can be considerably extended to molecules containing nitrogen, chlorine, fluorine or oxygen and that a reliable lattice-dynamical model can be obtained with atom–atom parameter sets taken from the literature, provided that information about the electric charge is available. This last point is not a serious problem nowadays, because quantum-mechanical calculation of atomic point charges, even for complex molecules, is not a forbidding task with modern computers.

It is also stimulating to notice how atom–atom potentials derived from static properties give a good performance in lattice-dynamical calculations.

Future work will be devoted to obtaining more reliable experimental atomic displacement parameters for comparisons. In particular, work is in progress on the correction of experimental atomic displacement systematic parameters for systematic thermal diffuse scattering (TDS) errors with lattice-dynamical models.

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# Solid-State and Solution Conformations of Isotiazofurin: Crystallographic, Computational and $^1$H NMR Studies

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### Abstract

Isotiazofurin ($C_9H_{12}N_2O_5S$, NSC363223) is a thiazole nucleoside analogue of the antitumour agent tiazofurin. The conformation of this analogue has been studied using a variety of experimental and computational techniques. The crystal and molecular structure of isotiazofurin has been determined using single-crystal X-ray diffraction techniques and refined to a conventional $R$ value of 0.030 for all data. Conformational features conserved in other thiazole nucleoside structures are also observed in the solid-state structure of isotiazofurin. The *C*-glycosidic torsion angle remains in the *anti* conformation and the carboxamide amino group remains *cis*-planar to the ring nitrogen. *Ab initio* calculations at the RHF/321G*//321G* level and natural bond orbital analysis of the results suggest that the carboxamide *cis*-planar conformation observed in the solid state is maintained in solution. However, semi-empirical calculations suggest that a *syn* conformation about the *C*-glycosidic bond is energetically favored. This is supported by $^1$H nuclear Overhauser enhancement (NOE) studies. Analyses of NOE results using both slow- and rapid-exchange models

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indicate a preference for the *syn* conformation in solution. Thus, the *anti* conformation observed in the crystal structures of isotiazofurin is not maintained by isotiazofurin in solution.

## Introduction

The *C*-glycosyl nucleoside tiazofurin (2-$\beta$-D-ribofuranosylthiazole-4-carboxamide, NSC286193, Fig. 1) exhibits a wide variety of biological activities. These include clinically effective antitumor activity, as well as the ability to induce differentiation in several human tumor cell lines (Goldstein *et al.*, 1991). Crystallographic studies of tiazofurin and its analogues have identified two conserved conformational features. In these structures, the conformations about the *C*-glycosidic bond is *anti*, producing a close intramolecular contact between the heterocyclic sulfur atom S and the furanose ring oxygen O1′ (Fig. 1) (Goldstein, Takusagawa, Berman, Srivastava & Robins, 1983; Goldstein, Mao & Marquez, 1988; Burling, Gabrielsen & Goldstein, 1991; Burling, Hallows, Phelan, Gabrielsen & Goldstein, 1992). Further, the carboxamide group is generally planar to the thiazole ring, the carboxamide amino nitrogen being *cis* to the thiazole ring nitrogen (Fig. 1).

Analysis of crystallographic results and computational studies indicate that the *C*-glycosyl bond conformation is produced by an attractive electrostatic interaction between the thiazole sulfur and the furanose oxygen (Burling & Goldstein, 1992). Crystallographic and database studies indicate that the carboxamide conformation results from a charge-transfer interaction between a carboxamide amino hydrogen and the thiazole nitrogen, and from an electrostatic interaction between the carbonyl oxygen and thiazole proton (Fig. 1) (Li & Goldstein, 1992).

These nonbonded interactions are of sufficient magnitude to constrain rotation about both the carboxamide and *C*-glycosidic bonds in solution. The *C*-glycosidic bond would be constrained in the *anti* range, and the carboxamide group would be constrained in the *cis*-planar conformation (Fig. 1). It has been suggested that these constraints are important in maintaining activity, either by enhancing conversion of tiazofurin to its active anabolite, and/or by reducing the free energy of binding of the active anabolite to its enzymatic target (Li & Goldstein, 1992).

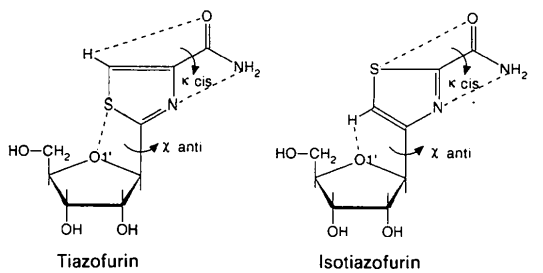


Fig. 1. Structural formula of tiazofurin and isotiazofurin. Dotted lines represent potential nonbonded interactions. In each structure, the *C*-glycosidic bond torsion angle $\chi$ is shown in the *anti* conformation and the carboxamide torsion angle $\kappa$ is shown in the *cis*-planar conformation.

Isotiazofurin (4-$\beta$-D-ribofuranosylthiazole-2-carboxamide, NSC363223) is an inactive tiazofurin analogue produced by transposition of the carboxamide and *C*-glycosidic bonds on the thiazole ring (Fig. 1). The subsequent displacement of the thiazole sulfur relative to the furanose oxygen precludes any sulfur–oxygen interaction of the type seen in tiazofurin. However, the thiazole H(C5) proton in isotiazofurin is well positioned to form an intramolecular interaction with O1′. This interaction would potentially constrain the *C*-glycosidic bond conformation of isotiazofurin to the *anti* range seen in tiazofurin (Fig. 1).

In order to investigate these potential conformational similarities between tiazofurin and isotiazofurin, the crystal structure of the analogue was examined. In order to determine whether or not nonbonded interactions could be expected to constrain rotation about the *C*-glycosyl and carboxamide bonds, quantum-mechanical-based computations were also performed on isotiazofurin. Finally, the conformation of isotiazofuin in solution was examined using high-resolution NMR spectroscopy. Results indicate that the conformation of isotiazofurin is analogous to that of tiazofurin in the solid state. However, not all of these conformational similarities are maintained in solution. This may in part explain the inactivity of isotiazofurin.

