vacuo, yielding 0.10 g (75%) of **10**: mp 240 °C. Anal. ($C_{28}H_{36}N_4Cl_2O$) C, H, N, Cl.

Quaternarization of 7*H*-Pyrido[4,3-*c*]carbazole with 10:11a and 12a. A 0.08-g (0.16-mM; 1-equiv) portion of **10** was solubilized in 2.5 mL of DMF. This solution was added dropwise to a stirred solution of 1.1 equiv of the appropriate 7*H*-pyrido-[4,3-*c*]carbazole in 2 mL of DMF. The solution was heated at 80 °C under nitrogen for 20 h. The resulting suspension was allowed to cool to room temperature and then evaporated to dryness in vacuo. The residue was flash chromatographed over a silica gel column (CH_2Cl_2-MeOH-NH_4OH, 8:2:0.5) and gave **11a**: yield 0.031 g (27%); R_f 0.11 (C). **12a**: yield 0.020 g (17%); R_f 0.32 (C).

Conversion of 11a and 12a into Their Dimethanesulfonate Salts 11b and 12b. One equivalent of **11a** or **12a** was solubilized in 3 mL of MeOH. Methanesulfonic acid (2 equiv) was added, and the solution was diluted with water (50 mL) and lyophilized. **11b**: yield 0.039 g (99%); mp 250 °C; FAB-MS (M^+) 727.35. **12b**: yield 0.025 g (99%); mp 206 °C; FAB-MS (M^+) 741.61.

Interaction with DNA. The K_{ap} values were determined at 25 °C in 0.1 M Tris·HCl, 0.1 M NaCl buffer pH 7.4, by fluorescence measurements based upon competition with ethidium dimer synthesized in our laboratory.[20] Excitation and emission were selected through a monochromator. The fluorescence of ethidium dimer was excited at 540 nm and emission recorded at 610 nm. Ethidium dimer (6.4×10^{-7} M) and calf thymus DNA (base pairs concentration: 1.6×10^{-6} M) were equilibrated for 24 h before measurements with different concentrations of drug. The concentration of bound ethidium dimer per base pair was deduced according to Gaugain et al.[16] The displacement curves were computed as described[16] and were compared to the experimental ones to evaluate the K_{ap} values. Viscometric measurements were performed at 25 °C by using the procedure already reported.[25] The intrinsic viscosity η of sonicated calf thymus DNA was measured in the absence (η_0) and presence (η) of increasing concentrations of drug. Plotting log (η/η_0) versus log ($1 + 2r$) where r is the number of bound ligand per nucleotide of DNA gives a slope ΔL, which accounts for monointercalation (ΔL between 2 and 3) or bisintercalation (ΔL between 4 and 6).

Biological Testing. Exponentially growing L1210 cells were treated with different drug concentrations.[25] Determinations of the dose effective in inhbiting 50% of the cell growth after 24-h exposure to the drug (ED_{50}) and of the dose required to inhibit the cloning efficiency to a factor of 0.37 after 24-h exposure to the drug (EC_{37}) were performed by using the method described in detail in our preceding paper.[6] Increases in life span (ILS) expressed as T/C × 100 values of DBA_2 mice inoculated with L1210 cells (10^5) were determined in the same way as reported elsewhere.[25] The statistical significance of the results was determined by the Student's t test.

Cytotoxicity Measurements. Bacteria were grown as reported in ref 11 and treated with various concentrations of the studied compounds. After 120 min of incubation at a temperature depending on the thermosensitivity of the *polA* mutation, bacteria were plated on LBT plates. Colonies were counted after an overnight incubation at 37 °C. This procedure was reported in detail by Lambert et al.[11]

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Supplementary Material Available: The NMR spectrum of heterodimer **11a** in Me_2SO-d_6 (in the presence of trifluoroacetic acid) is shown in Figure 1 (1 page). (Assignment of the protons was performed by using ^1H NMR COSY experiments.) Ordering information is given on any current masthead page.

