Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1997–2002

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

THE SYNTHESIS AND EVALUATION OF 6-ALKYLIDENE-2'β-SUBSTITUTED PENAM SULFONES AS β-LACTAMASE INHIBITORS

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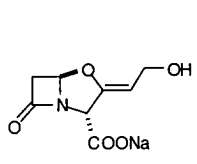
Received 24 April 1999; accepted 27 May 1999

Abstract: Penicillin sulfones, which structurally incorporate *both* a 6-position alkylidene substituent and a 2'β substituent, have been synthesized and evaluated as inhibitors of class C and class A serine β-lactamases. Incorporation of the 2'β-substituent generally improves inhibitory activity. Substituents that improve transport across the bacterial cell membrane have also been incorporated.

The most prevalent cause of bacterial resistance to antibiotic therapy is the acquisition of the ability to produce β-lactamase.[2] The hydrolytic destruction of the β-lactam may be either serine (class A, C, and D) or metalloenzyme (class B) catalyzed. Successful treatment of these infections can often be effected by the co-administration of an antibiotic and a β-lactamase inhibitor. Established inhibitors of the serine β-lactamases include clavulanate (**1**), sulbactam (**2**), and tazobactam (**3**). These compounds have no independent antibacterial activity. Due to the rapid spread and high rate of mutation of these enzymes, there is an urgent need for more effective, broader spectrum inhibitors.

We have recently reported new inhibitors **4**[3] and **5**[4] shown below. Within the 6-unsubstituted sulbactam-like molecules, it has proven advantageous to include a 2β-substituent (ex. **3**). We now report the synthesis and evaluation of a series of 2β-substituted-6-alkylidenepenams.



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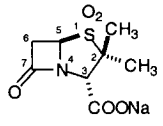

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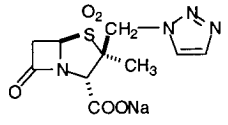

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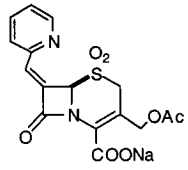

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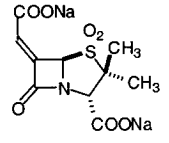

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Our first targets were the 2β-substituted analogs of our recently reported inhibitor, **5**. 6-Oxopenicillanate, **7**, which is now readily available from 6-APA,[5] was treated with one of three stabilized ylides at -78 °C to produce 6-alkylidene penicillinates which on reaction with 1 eq of MCPBA in CH_2Cl_2 afforded the sulfoxides **8**. These sulfoxides were heated to reflux in toluene in presence of 2-mercaptobenzothiazole to obtain the disulfides **9**.[6] The disulfides **9** were then treated with AgOAc in the presence of a large excess of selected

