Heteroatom-Activated β-Lactam Antibiotics: Considerations of Differences in the Biological Activity of [[3(S)-(Acylamino)-2-oxo-1-azetidinyl]oxy]acetic Acids (Oxamazins) and the Corresponding Sulfur Analogues (Thiamazins)

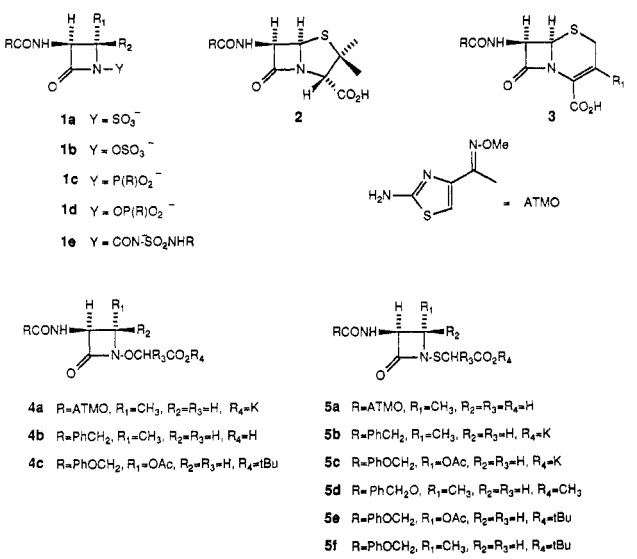
Donald B. Boyd,*† Charles Eigenbrot,*‡ Joseph M. Indelicato,*† Marvin J. Miller,*§ Carol E. Pasini,† and Steven R. Woulfe

Department of Chemistry, University of Notre Dame, Notre Dame, Indiana 46556, and Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. *Received June 2, 1986*

The considerable antibacterial activity of [[3(S)-(acylamino)-2-oxo-1-azetidinyl]oxy]acetic acids (oxamazins) in contrast to the lack of activity of the corresponding sulfur analogues (thiamazins) is examined in terms of physicochemical parameters, including electronegativity, IR carbonyl stretching frequencies, base hydrolysis rates, and three-dimensional molecular geometries. An X-ray structure determination of a protected thiamazin together with molecular graphics and molecular orbital calculations on model structures reveals that thiamazins would not fit as well as oxamazins in the active site of target bacterial transpeptidases. As a result of thiamazins' long N–S and S–C bond lengths, the pharmacophoric β-lactam ring and carboxylate functionality cannot adopt the spatial relationship they have in penicillins and cephalosporins. The β-lactam nitrogen of the monocyclic, crystalline thiamazin is 0.18 Å out of the plane of its three substituents, and this distance (*h*) is predicted by computational chemistry methods to be higher in oxamazins. The rates of β-lactam ring opening of an oxamazin, thiamazin, and aztreonam are comparable, even though the pyramidal character and IR data both indicate the electronegative oxygen analogue has reduced amide resonance. MNDO, AM1, and MINDO/3 correctly give a twofold potential for rotation about the N–S bond in model sulfenamides, with barrier heights ranging up to 12 kcal/mol.

Structure–activity studies have played an extensive role in the development of effective β-lactam antibiotics. Examination of all the different types of β-lactam antibiotics,[1] including the more recently discovered monocyclic β-lactams **1a** (monobactams[2]), now suggests that the essential pharmacophore for antibacterial activity is a reactive β-lactam ring with an ionizable acidic group located near the azetidinone nitrogen. In the more classical bicyclic β-lactams, such as the penicillins **2** and cephalosporins **3**, the fused ring provides the necessary reactivity through decreased amide resonance induced by the nonplanarity of the β-lactam nitrogen. In addition, enamine resonance promotes the reactivity of Δ^3-cephalosporins compared to Δ^2-cephalosporins[3,4] and of Δ^2-carbapenems compared to Δ^1-carbapenems.[5] The 3-position substituent of cephalosporins also contributes to the activation of the β-lactam by electron withdrawal.[1,6-8] The electron-withdrawing sulfonate group of the monobactams **1a** is primarily responsible for the enhanced reactivity at the β-lactam carbonyl of the monobactams. However, the crystal structure[9] of sulfazecin[10] shows even this monocyclic β-lactam has a pyramidal nitrogen (*h* = 0.13 Å). Extension of the concept of "alternative activation" of β-lactams has already led to the development of a number of novel antibiotics.[11-16] Herein we describe some of our studies related to the structure–activity relationships of two types of heteroatom-activated monocyclic β-lactams, the oxamazins **4**[13,14] and thiamazins **5**.[17,18]

The extensive efforts of the Squibb group have already helped define the structure–activity relationships associated with changes around the periphery of certain types of monobactams.[2] Effects on the biological activity induced by changing the ionizable group in a series of monobactams **1a**, monosulfactams **1b**, monophosphams **1c**, and monocarbams **1e** have also been investigated.[11] However, the effect of direct incorporation of a heteroatom spacer between the β-lactam nitrogen and a given ionizable group, e.g., COOH, has not been clearly demonstrated. Early in our development of a hydroxamate approach to the synthesis of β-lactams, we noted that the resulting substituted *N*-hydroxy-2-azetidinones **6** had unique properties.[19] For



† Lilly Research Laboratories.
‡ Molecular Structure Facility, University of Notre Dame.
§ Fellow of the Alfred P. Sloan Foundation (1981–1985). Recipient of an NIH Research Career Development Award (1983–1988).

(1) Boyd, D. B. In *Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Eds.; Academic: New York, 1982; Vol. 1, p 437.
(2) Koster, W. H.; Cimarusti, C. M.; Sykes, R. B. In *Chemistry and Biology of β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Eds.; Academic: New York, 1982; Vol. 3, p 339 and references therein.
(3) Sweet, R. M.; Dahl, L. F. *J. Am. Chem. Soc.* **1970**, *92*, 5489.
(4) Sweet, R. M. In *Cephalosporins and Penicillins: Chemistry and Biology*; Flynn, E. H., Ed.; Academic: New York, 1972; p 280.
(5) Pfaendler, H. R.; Gosteli, J.; Woodward, R. B.; Rihs, G. *J. Am. Chem. Soc.* **1981**, *103*, 4526.
(6) Boyd, D. B.; Lunn, W. H. W. *J. Med. Chem.* **1979**, *22*, 778.
(7) Boyd, D. B. *J. Med. Chem.* **1984**, *27*, 63.
(8) Boyd, D. B. *J. Org. Chem.* **1985**, *50*, 886.
(9) Kamiya, K.; Takamoto, M.; Wada, Y.; Asai, M. *Acta Crystallogr., Sect. B* **1981**, *B37*, 1626.
(10) Imada, A.; Kitano, K.; Kintaka, K.; Muroi, M.; Asai, M. *Nature (London)* **1981**, *289*, 590.
(11) Slusarchyk, W. A.; Dejneka, T.; Gordon, E. M.; Weaver, E. R.; Koster, W. H. *Heterocycles* **1984**, *21*, 191.
(12) Cimarusti, C. M.; Sykes, R. B. *Med. Res. Rev.* **1984**, *4*, 1.
(13) Woulfe, S. R.; Miller, M. J. *Tetrahedron Lett.* **1984**, *25*, 3293.
(14) Woulfe, S. R.; Miller, M. J. *J. Med. Chem.* **1985**, *28*, 1447.