Ara-tiazofurin: Conservation of Structural Features in an Unusual Thiazole Nucleoside

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Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193) is a *C*-glycosyl thiazole nucleoside with antitumor activity. Crystal structures of tiazofurin and its α,2′-deoxy and xylo analogues all show close contacts between the thiazole sulfur (S) and the furanose ring oxygen (O1′). These contacts have been interpreted in terms of an attractive intramolecular S–O interaction in the thiazole nucleosides. Ara-tiazofurin (2-β-D-arabinofuranosylthiazole-4-carboxamide, ara-T) is the inactive arabinose analogue of tiazofurin. The crystal structure of ara-T is reported. This structure provides evidence for an attractive S–O interaction not seen in the other thiazole nucleosides. The conformation about the *C*-glycosyl bond in ara-T is such that close contacts are formed between the thiazole sulfur and both O1′ *and* the 2′-hydroxyl oxygen O2′. This conformation is interpreted in terms of an additional attractive interaction between S and O2′. This interpretation is supported by comparison of the conformation of ara-T with those of other ara-nucleosides. These findings provide further evidence for an attractive S–O interaction in the thiazole nucleosides. Ara-T also demonstrates a second conformational feature found in these compounds: the carboxamide nitrogen remains cis to the thiazole nitrogen. Implications of these potentially constrained conformational features are discussed in terms of the mechanism of activity of tiazofurin.

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193, Figure 1a) is a novel *C*-glycosyl thiazole that has demonstrated significant antitumor activity in a number of model tumor systems. Tiazofurin is curative in vivo for the Lewis lung carcinoma in mice[1] and has demonstrated in vitro activity against both human lymphoid[2] and lung tumor[3] cell lines and in vivo activity

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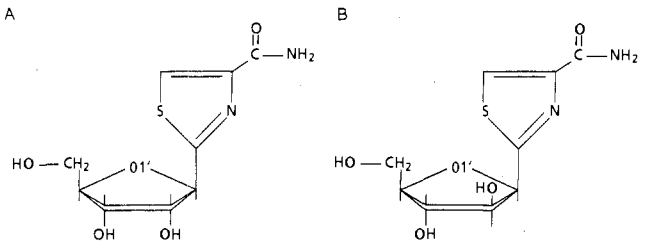

Figure 1. (A) Tiazofurin. (B) Ara-tiazofurin (ara-T)

against murine-implanted human ovarian cancers.[4] Phase I clinical trials have shown a variety of neurotoxic side effects to be dose limiting[5]. However, recent findings have demonstrated efficacy in the treatment of acute myeloid leukemia.[6] Clinical trials of tiazofurin are continuing.

Crystal structure of tiazofurin, as well as four inactive analogues, have been determined.[7,8] Two conformational features are conserved. In each structure, the carboxamide moiety on the heterocyclic base is oriented with the NH_2 group cis to the ring nitrogen. However, the most striking feature in these structures is the presence of an unusually close intramolecular contact between the sulfur atom and the furanose ring oxygen O1′. In each case, the distance between the heterocyclic atom and O1′ is significantly less than the sum of their van der Waals radii. These contacts have been interpreted in terms of an attractive interaction between the thiazole sulfur and the furanose oxygen.[8,9] This interaction would potentially limit rotation about the *C*-glycosyl bond, restricting the orientation of the heterocyclic base relative to the furanose ring. The orientation of the carboxamide moiety relative to the base may be similarly restricted.[8,9]

In vivo, tiazofurin is incorporated, via the 5′-monophosphate, into an analogue of nicotinamide adenine dinucleotide (NAD) in which the nicotinamide ring is replaced by the thiazole-4-carboxamide group.[10-12] This analogue, thiazole-4-carboxamide adenine dinucleotide (TAD), is a potent inhibitor of inosine monophosphate dehydrogenase (IMPd), the putative target enzyme.[10-13]

