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Cephalosporin-Derived Inhibitors of β-Lactamase. Part 4: The C3 Substituent

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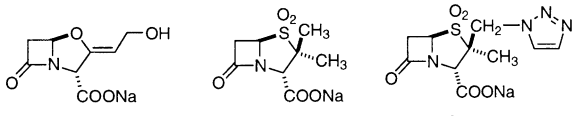
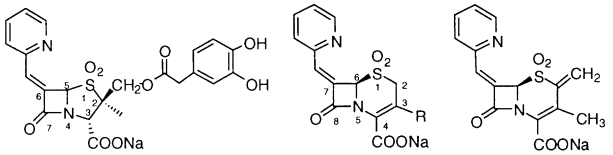
Abstract—New C3-substituted β-lactamase inhibitors were prepared and evaluated against representative class A and class C enzymes. It was possible to improve simultaneous inhibitory activity of both classes of serine hydrolase. Other inhibitors showed high selectivity for either the class C cephalosporinases or the class A penicillinases. This represents the first time that cephalosporin-derived inhibitors have demonstrated selectivity for the class A β-lactamases. © 2002 Elsevier Science Ltd. All rights reserved.

While the β-lactam antibiotics remain among the most commonly prescribed antimicrobial products,[1] the incidence of resistance to these safe and effective drugs is increasing. The most common form of such resistance is the bacterial ability to produce β-lactamase, an enzyme that hydrolyzes the β-lactam. More than 300 different β-lactamases have been identified.[2] These enzymes are divided into four groups (A, B, C, and D), based on their structure.[3] Both classes A and C are currently clinically relevant.[4] One partially effective method to overcome such resistance is to coadminister a combination of the antibiotic and a β-lactamase inhibitor.[5] Augmentin, the most commercially successful product, combines the inhibitor clavulanic acid (**1**), with the antibiotic amoxicillin. Other inhibitor/antibiotic combinations include sulbactam (**2**), which is coadministered with ampicillin in the form of Unasyn® (injectable) or Sultamicillin® (oral), and tazobactam (**3**), which is coadministered with piperacillin in the form of Zosyn®. However, these products have limitations. In particular, they are only active against class A-producing microorganisms, historically the most clinically relevant.[6] Recently the incidence of infections by class C producing organisms, in particular, has been rising (Fig. 1).[7]



1 **2** **3**

Figure 1. Commercial β-lactamase inhibitors.



4 **5**, R = CH_2OAc **6**, R = *E* CH=$CHCONH_2$ **7**

Figure 2. Recently reported inhibitors from our group.

Our group has recently reported inhibitors that possess the ability to simultaneously inactivate both class A penicillinases as well as the class C cephalosporinases.[8] A few of these inhibitors are shown in Figure 2. We have also demonstrated the synergy of these new compounds in combination with existing antimicrobial products in the treatment of resistant microorganisms.[8a] Recently, the crystal structure of the extended spectrum class C β-lactamase GC1, inhibited by **6** was obtained.[9] By contrast to the penicillin-derived inhibitors, the cephalosporin-derived series allows facile synthetic modification at C3. This permitted us to produce analogues with altered properties, including expanded inhibitory profile. In fact, our initial cephalosporin-derived

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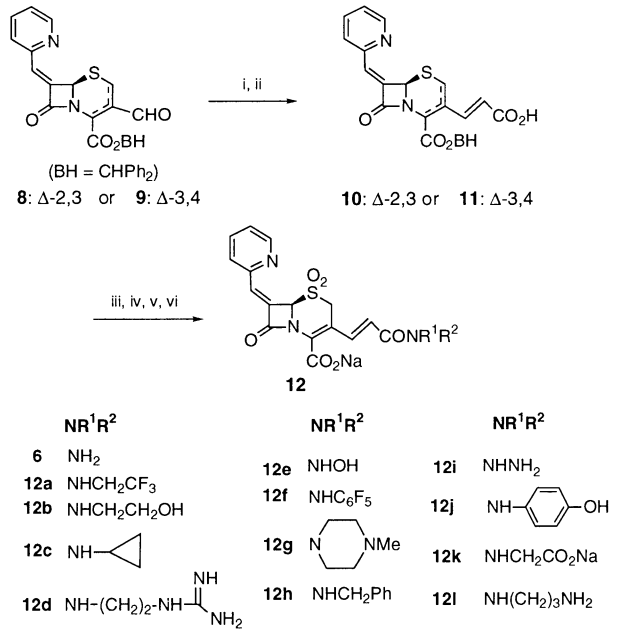
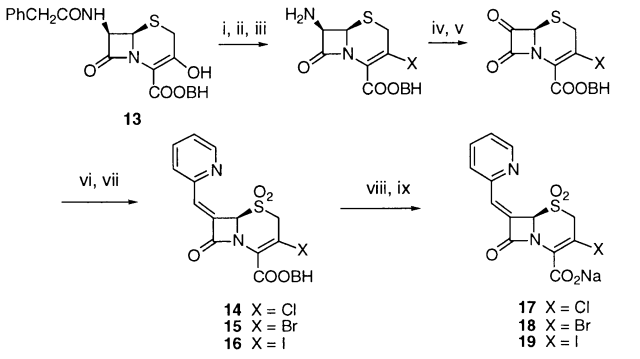


