Furanfurin and Thiophenfurin: Two Novel Tiazofurin Analogues. Synthesis, Structure, Antitumor Activity, and Interactions with Inosine Monophosphate Dehydrogenase

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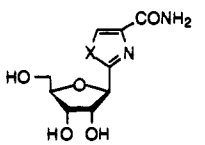
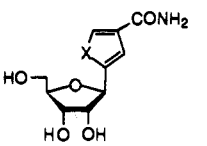
The syntheses of furan and thiophene analogues of tiazofurin (furanfurin and thiophenfurin, respectively) are described. Direct stannic chloride-catalyzed C-glycosylation of ethyl 3-furancarboxylate (**6**) or ethyl 3-thiophencarboxylate (**18**) with 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose gave 2- and 5-glycosylated regioisomers, as a mixture of α- and β-anomers, and the β-2,5-diglycosylated derivatives. Deprotection of ethyl 5-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)furan-3-carboxylate (**9β**) and ethyl 5-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)thiophene-3-carboxylate (**20β**) with sodium ethoxide afforded ethyl 5-β-D-ribofuranosylfuran-3-carboxylate (**12β**) and ethyl 5-β-D-ribofuranosylthiophene-3-carboxylate (**23β**) which were converted into 5-β-D-ribofuranosylfuran-3-carboxamide (furanfurin, **4**) and 5-β-D-ribofuranosylthiophene-3-carboxamide (thiophenfurin, **5**) by reaction with ammonium hydroxide. The anomeric configuration and the site of glycosylation were established by ^{1}H-NMR and proton–proton nuclear Overhauser effect difference spectroscopy. The structure of compound **23β** was confirmed by X-ray crystallography. Thiophenfurin was found to be cytotoxic *in vitro* toward murine lymphocytic leukemia P388 and L1210, human myelogenous leukemia K562, human promyelocytic leukemia HL-60, human colon adenocarcinoma LoVo, and B16 melanoma at concentrations similar to that of tiazofurin. In the same test furanfurin proved to be inactive. Thiophenfurin was found active *in vivo* in BD_2F_1 mice inoculated with L1210 cells with a % *T/C* of 168 at 25 mg/kg. K562 cells incubation with thiophenfurin resulted in inhibition of inosine monophosphate (IMP) dehydrogenase (63%) and an increase in IMP pools (6-fold) with a concurrent decrease in GTP levels (42%). Incubation of adenosine-labeled K562 cells with tiazofurin, thiophenfurin, and furanfurin resulted in a 2-fold higher NAD analogue formulation by thiophenfurin than by tiazofurin. Furanfurin was converted to the NAD analogue with only 10% efficiency. The results obtained support the hypothesis that the presence of S in the heterocycle in position 2 with respect to the glycosidic bond is essential for the cytotoxicity and IMP dehydrogenase activity of tiazofurin, while the N atom is not.

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC-286193, **1**) and selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide, NSC-340847, **2**) are two widely studied C-nucleosides endowed with several biological effects. These include effective antitumor activity both *in vitro* and *in vivo*[1a–f] and the ability to induce differentiation in neoplastic cells,[2] to inhibit the G protein-mediated cellular signaling mechanism,[3] and to downregulate oncogene activity.[4]

The biological effects of these nucleosides, which are structurally related to ribavirin,[5] appear to be due to inhibition of inosine monophosphate dehydrogenase (IMPDH), which induces the shutdown of guanine nucleotide synthesis.[1d,f,6,7] In sensitive cells, tiazo- and selenazofurin are converted into analogues of the cofactor nicotinamide adenine dinucleotide (NAD). These NAD analogues, called TAD (thiazole-4-carboxamide adenine dinucleotide) and SAD (selenazole-4-carboxamide adenine dinucleotide), are excellent inhibitors of IMPDH as well as of other dehydrogenases.[1f,7]



X = S Tiazofurin (1)
X = Se Selenazofurin (2)
X = O Oxazofurin (3)



X = O Furanfurin (4)
X = S Thiophenfurin (5)

Crystallographic studies of tiazofurin and selenazofurin showed close contacts between the thiazole S or selenazole Se heteroatoms and the furanose oxygen.[8] This close contact has been attributed to an electrostatic attractive interaction between the positively charged sulfur or selenium and the lone pair of electrons on the furanose oxygen, as supported by molecular orbital calculations.[8] This interaction would be expected to constrain rotation about the C-glycosidic bond in the active anabolites TAD and SAD, influencing the binding of these dinucleotide inhibitors to the target enzyme. This effect was recently observed in crystal structures of enzyme-bound analogues of TAD and SAD.[9]

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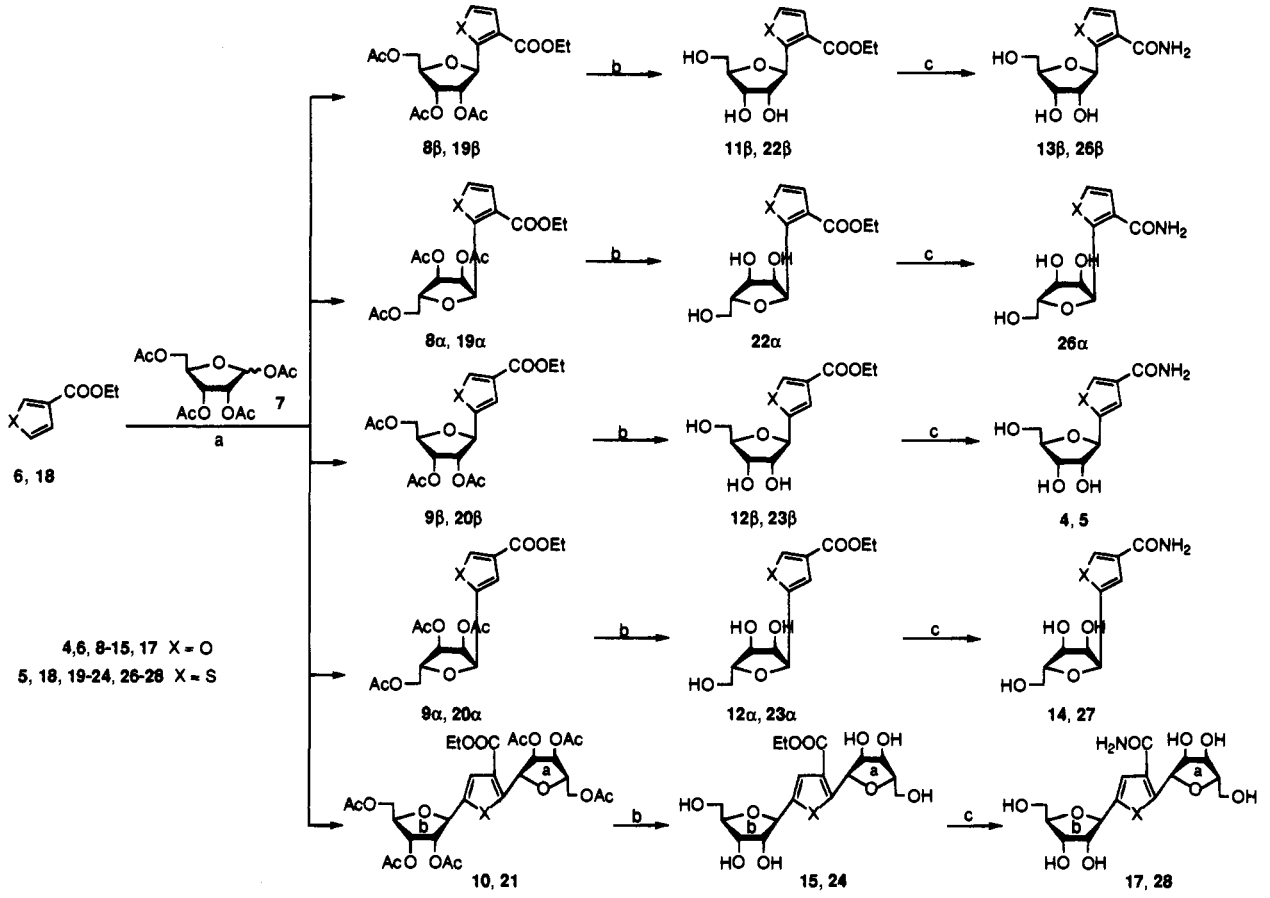
Scheme 1[a]



[a] Reagents: (a) $SnCl_4/ClCH_2CH_2Cl$; (b) EtONa, EtOH; (c) 30% NH_4OH.

Recently we have synthesized oxazofurin (**3**), an analogue of tiazofurin and selenazofurin in which the S and Se heteroatoms are substituted by oxygen. This compound was found to be inactive as an antitumor and antiviral agent.[10] The inactivity of oxazofurin might be due to the O–O-1′ repulsion which does not allow oxazole and furanose moieties in the putative anabolite oxazole-4-carboxamide adenine dinucleotide (OAD) to assume the right conformation to bind the enzyme. This hypothesis was supported by crystallographic data and quantum-mechanical-based computations.[11]

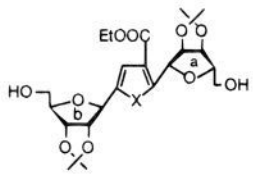
As part of our studies dealing with the structure–activity relationships of these types of C-nucleosides, we now report on the synthesis, structure, antitumor activity, and IMPDH inhibitory activity of oxazofurin and tiazofurin analogues in which the base is replaced by other five-membered ring heterocycles such as furan [furanfurin (**4**)] and thiophene [thiophenfurin (**5**)].

