Structural Studies of a New Antitumor Agent: Tiazofurin and Its Inactive Analogues

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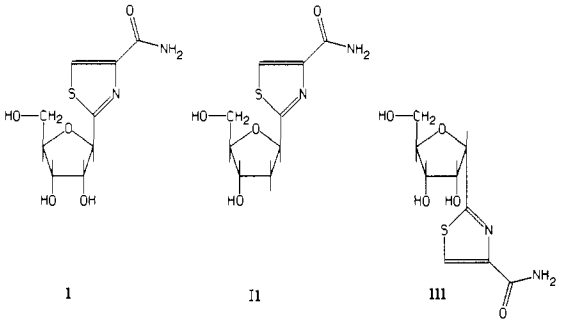
Abstract: The crystal and molecular structures of the novel antitumor agent tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide), $C_9H_{12}N_2O_5S$, its β-2′-deoxy derivative 2-(2-deoxy-β-D-*erythro*-pentofuranosyl)thiazole-4-carboxamide, $C_9H_{12}N_2O_4S$, and its α anomer 2-α-D-ribofuranosylthiazole-4-carboxamide, $C_9H_{12}N_2O_5S$, have been determined by using single-crystal X-ray diffraction techniques employing Cu Kα radiation. Tiazofurin crystallizes in space group $P2_12_12_1$ with cell dimensions a = 5.1053 (5) Å, b = 13.182 (1) Å, c = 16.268 (1) Å, and Z = 4. The structure was refined to a conventional R value of 0.032 for all data based on 4500 reflections. The β-2′-deoxy derivative crystallizes in space group $P2_1$ with cell dimensions a = 7.917 (1) Å, b = 6.7435 (5) Å, c = 10.531 (1) Å, β = 102.48 (1)°, and Z = 2 and was refined to R = 0.036 for all 1132 independent reflections. The α anomer crystallizes in space group $P2_1$ with cell dimensions a = 9.583 (3) Å, b = 5.255 (1) Å, c = 11.241 (1) Å, β = 102.34 (2)°, and Z = 2 and was refined to R = 0.037 for all 1142 independent reflections. The absolute configuration of each structure was confirmed by refinement of the corresponding enantiomorph and application of Hamilton's significance test. The thiazole ring in each structure is close to planar and shows some conjugation. The sugar moiety in tiazofurin shows a C2′ endo pucker, the β-2′-deoxy derivative shows a C3′ exo–C_2′ endo pucker, and a C3′ endo –C2′ exo pucker is seen in the α anomer. In each structure the conformation about the *C*-glycosyl bond is such that the thiazole sulfur forms a close intramolecular contact with the furanose ring oxygen O1′. The relationships between structural differences and differences in drug function are discussed and a model for drug activity is proposed.

The *C*-glycosyl thiazole tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC286193[1]) has demonstrated sig-



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nificant antitumor activity in several in vivo model tumor systems. In the mouse leukemias P388 and L1210, it prolongs mean survival time (T/C) by 245% and 230%, respectively.[2] Further, the drug is essentially curative for the Lewis lung carcinoma in mice over a broad dosage range.[3] Tiazofurin has also demonstrated in vitro antiviral activity.[1] In comparison, both the corresponding 2′-deoxynucleoside [2-(2-deoxy-β-D-*erythro*-pentofuronosyl)thiazole-4-carboxamide, II] and the α anomer (2-α-D-ribofuranosylthiozole-4-carboxamide, III) are inactive.[4] In order to investigate the structural requirements for drug activity, we have completed single-crystal X-ray diffraction studies of all three compounds. These results are presented and discused in light of the currently known biochemical functions of the active drug.

Experimental Section

X-ray Data Collection. (a) 2-β-D-Ribofuranosylthiazole-4-carboxamide (I). Colorless crystals were grown at room temperature by slow evaporation from a 10 mM aqueous solution of **I**. Precession photographs and a preliminary rapid data scan by diffractometer revealed an orthorhombic crystal system. The only observed systematic absences were $h00$ for $h = 2n + 1$, $0k0$ for $k = 2n + 1$, and $00l$ for $l = 2n + 1$, indicating unambiguously space group $P2_12_12_1$. Cell dimension and intensity data were collected at room temperature from a thick rectangular plate of approximate dimensions 0.15 × 0.10 × 0.05 mm mounted roughly parallel to the a axis. A Nonius CAD-4 diffractometer was employed by using a graphite monochromator and Cu Kα radiation. Lattice constants were obtained by least-squares refinement of the angular settings of 24 reflections in the range θ = 25–30°. These are indicated in Table I. Reflections were measured in the range $2° < \theta < 70°$ by using the $\omega - 2\theta$ scan method with a variable scan width $\Delta\omega = (1.0 + 0.15 \tan\theta)°$, this angle being extended 25% on each side for background measurements. The scan rate varied between 0.4 and 2.0 deg/min depending upon the value of $\sigma(I)/I$ for each reflection. Data were collected in four octants $(-h, \pm k, \pm l)$. Only identical redundantly measured intensities were averaged, yielding a total of 4500 reflections. Of these, 80 had values of $|F_o|^2 < 0.2\sigma(F_o^2)$ where $\sigma(F_o^2) = [\sigma^2(I) + (0.02|F_o|^2)^2]^{1/2}$ and were reset to $0.2\sigma(F_o^2)$. All data were used in the subsequent analysis and refinements (see paragraph at end of paper regarding supplementary material).

Table I. Crystal and Refinement Data

	I	II	III
space group	$P2_12_12_1$	$P2_1$	$P2_1$
a, Å	5.1053 (5)	7.917 (1)	9.583 (3)
b, Å	13.182 (1)	6.7435 (5)	5.255 (1)
c, Å	16.268 (1)	10.531 (1)	11.241 (1)
β, deg		102.48 (1)	102.34 (2)
Z	4	2	2
M_r	260.3	244.3	260.3
V, Å^3	1094.8	548.9	553.0
ρ(calcd), g/cm^3	1.579	1.478	1.563
no. of observations (m)	4500	1132	1142
no. of variables (n)	203	193	202
S	1.39	1.30	1.30
R, %	3.17	3.60	3.74
R_w	3.22	3.64	3.85
R_w(enantiomorph)	4.88	3.90	4.25

Three standard reflections measured every 2 h of X-ray exposure time showed no decline in intensity. Corrections for Lorentz and polarization factors were applied and, in addition, data were corrected for absorption

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by using the semiempirical ψ-scan technique.[5] A single ψ scan was employed, consisting of 36 measurements of a standard reflection in 10-deg steps of ϕ with the crystal in an approximate equiinclination setting.