Additionally, the isotiazofurin carboxamide oxygen is well placed to form an attractive 1,4 S···O interaction with the thiazole sulfur. The carboxamide amino group is similarly positioned to interact with the thiazole nitrogen (Fig. 1). These interactions would be expected to constrain the carboxamide group to the *cis*-planar conformation, also seen in tiazofurin.

## Methods

### *X-ray crystallographic study*

Colorless crystals of isotiazofurin were obtained by slow evaporation from a 7.5 m*M* isopropanol solution. The solution was initially heated to 353 K in order to fully dissolve the isotiazofurin, and then allowed to stand at room temperature. Crystal data, data collection and refinement variables are listed in Table 1. Full-sphere data were collected at room temperature and corrected for Lorentz and polarization factors. Monitoring of three standard reflec-

tions showed no decay and only 0.2% variation in intensity. Data were corrected for absorption using the semi-empirical $\psi$-scan technique (North, Phillips & Mathews, 1968). Equivalent reflections were averaged to give 1391 reflections, of which 1385 had $F_o > 3\sigma(F_o)$. However, all data were used in the subsequent refinements.

The structure was solved using standard Fourier techniques. The position of the S atom was determined from the Patterson map and positions of the non-H atoms determined from subsequent Fourier maps. The positions of all H atoms were obtained from successive difference Fourier maps, employing only low-angle data [$(\sin\theta)/\lambda < 0.4$ Å$^{-1}$].

The structure was refined using full-matrix least-squares techniques. The function minimized was $\sum w(\Delta F)^2$ where $\Delta F = |F_o| - |F_c|$. Weights were $w = 1/\sigma'^2$ where $\sigma' = [\sigma^2 + 0.5A|F_o|^2 + 0.5B(\sin\theta/\lambda)^2]^{1/2}$. Values of $\sigma^2$ were obtained from counting statistics. Values of $A$ and $B$ were obtained from least-squares minimization of the function $|\Delta F|^2 - \sigma'^2$ in 20 separate segments in $|F_o|$ and $(\sin\theta)/\lambda$ for the data set. Non-H atoms were refined anisotropically and positional parameters of all H atoms were refined with isotropic temperature factors. The final refinement included a type-I extinction correction (Coppens & Hamilton, 1970) and utilized all data. Atomic scattering factors and $f'$ and $f''$ for the S atom were taken from *International Tables for X-ray Crystallography* (1974, Vol. IV). All programs were from the *DNA* system (Takusagawa, 1981).

Table 1. *Crystal data, data collection and refinement variables for isotiazofurin*

| | |
|---|---|
| Formula | $C_9H_{12}N_2O_5S$ |
| $M_r$ | 260.3 |
| Space group | $P2_12_12_1$ |
| $a$ (Å) | 6.733 (1) |
| $b$ (Å) | 6.831 (1) |
| $c$ (Å) | 24.486 (2) |
| $Z$ | 4 |
| $V$ (Å$^3$) | 1126.2 |
| $D_x$ (g cm$^{-3}$) | 1.53 |
| Crystal size (mm) | 0.14 × 0.17 × 0.52 |
| Diffractometer | Enraf–Nonius CAD-4 |
| Scan type | $\omega$–$2\theta$ |
| Radiation | Cu $K\alpha$ ($\lambda$ = 1.54178 Å) |
| Lattice-parameter refinement | 24 reflections ($82 < 2\theta < 92.2°$) |
| Transmission factor (on $I$) | 0.720–1.000 |
| Data-collection range, $\theta$ (°) | $3 < 2\theta < 156$ |
| Data-collection range, $h$, $k$, $l$ | $-8 \le h \le 8$; $-8 \le k \le 8$; $-31 \le l \le 31$ |
| Total reflections | 4606 |
| Unique reflections | 1391 [$1385 > 3\sigma(F_o)$] |
| $R_{merge}$ ($I$) | 0.070 |
| $R(F)$ (all data) | 0.0304 |
| $wR(F)$ (all data) | 0.0395 |
| No. of variables | 203 |
| $S$ | 1.3798 |
| $(\Delta/\sigma)_{max}$ | 0.22 |
| $(\Delta\rho)_{max}$ (e Å$^{-3}$) | 0.26 |
| Extinction parameter $G$ ($\times 10^{-4}$) | 0.19 (4) |

*Computational study*

Energy as a function of carboxamide bond angle $\kappa$ was computed for the thiazole-2-carboxamide moiety using methods employed previously for the thiazole-4-carboxamide group (Li & Goldstein, 1992). *Ab initio* molecular orbital calculations were carried out, using the *GAUSSIAN*90 program (Frisch *et al.*, 1990) on the model fragment shown in Fig. 4(*a*). In this study, the torsion angle $\kappa$ was defined by atoms O6—C6—C2—S (Figs. 1, 2). The conformation for which $\kappa = 0°$ is illustrated in Fig. 4(*a*), and is referred to here as '*cis*-planar'. The conformation for which $\kappa = \pm 180°$ is described as '*trans*-planar'. Potential energies were obtained every 30° starting from $\kappa = -180°$ and ending at $\kappa = 0°$ by fully optimizing the fragment while fixing $\kappa$ at the desired value. The 3-21G* basis set was used for the geometry optimization and for the SCF energy values at each $\kappa$ point. Thus, each point represents a calculation at the RHF/3-21G*//RHF 3-21G* level. The computed results are plotted in Fig. 4(*a*). Note the symmetry with respect to the ring plane in the fragment, *i.e.* negative $\kappa$ values are equivalent to positive ones. In Fig. 4(*a*), the energy profile is symmetrized about $\kappa = 0°$. Profiles are presented from $\kappa = -180$ to 180° for comparison with results from tiazofurin (Li & Goldstein, 1992).

In order to chemically rationalize the computed energy profile we also performed natural bond orbital (NBO) analyses on the model fragment using the NBO program incorporated in *GAUSSIAN*90 (Glendening, Reed, Carpenter & Weinhold, 1990). NBO analysis allows one to isolate interactive energies due to electron density delocalization, or charge transfer, and to relate these interactions to either hydrogen bonding or other types of nonbonded interactions based on bond orbital interaction concepts (Reed, Curtiss & Weinhold, 1988). NBO analysis decomposes the internal energy of a chemical complex into a charge-transfer energy and a non-charge-transfer energy, the latter including electrostatic and exclusion-repulsion terms.

