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6-(1-HYDROXYALKYL)PENAM SULFONE DERIVATIVES AS INHIBITORS OF CLASS A AND CLASS C β-LACTAMASES I

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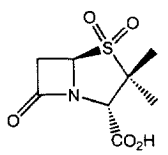
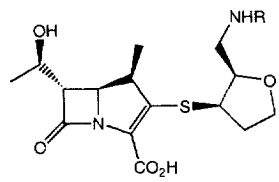
Abstract: Five 6-(1-hydroxyalkyl)penam sulfone derivatives and two 6-(hydroxymethyl)penams were synthesized for β-lactamase inhibitor screens. The substituent effects and stereochemical requirements of 6α- and 6β-(1-hydroxyalkyl) groups for the biological activity of penam sulfone derivatives were investigated. Of these substituents, only the 6β-hydroxymethyl group of **15** improved the activity of sulbactam against both TEM-1 and AmpC β-lactamases. The sulfone moiety is required for the enhancement of the β-lactamase inhibitory activity. 6β-Hydroxymethylsulbactam (**15**) was able to restore the activity of piperacillin in vitro and in vivo against various β-lactamase producing microorganisms.

Introduction

Sulbactam is one of the three currently marketed class A β-lactamase inhibitors.[1] It is used in combination with ampicillin and cefoperazone. This form of combination therapy appears to have advantages over single agent therapy, particularly with regard to the development of resistance. While the selection of resistant mutants following the use of broad-spectrum antibiotics is well documented, β-lactam antibiotic/β-lactamase inhibitor combinations seem much less of a problem with regard to the selection of resistance. Since bacteria which produce class C β-lactamases are increasing in prevalence among infectious organisms in nosocomial infections,[2] there is a need to develop a broad spectrum inhibitor which can inhibit the activity of both class A and class C β-lactamases.[3] Such an inhibitor would pose a clear clinical advantage over the three currently marketed class A β-lactamase inhibitors. As sulbactam has little activity against class C β-lactamase, it would be desirable to improve its activity against both class A and class C β-lactamases.



Sulbactam



CL191,121, R = H
OCA-983, R = L-Val

Recently, we reported oral tetrahydrofuranyl (THF) 1β-methylcarbapenems, of which OCA-983 and its parent compound, CL191,121, are representative members.[4] These carbapenems had the broad spectrum of activity against Gram-positive and Gram-negative organisms comparable to those of imipenem and meropenem with the exception of only moderate antipseudomonal activity. In addition, these carbapenems demonstrated

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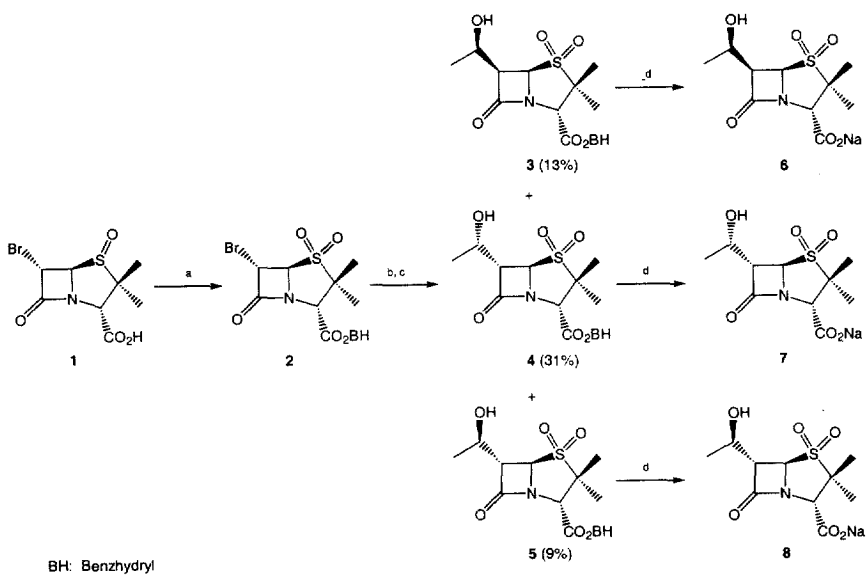
potent activity against both class A and class C β-lactamases (Table 1). Since the 6α-(1-hydroxyethyl) group of these carbapenems might be responsible for the high class A and class C β-lactamase inhibitory activity, we decided to introduce a (1-hydroxyalkyl) group onto the 6-position of sulbactam in order to explore the substituent effects and the stereochemical requirements of the 6-(1-hydroxyalkyl) group for the biological activity of sulbactam derivatives. Here we report the synthesis and biological activity of 6α- and 6β-(1-hydroxyethyl)- as well as 6α- and 6β-hydroxymethylsulbactams.