(b) **2-(2-Deoxy-β-D-*erythro*-pentofuranosyl)thiazole-4-carboxamide (II).** Crystals were obtained as described in ref 6. Preliminary photographic and counter measurements indicated a monoclinic system with systematic absences $0k0$ for $k = 2n + 1$, uniquely defining the space group as $P2_1$. Cell dimension and intensity data were obtained from a crystal of approximate dimensions 0.05 × 0.10 × 0.10 mm mounted roughly parallel to the [101] direction. The CAD-4 diffractometer was used with graphite monochromatized Cu Kα radiation. Lattice constants were obtained as described above and are listed in Table I. Intensity data were collected by using the parameters and methods described above with the exception that the variable scan width was $\Delta\omega = (1.17 + 0.15 \tan \theta)°$. A total of 2655 reflections (including standards) was measured in the two equivalent but non centrosymetrically related quadrants $(h,k,\pm l)$ and $(-h,k,\pm l)$. In this case the data were averaged, yielding 1132 unique reflections, of which 59 had values of $|F_o|^2 < 0.2\sigma(F_o^2)$ and were reset to $0.2\sigma(F_o^2)$. All unique data were used in the subsequent analysis. Standard reflections measured as above revealed an approximate 5% decline in intensity over the course of the data collection. A separate linear decay correction was applied to the set of reflections collected within each 2-h time period. Data were corrected for absorption as described above.

A preliminary report of the structure of II has been presented elsewhere.[6] However, both the crystal and the data set used in the present study were of higher quality than those used previously.

(c) **2-α-D-Ribofuranosylthiazole-4-carboxamide (III).** Colorless rod-shaped crystals were grown by vapor diffusion from a 20-μL drop of 10 mM aqueous solution of III in a 20% ethanol reservoir. Preliminary photographic and counter data indicated a monoclinic system with systematic absences $0k0$ for $k = 2n + 1$, uniquely defining the space group as $P2_1$. Cell dimensions and intensity data were obtained from a crystal of approximate dimensions 0.05 × 0.05 × 0.30 mm mounted parallel to the b axis. Lattice constants were obtained as described for I and are listed in Table I. Data were collected on the CAD-4 diffractometer by using Cu Kα radiation employing the methods described for I with the exceptions that the scan speed varied between 1.0 and 2.0°/min. Data were collected from one unique quadrant $(-h, -k, \pm l)$, yielding 1142 unique reflections, of which 25 had values of $|F_o|^2 < 0.2\sigma(F_o^2)$. These were reset to $0.2\sigma(F_o^2)$ and all unique data were used in the subsequent refinements. Three standard reflections measured every 2 h showed no decline in intensity. Lorentz and polarization factors were applied but in this case no correction for absorption was made.

Structure Solutions and Refinements. All structures were solved by the application of standard Fourier techniques. In each case the sulfur position was determined from the Patterson map and the positions of the remaining nonhydrogen atoms were obtained from subsequent least-squares refinements and Fourier maps. The positions of all hydrogen atoms were then obtained from successive difference Fourier maps, employing only low-angle data $[(\sin \theta)/\lambda < 0.4$ Å$^{-1}]$.

All structures were refined by using full-matrix least-squares techniques. The function minimized was $\sum w(\Delta F)^2$ where $\Delta F = |F_o| - |F_c|$ with weights $w = 1/\sigma^2$. In the early refinements, values of σ were calculated according to $\sigma = \sigma(F_o^2)/2|F_o|$, where $\sigma(F_o^2)$ is defined as above. In the later refinements, weights $w = 1/\sigma_{new}^2$ were used where $\sigma_{new}^2 = [\sigma^2 + 0.5A|F_o|^2 + 0.5B[(\sin \theta)/\lambda]^2]^{1/2}$. Values of A and B were obtained by a least-squares minimization of the function $|\Delta F|^2 - \sigma_{new}^2$ for 20 separate segments in $|F_o|$ and $(\sin \theta)/\lambda$ for each data set. Non-hydrogen atoms were refined anisotropically, the y coordinates of the sulfur atoms in the two monoclinic structures remaining fixed. Positional parameters of all hydrogen atoms were refined with isotropic temperature factors. The later refinements of each structure included a type I isotropic extinction correction and utilized all data. Final refinements converged to the values of $R = \sum|\Delta F|/\sum|F_o|$ and $R_w = [\sum w(\Delta F)^2/\sum w|F_o|^2]^{1/2}$ listed in Table I. Also listed for each structure is the discrepancy factor $S = [\sum w(\Delta F)^2/(m - n)]^{1/2}$. The largest final parameter shift observed in all three structures was 0.18σ; most were less than 0.10σ. The final difference Fourier maps were featureless. Atomic scattering factors for the non-hydrogen atoms and anomalous dispersion corrections for the sulfur atoms were from ref 7. Scattering factors for the hydrogen atoms were those of Stewart et al.[8]

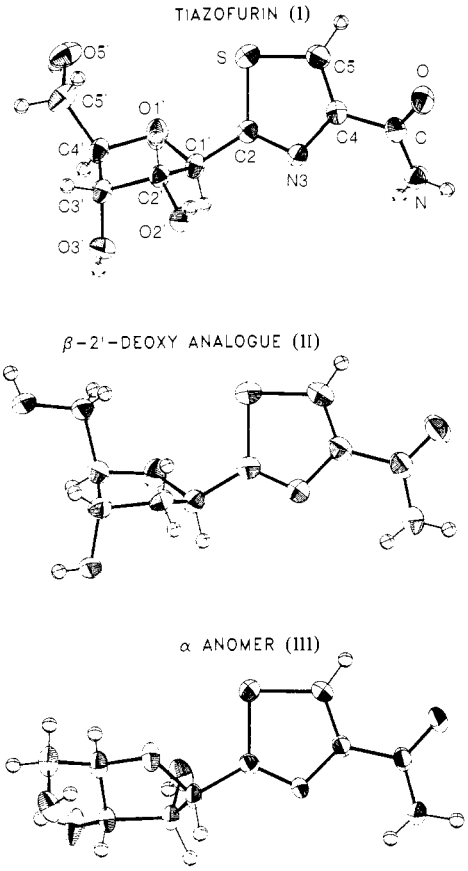

Figure 1. Conformations of the tiazofurin molecule and its analogues. Non-hydrogen atoms are represented by thermal ellipsoids at the 50% probability level.

