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Perspective

Dependence of β-Lactamase Stability on Substructures within β-Lactam Antibiotics

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Introduction

Bacteria and β-lactam antibiotics may be viewed as protagonists in a never-ending battle. Advantage on either side is always followed by a counterstroke that repositions the participants and sets the stage for the next advance. The chief weapons wielded by bacteria are β-lactamases—enzymes that inactivate β-lactam antibiotics by hydrolysis of the essential β-lactam bond.

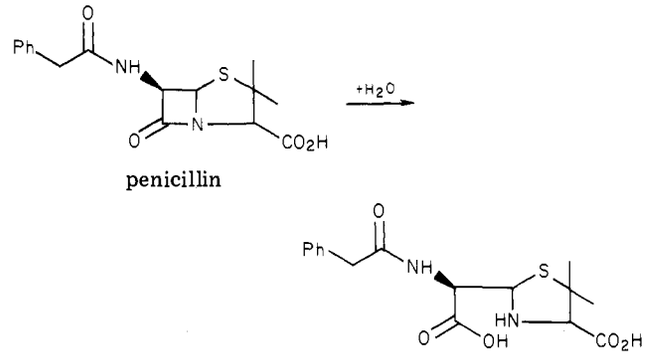
Fleming, by discovering penicillin,[1] also provided the β-lactam substrate necessary to detect β-lactamases. Abraham and Chain[2] later showed that some bacteria (*Escherichia coli*) elaborate enzymes capable of "destroying the growth-inhibitory property of penicillin". Following the structural elucidation of penicillin, Abraham[3] demonstrated that inactivation was a consequence of enzyme-catalyzed hydrolysis of the β-lactam bond to give penicilloic acid (Scheme I).

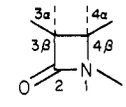
Some of these enzymes are extraordinarily efficient: a single P99 β-lactamase molecule can hydrolyze 100 000 molecules of cephaloridine in 1 min.[4] Development of β-lactam antibiotics stable to β-lactamase has been the most significant achievement since Fleming's initial observations in 1928. From the perspective of a medicinal chemist, the structure–activity relationships involved in the development of this diverse group of agents is fascinating. An understanding of the substructures permitting β-lactamase stability and high intrinsic activity should allow the design of new generations of β-lactams.

Substructures That Lead to β-Lactamase Stability

Substructure Classification. Discovery of monobactams[5] (monocyclic β-lactam antibiotics that derive chemical and biological activation from an N-1 sulfonate group) and the subsequent description of additional series of monocyclic β-lactams displaying alternate "activating" groups[6-8] have rendered the traditional term "nuclear modification" obsolete. Earlier discoveries of cephamycins, clavulanic acid, and carbapenems had similarly taxed the classical "side-chain" descriptor. For this discussion, numbering and substitution patterns on the simple azetidin-2-one substructure 1 (the least common denominator of bioactive β-lactams) will be followed. Thus, penicillin will be designated "1,4β-bridged", clavulanic acid as "3-

Scheme I





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(1) Fleming, A. *Br. J. Exp. Pathol.* **1929**, *56*, 344.
(2) Abraham, E. P.; Chain, E. *Nature (London)* **1940**, *146*, 837.
(3) Abraham, E. P.; Baker, W.; Boon, W. R.; Calam, C. T.; Carrington, H. C.; Chain, E. B.; Florey, H. W.; Freeman, G. G.; Robinson, R.; Sanders, A. G. In "The Chemistry of Penicillin"; Clark, T. H.; Johnson, J. R.; Robinson, R., Eds.; Princeton University Press, Princeton, NJ, 1949.
(4) Calculation performed by Dr. K. Bush, Squibb Institute for Medical Research.
(5) Cimarusti, C. M.; Sykes, R. B. *Chem. Br.* **1983**, 302.
(6) Monosulfactams: Gordon, E. M.; Ondetti, M. A.; Pluscec, J.; Cimarusti, C. M.; Bonner, D. P.; Sykes, R. B. *J. Am. Chem. Soc.* **1982**, *104*, 6053.
(7) Monophosphams: Koster, W. H.; Zahler, R.; Chang, H. W.; Cimarusti, C. M.; Jacobs, G. A.; Perri, M. *J. Am. Chem. Soc.* **1983**, *105*, 3743.
(8) Monocarbams: Slusarchyk, W. A.; Applegate, H. E.; Bonner, D. P.; Breuer, H.; Dejneka, T.; Koster, W. H. Interscience Conference on Antimicrobial Agents and Chemotherapy, 22nd, 1982; American Society for Microbiology: Washington, DC, 1982; Abstr 670.