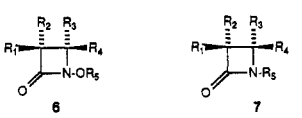
0022-2623/87/1830-0528$01.50/0

Table I. Selected β-Lactam Carbonyl Stretching Frequencies

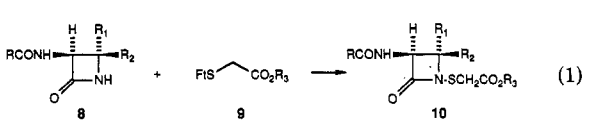
compound	C=O stretch, cm^{-1}	compound	C=O stretch, cm^{-1}
sulfazecin **1a**[a]	1775	oxamazin **4b**[c]	1770
penicillins **2**[b]	1770–1780	thiamazin **5a**[d]	1760
cephalosporins **3**[b]	1764–1776	thiamazin **5b**[d]	1750
oxamazin **4a**[c]	1770		

[a] Reference 10. [b] References 3, 4. [c] References 13–16. [d] References 17, 18.

example, the IR stretching frequencies of the corresponding β-lactam carbonyl group were consistently higher than the corresponding N-unsubstituted or N-alkylated β-lactams **7**. As suggested by this higher IR frequency, the substituted *N*-hydroxy-2-azetidinones **6** also appeared to be more susceptible to solvolytic ring opening. That this heteroatom chemical activation could also be used to develop new types of biologically active antibiotics was demonstrated by the preparation of the oxamazins **4**.[13-16] These compounds, similar to the monobactams, have considerable antibiotic activity, mostly against Gram-negative organisms.[14-16] Since the corresponding β-lactams without the oxygen spacer have no significant biological activity, it was initially assumed that, despite moving the ionizable group one atom farther from the β-lactam nitrogen, the increased electronegativity[20] of oxygen was responsible for the chemical and biological activity of the oxamazins. In order to test this hypothesis, many other types of alternately activated β-lactams might be prepared by simply incorporating other heteroatoms, that is, "electronegative spacers". In fact, this seemed to be the case with the other monobactams (**1a–e**) listed earlier. We decided to test this concept further by maintaining the same basic β-lactam framework and ionizable group, but simply varying the heteroatom. Our first choice was to substitute the oxygen of the oxamazins with sulfur and to study the corresponding thiamazins **5**.[17,18]



A variety of approaches to the synthesis of representative thiamazins were considered and tried. However, as described earlier,[17,18] direct sulfenylations of N-unsubstituted β-lactams **8** with substituted thiophthalimides **9** provided the basic nucleus most efficiently (eq 1). Sub-



sequent elaboration provided thiamazins suitable for biological screening. Interestingly, the various thiamazins tested (**5a–c**) were found to be devoid of antibacterial activity.[17,18] The following observations help explain this remarkable change in activity induced by changing the oxygen atom spacer of the oxamazins to the sulfur of the thiamazins.

Table II. Pseudo-First-Order Rate Constants for the Alkaline Hydrolysis of β-Lactams (35 °C, μ = 0.5) Based on Disappearance of Starting Material as Measured by HPLC

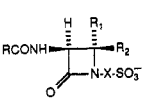
compound	pH 10		pH 11	
	$t_{1/2}$, h	k, h^{-1}	$t_{1/2}$, h	k, h^{-1}
1a (aztreonam)	1.7[a]	0.40 ± 0.03[a]		
4b	2.2	0.31 ± 0.02		
5b	0.95[b]	0.73 ± 0.01[b]		
8			24	0.029 ± 0.002

[a] Reference 29. [b] Based on disappearance of starting material by at least two processes (Scheme I).

Electronegativity and IR Considerations

Comparisons of the infrared spectra of structurally similar oxamazins **4** and thiamazins **5** revealed that the β-lactam carbonyl stretching frequencies of the oxamazins were consistently 10–20 cm^{-1} higher than those in the corresponding thiamazins. In addition, the carbonyl stretch of oxamazins falls in the range of other active β-lactams, such as the penicillins, cephalosporins, and monobactams, whereas the frequencies of the thiamazins are similar to unstrained and biologically inactive β-lactams (Table I). Thus, while it was apparent that the more electronegative oxygen atom spacer decreased the amide resonance of the oxamazins relative to sulfur's effect in thiamazins, it was not clear that a simple difference in electronegativity could completely explain the dramatic difference in biological activity.

Some supportive evidence for the electronegativity effect was provided by comparison of the biological properties of the monosulfactams **11a** (**1b**) and their nitrogen (**11b**) and sulfur (**11c**) analogues. Significant antibacterial activity of the monosulfactams **11a** has been well-documented.[21] From a recent patent describing the synthesis of *N*-amino-*N*-sulfated-2-azetidinones (**11b**), it can be inferred that these compounds are only moderately active.[22] The sulfur analogues **11c** are ineffective antimicrobial

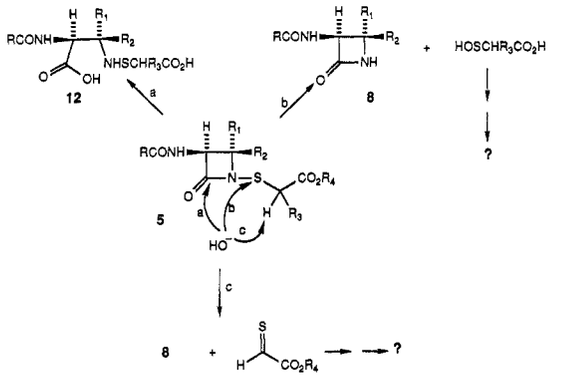


11a X=O (**1b**)
11b X=NR
11c X=S

agents.[23,24] Thus, within this class of compounds, there appears to be a distinct correlation between biological activity (**11a** > **11b** > **11c**) and electronegativity[20] of the heteroatom directly attached to the azetidinone nitrogen (O > N > S). However, it would be premature to ascribe differences in biological activity between these compounds and between the oxamazins and thiamazins to simply electronegativity differences. For example, would the chemical reactivity of the oxamazins and thiamazins also correlate with the differences in biological activity and electronegativity of the oxygen and sulfur? Would differences in their three-dimensional geometries affect affinity of the compounds to the active sites?

(15) Breuer, H.; Straub, H.; Treuner, U. D.; Drossard, J.-M.; Hohn, H.; Linder, K. R. *J. Antibiot.* **1985**, *38*, 813.
(16) Kishimoto, S.; Sendai, M.; Ochiai, M. PCT Int. Appl. WO 85 01,287.
(17) Woulfe, S. R.; Iwagami, H.; Miller, M. J. *Tetrahedron Lett.* **1985**, *26*, 3891.
(18) Woulfe, S. R.; Miller, M. J. *J. Org. Chem.* **1986**, *51*, 3133.
(19) Miller, M. J. *Acc. Chem. Res.* **1986**, *19*, 49.
(20) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960; p 90.
(21) Gordon, E. M.; Ondetti, M. A.; Pluscec, J.; Cimarusti, C. M.; Bonner, D. P.; Sykes, R. B. *J. Am. Chem. Soc.* **1982**, *104*, 6053.
(22) Breuer, H.; Straub, H.; Treuner, U. D. German Patent DE3328047, issued Feb 9, 1984.
(23) Woulfe, S. R. Ph.D. Dissertation, University of Notre Dame, Notre Dame, IN, 1985.
(24) Iwagami, H.; Woulfe, S. R.; Miller, M. J. *Tetrahedron Lett.* **1986**, *27*, 3095.

Scheme I

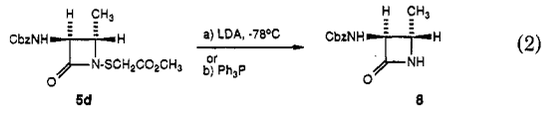


Chemical Stability Studies

Chemical reactivity of β-lactam antibiotics is an important parameter because a key step in the mode of action is acylation of a serine residue in the active site of bacterial cell wall enzymes.[25,26] Intrinsic reactivity of various β-lactam antibiotics has been examined in terms of the rate constants for alkaline hydrolysis of the β-lactam bond.[1,27-29] Rates of nucleophilic attack at the β-lactam carbonyl of oxamazins and thiamazins should be more direct indicators of acylating ability than are differences in electronegativity and IR stretching frequency of the β-lactam carbonyls.

The pseudo-first-order rate constants were determined for the reaction of oxamazin **4b** and the structurally similar thiamazin **5b** under alkaline conditions (Table II). The thiamazin actually seemed less stable to base than the corresponding oxamazin contrary to what might be expected from the biological activity and electronegativity. The reactivity of both the oxamazin and thiamazin is comparable to that of aztreonam (a form of **1a**), an active[15] monobactam antibiotic (Table II). Although the thiamazin is more susceptible to base than oxamazin, chemical instability does not account for the former's lack of biological activity. During a 7-h period no decomposition of thiamazin was observed by HPLC in solution at pH 7.2 ± 0.2 and 35 °C, the latter being similar to conditions of a microbiological assay.