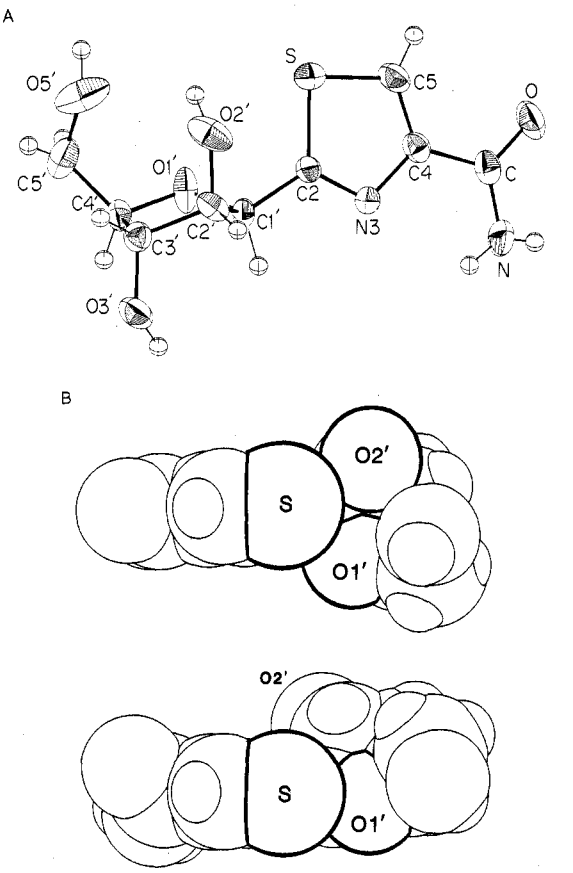

Figure 2. (A) Conformation of ara-tiazofurin. Non-hydrogen atoms are represented by thermal ellipsoids at the 50% probability level. Only the higher occupancy site of the disordered hydrogen on O3′ is shown. (B) Ara-tiazofurin (top) and tiazofurin (bottom). Atoms are drawn at their van der Waals radii. View down the S-C2 bond in ara-tiazofurin (top) shows the close contact between S and both O1′ and O2′. The same view in tiazofurin (bottom) shows only a close S–O1′ contact. In this case, O2′ lies "below" the furanose ring. H(O2′) has been omitted for clarity in both molecules.

Enzyme-inhibition studies indicate that TAD binds at the NAD binding sites in both alcohol and glutamate dehydrogenase.[14] Modeling studies of alcohol dehydrogenase bound TAD suggest that the thiazole ring on the dinucleotide occupies the pocket normally filled by the nicotinamide ring.[14] These studies suggest that the orientation of the heterocycle relative to the base is important in ensuring a proper fit of the dinucleotide analogue into the nicotinamide end of the cofactor pocket. Thus, restricted rotation about the *C*-glycosyl bond could potentially enhance or restrict dinucleotide binding, depending upon the specific stereochemical requirements of the cofactor binding site in the target enzyme(s). If the conformation of tiazofurin is restricted, it might suggest that this conformation is optimal for binding to the enzymes that convert tiazofurin to TAD. If TAD is itself conformationally restricted, it might alternatively suggest that this conformation is required for the very tight binding of the NAD analogue to IMPd. In either case, the presence of an attractive sulfur–oxygen interaction in the thiazole nucleosides would have important implications for drug

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Table I. Close S–O1′ Contacts in the Thiazole Nucleosides

structure	S–O1′ distance, Å	χ(O1′–C1′–C2–S), deg
α-tiazofurin	2.826 (3)	−20.8 (3)
xylo-tiazofurin (mol 1)	2.865 (2)	21.8 (4)
xylo-tiazofurin (mol 2)	2.929 (2)	30.1 (3)
tiazofurin	2.958 (1)	30.7 (1)
2′-deoxytiazofurin	3.018 (3)	40.8 (2)
ara-tiazofurin	3.158 (4)	55.2 (5)
	3.076 (4) (S–O2′ distance)	

binding and activity. This note presents additional evidence that close S–O contacts in the thiazole nucleosides result from an attractive sulfur–oxygen interaction and are not artifacts of crystal packing.

Ara-tiazofurin (2-β-D-arabinofuranosylthiazole-4-carboxamide, ara-T, Figure 1b) is the inactive arabinofuranosyl analogue of tiazofurin.[15] The structure of this compound provides evidence for an attractive S–O interaction not seen in other thiazole nucleosides. In ara-T, the 2′-hydroxyl lies above the furanose ring, placing it (as well as O1′) potentially close to the thiazole sulfur. In this position, O2′ "competes" with O1′ for the sulfur, distorting the conformation about the *C*-glycosyl bond relative to the other members of the series. Comparison of the ara-tiazofurin structure with those of other ara-nucleosides indicates that this distortion is greater than would be expected on purely steric grounds. This is consistent with the additional S–O2′ attraction and provides further support for the thesis of an attractive S–O interaction in the thiazole nucleosides. This structure also illustrates conservation of carboxamide group orientation in this series of compounds.

Results

The molecular structure of ara-T is shown in Figure 2. The feature of particular interest in this structure is the conformation about the *C*-glycosyl bond. As observed previously in the thiazole nucleosides, the thiazole sulfur remains cis to the furanose ring oxygen O1′. However, in ara-T, marginally close contacts are observed between the thiazole sulfur S and both O1′ *and* O2′. This is particularly well illustrated in the van der Waals representation (Figure 2b). The S–O1′ and S–O2′ contacts are, respectively, 0.142 and 0.224 Å less than the sum of the van der Waals radii for sulfur and oxygen.[16]