Energy as a function of *C*-glycosidic torsion angle $\chi$ was computed for isotiazofurin using methods employed previously for tiazofurin (Burling & Goldstein, 1992). By analogy with tiazofurin, $\chi$ is defined by atoms O1′—C1′—C4—C5. The terms *'anti'* and *'syn'* here refer to the ranges $-90 < \chi < 90$ and $90 < \chi < 270°$ respectively. These values are shifted 180° from the standard nucleotide nomenclature (Saenger, 1983), but more accurately represent the position of the carboxamide group relative to the ribose in the thiazole nucleosides. Semi-empirical methods were used as incorporated in the *MOPAC* 5.0 program with the MNDO parameter set (Stewart, 1985). The total energy was obtained by treating $\chi$ as the reaction coordinate. The value of $\chi$ was

incremented in 15–20° steps and fixed. The remainder of all geometry variables describing the entire molecule were then optimized. In addition to the full semi-empirical optimization, a molecular-mechanics correction on the carboxamide group was applied at each value of $\chi$ using the MMOK keyword. The minimized carboxamide group remained at about 30° with respect to the base ring. The computation was performed using both C2′-*endo* and C3′-*endo* sugar conformations as starting geometries with little difference in the final results. The reaction path of $\chi$ was also reversed in order to check for possible bias due to variation in the direction of the calculation. Energy profiles obtained from the two opposite reaction paths were consistent within computational errors. The computed energy profile is presented in Fig. 4(*b*) for an initial C2′-*endo* sugar pucker.

*Nuclear Overhauser enhancement study*

NOE measurements were performed on a GE Omega NMR spectrometer operating at a proton frequency of 400 MHz. The isotiazofurin sample consisted of a 5 m*M* solution in $D_2O$. Initial assignments were made *via* selective decoupling in the CW mode. The scheme for data acquisition was essentially that described by Derome for the one-dimensional experiment (Derome, 1987). 16 scans with irradiation on resonance were followed by 16 scans with irradiation off-resonance (9 p.p.m. downfield) and the whole sequence was repeated four times. The sample temperature was fixed at 303 K during the data acquisition. Spin-relaxation rates ($T_1$) for all protons were determined prior to the NOE measurement to allow the proper choice of saturation times. The shortest $T_1$ value (0.63 s) was observed for the two methylene protons H1(C5′) and H2(C5′). The longest $T_1$ value (6.4 s) was observed for the base proton H(C5). Saturation times of up to 30 s were thus used when irradiating selected resonances. In order to specify the conformation of isotiazofurin about the *C*-glycosidic bond, the H(C1′), H(C2′) and H(C5) resonances were saturated. NOEs were computed from the difference in integrated signals between on- and off-resonance spectra. Data were averaged over all experiments and resulting standard errors computed. Resulting NOE data are shown in Table 5.

Observed NOEs were compared with values computed for various conformations of isotiazofurin, taking into account both the indirect NOE effect and the internal motion of the molecule (Noggle & Schirmer, 1971). Expected NOEs were computed using the methods outlined by Schirmer, Davis, Noggle & Hart (1972). NOEs for a multispin system were estimated from the solution to McConell's equation (McConell, 1958). The specific method used to compute expected enhancements is determined by the exchange rate of molecules between conformations. Data were analyzed assuming rotational correlation times in the extreme narrowing limit under both the slow- and rapid-exchange models outlined below (Schirmer, Davis, Noggle & Hart, 1972).

If the exchange rate between the conformations of a molecule is slow compared to the relaxation rate ($R_d$) of a spin $d$, the steady-state solution $f_d(s,i)$ for the NOE enhancement of the spin $d$ when irradiating a single spin $s$ in conformation $i$ is given as (Noggle & Schirmer, 1971):

$$f_d(s,i) = \frac{\gamma_s \rho_{ds}(i)}{2\gamma_d R_d(i)} - \frac{1}{2\gamma_d R_d(i)} \sum_{n \neq d,s} \gamma_n \rho_{dn}(i) f_n(s,i) \quad (1)$$

where $R_d(i)$ is the total spin-lattice relaxation rate of spin $d$ in conformation $i$, $\rho_{dn}(i)$ is the spin relaxation rate between spins $d$ and $n$ in conformation $i$ and $f_n(s,i)$ is the enhancement on spin $n$ when irradiating spin $s$ in conformation $i$. In general, the observed NOEs under the slow-exchange approximation are assumed to represent population averages over all conformers $i$. Thus, enhancements computed at each conformation $i$, $f_d(s,i)$, are averaged, weighted by the fraction of molecules in conformation $i$ (Schirmer, Davis, Noggle & Hart 1972):

$$\langle f_d(s) \rangle = \sum_i P_i f_d(s,i)$$

where $P_i$ is the fraction of molecules in conformation $i$. The value of $P_i$ depends on the energy of conformation $i$, and is thus rigorously computed from the ensemble average *via* the Boltzmann distribution over all conformations (Cumming & Carver, 1987). In the case of isotiazofurin, energy calculations (below) suggested a two-state approximation consisting of a combination of *syn* and *anti* conformers. Thus,

$$\langle f_d(s) \rangle = P_{syn} f_d(s,syn) + P_{anti} f_d(s,anti) \quad (2)$$

where $P_{syn}$ and $P_{anti}$ are the populations of the *syn* and *anti* conformers respectively, and $P_{syn} + P_{anti} = 1$.

An iterative solution to (1) was obtained for each conformation of isotiazofurin in 15° increments of $\chi$ using both C2′ and C3′-*endo* sugar puckers. The resulting enhancements are plotted as a function of $\chi$ in Figs. 5(*a*) and 5(*b*), where they are compared with the observed NOEs shown in Table 5.

If the exchange rate between conformations is fast compared to the relaxation rate of the observed spin $d$, the relaxation rates $\rho(i)$ and $R_d(i)$ in (1), rather than the NOEs themselves, must be averaged over all conformational states. Resulting enhancements are then given by

$$f_d(s) = \frac{\gamma_s \langle \rho_{ds} \rangle}{2\gamma_d \langle R_d \rangle} - \frac{1}{2\gamma_d \langle R_d \rangle} \sum_{n \neq d,s} \gamma_n \langle \rho_{dn} \rangle f_n(s) \quad (3)$$

where $\langle\rangle$ denotes the population average over all conformational states $i$. These are given by:

$$\langle \rho_{ds} \rangle = \sum_i P_i \rho_{ds}(i) \quad \text{and} \quad \langle R_d \rangle = \sum_i P_i R_d(i) \qquad (4)$$

where $P_i$ is the fraction of molecules in conformation $i$. A two-site model was again employed. In this model, rapid interconversion between *syn* and *anti* conformers was assumed. Thus, the fractions of conformers in each of two states, $P_{syn}$ and $P_{anti}$, were used to obtain the population averages shown in (4). The averaged relaxation rates were used in (3).