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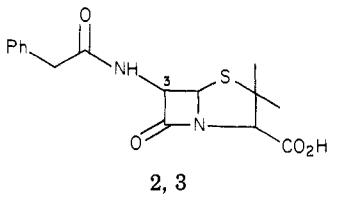
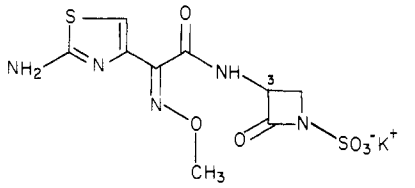
Table I. Dependence of β-Lactamase Stability on Acylamino Stereochemistry at C-3



2, 3

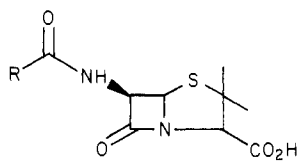


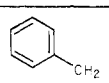
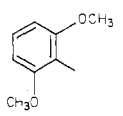
4, 5

compd	RHR:[a] *S. aureus* FS108	MIC,[b] μg/mL: *S. aureus* 209P	stereochem at C-3	no.	RHR: *E. coli* (RTEM+)	MIC, μg/mL: *P. rettgeri* (LAC–)[c]
2	100	0.065	β	4	106	<0.05
3	2.2	1.6	α	5	<0.02	>100

[a] RHR = relative hydrolysis rate. [b] MIC = minimum inhibitory concentration. [c] LAC– = β-lactamase-negative strain.

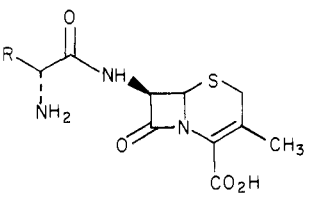
Table II. Dependence of β-Lactamase Stability on Steric Factors within the 3β-Acylamino Group



compd	R	K_m, μM	RV_{max}[a]	MIC, μg/mL: *S. aureus* (LAC+)/(LAC–)[b]
2		12	1	64/0.03
6		33	2	
7		5000	0.04	2/1

[a] RV_{max} = relative V_{max}. [b] LAC+ and LAC– = β-lactamase-positive and -negative strains, respectively.

Table III. Dependence of β-Lactamase Stability on the Aryl Moiety of the 3β-Acylamino Group



compd	R	zone reduction,[a] % *S. aureus*	*E. coli*
8		48	27
9		25	12

[a] Measured around a 10-μg disk in the presence of β-lactamase.

unsubstituted", cephamycins as "3α-methoxylated", and aztreonam as "4α-methylated".

3-Substitution. Acylamino Groups. Substrate binding to β-lactamase, as well as subsequent enzymatic processing to the hydrolysis product, is sensitive to the stereochemistry of the 3-substituent, particularly for 3-acylamino groups. When compared to benzylpenicillin (**2**), the 3α diastereomer **3** (Table I) is more β-lactamase stable and less active as an antibacterial agent (by approximately two orders of magnitude in each case).[9]

The pair of enantiomeric monobactams **4** and **5** illustrate an absolute requirement for 3β stereochemistry; the 3α analogue **5**, devoid of antibacterial activity, is also not a substrate for β-lactamases.[10]

Since the development of β-lactamase stable, but antibacterially inactive, β-lactams is a Pyrrhic victory, this perspective article will only deal with modifications consistent with good antibacterial activity.

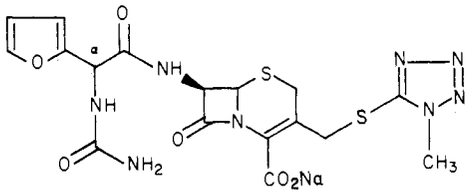
Emergence of resistant, β-lactamase-producing strains quickly followed the use of penicillin G against Gram-positive bacteria. Within a few years the incidence of β-lactamase-producing staphylococci in hospital practice was >50%. The solution to this problem had to await methodology for the commercial production of 6-APA (6-aminopenicillanic acid), however. Acylation of 6-APA with aromatic carboxylic acids, particularly those with sterically demanding ortho substituents, led to the group of penicillins exemplified by methicillin (**7**). The data in Table II demonstrate the contribution of the *o*-methyl groups to the β-lactamase stability of methicillin (compare **6** and **7**).[11]

Subtle structural changes also give rise to enhanced β-lactamase stability. Selwyn[12] has utilized a new test to demonstrate that cephradine (**9**) is more stable to β-lactamases than is the aromatic parent cephalexin (**8**) (Table III). Breuer et al.[13] reported the "L" side-chain diastereomer **11** was much more active against β-lactamase-producing bacteria than was the normally encountered "D" diastereomer **10** (Table IV).