The initial hydrolysis studies reflected only disappearance of starting materials. Although hydroxide attack at the β-lactam carbonyl might be assumed, earlier experimental evidence indicated that the thiamazins were susceptible to alternative base-initiated decomposition. For example, treatment of thiamazin **5d** with either LDA or triphenylphosphine resulted in formation of the N-unsubstituted β-lactam **8** (eq 2). The oxamazin nucleus is



(2)

stable to the same conditions. Thus we suspected that the basic hydrolysis of the thiamazins may proceed by several competitive routes: (a) attack at the carbonyl, (b) direct nucleophilic attack at the sulfur,[18] and/or (c) elimination to generate a thioaldehyde (Scheme I). Contributions from either or both of the latter two routes (b and c) could be determined by measuring formation of the resulting N-unsubstituted β-lactam **8** assuming that it was stable to the hydrolysis conditions. Indeed, hydrolysis of **8** (R = $PhCH_2$, R^1 = CH_3, R^2 = H, Scheme I) at pH 10 was so slow that a better measurement of reactivity of **8** had to be obtained by going to pH 11. With the indication that even at pH 11 the N-unsubstituted β-lactam **8** would be stable during the hydrolysis studies (Table II), the hydrolyses of thiamazin **5b** and oxamazin **4b** were reexamined looking for the appearance of **8** by HPLC. Basic hydrolysis of thiamazin **5b** did produce the N-unsubstituted β-lactam **8** at a rate constant of 0.35 ± 0.01 h^{-1}, indicating that approximately 50% of the thiamazin was decomposing by N–S cleavage, presumably by either path b or c (Scheme I). No N-unsubstituted β-lactam **8** was detected during the hydrolysis of the corresponding oxamazin **4b**. Considering that about half of the rate of disappearance of the thiamazin was due to N–S cleavage, the actual rates of hydrolysis of thiamazins and oxamazins by attack at the β-lactam carbonyl were nearly identical. Whether this situation is paralleled under microbiological conditions is not known.

Table III. Observed Interatomic Distances (in Å) from the β-Lactam Carbonyl Carbon and Oxygen to the Carboxyl Carbon of the Ionizable Group in β-Lactams[a]

	compd	C···C	O···C
biologically active	**13**	3.68[b]	4.26[b]
	14	3.11	3.20
	15	3.36	3.57
	16	3.38	3.61
biologically inactive	**17**	3.49	4.11
	18	3.72	4.28
	5e	3.91	4.21

[a] References 1, 35. [b] When the thiazolidine ring is flipped into a COOH-equatorial conformation, these distances decrease by 0.3–0.4 Å. The tabulated distances for **13** are from ref 30 rather than from ref 32 because of seriously inconsistent data in ref 32 itself, as well as between ref 32 and the Cambridge Structural Database (ref 40). The corresponding distances from ref 32 are about 0.1 Å longer than those from ref 30. References 1 and 35 used data from ref 30 also.

Goodness of Fit Considerations

The carbonyl stretching frequency and the chemical reactivity of the β-lactam carbonyl are not the only parameters to be considered as indicators of potential biological activity. Successful inhibition of bacterial transpeptidase enzymes also depends on the equilibrium constant for formation of a Michaelis complex. In other words, there must be a strong interaction between the β-lactam molecule and the active site within the enzyme prior to formation of the acyl–enzyme complex. The goodness of fit of a substrate is related to its three-dimensional structure. Comparisons of solid-state conformations from X-ray crystallographic studies of penicillin G[4,30-33] **13**, cephaloridine **14**, thienamycin (a Δ^2-carbapenem) **15**, penem **16**, and monobactam **1a** to those of Δ^2-cephalosporin **17** and Δ^1-carbapenem **18** reveal that active and inactive compounds have a different separation between the acidic group and β-lactam ring.[1,7,34-36] The ionizable group is

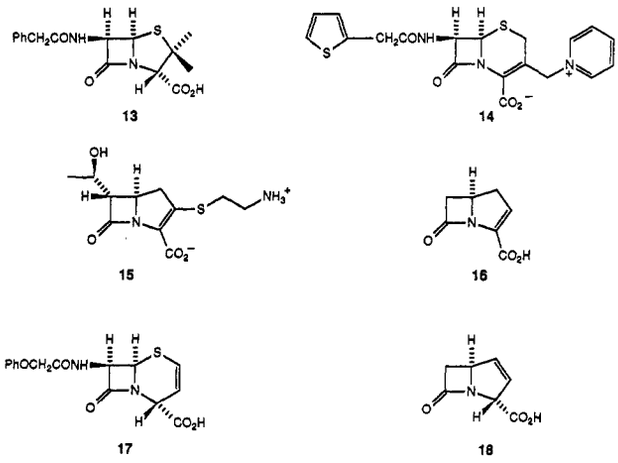
(25) Kelly, J. A.; Knox, J. R.; Moews, P. C.; Hite, G. J.; Bartolone, J. B.; Zhao, H.; Joris, B.; Frere, J.-M.; Ghuysen, J.-M. *J. Biol. Chem.* **1985**, *260*, 6449.
(26) Boyd, D. B.; Ott, J. L. *Antimicrob. Agents Chemother.* **1986**, *29*, 774 and references therein.
(27) Indelicato, J. M.; Dinner, A.; Peters, L. R.; Wilham, W. L. *J. Med. Chem.* **1977**, *20*, 961.
(28) Boyd, D. B. *J. Med. Chem.* **1983**, *26*, 1010.
(29) Indelicato, J. M.; Fisher, J. W.; Pasini, C. E. *J. Pharm. Sci.* **1986**, *75*, 304.
(30) Pitt, G. J. *Acta Crystallogr.* **1952**, *5*, 770.
(31) Vaciago, A. *Atti. Accad. Naz. Lincei. Cl. Sci. Fis., Mat. Nat., Rend.* **1960**, *28*, 851.
(32) Dexter, D. D.; van der Veen, J. M. *J. Chem. Soc., Perkin Trans. 1*, **1978**, 185.
(33) Boyd, D. B. *J. Med. Chem.* **1979**, *22*, 533.

Table IV. Interatomic Distances[a] (in Å) within Oxamazin **4c**[b] and Thiamazin **5e**[c]

atoms	4c	5e	range for active β-lactams[d]
O1–C6	3.79	4.21	3.2–4.1
N2–C6	5.75	6.18	5.4–5.8
C2–C6	3.48	3.91	3.1–3.6
N1–C6	2.80	3.18	2.4–2.8

[a] Refer to Figure 1 for the atom numbering system. [b] Estimated from mathematical replacement of S of **5e** with O and adjustment of the N–X–C bond lengths while holding the bond angles and torsional angles constant. The N–O and O–C distances adopted are 1.41 and 1.43 Å, respectively. [c] Determined from the X-ray structure. [d] For 11 active penicillins, cephalosporins, and other bicyclics from ref 1, p 518, plus **4c**. It is assumed that the COOH-equatorial conformation of penicillins is the one responsible for all or most of the activity.

generally closer to the β-lactam ring in the biologically active molecules (Table III).



Balsamo et al. have pointed out that penicillins that crystallize in a conformation with 3α-COOH in an axial position are less active against *some* organisms than are cephalosporins and COOH-equatorial penicillins.[34] A few exceptions to this idea have been noted, although to date no penicillins with "broader" activity[34] have been found that crystallize in the COOH-axial thiazolidine ring conformation.[1] In related work, the range of permissible dimensions of the pharmacophore of the then known active β-lactams was determined.[1] In a subsequent paper, Cohen[35] concluded that the distance between the β-lactam carbonyl oxygen and the carbon atom of the ionizable group (sulfur in the case of aztreonam, a form of **1a**) is shorter in the active compounds relative to the inactive molecules. In order to reach this conclusion, he classified penicillin G as "inactive", which, of course, is far from true.[37] However, it is likely that the thiazolidine ring of penicillins flips easily[35] between COOH-axial and COOH-equatorial conformations, so that the molecule can achieve a conformation required for activity.[1] A COOH-equatorial conformation would place the carboxyl carbon 0.3–0.4 Å closer to the β-lactam carbonyl. Thus, it is safe to conclude that the distance between the pharmacophoric groups is shorter in the active compounds when conformational flexibility is taken into account.

