# Spirocyclopropyl β-Lactams as Mechanism-Based Inhibitors of Serine β-Lactamases. Synthesis by Rhodium-Catalyzed Cyclopropanation of 6-Diazopenicillanate Sulfone

Vincent P. Sandanayaka, Amar S. Prashad, Youjun Yang, R. Thomas Williamson, Yang I. Lin, and Tarek S. Mansour*

*Chemical and Screening Sciences and Infectious Diseases, Wyeth Research, 401 North Middletown Road, Pearl River, New York 10965*

*Received March 3, 2003*

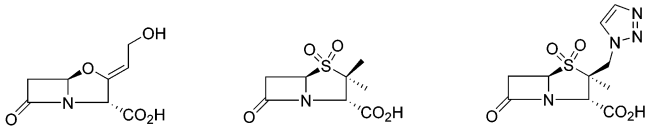
**Abstract:** Class A−class C mechanism-based β-lactamase inhibitors were designed on the basis of the intermediacy of an oxycarbenium species capable of cross-linking with amino acids residues in the active site. Penams **24** and **27** were very potent against AmpC in vitro. The MIC values of **24** in combination with piperacillin against class A and class C producing organisms showed improvement over clinically used tazobactam.

**Introduction.** Bacterial β-lactamases are enzymes that hydrolyze the β-lactam ring in all classes of β-lactam antibiotics before these drugs can exert their desired therapeutic advantage. These enzymes, which are the leading cause of resistance to β-lactam antibiotics,[1] now comprise a class of nearly 340 discrete β-lactamases and thus represent a significant challenge in combating bacterial infections.[2] In particular, serine enzymes classified as class A and class C are clinically relevant because of the emergence of extended spectrum β-lactamase (ESBLs) resistance and AmpC β-lactamases.[1,3] A therapeutically successful strategy to overcome the resistance problem relies on the administration of a β-lactamase inhibitor along with an antibiotic. Thus, in combination with antibiotics, clavulanic acid (**1**), sulbactam (**2**), and tazobactam (**3**) are β-lactamase inhibitors commercially used against class A β-lactamases but are not effective against the clinically relevant class C enzymes (Chart 1).

Very few broad-spectrum class A−class C inhibitors have been reported to date[4−6] that include borates as transition-state analogue inhibitors, acyl phosphonates, or phosphates that act by phosphorylation of the nucleophilic serine of the β-lactamase active site, cephalosporonic acid derivatives, and penem inhibitors such as **4** and **5** (Chart 2).

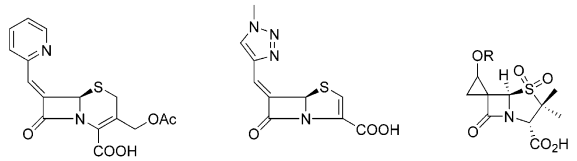
During the course of our continuing studies on β-lactamase inhibitors,[7] we hypothesized that the incorporation of a cyclopropyloxy group at the C6 position of **2** leading to compounds **6** should enhance the enzyme−inhibitor interactions within the active site and thus may lead to an inhibitor with a broad-spectrum activity against serine β-lactamases. Mechanistically, the cyclopropyloxy group can promote the subsequent chemical events after initial acylation of the enzyme to unravel the aldehyde or the oxycarbenium functionality

**Chart 1.** Structures of Clinically Approved β-Lactamases Inhibitors




**1** **2** **3**

**Chart 2.** Structure of Class A−Class C Inhibitors




**4** **5 (BRL-42715)** **6**

for further cross-linking with other active-site residues of the enzyme as an alternative to fragmentation[8] or rearrangement transformations[9,10] of the acyl-enzyme intermediate reported to date (Scheme 1). This concept of involving nucleophilic sites of the enzyme in addition to active serine has been postulated in both class A[8] and class C β-lactamases.[4] In this report, we communicate our preliminary results dealing with cyclopropanation reactions of 6-diazopenicillanate sulfone **10** and the utility of the resultant spirocyclopropyl sulfones as a novel class of β-lactamase inhibitors.

**Results and Discussion. Chemistry.** Retrosynthetically, we envisioned the use of Rh(II)-catalyzed cyclopropanation reaction of **10** as the key step to assemble the requisite tricyclic ring system based on literature precedents in the preparation of 6-spirocyclopropyl penicillanate.[11] Thus, the commercially available (+)-6-aminopenicillanic acid (**7**) was converted to the corresponding (+)-6-aminopenicillanic acid sulfone (**8**) in 78% yield by employing permanganate oxidation. The carboxylic acid group of **8** was protected as the diphenylmethyl ester, with concurrent formation of the ammonium salt for easy purification, leading to compound **9**. The salt was converted to the free amine, which was treated with $NaNO_2/HClO_4$ to obtain the novel diazosulfone **10** in 90% yield. The overall process is very efficient in that it does not require any chromatography and can be used to make multigram quantities of **10** in excellent overall yield (Scheme 2).

