Synthesis, Hydrolysis Rates, Supercomputer Modeling, and Antibacterial Activity of Bicyclic Tetrahydropyridazinones

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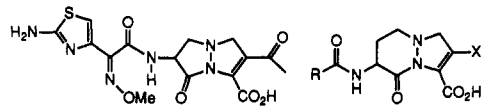
Bicyclic tetrahydropyridazinones, such as **13**, where X are strongly electron-withdrawing groups, were synthesized to investigate their antibacterial activity. These δ-lactams are homologues of bicyclic pyrazolidinones **15**, which were the first non-β-lactam-containing compounds reported to bind to penicillin-binding proteins (PBPs). The δ-lactam compounds exhibit poor antibacterial activity despite having chemical reactivity comparable to the γ-lactams. Molecular modeling, based on semiempirical molecular orbital calculations on a Cray X-MP supercomputer, predicted that the reason for the inactivity is steric bulk hindering high affinity of the compounds to PBPs, as well as high conformational flexibility of the tetrahydropyridazinone ring hampering effective alignment of the molecule in the active site. Subsequent PBP binding experiments confirmed that this class of compound does not bind to PBPs.

Recently we described a new class of synthetic γ-lactam antibacterial agents, the bicyclic pyrazolidinones, represented by **15b** (LY186826).[1] These compounds were

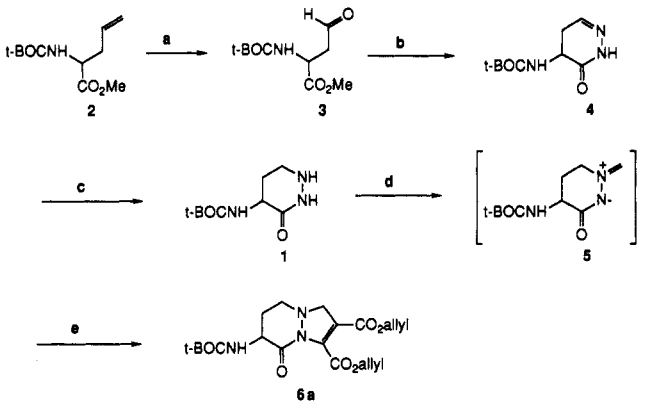


15b (LY186826) **13**

synthesized as part of a program aimed at preparing γ-lactam mimics of the well-studied β-lactam antibiotics. The bicyclic pyrazolidinones were found to exhibit broad-spectrum antibacterial activity against a variety of clinically important pathogens. It is noteworthy that the mechanism of action of the compounds was shown to be the same as that of the β-lactam antibiotics.[2] The β- and γ-lactam antibacterial agents act by binding to and inhibiting the action of several transpeptidase/carboxypeptidase enzymes (the so-called penicillin-binding proteins or PBPs) involved in the synthesis of bacterial cell wall peptidoglycan.[3] A good "fit" at the active site of the enzymes and a chemically reactive lactam ring are both necessary for acylation and inhibition of the PBPs.

Bicyclic pyrazolidinones synthesized in our laboratory were the first non-β-lactam-containing compounds reported to bind to PBPs.[2] In addition, the base-catalyzed hydrolysis rate of the pyrazolidinone lactam bond was found to be comparable to or greater than that of the β-lactam moiety of penicillins or cephalosporins.[4] Thus the criteria of good "fit" and sufficient chemical reactivity were met by **15** and its various analogues. The surprisingly high chemical reactivity of the bicyclic pyrazolidinone lactam suggested that a homologous bicyclic tetrahydropyridazinone (**13**) might also be sufficiently reactive to exhibit antibacterial activity. Herein, we report on the synthesis, hydrolysis rates, molecular modeling, and antibacterial evaluation of these "homo-pyrazolidinones".

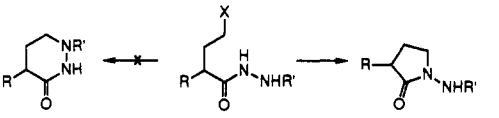
Scheme I[a]



[a] (a) O_3, CH_2Cl_2, then Me_2S, 96%; (b) hydrazine hydrate, EtOH; (c) $NaBH_3CN$, HOAc, MeOH, 36% from **3**; (d) H_2CO, CH_3CN; (e) diallyl acetylenedicarboxylate, $ClCH_2CH_2Cl$, reflux, 17%.

Results and Discussion

Synthesis. We envisioned the synthesis of the target bicyclic tetrahydropyridazinones to proceed via key intermediate **1** (Scheme I), utilizing chemistry previously developed for the preparation of bicyclic pyrazolidinones. A number of approaches to **1** were considered. The work of Taylor[5] and Barlos[6] suggested that cyclization of an acyl hydrazide, e.g. **16**, would preferentially give rise to a five-membered ring product rather than the desired six-membered ring.



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Tetrahydropyridazinone **1** was ultimately derived from allylglycine as outlined in Scheme I. Ozonolysis of the *t*-BOC protected methyl ester **2** gave rise to aldehyde **3**,[7] which was condensed with hydrazine in ethanol to provide imine **4**. Presumably hydrazine reacts with **3** first at the aldehyde moiety followed by ring closure to give the de-

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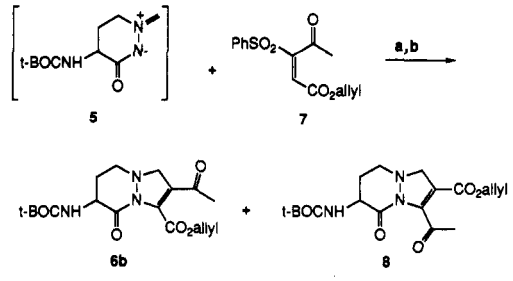
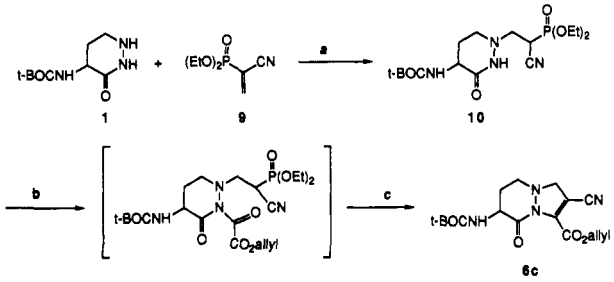
Scheme II[a]



[a] (a) $ClCH_2CH_2Cl$, reflux; (b) *N*-methylmorpholine, room temperature, 21%.

Scheme III[a]

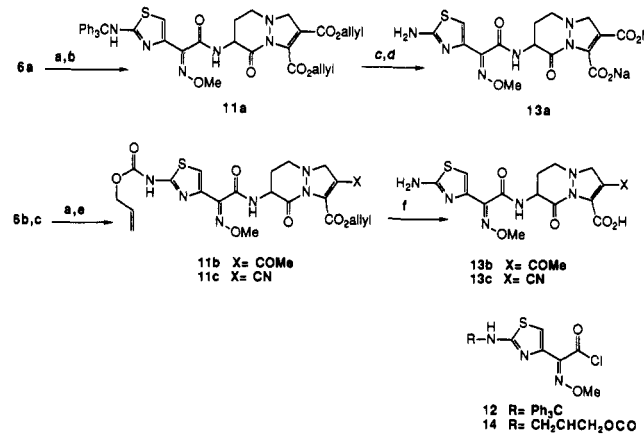


[a] (a) MeOH, room temperature; (b) $ClCOCO_2allyl$, *i*-Pr_2NEt, CH_2Cl_2; (c) *i*-Pr_2NEt, CH_2Cl_2, 11% from 1.

sired six-membered ring product. Reduction of the imine with sodium cyanoborohydride provided tetrahydropyridazinone **1** in good overall yield.