Scheme 1. Synthesis of amides **12a** to **12l**: (i) $Ph_3P{=}CHCO_2All$, CH_2Cl_2; (ii) *p*-$TolSO_2Na$, cat. $Pd(PPh_3)_4$, MeOH; (iii) R^1R^2NH, BOP reagent, TEA, CH_3CN, rt, 1–2 h, 70–80%; (iv) 2.5 equiv mCPBA; (v) TFA/anisole, 0 °C to rt; (vi) $NaHCO_3$.

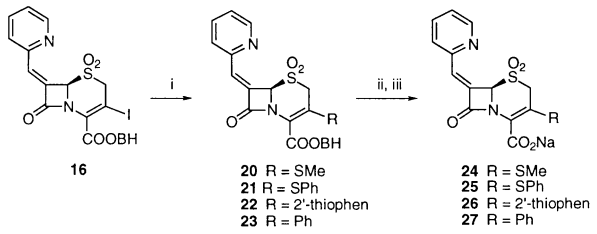


Scheme 2. Synthesis of C3 halides **17**, **18**, and **19**: (i) Tf_2O, DIPEA, DCM −78 °C, 85%; (ii) LiX (2.5 equiv), THF, rt 36 h, 70–90%; (iii) PCl_5, py, DCM/MeOH, 0 °C, 74%; (iv) *i*-prONO, cat. TFA, EtOAc; (v) propylene oxide, cat. $Rh_2(OOct)_4$, C_6H_6; (vi) α-py-CH_2–PPh_3Cl, KO-*t*-Bu, THF/DCM (30% overall for steps iv, v, and vi); (vii) mCPBA (2.5 equiv), DCM, rt, 30 min, 90%; (viii) TFA, anisole; (ix) $NaHCO_3$.

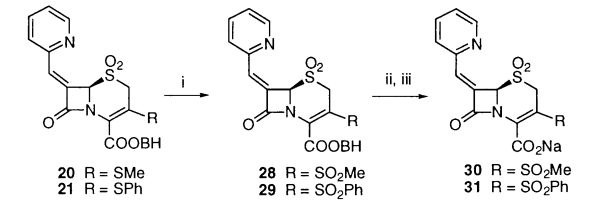
inhibitors (e.g., **5**) themselves were initially discovered as selective inactivators of the class C enzymes.[10] Only through modification at C3, was it possible to achieve the goal of dual inhibition of classes A and C.[8b,c] We have recently developed new synthetic methodology leading to the preparation of a wider range of substituted C3 derivatives in this series.[11] We now report the biological activity of these new materials.

The target molecules were prepared in a number of ways. As shown in Scheme 1, carboxylic acid **10** or **11** (prepared as described previously[8b]) could be coupled with a variety of amines to produce amides **12a**–**12l**.

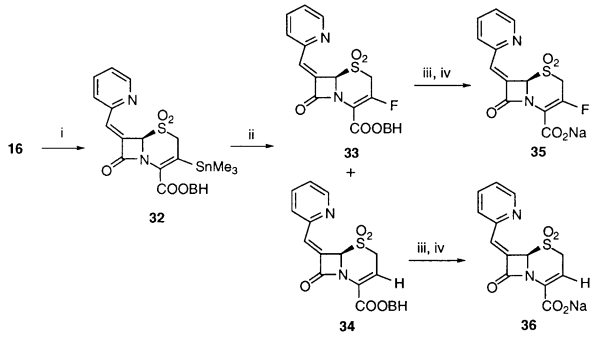
C3 halides, **17**, **18**, and **19** were prepared from the commercially available[12] **13** as shown in Scheme 2.



Scheme 3. Synthesis of **24** to **27**: (i) R-$SnBu_3$, cat. $Pd_2(dba)_3$; (ii) TFA, anisole; (iii) $NaHCO_3$.



Scheme 4. Synthesis of **30** and **31**: (i) 4 equiv mCPBA; (ii) TFA, anisole; (iii) $NaHCO_3$.