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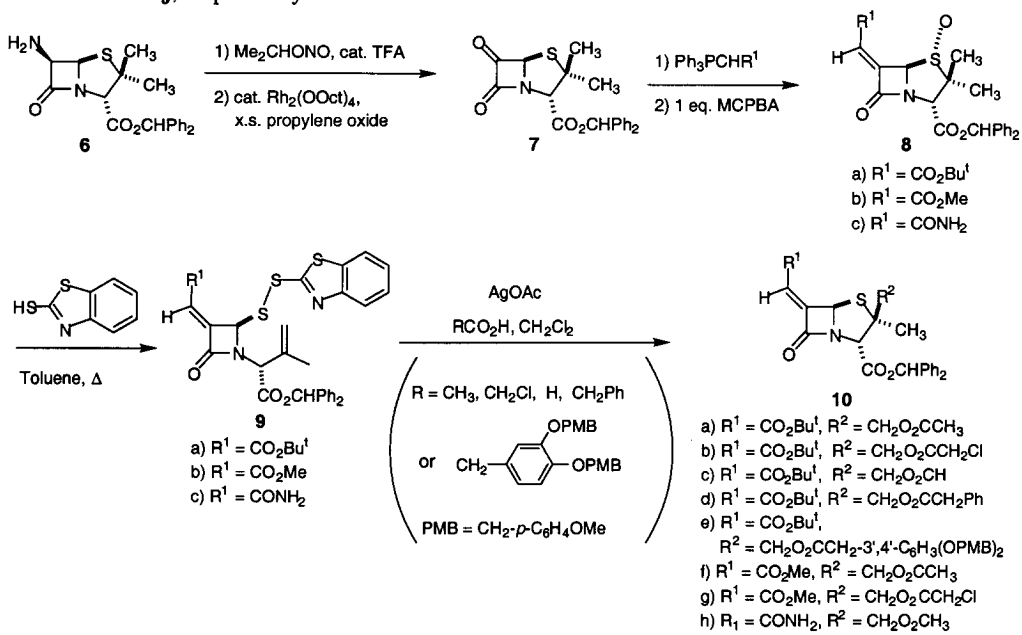
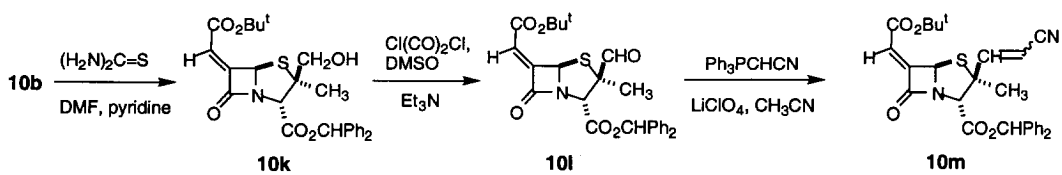
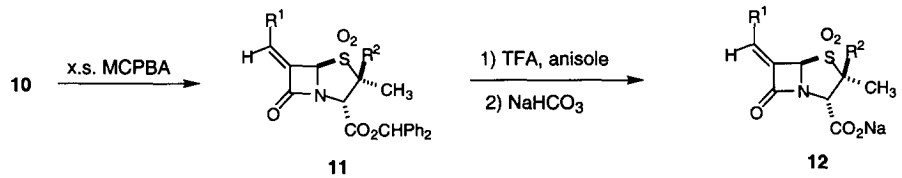
carboxylic acids to produce carboxylates **10**.[7] Uncharacteristically, the reaction cleanly produced a single product. It appears that the presence of the 6-alkylidene substituent prevents formation of the cepham side product usually observed in this type of reaction. As shown, the protected catecholic carboxylic acid was prepared from (3,4-dihyrdoxyphenyl)acetic acid. **9a** and **9b** could also be treated with $CuCl_2$ to produce chlorides **10i** and **10j**, respectively.



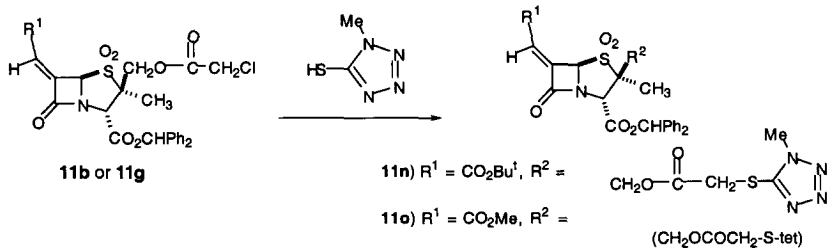
Following a protocol similar to that employed in the 6-unsubstituted series,[8] we were also able to create the nitrile **10m**. As shown, this process involves removal of the chloroacetate from **10b**, oxidation, and reaction with (cyanomethylene)triphenylphosphorane.



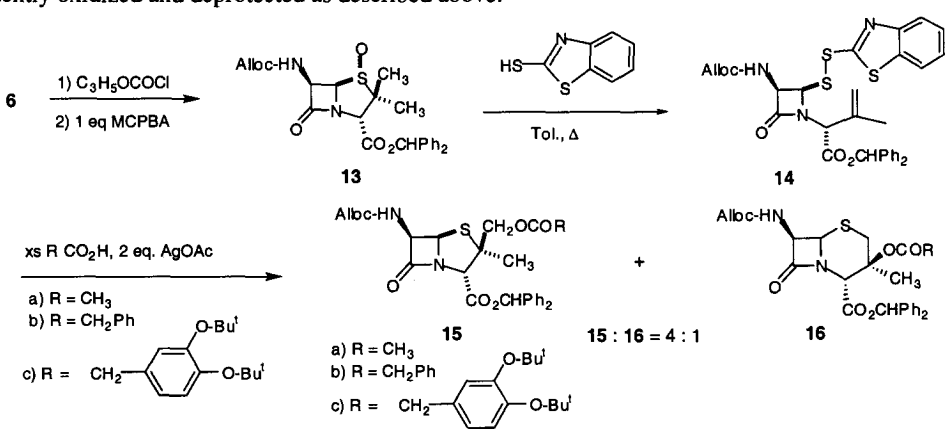
Oxidation of sulfides **10** with MCPBA produced the corresponding sulfones, which were readily deprotected to the corresponding carboxylate salts, **12**.



The chloroacetate sulfones **11b** and **11g** could also be substituted with nucleophiles as illustrated by the synthesis of **11n** and **11o** (and deprotected to produce the corresponding salts **12n** and **12o**).



