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Modifications of the C6-substituent of penicillin sulfones with the goal of improving inhibitor recognition and efficacy

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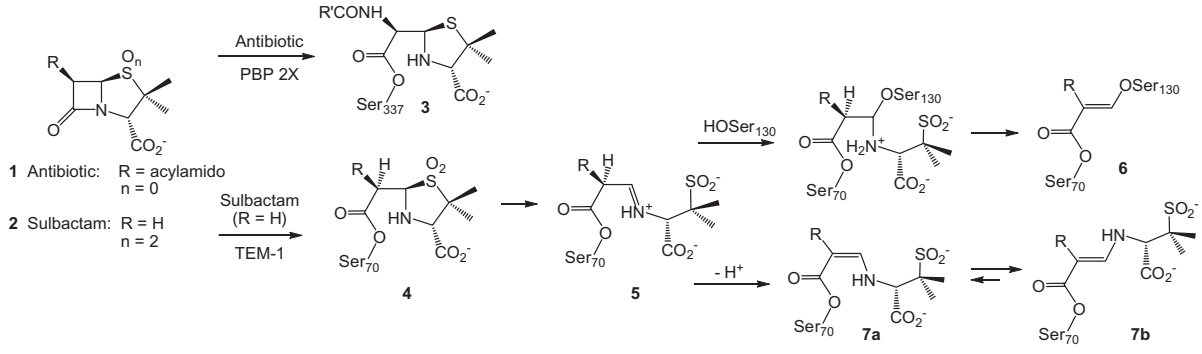
ABSTRACT

In order to evaluate the importance of a hydrogen-bond donating substituent in the design of β-lactamase inhibitors, a series of C6-substituted penicillin sulfones, lacking a C2′ substituent, and having an sp^3 hybridized C6, was prepared and evaluated against a representative classes A and C β-lactamases. It was found that a C6 hydrogen-bond donor is necessary for good inhibitory activity, but that this feature alone is not sufficient in this series of C6β-substituted penicillin sulfones. Other factors which may impact the potency of the inhibitor include the steric bulk of the C6 substituent (e.g., methicillin sulfone) which may hinder recognition in the class A β-lactamases, and also high similarity to the natural substrates (e.g., penicillin G sulfone) which may render the prospective inhibitor a good substrate of both classes of enzyme. The best inhibitors had non-directional hydrogen-bonding substituents, such as hydroxymethyl, which may allow sufficient conformational flexibility of the acyl-enzyme for abstraction of the C6 proton by E166 (class A), thus promoting isomerization to the β-aminoacrylate as a stabilized acyl-enzyme.

The past two decades have witnessed an exponential growth in the number of β-lactamases, now at more than 850. β-Lactamases are grouped into classes A–D and many of the newly reported enzymes have substantially broadened hydrolytic spectrum, including the appearance and dissemination of extended-spectrum β-lactamases (ESBLs)[1–4] and classes A and D carbapenemases[5–7]



Scheme 1. Interactions of a β-lactam antibiotic (**1**) or a β-lactamase inhibitor (**2**) with either a penicillin-binding protein (top) or a β-lactamase.

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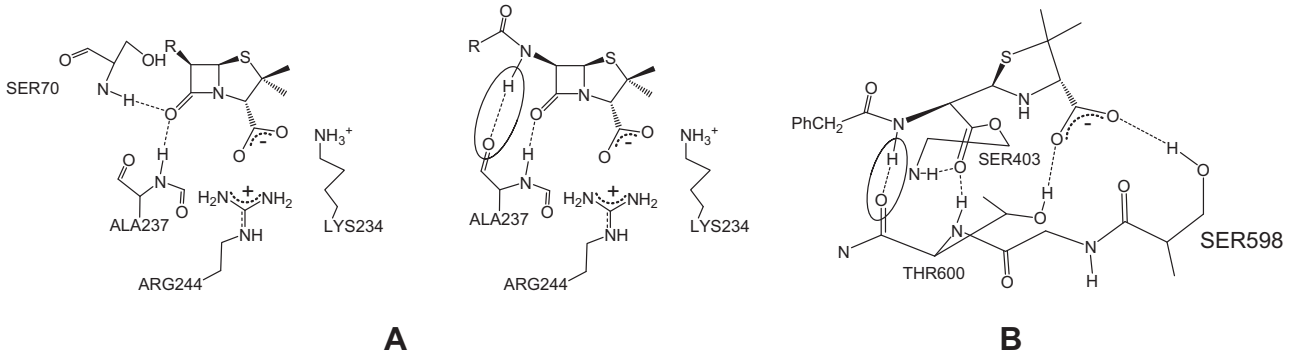
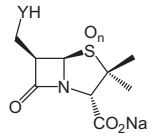


Figure 1. (A) Computer-assisted docking of penam substrate in TEM-1 β-lactamase (L shows H-bonding in oxyanion hole and R shows H-bonding of side C6 acylamido side chain); (**B**) Acyl-enzyme of *S. aureus* PBP 2a with Penicillin G (code 1MWT).

