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Understanding the longevity of the β-lactam antibiotics and of antibiotic/β-lactamase inhibitor combinations

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ABSTRACT

Microbial resistance necessitates the search for new targets and new antibiotics. However, it is likely that resistance problems will eventually threaten these new products and it may, therefore, be instructive to review the successful employment of β-lactam antibiotic/β-lactamase inhibitor combinations to combat penicillin resistance. These combination drugs have proven successful for more than two decades, with inhibitor resistance still being relatively rare. The β-lactamase inhibitors are mechanism-based irreversible inactivators. The ability of the inhibitors to avoid resistance may be due to the structural similarities between the substrate and inhibitor.

1. Introduction

The discovery of the β-lactam antibiotics in the early twentieth century represented a turning point in the struggle against pathogenic bacteria. These relatively inexpensive and highly efficient semi-synthetic products have been the mainstay of anti-infective chemotherapy for the past sixty years. However, the appearance and accelerating evolution of hydrolytic enzymes, the β-lactamases, now threaten these drugs, thus necessitating the genomic search for new bacterial targets and new classes of drugs. However, as we undertake this quest, it may be useful to review the successful counter-measures that have been so effective in the β-lactam antibiotic arena. Such strategies may be instructive in our efforts to forestall resistance to future antibiotic classes.

The two most common forms of resistance to β-lactam antibiotics are the production of (one or more) β-lactamases and the development of an altered target PBP (penicillin-binding-protein), such as PBP2a of methicillin-resistant *Staphylococcus aureus* (MRSA). Despite recent structural information on PBP2a [1], the former are the better understood (from a structural and mechanistic perspective) and more commonly observed of the resistance mechanisms, and will thus be the focus of this review. There are four different classes of β-lactamases, A through D. Classes A, C, and D β-lactamases are serine enzymes, while class B are zinc metalloenzymes. Their origin is ancient, presumably evolved to combat β-lactams of natural origin, but their development has undoubtedly been influenced by clinical administration of β-lactam antibiotics. The serine β-lactamases are believed to have evolved from the antibiotic target PBP's [2–4], through inclusion of water and other requisite hydrolytic machinery in the active site. β-lactamases are capable of hydrolyzing only activated carboxylic acid derivatives (e.g. structurally appropriate β-lactams and esters) while leaving more stabilized amide bonds unscathed. The precise structural and mechanistic reason for this selectivity is the subject of some speculation. Ambler [5] first proposed this sequence based

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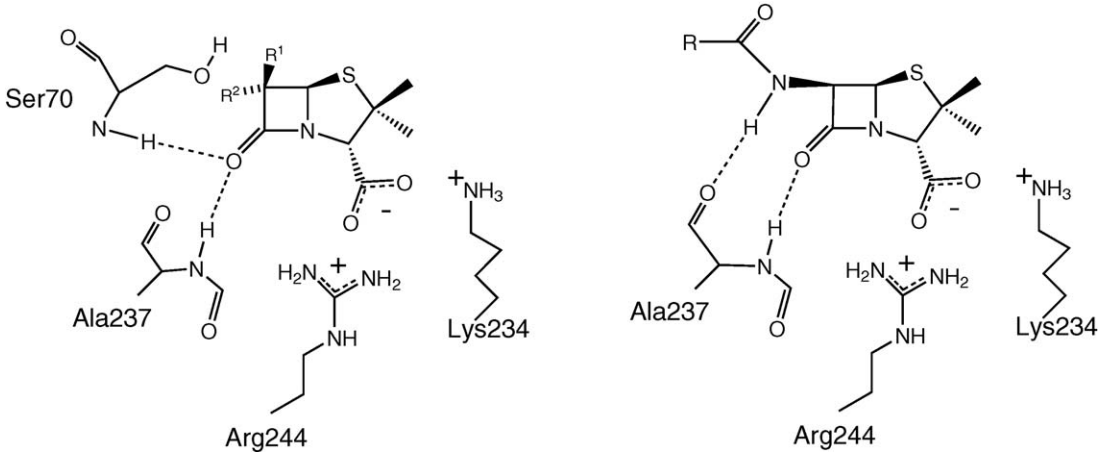


Fig. 1 – Simplified visualization of key interactions in the binding site of TEM-1 β-lactamase (two structures shown for clarity).

classification scheme, which initially involved dividing the known β-lactamases into the class A serine-β-lactamases and into class B metallo-β-lactamases, based on the then available sequences of only four enzymes. Within a few years, two new classes of serine β-lactamases, the class C cephalosporinases [6] and then the class D oxacillinases [7] were identified. Class A enzymes are usually plasmid-mediated, are known as penicillinases (since they tend to prefer penicillins over cephalosporins as substrates), and representative enzymes include (among others) TEM, SHV, and PC subclasses. Some class A β-lactamases have evolved to accept expanded spectrum cephalosporins as substrates and have become known as extended spectrum β-lactamases (ESBL's). Class B metallo-β-lactamases typically have an extremely broad substrate specificity, that includes not only penicillins and cephalosporins, but also the (usually β-lactamase resistant) carbapenems. These zinc metalloenzymes are typified by (among others) the IMP and VIM β-lactamases. Class C serine β-lactamases tend to be chromosomal (although several plasmid-borne class C β-lactamases have now been observed), are often referred to as AmpC β-lactamases, and tend to prefer cephalosporins as substrates. Class D β-lactamases have an unusually high substrate preference for oxacillin and related penicillins are thus referred to as oxacillinases. Functional classification schemes are also utilized [8].

One reasonably effective strategy for countering β-lactamase-mediated penicillin resistance has been the co-administration of the β-lactam antibiotic together with an inhibitor of one or more of the hydrolytic β-lactamase enzymes [9–18]. Current commercial inhibitors are effective only against the class A serine β-lactamases. Countering recent pharmaceutical product trends toward extremely tight binding reversible inhibitors, the known β-lactamase inhibitors are mechanism-based irreversible inactivators. Structurally, the relative successfulness of this approach probably stems from the resemblance of the inhibitors to the natural substrates themselves, thus making it difficult for the enzymes to mutate non-recognition of the inhibitors and still retain their full hydrolytic capability [19]. It should be noted that current commercial inhibitors share the bicyclic fused four-five membered ring system of the penicillins and also sp^3-hybridized nature of the C3 carbon, thus potentially explaining their preferential inactivation of class A penicillinases.

Key recognition elements of class A serine β-lactamases are illustrated in Fig. 1, which depicts the TEM-1 β-lactamase active site with a generic substrate. Such elements include a positively charged pocket to recognize the carboxylate, an oxyanion hole to recognize the β-lactam carbonyl oxygen and to accelerate formation of the tetrahedral intermediate, and well as a key hydrogen bonding interaction with the C6 acylamino group.

The serine β-lactamases operate via a multistep process involving the formation and hydrolytic destruction of an intermediate acyl-enzyme. Most β-lactam inhibitors of the serine β-lactamases function through the formation of a hydrolytically stabilized ester. Depending on the specific inhibitor, this acyl-enzyme may be stabilized electronically (i.e., by favorable resonance interactions that improve the stability of the acyl-enzyme ester bond to the serine), stabilized by covalently bonding to a second nucleophilic residue in the active site, or stabilized due to its position in the active site (e.g., the carbonyl of the bound inhibitor may be removed from the oxyanion hole and/or the hydrolytic water). This last type of stabilization may result from movement of the inhibitor or from a conformational change in the enzyme itself. β-lactam-containing compounds that inactivate serine β-lactamases are typically also substrates of the enzyme, with partitioning of the two processes (turnover and inhibition) at the acyl enzyme stage. For any particular inhibitor, the substrate/inhibitor (turnover) ratio can range from below 10 to nearly 10,000.

