Articles

Synthesis, Conformational Analysis, and Biological Activity of *C*-Thioribonucleosides Related to Tiazofurin

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The syntheses of furanthiofurin [5β-D-(4′-thioribofuranosyl)furan-3-carboxamide, **1**] and thiophenthiofurin [5β-D-(4′-thioribofuranosyl)thiophene-3-carboxamide, **2**], two *C*-thioribonucleoside analogues of tiazofurin, are described. Direct trifluoroacetic acid-catalyzed *C*-glycosylation of ethyl furan-3-carboxylate with 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-4-thio-D-ribofuranose gave 2- and 5-glycosylated regioisomers, as a mixture of α and β anomers. Ethyl 5-(2,3,5-tri-*O*-benzyl)-β-D-(4′-thioribofuranosyl)furan-3-carboxylate (**6**β) was debenzylated and then converted into the corresponding amide (furanthiofurin) by reaction with ammonium hydroxide. A similar *C*-glycosylation of ethyl thiophene-3-carboxylate with 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose catalyzed by stannic chloride afforded an anomeric mixture of 2- and 5-glycosylated regioisomers. Deacetylation of ethyl 5-(2,3,5-tri-*O*-acetyl)-β-D-(4′-thioribofuranosyl)-thiophene-3-carboxylate (**13**β) with methanolic ammonia and treatment of the ethyl ester with ammonium hydroxide gave thiophenthiofurin. The glycosylation site and anomeric configuration were established by ^{1}H NMR spectroscopy. Thiophenthiofurin was found to be cytotoxic in vitro toward human myelogenous leukemia K562, albeit 39-fold less than thiophenfurin, while furanthiofurin proved to be inactive. K562 cells incubated with thiophenthiofurin resulted in inhibition of inosine 5′-monophosphate dehydrogenase (IMPDH) and an increase in IMP pools with a concurrent decrease in GTP levels. From computational studies it was deduced that, among the *C*-nucleoside analogues of tiazofurin, activity requires an electrophilic sulfur adjacent to the *C*-glycosidic bond and an energetically favorable conformer around $\chi = 0°$. Among these, the more constrained (least flexible) compounds (tiazofurin and thiophenfurin) are more active than the less constrained thiophenthiofurin. Those compounds which contain a nucleophilic oxygen in place of the thiazole or thiophene (oxazofurin, furanfurin, and furanthiofurin) show the least activity.

Introduction

Tiazofurin is a synthetic thiazole *C*-nucleoside which has demonstrated clinical efficacy as an antitumor agent.[1] The antitumor activity of tiazofurin results from a combination of cytotoxic and maturation-inducing activities. It was found that tiazofurin inhibits inosine 5′-monophosphate dehydrogenase (IMPDH), decreases cellular GTP concentration, induces differentiation, down-regulates *ras* and *myc* oncogene expression, and causes apoptosis of K562 human erythroleukemia cells in a time- and dose-dependent fashion. The tiazofurin-mediated apoptosis may therefore be linked with the decrease of GTP and the consequent impairment of specific signal transduction pathways. The drug produces IMPDH inhibition after conversion in sensitive cells to the active metabolite thiazole-4-carboxamide adenine dinucleotide (TAD), which binds tightly at the NADH site and inhibits IMPDH activity. This enzyme catalyzes the NAD-dependent conversion of inosine 5′-monophosphate (IMP) to xanthosine monophosphate (XMP), which is the rate-limiting step in guanine nucleotide biosynthesis. Thus, IMPDH inhibitors reduce GTP and deoxyGTP pool levels, and they have been shown to possess antineoplastic, antiparasitic, antiviral, and immunosuppressive activity.[1]

In 1995, we reported the synthesis of thiophenfurin (5β-D-ribofuranosylthiophene-3-carboxamide) and furanfurin (5β-D-ribofuranosylfuran-3-carboxamide), two *C*-nucleoside isosters of tiazofurin, in which the thiazole ring was replaced by a thiophene and furan heterocycle, respectively. While thiophenfurin was found active as

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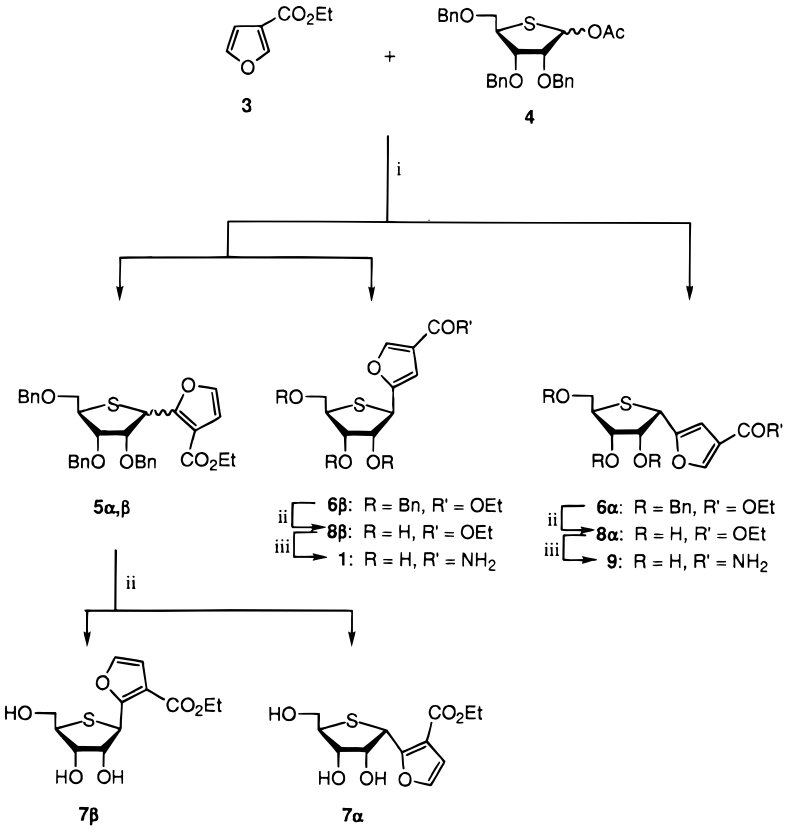
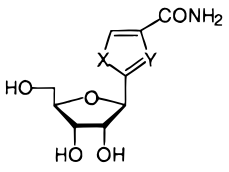
Scheme 1