Absolute Configuration Determinations. The enantiomorphs of structures I, II, and III were also refined by using the same techniques described above. The values of R_w thus obtained are listed in Table I. Application of Hamilton's significance test[9] to each of the three weighted R factor ratios confirmed the original absolute configuration assignments at the 99.5% significance level.

Results

The conformations of the three structures are illustrated in Figure 1. Atomic coordinates for I, II, and III are listed in Table II. Bond lengths involving non-hydrogen atoms are listed in Table III. Selected bond and torsion angles are given in Tables IV and V, respectively.

Thiazole Rings. Bond lengths and angles in the three thiazole rings are similar, most agreeing within 3σ. A somewhat larger difference in the S–C2 bonds may suggest contributions from slightly different resonance forms for each ring. However, in each case the S–C2 and S–C5 bond lengths are significantly shorter than the single S–C (sp^2) bond length (1.78 Å)[14] as well as unconjugated S–C (sp^2) bond lengths observed in several thiazolidines and thiazolidinones (cf. 1.771, 1.776, 1.777, and 1.782Å).[10-13] Similarly, all N3–C4 bond lengths are shorter than the C (sp^2)–N (sp^2) single bond length (1.47 Å).[14] Thus, the thiazole rings display some conjugation. This is similar to results observed in other thiazole structures, although comparison is complicated by the wide variations seen in ring dimensions upon changes in substituents. Nevertheless, bond lengths and angles observed here are within a standard deviation of the mean values obtained from

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Table II. Atomic Coordinates and Isotropic Thermal Parameters (for Non-H Atoms $B = (4/3)\Sigma_i\Sigma_j\beta_{ij}a_i \cdot a_j$)

atom	X	Y	Z	B	atom	X	Y	Z	B
			(a) Tiazofurin (I)						
S	−0.00896 (9)	0.30497 (3)	0.06386 (2)	2.96	O1′	0.3878 (3)	0.20349 (7)	0.16780 (6)	3.23
C2	0.2486 (3)	0.3623 (1)	0.11456 (8)	2.31	O2′	0.3155 (3)	0.41591 (8)	0.30379 (7)	2.79
N3	0.2946 (3)	0.45572 (8)	0.09311 (7)	2.44	O3′	0.6584 (2)	0.25902 (9)	0.33446 (7)	2.81
C4	0.1125 (3)	0.4864 (1)	0.03590 (8)	2.34	O5′	0.1739 (3)	0.00646 (9)	0.18599 (9)	3.86
C5	−0.0652 (3)	0.4158 (1)	0.01209 (9)	2.80	HC5	−0.196 (4)	0.421 (1)	−0.026 (1)	3.5
C	0.1207 (3)	0.5927 (1)	0.00501 (8)	2.39	H1N	0.493 (5)	0.605 (1)	0.009 (1)	3.9
O	−0.0847 (2)	0.63715 (8)	−0.01342 (7)	3.24	H2N	0.360 (5)	0.701 (1)	−0.010 (1)	4.4
N	0.3539 (3)	0.6346 (1)	−0.00067 (9)	2.85	HC1′	0.579 (4)	0.334 (1)	0.184 (1)	3.0
C1′	0.3916 (3)	0.3101 (1)	0.18227 (8)	2.37	HC2′	0.079 (3)	0.307 (1)	0.2630 (8)	2.1
C2′	0.2662 (3)	0.3207 (1)	0.26699 (8)	2.18	HC3′	0.303 (3)	0.211 (1)	0.3605 (9)	2.5
C3′	0.3992 (3)	0.2325 (1)	0.31205 (9)	2.35	HC4′	0.565 (4)	0.118 (1)	0.246 (1)	2.6
C4′	0.4078 (3)	0.1512 (1)	0.24521 (9)	2.39	H1C5′	0.203 (5)	0.039 (1)	0.308 (1)	5.6
C5′	0.1906 (4)	0.0755 (1)	0.2539 (1)	3.51	H2C5′	0.023 (5)	0.113 (1)	0.257 (1)	4.2
					HO2′	0.190 (6)	0.439 (2)	0.314 (2)	6.4
					HO3′	0.645 (4)	0.296 (2)	0.373 (1)	3.9
					HO5′	0.286 (6)	−0.022 (2)	0.185 (2)	6.3
			(b) 2-(2-Deoxy-β-D-*erythro*-pentofuranosyl)thiazole-4-carboxamide (II)						
S	0.0717 (1)	−0.00327	0.1793 (1)	4.61	HC5	0.262 (6)	0.032 (9)	0.042 (4)	6.3
C2	0.0449 (4)	0.2282 (5)	0.2443 (3)	2.95	H1N	0.312 (5)	0.633 (7)	0.179 (4)	5.5
N3	0.1327 (3)	0.3694 (4)	0.2049 (2)	2.99	H2N	0.447 (5)	0.693 (7)	0.087 (3)	4.4
C4	0.2233 (4)	0.2973 (5)	0.1167 (3)	2.95	HC1′	−0.047 (3)	0.386 (5)	0.381 (3)	2.1
C5	0.2045 (5)	0.1003 (6)	0.0906 (4)	3.99	HC2′	−0.288 (5)	0.178 (7)	0.174 (4)	4.6
C	0.3377 (4)	0.4298 (5)	0.0607 (3)	3.13	H2C2′	−0.308 (4)	0.401 (6)	0.215 (3)	3.6
O	0.4068 (3)	0.3754 (4)	−0.0268 (2)	4.09	HC3′	−0.480 (4)	0.148 (5)	0.316 (3)	2.7
N	0.3598 (4)	0.6109 (5)	0.1118 (3)	4.03	HC4′	−0.247 (4)	0.031 (6)	0.524 (3)	3.6
C1′	−0.0816 (4)	0.2594 (5)	0.3285 (3)	2.80	H1C5′	−0.286 (4)	−0.182 (6)	0.275 (3)	2.9
C2′	−0.2694 (4)	0.2667 (5)	0.2528 (3)	3.00	H2C5′	−0.160 (5)	−0.236 (7)	0.410 (4)	5.1
C3′	−0.3660 (4)	0.1924 (5)	0.3532 (3)	2.98	HO3′	−0.449 (4)	0.325 (7)	0.484 (4)	4.0
C4′	−0.2447 (4)	0.0342 (5)	0.4249 (3)	2.78	HO5′	−0.419 (7)	−0.37 (1)	0.398 (5)	7.2
C5′	−0.2736 (4)	−0.1718 (5)	0.3676 (3)	3.14					
O1′	−0.0736 (3)	0.0931 (4)	0.4144 (2)	3.30					
O3′	−0.3840 (4)	0.3550 (4)	0.4355 (3)	4.35					
O5′	−0.4296 (4)	−0.2536 (4)	0.3914 (3)	3.91					
			(c) 2-α-D-Ribofuranosylthiazole-4-carboxamide (III)						
S	0.36450 (9)	−0.25013	0.48924 (7)	2.83	HC5	0.496 (5)	−0.35 (1)	0.681 (4)	4.5
C2	0.2705 (3)	0.0243 (7)	0.5010 (3)	2.07	H1N	0.328 (5)	0.34 (1)	0.808 (4)	3.6
N3	0.2910 (3)	0.1209 (6)	0.6109 (2)	2.16	H2N	0.432 (4)	0.300 (8)	0.935 (4)	2.7
C4	0.3860 (3)	−0.0306 (7)	0.6908 (3)	2.16	HC1′	0.199 (5)	0.35 (1)	0.402 (4)	4.5
C5	0.4354 (3)	−0.2377 (8)	0.6419 (3)	2.52	HC2′	−0.037 (4)	0.201 (8)	0.407 (3)	2.0
C	0.4418 (3)	0.0424 (8)	0.8195 (3)	2.48	HC3′	−0.004 (6)	0.33 (2)	0.206 (5)	6.5
O	0.5306 (3)	−0.0968 (6)	0.8862 (2)	3.47	HC4′	0.101 (4)	−0.19 (1)	0.180 (4)	3.4
N	0.3966 (3)	0.2589 (9)	0.8570 (3)	3.56	H1C5′	0.244 (4)	0.004 (8)	0.062 (3)	2.2
C1′	0.1818 (4)	0.1458 (7)	0.3891 (3)	2.37	H2C5′	0.082 (4)	0.06 (1)	0.000 (4)	3.2
C2′	0.0230 (3)	0.0860 (7)	0.3587 (3)	2.40	HO2′	−0.083 (6)	−0.19 (1)	0.368 (4)	4.7
C3′	−0.0069 (4)	0.1342 (8)	0.2214 (3)	2.30	HO3′	−0.142 (5)	0.03 (1)	0.094 (4)	3.8
C4′	0.1189 (4)	0.0033 (7)	0.1861 (3)	2.56	HO5′	0.273 (7)	0.38 (2)	0.101 (5)	6.4
C5′	0.1589 (4)	0.0927 (9)	0.0702 (3)	3.02					
O1′	0.2366 (2)	0.0515 (6)	0.2871 (2)	2.89					
O2′	0.0029 (3)	−0.1754 (6)	0.3841 (2)	3.26					
O3′	−0.1426 (3)	0.0377 (8)	0.1658 (2)	3.76					
O5′	0.1815 (3)	0.3603 (6)	0.0692 (2)	3.28					