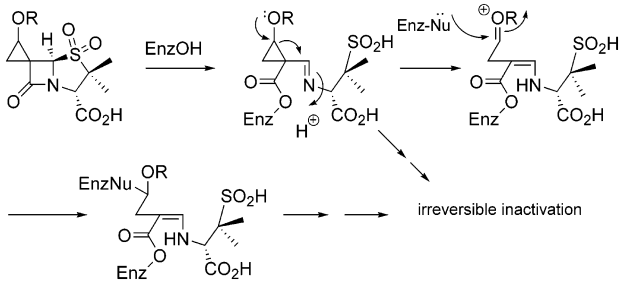
We next examined the insertion reactions between **10** and various monosubstituted olefins in the presence of rhodium(II) catalyst.[12] No attempts were made to improve the stereoselectivity in these reactions at this stage, since lack of stereochemical differentiation would be advantageous to gain more insights into the structural and mechanistic aspects of enzyme interactions. Some of the vinyl ether insertion partners were chosen such that the R moiety of **6** was a hydroxyl-protected group. If the hydroxycyclopropyl compound **6** (R = H) were stable enough to be prepared, it might give additional mechanistic implications of β-lactamase inhibition.[13]

Compound **10** was treated with 2 equiv of styrene in the presence of a catalytic amount of $Rh_2(OAc)_4$ to obtain

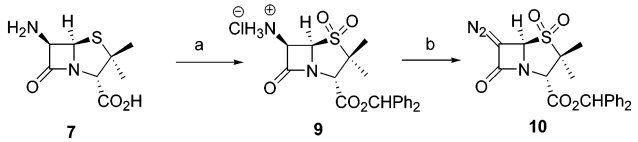
* To whom correspondence should be addressed. Phone: 845-602-3668. Fax: 845-602-5580. E-mail: mansout@wyeth.com.

10.1021/jm034056q CCC: $25.00 
Published on Web 05/23/2003

**Scheme 1.** Proposed Mechanism of Inactivation



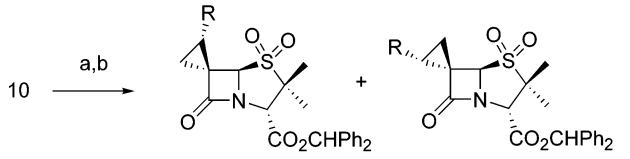

**Scheme 2.** Synthesis of Diazosulfone **10**[a]



**7** **9** **10**

[a] Conditions: (a) (i) $KMnO_4$, $H_2SO_4$, −10 °C (78%); (ii) $Ph_2CN_2$, 0 °C, then anhydrous HCl in ether (84%); (b) aqueous $NaNO_2$, 1 N $HClO_4$, 0 °C (90%).

**Scheme 3.** Insertion Reactions of **10** with Alkenes[a]

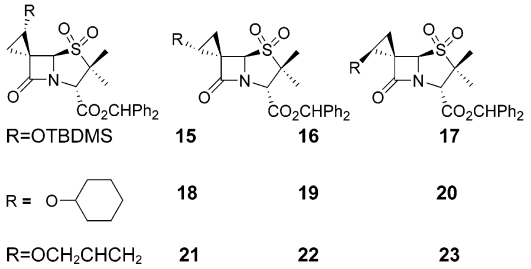


| | | |
|---|---|---|
| R=Ph | **11** | **12** |
| R=OCHPh$_2$ | **13** | **14** |

[a] Conditions: (a) 2 equiv of alkene in $CH_2Cl_2$ (0.2−4.0 M) added dropwise, $Rh_2(OAC)_4$ in $CH_2Cl_2$ (0.2 M) (46−60%); (b) flash chromatography on silica gel.

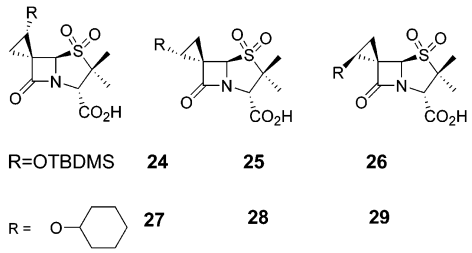
**11** and **12** in a ratio of 1.7:1 in 46% overall yield after separation by chromatography on silica gel. The stereochemical outcome of this transformation is controlled by the sterically demanding exo face of the bicyclic penam system. Importantly, diphenylmethyl vinyl ether resulted in the formation of **13** and **14** in a combined yield of 56% as a 1:1 mixture of diastereomers (Scheme 3).