We envisioned the conversion of **1** into the target bicyclic nuclei **6a–c** to be accomplished by utilizing three different routes: cycloaddition of the requisite azomethine imine **5** with an acetylene,[1c] or vinyl sulfone,[8] as well as via the intramolecular Horner–Emmons cyclization method of Ternansky.[9] We were pleased to find that azomethine imine **5** could be generated in situ and that it displayed reactivity comparable to the analogous pyrazolidinium ylides.[1,8] Thus, treatment of **1** with aqueous formaldehyde followed by heating to reflux in 1,2-dichloroethane generated ylide **5**, which was trapped in situ with diallyl acetylenedicarboxylate, giving rise to bicyclic tetrahydropyridazinone **6a** (Scheme I). Ylide **5** similarly underwent 1,3-dipolar cycloaddition with vinyl sulfone **7**, followed by base-catalyzed elimination of benzene sulfinic acid, to give bicyclic tetrahydropyridazinone **6b** in highly regioselective fashion (Scheme II). A small amount (<10%) of the isomeric cycloadduct **8** was evident in the NMR of the crude reaction mixture; however, this material was not isolated. The assignment of structure **6b** to the major cycloadduct is supported by the NMR chemical shifts observed for the $C(O)CH_3$ (δ 2.23) and $CO_2CH_2CHCH_2$ (δ 4.86) resonances which are in excellent agreement with the analogous shifts observed for the corresponding bicyclic pyrazolidinone nucleus of **15b** at δ 2.25 and 4.84. The preparation of the cyano-substituted nucleus **6c** via the methodology of Ternansky[9] is outlined in Scheme III. Treatment of **1** with known vinyl phosphonate **9**[10] provided conjugate adduct **10**. Acylation of **10** with allyl oxalyl chloride[11] in methylene chloride containing 3 equiv of

Scheme IV[a]



[a] (a) 3 N HCl(g) in HOAc; (b) **12**, CH_2Cl_2, *N*-methylmorpholine 62%; (c) TFA, Et_3SiH; (d) $Pd(OAc)_2$, Ph_3P, sodium 2-ethylhexanoate, 60%; (e) **14**, CH_2Cl_2, *N*-methylmorpholine, 51% for **11b**, 57% for **11c**; (f) $(Ph_3P)_2PdCl_2$, *n*-Bu_3SnH, CH_2Cl_2, 56% for **13b**, 90% for **13c**.

diisopropylethylamine, followed by intramolecular ring closure, gave rise to the desired bicyclic nucleus **6c**.

It remained for us to append an appropriate acylamino side chain onto the bicyclic tetrahydropyridazinone nuclei and remove any protecting groups (Scheme IV). The *t*-BOC groups on **6a–c** were hydrolyzed most efficiently with a solution of 3 N anhydrous hydrochloric acid in glacial acetic acid. The amine salt derived from **6a** was acylated with the acid chloride of 2-(tritylamino)-α-methoximino-4-thiazoleacetic acid (**12**) in the presence of *N*-methylmorpholine to give **11a**. The trityl group was hydrolyzed with neat trifluoroacetic acid and triethylsilane as a cation scavenger. Palladium(0)-catalyzed cleavage of the allyl esters[12] afforded bis-carboxylate analogue **13a**. The amine salts derived from **6b,c** were acylated with the acid chloride of 2-[[(allyloxy)carbonyl]amino]-α-methoximino-4-thiazoleacetic acid[1c] (**14**) in the presence of *N*-methylmorpholine to give **11b,c**, respectively. Palladium(0)-catalyzed cleavage of the allyl esters afforded the corresponding acids **13b,c**.

Antibacterial Activity. Bicyclic tetrahydropyridazinones **13a–c** were tested for activity against a battery of 36 Gram-positive and Gram-negative bacteria of clinical relevance and found to be inactive at concentrations up to 128 μg/mL.[13] These data suggested that perhaps the bicyclic tetrahydropyridazinones are not reactive enough to acylate the target PBPs and/or they do not "fit" the active site of these enzymes well. The first possibility is addressed in the next section.

Hydrolysis Rates. The acylating ability of the δ-lactam ring in bicyclic tetrahydropyridazinones was compared to

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(9) Ternansky, R. J.; Draheim, S. E. *Tetrahedron Lett.* **1990**, *31*, 2805.

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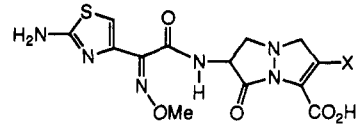
(13) Counter, F. T.; Wu, C.-Y. E. (Lilly Research Laboratories), unpublished data. The organisms tested were *Staphylococcus aureus* X1.1, V41, X400, S13E; *Staphylococcus epidermidis* 270, 222; *Streptococcus pyogenes* C203; *Streptococcus pneumoniae* Park; *Enterococcus faecium* X66; *Eeterococcus faecalis* 2041; *Haemophilis influenzae* C.L., 76; *E. coli* N10, EC14, TEM; *Klebsiella pneumoniae* X26, KAE, X68; *Enterobacter aerogenes* C32, EB17; *Enterobacter cloacae* EB5, 265A; *Salmonella typhi* X514; *Salmonella typhimurium* 1335; *Pseudomonas aeruginosa* X528, X239, PS18, PS72; *Serratia marcescens* X99, SE3; *Shigella sonnei* N9; *Morganella morganii* PR15; *Providencia alcalifacius* PR33; *Providencia rettgeri* C24; *Citrobacter freundii* CF17; and *Acinetobacter acetocoaelicus* AC12.

Table I. Pseudo-First-Order Rate Constants, k (HPLC), for the Hydrolysis of the δ- and γ-Lactams[a]

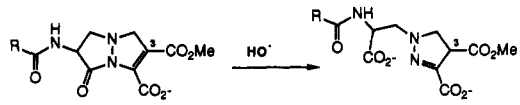


15a X= CO_2H
15b X= COMe
15c X= CN

compd	ring system	C-3 subst	pH 9 k, h^{-1}	pH 9 $T_{1/2}$, h	pH 10 k, h^{-1}	pH 10 $T_{1/2}$, h
13c	[4.3.0]	CN	1.75 ± 0.08	0.40		
15c	[3.3.0]	CN	1.73 ± 0.16[b]	0.40		
13b	[4.3.0]	COMe	3.16 ± 0.09	0.22		
15b	[3.3.0]	COMe	0.724 ± 0.04[b]	0.96		
13a	[4.3.0]	CO_2H	0.0144[c]	48.0	0.146[c]	4.75
15a	[3.3.0]	CO_2H			0.324 ± 0.01[b]	2.14
penicillin V	[2.3.0]				0.248[b]	

[a] pH stat, μ = 0.5, 35 °C. [b] Values taken from ref 4; experimental details are described therein. [c] Single determination. Rates were measured at pH 10 for the X = CO_2H derivatives due to their lower reactivity. As illustrated by the data, rates at pH 9 are about one-tenth those at pH 10.

that of the γ-lactam ring in bicyclic pyrazolidinones by measuring their chemical reactivity with hydroxide ion. We have previously established that hydroxide ion reacts selectively with bicyclic pyrazolidinones at the lactam linkage.[4] Furthermore, a correlation involving hydrolysis rate, antibacterial activity, and Hammett σ_p values for the C-3 substituent of bicyclic pyrazolidinones was observed,[4] which is analogous to that observed for cephalosporins[14-17] and carbacephalosporins.[18] The hydrolysis rates of the three tetrahydropyridazinones in question were found to be comparable to their analogous pyrazolidinones and other active β-lactam antibiotics (Table I). Thus the homologues should be chemically reactive enough to acylate bacterial cell wall synthesis proteins without being so reactive as to be unstable.