Table 1 Inhibition of β-Lactamases by CL191,121 and OCA-983

Compound	IC_{50} (nM) TEM-1 (class A)	AmpC (class C)
CL191,121	35	180
OCA-983	91	68
Sulbactam	1,400	65,900

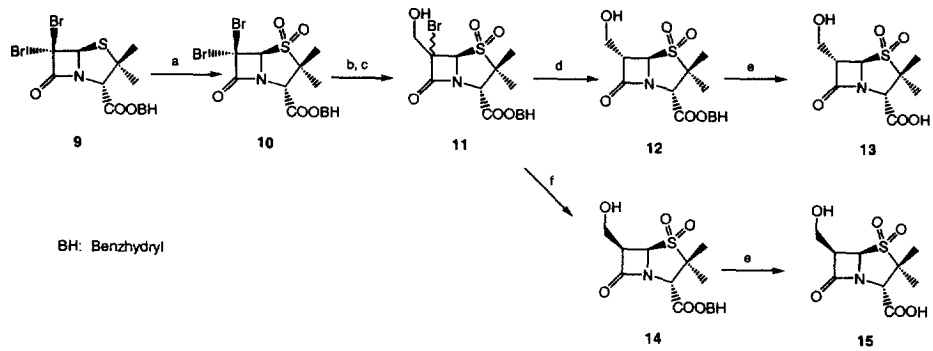
Chemistry

Three isomeric 6-(1-hydroxyethyl)sulbactams **6**, **7**, and **8** were synthesized as shown in Scheme 1 in order to determine stereochemical requirements of the 6-(1-hydroxyethyl) group for optimal biological activity.

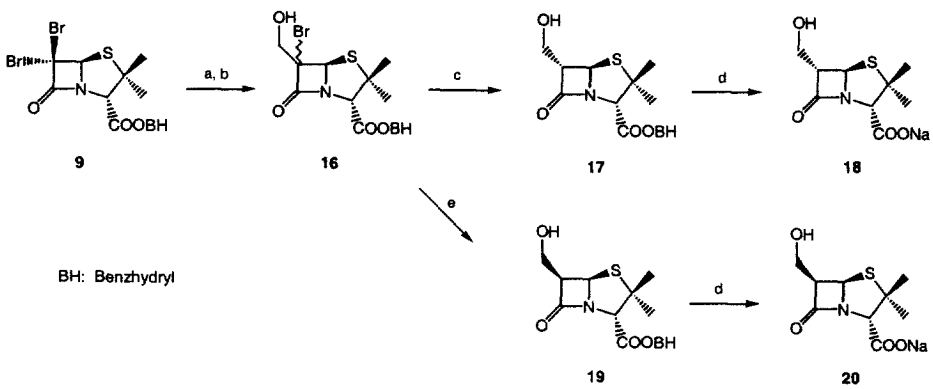


Scheme 1: (a) KMO_4/CH_2Cl_2, ~100%; (b) MeMgBr/THF; (c) CH_3CHO/THF, 53%; (d) *m*-cresol, 50 °C /$NaHCO_3$, 80%