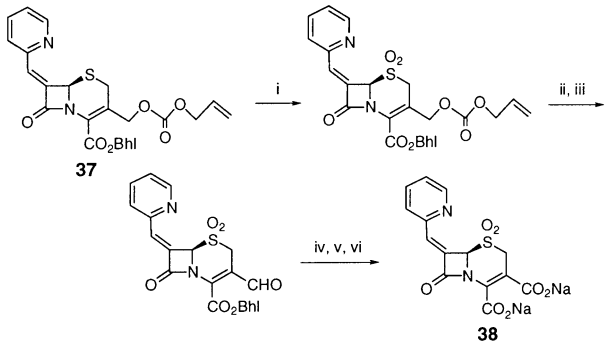
Scheme 5. Synthesis of **35** and **36**: (i) $(Me_3Sn)_2$, cat. $Pd_2(dba)_3$, THF; (ii) XeF_2, AgOTf, 0 °C, CH_2Cl_2, 5 min; (iii) TFA, anisole; (iv) $NaHCO_3$.

As shown in Scheme 3, iodosulfone **16** could be coupled with a number of organostannanes to produce new C3-substituted inhibitors. The sulfur side chain of intermediates **20** and **21** was also further oxidized to the sulfone oxidation state as shown in Scheme 4.

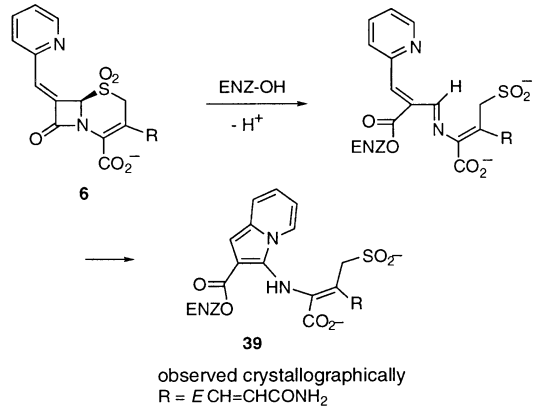
The fluoride **35** and the C3 unsubstituted compound **36** were prepared from the stannane **32** as shown in Scheme 5. The production of **34** is presumably produced by the presence of adventitious amounts of HF in the reaction.

Compound **38** was prepared from **37** as shown below in Scheme 6. Steps ii and iii are performed in the same pot to avoid formation of the lactone.

The biological activity of these new inhibitors against the representative class A enzyme TEM-1 and the class C enzyme P99 are reported in Table 1.[13] A survey of the data indicates that modification of the C3 substituent within this cephalosporin-derived group of inhibitors has a marked effect on the inhibitory activity. The ratios of the IC_{50} values in the case of the class A TEM-1: worst inhibitor/best inhibitor = 2437, and in the case of



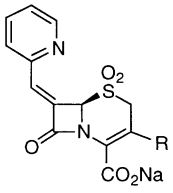
Scheme 6. Synthesis of **38**: (i) 2.5 equiv mCPBA, DCM, 30 min, 81%; (ii) Bu_3SnH, Pd, AcOH, DCM; (iii) Dess–Martin, DCM, $NaHCO_3$ (72% steps ii and iii); (iv) $NaClO_2$, H_2NSO_3H, $MeOH/H_2O = 2/1$, 78%; (v) TFA, anisole; (vi) $NaHCO_3$.



Scheme 7. Proposed mechanistic pathway leading to inhibition.

the class C P99 worst/best = 2172. While tazobactam is a specific inhibitor of the class A TEM-1 enzyme, several of these new inhibitors display outstanding inhibition of both enzymes. Included in this group are **12d**, **25**, **30**, and **31**. Others display a more highly specific inhibition of the class C P99 enzyme, including halides **17**, **18**, **19**, and **35**, the unsubstituted **36**, and the biscarboxylate **38**. This series of amides also demonstrates, for the first time, that it is possible to design cephalosporin-derived inhibitors that have substantially higher inhibitory activity against the class A penicillinases than against the class C cephalosporinases (e.g., **12g**).

Table 1. β-Lactamase inhibitory activity against representative class A (TEM-1) and class C (P99) enzymes[14]