Molecular Modeling. Why do bicyclic tetrahydropyridazinones (the δ-lactams) have poor antibacterial activity despite having chemical reactivity comparable to the corresponding biologically active bicyclic pyrazolidinones (the γ-lactams)? To try to answer this question, information is needed about the three-dimensional structure of the [4.3.0] ring system. X-ray crystallography could be very helpful in this regard, but requires the existence of suitable crystals. Computational chemistry experiments, on the other hand, can readily provide not only structural data but also conformational energies.

Three approaches to computational modeling can be considered.[16,19-22] The first, requiring the least computer time, is molecular mechanics. However, for a system such as the bicyclic tetrahydropyridazinones, so many force field parameters would have to be developed or guessed that this approach is not very efficient. The second approach is to use ab initio molecular orbital calculations.[23] These have the generality to treat bicyclic tetrahydropyridazinones, but require excessive computing time for a thorough conformational analysis of a molecule this large. The third approach, one which we adopt here, is to use semiempirical molecular orbital calculations.[24] Modern methods for these calculations are robust and reliable enough to treat complex heterocycles, yet are not inordinately demanding of computer time. With the availability of state-of-the-art supercomputing, these computational chemistry experiments become even easier.[20]

The MNDO molecular orbital method was selected because prior calculations have shown it to work generally well.[22,24-26] MINDO/3, another older semiempirical method,[27] is better for reproducing the amide C–N bond length of a β-lactam ring,[28,29] but MNDO is usually better at reproducing the pyramidal character of the bridgehead nitrogen in bicyclic β-lactams. To simplify the modeling, only the 6(*S*) diastereomer was considered for modeling because of its closer analogy to the biologically active [3.3.0] system and to penicillins and cephalosporins. For modeling, the *syn*-(2-amino-4-thiazolyl)methoximinoacetyl (ATMO) side chain was replaced by formyl, and the C-3 substituent was taken to be CO_2Me. These assumptions are acceptable because we are mainly interested in learning about the conformational characteristics of the nucleus with its six-membered tetrahydropyridazinone ring.

At the start of this work a number of conformational possibilities could be envisioned for the [4.3.0] ring system.

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(16) Boyd, D. B. *Drug Inf. J.* **1983**, *17*, 121.
(17) Boyd, D. B. *J. Med. Chem.* **1984**, *27*, 63.
(18) Blaszczak, L. C.; Brown, R. F.; Cook, G. K.; Hornback, W. J.; Hoying, R. C.; Indelicato, J. M.; Jordan, C. L.; Katner, A. S.; Kinnick, M. D.; McDonald, J. H., III; Morin, J. M., Jr.; Munroe, J. E.; Pasini, C. E. *J. Med. Chem.* **1990**, *33*, 1656.
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(29) Allen, N. E.; Boyd, D. B.; Campbell, J. B.; Deeter, J. B.; Elzey, T. K.; Foster, B. J.; Hatfield, L. D.; Hobbs, J. N., Jr.; Hornback, W. J.; Hunden, D. C.; Jones, N. D.; Kinnick, M. D.; Morin, J. M., Jr.; Munroe, J. E.; Swartzendruber, J. K.; Vogt, D. G. *Tetrahedron* **1989**, *45*, 1905.

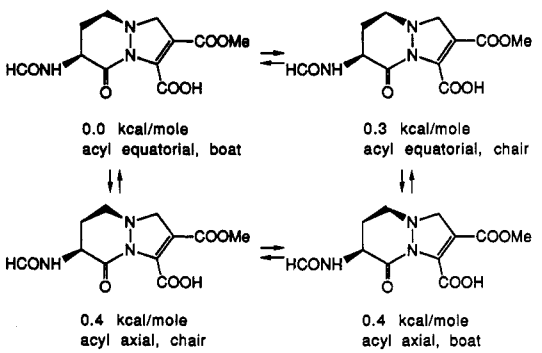


Figure 1. Relative conformational energies of the model of bicyclic tetrahydropyridazinones as computed from the MNDO heats of formation. The [4.3.0] ring system is shown in the four conformers found by energy minimization. The ΔH_f of the most stable conformer is −190.7 kcal/mol.

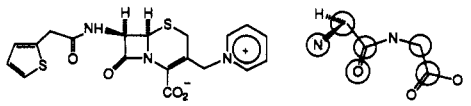
The initial strategy for generating these was to use a ring-making algorithm which could generate all conceivable conformers. However, our use of the SEARCH routine[30,31] in the SYBYL molecular modeling software package[32,33] generated fewer distinct conformers than expected. Hence an alternate strategy of building models using the usual chair, boat, and twist conformers of a six-membered ring was employed. The standard fragment library and standard bond lengths and angles in SYBYL were used in model building. The possible conformers of the tetrahydropyridazinone ring were fused to the five-membered pyrazolo ring, which is relatively flat and inflexible.

The models from SYBYL were then subjected to full energy minimization of all geometrical variables with the MNDO method in the computer program MOPAC.[34,35] In order to assure the reliability of these minimizations, they were run with the tight self-consistent field-convergence and energy-minimization criteria specified by the keywork PRECISE in the program.[28] The MOPAC calculations were run on the Cray X-MP/48 supercomputer at the National Center for Supercomputing (NCSA, University of Illinois, Urbana—Champaign). The computations took a total of 10 h of Cray CPU time, approximately one-tenth the time it would have taken to do the same computations on a VAX 8800. The results of the computations were transferred back to the Lilly Research VAX Cluster, postprocessed with in-house software,[36] and analyzed and visualized with SYBYL.

The [4.3.0] ring system is found to be in a dynamic equilibrium between four conformers. These are shown schematically in Figure 1 and graphically in Figure 2. (Torsional angles and other geometrical details can be obtained from the atomic coordinates in the supplementary material.) The energies of the four conformers are within 0.4 kcal/mol of each other, so a mixture of conformers can be expected to exist at room temperature. The calculated maximum barrier height for inversion of configuration at the amine-like bridgehead nitrogen is 8.5 kcal/mol, while the inversion barrier for the δ-lactam nitrogen would be much less. The barrier to conversion between the chairlike and boatlike conformers was not computed, but is expected to be comparable to the 10 kcal/mol energy required to flip between chair and boat cyclohexane.[37] Thus conformational change should occur readily at room temperature.

Analysis of X-ray data for β-lactam antibacterial agents has previously shown that a separation of 3.0–3.6 Å between carboxylate carbon and the lactam carbonyl carbon is associated with ability to inhibit cell growth.[38,39] In each of the four energy-minimized conformers of the bicyclic tetrahydropyridazinones, the distance between these carbons is close to 3.2 Å. This is within the range expected for biologically active lactam antibiotics, so these structures should meet at least this one requirement for being recognizable by the active site of bacterial transpeptidases.[40-43] Yet the compounds are inactive; why?

