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

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Abstract: Two stereoselective processes for the synthesis of novel 3,6-disubstituted penam sulfone derivatives were developed. One 6β-(1-hydroxyethyl) and four 6β-hydroxymethyl penam sulfone derivatives were synthesized. All four 6β-(hydroxymethyl)penam sulfone derivatives demonstrated good IC_{50} against both TEM-1 and AmpC β-lactamases. Of these, 6β-hydroxymethyl penam sulfone derivative **25** was the most active inhibitor which was able to restore the activity of piperacillin in vitro and in vivo against both TEM-1 and AmpC β-lactamases producing organisms.

Introduction

Penicillins and cephalosporins are the most frequently and widely used β-lactam antibiotics in the clinic. However, the development of bacterial resistance to these β-lactam antibiotics has had a damaging effect on maintaining the effective treatment of bacterial infections.[1] The most significant known mechanism related to the development of bacterial resistance to the β-lactam antibiotic is the production of class A and class C serine β-lactamases. These β-lactamases degrade the β-lactam antibiotics, resulting in a loss of antibacterial activity. class A β-lactamases have molecular weights of about 29 kDa and preferentially hydrolyze penicillins whereas class C β-lactamases have larger molecular weights of about 39 kDa and have a substrate profile favoring cephalosporin hydrolysis.[2] Bacterial resistance to these antibiotics could be greatly reduced by administering the β-lactam antibiotic in combination with a compound which inhibits these enzymes.

Three β-lactamase inhibitors in the market are clavulanic acid, sulbactam and tazobactam. They are all effective against class A β-lactamases, but have little or no activity against class C β-lactamases. Clavulanic acid is used in combination with amoxicillin and ticarcillin; similarly, sulbactam with ampicillin and tazobactam with piperacillin. Since bacteria producing class C β-lactamases are increasing in prevalence among infectious organisms in nosocomial infections,[3] there is a need to develop an inhibitor which can inhibit the activity of both class A and class C β-lactamases. As tazobactam has some activity against class C β-lactamases and its starting material, 6-aminopenicillanic acid, is readily available, we decided to use tazobactam as the lead for structural modifications. So far, the modification of either the 6-[4-5] or 3β-[6-9] position alone has not produced an

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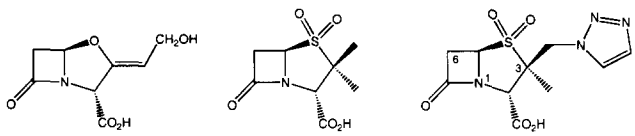
inhibitor with the desired activity against both class A and class C β-lactamases. Therefore, we decided to modify both positions simultaneously.

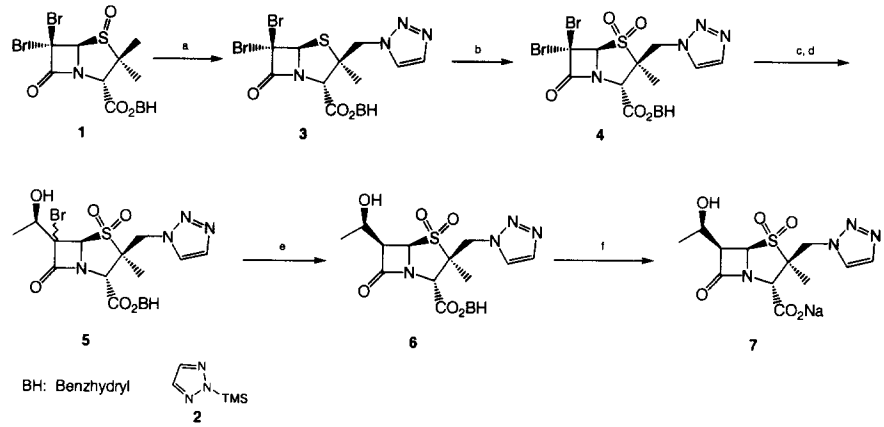


In the preceding publication,[10] we reported that the 6β-(1-hydroxyethyl) group improved the β-lactamase inhibitory activity of sulbactam against class C β-lactamases whereas the 6β-hydroxymethyl group increased the activity of sulbactam against both class A and class C β-lactamases. Therefore, we decided to introduce 6β-(1-hydroxyethyl) or 6β-hydroxymethyl group onto the 6-position of tazobactam in order to enhance the activity against class C β-lactamases, in particular. Here we report the synthesis and biological activity of a series of five 3,6-disubstituted penam sulfone derivatives.