Table III. Select Bond Distances (Å)

	I	II	III
C2-N3	1.301 (2)	1.299 (4)	1.311 (4)
N3-C4	1.376 (2)	1.379 (4)	1.385 (4)
C4-C5	1.356 (2)	1.358 (5)	1.350 (5)
C5-S	1.711 (2)	1.700 (4)	1.707 (3)
S-C2	1.727 (2)	1.736 (3)	1.720 (3)
C2-C1′	1.490 (2)	1.489 (5)	1.502 (4)
C4-C	1.489 (2)	1.483 (5)	1.482 (4)
C-O	1.239 (2)	1.225 (4)	1.244 (4)
C-N	1.316 (2)	1.331 (5)	1.317 (6)
C1′-C2′	1.526 (2)	1.528 (4)	1.520 (5)
C2′-C3′	1.533 (2)	1.518 (5)	1.530 (4)
C3′-C4′	1.528 (2)	1.522 (4)	1.512 (5)
C4′-C5′	1.499 (2)	1.512 (4)	1.510 (5)
C4′-O1′	1.439 (2)	1.439 (4)	1.442 (4)
O1′-C1′	1.426 (2)	1.434 (4)	1.447 (4)
C2′-O2′	1.413 (2)		1.424 (5)
C3′-O3′	1.417 (2)	1.424 (4)	1.412 (4)
C5′-O5′	1.433 (2)	1.424 (5)	1.423 (6)

Table IV. Select Bond Angles (Deg)

	I	II	III
C2-S-C5	89.4 (1)	89.4 (2)	89.6 (2)
S-C5-C4	109.4 (1)	110.1 (3)	110.0 (2)
C5-C4-N3	116.3 (1)	115.6 (3)	116.0 (3)
C4-N3-C2	109.7 (1)	110.4 (3)	109.5 (3)
N3-C2-S	115.1 (1)	114.5 (3)	114.9 (2)
C1′-C2-N3	123.1 (1)	124.2 (3)	124.7 (3)
C1′-C2-S	121.6 (1)	121.1 (2)	120.2 (2)
C-C4-N3	119.0 (1)	120.4 (3)	122.3 (3)
C-C4-C5	124.6 (1)	124.0 (3)	121.5 (3)
C4-C-O	120.2 (1)	122.0 (3)	119.5 (3)
C4-C-N	116.4 (1)	115.5 (3)	117.5 (3)
O-C-N	123.4 (1)	122.5 (3)	123.0 (3)