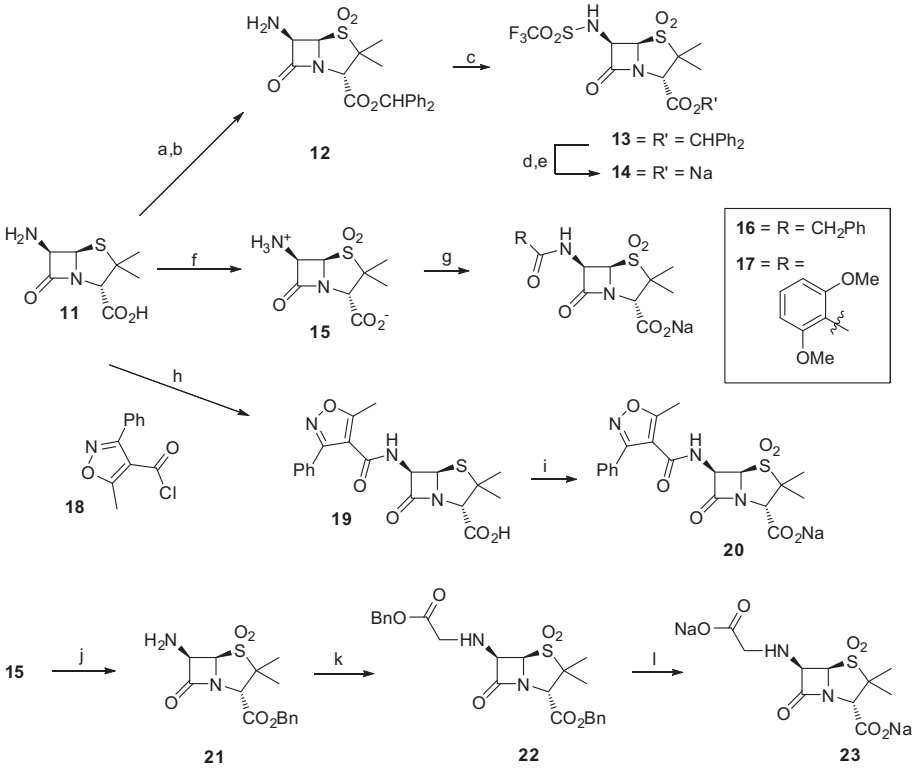


8 Y = O, n = 0
9 Y = O, n = 2
10 Y = S, n = 2

Figure 2. Inhibitors of β-lactamase possessing either a C6-hydroxymethyl or a C6-mercaptomethyl group.

Since β-lactam antibiotics continue to play a dominant role as first-line agents in the treatment of bacterial infections, and since the administration of antibiotic/β-lactamase inhibitor combinations remains a highly effective mechanism for overcoming β-lactamase-mediated resistance,[8] understanding the factors leading to optimal inhibitory efficacy is of utmost importance.

The β-lactamases are postulated to have evolved from the penicillin-binding proteins (PBPs), the transpeptidase targets of β-lactam antibiotics,[9] with the mechanistic difference that, while PBPs are inactivated by the antibiotics through formation of a stable acyl-enzyme, β-lactamases have evolved the ability to hydrolyze the corresponding covalent intermediate[10] and have also lost the ability to recognize peptidoglycan.[11] In general, β-lactamase inhibitors are designed to resemble the antibiotics, with minor structural modifications, such as the oxidation of the (penam) thiazolidine sulfide to the sulfone, and the omission of