Close S–O1′ contacts have been observed in tiazofurin and its 2′-deoxy derivative and α-anomer[8] as well as in two independent molecules of xylotiazofurin[7] (Table I). These contacts are significantly shorter than the S–O1′ distance observed in ara-T. Further, the value of the *C*-glycosyl torsion angle χ(S–C2–C1′–O1′) is significantly larger in this structure (Table I). These differences may be interpreted in terms of an additional S–O2′ interaction in ara-T. Comparison of ara-T with tiazofurin is shown in Figure 3, in which both structures are viewed down the *C*-glycosyl bond. The position of the heterocyclic base in ara-T

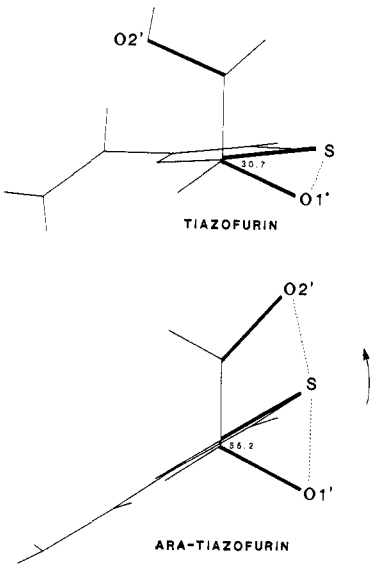

Figure 3. View down the *C*-glycosyl bond in tiazofurin and ara-tiazofurin. The thiazole rings are toward the viewer. Only part of each sugar moiety is shown for clarity. Dotted lines indicate close contacts. The putative S–O2′ interaction in ara-tiazofurin increases the glycosidic torsion angle.

suggests an attractive S–O interaction in which the sulfur has been "pulled" counterclockwise by O2′ until it rests roughly between O2′ and O1′. The S–O2′ contact is actually 0.082 Å shorter than the marginally close S–O1′ contact.

Close contacts between O1′ and/or O2′ and atoms in the base are common in crystal structures of *N*-glycosyl purine and pyrimidine ara-nucleosides. These contacts are often interpreted as purely steric, due to the unique position of O2′ in the arabinofuranosyl ring. However, the two ara-nucleosides having the highest *N*-glycosyl torsion angles, ara-adenine[17a] and ara-8-(*n*-butylamino)adenine,[17b] show attractive interactions between the base and sugar, which help stabilize the conformation about the *N*-glycosyl bond. In particular, ara-adenine shows a weak C8–H···O2′ H-bond.[17a]

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(16) The sum of van der Waals radii for S and O is 3.3 Å by use of the spherical van der Waals radii of Bondi (*J. Phys. Chem.* **1964**, *68*, 441–451). If recent anisotropic radii of Nyburg and Faerman (NF) are used (*Acta Crystallogr., Sect. B: Struct. Sci.* **1985**, *B41*, 274–279), the sum of S and O radii range between 3.14 and 3.57 Å, depending upon the geometry of the contact. However, the geometry of the contacts surveyed by NF is inappropriate for the *intra*molecular contacts between divalently bound atoms discussed here. Thus, the radii of Bondi are used.

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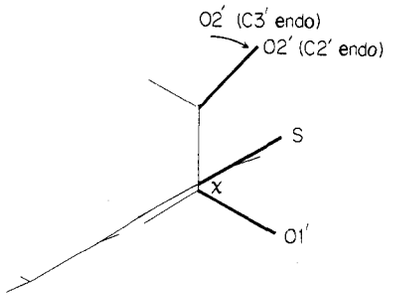

Figure 4. View down the *C*-glycosyl bond in ara-tiazofurin. Dashed bond indicates the position of O2′ in C3′ endo sugar pucker. The observed C2′ endo pucker brings O2′ closer to O1′.