2. Commercial β-lactamase inhibitors

Current commercial inhibitors include clavulanic acid, sulbactam and tazobactam, shown below which are administered in the antibiotic/inhibitor combinations: Augmentin™ (amoxicillin/clavulanic acid) [20,21], Timentin™ (ticarcillin/clavulanic acid) [22], Unasyn™ (ampicillin/sulbactam) [23], and Zosyn™ (piperacillin/tazobactam) [24] (Fig. 2). In particular, these commercial combinations are effective against

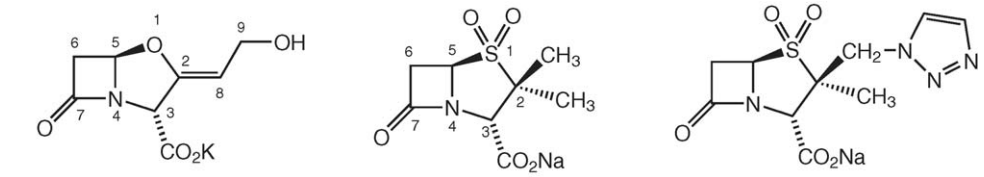


Fig. 2 – Current commercial β-lactamase inhibitors.

susceptible organisms expressing Ambler class A enzymes [25], which remain the most commonly encountered. These inhibitors are all unsubstituted at C6 and have related inhibitory mechanisms.

2.1. *Clavulanic acid*

The amoxicillin/clavulanate combination represents a broad-spectrum antibacterial, that has been in use for more than 20 years and continues to be widely utilized. The mechanism of inactivation of β-lactamases by clavulanic acid was initially studied by the Knowles group [26,27], and further refined by the Mobashery group [28]. Initial crystallographic studies were performed, on the class A PC1 β-lactamase from *Staphylococcus aureus*, by Chen and Herzberg [29], and ESIMS studies were performed by the Schofield group [30]. A scheme for this inhibition is shown in Scheme 1.

2.2. *Sulbactam*

Knowles also studied sulbactam [31] and proposed a nearly identical inhibitory mechanism, using the sulfone sulfur as a (sulfinate) leaving group in place of the enol ether oxygen of clavulanate as shown. Mobashery added data solidifying and elaborating this mechanism [32]. One should note the similarities between Schemes 1 and 2, in particular the formation of two structurally related iminium ions **4** and **15**, respectively, as well as their partitioning into transient (**7** and **16**, respectively) and irreversibly inhibited (**11** and **18**, respectively) species.

2.3. *Tazobactam*

Tazobactam is structurally related to sulbactam, the sole structural difference being the addition of a triazole group on the C2 β-methyl group. This modification improves activity against several β-lactamases (IC_{50} 2–100-fold better) and microorganisms (MIC values 4–64-fold lower). The partition ratios, against representative class A β-lactamases for clavulanate, sulbactam and tazobactam are 160, 10,000, and 125, respectively. Tazobactam also has modest activity against some class C β-lactamases, but not enough to be clinically useful. Crystallographic [33,34] and ESIMS [35,36] studies support a mechanism closely related to that of sulbactam and clavulanic acid.

A number of groups have continued to report improvements in β-lactamase inhibitory activity by substitution at (penicillin) C2′ [37,38]. Buynak et al. [39] have recently reported a C3 homologue of sulbactam with improved activity against class C β-lactamases.

3. Carbapenems

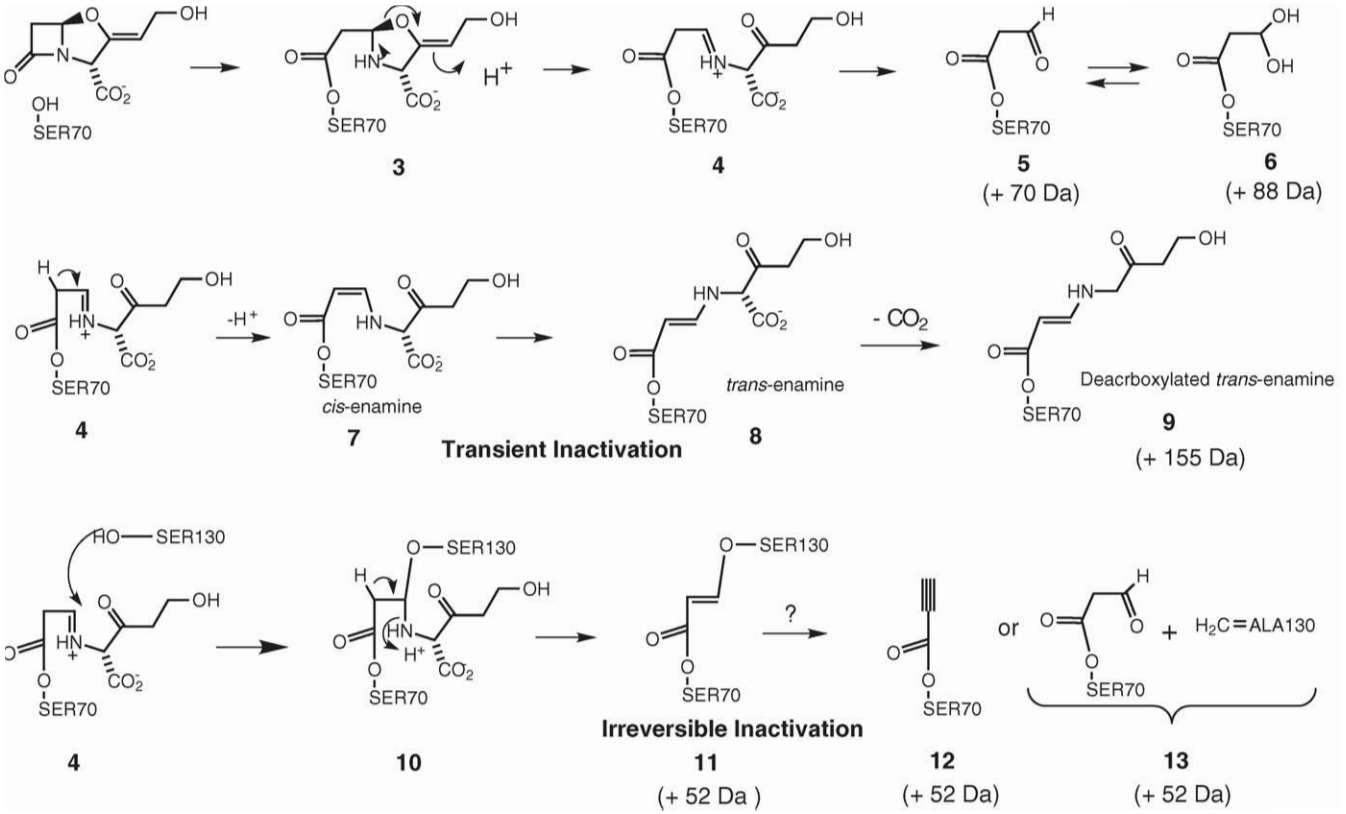
While most β-lactamase inhibitors lack significant antibacterial activity, the carbapenems, having a C6 hydroxyethyl side chain, are recognized as both broad-spectrum antibiotics and also as either very poor substrates or as competitive inhibitors of many serine β-lactamases. Commercial carbapenems include imipenem (**19**) /cilastatin (Primaxin™), Panipenem (**20**), Biapenem (**21**), Meropenem (**22**) (Merrem™), and Ertapenem (**23**) (Invanz™) (Fig. 3). The cilastatin is included in the imipenem mixture as an inhibitor of the renal metabolic enzyme, dehydropeptidase (DHP-1), whereas the C1 β-methyl group of later carbapenems renders the molecules poorer substrates of DHP.

The carbapenems are regarded as the β-lactam class with the broadest spectrum of antibacterial activity, with resistance being relatively rare. These antibiotics are also inhibitors (or very slow substrates [40]) of most serine β-lactamases. However, carbapenems are substrates of class B metallo-β-lactamases [41,42] and also substrates of a few specific serine β-lactamases [43,44]. All carbapenems of current commercial interest retain the C6 α-hydroxyethyl side chain of thienamycin.