Chemistry

The synthesis of furanfurin (**4**) was carried out as outlined in Scheme 1 by direct C-glycosylation of ethyl furan-3-carboxylate (**6**) under Friedel–Crafts conditions as described for heterocycle analogues.[12] The reaction of **6** with tetra-*O*-acetyl-β-D-ribofuranose (**7**) in 1,2-dichloroethane in the presence of $SnCl_4$ gave 2- and 5-glycosylated regioisomers as mixture of α- and β-anomers (**8**α, **8**β, **9**α, **9**β, 72%) and the 2,5-di-β-D-ribofuranosyl derivative **10** (12%). The isomers **8** and **9**, as anomers α and β, and compound **10** were separated by column chromatography. Compounds **8**β, **9**α, and **9**β were treated with a catalytic amount of sodium ethoxide in ethanol to give the corresponding deblocked ethyl esters **11**β, **12**α, and **12**β. The glycosylation position was determined by ^{1}H-NMR and proton–proton nuclear Overhauser effect (NOE) difference spectroscopy. The ^{1}H-NMR spectrum of **11**β showed that the signal of the H-2 proton of furan had disappeared, indicating that the glycosylation position was at C-2. On the other hand, in the ^{1}H-NMR spectra of compounds **12**α and **12**β, the signal of the H-5 proton was lacking, supporting C-5 glycosylation. The structures of compounds **12**α and **12**β were further supported by NOE experiments. In fact, when the H-1′ signal of these compounds was irradiated, a NOE effect was observed at H-4 confirming that the ribosyl moiety resides at C-5. The anomeric configuration was also assigned on the basis of NOE experiments. In fact, selective irradiation of the anomeric signal of **11**β and **12**β increases the intensity of the H-4′ signal (1.8% and 1.7%, respectively), while the intensity enhancement of the H-3′ signal was zero; this indicates that H-1′ and H-4′ are located on the same face of the ribosyl ring.[13] The anomeric configuration was further supported by studies of ^{13}C-NMR data. Thus, the finding that C-1′ in **12**β resonates upfield of C-1′ in **12**α is in accord with previous observation of anomeric chemical shifts in other C-ribofuranose compounds as well as N-nucleosides.[14]

Treatment of **12**β with ammonium hydroxide (30%) gave furanfurin (**4**). In a similar way, ethyl esters **11**β and **12**α were converted into the amides **13**β and **14**. As far as compound **10** is concerned, the glycosylation positions were established to be at C-2 and C-5 on the basis of the ^{1}H-NMR spectrum which showed that the

H-2 and H-5 signals were lacking. The anomeric configuration was established to be β on the basis of the observation that in the ^{13}C-NMR spectrum the chemical shift patterns of the bis-isopropylidene derivative of **15** (**16**), measured in $CDCl_3$, were very close to the ranges reported by Ohrui *et al.* and Cousineau and Secrist.[15] In fact, the spectrum exhibits methyl signals of the isopropylidene groups at 25.8 and 27.8 ppm, with a $\Delta\delta$ of 2.0 ppm, and the signals of the quaternary isopropylidene carbons at 114.8 and 115.1 ppm, clearly indicative of the β-configuration. Ester **15** was reacted with ammonium hydroxide to give amide **17**.



16 X = O
25 X = S

Thiophenfurin (**5**) was prepared in a similar way as furanfurin starting from ethyl thiophene-3-carboxylate (**18**) (Scheme 1). The reaction with **7** gave a mixture of 2- and 5-glycosylated regioisomers as a mixture of α- and β-anomers (**19**α, **19**β, **20**α, **20**β, 62%) and the 2,5-bis-glycosylated derivative **21** (6%). In this case, the mixture of anomers **19**α, **19**β, and **20**β was separated from **20**α and **21** by column chromatography. Treatment of the mixture of **19**α, **19**β, and **20**β with sodium ethoxide in ethanol gave the deblocked ethyl esters **22**α, **22**β, and **23**β, which were separated by chromatography. In a similar way, starting from **20**α and **21**, the esters **23**α and **24** were obtained. The glycosylation position and the anomeric configurations, assigned by ^{1}H- and ^{13}C-NMR measurements, were further supported by NOE experiments as described for compounds **11** and **12**. The anomeric configuration of **21** was assigned on the basis of the chemical shift patterns of the bis-isopropylidene derivative of **24** (**25**). Treatment of **23**β with 30% ammonium hydroxide gave thiophenfurin (**5**). In a similar way, starting from **22**α, **22**β, **23**α, and **24** were obtained the amides **26**α, **26**β, **27**, and **28**.

Crystallographic and Computational Studies

Owing to the impossibility of obtaining thiophenfurin and furanfurin in solid form, we have studied the crystal structure of the intermediate **23**β (ethyl 5-β-D-ribofuranosylthiophene-3-carboxylate) by X-ray analysis. The molecular structure of the thiophenfurin carboxylate intermediate shares several features observed in the structures of related thiazole and selenazole nucleosides (Figure 1). The thiophene ring is planar, with the ethyl carboxylate substituent at C-4 coplanar to the heterocycle. The C-glycosidic torsion angle is 46.5°, somewhat higher than that observed in the thiazole nucleosides. However, the thiophene sulfur remains *cis* to the furanose oxygen, with a marginally close nonbonded S–O contact of 3.04 Å. *Ab initio* computations (Figure 2) suggest that this conformation is stabilized by an electrostatic interaction between a positively charged thiophene sulfur and a negatively charged furanose oxygen; this effect is similar to that identified for tiazofurin, selenazofurin, and their analogues.[8]

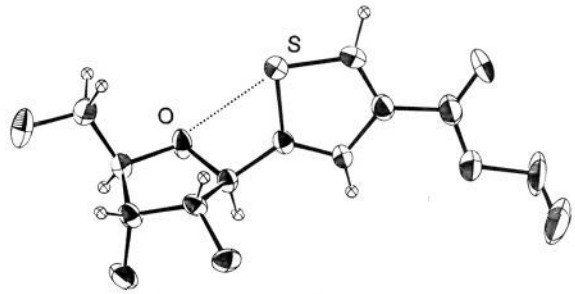


Figure 1. Molecular structure of thiophenfurin-4-carboxylate (**23**β). Non-hydrogen atoms are represented by thermal ellipsoids at the 30% probability level. The dotted line indicates the 3.04 Å nonbonded S–O contact.

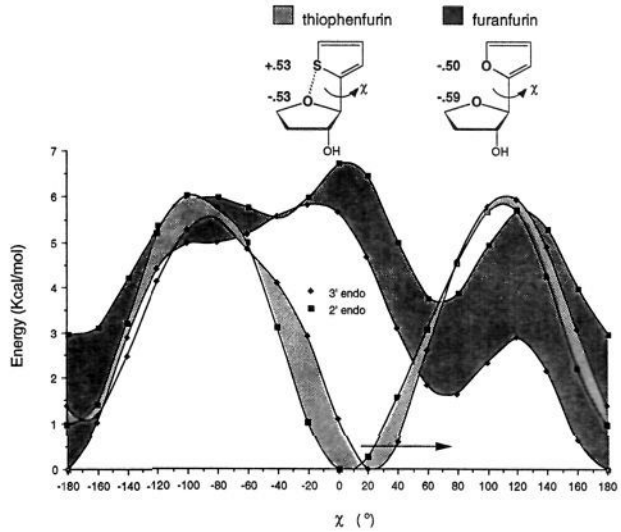


Figure 2. Energy versus C-glycosidic torsion angle χ in the thiophenfurin and furanfurin model fragments. The fragments are illustrated at top in the $\chi = 0°$ conformation. Numbers by the thiophene sulfur and the furan oxygen and both furanose oxygens are natural bond orbital point charges. Rotation about χ is indicated by the curved arrows. Points on each curve were obtained at the RHF/3-21G(*)//3-21G(*) level. Shaded areas for each model represent the span in energy between 2′-endo and 3′-endo conformations. The horizontal arrow indicates the shift to a higher value of χ predicted for furanfurin relative to thiophenfurin.

Calculation of energy versus C-glycosidic torsion angle for a thiophenfurin model fragment is shown in Figure 2. The thiophenfurin fragment shows a global minimum in the range $\chi \sim 0–25°$, depending upon the sugar conformation. A similar value is obtained for tiazofurin.[8] These results suggest that the minimum energy value of χ for thiophenfurin is somewhat lower than that observed in the carboxylate crystal structure. Conformers at lower values of χ are stabilized by an attractive sulfur–oxygen interaction. Donation of mobile sulfur valence electrons to the conjugated system of the thiophene ring results in a positive charge on the heteroatom (Figure 2). This effect is similar to that seen in the thiazole ring of tiazofurin[8] and accounts for the electrostatic attraction between the sulfur and the negatively charged furanose oxygen.