Results of both the slow- and rapid-exchange analyses are discussed below.

## Results

### *Crystallographic study*

The molecular structure of isotiazofurin in the solid state is illustrated in Fig. 2. Atomic coordinates for all non-H atoms are given in Table 2. Bond lengths, bond angles and selected torsion angles involving non-H atoms are given in Table 3. Intermolecular hydrogen bonds are illustrated in Fig. 3 and listed in Table 4.*

*Molecular packing and hydrogen bonding.* The isotiazofurin crystal structure shows a 'herringbone' network of primarily linear intermolecular hydrogen bonds linking thiazole ring to thiazole ring, furanose ring to furanose ring, and furanose ring to thiazole ring (Fig. 3, Table 4). Each hydroxyl oxygen acts as both a hydrogen-bond donor and acceptor. The carboxamide group participates in three hydrogen bonds. The carbonyl oxygen acts as an acceptor in one bond and the amino group acts as a donor in two bonds, one of which is of marginal length.

*Sugar moiety.* Bond lengths and angles for the isotiazofurin sugar moiety do not differ significantly from those in the other thiazole nucleosides. The calculated amplitude and phase angle of pseudorotation (Altona & Sundaralingam, 1972) are listed in Table 3. Isotiazofurin exhibits a C2′-*endo* C3′-*exo* sugar pucker ($^2T_3$). The conformation around the C4′—C5′ bond is *gauche*, *trans*.

*Thiazole ring.* Bond lengths and bond angles for the thiazole ring in isotiazofurin are listed in Table 3, along with the corresponding mean bond lengths and angles from 11 previously determined thiazole

* Lists of structure factors, anisotropic thermal parameters, deviations from thiazole-ring least-squares planes and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 55980 (11 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England. [CIF reference: GR0211]

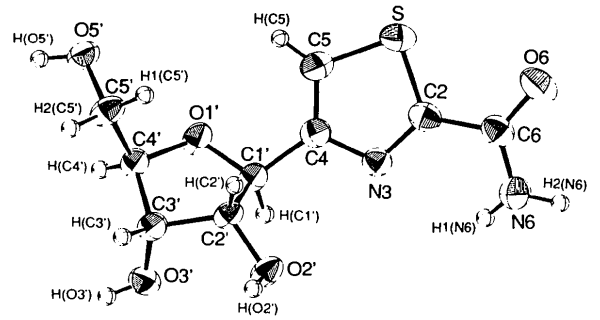


Fig. 2. The molecular structure of isotiazofurin, showing the atom-labeling scheme. Thermal ellipsoids of non-H atoms are drawn at the 50% probability level.

Table 2. *Fractional coordinates and equivalent isotropic thermal parameters for non-*H *atoms of isotiazofurin*

$B_{eq} = (4/3)\sum_i\sum_j \beta_{ij} \mathbf{a}_i.\mathbf{a}_j$.

| | $x$ | $y$ | $z$ | $B_{eq}$ |
|---|---|---|---|---|
| S | 0.9189 (1) | 0.6373 (1) | 0.46558 (2) | 4.39 (2) |
| C2 | 0.6977 (4) | 0.5668 (3) | 0.43715 (8) | 3.04 (8) |
| N3 | 0.6559 (3) | 0.6480 (3) | 0.39011 (7) | 2.76 (6) |
| C4 | 0.8025 (3) | 0.7767 (3) | 0.37572 (8) | 2.76 (7) |
| C5 | 0.9575 (4) | 0.7892 (4) | 0.4111 (1) | 3.87 (9) |
| C6 | 0.5678 (4) | 0.4253 (3) | 0.46612 (8) | 3.21 (8) |
| N6 | 0.4068 (4) | 0.3706 (4) | 0.43982 (9) | 4.35 (9) |
| O6 | 0.6137 (3) | 0.3681 (3) | 0.51239 (6) | 4.28 (7) |
| C1′ | 0.7755 (3) | 0.8924 (3) | 0.32417 (8) | 2.53 (7) |
| C2′ | 0.6208 (3) | 1.0554 (3) | 0.32863 (8) | 2.57 (7) |
| C3′ | 0.6930 (3) | 1.1991 (3) | 0.28518 (9) | 2.80 (7) |
| C4′ | 0.9175 (3) | 1.1850 (3) | 0.29139 (8) | 2.64 (7) |
| C5′ | 1.0076 (3) | 1.3323 (3) | 0.3299 (1) | 3.20 (8) |
| O1′ | 0.9574 (2) | 0.9890 (2) | 0.31047 (7) | 3.09 (6) |
| O2′ | 0.4289 (2) | 0.9781 (2) | 0.32133 (7) | 3.29 (6) |
| O3′ | 0.6320 (3) | 1.1320 (2) | 0.23257 (7) | 3.35 (6) |
| O5′ | 1.2193 (2) | 1.3266 (2) | 0.32822 (7) | 3.19 (6) |