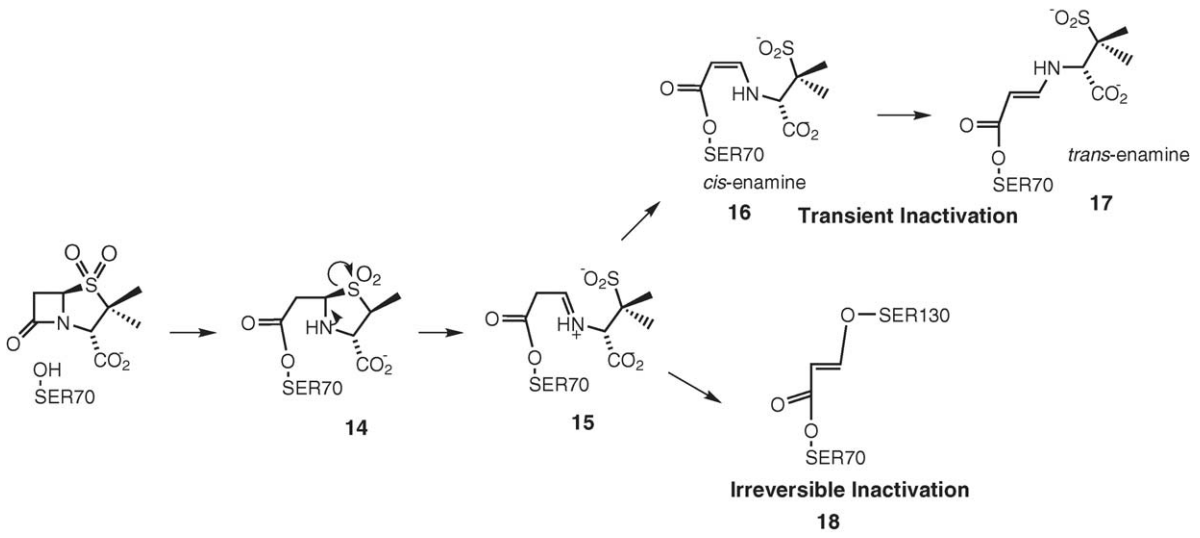
Kinetic investigations [40,45–48] and three crystallographic studies, including complexes of imipenem with TEM-1 (class A) [49] and AmpC (class C) [50], and a complex of meropenem with OXA-13 (class D) [51], have been published. In both imipenem structures, the reason for inhibition appears to be that the carbonyl oxygen of the resultant acyl-enzyme has been displaced from the oxyanion hole. In the case of the meropenem structure with the class D oxacillinase, the carbonyl oxygen is in the oxyanion hole, but a conformational change of the enzyme has displaced the hydrolytic water to a distance of nearly 5 Å from the carbonyl carbon. The C6 α-hydroxyalkyl side chain may play a role in the positional movements that occur subsequent to enzyme acylation.

4. 6-(Hydroxyalkyl)penicillanates

Given the potent dual (antibiotic/inhibitor) activity of the carbapenems, a number of researchers have investigated incorporating similar C6 functionality into the penicillins and



Scheme 1 – Mechanism for inhibition of class A β-lactamases by clavulanic acid.



Scheme 2 – Mechanism for the inactivation of class A β-lactamases by sulbactam.

their corresponding sulfones. Early researchers discovered that C6 hydroxyalkyl derivatives, such as **24**, were stabilized toward β-lactamase, with the loss of some antibacterial activity [52,53]. DiNinno et al., working at Merck, first prepared simple 6-(hydroxyethyl)penicillanates **25** (lacking the acylamino group; the hydroxyethyl group being analogous to the carbapenem side chain) and showed these compounds to be of modest antibacterial activity [54]. Subsequently, a number of companies simultaneously filed patents describing the generation of C6 hydroxyalkyl penicillanates, such as sulfide **26**, and sulfone **27**, and claimed their usefulness as inhibitors of β-lactamase [55,56] (Fig. 4). The Pfizer group attempted to further develop benzimidazole-substituted (hydroxymethyl)penicillanates, such as **28**, which display both antibacterial activity (against Gram-positive and a few Gram-negative strains) as well as β-lactamase inhibitory activity [57]. Recently, Mobashery designed [58] and, with Samama [59], provided structures of the class A TEM-1 β-lactamase from *E. coli* inhibited with the C6 α-hydroxymethylpenam **29**, the NMC-A class A carbapenemase inhibited with the C6 α-[(1′-hydroxy-1′-methyl)ethyl]-penam **30** [60] (the corresponding hydroxymethyl analog was a substrate) and the class D Oxa-10 β-lactamase inhibited with the C6α-[(1′-hydroxy-1′-methyl)ethyl]penam **31** [61]. The Mobashery group has been able to utilize the hydroxyalkylpenicillanates as probes to determine the direction of addition of water to the acyl-enzyme [62]. Wyeth researchers evaluated the epimeric C6-(hydroxymethyl)penicillanates, both as sulfides and as sulfones, presenting data that the best inhibitors in this series were the sulfones (e.g., C6 β-(hydroxymethyl)penicillin sulfone **27** exhibited an IC_{50} value of 8 nM against TEM-1) [63]. They also investigated the potential for the inhibitory activity to be modulated through manipulation of the C2 substituent, with their most active molecule being the acrylonitrile **32** [64]. Recently, the Buynak group has prepared the corresponding mercaptomethyl analogs **33** and has demonstrated their activity as dual inhibitors of both serine-β-lactamases and also certain class B metallo-β-lactamases [65].

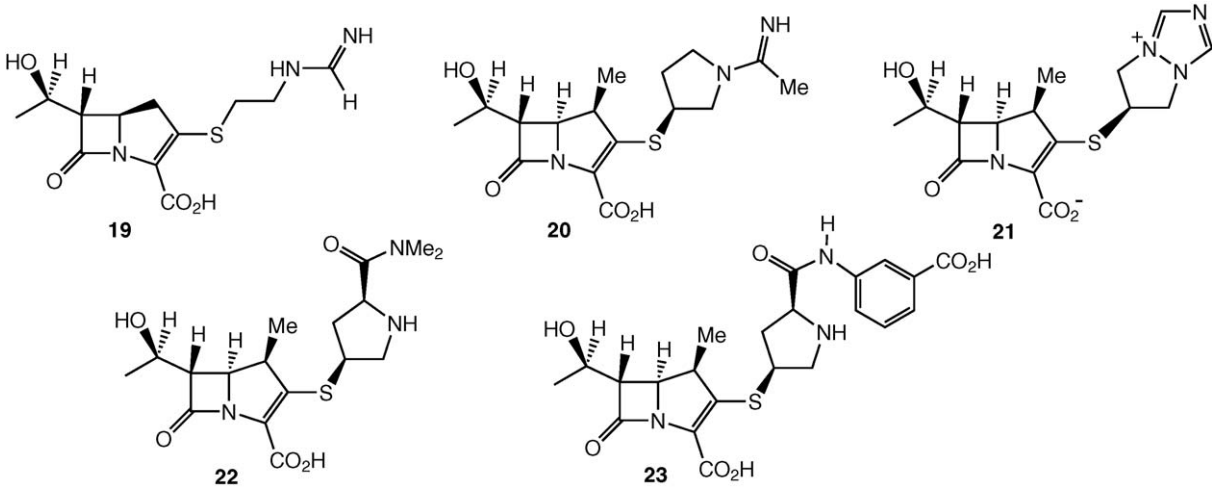


Fig. 3 – Commercial carbapenem antibiotics.

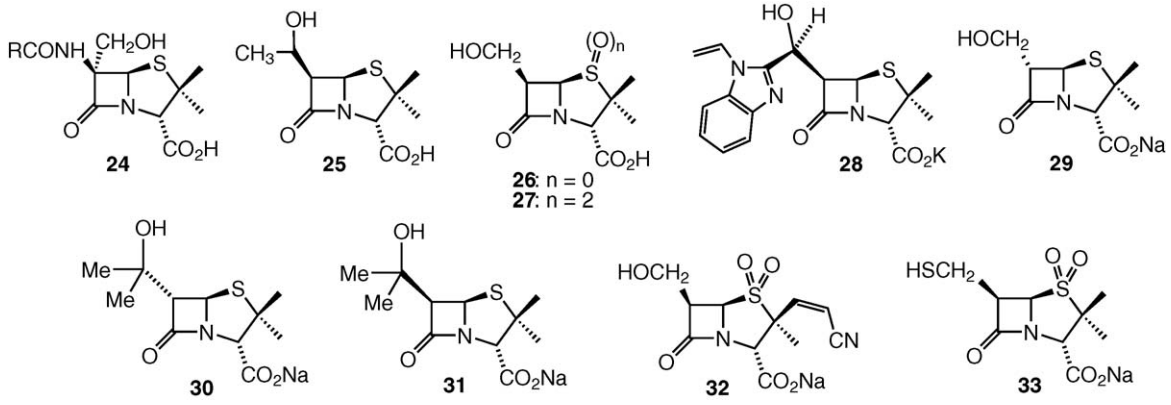


Fig. 4 – 6-(Hydroxyalkyl)penicillanates and corresponding sulfones.

