Structural Studies on Tazobactam

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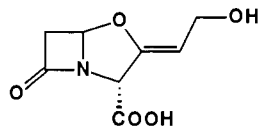
Tazobactam (3, $C_{10}H_{12}N_4O_5S$) is an effective inhibitor of bacterial β-lactamases. It crystallizes with unit cell dimensions a = 10.230 (2) Å, b = 14.396 (2) Å, and c = 17.291 (2) Å in space group $P2_12_12_1$. Compared to the related inhibitor sulbactam (2), which lacks the triazole ring, crystalline tazobactam exhibits very similar β-lactam geometry and the same S(1) envelope conformation of the thiazolidine ring. However, in both independent molecules of 3 a triazole ring nitrogen atom accepts an intermolecular hydrogen bond; similar interaction by this moiety of 3 with a hydrogen-bond donor on the enzyme, which is impossible for 2, could account for its enhanced inhibitory power. Semiempirical molecular orbital calculations show pronounced negative potential there. Molecular mechanics supports the hypothesis that the carboxyl group can rotate freely and the triazole ring can "flip".

The increasing threat posed by bacteria which have developed resistance to conventional chemotherapy as a consequence of β-lactamases has brought about the development of novel agents to fight this threat. One avenue of research has resulted in a series of β-lactamase inhibitors which have no inherent antibacterial activity, but which can be administered in conjunction with long-established antibiotics to provide an effective means of treating infections with β-lactamase producing strains.

Clavulanic acid[1] (1) and sulbactam[2] (2) have both been found to inhibit many clinically important β-lactamases and have been combined with β-lactamase-susceptible penicillins in clinical usage.[3] More recently,[4] the development of tazobactam (3, YTR-830, 3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid, 4,4-dioxide) has resulted in a β-lactamase inhibitor with a very low toxicity, wide range of inhibition, and weak induction of β-lactamases.[5]



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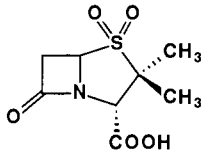

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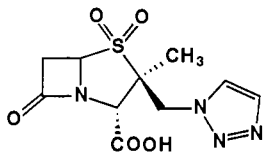

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As part of our continuing studies on the interaction between β-lactamases and their inhibitors, we wish to report the crystal structure of tazobactam and to present initial molecular modeling studies on the possible conformations of this molecule.

Crystal Stucture Determination

In order to facilitate computer-graphics modeling of this β-lactamase inhibitor, the crystal structure of 3 was determined. Crystallization from a 70:30 ethanol/water solution afforded colorless hexagonal prisms which were found to contain two independent molecules of 3. The structures (ORTEP[6] drawing) with their numbering scheme are shown in Figure 1, and the relevant experimental data are summarized in Table I.

The principal differences between the primed and unprimed molecules are observed in the positions of the triazolylmethyl and carboxylic acid moieties, and the main torsion angle differences between the two molecules are given in Table II. The thiazolidine rings are puckered into an envelope conformation, with S(1) and S(1′) out of the plane of the other four atoms by 0.813 (1) and 0.818 (1) Å, respectively, both with asymmetry parameters[7] ΔC_s of 1.3°. The alternative penicillin conformation with S(1) in plane and the β carbon C(2) out of plane would have positioned the sulfone oxygen atoms symmetrically. Nevertheless, the S(1) envelope conformation appears

Table I. Crystal Data for Compound 3

formula	$C_{10}H_{12}N_4O_5S$	system	orthorhombic
M_r	300.304	space group	$P2_12_12_1$
a	10.230 (2) Å	Z	8
b	14.396 (2) Å	wavelength	0.71069 Å
c	17.291 (2) Å	μ	0.23 mm^{-1}
V	2546.5 Å^3	D_x	1.57 g cm^{-3}
reflections collected	2849	index limits	$0 \le h \le 12$,
unique data ($I > 3\sigma$)	2464		$-17 \le k \le 2$,
parameters refined	394		$0 \le l \le 20$
extinction parameter[a]	0.000518	R, w_R	0.033, 0.045

[a] The value of X in the correction factor $[1 - 0.0001XF^2/\sin\theta]$ to be applied to the calculated structure factor.