Diazosulfone **10** was also treated with TBDMS vinyl ether under the same reaction conditions to afford a mixture of diastereomers **15**, **16**, and **17** in a total yield of 52% and a ratio of 2:2:1. The stereochemistry of the above isomers was assigned on the basis of $^1H-^{13}C$ heteronuclear multiple-bond correlation (HMBC), 2D nuclear Overhauser effect spectrometry (NOESY), and 1D NOE experiments. Correlation of chemical shift assignments by $^1H-^{13}C$ HMBC experiment and with 1D $^1H$ NMR spectra enabled the differentiation of the four methine protons present in the molecule. H5 proved to be the most important probe for the determination of the stereochemistry of these compounds because of its proximity to the cyclopropyl functionality in question. Moreover, the absolute configuration of **15** was confirmed by X-ray crystallography as shown in Figure 3b. Cyclohexyl vinyl ether under the above catalytic conditions gave the corresponding spiro compounds **18**, **19**, and **20** in 59% yield as a 3:3:2 mixture of diastereomers. The insertion reaction with allyl vinyl ether occurred chemoselectively at the more electron-rich olefinic carbon, providing isomers **21**, **22**, and **23** in a 1:1:0.75 ratio. In all cases, the diastereomers were readily separated by column chromatography (Figure 1).



| | | | |
|---|---|---|---|
| R=OTBDMS | **15** | **16** | **17** |
| R = O− | **18** | **19** | **20** |
| R=OCH$_2$CHCH$_2$ | **21** | **22** | **23** |

**Figure 1.** Products of Rh(II)-catalyzed insertion reactions of **10** with alkenes.



| | | | |
|---|---|---|---|
| R=OTBDMS | **24** | **25** | **26** |
| R = O− | **27** | **28** | **29** |

**Figure 2.** Structures of spirocyclopropylpenam sulfones.

**Table 1.** In Vitro Data for Selected Compounds

| | IC$_{50}$ [a] ($\mu$M) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| enzyme | **24** | **25** | **26** | **27** | **28** | **29** | **2** | **3** |
| TEM-1 | 0.65 | 12.0 | 17.0 | 0.51 | >13 | 1.7 | 1.4 | 0.1 |
| AmpC | 0.02 | 0.4 | 4.1 | 0.03 | >3.2 | 3.2 | 66.0 | 48.0 |

[a] Determined graphically from six different concentrations of the inhibitor.

Unmasking the protecting groups of **13** and **15** proved to be problematic with typical deprotection agents. Catalytic hydrogenolysis ($H_2$, 10% Pd/C, EtOAc) conditions were used to convert the benzhydryl esters to the corresponding acids. In this regard, the acid derivatives **24**−**29** corresponding to **15**−**20** were obtained in good yield (Figure 2). Despite the apparent instability of the parent hydroxycyclopropyl sulfone penam, the acids **24**−**29** seemed to be viable potential inhibitors based on the mechanistic proposal involving the oxycarbenium cation as outlined in Scheme 1.

**Enzyme and Cellular Assays.** Acids **24**−**29** were evaluated for their inhibitory activities[14] against two clinically important $\beta$-lactamases, TEM-1 and AmpC, representatives of class A and class C enzymes, respectively. As expected, alkoxycyclopropyl-containing compounds **24**−**29** showed good inhibitory activity against the $\beta$-lactamases tested (Table 1). In comparison to sulbactam, sulfone penams **24** and **27** exhibited excellent activity against TEM-1 and AmpC, respectively, with over a 2500-fold improvement against the class C enzyme. Within the same series, **24** and **27** are appreciably more potent than their corresponding diastereomers particularly against the AmpC enzyme. These penams have their H5 proton on the same side as the methylene moiety of the spirocyclopropyl ring. In the less active isomers, H5 is in proximity to the methine proton of the spirocyclopropyl ring, resulting in a 100- to 1000-fold reduction in potency. Such an outcome results in a stereochemical preference of the alkoxy substituent of the cyclopropyl ring that affects the