Table 3. *Bond distances* (Å), *angles* (°), *selected torsion angles* (°) *and pseudorotation parameters* (°)

| | | | | |
|---|---|---|---|---|
| C4—N3 | 1.368 (3) | 1.379 (4)* | C1′—C2′ | 1.529 (3) |
| N3—C2 | 1.309 (3) | 1.303 (8)* | C2′—C3′ | 1.527 (3) |
| C2—S | 1.714 (2) | 1.725 (8)* | C3′—C4′ | 1.522 (3) |
| S—C5 | 1.711 (3) | 1.706 (5)* | C4′—C5′ | 1.506 (3) |
| C5—C4 | 1.358 (3) | 1.349 (6)* | C4′—O1′ | 1.443 (2) |
| C4—C1′ | 1.500 (3) | 1.497 (5)* | O1′—C1′ | 1.431 (2) |
| C2—C6 | 1.484 (3) | 1.487 (7)* | C2′—O2′ | 1.407 (3) |
| C6—O6 | 1.237 (3) | 1.234 (7)* | C3′—O3′ | 1.428 (3) |
| C6—N6 | 1.315 (3) | 1.321 (6)* | C5′—O5′ | 1.426 (3) |
| C5—S—C2 | 89.2 (1) | 89.4 (3)* | C1′—C4—C5 | 126.6 (2) |
| S—C2—N3 | 115.2 (2) | 114.9 (3)* | C6—C2—N3 | 124.8 (2) |
| C2—N3—C4 | 110.1 (2) | 109.7 (5)* | C6—C2—S | 120.1 (2) |
| N3—C4—C5 | 115.5 (2) | 116.0 (5)* | C2—C6—O6 | 119.8 (2) |
| C4—C5—S | 110.0 (2) | 110.0 (5)* | C2—C6—N6 | 115.9 (2) |
| C1′—C4—N3 | 117.9 (2) | – | O6—C6—N6 | 124.4 (2) |
| | $\chi$ (O1′—C1′—C4—C5) | | 12.7 (3) | |
| | $\varphi$ (O5′—C5′—C4′—C3′) | | −172.5 (2) | |
| | $\kappa$ (N6—C6—C2—N3) | | 4.8 (3) | |
| | $\tau_m$ | | 37.9 | |
| | $P$ | | 164.0 | |

* Mean values obtained from 11 previously determined thiazole compounds.

nucleoside structures. Bond angles are similar to the mean values of the previously determined structures, and the thiazole-2-carboxamide ring is planar within experimental error (deviations from the least-squares plane are deposited).* The S—C2 and C4—N3 bonds in isotiazofurin are marginally shorter than the mean values seen in other thiazole nucleosides. In isotiazofurin, the S—C5 and S—C2 bonds are nearly equivalent in length. In other thiazole nucleosides, the S—C5 bond length is shorter than the S—C2 bond (Table 3). These differences, although of marginal significance, suggest that the electronic structure of the thiazole ring in isotiazofurin more closely approximates the classical resonance form illustrated in Fig. 1 (Burling, Hallows, Phelan, Gabrielsen & Goldstein, 1992).

*Carboxamide group.* The C6—O6 and C6—N6 bond lengths and the O6—C6—N6 bond angle in isotiazofurin are comparable with those seen in earlier thiazole nucleoside structures. This suggests that rearrangement of the heterocyclic ring does not change the resonance structure of the carboxamide group. The plane of the carboxamide group in isotiazofurin is rotated by only 4.8° relative to the thiazole ring plane (Table 3). Despite the fact that the carboxamide group forms three intermolecular hydrogen bonds, the carboxamide amino group remains *cis* to the ring nitrogen N3 and the carboxamide oxygen *cis* to the thiazole sulfur (Fig. 2). Computations (below) indicate that this conformation is highly stabilized by two *intra*molecular interactions: an electrostatic interaction between the thiazole S atom and the carboxamide oxygen O6 and a charge-transfer hydrogen-bonding interaction between the amino hydrogen H1(N6) and the lone-pair electrons on N3. The distance between the O6 atom and the S

* See deposition footnote.

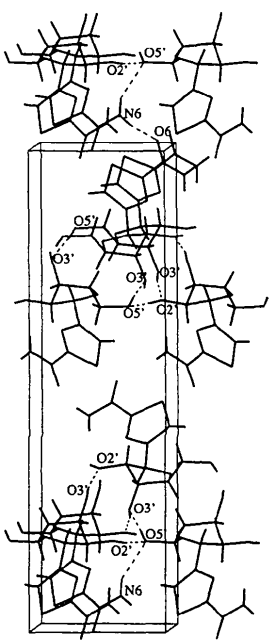


Fig. 3. Molecular packing of isotiazofurin in the solid state. Dotted lines indicate intermolecular hydrogen bonds. The *a* axis is horizontal; the *c* axis is vertical.

Table 4. *Intermolecular hydrogen-bond distances and angles*

| *D*—H···*A* | *D*···*A* (Å) | H···*A* (Å) | *D*—H···*A* (°) | Symmetry of acceptor *A* |
|---|---|---|---|---|
| O2′—H(O2′)···O5′ | 2.773 (2) | 1.98 (4) | 166 (4) | $-1+x, y, z$ |
| O3′—H(O3′)···O2′ | 2.739 (2) | 1.98 (5) | 161 (5) | $1-x, \frac{1}{2}+y, \frac{1}{2}-z$ |
| O5′—H(O5′)···O3′ | 2.751 (2) | 1.91 (4) | 175 (2) | $2-x, y+\frac{1}{2}, \frac{1}{2}-z$ |
| N6—H2(N6)···O5′ | 3.025 (3) | 2.34 (4) | 139 (4) | $-1+x, -1+y, z$ |
| N6—H1(N6)···O6 | 2.815 (3) | 1.91 (5) | 170 (4) | $-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$ |