Table II. Conformationally Significant Torsion Angles and Principal Differences between the Primed and Unprimed Crystal Structures of 3

S(1)-C(1)-C(8)-N(2)	-77.0 (3)°	S(1′)-C(1′)-C(8′)-N(2′)	-87.8 (3)°
C(1)-C(8)-N(2)-N(3)	-100.1 (3)°	C(1′)-C(8′)-N(2′)-N(3′)	-100.8 (4)°
C(1)-C(2)-C(6)-O(4)	39.9 (4)°	C(1′)-C(2′)-C(6′)-O(4′)	78.8 (4)°
C(1)-C(2)-C(6)-O(5)	-144.1 (3)°	C(1′)-C(2′)-C(6′)-O(5′)	-98.2 (4)°

Table III. Hydrogen-Bond Data for the Primed and Unprimed Structures of 3

	donor-acceptor distance, Å	donor-H-acceptor angle, deg
O(5)-H(12)···N(4)	2.667 (4)	170 (6)
O(5′)-H(12′)···N(4′)	2.682 (5)	170 (6)

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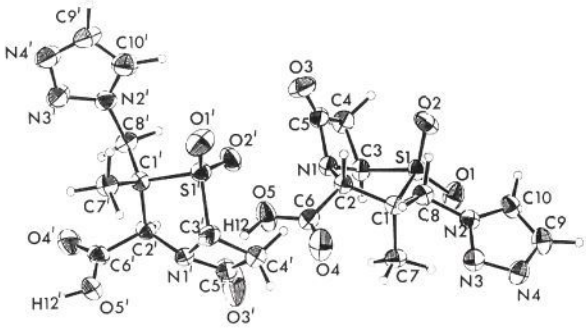

Figure 1. Computer-generated ORTEP drawing of the two independent molecules of **3**.

preferable since this is also observed in sulbactam[8] (**2**) (ΔC_s = 6.1°). This same thiazolidine conformation appears in X-ray and NMR studies on penicillin β-sulfoxides, where ΔC_s = 5.3° for penamicillin[9] and ΔC_s = 2.5° for cloxacillin[10] derivatives. It has been suggested[9] that this geometry is inimical toward binding to the target transpeptidase enzyme; however, this may aid in binding to β-lactamases.

An interesting feature apparent from the crystallographic study was the presence of intermolecular hydrogen bonding between the C(2)carboxylic acid proton and the N(4)triazolyl nitrogen. Examination of the Cambridge Crystallographic Database for compounds containing both triazole and carboxylic acid functionalities indicated literature precedent for this type of hydrogen bonding.[11,12] Table III shows the appropriate hydrogen-bond lengths and angles.

Molecular Modeling Studies

The enhanced β-lactamase inhibitory activity[5] of **3** compared to that of dimethyl analogue **2** is presumably due to the presence of the triazole function and thus we believe that this ring must participate in some favorable interaction within the active site of the β-lactamase. In particular we wish to investigate the molecular basis of enzyme inhibition as postulated by Knowles.[13] This involves acylation of an active site serine by the β-lactam, followed by a nucleophilic attack on the C(3) position of the β-lactam by another active site residue.

In order to study these hypotheses, it was necessary to establish the most favorable conformation of the molecule and to examine other possible conformations of the molecule which could be adopted. The optimum geometries of both independent molecules of **3** in the crystal structure were determined with MOPAC,[14] employing the MNDO/PM3 Hamiltonian. The independent structures optimized to virtually identical low-energy conformations, giving a final heat of formation of −76.8 kcal mol^{-1} for the unprimed structure and −76.5 kcal mol^{-1} for the primed structure. Before optimization, the corresponding figures were −38.0 and −32.8 kcal mol^{-1}.

The thiazolidine ring was less puckered after optimization than in the crystal, and as a result, the triazole ring occupied a slightly different region of space. The C(1)–S(1)–C(3)–C(4) torsion angles of 131.0 (2)° and 130.1 (3)° for the unprimed and primed molecules decreased to 123.5° and 119.4° upon optimization.

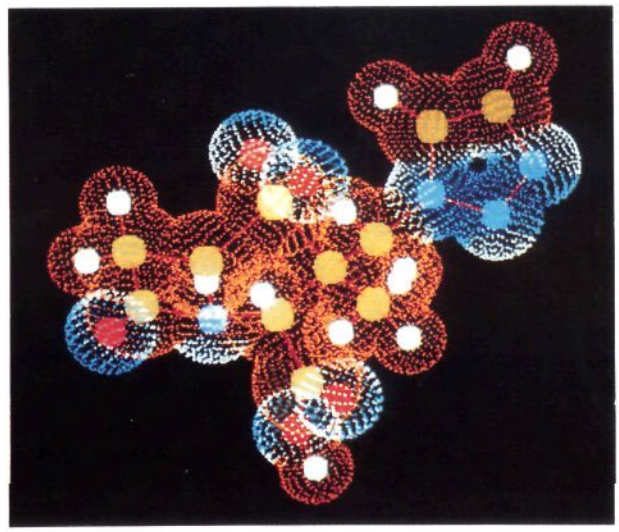

Figure 2. Electrostatic potential of the unprimed crystal structure of **3** after MOPAC optimization. The dot surface has been color coded as follows: dark blue <−55; light blue −55 to −45; white −45 to −25; orange >−25 kcal/mol.