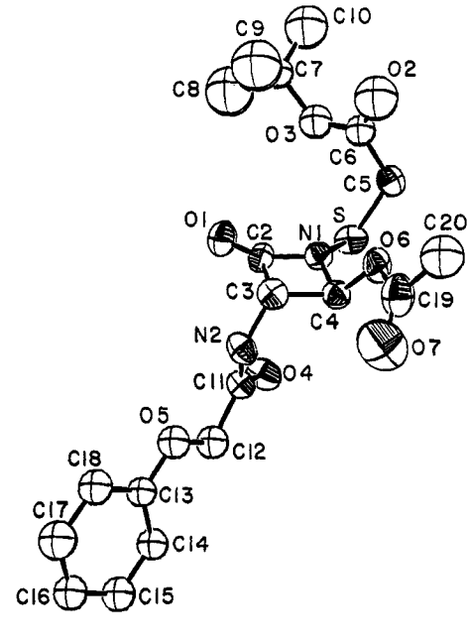
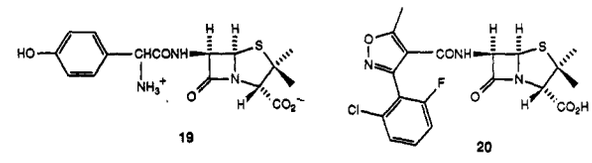


Figure 1. Crystalline state structure of thiamazin **5e**.

The structure of a crystalline thiamazin derivative **5e** (Figure 1) was determined by X-ray diffraction. Some of the interatomic distances for **5e** shown in Tables III and IV clearly fall outside the "active" range of the corresponding distances for the previously studied compounds.[1] For example, the 3.91 Å between the β-lactam carbonyl carbon and the carboxyl carbon is beyond the range of biologically active compounds (<3.6 Å). Although the corresponding interatomic distances have not been determined by a crystallographic study of an oxamazin, approximate values can be calculated as described in Table IV. These interatomic distances for hypothetical oxamazin analogue **4c** mostly fall *within* the ranges noted for biologically active β-lactams. These correlations suggest that the superior activity of the oxamazins may be a consequence of their better fit into the enzyme active site.

Besides the interatomic distances, further insight into geometrical properties was provided by molecular graphics. Figure 2 provides comparable stereoviews of the crystalline-state conformation of a cephalosporin (**14**, cephaloridine[3]), a penicillin in a 3α-COOH-equatorial conformation (**19**, amoxycillin[38]), and a penicillin in a 3α-COOH-axial conformation (**20**, flucloxicillin[39]), all with the side



chains stripped away to make the nuclei easier to see. The three structures were extracted from the Cambridge Structural Database.[40] Shown in Figures 3 and 4 are two similar views of thiamazin and oxamazin models. In no case was it possible to superpose exactly the β-lactam rings and the carboxyl groups of the first three antibiotics (Figure 2) with this conformation of the monocyclics. It

(34) Balsamo, A.; Domiano, P.; Macchia, B.; Macchia, F.; Nardelli, M. *Eur. J. Med. Chem., Chim. Ther.* **1980**, *15*, 559.
(35) Cohen, N. C. *J. Med. Chem.* **1983**, *26*, 259.
(36) Keith, D. D.; Tengi, J.; Rossman, P.; Todaro, L.; Weigele, M. *Tetrahedron* **1983**, *39*, 2445.
(37) Gorman, M.; Ryan, C. W. In *Cephalosporins and Penicillins: Chemistry and Biology*; Flynn, E. H., Ed.; Academic: New York, 1972; p 532.
(38) Boles, M. O.; Girven, R. J.; Gane, P. A. C. *Acta Crystallogr., Sect. B* **1979**, *B34*, 461.
(39) Blanpain, P. C.; Nagy, J. B.; Laurent, G. H.; Durant, F. V. *J. Med. Chem.* **1980**, *23*, 1283.
(40) Allen, F. H.; Kennard, O.; Taylor, R. *Acc. Chem. Res.* **1983**, *16*, 146.

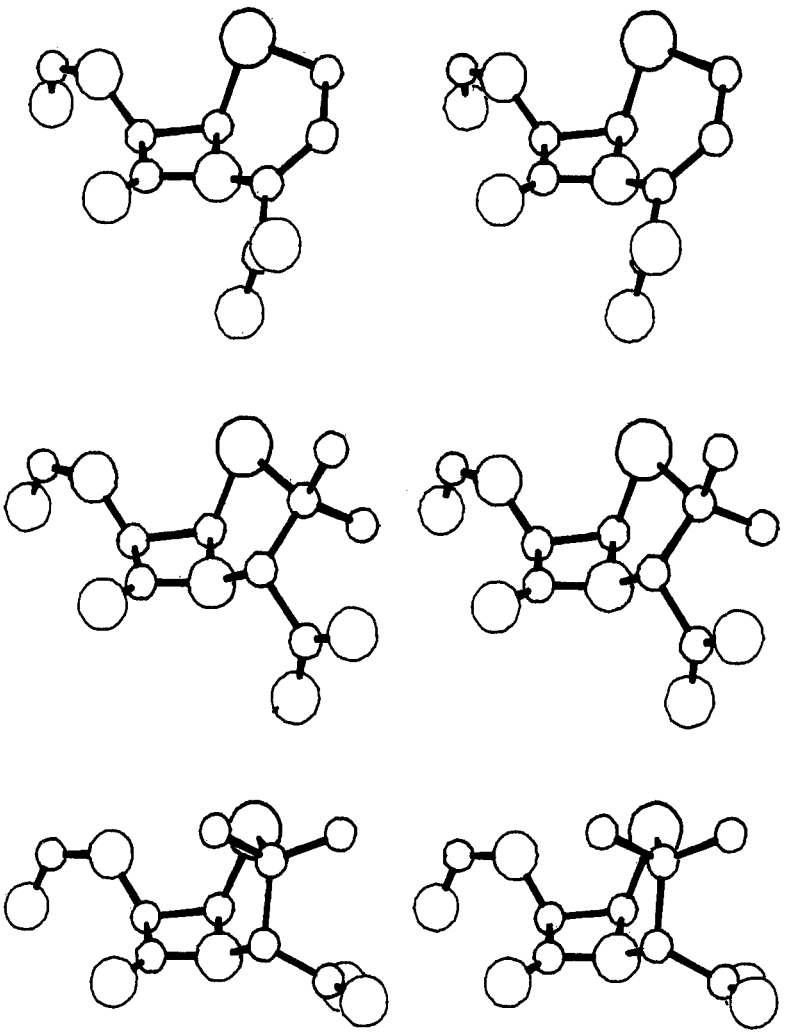


Figure 2. Comparable stereoviews of 14, 19, and 20. Parts of the side chains have been trimmed away for visual clarity.

is notable that, due to the way the thiamazin crystallizes, its carboxyl group is rather far "below" the plane of the β-lactam ring.

Because the chain attaching the ionizable group to the β-lactam nitrogen is flexible in oxamazins and thiamazins and because conformations of molecules in solution can differ from that in their crystalline form, computerized attempts were made with the SYBYL software package[41,42] to overlay the carboxyl group of the two monocyclics with the carboxyl group of the cephalosporin and penicillins while overlapping the β-lactam rings of each pair of molecules. In the overlaying process the rings were superimposed by a least-squares distance minimization of the amide O=C—N atoms and bond lengths and angles were held constant while freely rotating about the two bonds of N—X—CH_2. The shortest intermolecular distances that were achieved between the carboxyl carbons of the overlapped molecules are given in Table V. As discussed below, the resulting best fits to the active conformations of the reference antibiotics are with energetically accessible conformations of the monocyclics.