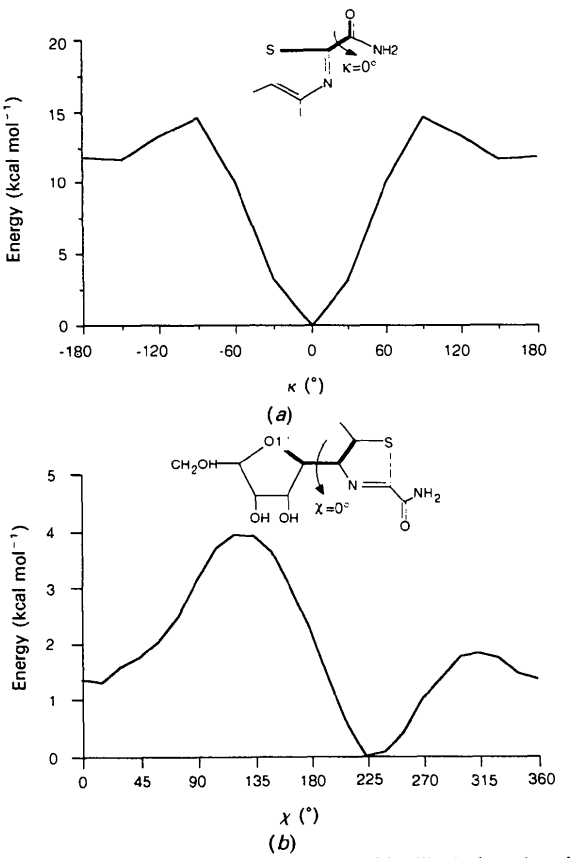


Fig. 4. (*a*) Energy profile for the carboxamide dihedral angle $\kappa$ in the thiazole-2-carboxamide ring. Each point was obtained by fixing $\kappa$ at the indicated value and computing the energy for the fully optimized fragment at the 3-21G*//3-21G* level. The global minimum is normalized to 0 kcal mol $^{-1}$. The fragment minimized is illustrated in the *cis*-planar conformation ($\kappa = 0°$) found at the global minimum. (*b*) Energy as a function of *C*-glycosidic torsion angle $\chi$ in isotiazofurin. The figure above the plot illustrates the molecule used in the computation at $\chi = 0°$. The arrow indicates the rotation about $\chi$ required to generate the curve. The value of $\chi$ was incremented in 15° steps, fixed, and the remainder of the fragment subjected to full geometry optimization using the MNDO Hamiltonian. (1 kcal mol $^{-1}$ = 4.1868 kJ mol $^{-1}$.)

Table 5. *Observed and calculated nuclear Overhauser enhancements* (%)

| $f_i[j]$[a] | Experimental | Slow exchange[b] | Fast exchange[c] |
|---|---|---|---|
| $f_{H(C5)}$[H(C1′)] | 15 (1) | 15 | 15 |
| $f_{H(C1')}$[H(C5)] | 10 (2) | 9 | 10 |
| $f_{H(C5)}$[H(C2′)] | 4 (2) | 6 | 5 |
| $f_{H(C2')}$[H(C5)] | 4 (3) | 2 | 2 |

Notes: (*a*) $f_i[j]$ is the NOE observed on the $i$th proton when irradiating the $j$th proton. (*b*) Computed NOEs based on the two-site slow-exchange model using equations (1) and (2) (see text). (*c*) Computed NOEs based on the two-site fast-exchange model using equations (3) and (4) (see text). Numbers in parentheses are standard errors in the measurements.

atom is 2.986 (2) Å and that between the N3 atom and H1(N6) is 2.43 (3) Å. The S···O6 interaction is qualitatively similar to the 1,4 S···O1′ interactions seen in those thiazole nucleosides with *C*2-glycosidic bonds (Burling & Goldstein, 1992). The N3···H1(N6) interaction is the same as that seen in thiazole nucleosides with thiazole-4-carboxamide moieties (Li & Goldstein, 1992).

*C-glycosidic bond.* The conformation around the *C*-glycosidic bond is of particular interest. The value of the O1′—C1′—C4—C5 *C*-glycosidic torsion angle $\chi$ is 12.7°, placing the base *anti* with respect to the furanose ring. If C5 in isotiazofurin is taken to be analogous to S in tiazofurin, the value of $\chi$ seen here is within the range of the O1′—C1′—C2—S *C*-glycosidic torsion angles seen in other thiazole nucleosides (Burling & Goldstein, 1992). In tiazofurin and its analogues, this *C*-glycosidic bond conformation is maintained by an attractive sulfur–O1′ interaction. The orientation of the thiazole ring relative to the base in isotiazofurin precludes this intramolecular S—O1′ interaction. However, the conformation observed here appears to be stabilized by an intramolecular H(C5)···O1′ interaction (below), the H(C5)—O1′ distance being 2.65 (4) Å.

## *Computational studies*

The crystal structure of isotiazofurin shows very similar conformational features to those seen in tiazofurin. Interatomic distances suggest that these features may also be constrained by intramolecular interactions analogous to those seen in tiazofurin. Given the potential steric and conformational similarities between isotiazofurin and tiazofurin, the question arises as to why isotiazofurin is inactive. In order to address this question, we first performed computational studies of the energy of isotiazofurin as a function of both carboxamide and *C*-glycosidic bond conformations. The purpose of these studies was to determine the degree to which the conformations observed in the solid state could be expected to be maintained *in vacuo*. Results of these studies are shown in Figs. 4(*a*) and 4(*b*) respectively.

*Carboxamide group.* Fig. 4(*a*) shows the energy of the thiazole-2-carboxamide moiety as a function of the O6—C6—C2—S torsion angle $\kappa$. The computational result shows a single minimum at $\kappa = 0°$, close to the value observed in the crystal structure. Results indicate that this conformation is quite stable, rotation about the carboxamide bond being constrained by a 15 kcal mol$^{-1}$ barrier. This barrier is similar to that observed for rotation of the thiazole-4-carboxamide group found in tiazofurin. Natural bond orbital (NBO) analysis shows that the *cis* conformation in isotiazofurin is stabilized by two strong interactions. The first is a charge-transfer