To address this question further, we need to consider the steric properties of the bicyclic tetrahydropyridazinones and compare their three-dimensional structures to that of a representative, biologically active cephalosporin. For the latter, the atomic coordinates for cephaloridine (17)[44,45] were retrieved via SYBYL from the



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Cambridge Crystallographic Database.[46] An appropriate spatial alignment of the four tetrahydropyridazinone conformers and the cephalosporin was achieved as follows. SYBYL's multifitting algorithm[47] was used to perform a root-mean-square fit of the models, which were held as rigid entities for this step. The fitting was done with harmonic potential functions having a force constant[48] of 5 kcal/Å^2 between the six pairs of circled atoms (18). These atoms are associated with the pharmacophore of cephalosporins and other lactam antibacterial agents. In effect, the procedure applies global translations and ro-

(30) Motoc, I.; Dammkoehler, R. A.; Mayer, D.; Labanowski, J. *Quant. Struct.-Act. Relat.* **1986**, *5*, 99.
(31) The parameters tried in the SYBYL SEARCH routine were a torsional angle increment of 1° for four rotatable bonds, a bond length tolerance of 0.3 Å, and a bond angle variance of 10°.
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(48) Although not critical for the present exercise, a force constant of 5 kcal/Å^2 has been found in our experience to achieve a good overlapping of flexible molecules in SYBYL without introducing unrealistic geometrical distortions (such as are obtained if the default force constant of 20 is used). In the modeling done here, the fitting was done with frozen structures, so the exact value of the force constant does not matter, but 5 kcal/Å^2 gives a good fit in a reasonable number of iterations.

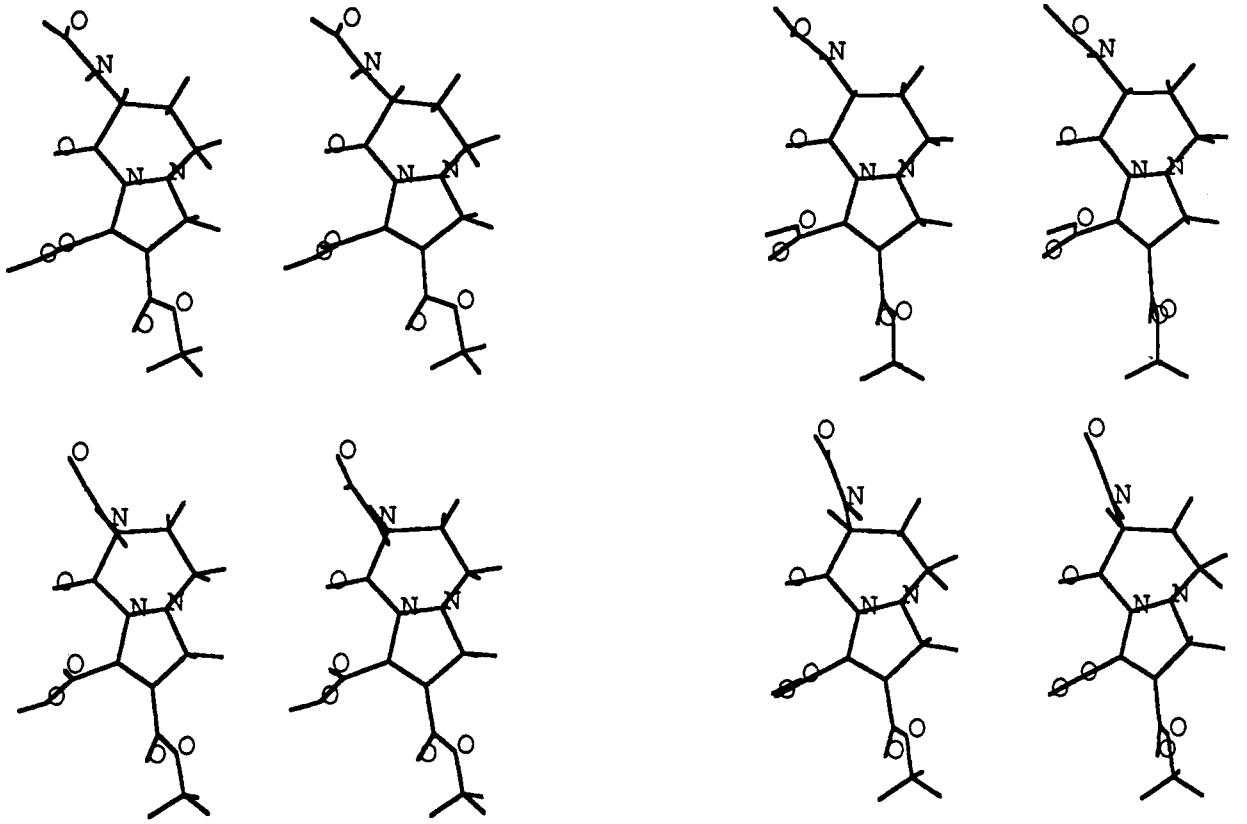

Figure 2. Stereo view of the four low-energy conformers of the tetrahydropyridazinone models with 6-(formylamino) and 2-(methoxycarbonyl) side chains. Heteroatoms are labeled. The layout is the same as in Figure 1. Clockwise from upper left, the *h* values (distance the δ-lactam nitrogen is above the plane of its three substituents) are +0.26, −0.28, −0.26, and +0.29 Å. The δ-lactam C–N bond length is overestimated at ca. 1.45 Å in each conformer by MNDO.

tations to provide the best geometrical congruence of the selected atoms in all four [4.3.0] conformers and the cephalosporin simultaneously.

As seen in Figure 3, the four tetrahydropyridazinone conformations show expected differences from the cephalosporin. Nevertheless, the fitting procedure achieved very good overlaps of the lactam amide, carboxylate, and side-chain amide functional groups, which are important to biological activity.

Molecular volumes can be computed on the basis of the "pseudo electron density" contour[49] surrounding a molecule, which SYBYL takes as an approximation to the van der Waals surface. Logical operations were done in SYBYL to form unions, intersections, and differences of the volume maps (Figure 3). The two resulting volume maps reveal that steric bulk of the tetrahydropyridazinone ring partially coincides with that occupied by a methyl group on the C-7 position of cephalosporins. This suggests that the bicyclic tetrahydropyridazinones may have a problem similar to that of the inactive 7-methylcephalosporins (and 6-methylpenicillins). In other words, the tetrahydropyridazinone ring, regardless of conformation, occupies a region of space known to be incompatible with good biological activity.

Even if a less stable conformation of the tetrahydropyridazinone ring can avoid having molecular volume impinge on the region of space apparently required for productive binding to the receptor site, another problem is possible with the new series of compounds. Protein crystallography suggests that penicillin-recognizing proteins use hydrogen bonding between a β-strand and the acylamino side chain of the ligand to help anchor the latter in the receptor pocket until acylation of the active site serine occurs.[40-42] Hence antibacterial activity depends in part on the probability of these intermolecular interactions being effective in orienting the antibacterial agent properly. The probability is lower in the bicyclic tetrahydropyridazinones than in cephalosporins because of the high flexibility of the tetrahydropyridazinone ring and the resulting entropy.

Experiments[50] were performed after the molecular modeling to determine the affinity of a tetrahydropyridazinone for the penicillin-binding proteins of *Escherichia coli* K-12. Competition studies between tetrahydropyridazinone **13c**, pyrazolidinone **15c** (see Table I), and iodinated penicillin V (IPV) revealed that **15c** blocked binding of IPV to PBP 3 by 50% at a concentration of 0.25 μg/mL, in good agreement with its MIC. In contrast, **13c** did not bind to any of the PBPs at concentrations up to 512 μg/mL. These results confirm the conclusions of the modeling studies. Cell wall permeability[51] is another factor essential to antibacterial activity, but it does not appear to be the limiting factor for the bicyclic tetrahydropyridazinones, as **13a–c** were also not active against a permeable mutant *E. coli* X580.

In summary, the compounds with the [4.3.0] ring system are inactive due to too much steric bulk and flexibility in the δ-lactam ring. This leads to poor affinity to the PBPs.