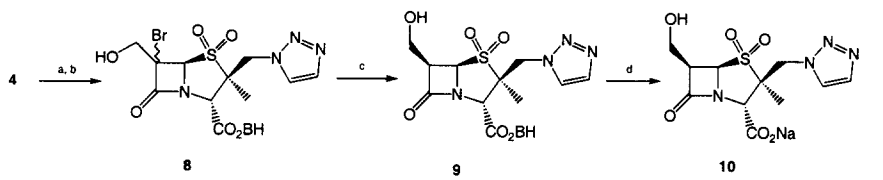
Chemistry

Two stereoselective processes (Schemes 1, 2, and 3) for the synthesis of five novel 3,6-disubstituted penam sulfone derivatives[11] were developed.

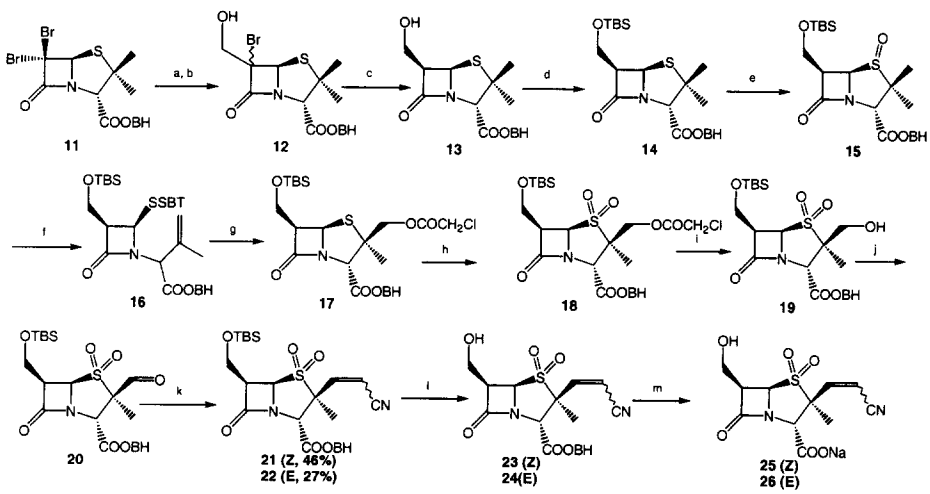
Reaction of dibromosulfoxide **1**[12] with 2-trimethylsilyl-2H-1,2,3-triazole (**2**)[13] in acetonitrile gave dibromotriazolylpenam **3** which was oxidized with $KMnO_4$ to give dibromotriazolylpenam sulfone **4**. Treatment of **4** with *t*-BuMgCl in THF, followed by reaction with acetaldehyde, provided a mixture of products **5**. Debromination of **5** with Bu_3SnH produced a pure product **6** in high yield.[14] The stereochemical assignment about the 6-position of **6** was confirmed by the ^1H NMR coupling constant $J_{H5\text{-}H6}$ = 4.6 Hz which is consistent with the cis configuration betweem H_5 and H_6.[10] Deprotection of the benzhydryl group[8] of **6** with *m*-cresol provided 6β-(1-hydroxyethyl)tazobactam (**7**) (Scheme 1). Similarly, 6β-hydroxymethyltazobactam (**10**) was stereoselectively prepared from the dibromotriazolylsulfone **4** (Scheme 2). 6β-Hydroxymethyl penam sulfones **25** and **26** were synthesized in 12 steps[15] from dibromosulfide **11**[16] which was prepared in 2 steps from 6-aminopenicillanic acid (Scheme 3). The intermediates, **21** and **22**, were separated by silica gel flash column chromatograhpy. 6β-Hydroxymethyl penam sulfone **29** was synthesized from **20** in 4 steps (Scheme 4).[15]