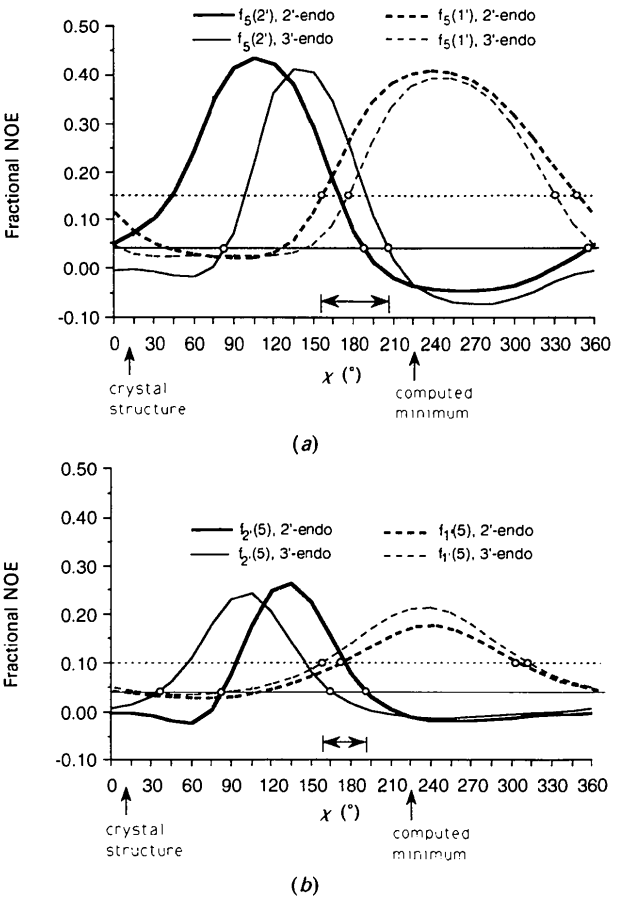


Fig. 5. NOEs computed between H(C1′) and H(C5) and between H(C2′) and H(C5) as a function of *C*-glycosidic torsion angle $\chi$ and sugar pucker. (*a*) NOEs computed for H(C5) upon irradiation of H(C1′) and H(C2′) [$f_5(1')$ and $f_5(2')$ respectively]. (*b*) NOEs computed for H(C1′) and H(C2′) upon irradiation of H(C5) [$f_{1'}(5)$ and $f_{2'}(5)$ respectively]. In each figure, a point on the dashed or solid curve represents an NOE computed for a given sugar pucker and glycosidic torsion angle under the assumption of slow conformational exchange. Horizontal lines in each figure indicate the values of the corresponding observed NOEs. Open circles indicate points at which observed and calculated NOEs agree. Horizontal arrows indicate the range of values of $\chi$ over which simultaneous agreement for the four NOEs is observed.

interaction from the N3 lone-pair electrons to the H1(N6) proton on the carboxamide amino group. An identical N—H···N interaction is seen in the thiazole-4-carboxamide group in tiazofurin (Li & Goldstein, 1992). The second interaction is an electrostatic attraction between the positively charged thiazole sulfur ($Q_S = 0.51$ e) and the negatively charged carbonyl oxygen O6 ($Q_{O6} = -0.61$ e). Analogous S···O interactions are seen in the thiazole nucleosides and elsewhere (Burling & Goldstein, 1992, 1993). These results suggest that it is unlikely that the thiazole-2-carboxamide group deviates substantially from the *cis* conformation observed in the crystal structure.

*C-glycosidic bond.* Fig. 4(*b*) shows the energy of the isotiazofurin molecule as a function of the O1′—C1′—C4—C5 torsion angle $\chi$. The resulting curve shows two minima. A local minimum at $\chi = 15°$ is close to the value of $\chi = 12.7°$ observed in the crystal structure. NBO analysis of this *anti* conformation shows that it is stabilized by an electrostatic interaction between the positively charged H(C5) and the negatively charged O1′. However, the computation also shows a global minimum at $\chi = 225°$, in the *syn* range. Both NBO analysis and examination of electrostatic isopotential maps suggest that this conformation is stabilized by electrostatic interactions between the carboxamide amino hydrogens and the O5′ hydroxyl oxygen. The energy of the *syn* conformer is approximately 1.3 kcal mol$^{-1}$ lower than that of the *anti* conformer. This suggests that, in solution, the *syn* conformer may be preferred over the *anti* conformer observed in the crystal structure. In order to test this hypothesis, NOE experiments were performed on isotiazofurin in solution. Results from these experiments are presented next.

## *NOE studies*

Results of $^1$H NOE measurements on isotiazofurin are shown in Table 5. The $^1$H NOE on one proton resonance obtained by presaturating a second proton is inversely proportional to the sixth power of the distance between the two protons, under the two-spin approximation (Noggle & Schirmer, 1971). Two distances that determine the conformation of isotiazofurin around the *C*-glycosidic bond are those from the base proton H(C5) to ribose protons H(C1′) and H(C2′) respectively. Table 5 shows that the largest NOEs are those observed between the base proton H(C5) and the ribose proton H(C1′). Specifically, the NOEs between H(C5) and H(C1′) [10 (2) and 15 (1)%] are significantly larger than the NOEs between H(C5) and H(C2′) [4 (2) and 4 (3)%]. This indicates that the distance between the base proton H(C5) and the ribose proton H(C1′) is shorter than that between H(C5) and H(C2′).

Recall that isotiazofurin is in the *anti* conformation in the crystal structure ($\chi = 12.7°$). In this conformation, the distance between H(C5) and H(C1′) (3.7 Å) is almost equal to that between H(C5) and H(C2′) (3.5 Å). However, at the computed global minimum ($\chi = 225°$) H(C5) is about 1.5 Å closer to H(C1′) than to H(C2′). The H(C5)—H(C1′) distance remains shorter than the H(5)—H(C2′) distance throughout the range in which the thiazole base is *syn* with respect to the ribose ($90 < \chi < 270°$). Thus, a qualitative analysis of the NOE data suggests that isotiazofurin adopts primarily the *syn* conformation in solution, as opposed to the *anti* conformation observed in the solid state.

In order to obtain more quantitative information about the distances between the protons of interest, the indirect NOE effect must be taken into account (Noggle & Schirmer, 1971). In general, the NOE between the observed and irradiated resonances is not simply proportional to the inverse sixth power of the distance between the two protons. Rather, the magnitude of the NOE on the observed proton is a function of the distances between the saturated proton and all protons to which it is dipole-coupled. This makes the observed NOE a more complex function of the conformation of the molecule. If the exchange rate between the conformations of a molecule is slow compared to the relaxation rate of the observed spin, equation (1) (above) may be used to estimate the expected enhancement.

Following the method of Schirmer, Davis, Noggle & Hart (1972) equation (1) was used to compute expected NOEs between the thiazole proton H(C5) and furanose protons H(C1′) and H(C2′). These NOEs were obtained for both C2′- and C3′-*endo* puckers and plotted as a function of the glycosidic torsion angle in Figs. 5(*a*) and 5(*b*). In each figure, a point on the dashed or solid curve represents an NOE computed for a given sugar pucker and glycosidic torsion angle under the assumption of slow conformational exchange. Horizontal lines in each figure indicate the values of the corresponding NOEs observed between H(C5) and H(C1′) and H(C2′) in Table 5.

