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REARRANGEMENT OF UNSYMMETRICAL AZETIDINONE DISULFIDES TO 2β-(HETEROCYCLYLTHIOMETHYL)PENAMS, A SYNTHETIC APPROACH TO NEW β-LACTAMASE INHIBITORS[1]

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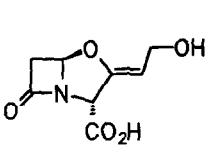
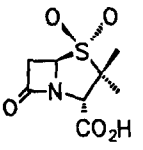
Abstract: The synthesis of 6,6-dibromo-2β-(heterocyclylthiomethyl)penam esters **4** and their conversion into compounds **7** and **10**, analogs of the β-lactamase inhibitors brobactam and sulbactam having a thio-substituted 2β-methyl group, is described. Biological activities of the new compounds are presented.

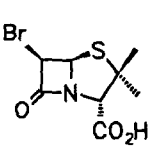
The successful clinical application of β-lactam antibiotic combinations incorporating clavulanic acid or sulbactam has firmly established that β-lactamase inhibitors can play an important role in the treatment of infections caused by β-lactamase producing organisms. The continuing search for more potent β-lactamase inhibitors has led to the synthesis of a variety of structurally modified β-lactam derivatives. Recent publications[2-4] have highlighted the discovery of potent β-lactamase inhibitors by modification of the 2β-methyl group in sulbactam. Similar modification of brobactam,[5] another effective β-lactamase inhibitor, has been less successful in providing compounds of superior activity.[6]



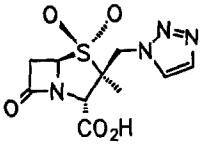
Clavulanic Acid



Sulbactam



Brobactam

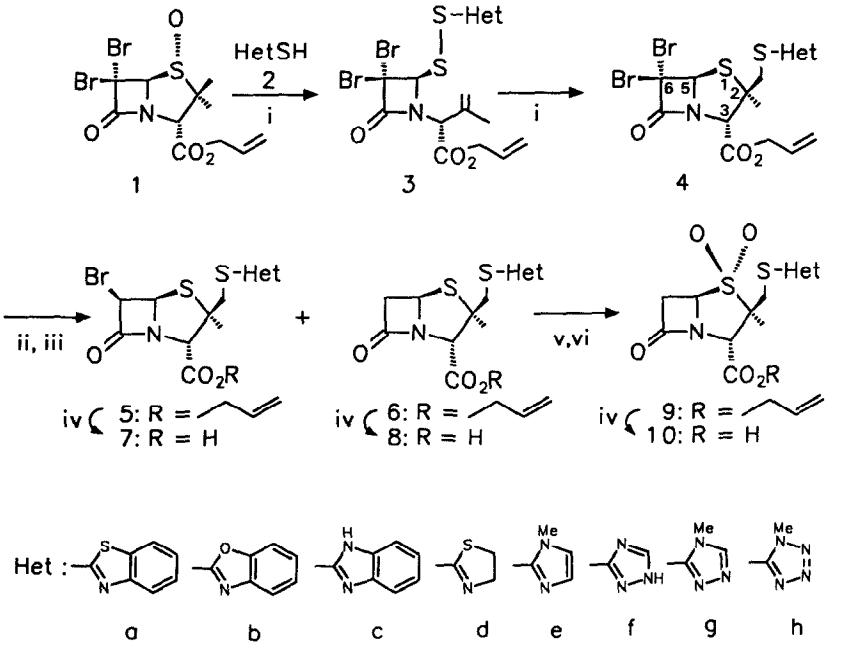


Tazobactam[3]

Our renewed interest in this area arose from a recent paper by Alpegiani *et al.*[7] who had discovered that selected azetidinyl benzothiazolyl disulfides[8] directly rearranged to the corresponding 2β-(benzothiazolylthiomethyl)penams upon thermolysis in refluxing toluene in the presence of an acid catalyst. We adopted this methodology and report herein on the synthesis of a series of 6,6-dibromo-2β-(heterocyclylthiomethyl)penam esters which, in turn, could be converted into analogs of sulbactam and brobactam having a heterocyclylthio-substituted 2β-methyl group. Comparative data on the β-lactamase inhibitory properties of the modified derivatives and their parent compounds are presented.