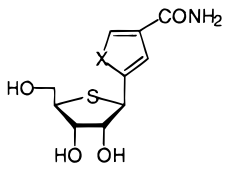


Reagents: (i) TFA, CH_2Cl_2; (ii) BBr_3/CH_2Cl_2, -78 °C; (iii) 30% NH_4OH.

an antitumor agent both in vitro and in vivo, furanfurin proved to be inactive.[2] In sensitive cells, thiophenfurin, like tiazofurin, is metabolized to a nicotinamide adenine dinucleotide (NAD) analogue, TFAD, which is a potent inhibitor of IMPDH and is more active than TAD.[3] We found that the inactivity of furanfurin was due both to its poor ability to be converted to furan-3-carboxamide adenine dinucleotide (FFAD) in target cells and to the poor affinity of this dinucleotide for IMPDH.[2,3]



Tiazofurin (X = S, Y = N)
Thiophenfurin (X = S, Y = CH)
Furanfurin (X = O, Y = CH)



Furanthiofurin (X = O)
Thiophenthiofurin (X = S)

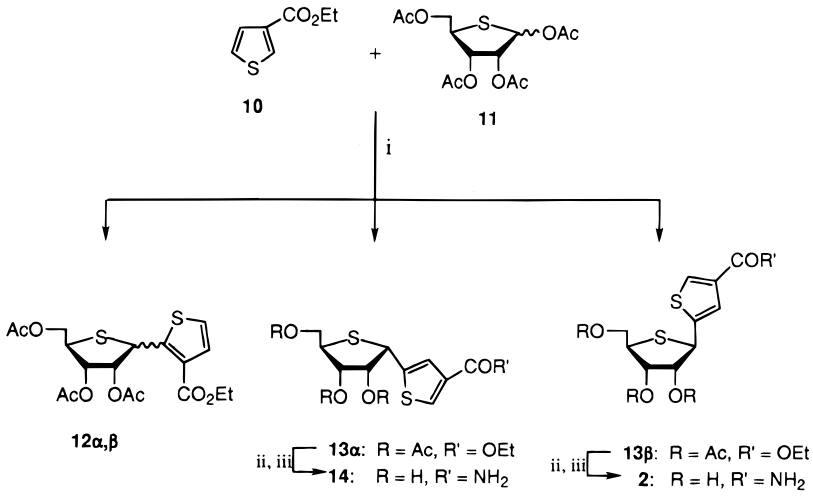
Crystallographic and ab initio computational studies showed that thiophenfurin, like tiazofurin, possesses a conformationally restricted glycosidic bond due to an energetically favorable intramolecular sulfur−oxygen interaction. This can be interpreted as an electrostatic interaction between the positively charged thiophene sulfur and negatively charged furanose oxygen. This interaction stabilizes the conformation in which these heteroatoms are adjacent. In contrast, in furanfurin the repulsive interaction between the negatively charged furan and furanose oxygens destabilizes the conformers in which such atoms are cis (Figure 1).[2]

To gain further information about the structure−activity relationships of this type of antitumor *C*-nucleosides, we investigated furanfurin and thiophenfurin analogues in which the furanose ring oxygen O-4′ was replaced by a sulfur atom (furanthiofurin, **1**, and thiophenthiofurin, **2**).

Chemistry

Furanthiofurin (**1**) was synthesized by direct *C*-glycosylation of ethyl furan-3-carboxylate (**3**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-4-thio-D-ribofuranose (**4**)[4] (Scheme 1). The glycosylation reaction performed in the presence of trifluoroacetic acid in methylene chloride gave 2- and 5-glycosylated regioisomers as a mixture of α and *β* anomers (**5**α,*β* and **6**α,*β*, 53%). Analytical samples of **5**α, **5***β*, **6**α, and **6***β* were obtained by semi-preparative HPLC. The mixture of compounds **5**α, **5***β*, and **6***β* was separated from **6**α by chromatography on silica gel. We found it more convenient to separate the mixture of the 2-glycosylated anomers **5**α, **5***β* and the isomer **6***β* after removal of benzyl groups using boron tribromide in methylene chloride at −78 °C. The deblocked ethyl esters **7**α, **7***β*, and **8***β* were separated by chromatography on silica gel. The glycosylation position of these nucleosides was determined by proton spectroscopic analysis. The ^{1}H NMR spectra of compounds **8**α and **8***β* showed that the signal of the H-5 proton of furan had disappeared, indicating that the glycosylation position was at C-5. On the other hand, in the ^{1}H NMR spectra of **7**α and **7***β*, the signal of the H-2 proton was lacking, supporting C-2 glycosylation. The stereostructures of these compounds were determined by compari-