The MOPAC calculations also suggest why **3** exhibits intermolecular hydrogen bonding between the C(4) triazole nitrogen and H(12) acid proton as described above. Figure 2 shows an electrostatic potential map of the optimized unprimed crystal structure of **3**. It can be seen that the areas of most negative potential (dark blue) are centered around the triazole ring nitrogens, and the carboxyl oxygens have a slightly less negative potential.

The conformational flexibility of both independent molecules was investigated by using molecular mechanical calculations with the modified MM2 parameters[15] available within Chem-X.[16] Separate rotation around the C(1)–C(8) and C(8)–N(2) bonds at ca. 10° intervals followed by calculation of the molecular mechanics energy shows that for the unprimed molecule, the energy barrier to rotation about either of these bonds is no greater than 50 kcal mol^{-1}. In the case of the primed molecule slightly more energy is required to "flip" the triazole ring through 180°. Figure 3, parts a and b, show plots of torsion angle against energy for rotation around the C(1)–C(8) and C(8)–N(2) bonds for both independent molecules. The equivalent torsion angles for the molecules in the crystal structure are −77.0° and −100.1° for the unprimed molecule and −87.8° and −100.8° for the primed molecule. Figure 4 shows the equivalent energy contour plot for the unprimed molecule. It can be seen that conformations of **3** with the triazole ring rotated through 180° occupy low-energy troughs, and it is possible that the energy required to overcome the barrier to rotation could be supplied to the molecules when in solution.

The only other part of the molecule which is able to undergo free rotation is the C(2) carboxylic acid group. Conformational analysis of rotation around the C(2)–C(6) bond as described above indicated that the energy barrier to rotation is very low, with the difference between the lowest and highest energy conformations of both molecules no greater than 9 kcal mol^{-1}. It is therefore reasonable to

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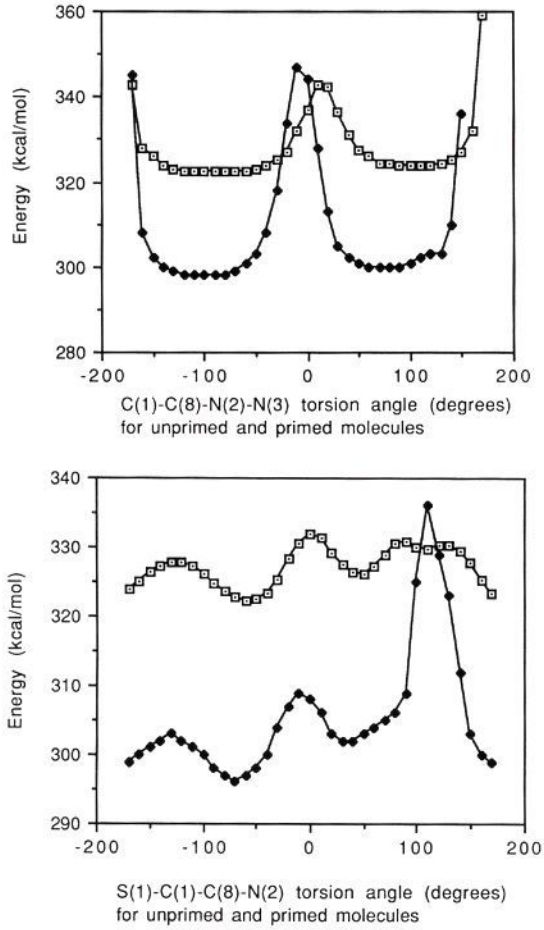

Figure 3. (a) Plots of MM2 molecular mechanics energy (kcal/mol) against torsion angle for rotation about the C(1)–C(8)–N(2)–N(3) bond for the unprimed (open squares) and primed (filled diamonds) molecules of **3**. (b) Similar plots for rotation about the S(1)–C(1)–C(8)–N(2) bond for the unprimed (open squares) and primed (filled diamonds) molecules of **3**.

assume that when in solution, sufficient energy can be obtained to rotate the carboxylic acid group into the most advantageous conformation for binding to the enzyme.