A single range of values of $\chi$ shows consistent agreement between all observed and calculated NOEs. The observed NOE values intersect each set of computed NOE curves over an ~30–50° span of *C*-glycosidic torsion angles centered about $\chi \simeq 180°$ (Fig. 5). These conformers are in the range $\chi \simeq 155$–$205°$, closer to the computed global minimum conformation ($\chi = 255°$), rather than to the conformation observed in the crystal structure ($\chi = 12.4°$). Under the slow-exchange approximation, these results can be interpreted to a first approximation as indicating the presence of a single *syn* conformer in solution, at $\chi \simeq 180°$.

A more quantitative interpretation of these data was obtained using a multi-conformational model. Under the slow-exchange approximation, expected NOEs between H(C5) and H(C1′) and H(C2′) were computed for each conformer from (1) and then averaged using (2). Based upon the observation of two distinct minima in the rotational-energy profile, a two-site model was employed. Thus, the assumption was made that isotiazofurin occupies two distinct conformations in solution: *syn* and *anti*. Conformations at the computed local and global minima, $\chi = 15$ and 225° respectively, were initially chosen (Fig. 4*b*). However, only partial agreement between computed and observed NOEs could be obtained for these conformers, even over a wide range of relative populations. A search for alternative conformers in the region of the observed minima was thus performed. Improved qualitative agreement between observed and calculated NOEs (Table 5) was obtained using $\chi = 15$ and 195° as alternative *anti* and *syn* conformations respectively. These results required relative populations of 35% *anti* and 65% *syn* in (2), employing a 3′-*endo* sugar pucker in each conformer.

In the slow-exchange model, it is assumed that the molecule spends a significant amount of time at or near these conformations. An alternative possibility is that rapid interconversion occurs among widely differing conformers. In this case the $\chi \simeq 15$ and 195° conformers would be 'virtual' in that the time spent in these conformations would be small (Cumming & Carver, 1987). In order to examine this possibility, expected NOEs between H(C5) and H(C1′) and H(C2′) were computed from (3) using averaged relaxation rates obtained from (4). As above, a two-site model was employed, in this case the assumption being that isotiazofurin undergoes rapid interconversion between the *syn* and *anti* conformations in solution. The best agreement between observed and calculated NOEs (Table 5) was again obtained assuming interconversion between $\chi = 15$ and 195°. These results were acquired using relative populations of 24% *anti* and 76% *syn* in (3) and (4). Extension of the model to allow two sugar puckers for each glycosidic angle achieved further agreement with the data based on relative populations of 70% 2′-*endo* and 30% 3′-*endo* (Table 5).

Thus, analyses of the NOE data using multi-conformational models under the assumption of either slow or rapid exchange still indicate a predominance of *syn* conformers in solution. The relative populations of these conformers are qualitatively consistent with the computational results, although the NOE data suggest that the minimum energy *syn* conformer is closer to $\chi = 195$ than 225°. Nevertheless, results from the NOE studies indicate that, in solution, isotiazofurin shows a clear preference for the *syn* conformation, rather than the *anti* conformation observed in the crystal structure.

## Summary and concluding remarks

The conformations about both the carboxamide and *C*-glycosidic bonds in tiazofurin have been identified as potentially important structural features in maintaining biological activity. Rotation about the carboxamide bond in tiazofurin is constrained to the conformation in which the carboxamide amino group is *cis* to the thiazole ring nitrogen. It has been suggested that this conformation, which is similar to that observed in dehydrogenase-bound cofactors, enhances enzyme binding by the active tiazofurin anabolite. Similarly, it has been suggested that constraint of rotation about the *C*-glycosidic bond to the *anti* conformation in tiazofurin may also enhance the fit of the active anabolite to the target enzyme.

The crystal structure of isotiazofurin shows very similar conformational features to those seen in tiazofurin. The carboxamide group is approximately *cis*-planar to the thiazole ring ($\kappa = 4.8°$) and the *C*-glycosidic bond is in the *anti* conformation ($\chi = 12.7°$). Interatomic distances suggest that the carboxamide conformation is maintained by an intramolecular N6—H1(N6)···N3 hydrogen bond and that the conformation about the *C*-glycosidic bond is stabilized by a C5—H(C5)···O1′ interaction. Computational results indicate that the carboxamide conformation observed in the crystal structure is likely to be maintained in solution. However, these results also show that the observed *C*-glycosidic bond conformation is at a local, rather than global, energy minimum. The predicted global minimum for this conformation is in the *syn* range. Analysis of NOE data supports this finding. Thus, the *anti* conformation observed in the crystal structures of both tiazofurin and isotiazofurin is probably not maintained by isotiazofurin in solution. This may in part explain the inactivity of the analogue.

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# A Database Study of Nonbonded Intramolecular Sulfur–Nucleophile Contacts

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## Abstract

A search of the Cambridge Structural Database (1991, version 4.5) was performed to investigate nonbonded intramolecular 1,4 S···O close contacts of the kind seen in the thiazole nucleoside tiazofurin and other classes of compounds. The search yielded 362 structures with 1,4 S···O connectivity. S···O distances in 70% of these structures were less than the sum of the sulfur and oxygen van der Waals radii. Findings indicate that 1,4 S···O close contacts are common and so probably result from intramolecular interactions rather than from external crystal packing forces. A structure containing a sulfur atom in a conjugated ring system is more likely to exhibit 1,4 S···O close contacts than a structure containing a sulfur atom in an unconjugated and/or acyclic environment. Sulfur–nitrogen contacts were also investigated and were found to show similar properties. These results are consistent with findings from previous quantum-chemical-based studies performed on model fragments [Burling & Goldstein (1992). *J. Am. Chem. Soc.* **114**, 2313–2320].

## Introduction

The crystallographic literature contains numerous examples of both intermolecular and intramolecular nonbonded sulfur–nucleophile close contacts, in which the sulfur–nucleophile distance is less than the sum of the sulfur and nucleophile van der Waals radii (Rosenfield, Parthasarathy & Dunitz, 1977; Kucsman & Kapovits, 1985, and references therein). A previous survey of the Cambridge Structural Database (Kucsman & Kapovits, 1985, and references therein) found 755 structures, with various intramolecular sulfur–oxygen connectivities, displaying nonbonded sulfur–oxygen contact distances between 2.00 and 3.25 Å. The observation of this conformational feature in a large number of compounds indicates that an intramolecular interaction is responsible for these close contacts.

We have been interested in the biological significance of an intramolecular 1,4 sulfur–oxygen close contact seen in the crystal structure of the thiazole

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