Scheme 2



Reagents; (i) $SnCl_4$, $ClCH_2CH_2Cl$; (ii) NH_3/CH_3OH; (iii) 30% NH_4OH.

son of the chemical shift of the anomeric proton. As reported in the case of other thioribonucleosides,[4] it was found that the chemical shift of H-1′ of the β anomer is upfield from the signal of the corresponding H-1′ proton of the α anomer. Treatment of **8**β with ammonium hydroxide (30%) gave furanthiofurin (**1**). In a similar way, ethyl ester **8**α was converted to the amide **9**, α anomer of **1**.

We found that thiophenthiofurin could not be synthesized by glycosylation of ethyl thiophene-3-carboxylate (**10**) with thioriboside **4**, probably owing to the reduced reactivity of the thiophene ring toward this electrophilic reagent. We succeeded in the synthesis of thiophenthiofurin using 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose (**11**) as a glycosylation reagent (Scheme 2). Compound **11** was synthesized starting from D-gulono-1,4-lactone as reported by Dukhan et al.[5] Reaction of **10** with **11** in 1,2-dichloroethane in the presence of $SnCl_4$ gave a mixture of 2- and 5-glycosylated regioisomers as α and β anomers (**12**α,β, **13**α,β, 56%). Isomer **13**α was separated from the mixture of **12**α, **12**β, and **13**β by column chromatography and converted into the amide **14** by reaction with methanolic ammonia and then with 30% ammonium hydroxide. Owing to the impossibility of separating by chromatography the mixture of **12**α, **12**β, and **13**β isomers, this mixture was deprotected with methanolic ammonia and then treated with ammonium hydroxide. Thiophenthiofurin (**2**) was separated from the mixture of C-2 isomers by chromatography on silica gel column.

The glycosylation position and the anomeric configuration of nucleosides **2** and **14** were determined by ^1H NMR data in DMSO-d_6 as reported above.

Biological Evaluation

Furanthiofurin, thiophenthiofurin, and their α anomers (**9** and **14**) were evaluated for their ability to inhibit the growth of human myelogenous leukemia K562 cells. Tumor cell proliferation was evaluated by incubating the cells continuously with either the compounds or saline for 48 h.[6] Thiophenfurin was used as a reference compound. The IC_{50} (μM) values for these *C*-nucleosides are summarized in Table 1. Furanthiofurin proved to be almost inactive until a maximum tested concentration of 100 μM. In contrast, thiophenthiofurin showed cytotoxic activity against K562 cells, albeit 39-fold lower than that of thiophenfurin. The α anomer of thiophenthiofurin was found inactive as an antitumor agent. This finding once more confirms that the β anomeric configuration of these *C*-nucleosides is important for cytotoxic activity.

Table 1. Cytotoxicity and Effect of Compounds on IMPDH Activity and on Purine Nucleotide Concentration in Human Myelogenous Leukemia K562 Cells in Culture

compd	cytotoxicity IC_{50} (μM)[a]	IMPDH inhibition[b] (% of control)	GTP pools[b] (% of control)	IMP pools[b] (% of control)
None	-	100.0	100.0	100.0
Thiophenfurin	1.7	76.0	96.6	88.2
Furanthiofurin	4700.0	75.2	66.1	105.0
Thiophenthiofurin	67.0	83.2	83.9	89.1

[a] Concentration required to inhibit 50% of cell proliferation at 48 h. [b] For studies related to IMPDH inhibition and nucleotide pools, cells were incubated with 2 μM thiophenfurin or 100 μM furanthiofurin or thiophenthiofurin.

Furanthiofurin and thiophenthiofurin were also tested for inhibitory properties against IMPDH from human myelogenous leukemia K562 cells in culture, as previously reported.[7] For these studies, K562 cells were exposed to 100 μM each of the inhibitors (2 μM in the case of thiophenfurin) or saline for 2 h at 37 °C. The results (Table 1) indicate that IMPDH activity was inhibited (25%) by the action of furanthiofurin with a potency comparable to that of thiophenthiofurin (17% inhibition) and that this inhibition was ~50-fold lower than that exhibited by thiophenfurin.

Since inhibition of IMPDH results in perturbation of nucleotide pools, we examined the influence of furanthiofurin and thiophenthiofurin on the ribonucleotide concentration in K562 cells. The results provided in Table 1 show an increase in IMP levels in the case of thiophenthiofurin (10.9%) and thiophenfurin (11.8%) with a concurrent decrease in guanylate concentration.

