. Volatile materials were evaporated, and the residue was chromatographed on a silica column (2 × 8 cm, eluted successively with $CHCl_3$, 2%, 4% MeOH/$CHCl_3$ v/v). Evaporation of the appropriate fractions gave 0.057 g (42%) of **24** as a white solid: MS (FAB) *m/z* 627 (34, MH^+ = 627); UV (MeOH)[36] λ max 245 nm, (MeOH, pH 1) λ max 244 nm, (MeOH, pH 11) λ max 248 nm; ^{1}H NMR

(DMSO-d_6) δ 8.13, 8.07 (2 overlaping br s, 2, NH_2), 4.80 (d, 1, 1'-H, $J_{1'-2'}$ = 7.0 Hz).

4-(Benzyloxy)-3(5)-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (4-*O*-Benzyl-2',3',5'-tri-*O*-acetylpyrazofurin) (27). A mixture of 18[28] (1.69 g, 4.838 mmol) in 30 mL of pyridine and 3.65 mL of acetic anhydride was stirred at room temperature for 5 h. MeOH (15 mL) was added and stirring was continued at room temperature for 20 h. Volatile materials were removed by evaporation and coevaporation with toluene (3×). The residue was chromatographed on a silica column (3.2 × 18 cm, eluted successively with pure $CHCl_3$, 1%, 2%, 3% MeOH/$CHCl_3$ v/v). Evaporation of the appropriate fractions gave 2.23 g (97%) of **27** as a white foam: MS (CI) *m/z* 476.1672 (100, MH^+ = 476.1669); UV (MeOH)[36] λ max ~250 nm (sh), (MeOH, pH 1) λ max ~250 nm (sh), (MeOH, pH 11) λ max ~250 nm (sh); ^1H NMR ($CDCl_3$) δ 12.15 [br s, 1, 1(or 2)-NH], 7.35 (m, 5, Ph), 6.74, 6.13 (2 × br s, 2, NH_2), 2.08, 2.05, 1.96 (3 × s, 9, 3 × Ac).

4-Hydroxy-3(5)-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazole-5(3)-carboxamide (2',3',5'-Tri-*O*-acetylpyrazofurin) (28). A solution of **27** (0.980 g, 2.061 mmol) in 20 mL of EtOH was hydrogenated at room temperature, under 50 psi of hydrogen for 5 h with 0.040 g of 20% Pd/C as catalyst. The reaction mixture was then filtered and the solid was washed with portions of MeOH. The filtrate and washings were combined and evaporated to give 0.765 g (96%) of **28** as a white foam: MS (CI) *m/z* 386.1198 (100, MH^+ = 386.1200); UV (MeOH)[36] λ max 224 and 264 nm, (MeOH, pH 1) λ max 225 and 264 nm, (MeOH, pH 11) λ max 309 nm; ^1H NMR ($CDCl_3$) δ 7.89 (br s, 1, 4-OH), 6.95, 6.33 (2 × br s, 2, NH_2), 2.08, 2.07, (2 × s, 9, 3 × Ac).

5-(Methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*e*][1,3]oxazin-7-one (29). NaH (0.044 g, 60% in oil, 1.1 mmol) was added to a solution of 2',3',5'-tri-*O*-acetylpyrazofurin (**28**) (0.385 g, 1.0 mmol) in 10 mL of THF at room temperature. After the bubbling had ceased, 0.214 g (1.2 mmol) of TCDI was added. The reaction mixture was stirred at room temperature for 1.5 h. Methyl iodide (10% by volume in THF, 0.747 mL, 1.2 mmol) was then added and stirring was continued at room temperature overnight. Volatile materials were removed by evaporation, and the residue was partitioned between H_2O/$CHCl_3$ (50 mL/50 mL). The $CHCl_3$ layer was washed with H_2O (50 mL × 2), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (2 × 8 cm, eluted successively with pure $CHCl_3$, 1%, MeOH/$CHCl_3$ v/v). Evaporation of the appropriate fractions gave 0.181 g (41%) of **29** as a white foam: MS (FAB) *m/z* 442 (73, MH^+ = 442); UV (MeOH)[36] λ max 234 and 260 nm, (MeOH, pH 1) λ max 234 and 261 nm, (MeOH, pH 11) λ max 240 and 271 nm; ^1H NMR ($CDCl_3$) δ 12.40 [br s, 1, 1(or 2)-NH], 2.63 (s, 3, MeS), 2.11, 2.08, 2.06 (3 × s, 9, 3 × Ac).