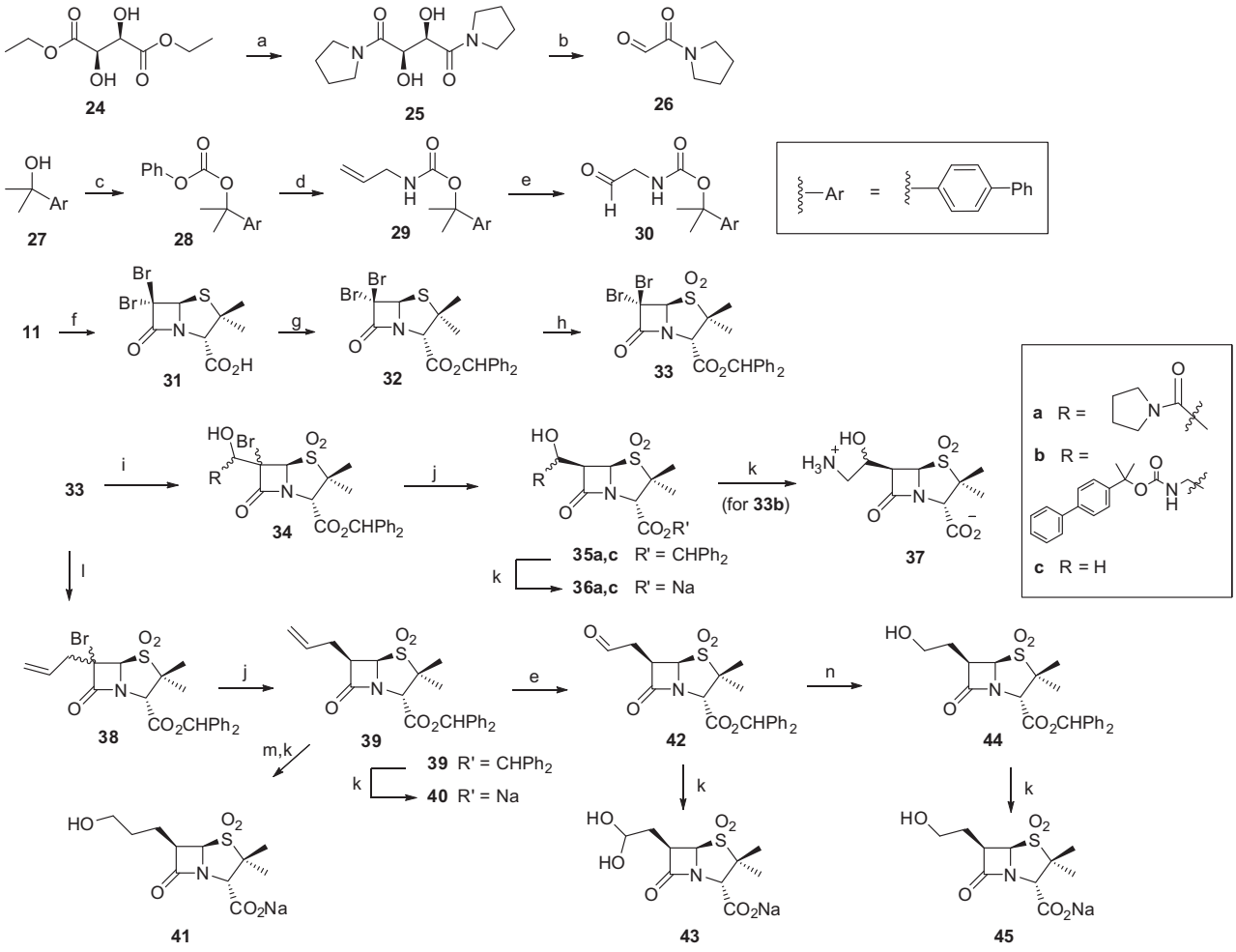


Scheme 2. Reagents and conditions:: (a) Ph_2CN_2, acetone, 0 °C to rt, 20 h; (b) 3 equiv mCPBA, DCM, rt, 1 h; (c) Tf_2O, TEA, 0 °C, 30 min; (d) anisole, CF_3CO_2H, −10 °C, 15 min; (e) $NaHCO_3$, EtOAc/H_2O, rt, 15 min; (f) $KMnO_4$, H_2SO_4, H_2O/MeCN, −10 °C, pH 3.2, 2 h; (g) (i) RCOCl, TEA, DCM, rt, 30 min; (ii) HCl; (iii) $NaHCO_3$; (h) (i) TEA, DCM, rt, 30 min; (ii) aq HCl; (i) (i) $KMnO_4$, H_3PO_4, H_2O, rt, 2 h; (ii) $NaHCO_3$; (j) $PhCHN_2$, acetone, rt, 30 min; (k) benzyl bromoacetate, DIPEA, DMF, 50 °C, 5 h; (l) (i) Pd/C, H_2, EtOH/EtOAc (4:1), 4 h, 40 psi; (ii) EtOAc/H_2O, $NaHCO_3$.