Experimental Section

General Procedure. All reactions were run under a positive pressure of dry nitrogen. Organic solutions of reaction products

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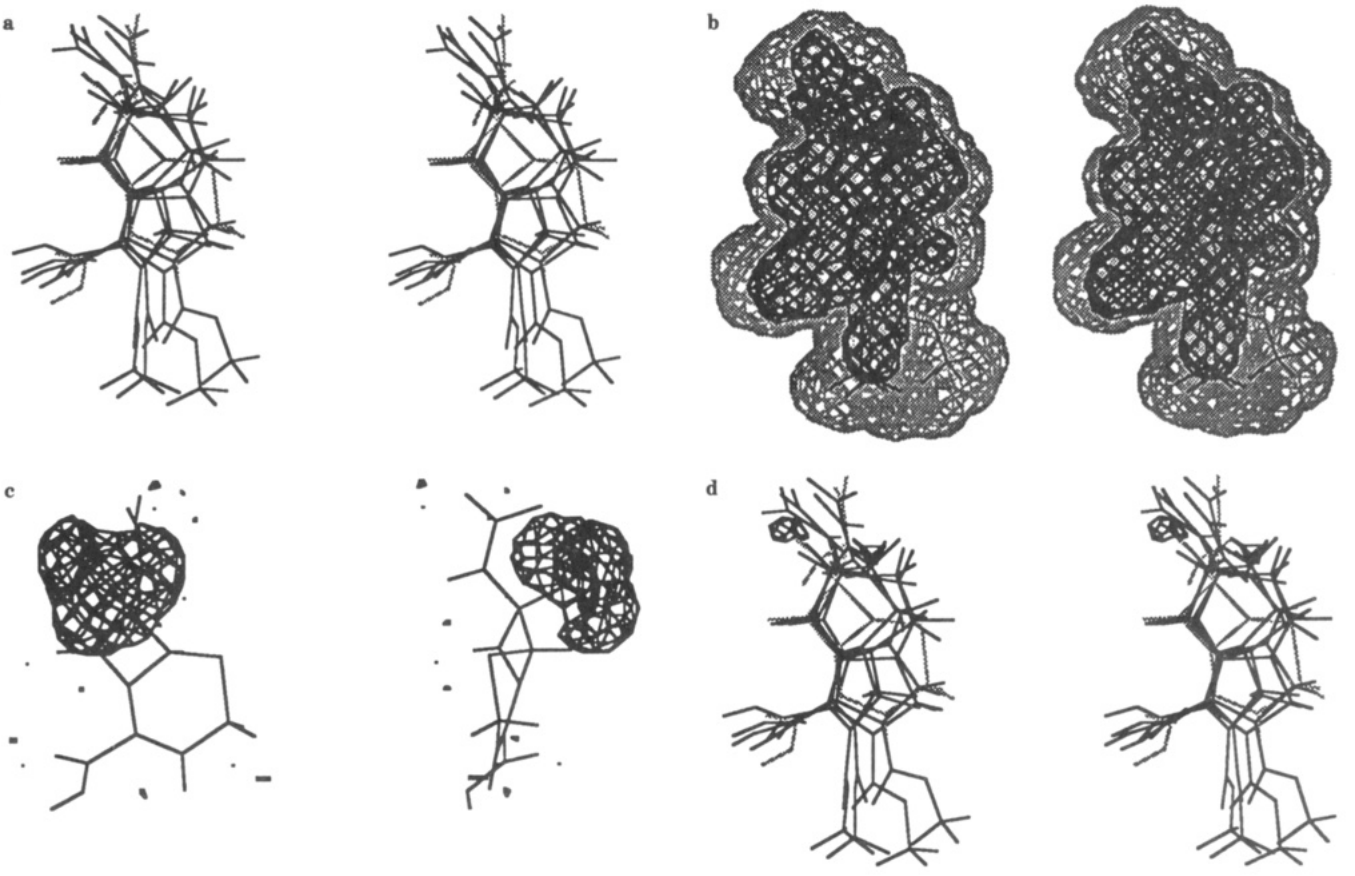

Figure 3. (a) Stereo view of the overlay of the crystallographic structure of cephaloridine (in gray) and the four tetrahydropyridazinone conformers (in black) as obtained from the SYBYL multifitting procedure. The internal geometry of each individual model was held frozen during this process. In the cephalosporin model, the 2-(thienylacetamido) side chain is replaced by formylamido, and the pyridinium methyl side chain is replaced by hydrogen to improve visual clarity. (b) Stereo view of the union of the molecular volumes of all four tetrahydropyridazinone conformers (in gray) and the intersection of these volumes (in black). The gray region describes all parts of space that these structures can reach, whereas the black region is the part which must be available to the [4.3.0] system for it to bind to the PBPs. (c) Orthogonal views of the volume occupied by the 7-methyl group of a cephalosporin. (d) Stereo view of volume maps showing the two regions of space that are common to both the intersection of the tetrahydropyridazinones and the volume of the 7-methyl group of cephalosporins.

were dried with $MgSO_4$ prior to being concentrated in vacuo on a rotary evaporator. Fast atom bombardment mass spectra (FABMS) were obtained on a VG ZAB-3 instrument. Flash chromatography was carried out on E. Merck Kieselgel 60 (230–400 mesh). Melting points are uncorrected. NMR J values are in hertz.

Preparation of 4,5-Dihydro-4-[(*tert*-butoxycarbonyl)amino]-3(2*H*)-pyridazinone (4). To a solution of aldehyde **3**[7] (9.7 g, 42 mmol) in EtOH (100 mL) was added dropwise hydrazine hydrate (2.47 g, 42 mmol) over 15 min. The resulting solution was stirred at room temperature for 2 h, refluxed for 3 h, then cooled. The solvent was removed in vacuo; the residue was taken up in toluene and reconcentrated in vacuo to give the title compound as sticky white solid. This material (250 mg) was purified by flash chromatography (5% $MeOH/CH_2Cl_2$) to give 130 mg of the title compound as a white powder. Mp: 121–122 °C. NMR ($CDCl_3$): δ 8.65 (br s, 1), 7.20 (m, 1), 5.24 (br s, 1), 4.23 (m, 1), 3.73 (m, 1), 3.18 (m, 1), 1.44 (s, 9). IR ($CHCl_3$): 3019, 1697, 1497 cm^{-1}. UV (EtOH): λ_{max} 242 nm (ϵ 4810). FABMS: calcd for $C_9H_{16}N_3O_3$ 214.1192, found 214.1200, M + 1.

Preparation of 1,4,5,6-Tetrahydro-4-[(*tert*-butoxycarbonyl)amino]-3(2*H*)-pyridazinone (1). To a solution of pyridazinone **4** (6.4 g, 30 mmol theoretical) in dry MeOH (60 mL) was added glacial AcOH (1.2 mL) followed by $NaBH_3CN$ (1 g). After 30 min and again after 1 h additional aliquots of AcOH (1.2 mL) and $NaBH_3CN$ (1 g) were added. The mixture was stirred at room temperature overnight. An additional 1 g of $NaBH_3CN$ was added and the mixture stirred for 24 h. Solid $NaHCO_3$ (5 g) was added in portions to the mixture, and then the solvent was removed in vacuo. The residue was partitioned between water and CH_2Cl_2, and the aqueous phase was extracted with additional methylene chloride. The combined organic extracts were dried and concentrated in vacuo to give 6 g of a crude white solid. Flash chromatography (5% $MeOH/CH_2Cl_2$) gave 2.32 g of the title compound as a white powder. Mp: 164–165 °C. Yield: 36% from aldehyde **3**. NMR ($CDCl_3$): δ 7.25 (br s, 1), 5.55 (br d, 1), 4.34 (m, 1, $NCH(CH_2)CO$), 4.10 (br m, 1), 3.14 (m, 2, CH_2CH_2NH), 2.68 (m, 1, CH_2CH_2NH), 1.60 (m, 1, CH_2CH_2NH), 1.43 (s, 9). IR ($CHCl_3$): 1687 cm^{-1}. FABMS: calcd for $C_9H_{18}N_3O_3$ 216.1348, found 216.1358, M + 1. Anal. ($C_9H_{17}N_3O_3$) C, H, N.