5-Amino-3-β-D-ribofuranosylpyrazolo[3,4-*e*][1,3]oxazin-7-one (25). A mixture of **29** (0.765 g, 1.733 mmol) in 20 mL of NH_3/MeOH was stirred at room temperature for 5 h. Volatile materials were removed by evaporation and coevaporation with MeOH. The residue was stirred in 5 mL of MeOH at room temperature overnight. The resulting suspension was filtered to give a yellowish solid. The solid product was dissolved in 5 mL of H_2O and was then chromatographed on a RP (C-18) silica column (2 × 28 cm, eluted with pure H_2O). Evaporation of the appropriate fractions and recrystallization of the residue from H_2O/MeOH gave 100 mg (20%) of **25** as a white crystalline powder: mp 230–235 °C dec; MS (FAB) *m/z* 285.0846 (100, MH^+ = 285.0835); UV (MeOH)[36] λ max 244 nm, (MeOH, pH 1) λ max 243 nm, (MeOH, pH 11) λ max 245 nm; ^1H NMR (DMSO-d_6) for the major tautomer, δ 13.85 [br s, 1, 1(or 2)-NH], 8.12, 8.08 (2 overlaping br s, 2, NH_2), 5.07 (d, 1, 2'-OH), 4.91 (d, 1, 3'-OH), 4.70 (m, 2, 5'-OH, 1'-H), 4.30 (m, 1, 2'-H), 3.94 (m, 1, 3'-H), 3.78 (m, 1, 4'-H), 3.50 (m, 1, 5'-H), 3.44 (m, 1, 5''-H). Anal. ($C_{10}H_{12}N_4O_6 \cdot 0.5H_2O$) C, H, N.

3(5)-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-4-hydroxypyrazole-5(3)-carboxamide (2',3'-*O*-Isopropylidenepyrazofurin) (30). 2,2-Dimethoxypropane (7 mL) and 70% perchloric acid (6 mL) were added into a 500-mL round-bottom flask containing 165 mL of acetone (predried over Na_2SO_4). The flask was stoppered and the solution was stirred at room temperature for 6 min. Pyrazofurin (**7**, 3.00 g, 11.573 mmol) was then added quickly, and the resulting clear yellow solution was stirred for 20 min. The reaction was terminated by adding 13.5 mL of pyridine, 60 mL of H_2O, and 6.5 mL of 58% NH_4OH. The reaction mixture was concentrated in vacuo until acetone was removed. A second portion of NH_4OH was added. The mixture was washed with CH_2Cl_2 (30 mL × 2) and then cooled to 0–5 °C. Glacial acetic acid was added dropwise to adjust the pH value of the solution to 5.5–6 and the solution was evaporated to dryness at 25–30 °C (1–2 mmHg). The residue was coevaporated with benzene (30 mL × 3), transfered into a Soxhlet apparatus and extracted with chloroform (300 mL × 3, 12 h each time). The first two fractions gave 2.77 g of a foamlike off-white residue. The third fraction furnished a slightly colored oil, which was dissolved in EtOAc, filtered through a Celite pad. Evaporation of the filtrate gave 0.45 g of **30** as a white foam. The three fractions were combined and dried in vacuo (40 °C, 0.5 mmHg) to furnish 3.2 g (87%) of **30**: R_f by TLC (20% MeOH/$CHCl_3$ v/v) = 0.7; ^1H NMR (DMSO-d_6) δ 1.55, 1.41 (2 × s, 6, 2 × CH_3); MS (CI) *m/z* 300 (MH^+ = 300). Anal. ($C_{12}H_{17}N_3O_6 \cdot H_2O$) C, H, N.