The energy profile for the thiophenfurin fragment also shows a local minimum at $\chi = \pm 180°$ (Figure 2). However, this conformer is separated from that at the global minimum by a 5–6 kcal/mol barrier to rotation. The local minimum at 180° is due primarily to interactions between a positively charged proton on C-3 of the

Table 1. Cytotoxicity of Tiazofurin, Furanfurin, and Thiophenfurin

analogue	cytotoxicity (IC_{50},[a] μM)						
	P388[b]	L1210[b]	K562[b]	HL-60[b]	LLC[b]	LoVo[b]	B16[b]
tiazofurin	2.2	7.4	4.5	8.7	1.5	>128	16.2
furanfurin	c	c	c	c	c	c	c
thiophenfurin	3.2	7.6	8.8	13.9	11.2	102	25.8

[a] The concentration of agent required to reduce cell viability by 50%. [b] P388 and L1210 murine lymphocytic leukemias, K562 human myelogenous leukemia, HL-60 human promyelocytic leukemia, Lewis lung carcinoma (LLC), LoVo human colon adenocarcinoma, and B16 melanoma. [c] Inactive until a maximum tested concentration of 200 μM.

thiophene ring and the furanose oxygen. This minimum is not observed in tiazofurin, whose negative thiazole nitrogen destabilizes the 180° conformer.[8]

Calculation of energy versus C-glycosidic torsion angle for a furanfurin model fragment is also shown in Figure 2. The furanfurin model shows a local minimum in the range $\chi \sim 70-80°$, depending upon the sugar conformation. This is ~60° higher than the global minimum computed for the thiophenfurin fragment (Figure 2) and is similar to values of χ observed in structures of the related inactive analogue oxazofurin.[11] Further, the local minimum for furanfurin at $\chi = 70-80°$ is itself 1–2 kcal/mol higher than a global minimum at $\chi = 180°$.

Antitumor Evaluation and Discussion

Furanfurin (**4**) and thiophenfurin (**5**) have been evaluated for their ability to inhibit the growth of murine lymphocytic leukemia P388 and L1210, human myelogenous leukemia K562, human promyelocytic leukemia HL-60, Lewis lung carcinoma (LLC), human colon adenocarcinoma LoVo, and B16 melanoma. Tumor growth inhibition was evaluated by the MTT assay after 72 h of cell–compound contact.[16] The IC_{50} (μM) values for these C-nucleosides and tiazofurin are summarized in Table 1.

Furanfurin was inactive until a maximum tested concentration of 200 μM. On the contrary, thiophenfurin demonstrated cytotoxicity against leukemic cells comparable to that of tiazofurin. Thiophenfurin exhibited a similar order of cytotoxicity against solid tumor cells as that observed for tiazofurin.

The cytotoxic activity of thiophenfurin was also tested against normal human bone marrow cells from five different healthy donors. The comparison of the results with those obtained with HL-60 cells (see ratio of the means, Table 2) indicates that thiophenfurin exhibits a good selectivity toward tumor cells. This is especially remarkable since in this system most of the cytotoxic compounds tested are equally or more toxic toward normal cells than tumor cells.

We also evaluated *in vivo* antitumor activity of thiophenfurin in BD_2F_1 mice inoculated intraperitoneally with 1×10^5 L1210 cells. Results reported in Table 3 indicate that thiophenfurin has antitumor activity comparable to that of tiazofurin at a dose of 25 mg/kg. However, the high-dose treatment, which gives a better antitumor activity using tiazofurin, cannot be adopted with thiophenfurin since this agent seems to be toxic. The relatively high toxicity of thiophenfurin was not seen in *in vitro* experiments (see Table 2). This discrepancy could be due to nonhematological toxicity.

As far as the structure–activity relationship is concerned, it was found that the glycosylation position and the anomeric configuration are important for the cytotoxicity. In fact, the 2-glycosylated isomers **26**β and **26**α and the α-anomer **27** were inactive (data not shown), as well as the bis-glycosylated isomer **28**.

The inactivity of furanfurin confirms the hypothesis that the presence of the sulfur in the five-membered ring heterocycle in position 2 with respect to the glycosydic bond is fundamental for the antitumor activity of this type of C-nucleosides, whereas the nitrogen atom contributes little or nothing to the activity of tiazofurin.

In furanfurin, the thiophene sulfur is replaced by the more electronegative furan oxygen, resulting in a negative charge in this region of the heterocycle. Thus, conformers in which the furan and furanose oxygens are *cis* (i.e., low values of χ) are destabilized by electrostatic repulsion between the two negatively charged atoms (Figure 2). A similar effect is seen in oxazofurin.[11] Like oxazofurin, furanfurin is inactive.

The activity of tiazofurin requires its enzymatic conversion to the active dinucleotide analogue TAD.[1,6,7] The activity of thiophenfurin likely requires a similar mechanism. Conversely, the inactivity of furanfurin may be due to its inability to be converted to the dinucleotide or failure of the dinucleotide to bind to the target. Either effect could result from the electronic and conformational differences between furanfurin and thiophenfurin discussed above.

In order to test these hypotheses, we evaluated the formation of NAD analogues by tiazofurin, thiophenfurin, and furanfurin in human myelogenous leukemia K562 cells. As showed in Table 4, incubation of adenosine-labeled cells with C-nucleosides resulted in a 2.5-fold higher NAD analogue formation by thiophenfurin than by tiazofurin. As expected, furanfurin was converted with difficulty to the NAD analogue with only 10% efficiency. These results are in accord with the inhibition of IMPDH activity. For this study, K562 cells in culture were exposed to thiophenfurin (10, 50, or 100 μM) or saline for 2 h at 37 °C, and then IMPDH activity was assayed (Table 5). The results indicate that IMPDH activity in K562 cells was inhibited by the action of thiophenfurin with a potency similar to that of tiazofurin.

Since inhibition of IMPDH results in perturbation of nucleotide pools, we examined the influence of this compound on the ribonucleotide concentration. The results provided in Table 6 show a dose-dependent increase in IMP levels (6-fold) with a concurrent decrease in guanilate concentration including GMP, GDP, and GTP. However, perturbation of adenylate pools was marginal.

In conclusion, these studies support the hypothesis that the presence of S in the heterocycle in position 2 with respect to the glycosidic bond is essential for cytotoxicity and IMPDH inhibition, while the N atom is not.

Experimental Section

Melting points were determined on a Buchi apparatus and are uncorrected. Elemental analyses were determined on a EA 1108 CHNS-O (Fisons Instruments) analyzer. Ultraviolet spectra were recorded on an HP 8452 A diode array spectrophotometer driven by an Olivetti M 24. Thin layer chromatography (TLC) was run on silica gel 60 F_{254} plates; silica gel 60 (70–230 and 230–400 mesh) (Merck) for column chromatography was used. ^{1}H- and ^{13}C-NMR spectra were deter-

Table 2. Relative Toxicity of Tiazofurin (1) and Thiophenfurin (**5**) *in Vitro*

	bond marrow samples		HL-60		ratio of the means[a]		
compd	range IC_{50}[b] (μM)	range IC_{90}[b] (μM)	range IC_{50}[b] (μM)	range IC_{90}[b] (μM)	IC_{50}(BM)/ IC_{50}(HL)	IC_{90}(BM)/ IC_{90}(HL)	IC_{50}(BM)/ IC_{90}(HL)
1	10.4–176.4	>128	2.5–13.7	7.6–67.7	7.3	>6	1.9
5	21.6–226.7	>128	2.4–4.3	8.3–17.2	21.9	>10	5.9

[a] Ratio of antilog (mean log IC_{50} or IC_{90}) on human bone marrow (BM) and antilog(mean log IC_{50} or IC_{90}) on human cell line HL-60 (HL). [b] The lower value is equal to antilog(mean log IC_{50} − 2SD), and the higher value is equal to antilog(mean log IC_{50} + 2SD).

Table 3. Antitumor Activity of Thiophenfurin and Tiazofurin *in Vivo*

compd	dose[a] (mg/kg)	avg Δwt[b] (%)	*T/C*[c] (%)
thiophenfurin	400	*d*	<100
	100	*d*	<100
	25	−9.8	168
	6.2	+8.5	155
tiazofurin	400	−13.1	260
	25	−4.3	177

[a] Female mice were injected intraperitoneally with L1210 cells as detailed in the methods. Treatment was started 24 h later, once a day for 9 consecutive days. [b] Averaged weight change of mice from onset to termination of drug treatment. [c] % *T/C* represents the ratio of the survival time of treated to control mice × 100. [d] Early death due to toxicity.