12 well-refined thiazole structures ($R < 0.10$) without protonated or substituted heterocyclic nitrogens [i.e., C2–N3 = 1.30 (1) Å; N3–C4 = 1.38 (1) Å; C_4–C5 = 1.35 (2) Å; C5–S = 1.72 (2) Å; S–C2 = 1.73 (2) Å; C2–S–C5 = 89 (1)°; S–C5–C4 = 110 (1)°;

Table V. Select Torsion Angles and Sugar Conformations (Deg)

	I	II	III
κ(N-C-C4-N3)	33.1 (1)	7.1 (2)	0.6 (2)
ϕ(O5'-C5'-C4'-C3')	172.3 (5)	−71.8 (2)	53.9 (3)
χ(O1'-C1'-C2-S)	30.67 (7)	40.8 (2)	−20.8 (3)
τ_0(C4'-O1'-C1'-C2')	−30.4 (1)	−16.3 (2)	15.1 (2)
τ_1(O1'-C1'-C2'-C3')	42.6 (1)	33.1 (2)	−36.7 (2)
τ_2(C1'-C2'-C3'-C4')	−38.4 (1)	−36.8 (2)	44.2 (2)
τ_3(C2'-C3'-C4'-O1')	21.8 (1)	28.1 (2)	−36.7 (2)
τ_4(C3'-C4'-O1'-C1')	5.2 (1)	−7.4 (2)	13.7 (2)
τ_m	43.4	37.7	45.0
P	154.9	173.1	−0.7

Table VI. Deviations from Thiazole Ring Least-Squares Planes (Å)

	I	II	III
S[a]	−0.007 (2)	−0.009 (2)	0.004 (4)
C2[a]	0.012 (2)	0.011 (5)	−0.003 (6)
N3[a]	−0.013 (2)	−0.007 (4)	0.001 (5)
C4[a]	0.006 (2)	−0.002 (5)	0.003 (6)
C5[a]	0.001 (2)	0.008 (6)	−0.004 (7)
C	0.040 (2)	−0.061 (5)	0.143 (7)
C1'	0.155 (2)	0.160 (5)	0.085 (7)

[a] Used in calculations of LS planes.

C5–C4–N3 = 116 (1)°; C4–N3–C2 = 110 (1)°; N3–C2–S = 115 (1)°].[14-18]

Deviations from the least-squares planes of the thiazole rings are listed in Table VI. In general, these are quite small. The thiazole ring in III is planar within experimental error. Deviations from the least-squares plane in II suggest a very slight pucker that may be significant. This is consistent with the 0.16-Å deviation of C1' above the ring plane observed in this structure. The thiazole ring in I shows a small but significant pucker similar to that suggested in II. In this structure the C2 atom lies slightly above the least-squares plane with flanking S and N3 atoms lying below the plane. This is also consistent with the bending of the C2–C1' bond above the ring plane (evidenced by the deviation in C1') and probably contributes to the differences seen in bond angles about C2.

Carboxamide Groups. The lengths of the C–O and C–N bonds in the carboxamide groups of I and III are similar. The β-deoxy structure shows a significantly shorter C–O bond and longer C–N bond, suggesting a greater contribution from the H_2N–C=O resonance form and a smaller contribution from the alternative H_2N^+=C–O^- form. This is consistent with the relatively small number of hydrogen bonds formed by this group in II (see below). The C4–C bond lengths are similar in all three structures. These are close to the values observed for extracyclic C (sp^2)–C (sp^2) bonds in other thiazoles that show relatively little conjugation with the heterocyclic ring (e.g., 1.48, 1.482, 1.49, and 1.49Å).[16-19] The plane of the carboxamide group in each structure is rotated relative to the thiazole ring plane. The N–C–C4–N3 torsion angle κ shows the smallest value (0.6°) in the alpha structure (III). However, the carboxamide carbon in this structure shows the largest displacement from the ring plane [0.143 (7) Å], indicating significant bending of the C4–C bond. This is seen to a lesser extent in the other two structures. The large (33°) rotation of the carboxamide group in tiazofurin (I) is probably the result of packing forces. The carboxamide group in this structure participates in a larger number of hydrogen bonds than in either II or III (see below).

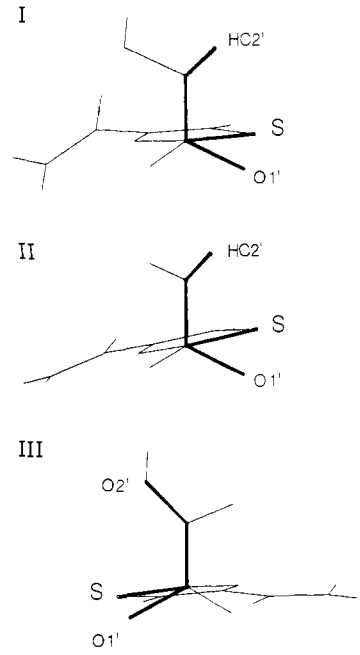

Figure 2. Views down the *C*-glycosyl bonds of the tiazofurin molecule and its analogues. Heavy bonds connect atoms involved in observed or potential close intramolecular contacts. Only part of each sugar moiety is shown for clarity.

Despite the variation in carboxamide group rotation seen among I, II, and III, in each structure the carboxamide amino group remains trans to the thiazole ring C5 and cis to the ring nitrogen N3 (Figure 1). Rotation of this group cis to C5 would produce unfavorable steric interactions between the amino hydrogen H1N and the ring hydrogen HC5. The geometry observed here avoids these as well as optimizing any electrostatic interactions between the amide H1N and the lone pair electrons of N3 and between the carbonyl oxygen and ring HC5. A similar effect is observed in the structures of several thiazole-containing antibiotics, all of which possess at least one free N-substituted carboxamide group on the 4-position of a 2–4-substituted thiazole ring.[20-22] In each case, the carboxamide nitrogen is found cis to the ring nitrogen and the carboxamide oxygen cis to HC5. The largest carboxamide rotation observed among those structures for which coordinates are available is about 15°.