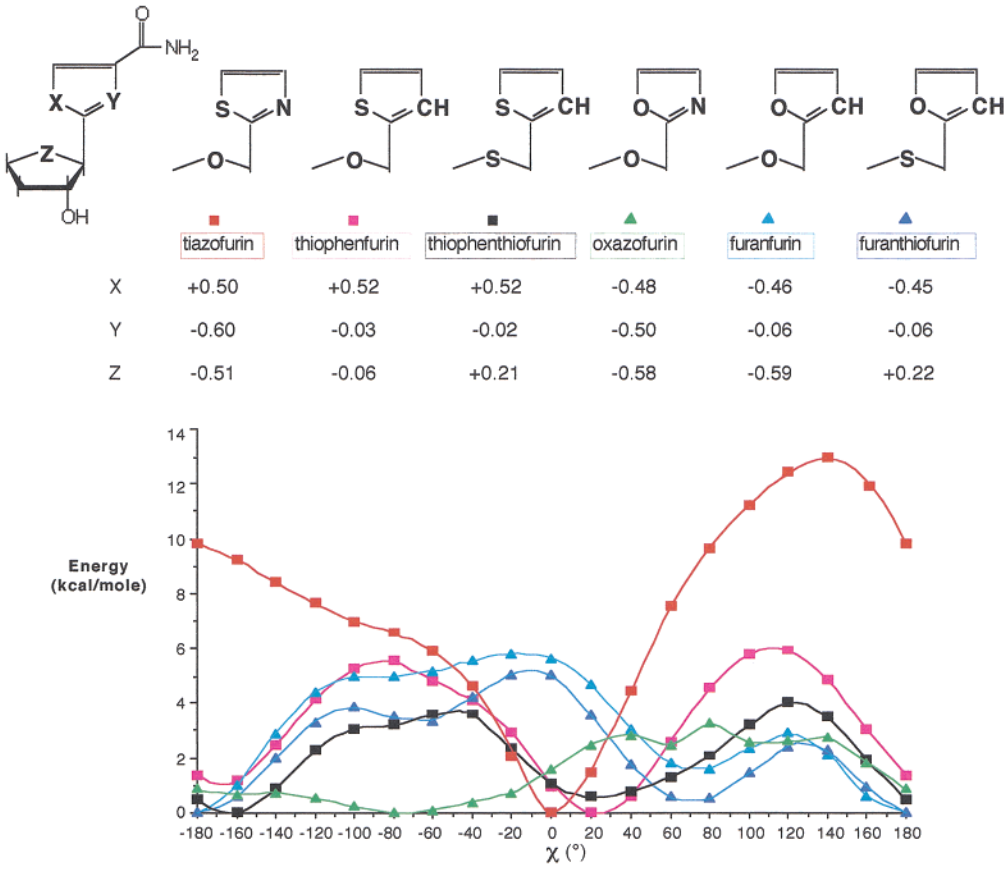


Figure 1. (Top) Comparison of heteroatom charges for tiazofurin, thiophenfurin, thiophenthiofurin, oxazofurin, furanfurin, and furanthiofurin. The heterocycle and portion of the ribofuranose or 4-thioribofuranose moiety corresponding to each agent is illustrated at the top. Charges were calculated at the DFT/6-31G**//RHF/3-21G* level of theory using the model fragment shown at the top left and partitioned using the NBO method. Where Y = C−H, the combined charge for the carbon and its attached proton is listed. (Bottom) Energy vs *C*-glycosidic torsion angle χ for model fragments of tiazofurin, thiophenfurin, thiophenthiofurin, oxazofurin, furanfurin, and furanthiofurin. The color of each curve matches that of the symbol above the corresponding agent's name. Each point on each curve was obtained at the RHF/3-21G*//3-21G* level. The fragment at the top left is shown in the χ = 0° conformation.

Computational Studies and Discussion

Heteroatom charges for tiazofurin, thiophenfurin, thiophenthiofurin, oxazofurin, furanfurin, and furanthiofurin are compared in Figure 1. Charges were obtained at the DFT/631G**//HF/321G* level of theory using natural bond order partitioning as implemented in Gaussian 98.[8−10] For those compounds having a C−H group adjacent to the *C*-glycosidic carbon, the combined charge of the carbon and its attached proton is indicated. While these charges depend to some degree on the particular computational method employed, relative values remain consistent over a variety of basis sets and partitioning methods. Thus, general observations may be made based on the values shown in Figure 1.

Tiazofurin, thiophenfurin, and thiophenthiofurin all contain a positively charged sulfur in the unsaturated five-membered heterocycle. Each of these compounds demonstrates activity, albeit to varying degrees. In oxazofurin, furanfurin, and furanthiofurin, the positively charged sulfur is replaced by a negatively charged oxygen. These compounds show minimal or no activity. These observations suggest that an electrophilic atom adjacent to the *C*-glycosidic carbon is required for activity. This requirement may result from one or a combination of several factors.[11,12]

The crystal structure of human IMPDH complexed with SAD, the selenium analogue of the active tiazofurin anabolite, has been recently determined.[13] The structure indicates an interaction between the SAD electrophilic heteroatom and the side chain of Gln 334 on the active site loop. Although the position of the loop is distorted in this complex by the presence of the 6-Cl analogue of the IMP substrate, comparison with other structures suggests that a similar interaction may be maintained between the electrophilic heteroatom and Gln 441 when complexed with native substrate.[13] The presence of a nucleophilic oxygen atom in place of the sulfur or selenium would destabilize this interaction. The presence of an electrophilic heteroatom would also be expected to influence binding of the parent compounds to anabolic enzymes. This may account for the variations in levels of NAD analogues observed between agents.