Table 4. Formation of NAD Analogues by Tiazofurin, Thiophenfurin, and Furanfurin in Human Myelogenous Leukemia K562 Cells in Culture[a]

compd	concentration (μM)	NAD analogue (pmol/10^9 cells)
tiazofurin	50	186.5 ± 19.3
	100	309.9 ± 28.9
thiophenfurin	50	504.9 ± 56.8
	100	885.5 ± 77.2
furanfurin	50	49.4 ± 2.1
	100	124.4 ± 17.2

[a] Cells in culture (1×10^7) were labeled with [^{3}H]adenosine for 60 min at 37 °C and then with saline, tiazofurin, thiophenfurin, or furanfurin for 2 h. Cells were centrifuged, extracted with trichloroacetic acid, and neutralized with 0.5 M tri-*n*-octylamine, and an aliquot was analyzed on HPLC.

Table 5. Effects of Thiophenfurin on IMPDH Activity of K562 Cells in Culture

compd	concentration (μM)	IMPDH activity (nmol of XMP formed/ h/mg of protein)	% of control
control		4.97 ± 0.13	100
thiophenfurin	10	2.80 ± 0.18	56[a]
	50	1.85 ± 0.15	37[a]
	100	1.07 ± 0.29	22[a]
tiazofurin	50	1.82 ± 0.08	37[a]

[a] Significantly different from control values ($p < 0.05$).

mined at 300 and 75 MHz, respectively, with a Varian VXR-300 spectrometer. The chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Stationary NOE experiments were run on degassed solutions at 25 °C. A presaturation delay of 1 s was used, during which the decoupler low power was set at 20 dB attenuation. Tiazofurin was obtained through the Drug Synthesis & Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.

Ethyl 2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)furan-3-carboxylate (8β), Ethyl 2-(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosyl)furan-3-carboxylate (8α), Ethyl 5-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)furan-3-carboxylate (9β), Ethyl 5-(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosyl)furan-3-carboxylate (9α), and Ethyl 2,5-Bis(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)furan-3-carboxylate (10). To a stirred solution of ethyl furan-3-carboxylate (**6**) (14 g, 99.9 mmol) in dry 1,2-dichloroethane (150 mL) were added 1,2,3,4-tetra-*O*-acetyl-β-D-ribofuranose (**7**) (32.4 g, 102 mmol) and $SnCl_4$ (6.4 mL), and the mixture was reacted at room temperature for 18 h. The black solution was diluted with H_2O, and the mixture was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The obtained residue was chromatographed on silica gel eluting with CH_2Cl_2–EtOAc (92:8). Compound **8β** was separated as the first fraction as a colorless oil (1.9 g, 4%). TLC (CH_2Cl_2–EtOAc, 92:8): $R_f = 0.28$. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 2.05–2.10 (3s, 9H, $OCOCH_3$), 4.10–4.40 (m, 5H, H-5′, H-4′, OCH_2CH_3), 5.33 (t, $J = 5.2$ Hz, 1H, H-3′), 5.45 (t, $J = 5.7$ Hz, 1H, H-2′), 5.60 (d, $J = 5.9$ Hz, 1H, H-1′), 6.81 (d, $J = 2.0$ Hz, 1H, H-4), 7.88 (d, $J = 2.0$ Hz, 1H, H-5). Anal. ($C_{18}H_{22}O_{10}$) C, H.

It was impossible to obtain a pure sample of compound **8α**, which was formed in very low yield (<0.5%). ^{1}H-NMR (DMSO-d_6) (deduced from the mixture **8α**, **8β**): δ 1.29 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 2.05, 2.06 (2s, 9H, $OCOCH_3$), 4.28 (m, 5H, H-5′, H-4′, OCH_2CH_3), 5.15–5.28 (m, 2H, H-3′, H-2′), 5.66 (d, $J = 4.9$ Hz, 1H, H-1′), 6.80 (d, $J = 2.0$ Hz, 1H, H-4), 7.85 (d, $J = 1.9$ Hz, 1H, H-5).

Evaporation of the following fraction gave **9β** as a colorless oil (27.36 g, 60%). TLC (CH_2Cl_2–EtOAc, 95:5): $R_f = 0.2$. ^{1}H-NMR (DMSO-d_6): δ 1.28 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 2.06, 2.08, 2.11 (3s, 9H, $OCOCH_3$), 4.10–4.40 (m, 5H, H-5′, H-4′, OCH_2CH_3), 5.02 (d, 4J = 6.4 Hz, 1H, H-1′), 5.28 (pseudo-t, $J = 5.5$ Hz, 1H, H-3′), 5.41 (pseudo-t, $J = 6.2$ Hz, 1H, H-2′), 6.90 (s, 1H, H-4), 8.46 (s, 1H, H-2). Anal. ($C_{18}H_{22}O_{10}$), C, H.

Compound **9α** was obtained from the third eluate as a white solid (3.18 g, 8%). Mp: 95–97 °C. TLC (CH_2Cl_2–EtOAc, 95:5): $R_f = 0.15$. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, 3H, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.98–2.08 (3s, 9H, $OCOCH_3$), 4.10–4.38 (m, 5H, H-5′, H-4′, OCH_2CH_3), 5.32 (dd, $J = 4.9$, 12.3 Hz, 1H, H-1′), 5.42 (d, $J = 4.2$ Hz, 1H, H-3′), 5.55 (pseudo-t, $J = 4.6$ Hz, 1H, H-2′), 6.70 (s, 1H, H-4), 8.38 (s, 1H, H-5). Anal. ($C_{18}H_{22}O_{10}$) C, H.

Further elution of the same column with CH_2Cl_2–EtOAc (85:15) as eluent yielded **10** as a colorless oil (7.87 g, 12%). TLC (CH_2Cl_2–EtOAc, 85:15): $R_f = 0.3$. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 2.0–2.12 (3s, 18H,

Table 6. Effect of Tiazofurin and Thiophenfurin on Ribonucleotide Levels of K562 Cells in Culture

		tiazofurin (% of control)		thiophenfurin (% of control)		
nucleotide	control (nmol/g)	50 μM	100 μM	10 μM	50 μM	100 μM
AMP	275 ± 14	100	103	156[a]	140[a]	131[a]
ADP	1399 ± 17	103	99	122[a]	114[a]	105
ATP	4356 ± 13	101	91	92	88[a]	81[a]
NAD	791 ± 43	98	84	81	66[a]	63[a]
IMP	131 ± 5	544[a]	578[a]	314[a]	594[a]	689[a]
GMP	83 ± 2	62[a]	55[a]	98	90	80[a]
GDP	289 ± 7	82	63[a]	97	79[a]	77[a]
GTP	1496 ± 70	64[a]	54[a]	71[a]	58[a]	58[a]
UMP	81 ± 16	113	96	110	115	107
UDP	146 ± 8	120	116	134[a]	134[a]	131[a]
UTP	1017 ± 15	115	90	91	99	87
CDP	358 ± 58	104	85	84	82	99
CTP	312 ± 9	92	32[a]	32[a]	32[a]	32[a]

[a] Significantly different from control values ($p < 0.05$). K562 cells in culture (1×10^7) were incubated with saline (control), tiazofurin, or thiophenfurin for 2 h at 37 °C. Cells were extracted with trichloroacetic acid and neutralized with tri-*n*-octylamine, and an aliquot was analyzed on HPLC.

OCOCH_3), 4.14–4.45 (m, 8H, H-5'a, H-5'b, H-4'a, H-4'b, OCH_2-CH_3), 5.05 (dd, J = 2.4, 6.1 Hz, 1H, H-1'b), 5.20–5.45 (m, 4H, H-3'a, H-3'b, H-2'a, H-2'b), 5.97 (d, J = 4.6 Hz, 1H, H-1'a), 6.87 (s, 1H, H-4). Anal. ($C_{29}H_{36}O_{17}$) C, H.

Ethyl 2-β-D-Ribofuranosylfuran-3-carboxylate (11β). Compound **8β** (1.5 g, 3.76 mmol) was treated with sodium ethoxide (33.1 mL, 15.12 mmol) for 1 h at room temperature. To the reaction mixture was added 0.85 g of Dowex 50w × 8 resin (H^+) (washed with ethanol), and the suspension was stirred for 1 h. The resin was filtered off, washed with ethanol, and discarded. The filtrate was evaporated to dryness, and the residue was chromatographed on silica gel with $CHCl_3$–MeOH (92:8) as eluent to give **11β** as a colorless oil (0.61 g, 60%). TLC ($CHCl_3$–MeOH, 92:8): R_f = 0.36. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.48 (dd, J = 5.7, 11.6 Hz, 2H, H-5'), 3.70 (dd, J = 4.5, 9.8 Hz, 1H, H-4'), 3.95 (dd, J = 5.0, 9.9 Hz, 1H, H-3'), 4.16 (dd, J = 6.3, 10.2 Hz, 1H, H-2'), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 4.75 (t, J = 5.7 Hz, 1H, OH), 5.02 (d, J = 5.1 Hz, 1H, OH), 5.10 (d, 1H, J = 6.3 Hz, 1H, OH), 5.30 (d, 1H, J = 6.2 Hz, H-1'), 6.73 (d, 1H, J = 2.0 Hz, H-4), 7.55 (d, J = 2.0 Hz, 1H, H-5). Anal. ($C_{12}H_{16}O_7$) C, H.