Preparation of Bicyclic Pyridazinone 6a via Cycloaddition with Diallyl Acetylenedicarboxylate. To a slurry of pyridazinone **1** (380 mg, 1.76 mmol) in CH_3CN (5 mL) was added a solution of 37% aqueous formaldehyde (143 mg, 1.76 mmol) in MeOH (1 mL). The mixture was stirred at room temperature for 1 h, at which time all of the pyridazinone had dissolved. The mixture was concentrated in vacuo and the residue taken up in 1,2-dichloroethane (5 mL). To this solution was added a solution of diallyl acetylenedicarboxylate (0.4 g, 2.06 mmol) in 1,2-dichloroethane (5 mL). The reaction mixture was heated to reflux for 4 h, cooled, and concentrated in vacuo. Flash chromatography of the residue (25% EtOAc/toluene) gave 125 mg (17%) of the cycloadduct **6a** as a yellow foam. NMR ($CDCl_3$): δ 6.07–5.80 (m, 2, OCH_2CHCH_2), 5.45–5.2 (m, 4, OCH_2CHCH_2), 4.82 (d, 2, J = 6, OCH_2CHCH_2), 4.63 (d, 2, J = 6, OCH_2CHCH_2), 4.55 (m, 1, $NCH(CH_2)CO$), 4.39 (d, 1, J = 13, NCH_2C), 3.96 (d, 1, J = 13, NCH_2C), 3.16 (dt, 1, J = 4, 10, CH_2CH_2N), 3.05 (m, 1, CH_2CH_2N), 2.76 (m, 1, CH_2CH_2N), 2.30 (m, 1, CH_2CH_2N), 1.40 (s, 9). IR ($CHCl_3$): 1749, 1713, 1691 cm^{-1}. UV (EtOH): λ_{max} 331 nm (ϵ 9070). FDMS: m/e 421, M+. Anal. ($C_{20}H_{27}N_3O_7$): C, H, N.

Preparation of Bicyclic Pyridazinone 11a. Acylation of Pyridazinone 6a. Bicyclic pyridazinone **6a** (200 mg, 0.47 mmol)

was dissolved in 5 mL of 3 N HCl(g) in glacial acetic acid. The mixture was allowed to stand for 5 min then concentrated in vacuo to remove the acetic acid. Toluene (25 mL) was added to the residue and after brief sonication was removed in vacuo. In the meantime, 2-(tritylamino)-α-methoximino-4-thiazoleacetic acid (288 mg, 0.6 mmol, Aldrich) was slurried in CH_2Cl_2 (5 mL) and cooled to 0 °C. *N*-Methylmorpholine (66 μL, 0.6 mmol) was added followed by $POCl_3$ (57 μL, 0.6 mmol) and the resulting solution was stirred at 0 °C for 30 min. Additional *N*-methylmorpholine (200 μL, 1.8 mmol) was added followed by a solution of the above prepared deblocked nucleus in CH_2Cl_2 (2 mL) and stirring was continued at 0 °C for 30 min and then at room temperature for 2 h. The mixture was diluted with EtOAc (50 mL) and washed with water, 1 N aqueous HCl solution, saturated aqueous $NaHCO_3$ solution, and brine. Drying followed by concentration in vacuo gave a yellow gum. Flash chromatography (50% EtOAc/toluene) gave 220 mg (62%) of the desired acylation product **11a** as a yellow foam. NMR ($CDCl_3$): δ 7.30 (m, 18), 6.96 (s, 1), 6.79 (d, 2, *J* = 6, CONH), 6.68 (s, 1), 6.05–5.80 (m, 2, OCH_2CHCH_2), 5.45–5.20 (m, 4, OCH_2CHCH_2), 4.88 (m, 1, $NCH(CH_2)CO$), 4.82 (d, 2, *J* = 6, OCH_2CHCH_2), 4.64 (d, 2, *J* = 6, OCH_2CHCH_2), 4.42 (d, 1, *J* = 14, NCH_2C), 4.03 (s, 3, OCH_3), 3.97 (d, 1, *J* = 14, NCH_2C), 3.25–3.00 (m, 3), 1.65 (m, 1, CH_2CH_2N). IR ($CHCl_3$): 1750, 1719, 1692, 1672, 1650 cm^{-1}. UV (EtOH): λ_{max} 311 nm (ε 12400). FDMS: *m/e* 746, M+. Anal. ($C_{40}H_{38}N_6O_7S$): C, H, N.

Preparation of 13a. Deprotection of Pyridazinone 11a. Acylated pyridazinone **11a** (200 mg, 0.26 mmol) was dissolved in a mixture of trifluoroacetic acid (TFA) (5 mL) and Et_3SiH (2 mL). After stirring for 5 min toluene (20 mL) was added and the mixture concentrated in vacuo. The solid residue was slurried in CH_2Cl_2 (25 mL), and several drops of *N*-methylmorpholine were added to dissolve the material. This solution was washed with pH 7 phosphate buffer and dried, and the solvent removed in vacuo to give a yellow gum. In the meantime $Pd(OAc)_2$ (5 mg, 0.022 mmol) and Ph_3P (23 mg, 0.089 mmol) were mixed together in EtOAc (1 mL) and stirred for 5 min. A solution of sodium 2-ethylhexanoate (108 mg, 0.65 mmol) in EtOAc (1 mL), followed by a solution of the above prepared detritylated material in CH_2Cl_2, (2 mL) was added, and after several minutes a precipitate began to form. After 1 h the precipitate was collected by centrifugation; the solid collected was triturated with CH_2Cl_2 and EtOAc and then dried in vacuo to give 117 mg of **13a** as tan powder. This material (60 mg) was purified on HP20SS ion-exchange resin (20 g) by elution with water to give after freeze-drying of the product-containing fractions (t_R = 2.14 min on a Waters C18-μ-Bondpak column (10% CH_3CN/1% NH_4OAc/water) 37 mg of the title compound as a white powder. NMR (D_2O): δ 7.19 (s, 1, SCHC), 4.85 (m, 1, $NCH(CH_2)CO$), 4.20 (d, 1, *J* = 13, NCH_2C), 4.00 (d, 1, *J* = 13, NCH_2C), 3.95 (s, 3, OCH_3), 3.27 (m, 2), 2.60 (m, 1), 1.95 (m, 1, CH_2CH_2N). IR (KBr): 3145, 1643, 1635, 1627 cm^{-1}. UV (EtOH): λ_{max} 299 nm (ε 4670), 227 (ε 5980). FABMS: calcd for $C_{15}H_{15}N_6O_7SNa_2$ 469.0518, found 469.1124, M + 1.