The structure of the IMPH−SAD complex also suggests that the conformation about the *C*-glycosidic bond in the bound ligand is such that the Se atom and furanose oxygen remain cis to each other. Thus, conformational studies were carried out by ab initio computations as previously reported,[2] and the results are compared in Figure 1 for tiazofurin, thiophenfurin, thiophenthiofurin, oxazofurin, furanfurin, and furanthiofurin.

Tiazofurin shows a well-defined minimum at χ = 0−20°, close to the conformation maintained in the enzyme-bound ligand. The barrier to rotation in tiazo-

furin is significantly higher than that found in the other agents, suggesting that the tiazofurin anabolite may gain an entropic advantage in binding the target. Among the active agents, tiazofurin remains the most potent and the most highly constrained.

Thiophenfurin also shows an energetic minimum close to the favorable cis conformer ($\chi = 20°$). However, replacement of the negatively charged thiazole nitrogen with the almost neutral C−H group also produces a local minimum at the unfavorable trans conformation ($\chi = 180°$). Replacement of the oxolane oxygen with a sulfur in thiophenthiofurin results in a broader, shallower minimum in the favored region. Thus, any entropic advantage becomes successively less significant in thiophenfurin and thiophenthiofurin, respectively, as conformational constraints diminish. In the inactive compounds, replacement of the positively charged thiazole or thiophene sulfur with a negatively charged oxygen results in generally lower barriers to rotation. What constraints do exist are associated with global or local minima shifted to higher, more unfavorable values of χ.

It is worth noting that the thiolane sulfur is electronically unique, mimicking neither the oxolane oxygen nor the thiazole or thiophene sulfur. Unlike the oxolane oxygen, the thiolane sulfur has a net positive charge. However, the magnitude of this charge is reduced to about one-half that of the more delocalized thiophene or thiazole sulfur. Thus, the conformational energy profiles for furanthiofurin and thiophenthiofurin are more heavily modulated by intramolecular steric and charge-transfer interactions. Nevertheless, the substitution of an electrophilic thiolane sulfur in place of the oxolane oxygen may be also be expected to influence binding of either the parent compounds or the NAD analogues to their targets. The net effect of this substitution is that neither furanthiofurin nor thiophenthiofurin appears to result in particularly effective IMPDH inhibition, despite wide variation in activity.

In summary, computational findings remain qualitatively consistent with biological results. Active compounds contain an electrophilic sulfur in a delocalized environment adjacent to the *C*-glycosidic bond and have an energetically favorable conformer around $\chi = 0°$. Among these, the more constrained (least flexible) compounds (tiazofurin and thiophenfurin) are more active than the less constrained thiophenthiofurin. Those compounds which contain a nucleophilic oxygen in place of the thiazole or thiophene sulfur appear less likely to adopt small angles of χ. These compounds (oxazofurin, furanfurin, and furanthiofurin) show the least activity.

Experimental Section

Melting points were determined on a Büchi 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 computer. Nuclear magnetic resonance ^{1}H spectra were determined at 300 MHz on a Varian VXR-300 spectrometer; chemical shift values are reported in δ values (ppm) relative to the internal standard tetramethylsilane. All exchangeable protons were confirmed by addition of D_2O. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates and RP-18 F_{254} S plates (Merck); silica gel 60 (Merck) (70−230 and 230−400 mesh) for column chromatography was used. High-performance liquid chromatography (HPLC) analysis was carried out on a Waters apparatus equipped with a model 600 system (detector 486). Beckman Ultrasphere Si 5U (10 mm × 25 cm) column was used.

Ethyl 2-(2,3,5-Tri-*O*-benzyl-4-thio-α-D-ribofuranosyl)-furan-3-carboxylate (5α), Ethyl 2-(2,3,5-Tri-*O*-benzyl-4-thio-*β*-D-ribofuranosyl)furan-3-carboxylate (5*β*), Ethyl 5-(2,3,5-Tri-*O*-benzyl-4-thio-α-D-ribofuranosyl)furan-3-carboxylate (6α), and Ethyl 5-(2,3,5-Tri-*O*-benzyl-4-thio-*β*-D-ribofuranosyl)furan-3-carboxylate (6*β*). To a stirred solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-4-thio-D-ribofuranose (**4**) (5 g, 10.44 mmol) in dry CH_2Cl_2 (60 mL) cooled at 0 °C was added trifluoroacetic acid (59 mmol, 4.5 mL). After 15 min under nitrogen atmosphere, the mixture was allowed to warm to room temperature, and ethyl furan-3-carboxylate (**3**) (2.13 g, 15.2 mmol) was added. The resulting solution was stirred for 8 h, neutralized with saturated $NaHCO_3$ (300 ml) and then extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to dryness to give an oily residue which was chromatographed on flash silica gel column eluting with cyclohexanes−EtOAc (97:3). A mixture of **5**α, **5***β* and **6***β* (1.16 g, 20%) was separated as first eluate. TLC (cyclohexanes−EtOAc, 90:10): R_f 0.75.