the acylamido side chains found at C6 in the antibiotics. Inhibitors are recognized in a similar fashion to the substrate β-lactam antibiotics themselves, and such structural similarity may advantageously render it difficult for the microorganism to evolve inhibitor resistance while maintaining substrate recognition. Mechanistically, β-lactam antibiotics and the β-lactamase inhibitors react with their respective enzymes to generate stabilized acyl-enzymes of differing structure. The penicillin sulfones, such as the commercial β-lactamase inhibitors, sulbactam and tazobactam, inactivate serine β-lactamases by the mechanism shown in Scheme 1 (bottom), leading to either the 'transiently inhibited' β-aminoacrylates **7a** and **7b**, or to the doubly covalently linked intermediates **6**, with both **6** and **7** having been observed crystallographically on the (soluble) β-lactamases. While crystallographic data on the membrane PBP transpeptidases has accumulated more slowly,[12] it is now established that the β-lactam antibiotics themselves, having sulfur in the sulfide oxidation state, maintain an unfragmented thiazolidine (or dihydrothiazole, in case of cephems) in the stabilized acyl-enzyme (**3**), as shown in Scheme 1 (top).[13]

The respective active sites of the PBPs and the serine β-lactamases have similar recognition elements, as shown in Figure 1, including a positively charged pocket to recognize the C3 carboxylate, an oxyanion hole (electrophilic center) to facilitate addition of serine to the β-lactam, and a suitably situated backbone carbonyl to serve as a hydrogen-bond acceptor for the C6 acylamido N–H (circled). While commercial inhibitors lack a hydrogen-bond donating C6 substituent, it is known that compounds which have an H-bond donor suitably positioned, such as the 6-hydroxymethylpenicillin sulfones (**9**, Fig. 2),[14–16] and the corresponding mercaptomethylpenicillin sulfones (**10**),[17] are recognized as potent β-lactamase inactivators. The analogous 6-(hydroxymethyl)penicillin sulfides (**8**), while less potent β-lactamase inhibitors,[15,17] are valuable mechanistic probes which can define the direction of approach of the active site water to the acyl-enzyme.[18,19] While covalent complexes of these 6-(hydroxymethyl)penicillin sulfides with representative β-lactamases are available,[20,21] corresponding structural data is unfortunately not available on the more potent 6-(hydroxymethyl)penicillin sulfones, which will undoubtedly exhibit a mechanistically dissimilar course of transformations subsequent to acylation. Although progress was recently made in understanding the recognition of the antibiotic substrates by the PBPs,[22] it is also not clear why, given their propensity to form stable acyl-enzymes of the β-lactamases, most β-lactamase inhibitors exhibit little or no binding to essential PBP transpeptidases,[23] with the notable exception of the binding of PBP 2 of *Acinetobacter baumannii* by sulbactam.[24,25]

In view of these factors, especially including the evolution of new enzymes and the lack of data of C6-substituted penicillin