Sugar Moieties. Bond lengths in the three ribose moieties are similar. Several possibly significant differences in bond lengths may reflect either the absence of the 2'-hydroxyl group or differences in sugar pucker.[23] Differences in bond lengths between I and III are comparable to those seen between other nucleoside anomeric pairs.[28,30,33,34] All bond lengths are within the range

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Table VII. Hydrogen Bond Distances and Angles

D-H···A	D-A, Å	H-A, Å	D-H-A, deg	symmetry of acceptor A
		I		
N-H1N···O	2.873 (2)	2.23 (3)	135 (2)	$1+x, y, z$
N-H2N···O	3.034 (2)	2.18 (2)	159 (2)	$^1/_2+x, ^3/_2-y, -z$
O2'-HO2'···O5'	2.774 (2)	2.06 (3)	167 (3)	$-x, ^1/_2+y, ^1/_2-z$
O3'-HO3'···O	2.853 (2)	2.07 (2)	167 (2)	$^1/_2-x, 1-y, ^1/_2+z$
O5'-HO5'···O2'	2.872 (2)	2.20 (3)	167 (3)	$1-x, -^1/_2+y, ^1/_2-z$
		II		
N-H2N···O	2.848 (4)	1.89 (4)	172 (3)	$1-x, ^1/_2+y, -z$
O3'-HO3'···O5'	2.683 (5)	1.86 (4)	173 (3)	$-1-x, ^1/_2+y, 1-z$
O5'-HO5'···O3'	2.691 (4)	1.91 (7)	173 (5)	$x, -1+y, z$
		III		
N-H2N···O	2.922 (4)	2.03 (4)	168 (4)	$1-x, ^1/_2+y, 2-z$
N-H1N···O3'	2.806 (5)	2.14 (5)	133 (4)	$-x, ^1/_2+y, 1-z$
O2'-HO2'···N3	3.025 (4)	2.29 (6)	152 (5)	$-x, -^1/_2+y, 1-z$
O3'-HO3'···O5'	2.750 (4)	1.99 (5)	155 (5)	$-x, -^1/_2+y, -z$
O5'-HO5'···O	2.706 (4)	1.86 (7)	160 (6)	$1-x, ^1/_2+y, 1-z$

of those seen in other β-ribo-,[24] β-deoxyribo-,[25-27] and α-ribonucleosides[28-30] with similar ribose puckering.

The amplitude τ_m and phase angle P of pseudorotation[31] for each structure is given in Table V. Conformations of the three ribose rings were standard. The tiazofurin structure (I) adopts a C2′ endo pucker (^{2}E) seen in a number of ribose structures.[24] The β-2′-deoxy analogue (II) shows a similar C3′ exo–C2′ endo pucker ($^2_3$T), falling within the range of conformations most commonly seen for deoxyribonucleosides.[32] The α anomer (III) adopts a pure C3′ endo–C2′ exo ($^3_2$T) pucker, essentially enantiomeric to that seen in the β structures I and II. This is not always the case among pairs of anomeric nucleosides,[30,33,34] although this particular conformation is seen in other α nucleosides.[28-30]

Conformations about the C4′–C5′ bond, defined by the torsion angle φ(O5′–C5′–C4′–C3′), are shown in Table V. These fall within the three commonly observed ranges, being gauche–trans (*t*), trans–gauche (*g*–), and gauche–gauche (*g*+) for I, II, and III, respectively. These conformations are generally associated with the observed ribofuranosyl puckering.[32]

***C*-Glycosyl Bonds: Conformational Stabilization.** The lengths of the *C*-glycosyl bonds C1′–C2 are similar to the mean values seen for other *C*-glycosyl nucleosides [1.50 (1) Å][35-37] as well as values for thiazole and thiazolium derivatives with an sp^3 hybridized carbon on the C2 position [1.50 (3) Å].[38-41] The conformations about this bond are defined by the torsion angles χ(O1′–C1′–C2–S), listed in Table V. A comparison of the conformations of the thiazole rings relative to the furanose moieties reveals several interesting features common to all three structures. The magnitudes of χ lie within the range $|\chi| = 30 \pm 11°$. In each case the thiazole sulfur is cis to the furanose oxygen O1′ (Figure 2). Further, the distance between the two atoms in each case is less than the sum of their van der Waals radii (3.30 Å). S···O1′ distances are 2.958 (1), 3.018 (3), and 2.826 (3) Å for structures I, II, and III, respectively. Very similar short intramolecular S···O contacts (2.749–2.901 Å) have been observed in four thiamin analogues containing substituents of the form $-CH_2OR$ on C2 of the thiazolium ring.[39-41] Analogous contacts (2.92–3.08 Å) have been observed in several thiazole derivatives as well.[21,42,43] Results from a number of molecular orbital calculations suggest that the thiazole sulfur has a net positive charge.[44,45] Thus, the S···O contact observed in each structure likely represents an electrostatic interaction between the heterocyclic sulfur and the lone pair electrons on the furanose oxygen. This interaction may stabilize the conformation of each structure about the *C*-glycosyl

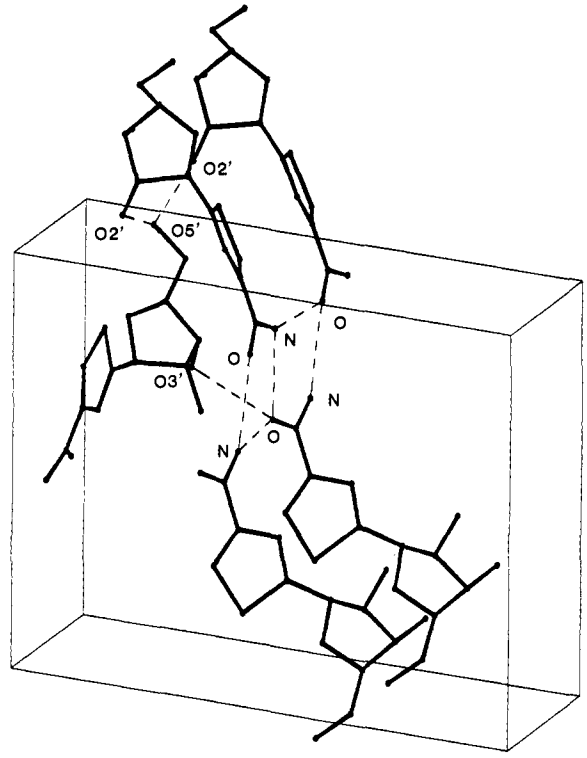

Figure 3. Molecular packing and hydrogen bonding in I viewed approximately down the crystallographic *a* axis. The *b* axis is vertical. Thin dashed lines are hydrogen bonds. Only part of the contents of the unit cell is shown and hydrogen atoms are omitted for clarity.