Ethyl 5-β-D-Ribofuranosylfuran-3-carboxylate (12β). The title compound was prepared from **9β** (26 g, 65.26 mmol) by the above method and purified by column chromatography using $CHCl_3$–MeOH (90:10) as eluent (colorless oil, 12.4 g, 70%). TLC ($CHCl_3$–MeOH, 90:10): R_f = 0.38. ^{1}H-NMR (DMSO-d_6): δ 1.28 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.45 (dd, J = 5.1, 10.1 Hz, 2H, H-5'), 3.79 (q, J = 4.4 Hz, 1H, H-4'), 3.91 (q, J = 5.0 Hz, 1H, H-3'), 4.08 (q, J = 5.3 Hz, 1H, H-2'), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 4.62 (d, J = 6.6 Hz, 1H, H-1'), 4.80 (t, J = 5.6 Hz, 1H, OH), 4.98 (d, J = 5.1 Hz, 1H, OH), 5.14 (d, J = 6.4 Hz, 1H, OH), 6.78 (s, 1H, H-4), 8.35 (s, 1H, H-2). Anal. ($C_{12}H_{16}O_7$) C, H.

Ethyl 5-α-D-Ribofuranosylfuran-3-carboxylate (12α). Compound **12α** was prepared from **9α** (3 g, 7.5 mmol) by the above method and chromatographed on silica gel ($CHCl_3$–MeOH, 90:10) as a white foam (1.3 g, 65%). TLC ($CHCl_3$–MeOH, 90:10): R_f = 0.36. ^{1}H-NMR (DMSO-d_6): δ 1.28 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.45 (pseudo-t, J = 5.4 Hz, 1H, H-5'), 3.58 (dd, J = 2.5, 5.4 Hz, 1H, H-5'), 3.79 (m, 1H, H-4'), 4.06 (m, 2H, H-2', H-3'), 4.23 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 4.70 (t, J = 5.6 Hz, 1H, OH), 4.95 (m, J = 2.8 Hz, 3H, H-1', 2 OH, changes to a d with deuterium oxide), 6.65 (s, 1H, H-4), 8.3 (s, 1H, H-2). Anal. ($C_{12}H_{16}O_7$) C, H.

Ethyl 2,5-Di-β-D-ribofuranosylfuran-3-carboxylate (15). Compound **15** was prepared from **10** (7.5 g, 11.42 mmol) by the above method. A foam (2.5 g, 55%) was obtained after purification by flash chromatography on silica gel ($CHCl_3$–MeOH, 90:10). TLC ($CHCl_3$–MeOH, 80:20): R_f = 0.24. ^{1}H-NMR (DMSO-d_6): δ 1.29 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.50 (m, 4H, H-5'a, H-5'b), 3.81 (m, 2H, H-4'a, H-4'b), 3.92 (m, 2H, H-3'a, H-3'b), 4.10 (m, 2H, H-2'a, H-2'b), 4.23 (q, J = 7.1 Hz, 2H, OCH_2CH$_3$), 4.61 (d, J = 6.2 Hz, 1H, H-1'b), 4.79, 5.0, and 5.15 (3m, 6H, OH), 5.30 (d, J = 5.1 Hz, 1H, H-1'a), 6.71 (s, 1H, H-4). Anal. ($C_{17}H_{24}O_{11}$) C, H.

Ethyl 2,5-Bis(2,3-isopropylidene-β-D-ribofuranosyl)furan-3-carboxylate (16). To stirring solution of **15** (1 g, 2.47 mmol) in acetone (25 mL) and dimethoxypropane (12 mL) cooled at 0 °C was added 0.08 mL of perchloric acid (70%) dropwise. The mixture was reacted for 1.5 h and neutralized to pH 7 by 1 N aqueous sodium hydroxyde. After concentration *in vacuo*, the residue was purified by flash chromatography on silica gel eluting with 35% acetone in *n*-hexane yielding **16** as an oil (0.59 g, 50%). ^{1}H-NMR ($CDCl_3$): δ 1.30 (t, J = 7.1 Hz, 3H, CH$_2$CH_3), 1.32 (s, 6H, CCH$_3$), 1.53, 1.57 (2s, 6H, CCH$_3$), 3.12 (br s, 1H, OH), 3.52–3.88 (m, 4H, H-5'a, H-5'b), 4.12–4.33 (m, 4H, CH_2CH$_3$, H-4'a, and H-4'b), 4.69–4.84 (m, 5H, H-1'b, H-2'a, H-2'b, H-3'a, H-3'b), 5.52 (d, J = 3.7 Hz, 1H, H-1'a), 6.68 (s, 1H, H-4). ^{13}C-NMR ($CDCl_3$): δ 14.7 (CH_2CH_3), 25.8, 27.8 (CH_3CCH_3), 61.4 (CH_2CH_3), 62.9, 63.1, 79.7, 80.1, 81.9, 84.2, 85.0, 85.7, 86.1 (carbons of the ribose skeleton), 110.0 (C-4), 114.8, 115.1 (CH_3CCH_3), 117.1 (C-2), 151.97 (C-5), 157.5 (C-3), 163.1 (C=O). Anal. ($C_{23}H_{32}O_7$) C, H.

2-β-D-Ribofuranosylfuran-3-carboxamide (13β). Treatment of compound **11β** (0.4 g, 1.5 mmol) with 30% ammonium hydroxide (16 mL) for 8 h at room temperature and evaporation to dryness afforded a product which was purified by application to the top of a silica gel column followed by elution with $CHCl_3$–MeOH (70:30). Evaporation of the homogeneous fractions gave 0.19 g (55%) of **13β** as white foam. TLC ($CHCl_3$–MeOH 70:30): R_f = 0.39. ^{1}H-NMR (DMSO-d_6): δ 3.5 (m, 2H, H-5'), 3.75 (q, J = 4.6 Hz, 1H, H-4'), 3.92 (q, J = 5.1 Hz, 1H, H-3'), 4.15 (q, J = 6.2 Hz, 1H, H-2'), 4.72 (t, 1H, J = 5.7 Hz, OH), 4.94 (d, J = 5.1 Hz, 1H, OH), 5.03 (d, J = 6.3 Hz, 1H, OH), 5.38 (d, J = 6.4 Hz, 1H, H-1'), 6.85 (d, J = 2.0 Hz, 1H, H-4), 7.3, 7.58 (2br s, 2H, NH_2), 7.65 (d, J = 2.0 Hz, 1H, H-5). ^{13}C-NMR (DMSO-d_6): δ 62.5 (C-5'), 71.8, 73.8, 75.4 (C-1', C-2', C-3'), 84.9 (C-4'), 110.3 (C-4), 122.8 (C-5), 142.3 (C-2), 155.1 (C-3), 164.4 (C=O). Anal. ($C_{10}H_{13}NO_6$) C, H, N.

5-β-D-Ribofuranosylfuran-3-carboxamide (4). Compound **4** was prepared as described for **13β** using **12β** (12 g, 44 mmol) (reaction time 4 h). The residue was chromatographed on silica gel with $CHCl_3$–MeOH (75:35) to give **4** as a white foam (6.4 g, 60%). TLC ($CHCl_3$–MeOH, 75:35): R_f = 0.36. UV (MeOH) λ_{max} 208 (ε 11 050), 242 nm (sh, ε 2050). ^{1}H-NMR (DMSO-d_6): δ 3.45 (m, 2H, H-5'), 3.75 (q, J = 4.7 Hz, 1H, H-4'), 3.90 (q, J = 4.5 Hz, 1H, H-3'), 4.05 (q, J = 6.1 Hz, 1H, H-2'), 4.58 (d, J = 6.8 Hz, 1H, H-1'), 4.78 (t, J = 5.6 Hz, 1H, OH), 4.98 (d, J = 4.8 Hz, 1H, OH), 5.1 (d, J = 6.4 Hz, 1H, OH), 6.79 (s, 1H, H-4), 7.2, 7.65 (2br s, 2H, NH_2), 8.12 (s, 1H, H-2). ^{13}C-NMR (DMSO-d_6): δ 62.3 (C-5'), 71.5, 74.1, 77.1 (C-1', C-2', C-3'), 85.3 (C-4'), 107.9 (C-4), 123.6 (C-2), 145.4 (C-5), 154.5 (C-3), 163.7 (C=O). Anal. ($C_{10}H_{13}NO_6$) C, H, N.