5. 6-(Hydroxyalkyl)penems

The penems were designed to be a cross between the fused 4- and 5-membered-ring system of the penicillins and the cephalosporins, which have an endocyclic double bond conjugated to the nitrogen [66] (Fig. 5). In terms of structure–activity relationships, penems most closely parallel carbapenems. Further reinforcement of this comparison, is evident in the identical 6*S*, 8*R* stereochemistry of the hydroxyethyl side chain, which, as in the carbapenems, was optimized for antibacterial activity [67]. Penems possess both antimicrobial activity and β-lactamase stability. However, several studies also document their β-lactamase inhibitory activity, which seems to be specific for class C cephalosporinases [68–70]. The precise inhibitory mechanism is unknown. Faropenem diooxylate is scheduled (2006) to become the first oral penem available outside Japan.

6. *N*-sulfonyl- and *N*-sulfonyloxy-β-lactams

Monocylic, *N*-sulfonated-β-lactams, **38**, were first reported as antibacterials in a 1979 Takeda patent [71], and such compounds were the subject an article from Takeda and an article from Squibb (where the name 'monobactam' was suggested), both published in 1981 [72,73] (Fig. 6). Squibb developed this series into the commercial product, aztreonam, **39**. Aztreonam is highly active against Gram-negative aerobic bacteria, but relatively inactive against Gram-positive bacteria or Gram-negative anaerobes [74,75]. It has high affinity for PBP 3. While initially discovered monobactams had poor affinities for class A β-lactamases, and were micromolar inhibitors of class C β-lactamases, aztreonam was described as forming a covalent complex with the class C P99 enzyme [76] and has been characterized as 'an efficient transient inactivator' of class C β-lactamases [77]. More recently, Syn 2190, **40**, a monobactam which is devoid of antibacterial activity, but highly efficient as a selective inhibitor of class C β-lactamases, has been described [78–82].

A structurally related series of compounds are the monosulfactams, represented by tigemonam (**41**). These compounds are synthetic in origin and were designed, synthesized, and evaluated at Squibb [82,83]. Structure–activity relationships demonstrate the importance of the substituent at C4 to β-lactamase stability and inhibition [84]. Tigemonam has a spectrum of activity similar to that of aztreonam, but is orally bioavailable. This monosulfactam is resistant to most β-lactamases, and is reported to be an inhibitor of the class C enzymes, such as *Enterobacter cloacae*, P99 [85].

Recently, new series of bridged monobactams [86,87] and bridged sulfactams [88] have been developed as β-lactamase inhibitors by Hoffmann-La Roche. Monobactam Ro 48-1256, **42**, had no antibacterial activity, but acted as a selective inhibitor of class C β-lactamases and displayed synergy with ceftazidime and piperacillin against strains derepressed for the AmpC enzyme [89]. Mechanistically, these compounds were designed to block access of the active site water to the intermediate acyl-enzyme [87]. Previously, the crystal structure of aztreonam bound to the class C β-lactamase from *Citrobacter freundii* [90] indicated that, subsequent to formation of the acyl-enzyme, a 70° rotation about the C3–C4 bond had to occur in order for the hydrolytic water to have access to the ester linkage. (The C4 α-methyl group of aztreonam is believed

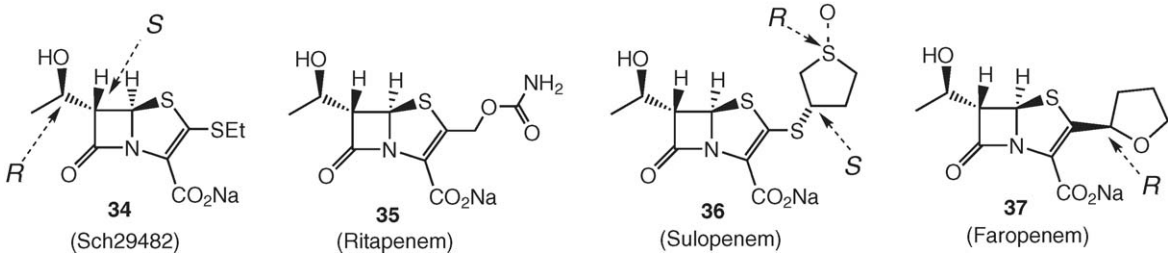


Fig. 5 – 6-(Hydroxyalkyl)penems and 6-(hydroxyalkyl)thiaclavams.

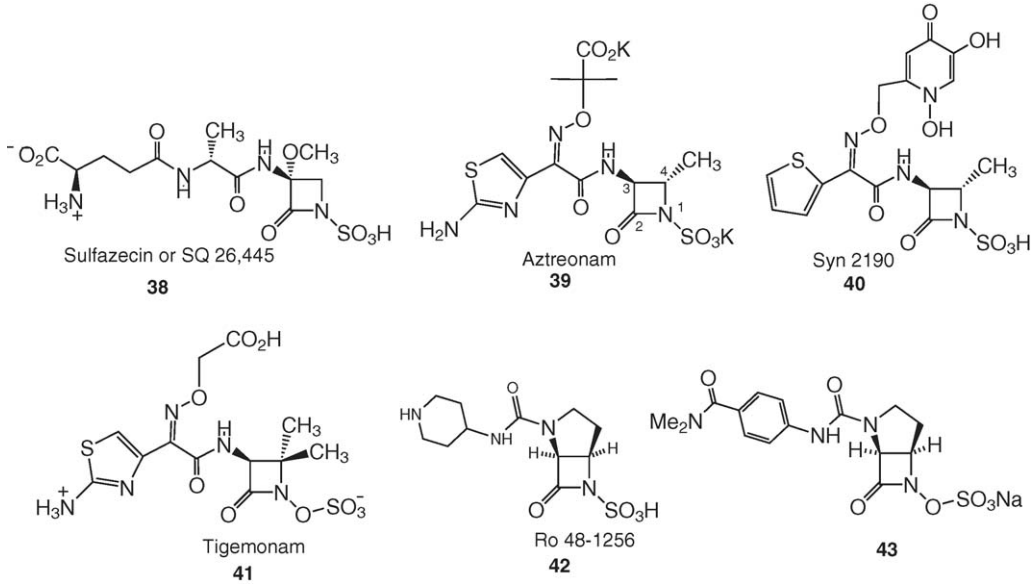


Fig. 6 – Monocyclic *N*-sulfated-β-lactams (monobactams) and *N*-sulfonyloxy-β-lactams (monosulfactams).

to interfere with the requisite rotation through a steric interaction with the aryl ring of Tyr150.) By structurally prohibiting this rotation through construction of a bridge between C3 and C4 (i.e., the five-membered ring), they were able to stabilize the acyl-enzyme toward attack of the hydrolytic water and inhibit the enzyme. The corresponding bridged sulfactams **43**, were also potent inhibitors, of both class C as well as class A β-lactamases. Analysis of the inhibition indicated that the inhibition by the bridged sulfactams was due to a high initial affinity for the enzymes and a high rate of acylation. However, the deacylation rate of the sulfactams was also high, rendering the overall synergy with ceftriaxone relatively weak, relative to the corresponding monobactams.

7. Inhibitor resistance

Given the frequency of clinical administration of antibiotic/inhibitor combinations, as well as the high reproduction rate and mutational frequency of bacteria, it is not surprising that inhibitor resistance has developed. The term 'inhibitor-resistant' usually refers to resistance to amoxicillin/clavulanate and does not necessarily imply resistance to other inhibitors. Even the (mechanistically) related penicillin sulfones are observed to have resistance profiles somewhat different from that of clavulanate [91] and no data is currently available on the effect of non-commercial β-lactamase inhibitors on 'inhibitor-resistant' enzymes. Such resistance may occur through hyperproduction of unmodified β-lactamase [92–94], by modification of outer membrane proteins [95], as well as through production of a mutant form of the β-lactamase which is more resilient toward the commercial inhibitors [96–99]. It is this last form that will be discussed.