Bromosulfoxide **1**[6] was oxidized with $KMnO_4$ to bromosulfone **2** in quantitative yield. Treatment of **2** with MeMgBr, followed by reaction with acetaldehyde, provided a mixture of three isomeric products, **3** (13%), **4** (31%) and **5** (9%).[7] Deprotection of the benzhydryl group[8] of **3**, **4** and **5** with *m*-cresol provided the desired products, **6**, **7** and **8**, respectively, in about 80% yield. For the synthesis of the hydroxymethyl derivatives, dibromosulfide **9**[9] was oxidized by $KMnO_4$ to dibromosulfone **10**. Treatment of **10** with *t*-BuMgBr, followed by reaction with formaldedye in THF,[10] provided **11** in about 30% yield. Debromination of **11** with Bu_3SnH produced pure **14** in high yield whereas the debromination with Bu_3P produced a mixture of **12** and **14** in a 1:1 ratio (Scheme 2).[11] The stereochemical assignments about the 6-positions of **12** and **14** were based on their ^1H NMR coupling constants (in deuterated chloroform) $J_{H5\text{-}H6}$ = 4.7 and 1.9 Hz, respectively, which are



Scheme 2: (a) $KMnO_4/CH_2Cl_2$, 94%; (b) *t*-BuMgCl/THF; (c) CH_2O/THF, 30%; (d) Bu_3P/CH_3OH, 20%; (e) *m*-cresol, 50 °C, 80%; (f) Bu_3SnH/AIBN, 85%



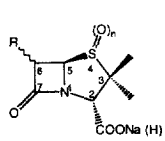
Scheme 3: (a) *t*-BuMgCl/THF; (b) CH_2O/THF, 30%; (c) Bu_3P/CH_3OH, 85%; (d) *m*-cresol/$NaHCO_3$, 50 °C, ~80%; (e) Bu_3SnH/AIBN, 85%

qualitatively in agreement with the Karplus equation.[12] Deprotection of the benzhydryl group[8] of **12** and **14** with *m*-cresol provided the desired products, **13** and **15**[13] in about 80% yield. Since 6α-hydroxymethylpenam (**18**) was reported to be a potent inhibitor of TEM-1 β-lactamase,[14] both isomeric 6-hydroxymethylpenams, **18** and **20**, were prepared (Scheme 3). It may be mentioned that in contrast to the debromination of **11**, the debromination of **16** with Bu_3P gave **17** as the only product in 85% yield.

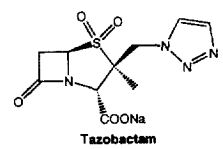
Results and Discussion

As is evident from Table 2, the 6β-(1-hydroxyethyl) group of **6** was much more effective than the 6α-(1-hydroxyethyl) group of **7** and **8** for improving the IC_{50} of sulbactam against the AmpC (class C) β-lactamase (549-fold vs 6- to 41-fold increase). However, both substantially decreased the activity (34- to 240-fold decrease) against the TEM-1 (class A) β-lactamase. The 6β-hydroxymethyl group of **15** was also more effective than the 6α-hydroxymethyl group of **13** for improving the activity of sulbactam against the AmpC β-lactamase (57-fold vs 11-fold increase). Most importantly, the 6β-hydroxymethyl group improved the activity against both TEM-1 (175-fold increase) and AmpC (57-fold increase) β-lactamases. It is also evident that the sulfone moiety (**13** and **15** vs **18** and **20**) is required for the enhancement of the β-lactamase inhibitory activity against both TEM-1 and AmpC β-lactamases.

Table 2 Inhibitory Activity of 6-(1-Hydroxyalkyl)penam Sulfone Derivatives



6: R = 8β,6β-$CH_3CH(OH)$, n = 2
7: R = 8α,6α-$CH_3CH(OH)$, n = 2
8: R = 8β,6α-$CH_3CH(OH)$, n = 2
13: R = 6α-$HOCH_2$, n = 2
15: R = 6β-$HOCH_2$, n = 2
18: R = 6α-$HOCH_2$, n = 0
20: R = 6β-$HOCH_2$, n = 0
Sulbactam: R = H, n = 2