Comparison of the conformation of ara-T with the conformations of other ara-nucleosides provides additional evidence for an attractive component to the S–O2′ contact. This becomes apparent when the effect of the sugar pucker on the glycosidic torsion angle is examined. The conformation of the arabinofuranosyl ring in ara-T is C2′ endo, C1′ exo (^2_1T, P = 140.7°, τ_m = 42.0°).[18] Although this particular pucker has not been seen, similar conformations in which C1′ is exo are not uncommon among other ara-nucleoside structures.[17h-j] This conformation places the base in an equatorial position, relieving the steric strain between N1 (or in this case C2) and O2′.[17a] This pucker is further stabilized by an intramolecular hydrogen bond between O2′ and O5′ (below), seen in ara-nucleosides in which C2′ is endo.[17j,17k,17p] NMR studies of a number of ara-nucleosides suggest that the ^2_1T twist conformation occurs in solution in equlibrium with the C3′ endo (^3E) envelope.[19] However the occurrence of a C2′ endo arabinofuranosyl pucker together with a glycosidic torsion angle as high as that observed in ara-T is unusual. A conformation in which C2′ is endo places the O2′ hydroxyl group in an axial position, bringing it closer to O1′ (Figure 4). Thus, the sugar conformation containing a C2′ endo carbon would be expected to place the greatest steric restriction on rotation about the glycosyl bond. Examination of a number of ara-nucleoside structures suggests that a relation between sugar pucker and glycosidic torsion angle does exist. A search of the Cambridge Structural Database[20] and the literature yielded 15 purine and pyrimidine nucleosides and one purine nucleotide containing arabinofuranosyl moieties with an unsubstituted hydroxyl group at the 2′-position.[17] Figure 5 shows a plot of the distribution of normalized glycosidic torsion angles[21] for these

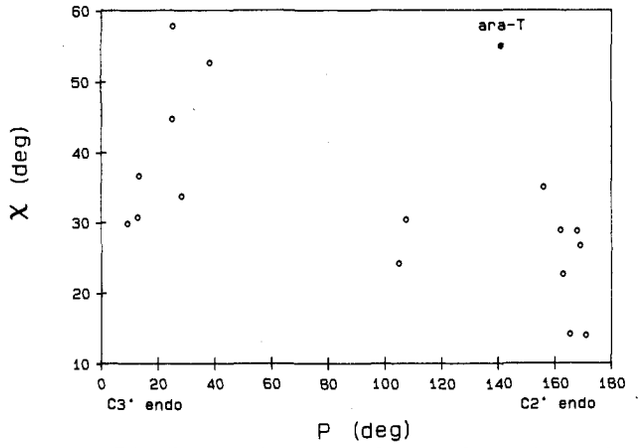

Figure 5. Distribution of normalized glycosidic torsion angles χ[21] versus phase angles of pseudorotation P for *N*-glycosyl ara-nucleosides. Values for the C-glycosyl thiazole ara-T (Table I) are plotted for comparison (filled circle). Structures examined and the corresponding values of χ and P are, respectively: (a) ara-adenine, 57.8°, 25°; (b) ara-8-*n*-(butylamino)adenine, 52.6°, 38°; (c) ara-8-morpholinoadenine, 44.7°, 25°; (d) ara-4-thiouracil, 36.6°, 13°; (e) ara-5-propynyluracil, 33.7°, 28°; (f) ara-cytosine 5′-monophosphate, 30.7°, 13°; (g) ara-adenine hydrochloride, 29.8°, 9°; (h) ara-5-bromouracil, 30.4°, 107°; (i) ara-thymine, 24.1°, 105°; (j) ara-uracil, 35.0°, 156°; (k) ara-cytosine, 28.8°, 162°; (l) 3′-*O*-methyl-ara-cytosine, 28.7°, 168°; (m) ara-cytosine hydrochloride, 26.7°, 169°; (n) ara-5-methylcytosine, 22.6°, 163°; (o) ara-5-fluorocytosine, 14.2°, 165°; (p) ara-cytosine (Pt(II) complex), 14.0°, 171°. Letters refer to the corresponding citation under ref 17.

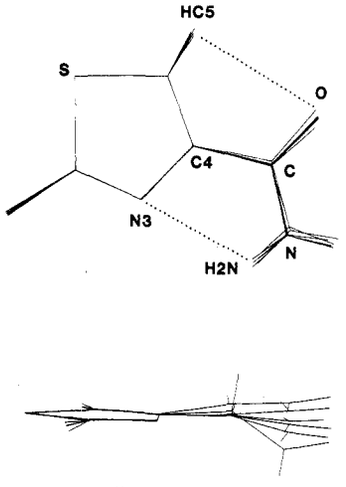

Figure 6. Least-squares fit of thiazole rings from the thiazole nucleosides listed in Table I. Values of the carboxamide torsion angle κ(N–C–C4–N3) vary between –1.3 (4)° (ara-tiazofurin) and 33.1 (1)° (tiazofurin). In each case the carboxamide NH_2 is cis to the thiazole ring nitrogen N3. Dotted lines indicate potential interactions that stabilize this conformation. Views are approximately normal to and parallel to the thiazole ring plane.