Preparation of Bicyclic Pyridazinone 6b via Cycloaddition with Vinyl Sulfone 7. To a slurry of pyridazinone **1** (1.08 g, 5 mmol) in CH_3CN (25 mL) was added a solution of 37% aqueous formaldehyde (405 mg, 5 mmol) in MeOH (5 mL). The mixture was stirred at room temperature for 1 h, at which time all of the pyridazinone had dissolved. The mixture was concentrated in vacuo, and the residue taken up in 1,2-dichloroethane (20 mL). To the resulting solution was added a solution of vinyl sulfone **7**[8] (1.47 g, 5 mmol) in 1,2-dichloroethane (5 mL) and the mixture was refluxed for 2 h. The mixture was cooled to room temperature and *N*-methylmorpholine (1.4 mL, 12.75 mmol) was added. After stirring overnight at room temperature the solvent was removed in vacuo, and the residue was subjected to flash chromatography (40% EtOAc/hexanes) to give 400 mg (21%) of the desired bicyclic nucleus **6b** as a yellow foam. NMR ($CDCl_3$): δ 6.02 (m, 1, OCH_2CHCH_2), 5.5–5.3 (m, 2, OCH_2CHCH_2), 5.25 (br, 1, NH), 4.86 (d, 2, *J* = 6, OCH_2CHCH_2), 4.55 (m, 1, $NCH(CH_2)CO$), 4.36 (d, 1, *J* = 13, NCH_2C), 3.98 (d, 1, *J* = 13, NCH_2C), 3.16 (dt, 1, *J* = 5, 10, CH_2CH_2N), 3.01 (m, 1, CH_2CH_2N), 2.75 (m, 1, CH_2CH_2N), 2.23 (s, 3, $COCH_3$), 1.60 (m, 1, CH_2CH_2N), 1.43 (s, 9). IR ($CHCl_3$): 1743, 1711, 1658 cm^{-1}. UV (EtOH): λ_{max} 347 nm (ε 6680), 224 (ε 6950). FABMS: calcd for $C_{18}H_{26}N_3O_6$ 380.1821, found 380.1820, M + 1.

Preparation of Bicyclic Pyridazinone 11b. Acylation of Pyridazinone 6b. Pyridazinone **6b** (340 mg, 0.9 mmol) was dissolved in 5 mL of 3 N HCl(g) in glacial acetic acid. The mixture was allowed to stand for 5 min then concentrated in vacuo to remove the acetic acid. Toluene (25 mL) was added to the residue and after brief sonication was removed in vacuo. In the meantime, 2-[[(allyloxy)carbonyl]amino]-α-methoximino-4-thiazoleacetic acid[1c] (285 mg, 1.0 mmol) was slurried in CH_2Cl_2 (5 mL) and cooled to 0 °C. *N*-Methylmorpholine (110 μL, 1.0 mmol) was added followed by $POCl_3$ (94 μL, 1.0 mmol), and the resulting solution was stirred at 0 °C for 25 min. Additional *N*-methylmorpholine (440 μL, 4.0 mmol) was added, followed by a solution of the above prepared deblocked nucleus in CH_2Cl_2 (3 mL). The cooling bath was removed and the mixture stirred at room temperature for 2 h. The mixture was diluted with EtOAc (50 mL) and washed with water, 1 N aqueous HCl solution, saturated aqueous $NaHCO_3$ solution, and brine. Drying followed by concentration in vacuo gave a yellow gum. Flash chromatography (3% MeOH/CH_2Cl_2) gave 250 mg (51%) of the desired acylation product **11b** as a yellow foam. NMR ($CDCl_3$): δ 9.30 (s, 1, NH), 7.90 (br, 1, NH), 7.16 (s, 1, SCHC), 6.00 (m, 2, OCH_2CHCH_2), 5.38 (m, 4, OCH_2CHCH_2), 5.13 (q, 1, *J* = 9, $NCH(CH_2)CO$), 4.84 (d, 2, *J* = 6, OCH_2CHCH_2), 4.75 (d, 2, *J* = 6, OCH_2CHCH_2), 4.42 (d, 1, *J* = 14, NCH_2C), 4.02 (d, 1, *J* = 14, NCH_2C), 3.96 (s, 3, $NOCH_3$), 3.24 (m, 1, CH_2CH_2N), 3.08 (m, 1, CH_2CH_2N), 2.85 (m, 1, CH_2CH_2N), 2.26 (s, 3, $COCH_3$), 1.85 (m, 1, CH_2CH_2N). IR ($CHCl_3$): 1743, 1734, 1662, 1627 cm^{-1}. UV (EtOH): λ_{max} 340 nm (ε 6940), 264 (ε 12700), 228 (ε 19700). FABMS: calcd for $C_{23}H_{27}N_6O_8S$ 547.1611, found 547.1612, M + 1.

Preparation of 13b. Deprotection of Pyridazinone 11b. To a solution of acylated pyridazinone **11b** (200 mg, 0.36 mmol) in CH_2Cl_2 (3 mL) was added $(Ph_3P)_2PdCl_2$ (14 mg, 0.02 mmol), glacial AcOH (58 μL, 1 mmol), and *n*-Bu_3SnH (0.22 mL, 0.8 mmol). This mixture was stirred at room temperature for 20 min, at which time some starting material was still evident by TLC (5% MeOH/CH_2Cl_2). An additional aliquot of *n*-Bu_3SnH (0.06 mL, 0.22 mmol) was added, and the mixture was stirred an additional 15 min. The solvent was removed in vacuo and the residue taken up in CH_3CN (40 mL) and washed with hexanes (3 × 15 mL). The CH_3CN layer was concentrated in vacuo and the residue purified by reverse-phase medium-pressure liquid chromatography (C18 Lobar column, eluted with 20% MeOH/1% AcOH/water) to give after freeze-drying 86 mg (56%) of **13b** as a yellow powder. Analytical HPLC on a Waters C18 μ-Bondpak column (30% MeOH/1% NH_4OAc/water) showed a single peak with t_R = 2.67 min. NMR (DMSO-d_6): δ 8.92 (d, 1, *J* = 9, NH), 7.20 (br, 1, NH), 7.06 (s, 1, SCHC), 4.62 (q, 1, *J* = 9, $NCH(CH_2)CO$), 4.20 (d, 1, *J* = 14, NCH_2C), 4.03 (d, 1, *J* = 14, NCH_2C), 3.79 (s, 3, $NOCH_3$), 3.20 (m, 1, CH_2CH_2N), 3.00 (m, 1, CH_2CH_2N), 2.30 (m, 1, CH_2CH_2N), 2.18 (s, 3, $COCH_3$), 1.82 (m, 1, CH_2CH_2N). IR (KBr): 1698, 1654, 1630 cm^{-1}. UV (EtOH): λ_{max} 336 nm (ε 11900), 235 (ε 14500). FABMS: calcd for $C_{16}H_{19}N_6O_6S$ 423.1087, found 423.1078, M + 1.

Preparation of Bicyclic Pyridazinone 6c via Michael Addition and Intramolecular Ring Closure. To a slurry of paraformaldehyde (400 mg, 13.3 mmol) in benzene (20 mL) was added glacial AcOH (4.5 mL) followed by pyrrolidine (0.11 mL, 1.33 mmol). The mixture was refluxed for 10 min and cooled to 0 °C, and diethyl (cyanomethyl)phosphonate (1.62 mL, 10 mmol) was added. The resulting solution was refluxed for 5 min, then a Dean–Stark trap was fitted to the flask and refluxing continued for 20 min. The mixture was cooled, and the solvent removed in vacuo. Toluene (2 × 50 mL) was added and removed in vacuo to assist in removing the excess AcOH. The residue was dissolved in MeOH (10 mL) and added to a solution of pyridazinone **1** (1.08 g, 5 mmol) in MeOH (15 mL). This solution was stirred at room temperature for 2.5 h and concentrated in vacuo, and the residue subjected to flash chromatography eluting with 5% MeOH/CH_2Cl_2. The material with polarity between the starting pyridazinone **1** and diethyl (cyanomethyl)phosphonate was collected to give 1.53 g of conjugate adduct **10** as a colorless oil. Partial NMR ($CDCl_3$): δ 4.25 (q, 4, *J* = 6), 1.44 (s, 9), 1.35 (t, 6, *J* = 6). Conjugate adduct **10** (1.53 g, 3.8 mmol theoretical) was dissolved in CH_2Cl_2 (30 mL), then *i*-Pr_2NEt (2 mL, 11.4 mL) and allyl oxalyl chloride[11] (566 mg, 3.8 mmol) were added. The resulting solution was stirred at room temperature overnight, diluted with CH_2Cl_2