In view of the high biological activity of the (2-unsubstituted) 6-[(α-pyridyl)methylene]penicillin sulfone,[9] we desired to explore 2β-substituted analogs of this inhibitor. Unfortunately our attempts to perform a transformation of **9** (R^1= α-pyridyl) to the corresponding analog **10** was unsuccessful. We therefore employed an alternative strategy, utilizing the *N*-protected 6-aminopenicillinate analog, **14**, in the transformation as shown. Unlike the 6-unsaturated case (i.e. the transformation from **9** to **10**), this cyclization was complicated by the presence of approximately 20% of the undesired cepham **16**. As shown below, an alternatively protected catechol was utilized as a trapping agent, due to the difficulty in purifying the PMB-protected version used above. Subsequent removal of the allyloxycarbonyl group, oxidation to the ketone under the extremely mild conditions we had earlier developed, followed by reaction with [(α-pyridyl)methylene]triphenylphosphorane produced the desired compounds **10p**, **10q**, and **10r** which could be subsequently oxidized and deprotected as described above.



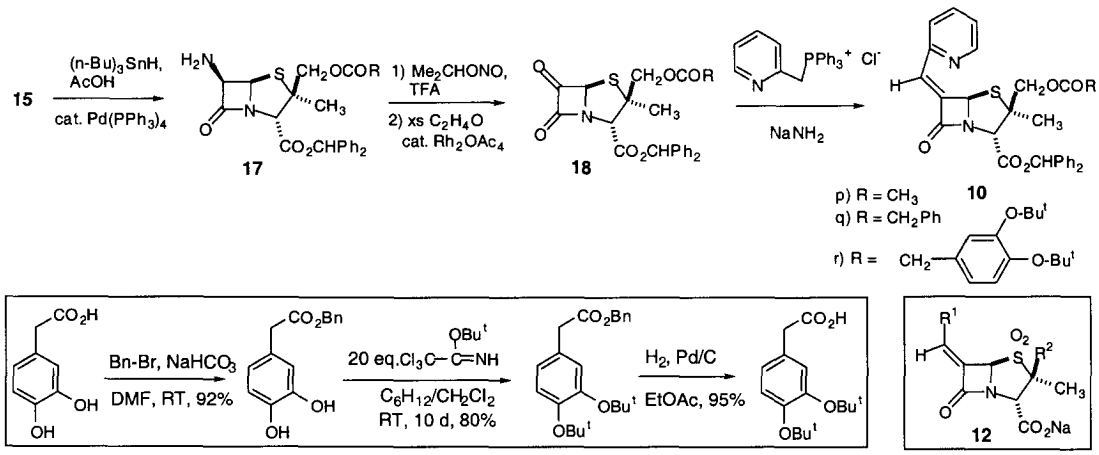


Table 1. Inhibitory activity of **12** on Three Representative Serine β-Lactamases IC_{50} (μM)

Compound	R^1	R^2	P99	TEM-1	PC1
3 (tazo)	(undefined)	$C_2H_2N_3$	51.9	0.297	2.57
5	CO_2Na	CH_3	4.50	1.8	108
12a	CO_2Na	$CH_2O_2CCH_3$	0.708	0.180	76.53
12b	CO_2Na	$CH_2O_2CCH_2Cl$	NT	0.196	7.2
12c	CO_2Na	CH_2O_2CH	0.592	1.84	173
12d	CO_2Na	$CH_2O_2CCH_2Ph$	0.54	0.0154	579.0
12e	CO_2Na	$CH_2O_2CCH_2$-3',4'-$C_6H_3(OH)_2$	0.37	0.105	116
12f	CO_2Me	$CH_2O_2CCH_3$	9.51	2.72	NT
12h	$CONH_2$	$CH_2O_2CH_3$	8.48	0.31	2.21
12i	CO_2Na	CH_2Cl	527.0	120.5	2100
12j	CO_2Me	CH_2Cl	13.91	44.51	432
12m	CO_2Na	=CHCN	6.76	21.67	504
12n	CO_2Na	$CH_2O_2CCH_2$-S-tet	0.64	0.233	NT
12o	CO_2Me	$CH_2O_2CCH_2$-S-tet	13.2	2.37	939.7
12p	α'-pyr	$CH_2O_2CCH_3$	0.062	0.004	0.66
12q	α'-pyr	$CH_2O_2CCH_2Ph$	0.001	0.04	0.39
12r	α'-pyr	$CH_2O_2CCH_2$-3',4'-$C_6H_3(OH)_2$	0.026	0.06	0.7

Discussion. In Table 1 is reported the relative inhibitory activity of these compounds against one class C, P99, and two class A, TEM-1 and PC1 (derived from *Staph. aureus*) β-lactamase. In general, the 6-alkylidenpenams (with or without a 2'-substituent) are better inhibitors of the isolated class C enzyme than is tazobactam.