structures relative to the conformations of their sugars as measured by their phase angles of pseudorotation.[18] Higher torsion angles are associated with sugar conformations in the C3′ endo range of the pseudorotation cycle. In these structures O2′ is equatorial, increasing the O1′–O2′ distance. Thus, a higher glycosidic angle can be achieved before steric interactions between the base and O2′ limit further rotation (Figure 4). As expected, the distribution of torsion angles shifts toward lower values when the 2′-position becomes endo and O2′ becomes axial. Values of χ over 35° are not observed in these conformations. Thus, the point representing the conformation of ara-T falls in an unusual region of the scatter plot. The value of χ for ara-T is outside the range of values seen for ara-nucleosides

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(20) (a) Allen, F. H.; Kennard, O.; Taylor, R. *Acc. Chem. Res.* **1983**, *16*, 146. (b) Cambridge Structural Database System (version 2), VAX/VMS Interim Documentation, Cambridge Crystallographic Data Centre, Cambridge, England, 1985. Structures were obtained by both bibliographic and connectivity searches, followed by visual screening to obtain only ara-nucleosides with unsubstituted 2′-hydroxyls. Functions for χ and P were defined, and values were extracted by employing the GEOSTAT routine. Values were checked with the literature where possible.
(21) The normalized glycosidic torsion angle is defined here as the absolute value of the O1′–C1′–Na–Cb torsion angle, where Cb is the base carbon closest to the furanose oxygen O1′, i.e., a = 1, b = 2 or 6 for pyrimidines; a = 9, b = 4 or 8 for purines. This geometry provides a measure of the approach of the plane of the base to O1′, regardless of whether the base is syn or anti. This definition yields the conventional value of χ[22] for all but two of the structures reported.[17c,o]
(22) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1983; pp 21–23.

with C2′ endo puckers. It is in fact comparable to the highest values seen in those ara-nucleosides with C3′ endo conformations.[17a,b] These observations further suggest that the S–O2′ contact has an attractive component.

Additional conformational features are observed in ara-T that are conserved in the other thiazole nucleosides. The carboxamide nitrogen (N) forms a close contact (2.771 Å) with the thiazole ring nitrogen N3 (Figures 2a and 6). This suggests a strong electrostatic interaction between one amide proton (H2N) and the lone pair electrons on N3. The observed geometry can be interpreted as a bent intramolecular N-H2N···N3 hydrogen bond. However, similar contacts (Figure 6) are observed in structures where N3 and H2N are both engaged in intermolecular H-bonding.[8] The N-H2N···N3 interaction may be further augmented by an electrostatic attraction between the thiazole proton on C5 (HC5) and the lone pair electrons on the carboxamide oxygen (Figure 6). The C4–C bond length (1.483 (7) Å) is comparable to that seen in other thiazole nucleosides and indicates little conjugation with the thiazole ring. The carboxamide group is rotated slightly relative to the plane of the thiazole ring, as indicated by the value of the N–C–C4–N3 torsion angle κ of −1.3 (4)°. Values of κ over 30° are observed in tiazofurin (Figure 6)[8] and its selenium analogue selenazofurin,[9] although in these structures the carboxamide moieties participate in the largest number of intermolecular hydrogen bonds. However, in the structures of all thiazole (and selenazole) nucleosides, the carboxamide group remains oriented with NH_2 cis to N3 (Figure 6).[7-9]

Other structural features common to the thiazole nucleosides are observed in ara-T. The thiazole ring is planar within experimental error. As observed previously, bond lengths in the heterocycle indicate some degree of aromaticity, with slightly greater contributions from the resonance forms containing the C5=S^+—C2 fragment.[7,8] The partial aromatic nature of the thiazole ring is of interest. This likely contributes to the heterocycles' resistance to reduction, hence its inability to accept hydride ion transfer when dehydrogenase bound.[8]

Discussion

Close sulfur–oxygen contacts have now been observed in five thiazole nucleosides (Table I).[7,8] The molecular environment in each structure differs. This implies that the heteroatom–oxygen contacts are not the result of external forces imposed by packing and may be observed in solution. This is further supported by the observation in ara-T of close contacts between the thiazole sulfur and both O1′ and O2′. The unusually high glycosidic torsion angle in this compound suggests a competing influence on the thiazole sulfur from the 2′-hydroxyl oxygen.

Close heteroatom–oxygen contacts have been attributed to both an electrostatic attraction between the sulfur and oxygen[8] and to an overlap of a nonbonding p orbital on the oxygen with an antibonding orbital along the backside of the S–C5 bond.[9] These interactions, in conjunction with steric factors,[8] could limit rotation about the *C*-glycosyl bond in the thiazole nucleosides and their nucleotide and dinucleotide anabolites. As discussed above, such constraints would have potential implications for binding and activity of tiazofurin and its active dinucleotide anabolite TAD.