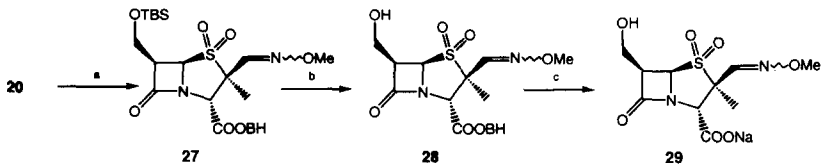
Scheme 1: (a) **2**/CH_3CN, ~20%; (b) $KMnO_4/CH_2Cl_2$, ~100%, ; (c) *t*-BuMgCl/THF; (d) CH_3CHO/THF, 53%; (e) Bu_3SnH, 85%,; (f) *m*-cresol, 50 °C /$NaHCO_3$, 80%



Scheme 2: (a) *t*-BuMgCl/THF; (b) CH_2O/THF, 30%; (c) Bu_3SnH, 85%; (d) *m*-cresol, 50 °C /$NaHCO_3$, 80%



Scheme 3: (a) *t*-BuMgCl/THF; (b) CH_2O/THF, 30-40%; (c) Bu_3SnH, 81-88%; (d) TBS-Tf, 86-90%; (e) HCO_2H/H_2O_2, 75-84%; (f) HSBT/toluene, ~100%; (g) $ClCH_2CO_2H$/AcOAg/CH_2Cl_2, 18-22%; (h) $KMnO_4$/AcOH, 79-88%; (i) thiourea/py/DMF, 97%; (j) PCC/silica gel, 64%; (k) Ph_3P=CHCN, 73%; (l) NH_4F.HF/DMF/NMP, 65%; (m) m-cresol, 50 °C /$NaHCO_3$, 80%

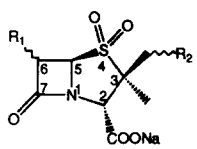


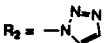
Scheme 4: (a) $MeONH_2.HCl/py/CH_2Cl_2$, 89%; (b) $NH_4F.HF/DMF/NMP$, 65%; (c) m-cresol, 50 °C /$NaHCO_3$, 80%

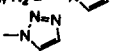
Results and Discussion

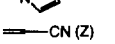
As is evident from Table 1, the 6β-(1-hydroxyethyl) group of **7** improved the IC_{50} of tazobactam by 397-fold against the AmpC (class C) β-lactamase but it decreased the IC_{50} by 42-fold against the TEM-1 (class A) β-lactamase. As expected, the 6β-hydroxymethyl group of **10** substantially improved the IC_{50} of tazobactam against both TEM-1 (ten fold) and AmpC (132-fold) β-lactamases. 6β-Hydroxymethyltazobactam (**10**) was also able to restore the activity of piperacillin in vitro and in vivo against the TEM-1 producing organism. At a 1:1 ratio of piperacillin to **10**, the MIC and ED_{50} values of piperacillin were reduced from >64 μg/mL and 256-512 mg/kg to 2 μg/mL and 3.6 mg/kg, respectively, against the TEM-1 producing organism. Disappointingly, 6β-hydroxymethyltazobactam (**10**) was almost as ineffective as tazobactam in reducing the MIC and ED_{50} values of piperacillin against the AmpC expressing bacterial isolate. Since Ro 48-1220 was reported to have better activity than tazobactam against AmpC β-lactamases,[8] 6β-hydroxymethyl derivative (**25**) of Ro 48-1220 and its related derivatives (**26** and **29**) were prepared. These three new 6β-(hydroxymethyl)penam sulfone derivatives, **25**, **26**, and **29**, all demonstrated good IC_{50} against both TEM-1 and AmpC β-lactamases. They were all able to restore the in vitro activity of piperacillin at a ratio of 1:1 of piperacillin to the inhibitor (**25**, **26**, or **29**) against TEM-1 and AmpC β-lactamases producing organisms. The activity of the Z-isomer **25** is little better than that of the E-isomer **26** and this observation is consistent with that of the 6-unsubstituted derivatives, Ro 48-1220 and its E-isomer.[8] Of these three derivatives, 6β-(hydroxymethyl)penam sulfone derivative **25** was the most active inhibitor which was selected for further in vivo evaluation. At a 2:1 ratio of piperacillin to **25**, the ED_{50} values for piperacillin were reduced from 256-512 mg/kg and 128-256 mg/kg to 4-8 mg/kg and 8-32 mg/kg against TEM-1 and AmpC expressing bacterial isolates, respectively.