Dramatic effects attend the introduction of α-alkoxyimino groups into the 3β-arylacetylamino moiety. The increased β-lactamase stability of cefuroxime, the prototype of this class, is attributable to its oxime moiety. Combination of 2-aminothiazol-4-yl as the aryl group and *syn*-methoxyimino as the α-substituent gives rise to the side chain of many β-lactamase-stable third-generation cephalosporins. Data[14] (Table V) for the pair **12** and **13** illustrate this effect. As indicated by the pair **14** and **15**,[15]

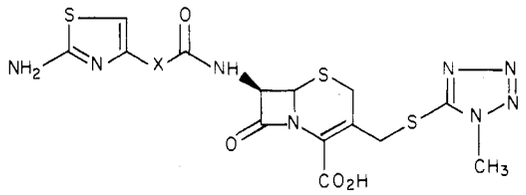
(9) Sawai, T.; Saito, T.; Mitsuhashi, S. *J. Antibiot.* **1970**, *10*, 488.
(10) Unpublished results, The Squibb Institute for Medical Research.
(11) Depue, R. H.; Moat, A. G.; Bondi, A. *Arch. Biochem. Biophys.* **1964**, *107*, 374.
(12) Selwyn, S. *J. Antimicrob. Chemother.* **1977**, *3*, 161.
(13) Breuer, H.; Treuner, U. D.; Schneider, H. J.; Young, M. G.; Basch, H. I. *J. Antibiot.* **1978**, *31*, 546.
(14) Moreta, K.; Normura, H.; Numata, J.; Ochiai, M.; Yoneda, M. *Philos. Trans. R. Soc. London, Ser. B* **1980**, *289*, 181.
(15) Gordon, E. M.; Sykes, R. B. In "Chemistry and Biology of β-Lactam Antibiotics"; Morin, R. B.; Gorman, M., Eds.; Academic Press: New York, 1982; Vol. 1.

Table IV. Dependence of β-Lactamase Stability on Stereochemistry within the 3β-Acylamino Group



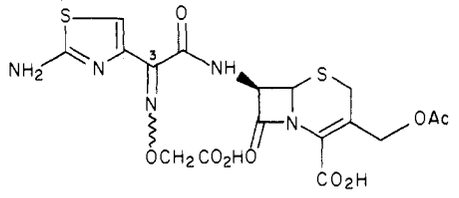
compd	stereochem at C-α	MIC, μg/mL: *E. cloacae* (LAC+)	MIC, μg/mL: *P. rettgeri* (LAC+)
10	D	25	12.5
11	L	0.6	0.4

Table V. Dependence of β-Lactamase Stability on the Presence of an α-*syn*-Methoxyimino Moiety within the 3β-Acylamino Group



compd	X	MIC, μg/mL: *Ent. cloacae* (LAC+)
12		>100
13		1.6

Table VI. Dependence of β-Lactamase Stability on Oxime Stereochemistry within the 3β-Acylamino Group



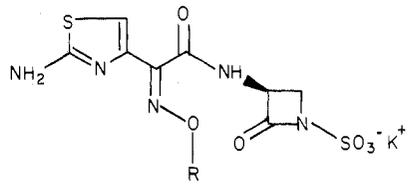
compd	stereochem at C-3	RHR: RTEM	RHR: K1
14	syn	<0.1	3
15	anti	2111	101
cefotaxime		100	100

the syn isomers are much more β-lactamase stable than the anti isomers (Table VI) and also display more antibacterial activity (data not shown).

Substituents within the oxime moiety influence both the spectrum of antibacterial activity and the β-lactamase stability, as indicated by comparison of **16**–**18**.[10] Note the modest increase in stability by the negatively charged carboxylate group (Table VII).

Incorporation of a 3α-methoxyl group leads to an increase in β-lactamase stability in both bicyclic[15] (penicillins, cephalosporins, and oxacephalosporins) and monocyclic (monobactam) β-lactam antibiotics. Recent observations that led to the development of moxalactam (**22**) illustrate this point.[16] Comparison of the pair **19** and **21** and the pair **20** and **22** clearly establishes increased stability to the broad-spectrum RTEM enzyme for methoxylated analogues. Stability to the cephalosporinase (as indicated by good antibacterial activity against *Enterobacter cloacae* 233) is largely due to the negatively charged carboxylate function (Table VIII). The excellent β-lactamase stability of BRL17421 (temocillin, **23**) is due to a similar cooperation

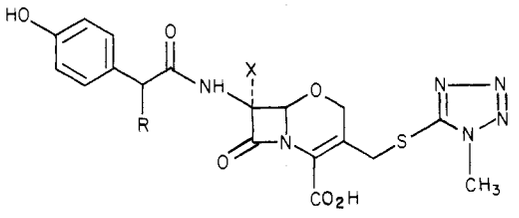
Table VII. Dependence of β-Lactamase Stability on Oxime Substitution within the 3β-Acylamino Group



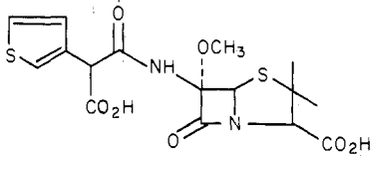
compd	R	RHR: RTEM	MIC, $10^4/10^6$ cfu,[a] μg/mL: *E. coli* (RTEM+)
16	CH_3	12	3.1/25
17	$C(CH_3)_2H$	7	25/100
18	$C(CH_3)_2CO_2^-K^+$	2.3	0.8/3.1
cephaloridine		100	

[a] cfu = colony forming units.