Selected 2'-substituents,in this series, improve the ability to inhibit TEM-1. The particular substituents which have resulted in improved activity are quite different from the corresponding substituents in the

Table 2. *In Vitro* Synergistic Activity of Inhibitor **12r**, in Combination with Piperacillin. (MIC μg/mL).

	Pip	PIP:TAZ	12r	PIP:12r	Ceftazidim	Imipenem
E. coli C600N (no β-lactamase)	2	2	>64	1	0.12	0.25
E. coli C600N +(TEM-1)	>64	4	>64	2	0.12	0.12
E. coli C600N + (TEM-10)	>64	2	>64	2	64	0.12
E. coli C600N + (SHV-1)	4	2	>64	2	0.50	0.12
E. coli C600N + (IRT – 2)	>64	8	>64	2	0.25	0.25
E. coli C600N + (SHV – 4)	>64	2	>64	2	>64	0.12
E. coli C600N + (PSE – 1)	32	1	>64	2	0.25	0.12
E. coli C600N + (OXA-10) {PSE-2}	>64	2	>64	2	0.12	0.12
E. coli C600N + (MIR-1)	64	8	>64	8	64	0.50
E. coli C600N + (ampRampC)	>64	8	>64	16	64	0.50
E. coli C600N + (P99)	>64	8	>64	16	64	0.25
E. coli C600N + (Sme-1)	>64	8	>64	8	0.50	64
E. coli C600N + (Imi-1)	>64	16	>64	8	1	4
E. coli C600N + (L1)	>64	>64	>64	64	32	2
E. coli 4100 (no β-lactamase)	<0.06	<0.06	>64	<0.06	<0.06	0.12
E. coli 4100 + (CcrA)	>64	>64	>64	>64	>64	16
E. coli 300 (no β-lactamase, imp mutant)	<0.06	<0.06	>64	<0.06	<0.06	<0.06
E. coli 300 + (TEM-1)	>64	4	>64	1	<0.06	<0.06
E. coli 300 + (ampRampC)	16	4	>64	2	4	<0.06
K. Pneumoniae KC 2 (TEM-10)	>64	2	>64	4	64	<0.06
E. cloacae SC12629 (AmpC inducible)	>64	>64	>64	64	>64	1
P. aeruginosa Ps505A1 (AmpC derepressed)	>64	16	>64	2	32	0.50
K. pneumoniae (ACT-1 + 3 blas, IPM=S)	>64	32	>64	32	>64	1
E. cloacae (Imi-1 + AmpC)	>64	16	>64	16	0.50	>64
A. sobria ((Asb A, OXA-12, AsbM)	64	64	>64	1	<0.06	>64
S. maltophila 1712 (L1)	>64	>64	>64	>64	>64	>64
S. aureus (PC 1)	>64	1	>64	1	4	<0.06
E. coli ATCC 25922 (no β-lactamase)	2	2	>64	1	<0.06	<0.06
E. coli ATCC 35218 (TEM-1)	>64	1	>64	1	<0.06	<0.06
S. marcescens GC 4132 (Amp C, *in vivo*)	64	32	>64	4	0.25	1
E. coli GC6265 (TEM-1, *in vivo*)	>64	4	>64	4	<0.06	<0.06
E. coli GC 6266 clinical isolate	>64	16	>64	16	0.25	0.25
E. coli GC 6267 clinical isolate	>64	16	>64	16	0.25	0.12
E. aerogenes GC 6268 clinical isolate	>64	32	>64	64	>64	1
K. pneumoniae GC 6269 clinical isolate	>64	8	>64	8	0.50	0.12

6-unsubstituted (i.e. sulbactam) series.[2d] For example, halides **12i** and **12j**, as well as nitrile **12m** are now almost completely inactive (in contrast to their relatively high activity in the 6-unsubstituted series), while the acetate **12a** and especially the phenylacetate **12d** display dramatically enhanced inhibition. Further studies will be required to determine the (mechanistic and/or recognition) significance of these differences.