Comparison with Sulbactam

In order to determine what effect, if any, the triazolylmethyl group exerts on the penam skeleton, the crystal structure of **3** was compared to that of the β-lactamase inhibitor sulbactam (**2**). Several parameters have been studied in an attempt to correlate the biological activity of β-lactams with structure. The chemical reactivity of the amide bond[17] and a suitable distance between the β-lactam oxygen and the carboxylic acid carbon[18] (the Cohen distance) have both been used in structure–activity relationships. The reactivity of the amide bond has been related to the C=O/C—N bond lengths and the pyramidality of the β-lactam nitrogen.[17]

These parameters were determined from the crystal structures of **2**[8] and **3** and are summarized in Table IV. It can be seen that values for the two independent molecules of **3** are very similar to those of **2** although the Cohen distance is at the upper limit of the range ascribed to active compounds[19] (3.0–3.9 Å). The pyramidality of the β-lactam

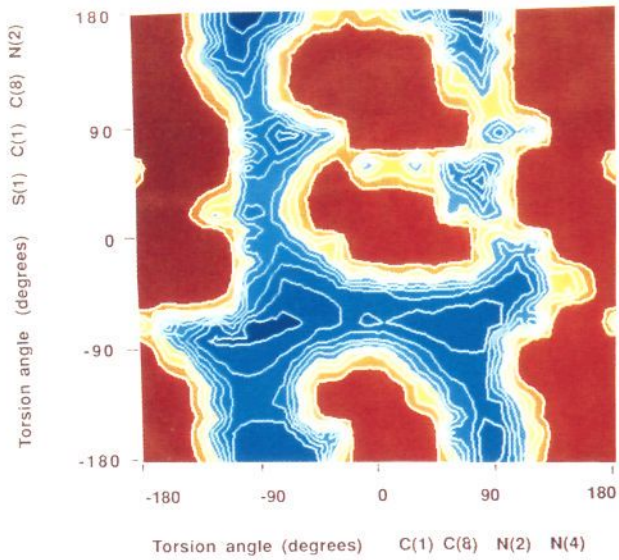

Figure 4. Energy contour map of Chem-X[16] molecular mechanics energy (kcal/mol) for rotations around S(1)–C(1)–C(8)–N(2) (*y* axis) and C(1)–C(8)–N(2)–N(4) (*x* axis). Energy levels (kcal/mol) are color coded as follows: blue, 405 (darkest shade) to 411 (lightest shade); yellow, 419; orange 421; red 423.

Table IV. β-Lactam Ring Parameters for **2** and **3**[a]

	2[7]	**3** unprimed	**3** primed
distance of β-lactam N from plane, Å	0.377	0.418 (4)	0.387 (4)
C=O bond length, Å	1.202	1.185 (5)	1.188 (6)
C—N bond length, Å	1.388	1.410 (5)	1.382 (5)
distance from β-lactam O to carboxyl C, Å	3.904	4.021 (4)	4.026 (5)

[a] Estimated standard deviations in parentheses.

nitrogen was expressed as the distance from this atom to the plane through C(2), C(3), and C(5). These figures indicate that the β-lactam N lies slightly further out of the plane of the three atoms surrounding it than the corresponding atom in **2**. Consequently, amide resonance is hindered and, as has been postulated by Woodward,[17] the susceptibility of the β-lactam to nucleophilic attack should be slightly greater.

It does not, therefore, appear that the triazolyl group exerts any major effect on the geometry of the β-lactam system of **3** (compared to **2**), indicating that its enhanced β-lactamase inhibitory activity does not stem from any altered chemical reactivity.

In general, the bond angles, bond distances, and torsion angles for the two independent molecules of **3** bear a very close similarity to the analogous data for **2**. The principal differences were noted around the carboxylic acid functionality, with the C–OH bond length decreased from 1.327 (6) Å in **2** to 1.288 (4) Å and 1.306 (5) Å in the unprimed and primed molecules, respectively.

Analysis of crystallographic data for **2** shows that an intermolecular hydrogen bond exists between the β-lactam carbonyl oxygen and the carboxylic acid proton. In the case of **3**, the enhanced electron density present on the triazolyl ring makes possible the hydrogen bonding described above. The possibility also exists for the formation of such a hydrogen bond between the triazole nitrogen and a suitable peptide residue within the enzyme active site. Work currently underway within these laboratories is

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directed toward identification of such a residue and examination of the binding of 3 to β-lactamases in an effort to rationalize its biological activity.

Experimental Section

Crystal Structure Determination. Tazobactam was crystallized from a 70:30 ethanol/water mixture as colorless stout hexagonal prisms. Crystals exhibit orthorhombic symmetry, space group $P2_12_12_1$. Unit cell parameters (Table I) were refined by least-squares analysis of setting angles of 25 reflections obtained on an Enraf-Nonius CAD4 diffractometer. A crystal 0.83 mm long × 0.33 mm wide × 0.40 mm thick was selected for collection of intensity data. Although a Ψ scan exhibited intensity variation of less than ±6% from the mean, an empirical absorption correction was made with DIFABS.[20] By the $\omega-2\theta$ scan technique a total of 2464 observed reflections with $I \geq 3\sigma$ were collected in the range $2° \leq \theta \leq 25°$ for graphite-monochromated Mo Kα radiation.