Table VIII. Dependence of β-Lactamase Stability on the Presence of a 3α-Methoxyl Group



compd	R	X	MIC, μg/mL: *E. coli* (RTEM+)	MIC, μg/mL: *E. coli* JC-S (LAC-)	MIC, μg/mL: *Ent. cloacae* 233 (LAC+)
19	H	H	>100	0.8	>100
20	CO_2H	H	50	0.2	0.4
21	H	OCH_3	0.8	0.1	100
22	CO_2H	OCH_3	0.4	0.2	0.2



23

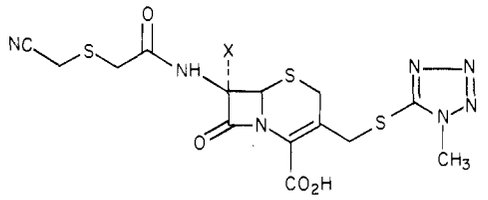
of a 3α-methoxyl group and a negatively charged 3β-acylamino group.

Larger alkoxyl groups also impart stability to β-lactamase, but at the expense of intrinsic activity (compare **24**–**27**, Table IX).[17]

Incorporation of a 3α-methoxyl group in penicillins and cephalosporins also leads to increased stability to chemical hydrolysis (ca. 500 and 30%, respectively).[18] The 3α-methoxyl group in monobactams, on the other hand, lends

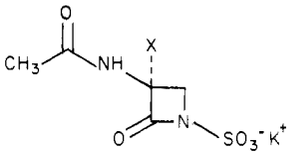
(16) Murakami, K.; Takasuka, M.; Motokawa, K.; Yoshida, T. *J. Med. Chem.* **1981**, *24*, 88.
(17) Nakao, H.; Yanagisawa, H.; Ishihara, S.; Nakayama, E.; Ando, A.; Nakazawa, J.; Shimizu, B.; Kaneko, M.; Nagano, M.; Sugawara, S. *J. Antibiot.* **1979**, *32*, 320.
(18) Indelicato, J. M.; Wilham, W. L. *J. Med. Chem.* **1974**, *17*, 528.

Table IX. Dependence of β-Lactamase Stability and Intrinsic Activity on the Size of the 3α-Alkoxy Group



compd	X	MIC, μg/mL: *E. coli* (RTEM+)	*E. coli* NIHJ (LAC–)
24	H	12.5	0.8
25	CH_3O	0.8	0.8
26	CH_3CH_2O	50	50
27	$CH_3(CH_2)_2O$	200	>200

Table X. Dependence of β-Lactamase Stability and Chemical Stability of Monobactams on the Presence of a 3α-Methoxyl Group



compd	X	RV_{max}: RTEM	% remaining after 24 h (pH 7, 37 °C)
28	H	12	>80
29	CH_3O	<0.01	<1
cephaloridine		100	

increased stability to β-lactamase but dramatically decreased stability to chemical hydrolysis (compare **28** and **29**, Table X).

α-Hydroxyalkyl Substituents. The discovery of thienamycin and related carbapenems added another dimension to β-lactam structure–activity relationships and served to redirect ongoing penem research to include α-hydroxyalkyl groups. Data (Table XI) for penems **30–34**[19] illustrate several interesting points: (1) 3α-hydroxyalkyl substituents confer significantly more β-lactamase stability than do the diastereomeric 3β analogues (compare **31** and **32** with **30**), although both diastereomers have intrinsic activity comparable to the 3-unsubstituted, β-lactamase-sensitive parent **30**; (2) the 3α(*R*)- and -(*S*)-(α-hydroxyethyl) diastereomers **33** and **34**, respectively, are of comparable β-lactamase stability, but the *R* diastereomer **33** has much more intrinsic activity.

Within the "parent" carbapenem series, similar relationships are demonstrated by comparison of **35–38**.[20] Additionally, it is apparent that incorporation of a negatively charged sulfate moiety significantly enhances β-lactamase stability (compare **35** and **36**, Table XII).