Synthesis

Analogs of brobactam and sulbactam modified in the 2β-methyl group by a variety of heterocyclic thio substituents were prepared by the reaction sequence outlined in Scheme 1. Introduction of the thio substituents was accomplished by treatment of allyl 6,6-dibromopenicillanate 1α-oxide[9] (**1**) with an appropriate heterocyclic thiol **2** in toluene at 90-95°C (or in refluxing dioxane) in the presence of a catalytic amount of *p*-toluenesulfonic acid to give, via rearrangement of the initially formed disulfide **3**, the desired 6,6-dibromo-2β-(heterocyclylthiomethyl)penam ester **4** (30-90%).[10] Stereoselective dehalogenation of **4** with trineophyltin hydride[11] in refluxing ether produced an approximate 70:20:10 mixture of 6β-bromopenam **5**, 6,6-dihydropenam **6** and unreacted starting material **4** from which the pure compounds could be isolated by column chromatography on silica gel. When **4** was treated with excess tributyltin hydride in ether-toluene 1:1 at 40°C, **6** was obtained as the only product. Deallylation of esters **5** and **6** to afford the corresponding acids **7** and **8** was performed by the method of Jeffrey and McCombie[12] (80-90%). Finally, oxidation of acid **8** at 0-5°C with potassium permanganate in neutral aqueous solution[13] provided the modified penam sulfone **10** (50-90%). Alternatively, **10** could be prepared, albeit in low yield, by oxidation of **6** with *m*-chloroperbenzoic acid in dichloromethane at 0-5°C, followed by deallylation of the resulting sulfone ester **9**.



Scheme 1: (i) HetSH (**2**, 1 eq), *p*-TsOH (0.05 eq), toluene, 90-95°C (or dioxane, reflux), 2-16 h; (ii) Neophyl$_3$SnH (1.2 eq), Et$_2$O, reflux, 0.5-2 h: **4** → **5** + **6**; (iii) Bu$_3$SnH (2.5 eq), Et$_2$O-toluene 1:1, 40°C, 0.5-4 h: **4** → **6**; (iv) PPh$_3$, Pd(PPh$_3$)$_4$, CH$_3$(CH$_2$)$_3$CH(C$_2$H$_5$)CO$_2$K, EtOAc, rt, 0.5-1 h; (v) KMnO$_4$/H$_3$PO$_4$, H$_2$O, 0-5°C, 0.5-1h: **8** → **10**; (vi) MCPBA (2.5 eq), CH$_2$Cl$_2$, 0-5°C, 0.5 h, then rt, 2-4 h: **6** → **9**.

Spectral data[14] obtained for compounds **4** as well as their derivatives in series **5** to **10** were consistent with the proposed 2β-(thio-subsitituted methyl)penam structures shown in Scheme 1. In particular, 2D hetero-nuclear correlation (COLOC) experiments demonstrated long range coupling between the heterocyclic carbon atom attached to sulfur and the CH_2S protons which is in agreement with penam structure **4**, but not with the cepham alternative **12.**[10] In a similar experiment with cepham **12h,**[10] long range coupling between C-6 and the methylene protons at C-2 was observed. The stereochemistry of the thio-substituted methyl group in penams **4** was determined to be 2β by NOE difference spectroscopy: Upon irradiation of the CH_2S protons, **4f** and **4h** exhibited NOEs at 3-H but not at 5-H, whereas NOEs at 5-H plus minor enhancements of the 3-H signals were detected upon irradiation of the CH_3 protons.