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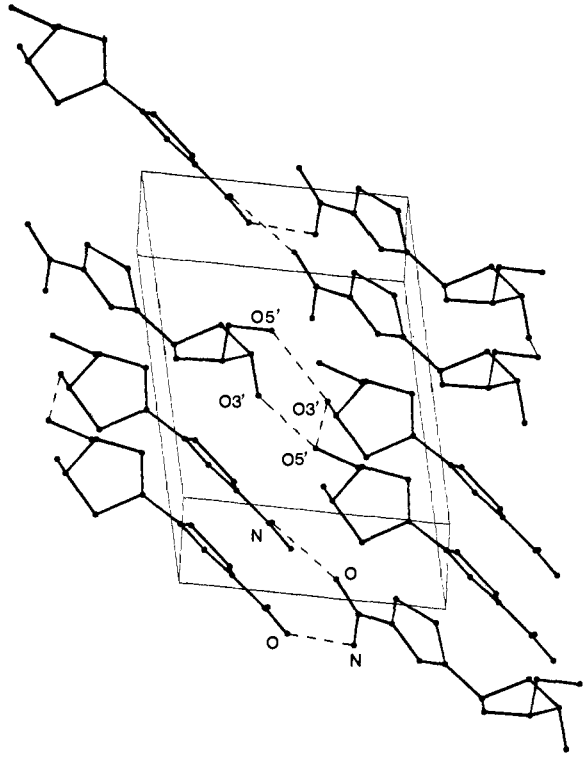

Figure 4. Molecular packing and hydrogen bonding in II viewed approximately down the crystallographic *b* axis. The *c* axis is vertical. Thin dashed lines are hydrogen bonds. Hydrogen atoms are omitted for clarity.

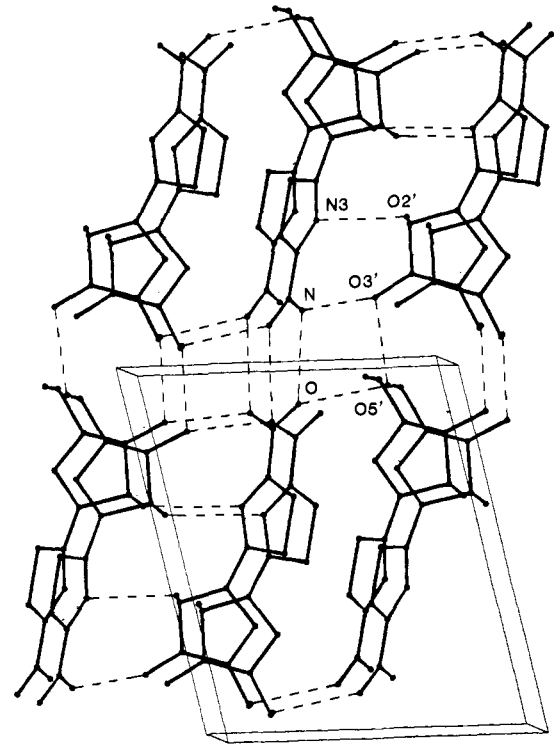

Figure 5. Molecular packing and hydrogen bonding in III viewed approximately down the crystallographic *b* axis. The *a* axis is horizontal. Thin dashed lines are hydrogen bonds. Hydrogen atoms are omitted for clarity.

bond. Further, rotation of the thiazole ring about this bond may be hindered by steric effects. In structures I and II, rotation of the ring to more positive values of χ (counterclockwise in Figure 2) would produce short contacts between the sulfur atom and the furanose hydrogen HC2′ (2.64 and 2.50 Å at $\chi = 100°$ for I and II, respectively). In structure III, rotation of the ring to more negative values of χ (clockwise in Figure 2) would bring the thiazole sulfur into very short contact with the hydroxyl oxygen O2′ (2.521 Å at $\chi = -100°$). Rotation of the rings in all three structures toward $\chi = 0°$ would maximize the steric component of the S···O1′ interaction described above. Some relief from these close contacts could be expected from simultaneous changes in ring pucker. However, it is possible that the favored conformations about the *C*-glycosyl bond are those in which the thiazole sulfur is generally cis to the furanose O1′.

Molecular Packing and Hydrogen Bonding. Crystal packing and intermolecular hydrogen bonding for I, II, and III are illustrated in Figures 3, 4, and 5. Hydrogen-bond distances and angles are listed in Table VII. In general, the crystal structures are characterized by a rich network of primarily linear hydrogen bonds linking thiazole ring to thiazole ring, furanose ring to furanose ring, and thiazole ring to furanose ring. There are no thiazole ring stacking interactions. All hydroxyl oxygens act as hydrogen-bond donors. In addition, all exocyclic O5′ oxygens act as hydrogen acceptors. One furanose hydroxyl oxygen in each structure also acts as an acceptor. The carboxamide group is a highly versatile hydrogen bond forming substituent. In acts as both donor and acceptor, the number of bonds formed varying with each structure.

In III, the heterocyclic nitrogen N3 acts as an acceptor in an O2′–HO2′···N3 bond. This is the only structure in which this nitrogen clearly participates in any hydrogen bonding. The carboxamide hydrogens H1N and H2N both act as hydrogen donors, while the carboxamide oxygen O acts as an acceptor in two hydrogen bonds. The fewest number of intermolecular hydrogen bonds are found in the β-deoxy structure, II. In this case, only one carboxamide hydrogen serves as a donor while the carboxamide oxygen accepts only one hydrogen in a complementary N–H2N···O bond. In contrast, both carboxamide hydrogens in I participate in H-bond formation. Further, the carboxamide oxygen in this structure acts as an acceptor in three bonds, the donor atoms being situated in a distorted tetrahedral arrangement around the acceptor oxygen.

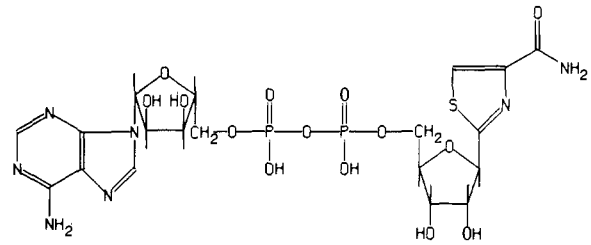

Figure 6. The NAD analogue TAD.