5-α-D-Ribofuranosylfuran-3-carboxamide (14). The title compound was prepared from **12α** (1.2 g, 4.4 mmol). Chromatographic purification on a silica gel column eluting with $CHCl_3$–MeOH (70:30) afforded **14** as a white foam (0.55 g, 52%). TLC ($CHCl_3$–MeOH, 70:30): R_f = 0.24. ^{1}H-NMR (DMSO-d_6): δ 3.42 (m, 1H, H-5'), 3.56, 3.64 (2dd, J = 2.4, 5.4 Hz, 1H, H-5'), 3.80 (m, 1H, H-4'), 4.06 (m, 2H, H-2', H-3'), 4.73 (t, J = 5.7 Hz, 1H, OH), 4.92 (3d, J = 3.5 Hz, 3H, H-1', 2 OH, changes to a d with deuterium oxide), 6.75 (s, 1H, H-4), 7.15, 7.64 (2br s, 2H, NH_2), 8.07 (s, 1H, H-2). ^{13}C-NMR (DMSO-d_6): δ 61.6 (C-5'), 72.1, 72.3, 76.3 (C-1', C-2', C-3'), 82.3 (C-4'), 107.7 (C-4), 123.4 (C-2), 144.5 (C-5), 153.6 (C-3), 163.6 (C=O). Anal. ($C_{10}H_{13}NO_6$) C, H, N.

2,5-Di-β-D-ribofuranosylfuran-3-carboxamide (17). Compound **17** was prepared from **15** (2 g, 4.9 mmol) as described for **13β**. The reaction residue was purified by flash chromatography on silica gel using $CHCl_3$–MeOH (65:35) to give **17** as a foam (0.69 g, 56%). TLC ($CHCl_3$–MeOH, 65:35): R_f = 0.18. ^{1}H-NMR (DMSO-d_6): δ 3.46–4.15 (m, 10H, H-5'a, H-5'b, H-4'a, H-4'b, H-3'a, H-3'b, H-2'a, H-2'b), 4.52 (d, J = 6.0, 1H, H-1'b), 4.72 (q, J = 5.6 Hz, 2H, 2 OH), 4.87 (d, J = 6.7 Hz, 1H, OH), 4.97 (d, J = 5.5 Hz, 1H, OH), 5.08 (d, J = 6.3 Hz, 1H, OH), 5.16 (d, J = 4.7 Hz, 1H, OH), 5.52 (d, J = 3.4 Hz, 1H, H-1'a), 6.79 (s, 1H, H-4), 7.21, 7.7 (2br s, 2H, NH_2). Anal. ($C_{15}H_{21}NO_{10}$) C, H, N.

Ethyl 2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)thiophene-3-carboxylate (19β), Ethyl 2-(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosyl)thiophene-3-carboxylate (19α), Ethyl 5-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)thiophene-3-carboxylate (20β), Ethyl 5-(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosyl)thiophene-3-carboxylate (20α), and Ethyl 2,5-Bis-(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl)thiophene-3-carboxylate (21). The title compounds were obtained from ethyl thiophene-3-carboxylate (**18**)[17] (15 g, 96 mmol) utilizing the same procedure used for preparation of **8–10**. The residue of $CHCl_3$ extracts was chromatographed on silica gel eluting with CH_2Cl_2–EtOAc (95:5). Three main fractions were separated. From the fast eluate, a mixture of **19β**, **19α**, and **20β** was obtained as a colorless oil (24.7 g, 62%). TLC (CH_2Cl_2–EtOAc, 95:5): R_f = 0.43.

Evaporation of the following fraction gave **20α** as an oil (3.06 g, 7.2%). TLC (CH_2Cl_2–EtOAc, 95:5): R_f = 0.38. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.00–2.10 (3s, 9H, OCOCH_3), 4.18–4.32 (m, 5H, H-5', H-4', OCH_2CH$_3$), 5.40 (dd, J = 4.6, 7.2 Hz, 1H, H-3'), 5.55 (t, J = 3.9 Hz, 1H, H-2'), 5.68 (d, J = 3.1 Hz, 1H, H-1'), 7.40 (d, J = 1.3 Hz, 1H, H-4), 8.34 (d, J = 1.3 Hz, 1H, H-2). Anal. ($C_{18}H_{22}O_9S$) C, H.

Evaporation of the last fraction gave ethyl 2,5-bis(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)thiophene-3-carboxylate (**21**) as a colorless oil (3.9 g, 6%). TLC (CH_2Cl_2–EtOAc, 88:12): R_f = 0.1. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 1.98–2.18 (m, 18H, $OCOCH_3$), 4.15–4.40 (m, 8H, H-3′a, H-3′b, H-5′a, H-5′b, OCH_2CH_3), 5.20 (m, 3H, H-2′a, H-4′a, H-4′b), 5.40 (m, 1H, H-2′b), 5.83 (pseudo-t, J = 3.4 Hz, 1H, H-1′b), 6.07 (d, J = 3.3 Hz, 1H, H-1′a), 7.38 (s, 1H, H-4). Anal. ($C_{29}H_{36}O_{16}S$) C, H.

Ethyl 2-β-D-Ribofuranosylthiophene-3-carboxylate (22β), Ethyl 2-α-D-Ribofuranosylthiophene-3-carboxylate (22α), and Ethyl 5-β-D-Ribofuranosylthiophene-3-carboxylate (23β). The mixture of **19β**, **19α**, and **20β** (20 g) was treated with sodium ethoxide as described for the preparation of **11β**. Purification of the reaction residue by chromatography on silica gel ($CHCl_3$–MeOH, 93:7) gave three main fractions which were separated. From the fast eluted fraction, **22β** (2.22 g, 16%) was obtained as a colorless oil. TLC ($CHCl_3$–MeOH, 93:7): R_f = 0.47. ^{1}H-NMR (DMSO-d_6): δ 1.32 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.6 (m, 2H, H-5′), 3.82 (m, 3H, H-2′, H-3′, H-4′), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.81 (t, J = 5.5 Hz, 1H, OH), 4.97 (d, J = 5.0 Hz, 1H, OH), 5.05 (d, J = 5.1 Hz, OH), 5.6 (d, J = 3.6 Hz, 1H, H-1′), 7.34 (d, J = 5.3 Hz, 1H, H-4), 7.5 (d, J = 5.3 Hz, 1H, H-5). Anal. ($C_{12}H_{16}O_6S$) C, H.

Evaporation of the following fraction gave **22α** (1.25 g, 9%) as a colorless oil. TLC ($CHCl_3$–MeOH, 93:7): R_f = 0.42. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.47, 3.65 (2m, 2H, H-5′), 3.88 (m, 1H, H-4′), 4.10 (m, 2H, H-2′, H-3′), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.75 (t, J = 5.8 Hz, 1H, OH), 4.81 (d, J = 4.7 Hz, 1H, OH), 4.85 (d, J = 7.3 Hz, OH), 5.71 (d, J = 3.1 Hz, 1H, H-1′), 7.33 (d, J = 5.3 Hz, 1H, H-4), 7.45 (d, J = 5.1 Hz, 1H, H-5). Anal. ($C_{12}H_{16}O_6S$) C, H.

Evaporation of the last fraction gave **23β** (8.62 g, 62%) as white needles (crystallized from $CHCl_3$). Mp: 117–119 °C. TLC ($CHCl_3$–MeOH, 90:10) R_f = 0.45. ^{1}H-NMR (DMSO-d_6): δ 1.32 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.60 (m, 2H, H-5′), 3.85 (m, 3H, H-2′, H-3′, H-4′), 4.27 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.83 (t, J = 5.5 Hz, 1H, OH-5′), 5.02 (d, J = 4.0 Hz, 1H, OH), 5.08 (d, J = 5.0 Hz, 1H, OH), 5.60 (d, J = 3.4 Hz, 1H, H-1′), 7.35 (d, J = 5.3 Hz, 1H, H-4), 7.5 (d, J = 5.3 Hz, 1H, H-5). Anal. ($C_{12}H_{16}O_6S$) C, H.

Ethyl 5-α-D-Ribofuranosylthiophene-3-carboxylate (23α). Compound **23α** was obtained from **20α** (3 g, 7.23 mmol) as described for **22β**, as a colorless oil (1.4 g, 67%), after chromatography on silica gel with $CHCl_3$–MeOH (94:6). TLC ($CHCl_3$–MeOH, 96:4) R_f = 0.20. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.45 (m, 1H, H-5′), 3.55–3.65 (2dd, J = 4.7, 11.9 Hz, 1H, H-5′), 3.83 (m, 1H, H-4′), 3.96 (dd, J = 4.0, 7.5 Hz, 1H, H-3′), 4.11 (m, 1H, H-2′), 4.25 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.73 (t, J = 5.7 Hz, 1H, OH), 4.95 (d, J = 6.3 Hz, 1H, OH), 5.12 (d, J = 4.4 Hz, 1H, OH), 5.20 (d, J = 2.9 Hz, 1H, H-1′), 7.33 (d, J = 1.4 Hz, 1H, H-4), 8.23 (d, J = 1.4 Hz, 1H, H-2). Anal. ($C_{12}H_{16}O_6S$) C, H.