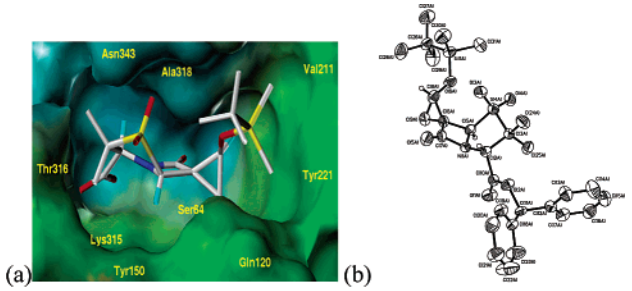



**Figure 3.** (a) Molecular modeling for **24**. (b) X-ray ORTEP diagram for **15.**

**Table 2.** MIC Data for **24** and **27** with Piperacillin and Tazobactam

| | MIC [c] ($\mu$g/mL) | | | |
|---|---|---|---|---|
| organism | pip | pip + **24** | pip + **27** | pip + **3** |
| *E. coli* GC 4206[a] | >64 | 4 | 8 | 4 |
| *E. coli* GC6265 | >64 | <0.06 | 64 | |
| *S. marcescens* GC 4132[b] | 32 | 8 | 32 | 32 |
| *P. aeroginosa*[b] | >64 | 8 | 32 | |

[a] TEM-1 (class A). [b] AmpC (class C). [c] Piperacillin (pip) to inhibitor ratio is 1:1.

binding affinities of these inhibitors. Within the silyloxy derivatives, **24** is appreciably more potent than **25** or **26** against both enzymes. The difference in potency of **25** versus **26** of ca. 10-fold against AmpC appears to reaffirm the stereochemical preference of the alkoxy substituent of the cyclopropyl ring affecting the binding affinity of these inhibitors.

Further support for the strong binding of **24** to AmpC was deduced from molecular modeling studies based on the reported crystal structure of class C enzymes. The model revealed that silyloxy penam **24** was well accommodated in the AmpC active site for initial binding compared to its isomers **25** and **26**. The cyclopropyl moiety benefits from hydrophobic interactions with cyclopropyl-$CH_2$-Tyr-221, *tert*-Bu-Leu-119, and dimethylsilyl-Val-211 in addition to lactam CO interaction with Ser-64 and the salt bridge between COOH and Lys-315 (Figure 3a).

Further in vitro evaluations in a cell-based assay (MIC) established the effectiveness of **24** as a potent broad-spectrum $\beta$-lactamase inhibitor (Table 2). Silyloxy sulfone **24**, in combination with piperacillin at a ratio of 1:1, was two dilutions better than tazobactam against class C producing organisms, consistent with its more potent AmpC enzyme activity. Moreover, the combination of **24** with cefotaxime increases the antimicrobial activity of cefotaxime against Gram-negative and Gram-positive organisms.[15] The relatively higher MIC values of **27** against class C producing organisms may be attributable to piperacillin not being the best partner with it or simply due to poor penetration into the cell.

In conclusion, a new class of mechanism-based class A and class C $\beta$-lactamase inhibitors has been synthesized using a Rh(II)-catalyzed cyclopropanation reaction of diazosulfone **10**.

Sulfone penam **24** emerged with good MIC activity against class A and class C producing microorganisms. The cyclopropyloxy structural motif, especially with an acceptor at the vicinal position on the cyclopropane ring, should find applications in the design of other mechanism-based inhibitors and as an internal mechanistic probe for the investigation of enzyme mechanisms.[16]

**Acknowledgment.** We thank Mr. Peter Petersen for MIC for determinations, Dr. D. Ho (Princeton University) for X-ray studies, and Drs. Hollinger and A. Agarwal for modeling studies.