Constraints on *C*-glycosyl bond conformation imposed in part by an S–O1′ interaction may be required for either conversion of tiazofurin to TAD or for the very tight binding of TAD to the target enzyme IMPd. Replacement of the thiazole sulfur in tiazofurin by an oxadiazole nitrogen results in loss of activity.[23] Replacement of the thiazole ring in TAD itself by a 1,2,4-triazole ring reduces IMPd binding of the resulting dinucleotide by 3 orders of magnitude.[24] Heterocycles on these analogues have steric and hydrogen bonding properties similar to those of the thiazole-4-carboxamide moiety. However, absence of the S–O1′ interaction precludes the type of conformational constraints about the glycosidic bond proposed for the thiazole nucleosides.

The second conformational feature conserved in the thiazole and selenazole nucleosides is the orientation of the carboxamide group relative to the heterocycle. In each structure, an N-H2N···N3 interaction maintains the amide group cis to N3. Again, appearance of this geometry in a variety of different packing environments suggests that its represents a low-energy conformation in solution. Tiazofurin requires the carboxamide group in the 4-position for activity.[23] Modeling studies of dehydrogenase binding by TAD suggest that carboxamide side chain hydrogen bonds can anchore the thiazole ring to the nicotinamide end of a cofactor binding site.[14] Thus, stabilization of the conformation of the carboxamide group relative to the heterocycle may contribute to the highly effective binding of TAD to the target enzyme.

Conformational features discussed above are not by themselves sufficient to insure activity. This is clear from the fact the tiazofurin is the only oncolytic thiazole nucleoside in Table I. Reasons for inactivity in the α-anomer and 2′-deoxy derivative have been proposed.[8] Hydrogen bonding groups in these compounds are either missing or sterically distorted relative to the geometry found in tiazofurin. This is also true of the inactive xylo and ara analogues, although the unusual *C*-glycosyl bond conformation in ara-T may contribute to its inactivity. These analogues suggest that the β-linked ribofuranose moiety is a necessary (albeit not sufficient) feature of the active compound. The question remains whether potential constraints on carboxamide and/or *C*-glycosyl bond conformation are among the more subtle requirements for activity.

Experimental Section

X-ray Data Collection. Crystals were obtained as described in ref 15a. Preliminary photographic and counter measurements indicated a monoclinic system with systemic absences $0k0$ for $k = 2n + 1$, uniquely defining the space group as $P2_1$. Cell dimension and intensity data were collected at room temperature from a colorless needle of approximate dimensions 0.2 × 0.02 × 0.02 mm mounted roughly parallel to the *b* axis. A nonius CAD-4 diffractometer was employed with a graphite monochromator and Cu Kα radiation. Lattice constants were obtained by least-squares refinement of the angular settings of 25 reflections in the range θ = 25–28°. These are as follows: a = 9.462 (1) Å, b = 5.3715 (6) Å and c = 10.710 (2) Å and β = 99.42 (1)°. Other crystal data are Z = 2, M_r = 260.3, V = 537.0 Å^3, and ρ(calcd) = 1.610 g/cm^3.

Reflections were measured in the range $2° < \theta < 70°$ by using the ω–2θ scan method with a variable scan width $\Delta\omega = (0.77 + 0.15 \tan \theta)°$, this angle being extended 25% on each side for background measurements. The scan rate varied between 0.8 and 2.0 deg/min depending upon the value of $\sigma(I)/I$ for each reflection. A total of 2509 reflections (including standards) were measured in the two equivalent but noncentrosymetrically related quadrants $(\pm h,-k,l)$ and $(\pm h,-k,-l)$. These data were averaged, yielding 1122 unique reflections, of which 77 had values of $|F_o|^2 < 0.3\sigma(F_o^2)$,

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where $\sigma(F_o^2) = [\sigma^2(I) + (0.02|F_o|^2)^2]^{1/2}$, and were reset to $0.3\sigma(F_o^2)$. All data were used in the subsequent analysis and refinements.

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 correted for absorption by using the semiemprical ψ-scan technique.[25] 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.

Struture Solution and Refinement. The structure was solved by direct methods by employing MULTAN78.[26] Starting sets based on 264 reflections with $E > 1.2$ were subjected to tangent refinement. An E map calculated from the set of phases with the highest combined figure of merit yielded all non-hydrogen atoms. The positions of all hydrogen atoms were then obtained from subsequent least-squares refinements and difference Fourier maps, employing only the low-angle data [$(\sin\theta)/\lambda < 0.4$ Å^{-1}].