Ethyl 2,5-Di-β-D-ribofuranosylthiophene-3-carboxylate (24). Compound **24** was prepared from **21** (3.8 g, 5.65 mmol) by the above method. The reaction residue was purified by flash chromatography, using $CHCl_3$–MeOH (80:20), to give **24** as a foam (1.28 g, 55%). TLC ($CHCl_3$–MeOH, 80:20): R_f = 0.33. ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 3.48 (t, J = 5.1 Hz, 2H, H-5′b), 3.58 (m, 2H, H-5′a), 3.80 (m, 4H, H-4′a, H-4′b, H-3′a, H-3′b), 3.92 (m, 2H, H-2′a, H-2′b), 4.23 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.72 (d, J = 7.2 Hz, 1H, H-1′b), 4.80–5.21 (m, 6H, OH), 5.60 (d, J = 3.8 Hz, 1H, H-1′a), 7.28 (s, 1H, H-4). Anal. ($C_{17}H_{24}O_{10}S$) C, H.

Ethyl 2,5-Bis(2,3-isopropylidene-β-D-ribofuranosyl)-thiophene-3-carboxylate (25). The title compound was prepared from **24** (1 g, 2.38 mmol) as described for **16**. The reaction mixture was purified by flash chromatography eluting with 35% acetone in *n*-hexane to give **25** as an oil (0.59 g, 50%). ^{1}H-NMR ($CDCl_3$): δ 1.30 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.31 (s, 6H, CCH_3), 1.43, 1.46 (2s, 6H, CCH_3), 3.15 (br s, 1H, OH), 3.54–3.94 (m, 4H, H-5′a, H-5′b), 4.06–4.33 (m, 4H, CH_2CH_3, H-4′a, H-4′b), 4.60–4.75 (m, 3H, H-2′b, H-3′a, H-3′b), 4.90–5.01 (m, 2H, H-1′b, H-2′a), 5.81 (d, J = 3.7 Hz, 1H, H-1′a), 7.18 (s, 1H, H-4). ^{13}C-NMR ($CDCl_3$): δ 14.7 (CH_2CH_3), 26.0, 28.1 (CH_3CCH_3), 61.4 (CH_2CH_3), 63.0, 63.2, 81.4, 82.1, 82.2, 82.3, 84.7, 86.1, 86.2, 87.0 (carbons of the ribose skeleton), 114.6, 115.5 (CH_3CCH_3), 124.7 (C-4), 128.4 (C-2), 141.7 (C-5), 148.0 (C-3), 163.4 (C=O). Anal. ($C_{23}H_{32}O_6S$) C, H.

2-β-D-Ribofuranosylthiophene-3-carboxamide (26β). Compound **26β** was obtained from **22β** (2 g, 6.93 mmol) by the above method (reaction time 8 h). The reaction residue was chromatographed on silica gel with $CHCl_3$–MeOH (80:20) to give **26β** as a white foam (1.1 g, 62%). TLC ($CHCl_3$–MeOH, 80:20): R_f = 0.21. ^{1}H-NMR (DMSO-d_6): δ 3.4 (m, 2H, H-5′), 3.6 (m, 1H, H-4′), 3.85 (m, 1H, H-3′), 4.12 (m, 1H, H-2′), 4.9 (br s, 2H, OH), 5.17 (dd, J = 5.1, 9.1 Hz, 1H, H-1′), 5.20 (d, J = 5.1 Hz, 1H, OH), 7.38 (d, J = 9.16 Hz, 1H, NH), 7.38 (s, 2H, H-4, H-5), 8.31 (d, J = 9.16 Hz, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ 61.7 (C-5′), 72.6, 73.1, 78.4 (C-1′, C-2′, C-3′), 82.3 (C-4′), 125.3 (C-5), 129.6 (C-4), 136.1 (C-2), 142.6 (C-3), 164.4 (C=O). Anal. ($C_{10}H_{13}NO_5S$) C, H, N.

2-α-D-Ribofuranosylthiophene-3-carboxamide (26α). Compound **26α** was prepared from **22α** (1 g, 3.46 mmol) (reaction time 9 h) and purified by chromatography on silica gel ($CHCl_3$–MeOH, 75:25) to give a white foam (0.52 g, 58%). TLC ($CHCl_3$–MeOH, 75:25): R_f = 0.41. ^{1}H-NMR (DMSO-d_6): δ 3.45 (m, H, H-5′), 3.62 (m, 1H, H-5′), 3.82 (m, 1H, H-4′), 4.08–4.24 (m, 2H, H-2′, H-3′), 4.72 (pseudo-t, 2H, OH), 4.85 (d, J = 6.0 Hz, 1H, OH), 5.77 (d, J = 2.7 Hz, 1H, H-1′), 7.2 (br s, 1H, NH), 7.4 (s, 2H, H-4, H-5), 7.65 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ 61.5 (C-5′), 72.5, 72.6, 78.9 (C-1′, C-2′, C-3′), 82.3 (C-4′), 124.2 (C-5), 126.8 (C-4), 130.9 (C-2), 149.7 (C-3), 165.2 (C=O). Anal. ($C_{10}H_{13}NO_5S$) C, H, N.

5-β-D-Ribofuranosylthiophene-3-carboxamide (5). The title compound was obtained from **23β** (5 g, 17.3 mmol) (reaction time 9 h) by the above method. After chromatographic purification of the reaction mixture ($CHCl_3$–MeOH, 80:20), **5** was obtained as a white foam (3.1 g, 70%). TLC ($CHCl_3$–MeOH, 80:20): R_f = 0.31. UV (MeOH) λ_{max} 210 (ε 35 000), 240 nm (ε 10 500). ^{1}H-NMR (DMSO-d_6): δ 3.50 (t, J = 5.1 Hz, 2H, H-5′), 3.81 (m, 2H, H-4′, H-3′), 3.93 (m, 1H, H-2′), 4.76 (d, J = 8.0 Hz, 1H, H-1′), 4.80 (t, J = 5.5 Hz, 1H, OH), 5.01 (d, J = 4.7 Hz, 1H, OH), 5.18 (d, J = 6.8 Hz, 1H, OH), 7.20 (br s, 1H, NH), 7.42 (s, 1H, H-4), 7.75 (br s, 1H, NH), 8.02 (s, 1H, H-2). ^{13}C-NMR (DMSO-d_6): δ 62.3 (C-5′), 71.6 (C-3′), 77.7 (C-2′), 79.3 (C-1′), 85.6 (C-4′), 124.5 (C-4), 128.3 (C-2), 137.4 (C-5), 145.3 (C-3), 165.2 (C=O). Anal. ($C_{10}H_{13}NO_5S$) C, H, N.

5-α-D-Ribofuranosyltiophene-3-carboxamide (27). Compound **27** was prepared from **23α** (1 g, 3.46 mmol) (reaction time 9 h) by the above method. The reaction residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (80:20) to give **27** (0.49 g, 55%) as a white solid. Mp: 155–158 °C. TLC ($CHCl_3$–MeOH, 80:20): R_f = 0.26. ^{1}H-NMR (DMSO-d_6): δ 3.46 (m, 1H, H-5′), 3.58–3.65 (2 m, 1H, H-5′), 3.81 (m, 1H, H-4′), 3.96 (dd, J = 4.3, 7.6 Hz, 1H, H-3′), 4.12 (m, 1H, H-2′), 4.73 (t, J = 5.7 Hz, 1H, OH), 4.92 (d, J = 7.4 Hz, 1H, OH), 5.08 (d, J = 4.7 Hz, 1H, OH), 5.18 (d, J = 2.8 Hz, 1H, H-1′), 7.15 (br s, 1H, NH), 7.35 (s, 1H, H-4), 7.72 (br s, 1H, NH), 8.04 (d, J = 1.3 Hz, 1H, H-2). ^{13}C-NMR (DMSO-d_6): δ 61.7 (C-5′), 72.6 (C-3′), 73.1 (C-2′), 78.3 (C-1′), 82.3 (C-4′), 125.3 (C-4), 129.6 (C-2), 136.1 (C-5), 142.6 (C-3), 164.4 (C=O). Anal. ($C_{10}H_{13}NO_5S$) C, H, N.

2,5-Di-β-D-ribofuranosylthiophene-3-carboxamide (28). Compound **28** was obtained from **24** (1 g, 2.37 mmol) by the above method and purified by flash chromatography ($CHCl_3$–MeOH, 80:20) as a foam (0.45 g, 50%). TLC ($CHCl_3$–MeOH, 60:40): R_f = 0.16. ^{1}H-NMR (DMSO-d_6) (some signals are doubled for the presence of two conformers): δ 3.46, 3.63 (2m, 4H, H-5′a, H-5′b), 3.83 (m, 4H, H-4′a, H-4′b, H-3′a, H-3′b), 4.12, 4.20 (2m, 2H, H-2′a, H-2′b), 4.65–5.0 (m, 6H, OH), 4.70, 5.12 (2d, 1H, H-1′b), 5.72, 5.80 (2d, 1H, H-1′a), 7.10, 7.17 (2br s, 1H, NH), 7.25, 7.30 (2s, 1H, H-4), 7.57, 7.62 (2br s, 1H, NH). Anal. ($C_{15}H_{21}NO_9S$) C, H, N.