Since commercial inhibitors target the clinically prevalent, plasmid-mediated class A β-lactamases, it is logical that the inhibitor-resistant enzymes are also of class A, specifically the inhibitor resistant TEM β-lactamases (IRT's), as well as resistant SHV β-lactamases, close structural relatives of TEM. The frequency of IRT production among resistant strains is a matter of current study. A 1993 French study examined nearly 3000 *E. coli* isolates and found the amoxicillin–clavulanate resistance rate to be 25.0% (hospital) and 10.0% (community). Of these resistant strains, 27.5% (hospital) and 45.0% (community) showed an unusual β-lactam resistance pattern, suggestive of IRT production. Thus, they estimate that the overall IRT production rate, among *E. coli* to be around 4.9%, indicating the IRT producing strains may be beginning to make an important contribution [100]. A recent U.S. study found the overall incidence of amoxicillin/clavulanate resistance was 3–5%, but although IRT-production was detected in some of these resistant strains, the precise frequency of such IRT production was not determined [101]. A study of clinical *E. coli* strains from a Spanish hospital showed that about 7% had reduced susceptibility to amoxicillin/clavulanate, with the major cause (75% of those resistant strains) being probable overproduction of class C β-lactamase, with only approximately 5% of the amoxicillin/clavulanate-resistant strains producing IRT's (i.e., 0.35% of the total resistant strains) [102]. The overall IRT incidence may be higher than recognized, since inhibitor-resistant strains cannot be detected by routine susceptibility tests and are identified by iso-electric points and kinetic investigation of the produced β-lactamases, as well as by molecular biology techniques [103,104]. Obviously, this can lead to discrepancies in determining their current clinical significance.

Several studies of selected inhibitor-resistant enzymes were recently completed and some of these are briefly summarized. In the class A TEM β-lactamases, active site mutations leading to inhibitor resistance include single amino acid substitutions at position 69 (M69I, M69L, M69V), at position 130 (S130G), at position 244 (R244S, R244C, R244H, and R244G), at position 275 (R275L), and at position 276

(N276D). Given the structural similarity between the inhibitors and the substrates, the key mechanistic question involves determining how the enzyme is able to maintain its ability to hydrolyze the antibiotic while reducing the efficacy of inhibitors. Indeed, in most mutations, some attenuation of catalytic efficacy is observed.

The mutations of Met69 are commonly observed in inhibitor-resistant enzymes and are poorly understood mechanistically. The three mutations lead to enzymes with an approximate ten-fold increase in the apparent K_i and also an approximate ten-fold decrease in the k_{inact} toward all three commercial inhibitors (and consequent hundred-fold degradation of the inhibitory efficacy, k_{inact}/K_i) and with a comparatively less dramatic effects on either the k_{cat} or K_m of the antibiotics [105,106]. This side chain lies directly behind the oxyanion hole. Recent hypotheses include hydrophobicity and steric constraints [106], a distortion in the conformation of Ser70 [107], and a difference in the dynamic motions of the residues in the vicinity of the active site [108]. It is interesting that replacement of the methionine by less sterically demanding residues (i.e., M69A and M69G) results in an enzyme more susceptible toward inhibitors [109].

Ser130 is currently believed to play key roles in recognition of the C3 (C4) carboxylate of the penams and cephems, respectively, providing a proton to the departing nitrogen during initial acylation of Ser70, as well as serving as the second nucleophile in irreversible inactivation (attack of the intermediate imine, resulting in double attachment to the enzyme) of the β-lactamase by commercial inhibitors, clavulanate and the penicillin sulfones. Particularly in view of this last factor, it might be anticipated that an S130G mutant would be entirely resistant to inhibitors. The fact that commercial inhibitors still have (albeit diminished) efficacy [110] against such a mutant underscores the importance of the transiently stabilized intermediates (see Scheme 2) on the in vitro inactivation of the enzyme (particularly considering the 20 min replication cycle of the bacterium). A recent structure of an S130G mutant of SHV-1, complexed with tazobactam as the cis-vinylogous urethane further emphasizes this fact [111]. In a study of this SHV-1 mutant, it was found that the mutation increased the K_m for the substrate ampicillin (approx. 2-fold) and the apparent K_i values for the inhibitors (approx. 40–300-fold) [112]. The k_{cat} values for ampicillin was decreased (approx. 10-fold), while k_{inact} of the inhibitors was relatively unchanged. As a measure of efficiency, the S130G k_{cat}/K_m value for ampicillin was 3.6% that of the wild type, while the k_{inact}/K_i values for clavulanate and tazobactam, were 0.24 and 1% that of the wild type, respectively. The partition ratios for the two inhibitors were approximately the same for the wild type and the S130G mutant. Thus, the enzyme loses efficiency in the hydrolysis of ampicillin, to enjoy a larger enhancment in inhibitor resistance. In both cases, the dominating factor is the increase in the (apparent) dissociation constants for the preacylation complex of either the inhibitor or the antibiotic. Similar results were obtained in a study of a TEM 1 S130G mutant [113]. In both SHV and TEM S130G mutants, a water molecule is believed to be taking the place of Ser130 in the donation of a proton to the departing nitrogen.

The IRT data obtained thus far seem to indicate that the inhibitors, with their smaller structures and two point recognition (i.e., C3 (C4) carboxylate and β-lactam carbonyl oxygen, via the oxyanion hole) are more susceptible to mutations affecting these two critical points than are the β-lactam antibiotics, which have additional hydrogen bonding recognition at the C6 (C7) acylamino side chain (see Fig. 1).

8. Conclusion and analysis

β-Lactam antibiotic/β-lactamase inhibitor combinations are one of the success stories of the anti-infectives area. These drugs have been successful in circumventing the bacterial evolutionary drive toward resistance. There are still several important questions to be addressed, the most important of which remains the identification of a suitable inhibitor which can simultaneously inactivate both class A and class C serine β-lactamases. Additionally, the appearance of class B metallo-β-lactamases, and carbapenem-hydrolyzing class A enzymes now threaten the carbapenems, which represent one of the remaining generally effective β-lactam antibiotics. A key to the success of β-lactamase inhibitors has been their close structural resemblance to the substrate antibiotics themselves. Another, related, feature is the mechanism-based irreversible nature of the inactivation. It is reasonable to suggest that the prerequisite for substrate-like acylation of the enzyme improves their selectivity and that such formation of covalent bonds between the inhibitor and the target enzyme renders it still more difficult for small mutations to lessen the binding efficacy.

Pathogenic bacteria are adaptable and resourceful opponents. Our search for new antibiotics must be relentless, utilizing all available technology. However, given the historical precedent of the appearance of resistance within a few years of the introduction of the antibiotic class, it seems inevitable that the new antibiotics developed to hit novel targets will also encounter resistance. The existing bacterial 'resistome' may be more highly developed than previously recognized, including mechanisms such as efflux, permeability barriers, target modification, and drug inactivation. Bacterial cell wall machinery has proven a useful target, that, due to differences between bacterial and mammalian cells, allows the administration of a number of highly selective (i.e. non-toxic) small molecules. At the present moment, it seems likely that we will be forced to utilize the entire armamentarium of antibiotics (particularly including β-lactams) for the foreseeable future. Knowledge of previous success stories, such as the β-lactams, may assist in the design and development of new classes of drugs.

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REFERENCES

[1] Lim D, Strynadka NCJ. Structural basis for the beta-lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. Nat Struct Biol 2002;9:870–6.