The conclusions to be drawn from these molecular modeling[43,44] studies are that (a) it is impossible for the acidic functions of oxamazin or thiamazin to get in the same spatial position relative to the β-lactam ring as for cephalosporins and penicillins, (b) the closest the carboxyl groups of the monocyclics can come to the corresponding positions in the bicyclic molecules is about 1 Å, and (c) the active monocyclic, oxamazin, fits the distance separation requirement 0.3–0.4 Å better than does the inactive thiamazin. Protein receptors have considerable vibrational motion, but if the functional groups were stretched near

Table V. Shortest Intermolecular Distances[a] (in Å) between Carboxyl Carbons and Corresponding C2–N1–X–C and N1–X–C–C Torsional Angles (in Deg) in the N–R Side Chain from Interactive Molecular Graphics Fitting of N–X–CH_2–COOH-azetidin-2-ones to Classical Bicyclic β-Lactams

X	CR_2	NR	O	S
Δ^3-cephalosporin **14**	1.09	1.02	0.97	1.37
ϕ_1[b]	78	80	80	79
ϕ_2[b]	246	243	243	262
penicillin (COOH-equatorial) **19**	1.39	1.31	1.25	1.74
ϕ_1	102	101	102	100
ϕ_2	182	179	184	180
penicillin (COOH-axial) **20**	1.30	1.22	1.19	1.64
ϕ_1	154	150	141	146
ϕ_2	158	155	140	137

[a] For X = CR_2, NR, and O, possible changes in bond angles at X and at the β-lactam nitrogen from their values in crystalline thiamazin would introduce uncertainties in these distance of perhaps 10%. [b] ϕ_1 and ϕ_2 are C2–N1–X–C and N1–X–C–C torsional angles, respectively, which give the tabulated distance. A 0° angle corresponds to cis. Torsional angle for A–B–C–D is measured clockwise from the A–B–C plane to the B–C–D plane looking from B to C.

(41) SYBYL Manual, Tripos Associates, St. Louis, MO, 1985. Appendix 2 for standard bond lengths.
(42) Van Opdenbosch, N.; Cramer, R., III; Giarrusso, F. F. *J. Mol. Graphics* **1985**, *3*, 110.
(43) Hopfinger, A. J. *J. Med. Chem.* **1985**, *28*, 1133.
(44) Boyd, D. B. *Quantum Chem. Prog. Exchange Bull.* **1985**, *5*, 85.

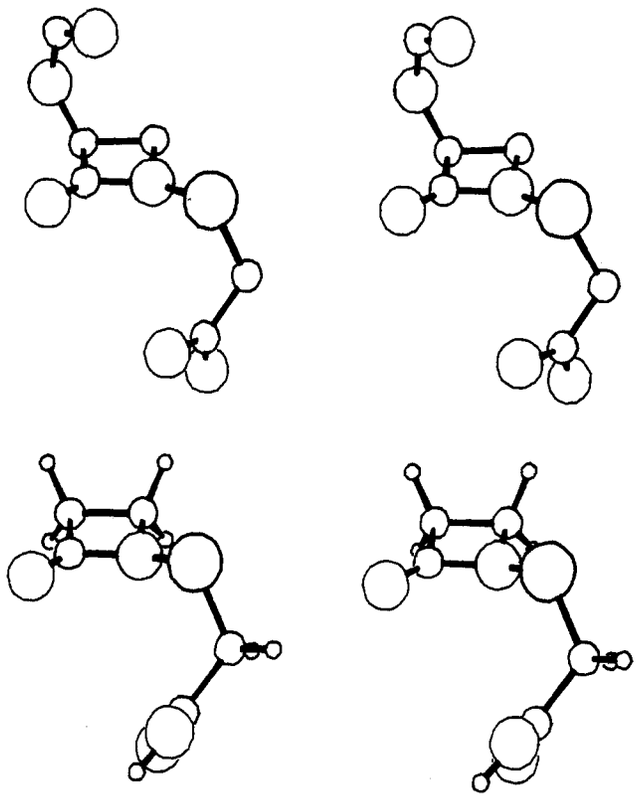


Figure 3. Comparable stereoviews of models of thiamazin as observed in the crystal (upper) and optimized by MNDO (lower). Side chains of **5e** have been trimmed away for visual clarity. Note that the acyl side chain is turned away from the usual position observed in penicillins and cephalosporins so that the acyl oxygen lies over the β-lactam ring. This solid-state conformation of 3-R is not significant in terms of biological activity because rotation about the C3–N bond is possible in solution. The MNDO geometry optimization was done with hydrogens at positions 3 and 4 of the azetidin-2-one as shown.

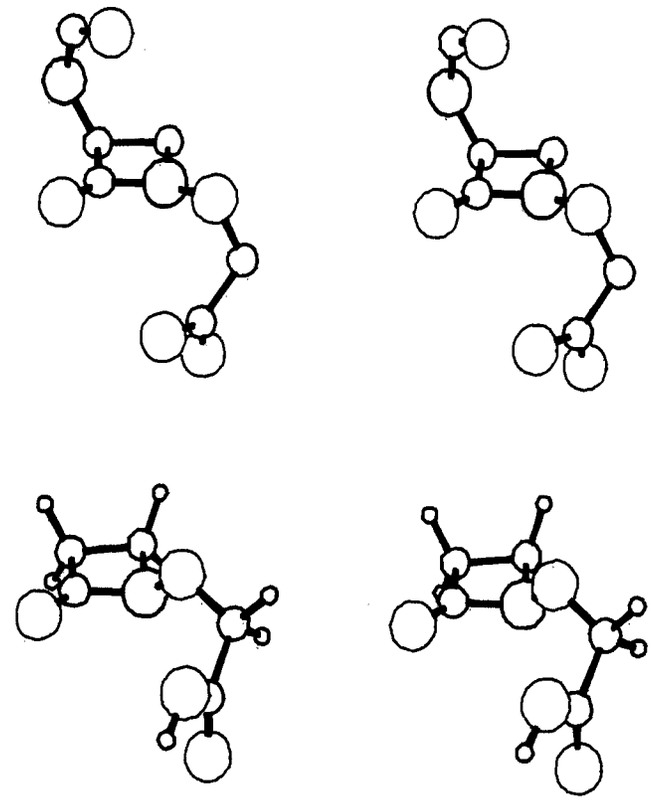
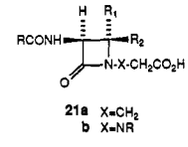


Figure 4. Comparable stereoviews of oxamazin as mathematically modeled from **5e** (upper) and optimized by MNDO (lower). The starting geometry for the optimization was that of the MNDO-optimized thiamazin model in Figure 3.

their maximum to accommodate oxamazin, then thiamazin would be too large for a very productive interaction. Thus, the better activity of the oxamazin relative to the thiamazin is compatible with the concept of a better receptor-site fit.

Similar molecular overlay studies were performed on additional monocyclic analogues **21** (**a**, X = CH_2 and **b**, X = NR) by constructing the molecular structures with standard N–X–C bond lengths and other geometrical data from the thiamazin X-ray structure. The N–C–C bond



21a X=CH_2
b X=NR

lengths used were 1.47 and 1.54 Å, which are SYBYL standard values;[41] N–N–C bond lengths were 1.42 and 1.48 Å.[45] By the same fitting procedure as in the thiamazin (X = S) and oxamazin (X = O) cases, the shortest intermolecular distances between the carboxyl carbons that could be achieved are included in Table V. From the point of view of receptor site fit, the oxygen and nitrogen analogues appear to be the best of the four (X = O, S, NR, CH_2). Although the synthesis of such a nitrogen analogue (X = NR) has not yet been reported, other factors, including potential NR protonation under physiological conditions, might alter its activity compared to oxamazin.