Crystallographic Study. Small colorless crystals of the carboxylate precursor of thiophenfurin [ethyl 5-β-D-ribofuranosylthiophene-3-carboxylate, $C_{12}H_{16}O_6S$ (**23β**)] were obtained from chloroform. Crystals are orthorombic, space group $P2_12_12_1$, with cell dimensions a = 4.893(2) Å, b = 12.764(11) Å, and Z = 4. Diffraction data were collected on an Enraf-Nonius FAST detector using graphite monochromatized Mo

Kα radiation from a rotating anode source. A total of 14 132 reflections were measured to $\theta = 25.3°$ and averaged to yield 1897 unique reflections. The structure was solved by routine application of direct methods and refined to $R = 6.6\%$ ($R_w = 6.5\%$) for the 1312 reflections with values of $F_o > 2\sigma(F_o)$. All non-hydrogen atoms were refined with anisotropic temperature factors using full-matrix least-squares techniques. Thiophene and ribose methylene protons were added in idealized positions and then refined with isotropic temperature factors. High thermal parameters in the ethyl carbons indicated some disorder in this portion of the carboxylate substituent.

Computational Study. Energy as a function of C-glycosidic torsion angle χ was computed for thiophenfurin and furanfurin model fragments using methods similar to those described previously.[8,11] *Ab initio* calculations were performed using the Gaussian 92 system of programs and internal basis sets.[18]

Model fragments for thiophenfurin and furanfurin contained thiophene and furan heterocycles, respectively, with the carboxamide substituent omitted. The heterocycle was connected via a C-glycosidic bond to a 3′-deoxyfuranose ring (Figure 2). By analogy to tiazofurin, χ was defined by atoms O-1′−C-1′−C-2−S-1 and O-1′−C-1′−C-2−O-1 for thiophenfurin and furanfurin, respectively. A value of $\chi = 0°$ refers to the conformation in which the furanose oxygen is *cis* planar to either the furan oxygen or the thiophene sulfur. A positive value of χ indicates a counterclockwise rotation of the C-2−O-1/S bond relative to the C-1′−O-1′ bond when viewed down the C-glycosidic bond from C-2 to C-1′.

Energy profiles for the thiophenfurin and furanfurin model compounds were obtained with the furanose ring fixed in both 3′-endo and 2′-endo puckers. The global energy minimum for each compound was normalized to 0 kcal/mol. Energies for each model were obtained for conformers between $\chi_0 = -180°$ and 180°. In each case, the value of χ was incremented in 20° steps and fixed. All remaining geometry variables describing the fragment were then optimized, with the exception of the furanose torsion angles. The starting geometry at each value of χ was the optimized geometry obtained at the previous value. All geometry optimizations used the analytical gradient method.[19]

Optimized geometries and associated SCF energies were obtained using the 3-21G basis set for the furanfurin model and the 3-21G* basis set for the sulfur-containing thiophenfurin model. Thus, each point in Figure 2 represents a calculation at either the RHF/3-21G//3-21G or RHF/3-21G*//3-21G* levels.[18]

Point charges were obtained from both thiazole and oxazole fragments using the natural population analysis method incorporated in Gaussian 92.[16,18] This method of charge partitioning uses fewer assumptions than that of the traditional Mulliken population analysis.[19,20] Charges were obtained from optimized geometries at the RHF level of theory using a 3-21G basis set for the furanfurin model and a 3-21G* basis set for the thiophenfurin fragment.[19,20]

Antitumor Evaluation. The following cell lines were used, L1210 and P388 murine leukemia, HL-60 human promyelocytic leukemia, K562 human myelogenous leukemia, Lewis lung carcinoma (LLC), B16 murine melanoma, and LoVo human colon adenocarcinoma.

With the exception of LoVo, cell lines were grown in RPMI 1640 medium, supplemented with 10 mM HEPES buffer and 15% new born calf serum (L1210 and P388) or 5% (LLC) or 10% (K562 and B16) or 15% (HL-60) fetal calf serum. LoVo cells were maintained in HAM's F12 medium supplemented with 10% fetal calf serum. Antibiotics were added to all cell culture media. 2-Mercaptoethanol (10 nM) was also added to P388, L1210, and HL-60, while 1% vitamins and nonessential amino acids were added to LoVo and B16. The cells were growth at 37 °C in a CO_2 incubator and maintained in logarithmic phase of growth.

Various concentrations of each drug were placed with tumor cell suspension (B16, 5×10^3 cells/well; P388, L1210, and LLC, 10^4 cells/well; HL-60, K562, and LoVo, 2.5×10^5 cells/well). Cell growth was evaluated after 72 h of cell−drug contact using a modified MTT assay.[16,21] The IC_{50} and IC_{90} (concentration reducing optical absorption by 50% or 90%, respectively) were calculated by the regression analysis proposed by Chou.[22]

Relative Toxicity Evaluation *in Vitro*. The evaluation of *in vitro* relative toxicity as a new prescreening model for agents potentially active against tumors is described in a recent paper.[23] Briefly, the cytoxicity of a test drug is evaluated by using an antiproliferative assay which was developed to evaluate *in vitro* antitumor activity.[10] This measures [^{125}I]-5-iodo-2′-deoxyuridine ([^{125}I]UdR) incorporation in DNA after a 72 h drug−cell contact. The potential antitumor activity of new agents is estimated by comparing the cytotoxic activity on normal (human bone marrow) and tumor (human cell line HL-60) cells. In this system the ratios of the mean IC_{50}(BM)/IC_{50}(HL-60) and the mean IC_{90}(BM)/IC_{90}(HL-60) were greater than 1 for drugs in clinical use. In contrast, this ratio is less than 1 for compounds which have demonstrated only good cytotoxic activity toward tumor cell lines but are not clinically used. Thus, a new compound which has a ratio greater than 1 is considered to have a greater probability of becoming a clinically useful antitumor agent than a compound with a ratio lower than 1. In these sets of experiments, assays on BM samples were conducted from five different donors and three assays on HL-60 cells were performed.

Antitumor Activity *in Vivo*. The test animals used in this experiment were female BD_2F_1 mice (8 weeks old, 20−22 g), purchased from Charles River, Italy. Each experimental group contained eight mice which were injected ip with 10^5 L1210 cells on day 0. Twenty-four hours later, drugs were solubilized in a 0.9% NaCl solution and injected ip once a day for 9 days.

Formation of NAD Analogues. NAD analogues of tiazofurin, thiophenfurin, and furanfurin were synthesized by the method cited.[24] Briefly, human myelogenous leukemia K562 cells (1×10^7 cells/10 mL) were incubated with [2,8-^{3}H]-adenosine (185 nM, specific radioactivity 200 mCi/mmol) for 1 h at 37 °C. Saline or indicated concentration of analogue was then added, incubated further for 2 h, and then centrifuged at 400*g* for 5 min. The cell pellet was washed once with phosphate-buffered saline, homogenized in cold 10% trichloroacetic acid, and neutralized with 0.5 M tri-*n*-octylamine in freon and an aliquot analyzed on a Partisil 10-SAX column using a RCM-10 module employing high-pressure liquid chromatography (HPLC) utilizing an ammonium phosphate buffer system.[25]

IMPDH Assay. K562 cells (1×10^6 cells/ mL, 10 mL) in logarithmic phase of growth were incubated with saline or indicated concentrations of the agents for 2 h at 37 °C. The cells were then harvested by centrifugation, washed once with cold phosphate-buffered saline, and lysed in buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM $MgCl_2$, 0.5% NP-40, and 2 μg/mL aprotinin. The lysate was kept for 20 min on ice and centrifuged at 13000*g* for 20 min, and the supernatant was used as a source of enzyme.

The enzyme activity was measured according to the published methodology.[26] Briefly, 5 μL aliquots of 0.5 M KCl containing 20 mM allopurinol were dispensed into the apex of Eppendorf tubes and dried at room temperature (25 °C). For the conduct of the assay, in a total volume of 10 μL, tubes contained 5 μL of the substrate mixture containing 286 μM [2,8-^{3}H]IMP (200 μCi/mL) and 1 mM NAD. The reaction was initiated by the addition of a 5 μL aliquot of enzyme extract. After 30 min at 37 °C, the reaction was terminated by heating for 1 min at 95 °C. Tubes were centrifuged at 13000*g* for 0.5 min, 5 μL of 100% KOH was deposited on the underside of the cap, and the tubes were closed and incubated at room temperature overnight (16 h). The caps containing the droplet were cut, and the radioactivity was determined by scintillation spectrometry. IMPDH activity was expressed as nmol of XMP formed/mg of protein/h.

Determination of the Concentration of Intracellular Ribonucleotides. Cells in culture were treated with saline or agents for 2 h at 37 °C; cells were harvested by centrifugation and washed once with cold saline. Cells were extracted with cold 10% trichloroacetic acid and centrifuged for 0.5 min;

the supernatant was immediately neutralized with 0.5 M tri-*n*-octylamine in freon. An aliquot of the neutralized extract was analyzed on HPLC using a Partisil 10-SAX column as described above.

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Supporting Information Available: Coordinates, thermal parameters, and bond lengths and angles (3 pages); table of observed and calculated structure factors for thiophenfurin-4-carboxylate (**23**β) (10 pages). Ordering information is given on any current masthead page.

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