(30 mL), washed with 1 N HCl solution, saturated aqueous $NaHCO_3$ solution, and brine, and dried; the solvent was removed in vacuo. Flash chromatography (33% EtOAc/toluene) gave 210 mg (11%) of the desired bicyclic nucleus **6c** as a yellow oil. NMR ($CDCl_3$): δ 5.98 (m, 1, OCH_2CHCH_2), 5.5–5.2 (m, 2, OCH_2CHCH_2), 5.25 (br, 1, NH), 4.85 (d, 2, J = 6, OCH_2CHCH_2), 4.56 (m, 1, $NCH(CH_2)CO$), 4.38 (d, 1, J = 13, NCH_2C), 3.95 (d, 1, J = 13, NCH_2C), 3.15 (m, 2, CH_2CH_2N), 2.80 (m, 1, CH_2CH_2N), 1.65 (m, 1, CH_2CH_2N), 1.45 (s, 9). IR ($CHCl_3$): 2230, 1747, 1704 cm^{-1}. UV (EtOH): λ_{max} 321 nm (ε 5490). FABMS: calcd for $C_{17}H_{23}N_4O_5$ 363.1668, found 363.1638, M + 1. Anal. ($C_{17}H_{22}N_4O_5$): C, H, N.

Preparation of Bicyclic Pyridazinone 11c. Acylation of Pyridazinone 6c. Pyridazinone **6c** (181 mg, 0.5 mmol) was dissolved in 5 mL of 3 N HCl(g) in glacial acetic acid. The mixture was allowed to stand for 10 min then concentrated in vacuo to remove the acetic acid. Toluene (25 mL) was added to the residue and after brief sonication was removed in vacuo. In the meantime, 2-[[(allyloxy)carbonyl]amino]-α-methoximino-4-thiazoleacetic acid[1c] (171 mg, 0.6 mmol) was slurried in CH_2Cl_2 (5 mL) and cooled to 0 °C. *N*-Methylmorpholine (66 μL, 0.6 mmol) was added followed by $POCl_3$ (57 μL, 0.6 mmol), and the resulting solution was stirred at 0 °C for 20 min. Additional *N*-methylmorpholine (200 μL, 1.8 mmol) was added followed by a solution of the above prepared deblocked nucleus in CH_2Cl_2 (3 mL). The mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. The mixture was diluted with EtOAc (50 mL) and washed with water, 1 N HCl solution, saturated aqueous $NaHCO_3$ solution, and brine. Drying followed by concentration in vacuo gave a yellow powder. Flash chromatography (3% MeOH/CH_2Cl_2) gave 150 mg (57%) of the desired acylation product **11c** as a yellow powder. NMR ($CDCl_3$): δ 9.20 (s, 1, NH), 7.86 (br d, 1, J = 9, NH), 7.18 (s, 1, SCHC), 5.95 (m, 2, OCH_2CHCH_2), 5.35 (m, 4, OCH_2CHCH_2), 5.13 (q, 1, J = 9, $NCH(CH_2)CO$), 4.83 (d, 2, J = 6, OCH_2CHCH_2), 4.74 (d, 2, J = 6, OCH_2CHCH_2), 4.44 (d, 1, J = 13, NCH_2C), 4.00 (d, 1, J = 13, NCH_2C), 3.97 (s, 3, $NOCH_3$), 3.20 (m, 2, CH_2CH_2N), 2.85 (m, 1, CH_2CH_2N), 1.88 (m, 1, CH_2CH_2N). IR ($CHCl_3$): 2225, 1747, 1733, 1694, 1673 cm^{-1}. UV (EtOH): λ_{max} 313 nm (ε 8010), 266 (ε 14100), 225 (ε 21100). FDMS: m/e 529, M+.

Preparation of 13c. Deprotection of Pyridazinone 11c. To a solution of acylated pyridazinone **11c** (140 mg, 0.26 mmol) in CH_2Cl_2 (3 mL) was added $(Ph_3P)_2PdCl_2$ (14 mg, 0.02 mmol), glacial AcOH (58 μL, 1 mmol), and *n*-Bu_3SnH (157 μL, 0.58 mmol). This mixture was stirred at room temperature overnight at which time a precipitate had formed. This material was collected by filtration and then purified by reverse-phase medium-pressure liquid chromatography (C18 Lobar column, material was dissolved in water containing 1 mmol of $NaHCO_3$ and eluted with 20% MeOH/water) to give after freeze-drying: 100 mg (90%) of **13c** as a light tan powder. Analytical HPLC on a Waters C18 μ-Bondpak column (30% MeOH/1% AcOH/water) showed a single peak with t_R = 2.48 min. NMR (D_2O) partial: δ 7.16 (s, 1, SCHC), 4.27 (d, 1, J = 13, NCH_2C), 4.09 (d, 1, J = 13, NCH_2C), 3.94 (s, 3, $NOCH_3$), 3.25 (m, 2, CH_2CH_2N), 2.59 (m, 1, CH_2CH_2N), 1.95 (m, 1, CH_2CH_2N). IR (KBr): 2220, 1646, 1642 cm^{-1}. UV (EtOH): λ_{max} 299 nm (ε 10600), 232 (ε 13000). FABMS: calcd for $C_{15}H_{15}N_7O_5SNa$ 428.0753, found 428.0752, M + 1.

Computational Chemistry. The SYBYL molecular modeling software (versions 3.5 and 5.3) was run on a VAX 8800 minisupercomputer. Macintosh II, Modgraph GX1000, and Evans and Sutherland PS330 terminals were used for molecular graphics. MOPAC (Version 4.0) was run on the Cray X-MP/48 supercomputer at the National Center for Supercomputing Applications (University of Illinois, Urbana—Champaign).

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Supplementary Material Available: Four tables listing the MNDO-optimized Cartesian atomic coordinates (4 pages). Ordering information is given on any current masthead page.

Synthesis and Quantitative Structure–Activity Relationship Analysis of 2-(Aryl or Heteroaryl)quinolin-4-amines, a New Class of Anti-HIV-1 Agents

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Thirty-eight 2-(aryl or heteroaryl)quinolin-4-amines, N,N-disubstituted, N-monosubstituted, and without a substituent at the amino group have been synthesized with use of novel chemistries developed by us recently. Some of these derivatives show anti-HIV-1 activity at a concentration level of 1 μM and low cell toxicity in vitro. The most active and least toxic compounds are derivatives of 2-(3-pyridyl)quinoline. The results of the quantitative structure–activity relationship analyses, including several classical, linear regression correlations and a Free–Wilson approach of de novo model, provide guidelines for the design of new active compounds of this class.

Recently we analyzed a short series of heteropolyaromatic compounds as potential anti-HIV-1 agents.[1] Several moderately active derivatives were identified which contained alkylamino, dialkylamino, or alkoxy substituents located ortho or para to the ring nitrogen atoms. By contrast, the alkylthio-substituted analogues and non-substituted parent heteropolyaromatic systems [e.g., 4,6-di-2-thienylpyrimidine] generally were inactive. These results were interpreted in terms of different electronic effects in the two sets of molecules. It is known that the amino and alkoxy groups are strongly conjugated with an aromatic ring system while this conjugation is relatively

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