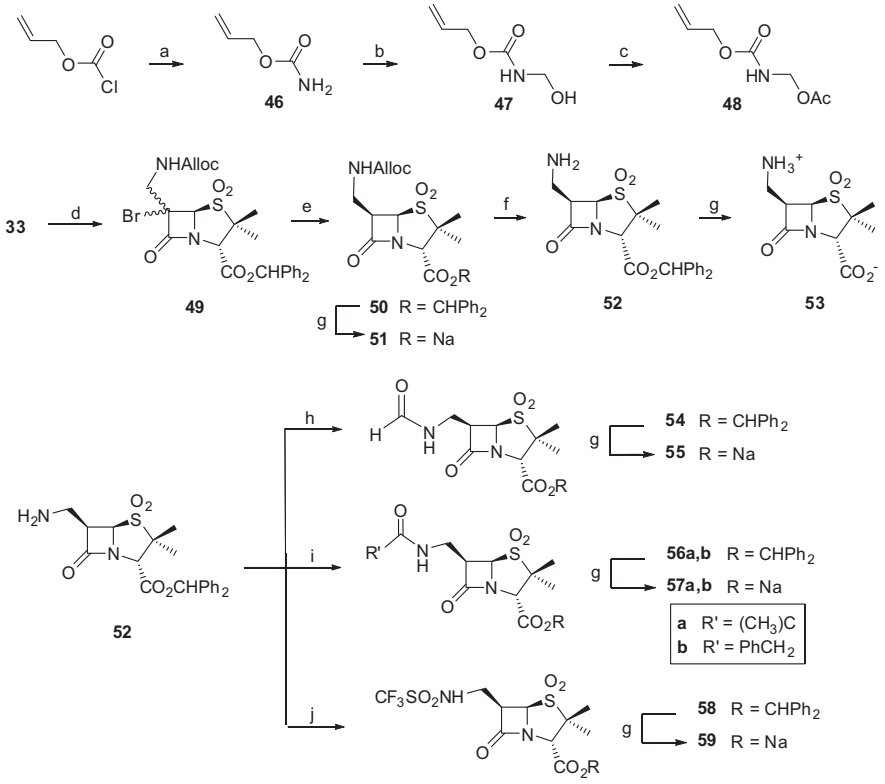


Scheme 3. Reagents and conditions: (a) pyrrolidine, 70 °C, 18 h; (b) HIO_4, Et_2O, 0 °C, 4 h; (c) PhOCOCl, pyr, DCM, 0 °C, 1 h; (d) allylamine, $CHCl_3$, 50 °C, 12 h; (e) (i) O_3, DCM, −78 °C, 30 min; (ii) Me_2S, DCM, −78 °C to rt, 2 h; (f) Ref. 27; (g) Ph_2CN_2, acetone, 0 °C to rt; (h) mCPBA, DCM, rt, 1 h; (i) (i) MeMgBr, THF, −78 °C, 30 min; (ii) RCHO (**26**, **30**, or CH_2O), THF, −78 °C to rt; 2 h; (j) *n*-Bu_3SnH, cat. AIBN, tol, reflux, 1 h; (k) (i) anisole, CF_3CO_2H, −10 °C, 15 min; (ii) $NaHCO_3$, EtOAc/H_2O, rt, 15 min; (l) allyltributyltin, cat. AIBN, tol, reflux, 1 h; (m) (i) 9-BBN, THF, rt, 1 h; (ii) H_2O_2, NaOAc, MeOH; (n) $NaBH_3CN$, HOAc–THF, rt, 30 min.

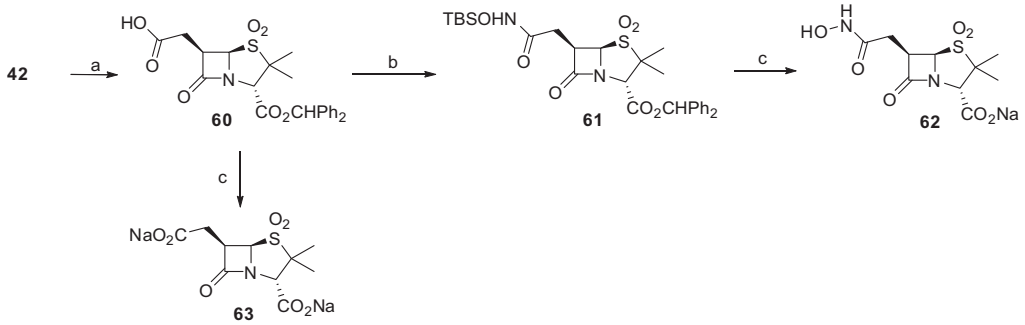
sulfones on these new serine β-lactamases, we proceeded to systematically study the inhibitory activity of a series of structurally modified penicillin sulfones (i.e., penicillin sulfones possessing an sp^3-hybridized carbon at C6 and lacking a C2′ substituent) with representative classes A and C β-lactamases, and to compare them with the prototypical C2′-unsubstituted penicillin sulfone, sulbactam. All compounds possess C6β geometry, analogous to the natural penicillin series.

As shown in Scheme 2, commercial 6-aminopenicillanic acid (6-APA, **11**) was esterified and oxidized to produce sulfone **12**, which was sulfonylated and deprotected to produce inhibitor **14**. Depending on the bulkiness of the acylamido side chain the 6-acylamido inhibitors were prepared either by an oxidation-acylation or an acylation-oxidation sequence. Thus **11** was oxidized to the corresponding sulfone with potassium permanganate,[26] and subsequently acylated to produce inhibitors **16** and **17**. The more bulky **20**, however, was prepared in the converse fashion as shown. Inhibitor **23** was generated from the corresponding benzyl ester as shown.