[2] Massova I, Mobashery S. Kinship and diversification of bacterial penicillin-binding proteins and β-lactamases. Antimicrob Agents Chemother 1998;42:1–17.
[3] Knox JR, Moews PC, Frère J-M. Molecular evolution of bacterial β-lactam resistance. Chem Biol 1996;3:937–47.
[4] Pratt RF. Functional evolution of the serine β-lactamase active site. J Chem Soc Perkin Trans 2002;2:851–61.
[5] Ambler RP. The structure of β-lactamases. Phil Trans R Soc Lond B 1980;289:321–31.
[6] Jaurin B, Grundström T. AmpC cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of β-lactamases of the penicillinase type. Proc Natl Acad Sci USA 1981;78:4897–901.
[7] Dale JW, Godwin D, Mossakowska D, Stephenson P, Wall S. Sequence of the OXA2 β-lactamase: comparison with other penicillin-reactive enzymes. FEBS Lett 1985;191:39–44.
[8] Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 1995;39:1211–33.
[9] Maiti SN, Phillips OA, Micetich RG, Livermore DM. β-Lactamase inhibitors: agents to overcome bacterial resistance. Curr Med Chem 1998;5:441–56.
[10] Miller LA, Ratnam K, Payne DJ. β-lactamase-inhibitor combinations in the 21st century: current agents and new developments. Curr Opin Pharm 2001;1:451–8.
[11] Micetich RG, Salama SM, Maiti SN, Reddy AVN, Singh R. β-lactamases and their inhibitors: an update. Curr Med Chem Anti Infect Agents 2002;1:193–213.
[12] Sandanayaka VP, Prashad AS. Resistance to β-lactam antibiotics: structure and mechanism based design of β-lactamase inhibitors. Curr Med Chem 2002;9:1145–65.
[13] Page MGP. β-Lactamase inhibitors. Drug Resist Updates 2000;3:109–25.
[14] Theuretzbacher U. β-Lactamases and β-lactamase inhibitors. Chemother J 2004;13:206–17.
[15] Georgopapadakou NH. β-Lactamase inhibitors: evolving compounds for evolving resistance targets. Expert Opin Investig Drugs 2004;13:1307–18.
[16] Bush K. The impact of β-lactamases on the development of novel antimicrobial agents. Curr Opin Investig Drugs 2002;3:1284–90.
[17] Coleman K. An update on β-lactamases and β-lactamase inhibitors. Expert Opin Investig Drugs 1995;4:693–704.
[18] Pratt RF. β-Lactamase: inhibition. In: Page MI, editor. Chemistry of β-lactams. New York: Springer; 1992. p. 229–71.
[19] Varghese JN, Smith PW, Sollis SL, Blick TJ, Sahasrabudhe A, McKimm-Breschkin JL, et al. Drug design against a shifting target: a structural basis for resistance to inhibitors in a variant of influenza virus neuraminidase. Structure 1998;6:735–46.
[20] White AR, Kaye C, Poupard J, Pypstra R, Woodnutt G, Wynne B. Augmentin (amoxicillin/clavulanate) in the treatment of community-acquired respiratory tract infection: a review of the continuing development of an innovative antimicrobial agent. J Antimicrob Chemother 2004;53(S1):i3–20.
[21] Stein GE, Gurwith MJ. Amoxicillin-potassium clavulanate, a β-lactamase-resistant antibiotic combination. Clin Pharm 1984;3:591–9.
[22] Reed MD. Rational prescribing of extended-spectrum penicillin β-lactamase inhibitor combinations: focus on ticarcillin/clavulanic acid. Annal Pharmacother 1998;32:S17–21.
[23] Lode H. Role of sultamicillin and ampicillin/sulbactam in the treatment of upper and lower bacterial respiratory tract infections. Int J Antimicrob Agents 2001;18:199–209.
[24] For a review, see: Bryson HM, Brogden RN. Piperacillin/tazobactam. a review of its antibacterial activity, pharmacokinetic properties and therapeutic potential. Drugs 1994;47:506–35.
[25] Bush K, Mobashery S. How β-lactamases have driven pharmaceutical drug discovery. From mechanistic knowledge to clinical circumvention. Adv Exp Med Biol 1998;456:71–98.
[26] Charnas RL, Fisher J, Knowles JR. Chemical studies on the inactivation of *Escherichia coli* RTEM β-lactamase by clavulanic acid. Biochemistry 1978;17:2185–9.
[27] Charnas RL, Knowles JR. Inactivation of RTEM β-lactamase from *Escherichia coli* by clavulanic acid and 9-deoxyclavulanic acid. Biochemistry 1981;20:3214–9.
[28] Imtiaz U, Billings E, Knox JR, Manavathu EK, Lerner SA, Mobashery S. Inactivation of class A β-lactamases by clavulanic acid: the role of arginine-244 in a proposed nonconcerted sequence of events. J Am Chem Soc 1993;115:4435–42.
[29] Chen CCH, Herzberg O. Inhibition of β-lactamase by clavulanate. Trapped intermediates in cryocrystallographic studies. Mol Biol 1992;224:1103–13.
[30] Brown RPA, Aplin RT, Schofield CJ. Inhibition of TEM-2 β-lactamase from *Escherichia coli* by clavulanic acid: observation of intermediates by electrospray ionization mass spectrometry. Biochemistry 1996;35:12421–32.
[31] Fisher J, Charnas RL, Bradley SM, Knowles JR. Inactivation of the RTEM β-lactamase from *Escherichia coli*. Interaction of penam sulfones with enzyme. Biochemistry 1981;20:2726–31.
[32] Imtiaz U, Billings EM, Knox JR, Mobashery S. A structure-based analysis of the inhibition of the class A β-lactamases by sulbactam. Biochemistry 1994;33:5728–38.
[33] Kuzin AP, Nukaga M, Nukaga Y, Hujer A, Bonomo RA, Knox JR. Inhibition of the SHV-1 β-lactamase by sulfones: crystallographic observation of two reaction intermediates with tazobactam. Biochemistry 2001;40:1861–6.
[34] Padayatti PS, Helfand MS, Totir MA, Carey MP, Hujer AM, Carey PR, et al. Tazobactam forms a stoichiometric trans-enamine intermediate in the E166A variant of SHV-1 β-lactamase: 1.63 Å crystal structure. Biochemistry 2004;43:843–8.
[35] Yang Y, Janota K, Tabei K, Huang N, Siegel MM, Lin Y-I, et al. Mechanism of inhibition of the class A β-lactamases PC1 and TEM-1 by tazobactam. Observation of reaction products by electrospray ionization mass spectrometry. J Biol Chem 2000;275:26674–82.
[36] Pagan-Rodriguez D, Zhou X, Simmons R, Bethel CR, Hujer AM, Helfand MS, et al. Tazobactam inactivation of SHV-1 and the inhibitor-resistant Ser130 → Gly SHV-1 β-lactamase: insight into the mechanism of inhibition. J Biol Chem 2004;279:19494–501.
[37] Richter HGF, Angehrn P, Hubschwerlen C, Kania M, Page MGP, Specklin J-L, et al. Design, synthesis, and evaluation of 2β-alkenyl penam sulfone acids as inhibitors of β-lactamases. J Med Chem 1996;39:3712–22.
[38] Tzouvelekis L, Gazouli M, Prinarakis EE, Tzelepi E, Legakis NJ. Comparative evaluation of the inhibitory activities of the novel penicillanic acid sulfone Ro 48-1220 against β-lactamases that belong to groups 1, 2b, and 2be. Antimicrob Agents Chemother 1997;41:475–7.
[39] Buynak JD, Gadhachanda VR, Vogeti L, Zhang H, Chen H. Synthesis and evaluation of 3-(carboxymethylidene)- and 3-(carboxymethyl)penicillinates as inhibitors of β-lactamase. J Org Chem 2005;70:4510–3.
[40] Monks J, Waley SG. Imipenem as substrate and inhibitor of β-lactamases. Biochem J 1988;253:323–8.