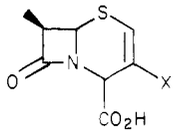
4-Substitution. In bioactive bicyclic β-lactams, all 4-substituents are necessarily β; epimeric 1,4α-bridged compounds are devoid of antibacterial activity. In the cephalosporin series, variation of the oxidation state of the 4β-sulfur atom leads to dramatic changes in β-lactamase stability (compare **39–41**). Of particular interest is the dependence of increased stability on (*S*)-sulfoxide stereochemistry (compare **40** and **41**, Table XIII).[10,21] The exchange of oxygen for sulfur leads to a decrease in β-lactamase stability and an increase in intrinsic activity attributed to increased chemical reactivity (compare **42** and **43**,[16] Table XIV).

4-Substitution in monocyclic β-lactams has extraordinary effects on β-lactamase stability. Introduction of either a 4β- or 4α-methyl group leads to increased β-lactamase stability and increased intrinsic activity. This is most pronounced for monobactams, such as **44–46** (Table XV), which also contain 3β-acylamino moieties incorporating aminothiazole oximes.[10] Comparison of **45** to **44** and **46** demonstrates that 4β-methylation leads to more β-lactamase stability than 4α-methylation. As expected, these formal β-lactamase stability studies are mirrored in the MIC data, which also indicate increased intrinsic activity (compare the activities of **44–46** against the *lac*-strains) for 4-methylated analogues.

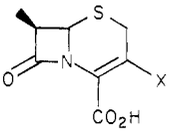
The steric nature of the effect is indicated by reference to **47–49**; the 4β-ethyl analogue **47** is the most β-lactamase stable against the K1 enzyme (Table XVI; note that activities against the RTEM+ organism are identical, however).[10]

Examination of the monosulfactam triad **50–52** reveals the opposite effect of 4-methylation. Both 4-methyl analogues are less β-lactamase stable than the unsubstituted parent **50** (Table XVII).[10]

1-Substitution. Certain substructures within bicyclic β-lactams may be formally viewed as 1-substituted variants; these are exemplified by the Δ^2/Δ^3 cephem pair A and B. The Δ^2 analogue A contains an allylic amide



A



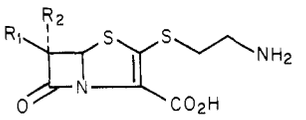
B

function, while the Δ^3 parent B has an enamide function in which the β-lactam nitrogen lone pair can be delocalized. Depending on the nature of X, further electron withdrawal (donation) by resonance and/or inductive effects may be possible. Frère et al.[22] have recently discussed these effects in terms of the relationship between intrinsic chemical reactivity and enzymatic processing. In these cases the bicyclic ring system presents a flexible framework for the interplay of steric, electronic, and geometric factors. The monocyclic β-lactams eliminate one of these parameters: in the cases examined by X-ray crystallography, the β-lactam amide bond and the first atom of the N-1 activating group are coplanar.

The report[23] that both nocardicin A and dethiabenzylpenicillin are poor substrates for certain β-lactamases was the first indication that monocyclic β-lactams would bind and be processed by these enzymes. Recent results dem-

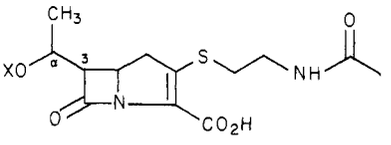
(19) Obya, S.; Utsui, Y.; Sugawara, S.; Yamazaki, M. *Antimicrob. Agents Chemother.* **1982**, *21*, 492.
(20) Basker, M. J.; Boon, R. J.; Hunter, P. A. *J. Antibiot.* **1980**, *32*, 878.
(21) When a more β-lactamase-sensitive reference substrate (cephaloridine) was used, essentially no differences in β-lactamase stability among these compounds was reported: Durckheimer, W.; Klesel, N.; Limbert, M.; Schrinner, E.; Seeger, K.; Seliger, H. in "Recent Advances in the Chemistry of β-Lactam Antibiotics" (*Spec. Publ. Chem. Soc. no. 38*); Gregory, G. I., Ed.; The Royal Society of Chemistry, London, 1981, Chapter 4.
(22) Frère, J.-M.; Kelley, J. A.; Klein, D.; Ghuysen, J.-M.; Claes, P.; Vanderhaeghe, H. *Biochem. J.* **1982**, *203*, 223.
(23) Pratt, R. F.; Anderson, E. G.; Odeh, I. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 1266.