**Supporting Information Available:** Experimental procedures, spectral data for all relevant compounds, and NOE studies. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Bradford, P. A. Extended-Spectrum $\beta$-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat. *Clin. Microbiol. Rev.* **2001**, *4*, 933−951.
(2) Bush, K. $\beta$-Lactamases of Increasing Clinical Importance. *Curr. Pharm. Des.* **1999**, *5*, 839−845.
(3) Bradford, P. A. What's New in $\beta$-Lactamases? *Curr. Inf. Dis. Rep.* **2001**, *3*, 13−19.
(4) Sandanayaka, V. P.; Prashad, A. S. Resistance to $\beta$-Lactam Antibiotics: Structure and Mechanism Based Design of $\beta$-Lactamase Inhibitors. *Curr. Med. Chem.* **2002**, *9*, 1145−1165.
(5) Micetich, R. G.; Salama, S. M.; Maiti, S. N.; Reddy, A. V. N.; Singh, R. $\beta$-Lactamases and Their Inhibitors: An Update. *Curr. Med Chem.: Anti-Infect. Agents* **2002**, *1*, 193−213.
(6) Payne, D. J.; Wensheng, D.; Bateson, J. H. $\beta$-Lactamase Epidemiology and the Utility of Established and Novel $\beta$-Lactamase Inhibitors. *Expert Opin. Invest. Drugs* **2000**, *9*, 247−261.
(7) (a) Sandanayaka, V. P.; Yang, Y. Dipolar Cycloaddition of Novel 6-(Nitrileoxidomethyl) Penam Sulfone: An Efficient Route to a New Class of $\beta$-Lactamase Inhibitors. *Org. Lett.* **2000**, *2*, 3087−3090. (b) Sandanayaka, V. P. Allyl and Propargyl Substituted PenamSulfones as Versatile Intermediates toward the Syntheses of New $\beta$-Lactamase Inhibitors. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 997−1000 (c) Bitha, P.; Zhong, L.; Francisco, G. D.; Rasmussen, B.; Lin, Y. I. 6-(1-Hydroxyalkyl) Penam Sulfone Derivatives as Inhibitors of Class A and Class C $\beta$-Lactamases I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 991−996. (d) Lin, Y. I.; Bitha, P.; Zhong, L.; Francisco, G. D.; Yang, Y.; Petersen, P. J. 6-(1-Hydroxyalkyl) Penam Sulfone Derivatives as Inhibitors of Class A and Class C $\beta$-Lactamases II. *Bioorg Med. Chem. Lett.* **1999**, *9*, 997−1002.
(8) Yang, Y.; Janota, K.; Tabei, K.; Huang, N.; Siegel, M.; Lin, Y. I.; Rasmussen, B. A.; Shlaes, D. M. Mechanism of Inhibition of the Class A $\beta$-Lactamases PC1 and TEM-1 by Tazobactam. *J. Biol. Chem.* **2000**, *35*, 26674−26682.
(9) Bulychev, A.; Massova, I.; Lerner, S. A.; Mobashery, S. Penem BRL 42715: An Effective Inactivator for $\beta$-Lactamases. *J. Am. Chem. Soc.* **1995**, *117*, 4797−4801.
(10) Richter, H. G. F.; Angehrn, P.; Hubschwerien, C.; Kania, M.; Page, M. G. P.; Specklin, J.-L.; Winkler, F. K. Design, Synthesis, and Evaluation of 2$\beta$-Alkenyl Penam Sulfone Acids as Inhibitors of $\beta$-Lactamases. *J. Med Chem.* **1996**, *39*, 3712−3722.
(11) (a) Campbell, M. M.; Harcus, R. G. Reactions of 6-Diazopenams and 7-Diazocephems: Spirocyclopropyl and Spiropyrazolinyl $\beta$-Lactams. *Tetrahedron Lett.* **1979**, *20*, 1441−1444. (b) Arrowsmith, J. E.; Greengrass, C. W.; Newmann, M. J. Copper(I) Catalysed Reactions of 6-Bromopenicillanolyl Magnesium Bromide. *Tetrahedron* **1983**, *39*, 2469−2475.
(12) (a) Davies, H. M. L.; Nagashima, T.; Klino, J. L., III. Stereoselectivity of Methyl Aryldiazoacetate Cyclopropanations of 1,1-Diarylethylene. Asymmetric Synthesis of a Cyclopropyl Analogue of Tamoxifen. *Org. Lett.* **2000**, *2*, 823−826. (b) Bauta, W.; Dodd, J.; Bullington, J.; Gauthier, D.; Leo, G.; McDonnell, P. Stereoselectivity in the Rhodium(II) Acetate Catalysed Cyclopropanations of 2-Diazo-1-indanone with Styrenes. *Tetrahedron Lett.* **2000**, *41*, 1491−1494.
(13) Crichlow, G. V.; Michiyoshi, N.; Doppalapudi, V. R.; Buynak, J. D.; Knox, J. R. Inhibition of Class C $\beta$-Lactamases: Structure of a Reaction Intermediate with Cephem Sulfone. *Biochemistry* **2001**, *40*, 6233−6239.
(14) Bush, K.; Macalintal, C.; Rasmussen, B. A.; Lee, V. J.; Yang, Y. Kinetic Interactions of Tazobactam with $\beta$-Lactamases from All Major Structural Classes. *Antimicrob. Agents Chemother.* **1993**, *37*, 851−858.
(15) Sandanayaka, V.; Prashad, A. 6-(Spirocyclopropyl) penicillanic acid 4,4-dioxides. U.S. Patent 6,489,316, 2002.
(16) Bycroft, B. W.; Shute, R. Preparation of beta-lactam epoxides as beta-lactamase inhibitors. G.BB Patent 2202533, 1988. 6-Spiroepoxycephems have been reported to be weak inhibitors of $\beta$-lactamases.

JM034056Q