Evaporation of the following fraction gave 1.56 g (27%) of **6**α. TLC (cyclohexanes−EtOAc, 90:10): R_f 0.72. ^{1}H NMR (Me_2SO-d_6): δ 1.30 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); 3.60 (dd, $J = 5.9, 9.9$ Hz, 1H, H5′b); 3.75 (dd, $J = 3.7, 10.0$ Hz, 2H, H4′, H5′a); 3.95 (m, 1H, H3′); 4.22 (m, 1H, H2′); 4.25 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3); 4.40−4.55 (m, 7H, $CH_2C_6H_5$, H1′); 5.80 (s, 1H, H4); 7.20−7.35 (m, 15H, arom.); 7.90 (s, 1H, H2). Anal. C, H.

Compounds **5**α, **5***β*, **6**α and **6***β* were separated by HPLC using cyclohexanes−EtOAc (95:5) as eluents (F 4.4 mL/min, P1600 psi). HPLC retention times (t_R min): **5***β*, 12.70 min; **5**α, 13.48 min; **6***β*, 14.09 min; **6**α, 15.43 min.

Ethyl 2-(4-Thio-α-D-ribofuranosyl)furan-3-carboxylate (7α), Ethyl 2-(4-Thio-*β*-D-ribofuranosyl)furan-3-carboxylate (7*β*), and Ethyl 5-(4-Thio-*β*-D-ribofuranosyl)furan-3-carboxylate (8*β*). To a solution of 1 M BBr_3 in dry CH_2Cl_2 (1.5 mL) cooled at −78 °C was added dropwise a solution of the mixture of **5**α, **5***β* and **6***β* (1.1 g, 1.96 mmol) in dry CH_2Cl_2 (50 mL). The reaction mixture was stirred for 1 h under nitrogen atmosphere at the same temperature and then evaporated to dryness. The residue was coevaporated with cooled dry CH_2Cl_2/MeOH (2:1, v/v). After neutralization with saturated $NaHCO_3$ the mixture was evaporated to obtain a residue which was washed with cooled anhydrous EtOH and then filtered off. The filtrate was evaporated to dryness to give an oily residue which was chromatographed on silica gel column eluting with $CHCl_3$−MeOH (97:3). Evaporation of the first fraction gave a mixture of **7**α and **7***β*, which was separated by preparative TLC using $CHCl_3$−MeOH (90:10) as eluent in very low yield.

Compound **7**α**:** R_f 0.28. ^{1}H NMR (Me_2SO-d_6): δ 1.30 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); 3.50−3.65 (m, 2H, H4′, H5′b); 3.90 (m, 2H, H5′a, H3′); 4.25 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3); 4.28 (m, 1H, H2′); 4.85 (t, $J = 5.3$ Hz, 1H, OH); 5.07 (d, $J = 5.1$ Hz, 1H, OH); 5.10 (d, $J = 4.7$ Hz, 1H, OH); 5.22 (d, $J = 3.9$ Hz, 1H, H1′); 6.68 (d, $J = 2.0$ Hz, 1H, H4); 7.70 (d, $J = 2.0$ Hz, 1H, H5). Anal. C, H.

Compound **7***β***:** R_f 0.27. ^{1}H NMR (Me_2SO-d_6): δ 1.32 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); 3.45 (m, 1H, H4′); 3.60 (m, 1H, H5′b); 3.75 (m, 1H, H5′a); 4.18 (m, 1H, H3′); 4.41 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3); 4.45 (m, 1H, H2′); 4.70 (t, $J = 5.2$ Hz, 1H, OH); 5.10 (d, $J = 5.2$ Hz, 1H, H1′); 5.13 (d, $J = 4.3$ Hz, 1H, OH); 5.20 (d, $J = 7.1$ Hz, 1H, OH); 6.66 (d, $J = 2.0$ Hz, 1H, H4); 7.70 (d, $J = 2.0$ Hz, 1H, H5). Anal. C, H.

Evaporation of the following fraction of column chromatography gave **8***β* as a white solid (255 mg, 45%). Mp: 113−118 °C dec. TLC ($CHCl_3$−MeOH, 90:10): R_f 0.25. ^{1}H NMR (Me_2SO-d_6): δ 1.27 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); 3.22 (m, 2H, H4′, H5′b); 3.40 (m, 1H, H5′a); 3.58 (m, 1H, H3′); 4.05 (m, 1H, H2′); 4.25 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3); 4.32 (d, $J = 7.4$ Hz,

1H, H1′); 5.05 (t, J = 5.3 Hz, 1H, OH); 5.35 (d, J = 4.3 Hz, 1H, OH); 5.42 (d, J = 7.0 Hz, 1H, OH); 6.62 (s, 1H, H4); 8.30 (s, 1H, H2). Anal. C, H.

Ethyl 5-(4-Thio-α-D-ribofuranosyl)furan-3-carboxylate (8α). The title compound was obtained from **6**α (1.5 g, 2.68 mmol) as reported for **8**β as a white solid (435 mg, 56%). Mp: 125−127 °C. TLC ($CHCl_3$−MeOH, 90:10): R_f 0.31. ^{1}H NMR (Me_2SO-d_6): δ 1.30 (t, J = 7.1 Hz, 3H, OCH_2CH_3); 3.45 (m, 2H, H4′, H5′b); 3.82 (m, 2H, H5′a, H3′); 4.15 (m, 1H, H2′); 4.22 (q, J = 7.0 Hz, 2H, OCH_2CH_3); 4.58 (d, J = 3.6 Hz, 1H, H1′); 4.85 (t, J = 5.3 Hz, 1H, OH); 5.10 (d, J = 6.9 Hz, 1H, OH); 5.16 (d, J = 5.0 Hz, 1H, OH); 6.62 (s, 1H, H4); 8.28 (s, 1H, H2). Anal. C, H.