Table XI. Dependence of β-Lactamase Stability on the Nature and Stereochemistry of 3-(α-Hydroxyalkyl) Groups in Penems



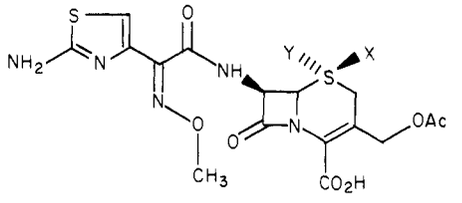
compd	R_1	R_2	RV_{max}: *E. coli* 609 (LAC+)	MIC, μg/mL: *E. coli* 609 (LAC+)	MIC, μg/mL: *E. coli* NIHJ (LAC–)
30	H	H	75	50	0.8
31	CH_2OH	H	10	25	3.1
32	H	CH_2OH	<0.1	3.1	1.5
33	H	$CH(OH)CH_3$ (R)	<0.1	1.5	0.8
34	H	$CH(OH)CH_3$ (S)	<0.1	25	12.5
cephaloridine			100		

Table XII. Dependence of β-Lactamase Stability on the Nature and Stereochemistry of the 3-(α-Hydroxyalkyl) Group in Carbapenems



compd	stereochem at the following positions: 3	α	X	MIC, μg/mL: *E. coli* (RTEM+)	MIC, μg/mL: *E. coli* (LAC–)
35	β	*R*	H	25	0.2
36	β	*R*	$SO_3^-K^+$	1.6	0.2
37	α	*R*	H	6.2	6.2
38	α	*S*	H	0.8	0.8

Table XIII. Dependence of β-Lactamase Stability on the Oxidation State of Sulfur in Cephalosporins

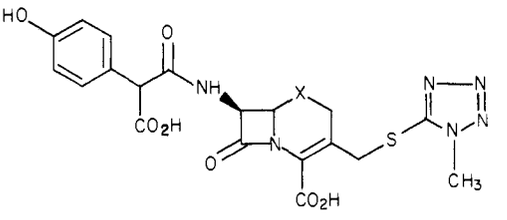


compd	X	Y	RHR: RTEM	MIC, 10^6 cfu, μg/mL: *E. coli* (RTEM+)
39			100	0.1
40	O		0	0.1
41		O	160	1.6

onstrate that nonmethoxylated monobactams can serve as good substrates. In the case of aztreonam (**53**, a poor substrate), the simple hydrolysis product **54** has been identified[24] as the reaction product of aztreonam, water, and RTEM, K1, or P99 β-lactamases (Scheme II).

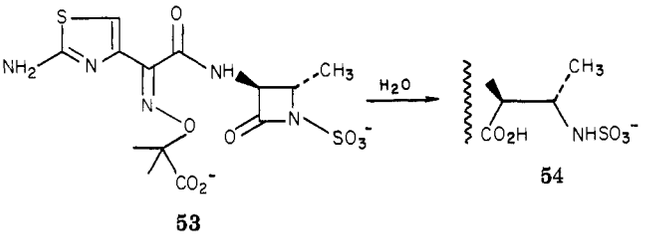
Consideration of the homologous series **55-58** (Table XVIII) with reference to the monobactam parent **53** demonstrates a wide range of stability to the RTEM enzyme, accompanied by essentially equivalent intrinsic activity.[10] The possibility of combining increased β-lactamase stability and intrinsic activity (noted most remarkably with 4-methylated monobactams) is one motivation for the

Table XIV. Dependence of β-Lactamase Stability on the 3β-Heteroatom in Cephems



compd	X	RHR: RTEM	MIC, μg/mL: *E. coli* NIHJ (LAC–)
42	O	19	0.2
43	S	0.96	3.13
cephaloridine		100	

Scheme II



53 **54**

continuing effort with monocyclic, as well as bicyclic, β-lactams.

Conclusion

One objective of collecting substructural information of this type is to synthesize it into a detailed model of enzyme–substrate interaction. For β-lactamases and their substrates, the β-lactams, this is not yet possible. Except for the demonstration[25] that they are "serine proteases", little is actually known about the active site of Gram-negative β-lactamases. Fitting various β-lactams, with their appended 1-, 3-, and 4-substituents, to a hypothetical binding site is a task beyond the imagination of the present reviewer.

An interesting aspect of this question is the preferred direction of attack of nucleophiles (enzymatic or chemical) on the β-lactam bond. Stereoelectronic theory[26,27] predicts 2β-nucleophilic attack for 1,4β-bridged β-lactams, although experimental evidence[28] and common assumption[29,30]

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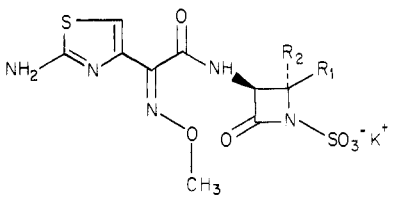
(25) Fisher, J.; Charnas, R. L.; Bradley, S. M.; Knowles, J. R. *Biochemistry* **1981**, *20*, 2726.