The structure was refined by using full-matrix least-squares techniques. The function minimized was $\sum w(\Delta F)^2$ where $\Delta F = |F_o| - |F_c|$. Weights $w = 1/\sigma_{new}^2$ were used where $\sigma_{new}^2 = [\sigma^2 + 0.5A|F_o|^2 + 0.5B[(\sin\theta)/\lambda]^2]^{1/2}$ and $\sigma = \sigma(F_o^2)/2|F_o|$. 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 $(\sin\theta)/\lambda$. Non-hydrogen atoms were refined anisotropically, the y coordinate of the sulfur atom remaining fixed. Positional parameters of all hydrogen atoms were refined with isotropic temperature factors.

Early refinements and difference maps indicated unambiguously that HO3′ was disordered over two sites. Occupanices were refined and then fixed at 0.6 and 0.4 with thermal parameters fixed at $B = 10$. This provided good convergence of the positional parameters at both sites. Refinement of the position of HO2′ was complicated by proximity to the sulfur atom density. Good convergence was obtained by fixing the thermal parameter of this proton as well.

Final refinements included a type I isotropic extinction correction and utilized all data. These converged to the values of $R = \sum||\Delta F|/\sum|F_o| = 0.052$ and $R_w = [\sum w(\Delta F)^2/\sum w|F_o|^2]^{1/2} = 0.051$ for 1122 observations m and 203 variables n. The discrepancy factor $S = [\sum w(\Delta F)^2/(m-n)]^{1/2} = 1.24$. The largest final parameter shift observed was 0.05σ. Atomic scattering factors for the non-hydrogen atoms and anomalous dispersion corrections for the sulfur atom were from ref 27. Scattering factors for the hydrogen atoms were those of Stewart et al.[28] The DNA system of programs[29] was used throughout. Final fractional atomic coordinates and thermal parameters are deposited.

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Supplementary Material Available: Tables of fractional atomic coordinates and thermal parameters, select bond lengths, angles and torsion angles, and hydrogen bond distances and angles (3 pages); table of observed and calculated structure factors (6 pages). Ordering information is given on any current masthead page.

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2,4-Diamino-6,7-dimethoxyquinoline Derivatives as α_1-Adrenoceptor Antagonists and Antihypertensive Agents

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A series of 2,4-diamino-6,7-dimethoxyquinoline derivaties (**2**), prepared by LDA- or $ZnCl_2$-mediated intramolecular cyclization of an *N*-[1-(dialkylamino)ethylidene]-2-cyano-4,5-dimethoxyaniline (**3**), was evaluated for α-adrenoceptor affinity and antihypertensive activity. Most compounds displayed high in vitro binding affinities (K_i's, 10^{-10} M) for α_1-adrenoceptors with α_1-/α_2-selectivity ratios of at least 10 000. 4-Amino-2-[4-(2-furoyl)piperazin-1-yl]-6,7-dimethoxyquinoline (**14**) proved to be the most potent member ($K_i = 1.4 \times 10^{-10}$ M) of series **2**, and displayed no activity at α_2-adrenoceptor binding sites at concentrations up to 10^{-6} M. In the rabbit pulmonary artery, **14** was a highly potent ($pA_2 = 9.76 \pm 0.26$) competitive antagonist of the α_1-mediated vasoconstrictor action of noradrenaline and was some 20 times more active than prazosin. pK_a measurements confirmed that, at physiological pH, N-1 protonation of series **2** would efficiently provide **1b**, a key pharmacophore for α_1-adrenoceptor recognition. Antihypertensive activity for series **2** was evaluated after oral administration (3 mg/kg) to spontaneously hypertensive rats (SHR) and falls in blood pressure were determined at 1 and 4.5 h. Various quinoline derivatives (**2**) proved to be effective antihypertensive agents in SHR, with both efficacy and duration of action at least equivalent to prazosin, and **14** displayed the most favorable overall profile. These observations are consistent with the high affinity and selectivity displayed by series **2** for postjunctional α_1-adrenoceptors.

Previous reports from these laboratories have presented detailed structure–activity relationships (SAR) with respect to α_1-adrenoceptor affinity and antihypertensive activity for various series of 2,4-diamino-6,7-dimethoxyquinazoline derivatives.[1-3] In these studies, attention was concentrated on modification of the quinazoline 2-substituent in order to optimize both in vitro and in vivo performance, and as a result, doxazosin was selected for clinical evaluation.[4] This compound is a potent, highly

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