5-(4-Thio-β-D-ribofuranosyl)furan-3-carboxamide (1). Compound **8**β (250 mg, 0.86 mmol) was reacted with 30% ammonium hydroxide (10 mL) and the mixture was stirred at room temperature for 8 h. Evaporation of the reaction mixture gave a solid residue which was purified by chromatography on silica gel column eluting with $CHCl_3$−MeOH−NH_4OH (80:15:5). Compound **1** was obtained as a white solid (190 mg, 83%). Mp: 131−134 °C. TLC (*i*PrOH−NH_4OH, 80:20): R_f 0.6. UV (MeOH): λ_{max} 208 nm (ϵ 12500), 262 nm (sh, ϵ 3100). ^{1}H NMR (Me_2SO-d_6): δ 3.20 (m, 1H, H4′); 3.40, 3.60 (2m, 2H, H5′b, H5′a); 4.05 (m, 1H, H3′); 4.15 (m, 1H, H2′); 4.30 (d, J = 7.3 Hz, 1H, H1′); 5.05 (t, J = 5.3 Hz, 1H, OH); 5.10 (d, J = 4.1 Hz, 1H, OH); 5.25 (d, J = 7.0 Hz, 1H, OH); 6.67 (s, 1H, H4); 7.12, 7.62 (2 brs, 2H, NH_2); 8.10 (s, 1H, H2). Anal. C, H, N.

5-(4-Thio-α-D-ribofuranosyl)furan-3-carboxamide (9). The title compound was obtained from **8**α (400 mg, 1.38 mmol) as reported for **1** as a white foam (300 mg, 80%). TLC (*i*PrOH−NH_4OH, 80:20): R_f 0.57. ^{1}H NMR (Me_2SO-d_6): δ 3.48 (m, 2H, H4′, H5′b); 3.85 (m, 2H, H5′a, H3′); 4.15 (t, J = 3.1 Hz, 1H, H2′); 4.54 (d, J = 3.4 Hz, 1H, H1′); 4.85 (brs, 1H, OH); 5.18 (brs, 1H, OH); 6.72 (s, 1H, H4); 7.12, 7.65 (2 brs, 2H, NH_2); 8.03 (s, 1H, H2). Anal. C, H, N.

Ethyl 2-(2,3,5-Tri-*O*-acetyl-4-thio-α-D-ribofuranosyl)-thiophene-3-carboxylate (12α), Ethyl 2-(2,3,5-Tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)thiophene-3-carboxylate (12β), Ethyl 5-(2,3,5-Tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)thiophene-3-carboxylate (13β), and Ethyl 5-(2,3,5-Tri-*O*-acetyl-4-thio-α-D-ribofuranosyl)thiophene-3-carboxylate (13α). To a cooled solution of ethyl thiophene-3-carboxylate (**10**) (1 g, 6.40 mmol) in 60 mL of dry 1,2-dichloroethane were added 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose (**11**) (2.14 g, 6.40 mmol) and then $SnCl_4$ (1.6 mL, 6.40 mmol). The mixture was reacted at 0 °C for 0.5 h and then at room temperature for 24 h. After dilution with H_2O, the mixture was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2 (50 mL × 3). The organic layers were dried (Na_2SO_4) and concentrated in vacuo to obtained a brown oily residue which was chromatographed on silica gel column eluting with $CHCl_3$−hexane (55:45). From the first eluate, a mixture of **12**α, **12**β and **13**β was obtained (800 mg, 31%). TLC (diethyl ether−hexane, 70:30): R_f 0.50.

From the last fraction **13**α was separated as an oil (650 mg, 26%). TLC (diethyl ether−hexane, 70:30): R_f 0.43. ^{1}H NMR ($CDCl_3$): δ 1.35 (t, J = 7.1 Hz, 3H, OCH_2CH_3); 2.05−2.15 (3s, 9H, $COCH_3$); 4.10 (m, 1H, H4′); 4.15 (m, 1H, H5′b); 4.30 (q, J = 7.0 Hz, 2H, OCH_2CH_3); 4.42 (m, 1H, H5′a); 5.10 (d, J = 3.9 Hz, 1H, H1′); 5.26 (dd, J = 3.1, 8.9 Hz, 1H, H3′); 5.63 (t, J = 3.6 Hz, 1H, H2′); 7.45 (s, 1H, H4); 8.05 (s, 1H, H2). Anal. C, H.