(26) Mock, W. L. *Biorg. Chem.* **1975**, *4*, 270.

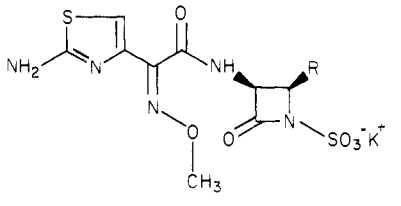
(27) Deslongchamps, P. *Tetrahedron*, **1975**, *31*, 2463.

Table XV. Dependence of β-Lactamase Stability on 4-Substitution in Monobactams



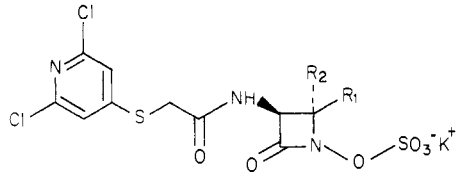
compd	R_1	R_2	RHR RTEM	RHR K1	MIC, $10^4/10^6$ cfu, μg/mL *E. coli* (RTEM+)	*E. coli* (RTEM−)	*K. aerogenes* (K1+)	*K. aerogenes* (K1−)
44	H	H	12	106	6.3/>100	0.4/1.6	100/>100	0.4/1.6
45	CH_3	H	0.3	6.4	0.1/0.1	<0.05/0.1	12.5/>100	0.05/0.1
46	H	CH_3	1	20	0.4/1.6	0.1/0.2	100/>100	0.2/0.2
cephaloridine			100	100				

Table XVI. Dependence of β-Lactamase Stability on 4β-Alkyl Substitution in Monobactams



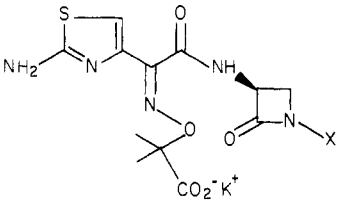
compd	R	MIC, $10^4/10^6$ cfu, μg/mL *E. coli* (RTEM+)	*E. coli* (RTEM−)	*K. aerogenes* (K1+)	*K. aerogenes* (K1−)
47	CH_3CH_2	0.1/0.4	0.2/0.4	0.2/0.8	0.1/0.4
48	CH_2=CH	0.1/0.2	0.2/0.2	6.3/>100	<0.05/0.1
49	CH≡C	0.4/0.2	0.1/0.4	100/>100	0.8/0.8

Table XVII. Dependence of β-Lactamase Stability on 4-Substitution in Monosulfactams



compd	R_1	R_2	RHR: *S. aureus* (PC1)	MIC, μg/mL *S. aureus* (LAC+)	*S. aureus* (LAC−)
50	H	H	≤0.5	0.2	0.1
51	CH_3	H	1.0	1.6	0.1
52	H	CH_3	2.6	6.3	0.2

Table XVIII. Dependence of β-Lactamase Stability on 1-Substitution in Monocyclic β-Lactams



compd	X	MIC, $10^4/10^6$ cfu, μg/mL *E. coli* (RTEM+)	*E. coli* (RTEM−)
53	SO_3^-	3.1/25	0.4/0.4
55	OSO_3^-	50/>100	0.4/1.6
56	$PO_3CH_3^-$	0.8/3.1	1.6/1.6
57	$OPO_2CH_3^-$	12.5/50	0.8/1.6
58	$CONSO_2CH_3^-$	0.8/3.1	1.6/1.6

suggest the opposite. The evidence gathered here demonstrates marked effects on β-lactamase stability for 3β- and 4β-substitution. One interpretation of this could involve "warding off" the enzymatic nucleophile as it approaches from the β-side. Another interpretation involves subtle effects of α- and β-substitution on the exact positioning of enzyme and substrate.

A second objective is to be able to predict the intrinsic activity and β-lactamase stability of novel β-lactam structures. The relationships among intrinsic activity, β-lactamase stability, and intrinsic chemical reactivity have been recently analyzed.[22] The conclusion reached is that "goodness of fit" of the substrate β-lactam to the enzyme is more important than intrinsic chemical reactivity. This is clearly illustrated by data for **28** and **29**: the chemically more reactive substance **29** is the more β-lactamase stable; interestingly, for this pair, methoxylated analogue **29** also exhibits higher intrinsic activity against Gram-negative bacteria. In the penicillin series, methoxylation increases chemical stability and β-lactamase stability and markedly decreases intrinsic activity (temocillin and related 3β-(α-carboxyarylacetylamino) derivatives retain useful activity against Gram-negative bacteria, however). In the absence of detailed information about β-lactamase and transpeptidase binding sites, a priori evaluation of "goodness of fit" becomes problematic.