4-Hydroxy-3-[2,3-*O*-isopropylidene-5-*O*-(tetrahydropyran-2-yl)-β-D-ribofuranosyl]-1-(tetrahydropyran-2-yl)-pyrazole-5-carboxamide and 4-Hydroxy-5-[2,3-*O*-isopropylidene-5-*O*-(tetrahydropyran-2-yl)-β-D-ribofuranosyl]-1-(tetrahydropyran-2-yl)pyrazole-3-carboxamide [2',3'-*O*-Isopropylidene-1(and 2),5'-*O*-bis(tetrahydropyran-2-yl)pyrazofurin] (31). 3,4-Dihydro-2*H*-pyran (5.5 mL, 53.5 mmol) in 50 mL of EtOAc was added dropwise to a solution of **30** (2.0 g, 6.303 mmol) and *p*-toluenesulfonic acid (0.254 g, 1.34 mmol) in 450 mL of EtOAc at 60 °C over 20 min. After the addition, stirring was continued at 60 °C for an additional 20 min. The mixture was then cooled to 10 °C and 4 mL of 3 N NH_4OH solution was added. The mixture was transferred into a separatory funnel and washed with cold water (~10 °C, 5 mL × 4). The washings were additionally extracted with 50 mL of EtOAc. The combined organic layers were dried (Na_2SO_4) and evaporated to give a yellow thick oil, which was then dried in vacuo (40 °C, 0.3 mmHg, 15 h). Purification by silica column chromatography (2.5 × 40 cm, eluted with 2% MeOH/$CHCl_3$ v/v) furnished 1.348 g (46%) of **31** as a white foam after drying (40 °C, 0.1 mmHg, 10 h): TLC (5% MeOH/$CHCl_3$ v/v) showed two spots with R_f 0.44 and 0.39; $FeCl_3$ test was positive; MS (CI) *m/z* 468 (MH^+ = 468). Anal. ($C_{22}H_{33}N_3O_8$) C, H, N.

3-[2,3-*O*-Isopropylidene-5-(tetrahydropyran-2-yl)-β-D-ribofuranosyl]-1(and 2)-(tetrahydropyran-2-yl)pyrazolo-[3,4-*e*][1,3]oxazine-5,7-dione (32). Solid NaH (0.062 g, 2.58 mmol) and compound **31** (0.40 g, 0.856 mmol) were added to 50 mL of dry THF under nitrogen. The mixture was stirred at 45 °C for 10 min and cooled to 25 °C, and CDI (0.278 g, 1.713 mmol) was then added. The reaction mixture was heated at reflux for 100 min, cooled to 10 °C, filtered under N_2 through glass wool into a round-bottom flask, and evaporated in vacuo to dryness. The yellow solid was treated with 50 mL of EtOAc and 20 mL of saturated NH_4Cl solution while being chilled in an ice bath. The suspension was filtered into a separatory funnel. The EtOAc solution was washed successively with saturated NH_4Cl solution (20 mL) and water (15 mL × 4), dried (Na_2SO_4), evaporated, and coevaporated with benzene (15 mL × 2). The resulting foam was dried in vacuo (40 °C, 0.1 mmHg, 15 h). Purification by LPLC (Michel-Miller column, 125 mL, SiO_2, eluted with 2% MeOH/$CHCl_3$ v/v) furnished 0.34 g (80%) of **32** as a white foam: R_f by TLC (5% MeOH/$CHCl_3$ v/v) = 0.55; MS (CI) *m/z* 493 (MH^+ = 493); UV (EtOH) λ max 232 nm (ε 7650), 266 nm (ε 4110); IR (KBr) 3240, 3130, 2940, 2850, 1780–1750 (br), 1605 cm^{-1}. Anal. ($C_{23}H_{31}N_3O_9$) C, H, N.

3-β-D-Ribofuranosylpyrazolo[3,4-*e*][1,3]oxazine-5,7-dione (33). Compound **32** (0.197 g, 0.399 mmol) was dissolved in 50 mL of dry CH_2Cl_2 and HCl (gas) was bubbled through the solution at 0 °C for 5 min. The reaction mixture was stirred at 0 °C for an additional 2 h 20 min, and the resulting suspension was filtered. The white precipitate collected by filtration was washed with hexane (5 mL × 2), hexane/ether (1:1, 5 mL × 2), and dried in vacuum at 40 °C (0.5 mmHg) for 14 h to give 0.084 g (72%) of **33**. The sample was homogeneous by TLC and showed one major peak by HPLC (MicroPac, M CH-5, 15 cm, RP-18, UV at λ 254, 2% MeOH in H_2O, retention time 12.31 min). A 0.054-g sample was additionally purified by LPLC (Lobar "B", RP-8, H_2O) to give 0.043 g of analytically pure **33** after drying in vacuo (60 °C,