Discussion

Work by several groups suggests that the oncolytic activity of tiazofurin (I) is related to its ability to create a state of cellular guanine nucleotide depression with a subsequent interruption of the cell cycle in the S phase. This is a result of a decline in the guanosine precurser xanthosine monophosphate due to the inhibition of inosine monophosphate dehydrogenase (IMPd).[4,46] It has been convincingly demonstrated, both in vitro and in vivo, that the major IMPd inhibitor is an anabolite of I. The tiazofurin molecule is incorporated into an analogue of the cofactor nicotinamide adenine dinucleotide (NAD) in which the nicotinamide ring is replaced by the thiazole-4-carboxamide group (Figure 6).[46,47] This NAD analogue (TAD) is a more potent IMPd inhibitor ($K_i \sim 0.5$ μM) than either tiazofurin 5′-monophosphate ($K_1 \sim 0.4$ mM) or tiazofurin itself ($K_i \sim 8.2$ mM).[4,47]

A comparison of both the steric and hydrogen bonding properties of the thiazole-4-carboxamide ring with those of the nicotinamide ring suggests a mechanism for the observed IMPd in-

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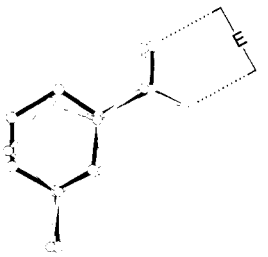

Figure 7. Overlay of the thiazole-4-carboxamide moiety from the tiazofurin structure (light bonds) onto a nicotinamide moiety from NAD (dark bonds).[61] Hydrogen atoms are omitted. The potential similarity in IMPd (E) binding between the nicotinamide group in NAD and the thiazole-4-carboxamide group in the dinucleotide analogue TAD is illustrated schematically.

hibition. The TAD molecule would bind at the NAD cofactor binding site, the thiazole-4-carboxamide group occupying the pocket normally filled by the nicotinamide ring. Although the structure of IMPd is unknown, single-crystal structures of five other dehydrogenase–cofactor complexes have shown a conservation of carboxamide–enzyme hydrogen bonding at the nicotinamide end of the cofactor binding site.[48] Similarly, enzyme binding studies using NAD analogues have emphasized the necessity of maintaining both the hydrogen-bonding abilities of the carboxamide group and the general steric characteristics of the nicotinamide ring in forming binary or tertiary complexes in several dehydrogenase systems.[49] The similar size of the thiazole ring and location of its carboxamide group suggest that it could successfully mimic the nicotinamide moiety in the binding of TAD to IMPd (Figure 7). However, once bound, the thiazole ring would be unable to accept a hydride ion from the enzyme substrate, as the uncharged ring is highly resistant to reduction.[45,51] This mechanism is consistent with the finding that the carboxamide group in the thiazole 4-position is a requirement for cytotoxicity in the P388 system.[4]

It has been suggested above that preferred conformations about the *C*-glycosyl bond may be those in which the thiazole sulfur is generally cis to the ribose oxygen O1′. In this case the thiazole carboxamide group would be found in generally trans conformations relative to O1′ (Figure 2). If binding of TAD to IMPd were analogous to that of NAD, then the carboxamide moiety in NAD might be expected to occupy a similar location relative to the nicotinamide ribose, implying some restrictions on the stereochemistry of cofactor binding in IMPd.[52]

The inactivity of both the α anomer (III) and the β-deoxy derivative (II) suggests that either these species are not converted to the corresponding NAD analogues or, if converted, the analogues do not effectively bind IMPd. The proposed mechanism for the metabolic synthesis of TAD involves the formation of tiazofurin 5′-monophosphate from I via a kinase followed by reaction with ATP via NAD pyrophosphorylase.[55] It is possible that either of these enzymes is unable to utilize the tiazofurin analogues as substrates. As seen above, the solid-state conformation of the α anomer ribose is different from that of tiazofurin. If some population of the α anomer in solution could adopt the same ribose conformation, the sterochemical relationship of the heterocycle to the sugar would still remain distinct from that in tiazofurin. Although the β-deoxy analogue adopts a similar conformation to that seen in I, it lacks the hydrogen-bonding capability of the 2′-hydroxyl group demonstrated in I and III.

The possibility does exist that either III or II is converted to α-TAD or β-2′-deoxy TAD analogues respectively. However, the same structural differences discussed above could adversely influence IMPd binding as well. This is suggested by the behaviors of similar NAD analogues. α-NADH does exist in mammalian and microbial systems, although it probably results from a nonenzymatic anomerization of β-NADH.[50,56] The α coenzyme is oxidized by several dehydrogenases, but at rates 3–4 orders of magnitude slower than that of β-NADH.[56] α-NAD is enzymatically inactive.[57] Similarly, an NAD analogue containing a β-2′,3′-dideoxynicotinamide ribose is reduced more slowly than NAD by both alcohol and lactate dehydrogenase.[58] The importance of the nicotinamide ribose 2′-hydroxyl group is also suggested by the conservation of an enzyme···O2′ hydrogen bond in a number of dehydrogenase–coenzyme crystal structures.[48] Thus, if binding of TAD to IMPd were analogous to that of NAD, an α-TAD or β-2′-deoxy-TAD might well be inefficient enzyme inhibitors.

Finally, it is worth adding that the thiazole-4-carboxamide group is also stereochemically similar to the base in inosine, suggesting that tiazofurin 5′-monophosphate (TMP) might be capable of acting as an analogue of the IMPd substrate inosine 5′-monophosphate. In this case the mechanism for enzyme inhibition would be similar to that proposed for the antiviral agent virazole.[59,60] Although TMP is not as potent an inhibitor as TAD, the possible dual role of both a coenzyme and a substrate analogue in IMPd inhibition has not been ruled out.

Acknowledgment. This research was supported by the National Institutes of Health through Grants GM 21589, RR 05539, CA 06927, and CA 09035-08 and through an appropriation by the Commonwealth of Pennsylvania.

Supplementary Material Available: Aniosotropic thermal parameters and listings of observed and calculated structure amplitudes for all three structures (Tables VIII and IX, respectively) (37 pages). Ordering information is given on any current masthead page.

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