Tazobactam

Compound	IC_{50} (nM) TEM-1 (class A)	AmpC (class C)
6	204,000	120
7	47,900	10,200
8	>334,000	1,600
13	8,000	5,800
15	8	1,160
18	59,000	220,000
20	31,000	100,000
Sulbactam	1,400	65,900
Tazobactam	60	47,700

Of these seven 6-(1-hydroxyalkyl)penam sulfone derivatives, only 6β-hydroxymethylsulbactam (**15**) demonstrated good β-lactamase inhibitory activity (IC_{50}) against both class A and class C β-lactamases and was selected for further in vitro and in vivo evaluation against various β-lactamase producing microorganisms (Table 3). 6β-Hydroxymethylsulbactam (**15**) was able to restore the activity of piperacillin in vitro and in vivo against various β-lactamase producing microorganisms. At a 1:1 ratio of piperacillin to **15**, the MIC values of piperacillin were reduced from >64 μg/mL and 32 μg/mL to 4 μg/mL and 8 μg/mL against TEM-1 and AmpC expressing bacterial isolates (GC6265 and GC4132), respectively. At a 2:1 ratio of piperacillin to **15**, the ED_{50} values of piperacillin were reduced from 256-512 mg/kg and 128-256 mg/kg to 8 mg/kg and 30 mg/kg against TEM-1 and AmpC expressing bacterial isolates (GC6265 and GC4132), respectively. In comparison, tazobactam was able to reduce the MIC and ED_{50} values of piperacillin from >64 μg/mL and 256-512 mg/kg to 2 μg/mL and 7.7 mg/kg, respectively, against the TEM-1 expressing bacterial isolate. However, tazobactam was not effective in reducing the MIC and ED_{50} values of piperacillin against the AmpC expressing bacterial isolate.

Table 3 Biological Activity of **15** and Tazobactam

	MIC (μg/mL; 1:1[d])		ED_{50} (mg/kg; 2:1[d]; mice)	
Compound	*E. coli*[a]	*S. marcescens*[b]	*E. coli*[a]	*S. marcescens*[b]
15	4[c]	8[e]	8	30
Tazobactam	2	32	7.7	144
Piperacillin	>64	32	256-512	128-256

[a]GC6265, TEM-1 (class A); [b]GC4132, AmpC (class C); [c]GC2847, TEM-1 (class A); [d]piperacillin:inhibitor ratio; [e]GC2894; AmpC (class C).

Both sulbactam and tazobactam are effective in the inhibition of class A β-lactamases for mechanistic reasons that have been elucidated.[15] Recently, Mobashery et al showed that the hydrolytic water of class C β-lactamases approaches the acyl-enzyme intermediate from the β face of the ester (opposite of that for class A β-lactamases).[16] Presumably, 6β-hydroxymethylsulbactam **15** works in the same way that sulbactam and tazobactam work against class A β-lactamases. Yet, by having the 6β-hydroxymethyl group crowd the β face of the ester in the acyl-enzyme intermediate, the approach of the hydrolytic water in class C β-lactamases to the ester is impaired. This would impart relative longevity to the acyl-enzyme intermediate in class C β-lactamases, accounting for the onset of inhibition.

In summary, the substituent effects and stereochemical requirements for the biological activity of 6α- and 6β-(1-hydroxyalkyl) groups of penam sulfone derivatives were investigated. Of these substituents, only the 6β-hydroxymethyl group of **15** improved the inhibitory activity (IC_{50}) of sulbactam against both TEM-1 and AmpC β-lactamases. The sulfone moiety is required for the enhancement of the β-lactamase inhibitory activity. 6β-hydromethylsulbactam (**15**) was able to restore the activity of piperacillin in vitro (MIC) and in vivo (ED_{50}) against various β-lactamase producing microorganisms.

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δ: 4.94 (1H, d; J = 4.5 Hz), 4.2 (1H, s), 4.2–4.14 (1H, m), 4.15–3.92 (2H, m), 1.46 (3H, s), 1.34 (3H, s).
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