0.05 mmHg, 10 h): R_f by TLC (EtOAc/Me_2CO/MeOH/H_2O 7:1:0.5:0.5 v/v/v/v) = 0.45; MS (CI) m/z 286 (MH^+ = 286); UV λ max (pH 1) 231 nm (ε 8700), 263 nm (ε 5600); (pH 7) 232 nm (ε 9000), 260 nm (sh); (pH 11) 226 nm (ε 11100), 242 nm (ε 10800), ~265 nm (br sh); IR (KBr) 3450–3000 (br), 2920 (sh), 2850 (sh), 1750 (br), 1610 cm^{-1}; ^1H NMR (D_2O) δ 4.90 (d, 1, 1′-H, $J_{1'-2'}$ = 6.8 Hz), 4.30 (pst, 1, 2′-H), 4.09 (t, 1, 3′-H), 3.95 (q, 1, 4′-H), 3.63 (m, 2, 5′-H and 5″-H). Anal. ($C_{10}H_{11}N_3O_7 \cdot 0.5H_2O$) C, H, N.

Antitumor Studies. The in vitro cytotoxicity against L1210 was evaluated as described previously.[34] L1210 cells were grown in static suspension culture at 38 °C using Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the continuous presence of various concentrations of the test compound. For studies on protection from growth inhibition, the cytotoxic compound and the compound being tested for protective capacity were added to the cultures simultaneously. Both compounds were present continuously during the 3-day period of growth-rate determination. Growth rate was defined as the slope of the semilogarithmic plot of cell number against time for the treated culture, as a percent of the slope for the control culture. Experimentally this parameter was determined by calculating the ratio of the population doubling time of control cells to the population doubling time of treated cells. When the growth rate slowed during the experiment, the rate used was the final rate attained at the end of the 3-day period. The IC_{50} was defined as the concentration required to reduce the growth rate to 50% of the control.

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Synthesis and Biological Evaluation of Quinocarcin Derivatives: Thioalkyl-Substituted Quinones and Hydroquinones

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Varieties of thioalkyl-containing quinone and hydroquinone analogues of quinocarcin (**1a**) were prepared effectively, by addition of mercaptan to **3a–c**, which were derived from **1a** via DX-52-1 (**1b**). Antitumor activities of these analogues were preliminarily evaluated by growth inhibition of HeLa S_3 cells (in vitro) and increased life span of P388 implanted mice (in vivo). Bis(alkylthio)quinones **4a–d** and **5a–d**, and corresponding hydroquinones **9b–d** exhibited high activities both in vitro and in vivo. They were superior to **1a** especially in single administration. Selected compounds **4a**, **4d**, **5a**, **5d**, and **9b** were subjected to further evaluation, and bis(methylthio)quinone **5a** was revealed to possess broad-spectrum activity toward human xenographted carcinomas MX-1, Co-3, St-4, and LC-06.

Quinocarcin (**1a**)[1] is an antitumor antibiotic isolated from the culture broths of *Streptomyces melanovinaceus*.[1a] Its structure was elucidated by NMR analysis[1b] and X-ray crystallography[1d] of quinocarcinol (**1c**), which was produced by the same organism. Quinocarcin is active against several experimental tumor systems[1e,f] and thought to exert its activity via inhibition of DNA[1c] and RNA[1e] synthesis. In spite of little information on the mode of action of **1a**, a plausible mechanism is alkylation of DNA by opening of the oxazolidine ring, which would give a highly reactive iminium ion susceptible to a nucleophilic moiety of DNA. **1a** has a significant activity against P388 leukemia and human xenograft MX-1; however, repeated daily administration is required for high efficacy. To enhance the antitumor activity and to broaden the spectrum of **1a**, we attempted the synthetic studies of its analogues.[2] Among the neoplastic agents, quinone-containing compounds continue to receive considerable attention,[3] both for clinical use and under preclinical studies (e.g. mytomycin,[4] adriamycin,[5] mitoxantrone,[6] streptonigrin,[7] saframycin,[8] and their derivatives, etc.). Additionally saframycin and

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