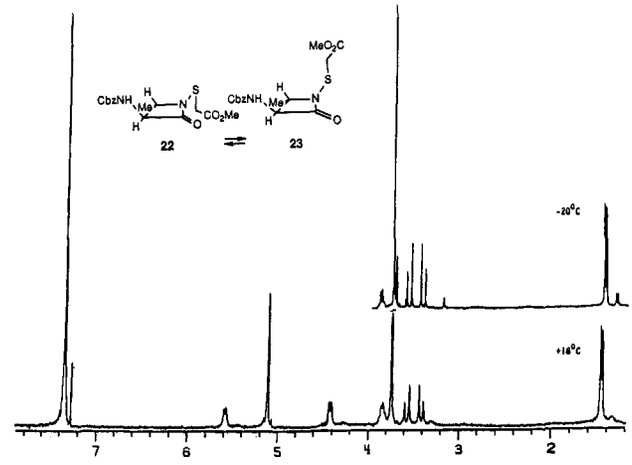


Figure 5. ^{1}NMR spectra of **5d** at −20 and 18 °C. The spectrum at lower temperature clearly shows the mixture of conformational isomers presumably **22** and **23**.

Another consideration for the biological inactivity of the thiamazins was that restricted rotation around the bonds in the carboxyl-bearing side chain might further prevent the ionized group from adopting a necessary conformation. Restricted rotation about the N–O bond in hydroxylamines and the N–S bond in thiohydroxylamines (sulfenamides) is well-documented.[46] No restricted rotation about the N–O bond of various oxamazins and related model compounds was revealed by variable-temperature NMR

(45) Kennard, O.; Watson, D. G.; Allen, F. H.; Isaacs, N. W.; Motherwell, W. D. S.; Pettersen, R. C.; Town, W. G. *Molecular Structures and Dimensions*; Cambridge Data Centre: Cambridge, U.K., 1972; Vol. A1, pp 63 (*n*-nonanoic acid hydrazide), S2 (paraffinic carbon–quaternary nitrogen).

(46) Raban, M.; Kost, D. *Tetrahedron* **1984**, *40*, 3345.

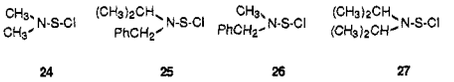
studies. However, the NMR spectrum of a 4α-methylthiamazin derivative **5d** indicates restricted rotation about the N–S bond. NMR spectra were taken at several different temperatures ranging from −40 to 60 °C. At −20 °C, a 9:1 mixture of conformational isomers (presumably **22** and **23**) was clearly discernible as seen in Figure 5. Warming the NMR sample revealed a coalescence temperature near room temperature, and at 30 °C the NMR was very sharp apparently due to the rapid rotation about the N–S bond relative to the NMR time scale.

On the other hand, the 300-MHz NMR spectrum of thiamazin salt **5b** in D_2O at room temperature was sharp and displayed nothing to indicate any type of conformational isomerism. Low-temperature studies could not be performed with D_2O as solvent. Restricted rotation at room temperature and at the temperature of biological testing (37 °C) is probably not a factor in the inactivity of this molecule because the implication is that the N–S rotational barriers for **5b** are low. However, the possibility of completely restricted rotation or a locked conformation of one form of the salt cannot be ruled out. Alternatively, if the equilibrium constant between two conformational isomers is greater than 20, the NMR signals corresponding to the minor isomer might have been too weak to be observed. Furthermore, if the minor conformer fits into the enzyme active site better than the major one, the rate of rotation might become a factor in the affinity of the β-lactam for the enzyme.

Indications are that sulfenamides have barriers to rotation around the N–S bond of 9–23 kcal/mol.[46] Acylation of the nitrogen presumably lowers the barrier, but no data for β-lactams have been reported. Computational chemistry[43,44] offers the best approach to the question of the nature of the N–S rotational barrier in thiamazins. To begin, the computational methods should be tested in regard to their ability to treat the structures of interest.

Quantum mechanical calculations[47] by the MNDO[48] method gave energy-minimized geometries for a thiamazin (Figure 3) and an oxamazin (Figure 4). The crystalline and optimized bond lengths and bond angles for the nuclei of **5e** and the model thiamazin are similar. The β-lactam nitrogen is 0.18 Å out of the plane of its three substituents in crystalline **5e**, whereas the MNDO calculation predicts this distance, *h*, to be 0.25 Å in the unsubstituted structure and 0.31 Å in the 4α-methyl structure. We have found that MNDO characteristically overestimates pyramidal character at a β-lactam nitrogen. Hence one might expect that the predicted *h* value for the oxamazin structure of 0.46 Å is on the high side, but in the correct direction relative to thiamazin. Energy minimization gives an N–S–CH2–COOH side chain conformation very similar to the starting, crystalline conformation: MNDO yields C2–N1–S–C5 and N1–S–C5–C6 torsional angles of 114° and −64°, respectively, in the 4α-methyl model (experimental 119° and −61° for **5e**). The analogous C–N–O–C and N–O–C–C torsional angles in the unsubstituted oxamazin model are predicted to be 131° and −72°, respectively.

Next consider the ability of the MNDO, MINDO/3,[49] and AM1[50,51] molecular orbital methods to predict potential energies for rotation around the N–S bond. Me_2N–S–Cl **24** is a simple model of previously reported[46] sulfenamides **25–27** and serves as another good test of the



24 **25** **26** **27**

methods. As shown in Figure 6, MNDO does quite well: the predicted rotational barrier heights for Me_2N–S–Cl are up to 12.3 kcal/mol, which is in good agreement with the reported NMR experiments[46] indicating barriers of 14.5–15.5 kcal/mol for **25–27**. The maximum barrier height is more seriously underestimated by AM1 (10.5 kcal/mol) and MINDO/3 (7.3 kcal/mol). All the MO calculations give a twofold potential with minima in the

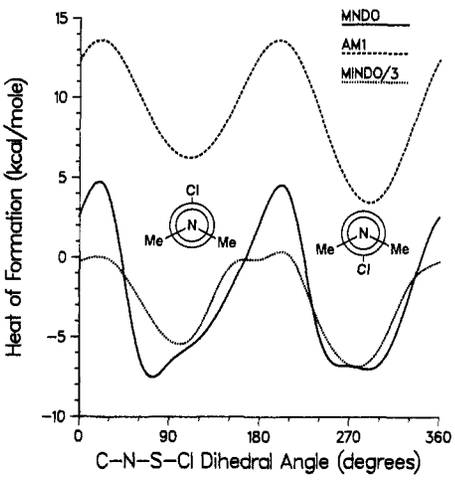


Figure 6. Rotational potential energy curves for sulfenamide **24** as predicted by semiempirical MO methods. In order to prevent inversion during the rotation process, the difference in the two C–N–S–Cl torsional angles were held constant at the optimized value predicted by each method. Other geometrical variables were free to optimize.

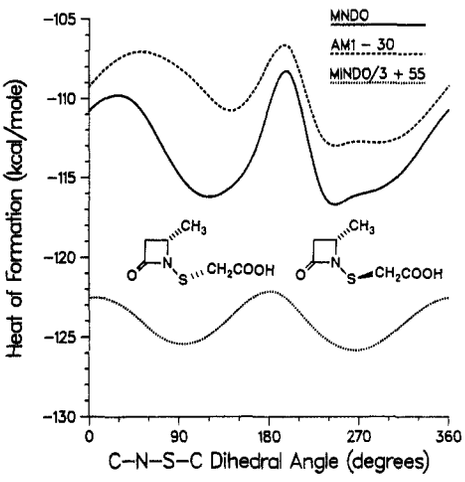


Figure 7. Rotational potential energy curves for N-SCH_2COOH-4α-CH_3-azetidin-2-one as predicted by semiempirical MO methods. Free geometry optimization was done on each rotamer, starting with an optimized geometry predicted by that MO method. For purposes of having all curves to fit on this same graph, the constants shown in the legend were subtracted from or added to the actual computed heats of formation for AM1 and MINDO/3, respectively. AM1 predicts ΔH_f values to be less negative than MNDO, whereas MINDO/3 predicts them to be much more negative. Rotations about the S–CH_2 and CH_2–COOH bonds were permitted during N1–S rotation; the conformation of the N–R side chain changes as it sweeps past the 4α-Me group.