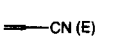
Table 1: Biological Activity of 3,6-Disubstituted Penam Sulfone Derivatives

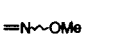


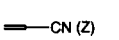
7: R_1 = 8β,6β-$CH_3CH(OH)$, R_2 = 

10: R_1 = 6β-$HOCH_2$, R_2 = 

25: R_1 = 6β-$HOCH_2$, R_2 = 

26: R_1 = 6β-$HOCH_2$, R_2 = 

29: R_1 = 6β-$HOCH_2$, R_2 = 

Ro 48-1220: R_1 = H, R_2 = 

	IC_{50} (nM)		MIC (μg/mL; 1:1[d])		ED_{50} (mg/kg; 2:1[d]; mice)	
Compound	TEM-1	AmpC	*E. coli*[a]	*S. marcescens*[b]	*E. coli*[a]	*S. marcescens*[b]
7	2,500	120	>64[c]	16[e]	--	--
10	6	360	2[c]	16[e]	3.6	125
25	19	270	4	8	4-8	16-32
26	74	280	16	4	--	--
29	64	280	8	16	--	--
Ro 48-1220	42	1,133	4[c]	4[e]	15	82
Sulbactam	1,400	65,900	--	--	--	--
Tazobactam	60	47,700	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).

In conclusion, a series of one 6β-(1-hydroxyethyl) and four 6β-hydroxymethyl penam sulfone derivatives have been synthesized and evaluated for their potency as inhibitors of β-lactamases and as partners for piperacillin. The four 6β-hydroxymethyl penam sulfone derivatives all demonstrated good IC_{50} against both TEM-1 and AmpC β-lactamases. Of these, 6β-hydroxymethyl penam sulfone derivative **25** was the most active inhibitor which was able to restore the activity of piperacillin in vitro and in vivo against both TEM-1 and AmpC β-lactamases producing organisms.

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11. ^{1}H NMR data in D_2O of these five new derivatives are summarized as follows: **7**, δ: 8.11 (1H, s), 7.84 (1H, s), 5.36 (1H, d; J = 15.4 Hz), 5.17 (1H, d; J = 15.4 Hz), 4.80 (1H, d; J = 4.8 Hz), 4.74 (1H, m), 4.57 (1H, s), 4.03 (1H, dd; J = 4.8 Hz), 1.42 (3H, s), 1.34 (3H, s); **10**, δ: 8.12 (1H, s), 7.85 (1H, s), 5.37 (1H, d; J = 15.4 Hz), 5.16 (1H, d; J = 15.4 Hz), 5.08 (1H, d; J = 4.6 Hz), 4.57 (1H, s), 4.38-4.31 (1H, m), 4.28-3.98 (2H, m), 1.41 (3H, s); **25**, δ: 6.68 (1H, d; J = 12.4 Hz), 6.16 (1H, d; J = 12.4 Hz), 5.25 (1H, d; J = 4.7 Hz), 4.36 (1H, m), 4.22 1H, dd; J = 8.16 Hz), 4.08 (1H, dd; J = 8.16 Hz), 1.94 (3H, s); **26**, δ: 7.08 (1H, d; J = 16.5 Hz), 6.07 (1H, d; J = 16.5 Hz), 5.2 (1H, s), 4.36 (1H, m), 4.21 (1H, dd; J = 8.10 Hz), 4.07 (1H, dd; J = 8.10 Hz), 1.64 (3H, s); **29**, δ: 7.68 (1H, s), 5.18 (1H, d; J = 4.5 Hz), 4.88 (1H, s), 4.35 (1H, m), 4.2 (1H, t), 4.08 (1H, dd), 3.97 (3H, s), 1.66 (3H, s).
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17. Molecular modelling studies using MacroModel v6.0 showed that good binding ligands fit well in the enzyme active site and remained there during the molecular dynamics simulation. Details of the molecular modelling studies will be published elsewhere.