6β-(Hydroxyalkyl)penicillanic acids were prepared as shown in Scheme 3. In preparation for linking to the penicillin, 6-APA was converted to 6,6-dibromopenicillanic acid (**31**) by the method of Volkmann,[27] esterified, and oxidized to sulfone **33** as shown. **33** was metallated and reacted with aldehydes to produce bromoalcohols **34**, which were reduced and deprotected to produce inhibitors **36a**, **36c**, and **37**. Dibromide **33** was reacted with allyltributylstannane in the presence of a catalytic amount of AIBN to produce **38** as a mixture of epimers. These were reduced with tributyltin



Scheme 4. Reagents and conditions: (a) aq NH_4OH, THF, 0 °C, 1 h; (b) Na_2CO_3, paraformaldehyde, H_2O, 24 h; (c) pyr, Ac_2O, 0 °C, 18 h; (d) (i) MeMgBr, THF, −78 °C, 30 min; (ii) **48**, THF, −78 °C, 2 h; (e) nBu$_3$SnH, cat AIBN, tol, reflux; 1 h; (f) n-Bu$_3$SnH, PPh_3, cat. Pd(PPh_3)$_4$, DCM/H_2O, rt, 90 min; (g) (i) TFA, anisole, −10 °C, 1 h; (ii) $NaHCO_3$, EtOAc/H_2O, rt, 15 min; (h) AcOCHO, pyr, DCM, 0 °C, 15 min; (i) RCOCl, pyr, DCM, 0 °C, 15 min; (j) Tf_2O, pyr, THF, −78 °C, 20 min.



Scheme 5. Reagents and conditions: (a) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, tBuOH/THF/H_2O, 0 °C, 1 h; (b) (i) EtOCOCl, TEA, THF, 0 °C, 1 h; (ii) $TBSONH_2$, DCM, 0 °C, 30 min; (c) (i) TFA, anisole, −10 °C, 1 h; (ii) $NaHCO_3$, EtOAc/H_2O, rt, 15 min.

hydride to stereoselectively generate the 6β-allylpenicillin sulfone **39** by addition of the hydrogen atom to the intermediate radical from the less-hindered α-face. Compound **39** was deprotected to produce inhibitor **40** and also reacted with 9-BBN, followed by hydrogen peroxide, and deprotected to produce **41**, or alternatively, subjected to ozonolysis and deprotected to produce the aldehyde hydrate **43**, as shown. Intermediate aldehyde **42** was reduced to the corresponding alcohol **44** and deprotected to produce the 6β-(hydroxyethyl)penicillin sulfone **45**.

The 6β-(aminomethyl)penicillanic acid sulfones were prepared as shown in Scheme 4. Dibromide **33** was aminomethylated using the alloc-protected *N,O*-acetal **48** (prepared from allycarbamate **46**, as shown) and stereoselectively reduced to produce **50**. This acylamidomethylpenicillinate was deprotected to produce inhibitor **51**. The allyloxycarbonyl protecting group was removed to produce the parent 6β-aminomethylpenicillin sulfone **52**, which was further deprotected to produce inhibitor **53** as shown. Then **52** was reacted with formic acetic anhydride, pivaloyl chloride, phenylacetyl chloride, and triflic anhydride, to produce, after subsequent depotection, inhibitors **55**, **57a**, **57b**, and **59**, respectively. Lastly, the hydroxamate **62** was prepared, through oxidation of aldehyde **42** to generate acid **60**, (which was also deprotected to produce diacid **63**) followed by coupling with *O*-TBS hydroxylamine and deprotection, as shown in Scheme 5.

As seen in Table 1 and Figure 3, the most active inhibitors in this series have an hydroxymethyl group at C6, including inhibitors **36a** and **36c**, which result in improvements in inhibitory activity against both the representative class A and the class C β-lactamase, relative to sulbactam. Related compounds **37** and **53**, which also have hydrogen bonding potential at the C6′ position, are also capable of maintaining inhibitory activity of the class A TEM-1 enzyme. Surprisingly, the inhibitors most similar to the natural antibiotics and possessing C6 substituents able to mimic their hydrogen bond-donating capability through a directly attached C6 acylamino group (e.g., **16**, **17**, **20**, and **14**) have little activity as inhibitors of the class A enzyme.