[41] Livermore DM, Neil W. Carbapenemases: a problem in waiting? Curr Opin Microbiol 2000;3:489–95.
[42] Rice LB, Bonomo RA. β-lactamases: which ones are clinically important? Drug Resist Updates 2000;3:178–89.
[43] Rasmussen BA, Bush K. Carbapenem-hydrolyzing β-lactamases. Antimicrob Agents Chemother 1997; 41:223–32.
[44] Yigit H, Queenan AM, Rasheed JK, Biddle JW, Domenech-Sanchez A, Alberti S, et al. Carbapenem-resistant strain of Klebsiella oxytoca harboring carbapenem-hydrolyzing β-lactamase KPC-2. Antimicrob Agents Chemother 2003;47:3881–9.
[45] Matagne A, Ghuyesen M, Frère JM. Interactions between active-site-serine β-lactamases and mechanism-based inactivators: a kinetic study and an overview. Biochem J 1993;295:705–11.
[46] Galleni M, Amicosante G, Frère JM. A survey of the kinetic parameters of class C β-lactamases. Cephalosporins and other β-lactam compounds. Biochem J 1988;255:123–9.
[47] Babini GS, Yuan M, Livermore DM. Interactions of β-lactamases with sanfetrinem (GV 104326) compared to those with imipenem and with oral β-lactams. Antimicrob Agents Chemother 1998;42:1168–75.
[48] Hashizume T, Yamaguchi A, Hirata T, Sawai T. Kinetic studies on the inhibition of *Proteus vulgaris* β-lactamase by imipenem. Antimicrob Agents Chemother 1984;25:149–51.
[49] Maveyraud L, Mourey L, Kotra LP, Pedelacq J-D, Guillet V, Mobashery S, et al. Structural basis for clinical longevity of carbapenem antibiotics in the face of challenge by the common class A β-lactamases from the antibiotic-resistant bacteria. J Am Chem Soc 1998;120:9748–52.
[50] Beadle BM, Shoichet BK. Structural basis for imipenem inhibition of class C β-lactamases. Antimicrob Agents Chemother 2002;46:3978–80.
[51] Pernot L, Frenois F, Rybkine T, L'Hermite G, Petrella S, Delettre J, et al. Crystal structures of the class D β-lactamase OXA-13 in the native form and in complex with meropenem. J Mol Biol 2001;310:859–74.
[52] Dixon RA, Edmondson RA, Hardy KD, Milner PH. The synthesis and antibacterial activity of some β-lactamase stable 6α-(hydroxymethyl)penicillins. J Antibiot 1984;37:1729–31.
[53] Reiner R, Zeller P. Substitution of 6-aminopenicillanic acid at the 6th carbon atom. Helv Chim Acta 1968;51:1905–18.
[54] DiNinno F, Beattie TR, Christensen BG. Aldol condensations of regiospecific penicillanate and cephalosporanate enolates. Hydroxyethylation at C-6 and C-7. J Org Chem 1977;42:2960–5.
[55] Kellogg MS. Derivatives of 6β-hydroxyalkylpenicillanic acids as β-lactamase inhibitors. U.S. Patent 4,287,181, Oct 22, 1979.
[56] Schneider P, Scartazzini R. Penam-dioxide compounds, processes for their manufacture and their use. British Patent GB 2076812A, May 15, 1981.
[57] Chen YL, Hedberg K, Guarino K, Retsema JA, Anderson M, Manousos M, et al. (6R,8S)-(2-Benzimidazolyl)hydroxymethylpenicillanic acids as potent antibacterial agents and β-lactamase inhibitors. J Antibiot 1991;44:870–84.
[58] Miyashita K, Massova I, Taibi P, Mobashery S. Design, synthesis, and evaluation of a potent mechanism-based inhibitor for the TEM β-lactamase with implications for the Enzyme Mechanism. J Am Chem Soc 1995;117:11055–9.
[59] Maveyraud L, Massova I, Birck C, Miyashita K, Samama J-P, Mobashery S. Crystal structure of 6α-(hydroxymethyl)penicillanate complexed to the TEM-1 β-lactamase from *Escherichia coli*: Evidence on the mechanism of action of a novel inhibitor designed by a computer-aided process. J Am Chem Soc 1996;118:7435–40.
[60] Mourey L, Miyashita K, Swaren P, Bulychev A, Samama J-P, Mobashery S. Inhibition of the NMC-A β-lactamase by a penicillanic acid derivative and the structural bases for the increase in substrate profile of this antibiotic resistance enzyme. J Am Chem Soc 1998;120:9382–3.
[61] Maveyraud L, Golemi-Kotra D, Ishiwata A, Meroueh O, Mobashery S, Samama J-P. High-resolution X-ray structure of an acyl-enzyme species for the class D OXA-10 β-lactamase. J Am Chem Soc 2002;124:2461–4.
[62] Golemi D, Maveyraud L, Ishiwata A, Tranier S, Miyashita K, Nagase T, et al. 6-(hydroxyalkyl)penicillanates as probes for mechanisms of β-lactamases. J Antibiot 2000;53: 1022–7.
[63] Bitha P, Li Z, Francisco GD, Rasmussen BA, Lin Y-I. 6-(1-hydroxyalkyl)penam sulfone derivatives as inhibitors of class A and class C β-lactamases I. Bioorg Med Chem Lett 1999;9:991–6.
[64] Bitha P, Li Z, Francisco GD, Yang Y, Petersen PJ, Lenoy E, et al. 6-(1-hydroxyalkyl)penam sulfone derivatives as inhibitors of class A and class C β-lactamases II. Bioorg Med Chem Lett 1999;9:997–1002.
[65] Buynak JD, Chen H, Vogeti L, Gadhachanda VR, Buchanan CA, Palzkill T, et al. Penicillin-derived inhibitors that simultaneously target both metallo- and serine-β-lactamases. Bioorg Med Chem Lett 2004;14:1299–304.
[66] Ernest I, Gosteli J, Greengrass CW, Holick W, Pfaendler HR, Woodward RB. The penems, a new class of β-lactam antibiotics: 6-acylaminopenem-3-carboxylic acids. J Am Chem Soc 1978;100:8214–22.
[67] Ganguly AK, Girijavallabhan VM, McCombie S, Pinto P, Rizvi R, Jeffrey PD, et al. Synthesis of Sch 29482 - a novel penem antibiotic. J Antimicrob Chemother 1982;9(Suppl. C):1–5.
[68] Barry AL, Jones RN, Wilson HW, Badal RE, Thornsberry C. Sch 29482, a new oral penem: comparative in vitro activity, β-lactamase stability and inhibition. J Antimicrob Chemother 1982;9(Suppl. C):97–112.
[69] Pechere JC, Letarte R, Guay R, Asselin C, Morin C. Sch 29482: stability and inhibitory potency towards β-lactamases from gram-negative bacteria. J Antimicrob Chemother 1982;9(Suppl. C):123–32.
[70] Inoue E, Mitsuhashi S. In vitro antibacterial activity and β-lactamase stability of SY5555, a new oral penem antibiotic. Antimicrob Agents Chemother 1994;38:1974–9.
[71] Imada A, Kitano K, Asai M. Antibiotic G-6320. German Patent DE 2855949 19790705, 1979.
[72] Imada A, Kitano K, Kintaka K, Muroi M, Asai M. Sulfazecin and isosulfazecin, novel β-lactam antibiotics of bacterial origin. Nature 1981;289:590–1.
[73] Sykes RB, Cimarusti CM, Bonner DP, Bush K, Floyd DM, Georgopapadakou NH, et al. Monocyclic β-lactam antibiotics produced by bacteria. Nature 1981;291:489–91.
[74] Georgopapadakou NH, Smith SA, Sykes RB. Mode of action of azthreonam. Antimicrob Agents Chemother 1982;21:950–6.
[75] Jacobus NV, Ferreira MC, Barza M. In vitro activity of azthreonam, a monobactam antibiotic. Antimicrob Agents Chemother 1982;22:832–8.
[76] Bush K, Freudenberger JS, Sykes RB. Interaction of azthreonam and related monobactams with β-lactamases from gram-negative bacteria. Antimicrob Agents Chemother 1982;22:414–20.
[77] Galleni M, Amicosante G, Frère J-M. A survey of the kinetic parameters of class C β-lactamases. Cephalosporins and other β-lactam compounds. Biochem J 1988;255:123–9.
[78] Maiti SN, Setti EL, Phillips OA, Reddy AVN, Micetich RG, Singh R, et al. Preparation of 2-oso-1-azetidine sulfonic acid derivatives as potent β-lactamase inhibitors. World Patent WO 9847895 A1 19981029, 1998.