Another mechanism of bacterial antibiotic resistance involves prevention of access to the target. This is particularly important with certain strains of gram-negative bacteria, such as *Pseudomonas aeruginosa*. In the

area of the *antibiotics* themselves (as opposed to the β-lactamase inhibitors), improved potency has been achieved by appending catecholic groups to the C-6 of penicillins, as well as C-7 and C-3 of cephalosporins.[10] It is believed that such iron-chelating functionality allows the antibiotic to enter the cell via an iron transport pathway.[11] Such modified antibiotics typically show improved profiles in treatment of gram negative strains.

Table 2 shows *in vitro* synergistic studies on **12r**, in combination with the antibiotic piperacillin, in the treatment of intact penicillin-resistant microorganisms. Structurally, **12r** incorporates a catechol functionality into the 2'-side chain. This work represents the first time a catechol has been incorporated at C-2 of the penicillins and the first time such functionality has been appended to any β-lactamase inhibitor. This addition improves the synergistic activity of this mixture against gram negative strains, including *P. aeruginosa*, *Aeromonas sobria,* and *Serratia marcescens.* It is interesting that the effect was attained despite the fact that the catechol was attached only to the inhibitor and not to the antibiotic.

In conclusion, it should be noted that these inhibitors are especially valuable due to their ability to inhibit both the class A and class C enzymes (AmpC). Such class C enzymes (which prefer cephalosporins as substrates) are not inhibited by clavulanate and have become increasingly clinically significant.[12]

Acknowledgment: We thank the Robert A.Welch Foundation and the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. The *S. aureus* and TEM-1 β-lactamases were generously provided by Dr. O. Herzberg and Dr. N. Strynadka, respectively. We thank Wyeth-Ayerst Research for providing synergistic studies and Dr. Stan Lang (Wyeth-Ayerst) for his continued support.

References

1. Wyeth-Ayerst Research, 401 N. Middletown Rd., Pearl River, NY 10913
2. a) Knowles, J. R.. *Acc. Chem. Res.* **1985**, *18*, 97-104. b) Sutherland, R. *Trends Pharmacol. Sci.* **1991**, *12*, 227. c) Medeiros, A. A. *Clin. Infect. Dis.* **1997**, *24*, S19. d) Maiti, S. N.; Phillips, O A.; Micetich, R. G.; Livermore, D. M. *Curr. Med. Chem.* **1998**, *5*, 441.
3. Buynak, J. D.; Wu, K.; Bachmann, B.; Khasnis, D.; Hua, L.; Nguyen, H. K.; Carver, C. L. *J. Med. Chem.* **1995**, *38*, 1022.
4. Buynak, J. D.; Geng, B; Bachmann, B.; Hua, L. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1513.
5. Buynak, J. D.; Rao, A. S.; Nidamarthy, S. D. *Tetrahedron Lett.* **1998**, *39*, 4945.
6. Kamiya, T.; Teraji, T.; Saito, Y.; Hashimoto, M.; Nakaguchi, O.; Oku, T. *Tetrahedron Lett.* **1973**, *14*, 3001.
7. For leading references to this and related transformations, see: a) Cooper, R. D. G.; Hatfield, L. D.; Spry, D. O. *Acc. Chem. Res.* **1973**, *6*, 32. b) Morin, R. B.; Jackson, B. G.; Mueller, R. A.; Lavagnino, E. R.; Scanlon, W. B.; Andrews, S. L. *J. Am. Chem. Soc.* **1969**, *91*, 1401.
8. Richter, H. G. F.; Angehrn, P.; Hubschwerlen, C.; Kania, M.; Page, M. G. P.; Specklin, J. L.; Winkler, F. K. *J. Med. Chem.* **1996**, *39*, 3712.
9. Chen, Y. L.; Chang, C. W.; Hedberg, K.; Guarino, K.; Welch, W. M.; Kiessling, L.; Retsema, J. A.; Haskell, S. L.; Anderson, M.; Manousos, M.; Barrett, J. F. *J. Antibiot.* **1987**, *40*, 803.
10. For an overview, see: a) Arnould, J. C.; Bertrandie, A.; Bird, T. G. C.; Boucherot, D.; Jung, F.; Lohmann, J. J.; Olivier, A.; Bailey, J. P.; Bell, W.; Davies, G. M. *J. Med. Chem.* **1992**, *35*, 2631. b) Bird, T. G. C.; Arnould, J. C.; Bertrandie, A.; Jung, F. H. *J. Med. Chem.* **1992**, *35*, 2643.
11. Watanabe, N. A.; Nagasu, T.; Katsu, K.; Kitsch, K. *Antimicrob. Agents Chemother.* **1987**, *31*, 497.
12. Bauernfeind, A.; Chong, Y.; Lee, K. *Yonsei Med. J.* **1998**, *39*, 520.