Like other serine hydrolases (albeit lacking the usual catalytic triad), classes A and C β-lactamases function through a three-step process involving formation of the Michaelis complex (EC) of the enzyme (E) with the antibiotic/inhibitor (C), acylation of the active site serine to form the acyl-enzyme (EC^*), and deacylation, as shown in Scheme 6. After acylating the active site serine, inhibitors partition, via subsequent fragmentation of the dioxothiazolidinone (Scheme 1 and **4**→**5**) to produce a more stabilized acyl-enzyme (Scheme 6, EC^{**}; Scheme 1, **6** or **7**) with a dramatically reduced rate of hydrolysis (k_5). Classes A and C β-lactamase differ kinetically. Class A enzymes are less able to recognize (higher K_m) sterically bulky β-lactams (such as third generation cephalosporins and methicillin[30]), but possess enhanced deacylation capability due to the presence of Glu166, which activates the hydrolytic water.[31] Class C β-lactamases, while having a broader recognition of substrates (lower K_m) and rapid acylation (k_2), have reduced deacylation rates (k_3),[32,33] and thus the k_{cat} for many class C enzymes is equal to the rate of this final step of the hydrolytic process.[10]

As a result of these arguments, we advance the following hypotheses:

(1) In this series of inhibitors, an H-bond donor, at either C6, or at the adjacent carbon (C6′) is needed for activity at either the class A or the class C β-lactamase (e.g., low activity of **41**, **43**, **45**, **62**, and **63** against both enzymes).

(2) Branched, bulky side chains, combined with a C6 H-bond donor, lower activity against the class A β-lactamase, but improve activity against class C. This is probably due to the smaller, more well-defined active site of the class A enzyme as a consequence of the presence of the omega loop thus leading to poor recognition (high K_m) for branched inhibitors (Fig. 4). This same bulkiness possibly leads to a longer lifetime of the acyl-enzyme in the class C β-lactamase (e.g., low activity of **17** and **20** against the class A enzyme, but improved inhibition of class C).

(3) Inhibitors which closely resemble good substrates (e.g., **16**) have little potency. This is probably due to their functioning as substrates of both enzymes, and thus having a large partition ratio (k_3/k_4, i.e., k_{cat}/k_{inact}).[34]

(4) Inhibitors having a non-directional C6′ H-bond donating group (OH) (e.g., **36a**, **36c**, and **37**) are the best overall inhibitors. In these inhibitors, the side chain CH_2O–H group may be improving recognition (lowering K_m), and also lowering the partition ration (k_3/k_4) by providing sufficient conformational freedom in the acyl-enzyme to allow the catalytic base (E166, in the case of Class A) to abstract a proton directly from C6, leading to formation of the β-aminoacrylate, (EC^{**}, **7**) rather than abstracting a proton from the hydrolytic water (to form E and P) as shown in Scheme 7.[35]

Table 1
Inhibitory activity against TEM-1 (class A) and PDC-3 (extended spectrum class C[28]) β-lactamase (IC_{50}, μM, ±10%)[29]

Inhibitor	-R	TEM-1	PDC-3
Sulbactam	–H	1.3	4.3
14	$-NHSO_2CF_3$	>50	7.0
15	$-NH_3^+$	8.4	>50
16	$-NHCOCH_2Ph$	>50	>50
17	$-NHCO(2,6\text{-}C_6H_3(OCH_3)_2)$	>50	1.1
20	$-NHCO(5\text{-Me-3-Ph-}(1,2\text{-}C_3NO)$	>50	1.7
23	$-NHCH_2CO_2Na$	>50	13.8
36a	$-CH(OH)CO(NC_4H_8)$	0.18	0.16
36c	$-CH_2OH$	0.012	0.18
37	$-CH(OH)CH_2NH_3^+$	1.2	1.2
40	$-CH_2CH{=}CH_2$	35	1.2
41	$-CH_2CH_2CH_2OH$	>50	30
43	$-CH_2CH(OH)_2$	>50	>50
45	$-CH_2CH_2OH$	>50	43
51	$-CH_2NHCO_2CH_2CH{=}CH_2$	>50	45
53	$-CH_2NH_3^+$	0.45	>50
55	$-CH_2NHCHO$	9.9	10
57a	$-CH_2COBu^t$	>50	1.7
57b	$-CH_2COCH_2Ph$	>50	4.2
59	$-CH_2NHSO_2CF_3$	6.1	1.8
62	$-CH_2CONHOH$	>50	>50
63	$-CH_2CO_2Na$	>50	12.7

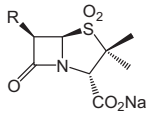
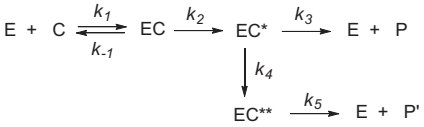


Figure 3. General inhibitor structure for Table 1.