[79] Nishida K, Kunugita C, Uji T, Higashitani F, Hyodo A, Unemi N, et al. In vitro and in vivo activities of Syn2190, a novel β-lactamase inhibitor. Antimicrob Agents Chemother 1999;43:1895–900.
[80] Babini GS, Livermore DM. Effect of conalbumin on the activity of Syn 2190, a 1,5 dihydroxy-4-pyridon monobactam inhibitor of AmpC β-lactamases. J Antimicrob Chemother 2000;45:105–9.
[81] Danes C, Navia MM, Ruiz J, Marco F, Jurado A, Jimenez de Anta MT, et al. Distribution of β-lactamases in *Acinetobacter baumannii* clinical isolates and the effect of Syn 2190 (AmpC inhibitor) on the MICs of different β-lactam antibiotics. J Antimicrob Chemother 2002;50:261–4.
[82] Gordon EM, Ondetti MA. O-Sulfated β-lactam hydroxamic acids. European Patent EP 51381 A1 19820512, 1982.
[83] Gordon EM, Ondetti MA, Pluscec J, Cimarusti CM, Bonner DP, Sykes RB. O-Sulfated β-lactam hydroxamic acids (monosulfactams). Novel monocyclic β-lactam antibiotics of synthetic origin. J Am Chem Soc 1982;104:6053–60.
[84] Yoshida C, Hori T, Momonoi K, Nagumo K, Nakano J, Kitani T, et al. Studies on monocyclic β-lactam antibiotics. II. Synthesis and antibacterial activity of 3-acylamino-2-azetidinone-1-oxysulfonic acids. J Antibiot 1985;38:1536–49.
[85] Chin N-X, Neu HC. Tigemonam, an oral monobactam. Antimicrob Agents Chemother 1988;32:84–91.
[86] Charnas R, Gubernator K, Heinze I, Hubschwerlen C. Preparation of β-lactams as β-lactamase inhibitors. European patent EP 508234 A2 19921014, 1992.
[87] Heinze-Krauss I, Angehrn P, Charnas RL, Gubernator K, Gutknecht E-M, Hubschwerlen C, et al. Structure-based design of β-lactamase inhibitors. 1. Synthesis and evaluation of bridged monobactams. J Med Chem 1998;41:3961–71.
[88] Hubschwerlen C, Angehrn P, Gubernator K, Page MGP, Specklin J-L. Structure-based design of β-lactamase inhibitors. 2. Synthesis and evaluation of bridged sulfactams and oxamazins. J Med Chem 1998;41:3972–5.
[89] Livermore DM, Chen HY. Potentiation of β-lactams against *Pseudomonas aeruginosa* strains by Ro 48-1256, a bridged monobactam inhibitor of AmpC β-lactamases. J Antimicrob Chemother 1997;40:335–43.
[90] Oefner C, D'Arcy A, Daly JJ, Gubernator K, Charnas RL, Heinze I, et al. Refined crystal structure of β-lactamase from *Citrobacter freundii* indicates a mechanism for beta-lactam hydrolysis. Nature 1990;343:284–8.
[91] Bonomo RA, Rudin SA, Shlaes DM. Tazobactam is a potent inactivator of selected inhibitor-resistant class A β-lactamases. FEBS Microbiol Lett 1997;148:59–62.
[92] Martinez JL, Vicente MF, Delgado-Iribarren A, Perez. Diaz JC, Baquoro F. Small plasmids are involved in amoxicillin-clavulanate resistance in *Escherichia coli*. Antimicrob Agents Chemother 1989;33:595.
[93] Wu P-J, Shannon K, Phillips I. Effect of hyperproduction of TEM-1 β-lactamase on in vitro susceptibility of *Escherichia coli* to β-lactam antibiotics. Antimicrob Agents Chemother 1994;38:494–8.
[94] Wu P-J, Shannon K, Phillips IJ. Mechanisms of hyperproduction of TEM-1 β-lactamase by clinical isolates of *Escherichia coli*. J Antimicrob Chemother 1995;36:927–39.
[95] Reguera JA, Baquero F, Perez-Diaz JC, Martinez JL. Factors determining resistance to β-lactam combined with β-lactamase inhibitors in *Escherichia coli*. J Antimicrob Chemother 1991;27:569–75.
[96] Bonomo RA, Rice LB. Inhibitor resistant class A beta-lactamases. Front Biosci 1999;4:e34–41.
[97] Nicolas-Chanoine MH. Inhibitor-resistant β-lactamases. J Antimicrob Chemother 1997;40:1–3.
[98] Chaïbi EB, Sirot D, Paul G, Labia R. Inhibitor-resistant TEM β-lactamases: phenotypic, genetic and biochemical characteristics. J Antimicrob Chemother 1999;43:447–58.
[99] Yang Y, Rasmussen BA, Shlaes DM. Class A β-lactamases-enzyme-inhibitor interactions and resistance. Pharmacol Ther 1999;83:141–51.
[100] Henquell C, Sirot D, Chanal C, De Champs C, Chatron P, Lafeuille B, et al. Frequency of inhibitor-resistant TEM β-lactamases in *Escherichia coli* isolates from urinary tract infections in France. J Antimicrob Chemother 1994;34:707–14.
[101] Kaye KS, Gold HS, Schwaber MJ, Venkataraman L, Qi Y, De Girolami PC, et al. Variety of β-lactamases produced by amoxicillin-clavulanate-resistant *Escherichia coli* isolated in the northeastern United States. Antimicrob Agents Chemother 2004;48:1520–5.
[102] Miro E, Navarro F, Mirelis B, Sabate M, Rivera A, Coll P, et al. Prevalence of clinical isolates of *Escherichia coli* producing inhibitor-resistant β-lactamases at a university hospital in Barcelona, Spain, over a 3-year period. Antimicrob Agents Chemother 2002;46:3991–4.
[103] Chaïbi EB, Farzaneh S, Morand A, Péduzzi J, Barthélémy M, Sirot D, et al. J Antimicrob Chemother 1996;37:190–1.
[104] Chardon H, Farzaneh S, Labia R, Jarlier V, Nicolas MH, Paul G, et al. Analysis of β-lactamases produced by cephalothin-susceptible *Escherichia coli* clinical isolates resistant to co-amoxiclav and ticarcillin-clavulanic acid. J Antimicrob Chemother 1995;36:267–9.
[105] Lin S, Thomas M, Shlaes DM, Rudin SD, Knox JR, Anderson V, et al. Kinetic analysis of an inhibitor-resistant variant of the OHIO-1 β-lactamase, an SHV-family class A enzyme. Biochem J 1998;333:395–400.
[106] Chaïbi EB, Péduzzi J, Farzaneh S, Barthélémy M, Sirot D, Labia R. Clinical inhibitor-resistant mutants of the β-lactamase TEM-1 at amino-acid position 69. Kinetic analysis and molecular modeling. Biochim Biophys Acta 1998;1382:38–46.
[107] Wang X, Minasov G, Shoichet BK. The structural bases of antibiotic resistance in the clinically derived mutant β-lactamases TEM-30, TEM-32, and TEM-34. J Biol Chem 2002;277:32149–56.
[108] Meroueh SO, Roblin P, Golemi D, Maveyraud L, Vakulenko SB, Zhang Y, et al. Molecular dynamics at the root of expansion of function in the M69L inhibitor-resistant TEM β-lactamase from *Escherichia coli*. J Am Chem Soc 2002;124:9422–30.
[109] Madec S, Blin C, Krishnamoorthy R, Picard B, Chaïbi EB, Fouchereau-Peron M, et al. Substitution of Met-69 by Ala or Gly in TEM-1 β-lactamase confer an increased susceptibility to clavulanic acid and other inhibitors. FEMS Microbiol Lett 2002;211:13–6.
[110] Pagan-Rodriguez D, Zhou X, Simmons R, Bethel CR, Hujer AM, Helfand MS, et al. Tazobactam inactivation of SHV-1 and the inhibitor-resistant Ser130 → Gly SHV-1 β-lactamase: Insight into the mechanism of inhibition. J Biol Chem 2004;279:19494–501.
[111] Sun T, Bethel CR, Bonomo RA, Knox JR. Inhibitor-resistant class A β-lactamases: consequences of the Ser130-to-Gly mutation seen in apo and tazobactam structures of the SHV-1 variant. Biochemistry 2004;43:14111–7.
[112] Helfand MS, Bethel CR, Hujer AM, Hujer KM, Anderson VE, Bonomo RA. Understanding resistance to β-lactams and β-lactamase inhibitors in the SHV β-lactamase: lessons from the mutagenesis of SER-130. J Biol Chem 2003;278:52724–9.
[113] Thomas VL, Golemi-Kotra D, Kim C, Vakulenko SB, Mobashery S, Shoichet BK. Structural consequences of the inhibitor-resistant Ser130Gly substitution in TEM β-lactamase. Biochemistry 2005;44:9330–8.