5-(4-Thio-β-D-ribofuranosyl)thiophene-3-carboxamide (2). The mixture of **12**α, **12**β, and **13**β (700 mg, 1.75 mmol) was treated with methanolic ammonia (30 mL) at room temperature. A TLC analysis showed complete consumption of the starting material after 30 h. Evaporation to dryness of the reaction mixture gave a yellow oily residue which was treated with 30% ammonium hydroxide (30 mL) at room temperature for 24 h. Evaporation of the reaction mixture gave a solid residue which was chromatographed on silica gel column eluting with $CHCl_3$−MeOH−NH_4OH (82:16:2) to give **2** (300 mg, 63%) as a white solid. Mp: 137−141 °C. TLC ($CHCl_3$−MeOH, 80:20): R_f 0.13. UV (MeOH): λ_{max} 210 nm (ϵ 11500), 244 nm (sh, ϵ 3600). ^{1}H NMR (Me_2SO-d_6): δ 3.20 (m, 1H, H4′); 3.45, 3.55 (2m, 2H, H5′b, H5′a); 3.85 (m, 1H, H3′); 4.08 (m, 1H, H2′); 4.46 (d, J = 7.8 Hz, 1H, H1′); 5.06 (t, J = 5.4 Hz, 1H, OH); 5.18 (d, J = 4.5 Hz, 1H, OH); 5.26 (d, J = 6.6 Hz, 1H, OH); 7.15, 7.75 (2 br s, 2H, NH_2); 7.35 (s, 1H, H4); 8.02 (s, 1H, H2). Anal. C, H, N.

As a second fraction a very low amount (<0.5%) of C-2 amides as an α,β mixture was separated.

5-(4-Thio-α-D-ribofuranosyl)thiophene-3-carboxamide (14). The title compound was obtained from **13**α (600 mg, 1.5 mmol) as reported for **2** as a white solid (230 mg, 57%). Mp: 137−141 °C. TLC ($CHCl_3$−MeOH, 80:20): R_f 0.11. ^{1}H NMR (Me_2SO-d_6): δ 3.50 (m, 2H, H4′, H5′b); 3.80−4.05 (m, 3H, H5′a, H3′, H2′); 4.78 (t, J = 4.3 Hz, 1H, OH); 4.86 (d, J = 3.3 Hz, 1H, H1′); 5.06 (d, J = 7.2 Hz, 1H, OH); 5.34 (d, J = 4.9 Hz, 1H, OH); 7.12, 7.70 (2 br s, 2H, NH_2); 7.33 (s, 1H, H4); 8.0 (s, 1H, H2). Anal. C, H, N.

Computational Study. Point charges and energy profiles for tiazofurin, thiophenfurin, thiophenthiofurin, oxazofurin, furanfurin and furanthiofurin were obtained using Gaussian 98.[8] Charges using the model fragment shown in Figure 1 were obtained at the DFT/6-31G**//RHF/3-21G* level of theory and partitioned using the natural bond orbital (NBO) method incorporated in Gaussian 98.[8,9] The density functional single-point component of the calculation employed the B3LYP three-parameter hybrid functional.[8,10] Charges shown in Figure 1 were also calculated using a variety of basis sets and partitioning methods. While magnitudes of individual charges varied depending on the basis set and partitioning function used, signs and relative magnitudes of charges remained consistent with those shown.

Energy profiles were obtained in a manner similar to that described previously.[2] The starting geometry for each model placed the 3′-deoxy ribose or thioribofuranose ring in a 3′-endo conformation, the *C*-glycosidic torsion angle χ at 0°, and the 2′-hydroxyl oxygen proton trans to H-1′. Energies for each conformer were obtained for values of χ between ± 180°. In each case, the value of χ was incremented in 20° steps and fixed. All remaining geometry variables describing the fragment were then fully optimized. 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 and reduntant internal coordinates.[8] Optimized geometries and associated SCF energies were obtained using the 3-21G* basis set.[8] Thus, each point in Figure 1 represents a calculation at the RHF/3-21G*//3-21G* level.[8]

Antiproliferative Assay. Cytotoxicity of tiazofurin and its analogues against human myelogenous leukemia K562 cells growing in logarithmic phase in RPMI 1640 medium containing 10% fetal bovine serum in an atmosphere of air with 5% CO_2 at 37 °C was examined as cited.[6] In short, cell suspensions (0.2 mL; 1 × 10^5 cells/mL) were transferred into 96-well plates, appropriate concentration (0.5−100 μM) of compounds prepared in sterile saline or saline was added, mixed and incubated under conditions described above for 48 h, and then an aliquot was counted using a Coulter counter. The percent inhibition of cell proliferation at 48 h in the presence of compounds compared to saline was expressed as the inhibition of cell growth. IC_{50} concentration is expressed as the drug concentration required to inhibit 50% of cell proliferation.

IMP Dehydrogenase Assay. K562 cells (1 × 10^6 /mL; 10 mL) in logarithmic phase of growth were incubated with saline or indicated concentrations of compounds for 2 h at 37 °C. The cells were harvested by centrifugation, lysed and assayed for IMPDH activity as described.[7] In short, cells were extracted in 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM $MgCl_2$, 0.5% NP-40, and 2 μg/mL aprotinin, and the supernate after centrifugation was used as the source of IMPDH. The enzyme activity was measured according to the cited method.[14] Briefly, 5-μL aliquots of 0.5 M KCl containing 20 mM allopurinol were dispensed into the apex of Eppendorf tubes and dried at 25 °C. For 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 and incubated at 37 °C for 30 min, and the reaction was terminated by heating at 95 °C for 1 min. Tubes were centrifuged, 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 were cut, and the radioactivity was determined by scintillation spectrometry. IMP dehydrogenase 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 compounds at the concentrations specified in Table 1 for 2 h at 37 °C, 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.[15]

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