Biological Activity

The β-lactamase inhibitory activities of the compounds in series **7** and **10** were determined against a broad range of cell-free enzyme preparations and compared with those of other β-lactamase inhibitors. The results in Table 1 show that the modified 6β-bromopenams in series **7** were less effective inhibitors than brobactam, with IC_{50} values generally 5 to 10 fold higher than those observed for the parent compound. In contrast, the sulfone analogs in series **10** were significantly more active than sulbactam, as apparent from Table 2. In fact, the inhibitory potency of several of these derivatives, eg **10b**, **10d**, **10g** and **10h**, compared favorably with that of tazobactam against a broad range of enzymes tested. However, in combination with ampicillin, compounds in both series **7** and **10** failed to demonstrate useful synergistic activity against β-lactamase producing *Enterobacteriaceae*, but were able to potentiate ampicillin activity against β-lactamase positive strains of *Straphylococcus aureus*. This could be due to poor penetration of the modified inhibitors through the porin channels in the outer cell membrane of Gram-negative enteric bacteria.

Conclusion

A series of 2β-(heterocyclylthiomethyl)penam esters, prepared by direct rearrangement of *unsym*-azetidinone disulfides in analogy to a published method, was converted into derivatives of the β-lactamase inhibitors brobactam and sulbactam bearing a 2β-heterocyclylthiomethyl group. Many of the new compounds, in particular the sulfone analogs, showed potent inhibitory activity against a broad range of cell-free β-lactamases. However, they were unable to potentiate ampicillin activity against most β-lactamase producing *Enterobacteriaceae*.

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Table 1. Comparative β-lactamase inhibitory activities of 6β-bromo-2β-(heterocyclylthiomethyl)penams **7**, brobactam (BRO), and clavulanic acid (CLA).

β-Lactamase			IC_{50} (μg/ml)[b]							
Source	Class[a]	Type	**7a**	**7b**	**7d**	**7e**	**7f**	**7h**	BRO	CLA
C.freundii 87470	I		2.5	6.3	20	32	4.0	40	0.50	3.2
E.cloacae HC8	I	P99	1.6	7.9	13	10	16	79	0.50	6.3
P.vulgaris HJ33C	I		0.001	0.001	0.003	0.010	0.003	0.003	0.001	0.013
B.fragilis 88854	I		0.003	0.010	0.013	0.032	0.025	0.016	0.013	0.050
K.oxytoca HC7	IV	K1	0.63	0.50	1.0	0.63	0.50	0.25	0.080	0.006
E.coli HA58R	III	TEM-1	0.016	0.013	0.016	0.006	0.008	0.040	0.004	0.008
E.coli HA208	III	SHV-1	0.063	0.063	0.20	0.020	0.010	0.40	0.005	0.004
B.catarr. 89001	III	BRO-1	0.025	0.010	0.032	0.025	0.025	0.020	0.003	0.006
E.coli HA209	V	OXA-1	0.63	1.0	0.63	4.0	2.0	3.2	0.20	0.080
S.aureus CJ8			0.032	0.050	0.050	0.20	0.13	0.63	0.013	0.005

[a] Classification of Richmond and Sykes (*Adv.Microb.Physiol.*; **1973**, *9*, pp 31-88).
[b] Concentration giving 50% inhibition of nitrocefin hydrolysis after preincubation of enzyme and inhibitor for 30 min at 37°C. Preincubation of *P.vulgaris* enzyme for 10 min, no preincubation of *B.fragilis* enzyme. See reference 5 for the assay method.

Table 2. Comparative β-lactamase inhibitory activities of 2β-(heterocyclylthiomethyl)penam sulfones **10**, sulbactam (SUL), and tazobactam (TAZ).