The structure was solved by direct methods using the RANTAN procedure within MULTAN.[21] Two independent molecules of 3 are present. After missing non-hydrogen atoms were located in an electron density map, least-squares refinement was carried out with SHELX.[22] All hydrogen atoms except those in carboxyl groups were introduced in calculated positions determined by the molecular geometry, methyl groups were treated as rotatable rigid bodies, and other H atoms were assumed to ride on their attached atoms. Carboxyl hydrogen atom H(12) was located unambiguously in a difference electron density map. Two plausible alternatives were found for H(12′), but one was selected because it gave the molecule a heat of formation from MOPAC[14] 20 kcal mol^{-1} lower than the other, its intramolecular geometry was better, and only it engaged in reasonable hydrogen bonding. Coordinates and isotropic temperature factors were refined for H(12) and H(12′) in the final cycles, along with isotropic temperature factors for other H atoms, coordinates and anisotropic thermal parameters for non-hydrogen atoms, and an empirical extinction correction. The observed reflections were weighted by $1/[\sigma^2(F) + 0.000519F^2]$. At termination no parameter shifted by more than 0.03 ESD, the final discrepancy index was $R = 0.033$, and no peak in a difference electron density map exceeded 0.30 eÅ^{-3}. Fractional coordinates and thermal parameters have been deposited as supplementary material.

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Supplementary Material Available: Tables listing fractional coordinates and thermal parameters, bond distances, bond angles, and torsion angles for tazobactam (7 pages). Ordering information is given on any current masthead page.

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Additional Nucleotide Derivatives of Mitosenes. Synthesis and Activity against Parental and Multidrug Resistant L1210 Leukemia

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Cytidine 5′-monophosphate and 5′-*ara*-CMP conjugates of 2,7-diaminomitosene, with the phosphate groups linked to C-1, were prepared by treating mitomycin C with the appropriate nucleotides. 5′-UMP conjugates were prepared from mitomycin A, 7 (M-83), and 8 (BMY-25282) by similar procedures. A conjugate could not be prepared from mitomycin C and 6-MPRP, but a sulfur-linked derivative was made with 6-MP ribonucleoside. The corresponding 1-hydroxy-2-aminomitosenes were prepared from the parent mitomycin analogues for structure–activity comparisons. All compounds were tested against L1210 murine leukemia in the MTT tetrazolium dye assay. In general, the conjugates were less potent than the parent mitomycins; however 5′-*ara*-CMP conjugate 14 derived from mitomycin C was more potent than the parent compound or any mitomycin tested except mitomycin A. It also was more potent than *ara*-C. This result establishes the value of this approach to prodrugs, at least in cell culture. Against a multidrug-resistant L1210 cell line, all of the conjugates derived from mitomycin C were more potent than the parent compound. 6-Mercaptopurine ribonucleoside conjugate 15 was more active against the resistant cells than it was against the parental cell line.

In a previous article on nucleotide conjugates of 2,7-diaminomitosene, we reported that treatment of mitomycin C (1) with 5′-UMP under acidic conditions gave the cis isomer of a product (2) in which C-1 of the mitosene was substituted by the phosphate group of the uridylate[1] (Scheme I). Catalytic reduction of 2 in $H_2{}^{18}O$ resulted in release of the nucleotide and formation of 2,7-diamino-1-hydroxymitosene (3) with 60% of the ^{18}O incorporated at C-1. This process was thought to occur by way of intermediate 5. Reduction of the conjugate by sodium dithionite in the presence of 2′-deoxyguanosine gave bifunctional alkylation involving C-1 and C-10 of the resulting decarbamoylmitosene and the 2-amino groups of two 2′-deoxyguanosines. This is the same product obtained from mitomycin C under the same conditions.[1] Catalytic reduction of 2 in the presence of calf thymus DNA resulted in covalent binding to an extent slightly less than that obtained with mitomycin C. A product of monoalkylation at C-1 (4) is expected under these conditions.[2]

5′-UMP derivative 2 was readily taken up by L1210 leukemia cells in culture and it was highly cytotoxic to these cells: about $^1/_5$ as potent as mitomycin C on 1-h exposure, but equally potent on 8–9-h exposure. It also prolonged the life span of mice bearing P388 lymphocytic

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