If the information collected here is utilized, it should be possible to modify a given structure, whether generated

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(29) Topp, W. C.; Christensen, B. G. *J. Med. Chem.* **1974**, *17*, 242.
(30) Boyd, D. B.; Lunn, W. H. *J. Antibiot.* **1979**, *32*, 855.

as part of a synthetic program or by fermentation, in order to increase β-lactamase stability. For the case of penicillin, however, no general solution to combining both high intrinsic activity and β-lactamase stability has yet emerged. These data should also be useful as a catalogue of substructures to be correlated with other aspects of the interactions among β-lactams, β-lactamases, and β-lactamase-producing bacteria. These include the ability to induce the formation of β-lactamase by Gram-negative bacteria, to bind tightly to the inducible β-lactamases, and to be excluded, by permeability changes, from entry into Gram-negative organisms. Each of these phenomena has been implicated as a mechanism of resistance of Gram-negative bacteria to β-lactamase-stable β-lactam antibiotics.[31]

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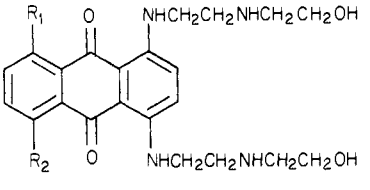
Communications to the Editor

5-[(Aminoalkyl)amino]-Substituted Anthra[1,9-*cd*]pyrazol-6(2*H*)-ones as Novel Anticancer Agents. Synthesis and Biological Evaluation

Sir:

The anthracycline antitumor antibiotics daunorubicin and doxorubicin have a well-established place in the clinical treatment of various malignant diseases.[1] Unfortunately, these drugs possess some serious toxicities, especially cardiotoxicity, ranging from delayed and insidious cardiomyopathy to irreversible congestive heart failure. Recent research efforts have directed attention toward developing new DNA-complexing agents that minimize this side effect, notably second-generation anthracyclines, such as aclacinomycin A,[1b] and several anthracenediones.[2,3] Of the latter, ametantrone (1) and



1, $R_1 = R_2 = H$ (ametantrone)
2, $R_1 = R_2 = OH$ (mitoxantrone)

mitoxantrone (2) are currently undergoing phase II clinical trials. In-depth studies on mitoxantrone (2)[4] have suggested activity principally in the treatment of breast cancer and the acute leukemias, with less encouraging results against other tumors when compared to doxorubicin. However, cardiotoxicity studies show that while occasional episodes have been reported in patients on prolonged treatment with 2, the overall incidence of cardiac failure has been relatively low.

We now report the synthesis and initial biological evaluation of two representatives, compounds 8 and 10, of a novel class of highly active anticancer DNA-complexing agents, namely, 5-[(aminoalkyl)amino]anthra[1,9-*cd*]pyrazol-6(2*H*)-ones (hereafter referred to as anthrapyrazoles). It was anticipated that such chromophore modification of the anthracenedione nucleus might provide a unique class of DNA-complexing agents with diminished or absent cardiotoxicity vis-a-vis a reduced tendency to semiquinone free radical formation. Such rationale has been applied to the synthesis of chromophore-modified anthracyclines.[5-7]

Chemistry. Scheme I delineates the synthetic pathway to the target anthrapyrazoles, 8 and 10.[8]

Alkylation of 1,4-dichloro-5-hydroxy-9,10-anthracenedione (3)[9] with powdered K_2CO_3 and benzyl bromide in refluxing acetone gave a 93% yield of the benzyl ether 4, mp 124–127 °C. Condensation of dichloroether 4 with 3 equiv of 2-[(2-hydrazinoethyl)amino]ethanol[10] in anhydrous Me_2SO afforded a 50% crude yield of regioisomers 5 and 6 in ca. 4:1 ratio, respectively, by HPLC. Partial separation of isomers was achieved by flash silica gel chromatography. The faster eluting component was the minor isomer, 6, mp 172–175 °C, and the slower eluting component was the major isomer, 5, mp 142–143 °C. Reaction of 5 with an excess of 2-[(2-aminoethyl)amino]ethanol in refluxing pyridine gave the "two-armed" compound 7, mp 157–159 °C. Hydrogenolysis of the benzyl protecting group with Pearlman's catalyst[11] in glacial AcOH, followed by dihydrochloride salt formation, gave the target 7-hydroxy compound 8 in 88% yield, mp 265–270 °C dec. Application of the same transformations to 6 afforded the 10-hydroxy isomer 10, mp 260–267 °C dec, via benzylated intermediate 9, mp 178–180 °C.

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(4) Smith, I. E. *Cancer Treat. Rev.* 1983, *10*, 103–115.
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(11) Pearlman, W. M. *Tetrahedron Lett.* 1967, 1663–1664.

0022-2623/84/1827-0253$01.50/0