β-Lactamase			IC_{50} (μg/ml)[b]							
Source	Class[a]	Type	**10b**	**10c**	**10d**	**10e**	**10g**	**10h**	SUL	TAZ
C.freundii 87470	I		0.050	0.20	0.16	0.16	0.040	0.013	0.20	0.050
E.cloacae HC8	I	P99	0.32	1.0	0.063	0.25	0.16	0.13	0.80	0.10
P.vulgaris HJ33C	I		0.002	0.008	0.013	0.025	0.013	0.008	0.080	0.006
B.fragilis 88854	I		0.40	0.50	0.32	1.0	0.25	0.40	0.50	0.80
K.oxytoca HC7	IV	K1	0.032	0.20	0.13	0.13	0.080	0.025	0.40	0.032
E.coli HA58R	III	TEM-1	0.004	0.025	0.008	0.10	0.025	0.006	0.13	0.006
E.coli HA208	III	SHV-1	0.040	0.25	0.13	0.32	0.20	0.025	0.40	0.040
B.catarr. 89001	III	BRO-1	0.002	0.005	0.001	0.001	0.001	0.003	0.025	0.010
E.coli HA209	V	OXA-1	0.032	0.050	0.040	0.16	0.13	0.032	0.32	0.13
S.aureus CJ8			0.040	0.16	0.20	0.10	0.16	0.063	0.80	0.050

[a+b] See corresponding notes in Table 1.

References and Notes

1. Part of this work was presented at the 3rd International Symposium on the Chemical Synthesis of Antibiotics and Related Microbial Products, Kloster Banz, Germany, September 20-25, 1992; Poster Abstract No. 2.

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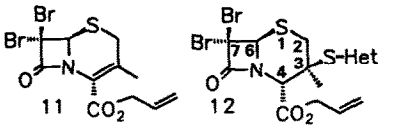
9. Chen, Y.L.; Hedberg, K.; Guarino, K.; Retsema, J.A.; Anderson, M.; Manousos, M.; Barrett, J. *J.Antibiot.* **1991**, *44*, 870. Compounds **1** and **11** obtained as white crystals from ether: **1**, mp 73-74°C, $[\alpha]^{20}_D$ +107.8° (c 0.5, $CHCl_3$); **11**, mp 71-72°C, $[\alpha]^{20}_D$ +64.5° (c 0.5, $CHCl_3$).

10. The course of the reaction was monitored by TLC. Apparently, the formation of penam **4** by acid-catalyzed rearrangement of disulfide **3** is affected by both the nature of the heterocyclic thiol **2** and the stability of the generated disulfide **3** under the reaction conditions. For example, treatment of sulfoxide **1** with 2-mercaptopiperidine or 2-mercaptopyridine under standard conditions (Scheme 1) readily produced the corresponding disulfides **3**, but rearrangement to the thio-substituted penams **4** could not be observed. Crude compounds **4** were generally purified by column chromatography or by fractional crystallization to leave a number of by-products, including unreacted disulfide **3**, its conjugated ester isomer, the 7,7-dibromo-3-cephem **11**[9] and, on one occasion, a 3β-(heterocyclylthio)cepham **12**. The latter was obtained by treatment of sulfoxide **1** (16.61 g, 40 mmol) with 5-mercapto-1-methyltetrazole (**2h**; 4.65 g, 40 mmol) for 16 h in toluene (320 mL) at 90°C in the presence of *p*-toluenesulfonic acid (0.38 g, 2 mmol) to give, after wash with aq $NaHCO_3$, drying, decolorization with charcoal and evaporation of solvent, a yellowish oil which crystallized from ether to afford penam **4h** (15.06 g, 73.4%). Concentration of the mother liquor gave crystalline cepham **12h** (0.32 g, 1.6%). For physical data of compounds **4h** and **12h**, see reference 14.