(47) Boyd, D. B. *Drug. Inf. J.* **1983**, *17*, 121.
(48) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899, 4907.
(49) Bingham, R. C.; Dewar, M. J. S.; Lo, D. H. *J. Am. Chem. Soc.* **1975**, *97*, 1285, 1302, 1307.
(50) Dewar, M. J. S.; Zoebisch, E. V.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(51) Stewart, J. J. P. *Quantum Chem. Prog. Exchange Bull.* **1985**, *5*, 133; QCPE Program 455.

two gauche regions. This agrees with available evidence.[46]

Corresponding to the NMR experiments on **5d**, the three semiempirical MO methods were used to determine the rotational potential in a 4α-methylthiamazin analogue. The acylamino side chain and methyl ester of **5d** were replaced by hydrogens in order to keep computer times low; these substitutions should have no significant effect on N–S rotation. The preferred conformations are gauche (Figure 7) and thus compatible with the crystalline-state conformation of thiamazin **5e**. Also, a gauche conformation is appropriate for mimicking the pharmacophore of Δ^3-cephalosporins and COOH-equatorial penicillins (see ϕ_1 values in Table V). The highest N–S rotational barriers are 8.6 kcal/mol by MNDO and 5.4 kcal/mol by AM1 (Figure 7). Supposing that MNDO systematically underestimates the rotational barrier by 20% (see above), then the actual N–S barrier in the model thiamazin would be about 11 kcal/mol. As expected from other sulfenamides compared to acylated sulfenamides,[46] a lowered barrier is consistent with the less pyramidal geometry and greater lone-pair delocalization at the β-lactam nitrogen compared to an amine nitrogen. On the basis of previously reported data,[46] freezing out the N–S rotamers in the model thiamazin would be possible only at temperatures lower than −20 °C. Thus, the NMR spectrum of **5d** at room temperature is indeed consistent with relatively free rotation about the N–S bond.

In conclusion, several factors were considered to explain the remarkable differences in the biological activity of the oxamazins and thiamazins. The differences in the electronegativity of the oxygen and sulfur substituents were reflected in the difference in the IR stretching frequency of the oxamazins and thiamazins, but not significantly in their susceptibility toward nucleophilic attack at the β-lactam carbonyl (hydrolysis). The tested thiamazin was stable under in vitro conditions, but competitive attack at the sulfur under microbiological conditions to generate the inactive N-unsubstituted β-lactam cannot be completely ruled out. Smaller β-lactam antibiotics pass through porins in the outer membrane of Gram-negative bacteria more efficiently than large, rigid ones.[52,53] Although it is possible that the more compact structure of oxamazins allows them to reach higher concentrations in the periplasm compared to thiamazins, this aspect is not seen as a major distinction for two monocyclics. Restricted N–S rotation in the biologically tested thiamazin does not account for its lack of activity. An X-ray structure determination, molecular graphics, and quantum mechanical calculations suggest that a plausible explanation for thiamazin's lack of biological activity is a poor fit in the active site of the appropriate bacterial enzymes due to the molecule's inherent bond lengths and bond angles.

Experimental Section

The syntheses and complete characterization of all of the compounds described have been reported elsewhere.[13,14,17,18]

A clear, colorless regular parallellepiped crystal of thiamazin **5e** (recrystallized from $CHCl_3$/hexane) was mounted on a glass fiber in a random orientation on an Enraf-Nonius CAD4 diffractometer equipped with a graphite crystal, incident beam monochromator. Cell constants were obtained from the setting angles of 25 reflections in the range $20° < |2\theta| < 30°$. The orthorhombic cell parameters and calculated volume are presented in Table VI (supplementary material). The observed density (flotation in CCl_4/hexanes) indicated $Z = 4$. The systematic absences $h00$ ($h = 2n$), $0k0$ ($k = 2n$), and $00l$ ($l = 2n$) and subsequent least-squares refinement determined the space group to be $P2_12_12_1$ (No. 19). A total of 2844 $+h,+k,+l$ reflections were collected, of which 2816 were not systematically absent. The intensities of four representative reflections remeasured every 60 min of X-ray exposure remained constant within experimental error throughout data collection. Lorentz and polarization corrections were applied to the reduced data.[54-57] The standard deviation on intensities, $\sigma(F_o^2)$, is defined as $\sigma^2(F_o^2) = [S^2*(C + R^2B) + (pF_o^2)^2]/(LP)^2$, where S is the scan rate, C is the total integrated peak count, R is the ratio of scan time to background counting time (=2.0), B is the total background count, LP is the Lorentz–polarization factor, and the parameter p is a factor introduced to down weight intense reflections. Here p was set to 0.060. No absorption correction was made, the maximum possible error neglected thereby being 5% in intensity.

The structure was solved by direct methods (MULTAN) and developed by Fourier techniques. Hydrogen atoms were included in the refinement, but restrained to ride on the atom to which they are bonded. Hydrogen atoms were assigned isotropic temperature factors 1.0 Å^2 greater than the isotropic temperature factor of the heavy atom to which they are bonded. Sufficient data were available to refine 13 atoms with anisotropic thermal parameters. Those chosen include the central four-membered ring and proceeded outward from it. The model converged by full-matrix least-squares refinement on F to $R_1 = \sum||F_o| - |F_c||/\sum|F_o| = 0.078$ and $R_2 = (\sum w||F_o| - |F_c||^2/\sum wF_o^2)^{1/2} = 0.10884$. Least-squares weights were calculated as $w = 1/\sigma^2(F_o)$. The alternate enantiomer converged to $R_2 = 0.10902$, allowing rejection of this enantiomer at approximately the 0.025 level, in confirmation of the stereochemistry at C3 required by the synthesis of the compound.[17,18] The highest peak in the final difference Fourier was near atoms of the tertiary butyl group that terminates the substituent on N1. The residuals showed no unusual trends. The complete lists of atomic positions and thermal parameters, bond lengths, and angles are provided in Tables VII–X (supplementary material).

Computational chemistry experiments were run on a VAX cluster consisting of two 8600s and one 11/785. Modgraph GX1000 and Evans and Sutherland PS340 graphics terminals were used to run SYBYL. MOPAC was run with default options independently of SYBYL. Ball-and-stick structure drawings were produced with ORTEP[58] as adapted by Molecular Design Ltd., San Leandro, CA. Line drawings were produced with TELL-A-GRAF by Integrated Software Systems Corp., San Diego, CA. Chemical structure drawings were produced on a Macintosh computer with ChemDraw software by S. Rubenstein, Cambridge Scientific Computing, Inc., Cambridge, MA.

The hydrolysis rates were determined by following loss of parent compound, **4b** or **5b**, by HPLC. The system consisted of a Beckman 332 chromatograph, a Rheodyne 7125 injection valve fitted with a 20-μL loop, a Waters 450 variable-wavelength detector, and a Hewlett-Packard 3390A integrator. The stationary phase was a 4.6 × 250 mM Zorbax ODS (Dupont) reverse-phase column and the detector set at 254 nm. The flow rate was 1 mL/min. The mobile phase contained acetonitrile/0.025 M NH_4OAc (16:84, v/v). The rate of formation of 8 was determined on the same system. In this case the mobile phase contained acetonitrile/0.025 M NH_4OAc (24:76, v/v).