Scheme 6. Minimal kinetic scheme for interaction of a β-lactamase (E) with a substrate and/or inhibitor (C).

In conclusion, it is clear that inhibitor design in the penicillin sulfone series involves achieving a balance between inhibitor recognition and the hydrolytic stability of the resultant acyl-enzyme. One needs sufficient similarity to the natural substrate to ensure that the inhibitor is recognized and can efficiently acylate the

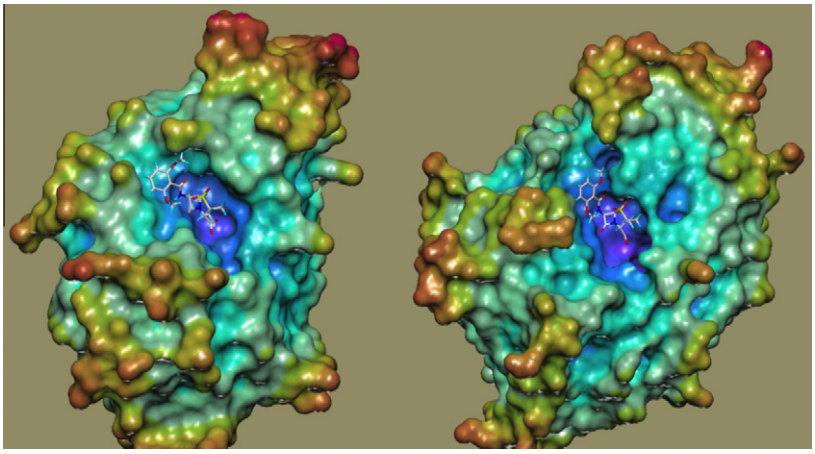
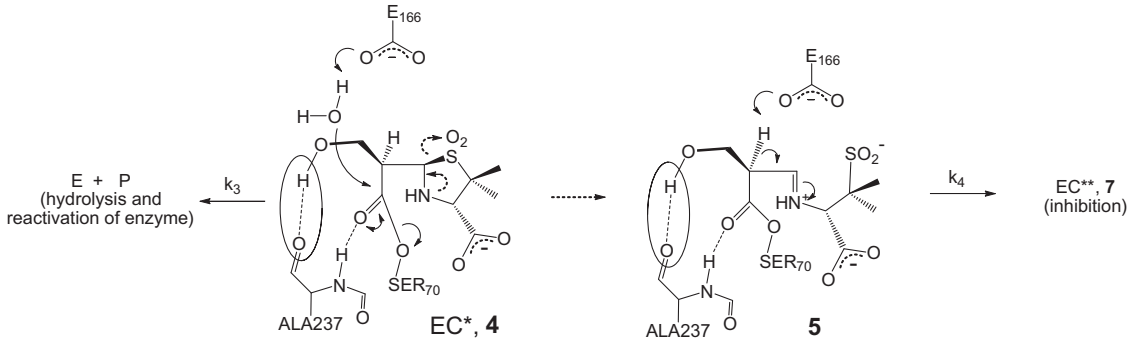


Figure 4. Computer-assisted docking of methicillin sulfone (**17**) in the active sites of a representative class A (TEM-1, 1ZG4, left) and class C (P99, 1XX2) β-lactamase. Electrostatic potential is mapped onto the surface of the enzyme, with blue being most positive and red most negative.



Scheme 7. Proposed interactions of the acyl-enzyme derived from inhibitor **36c** with the class A, TEM-1 β-lactamase, leading either to turnover (left) or inhibition.

enzyme, but excessive resemblance to natural substrates may result in poor activity due to turnover (i.e., high partition ratio). Substituents which, through their placement and interactions with the enzyme, facilitate the formation of the β-aminoacrylate and/or enhance its stability, improve inhibitory potency. The evolution of the β-lactamase active site necessitates the redesign of both β-lactam antibiotics and β-lactamase inhibitors to counter new modifications of the enzymes. This work, which systematically explores inhibitory activity of a series of penicillin sulfones having an sp^3 hybridized C6 substituent, demonstrates that recognition may be an important factor, with both the conformational freedom and the hydrogen-bond donating capability of the side chain at C6 being essential factors in attaining optimal activity.

Acknowledgments

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