Compd	R	TEM-1 Inhibition IC_{50} (μM)	P99 Inhibition IC_{50} (μM)
Tazobactam		0.25	101.6
6	CH=CH–$CONH_2$	0.2615	0.022
12a	CH=CH–$CONHCH_2CF_3$	0.078	1.18
12b	CH=CH–$CONHCH_2CH_2OH$	0.0701	0.212
12c	CH=CH–$CONHCH(CH_2)_2$	0.240	0.824
12d	CH=CH–CONH–CH_2CH_2 (CN_3H_4)	0.0083	0.0055
12e	CH=CH–CONHOH	7.69	0.128
12f	CH=CH–$CONHC_6F_5$	4.28	0.127
12g	CH=CH–$CON(CH_2CH_2)_2NMe$	0.053	6.34
12h	CH=CH–$CONHCH_2Ph$	1.4	0.11
12i	CH=CH–$CONHNH_2$	0.39	1.1
12j	CH=CH–CO-NHC_6H_4OH	0.11	0.035
12k	CH=CH–$CONHCH_2CO_2Na$	4.2	0.31
12l	CH=CH–$CONH(CH_2)_3NH_2$	1.59	4.2
17	Cl	1.82	0.003
18	Br	0.791	0.0029
19	I	1.28	0.0047
24	SMe	1.065	0.059
25	SPh	0.012	0.0217
26	Thiophen-2′-yl	3.54	0.0403
27	Ph	9.14	0.038
30	SO_2Me	0.0076	0.0907
31	SO_2Ph	0.0094	0.174
35	F	1.84	0.011
36	H	18.52	0.116
38	CO_2Na	10.44	0.032

In the case of inhibitor **6**, we have crystallographically observed the formation of stabilized acyl enzyme **39**, and proposed the mechanism shown in Scheme 7.[9] It is logical to assume that each of these compounds inactivates the enzymes in a similar manner. The observed individual IC_{50} values likely represent a complex interplay of factors. Included among these are: (1) recognition in the active site (i.e., formation of the Michaelis Complex), (2) the rate of initial acylation of the active site serine, (3) the rate of rearrangement of the initial acyl-enzyme to the stabilized acyl-enzyme, and (4) the stability of this resultant acyl enzyme toward hydrolysis (perhaps owing to its chemical structure, its placement in the active site, and/or induced conformational changes in the enzyme).

However, some generalizations regarding structure–activity relationships can be made. In our first paper in this series,[15] we observed that the prototypical cephalosporin C3 side chain (i.e., $R = CH_2OAc$) produced an inhibitor which solely targeted the class C P99 enzyme. In the second paper, we observed a dramatic enhancement of inhibitory activity (especially against the class A enzyme, TEM-1) by placing an electronegative group at C3 (e.g., R = CN or R = CH=CH–COR′). The present work illustrates that, while the trend toward improved activity with electronegative groups is still operative (e.g., the high activity of sulfones **30** and **31**), the effect of the C3 substituent is more complex. The unexpectedly high overall activity of **25**, having a (relatively) non-electronegative C3 side chain illustrates that, in addition to electronegativity, inhibitor–enzyme recognition at C3 probably plays a role in determining activity. Such recognition may be occurring during formation of the initial Michaelis complex or may be further enhancing binding of the chemically stabilized acyl-enzyme. This is further illustrated by the C3 substituent of **12d**, which has roughly the same electronegativity as that of the other (unsaturated) amides, but probably attains improved binding due to its positively charged side chain thereby leading to enhanced inhibition.

A close inspection of reported β-lactamase crystal structures reveals that, when the two enzymes (TEM-1 and P99) are superimposed, the P99 structure is more congested in the region of the cephalosporin C3 substituent.[16] This may partially explain the better activity of very small C3 side chain inhibitors like **36** against P99 and larger C3 side chain inhibitors like **12g** against TEM-1.

In summary, we have now demonstrated the profound effect of the C3 substituent upon the ability of selected cephalosporin sulfones to inhibit β-lactamase. Depending on the substituent, the inhibitor may select for inhibition of class C β-lactamases, inhibition of class A enzymes, or become a general inhibitor of both these serine classes. Although increasing the electronegativity at C3 seems to improve inhibition of both serine classes, other factors, including enzyme-inhibitor recognition, may also play a role.

Acknowledgements

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13. (a) The Class A TEM-1 β-lactamase, derived from *Escherichia coli*, was kindly provided by Dr. Timothy Palzkill (Baylor College of Medicine). It was purified to >90% homogeneity by the reported procedure: Cantu, C.; Huang, W.; Palzkill, T. *J. Biol. Chem.* **1997**, *272*, 29144. (b) The Class C enzyme, derived from *Enterobacter cloacae*, strain P99, was purchased from the Center for Applied Microbiology and Research (CAMR), Porton Down, Salisbury, UK.
14. Assay method involves 4 min incubation of a solution of inhibitor and enzyme (0.1–1 μM in enzyme), followed by transfer of an aliquot into a dilute solution of the substrate nitrocefin. Hydrolysis is monitored spectrophotometrically at 480 nm for 1 min. The rate is constant throughout this period. Error is +10%, based on multiple experiments. The purity of all compounds and intermediates was verified by NMR.
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16. This is true despite the fact that the class C enzymes are generally thought of as having a larger overall binding pocket than the class A β-lactamases. Indeed the region of the pocket in the vicinity of C7 is larger in class C enabling the enzymes to more readily accommodate C7-modified antibiotics, including the third and fourth generation cephalosporins.