All rates were determined at constant pH on a pH–stat consisting of a Metrohm 655 dosimat, 614 implusomat, and a 632 pH

(52) Yoshimura, F.; Nikaido, H. *Antimicrob. Agents Chemother.* **1985**, *27*, 84.
(53) See also: Boyd, D. B.; Ott, J. L. *J. Antibiot.* **1986**, *39*, 281.
(54) Frenz, B. A. In *Computing in Crystallography*; Schenk, H., Olthof-Hazelkamp, R., vanKonigsveld, H., Bassi, G. C., Eds.; Delft University Press: Delft, The Netherlands, 1978; pp 64–71.
(55) Scattering factors are from Cromer, D. T.; Waber, J. T. *International Tables for X-Ray Crystallography*; The Kynoch Press: Birmingham, U.K., 1974; Vol. IV, Table 2.2B.
(56) Anomalous dispersion effects were included in F_c: Ibers, J. A.; Hamilton, W. C. *Acta Crystallogr.* **1964**, *17*, 781–782.
(57) Values for Δ(f′) and Δ(f″) were from Cromer, D. T. *International Tables for X-Ray Crystallography*; The Kynoch Press: Birmingham, U.K., 1974; Vol. IV, Table 2.3.1.
(58) Johnson, C. K. *ORTEP: A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations*; Oak Ridge National Laboratory Report 3794; Oak Ridge, TN, 1965.

meter fitted with a combination electrode. The pH was maintained by the addition of NaOH. Experiments were carried out at 35 °C. The ionic strength was adjusted to 0.5 with potassium chloride. Initial β-lactam concentrations were 1×10^{-4} M.

Acknowledgment. We gratefully acknowledge the support of the National Institutes of Health and Eli Lilly and Co. to the University of Notre Dame and a Reilly Fellowship for S.R.W. We also thank Dr. Lowell D. Hatfield, Dr. Robin D. G. Cooper, Byron W. Daugherty, John L. Occolowitz, David W. Smith, and Dr. John L. Ott of Lilly Research Laboratories for their interest and help. Kathleen Peterson obtained the 300-MHz NMR spectra with a NMR spectrometer made available by grants from the NIH and the University of Notre Dame. We are grateful to James J. P. Stewart for making the MOPAC program available through the Quantum Chemistry Program Exchange, Bloomington, IN.

Registry No. **4a**, 93712-30-2; **4b**, 93712-27-7; **4c**, 106139-91-7; **5a**, 102652-83-5; **5b**, 102652-84-6; **5c**, 102652-85-7; **5d**, 100239-03-0; **5e**, 102652-81-3; **5f**, 106139-92-8.

Supplementary Material Available: Table VI–X, unit cell parameters, fractional atomic coordinates, bond lengths, bond angles, and anisotropic temperature factors, respectively (5 pages). Ordering information is given on any current masthead page.

α-Methylproline-Containing Renin Inhibitory Peptides: In Vivo Evaluation in an Anesthetized, Ganglion-Blocked, Hog Renin Infused Rat Model

Suvit Thaisrivongs,* Donald T. Pals, Judy A. Lawson, Steve R. Turner, and Douglas W. Harris

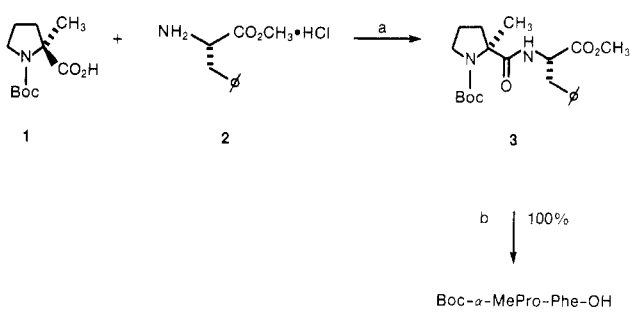
Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, Michigan 49001. *Received August 18, 1986*

A structure–activity analysis of peptides containing backbone C_α-methyl modification at the P_4 site of the angiotensinogen sequence led to the discovery of potent renin inhibitors with apparent in vitro metabolic stability. Boc-α-MePro-Phe-His-Leuψ[$CHOHCH_2$]Val-Ile-Amp dicitrate (Va) is a potent inhibitor of human plasma renin with an IC_{50} value of 1.8 nM. This peptide was shown not to be degraded in vitro by chymotrypsin, elastase, pepsin, and a rat liver homogenate preparation. It is also a potent inhibitor of hog renin with an IC_{50} value of 1.6 nM and was shown to elicit in vivo activity and cause dose-dependent hypotensive responses when given intravenously to anesthetized ganglion-blocked, hog renin infused rats.

The renin–angiotensin system has been implicated in several forms of hypertension.[1] Renin is an aspartyl protease that is produced mainly in the juxtaglomerular apparatus of the kidney.[2] It is a highly specific proteolytic enzyme and cleaves the circulating α-globulin angiotensinogen, produced by the liver, to form the decapeptide angiotensin I.[3] The N-terminal sequence of human angiotensinogen is shown in Figure 1, the cleavage site being the peptidic bond between amino acids 10 and 11.[4] Angiotensin I has no known biological activity, but it is converted to the octapeptide angiotensin II by the angiotensin-converting enzyme present in lungs and other organs, as a result of the removal of the C-terminal dipeptide histidylleucine. Angiotensin II is a very potent vasoconstrictor and also stimulates the release of aldosterone from the adrenal gland. This mineralocorticoid induces sodium and water retention, contributing to an increase in blood pressure.[3]

The antihypertensive activity of inhibitors of converting enzyme is not clear mechanistically due to its involvement in the kinin system. Renin, however, is an enzyme of high substrate specificity and inhibitors of renin should affect only the renin–angiotensin system.[5] Interest in the blockade of renin has led to rapid development of potent inhibitors based on the angiotensinogen sequence. The most successful approach has been based upon the concept of a transition-state analogue[6] of the amide hydrolysis.

Scheme I. Synthesis of Dipeptide Subunit **4**[a]



[a] (a) Et_3N, DCC, HOBT, CH_2Cl_2; (b) NaOH, H_2O, THF; aqueous HCl.

Modifications at the cleavage site to mimic the tetrahedral species have generated analogues of the minimum substrate with high inhibitory potency in vitro.[7]

Many renin inhibitors have been shown to lower blood pressure during intravenous infusion. However, blood pressure usually recovers within minutes after stopping an infusion.[8] Efforts to obtain renin inhibitors with longer duration of action have continued to make progress.[9,10]

(1) Davis, J. O. *Circ. Res.* **1977**, *40*, 439. Swales, J. D. *Pharmacol. Ther.* **1979**, *7*, 172.
(2) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313.
(3) Ondetti, M. A.; Cushman, D. W. *Annu. Rev. Biochem.* **1982**, *51*, 283.
(4) Skeggs, L.; Lentz, K.; Kahn, J.; Hochstrasser, H. *J. Exp. Med.* **1968**, *128*, 13.
(5) Haber, E. *N. Engl. J. Med.* **1984**, *311*, 1631.
(6) Pauling, L. *Am. Sci.* **1948**, *36*, 58. Jencks, W. P. *Current Aspects of Biochemical Energetics*; Kaplan, N. O., Kennedy, E. P., Eds.; Academic: New York, 1966; p 273. Wolfenden, R. *Nature (London)* **1969**, *223*, 704; *Acc. Chem. Res.* **1972**, *5*, 10.
(7) Szelke, M.; Jones, D. M.; Atrash, B.; Hallett, A.; Leckie, B. J. *Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; p 579. Boger, J. *Ibid.*, p 569.
(8) Szelke, M.; Leckie, B. J.; Tree, M.; Brown, A.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Lever, A. F. *Hypertension* **1982**, *4* (Suppl. II), II-59. Blaine, E. H.; Schorn, T. W.; Boger, J. *Ibid.* **1984**, *6*, I-111. Wood, J. M.; Forgiarini, P.; Hofbauer, K. G. *J. Hypertension* **1983**, *1* (Suppl. II), 189. Leckie, B. J.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Szelke, M.; Tree, M. *Scott. Med. J.* **1984**, *29*, 125.
(9) Wood, J. M.; Gulati, N.; Forgiarini, P.; Fuhrer, W.; Hofbauer, K. *Hypertension* **1985**, *7*, 797. DeClaviere, M.; Cazaubon, C.; Lacour, C.; Nisato, D.; Gagnol, J. P.; Evin, G.; Corvol, P. *J. Cardiovasc. Pharmacol.* **1985**, *7* (Suppl. 4), S58.

0022-2623/87/1830-0536$01.50/0