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14. All new compounds gave satisfactory ^{1}H NMR spectral data. Selected compounds were further characterized by ^{13}C NMR, IR, MS and/or combustion analysis. It should be noted that ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) chemical shift data for compounds within a series, eg **4a-4h**, were similar. Chemical shifts are given in ppm relative to TMS at 0.00 ppm and coupling constants in Hz. Representative physical data are as follows:
Compound **4h**: mp 117-118°C (ether); $[\alpha]^{20}_{D}$ +111.5° (c 0.5, $CHCl_3$); IR (KBr) 1790 (β-lactam CO), 1735 (ester CO) cm^{-1}; Anal calcd for $C_{13}H_{15}Br_2N_5O_3S_2$: C 30.42, H 2.95, Br 31.14, N 13.65, S 12.49%; found: C 30.57, H 3.02, Br 31.25, N 13.72, S 12.43%; ^{1}H NMR ($CDCl_3$) δ 1.56 (s, 3H, 2α-CH_3), 3.75 and 3.91 (2d, J=14, 2H, 2β-CH_2S), 3.98 (s, 3H, NCH_3), 4.72 (m, 2H, OCH_2), 4.87 (s, 1H, 3-*H*), 5.38 (m, 2H, CH=CH_2), 5.86 (s, 1H, 5-*H*), 5.90 (m, 1H, CH=CH_2); ^{13}C NMR ($CDCl_3$) δ 22.68 (2-CH_3), 33.60 (NCH_3), 46.38 (CH_2S), 58.25 (*C*-6), 66.81 (OCH_2), 66.89 (*C*-3), 69.56 (*C*-2), 80.50 (*C*-5), 120.47 (CH=CH_2), 130.60 (CH=CH_2), 153.52 (S-*C*=N), 163.80 (*C*-7), 165.62 (3-*C*O).
Compound **5h**: ^{1}H NMR ($CDCl_3$) δ 1.58 (s, 3H, 2α-CH_3), 3.85 and 3.96 (2d, J=14, 2H, 2β-CH_2S), 3.97 (s, 3H, NCH_3), 4.69 (m, 2H, OCH_2), 4.86 (s, 1H, 3-*H*), 5.36 (d, J=3.8, 1H, 6-*H*), 5.36 (m, 2H, CH=CH_2), 5.65 (d, J=3.8, 1H, 5-*H*), 5.92 (m, 1H, CH=CH_2).
Compound **6h**: ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 3H, 2α-CH_3), 3.16 (dd, J=16, J'=1.5, 1H, 6-*H*), 3.60 (dd, J=16, J'=4, 1H, 6-*H*), 3.83 and 3.99 (2d, J=14, 2H, 2β-CH_2S), 3.96 (s, 3H, NCH_3), 4.68 (m, 2H, OCH_2), 4.80 (s, 1H, 3-*H*), 5.35 (m, 2H, CH=CH_2), 5.36 (m, 1H, 5-*H*), 5.95 (m, 1H, CH=CH_2); CI-MS: MH^+ 356.
Compound **7h** (potassium salt): ^{1}H NMR (DMSO-d_6) δ 1.51 (s, 3H, 2α-CH_3), 3.67 and 3.77 (2d, J=13, 2H, 2β-CH_2S), 3.97 (s, 3H, NCH_3), 4.19 (s, 1H, 3-*H*), 5.52 (d, J=3.6, 1H, 5-*H*), 5.68 (d, J=3.6, 1H, 6-*H*).
Compound **8h** (potassium salt): $[\alpha]^{20}_{D}$ +123.9° (c 0.5, H_2O); IR (KBr) 1765 (β-lactam CO), 1600 (carboxylate CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 3H, 2α-CH_3), 2.83 (dd, J=16, J'=1.5, 1H, 6-*H*), 3.43 (dd, J=16, J'=4, 1H, 6-*H*), 3.79 (ABq, J=13, 2H, 2β-CH_2S), 3.96 (s, 3H, NCH_3), 4.12 (s, 1H, 3-*H*), 5.17 (m, 1H, 5-*H*).
Compound **9h**: IR (neat) 1795 (β-lactam CO), 1750 (ester CO), 1320 and 1135 (sulfone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.57 (s, 3H, 2α-CH_3), 3.48 (dd, J=16, J'=2, 1H, 6-*H*), 3.59 (dd, J=16, J'=4, 1H, 6-*H*), 3.97 (s, 3H, NCH_3), 4.06 and 4.24 (2d, J=14.7, 2H, 2β-CH_2S), 4.68 (m, 1H, 5-*H*), 4.73 (s, 1H, 3-*H*), 4.75 (m, 2H, OCH_2), 5.41 (m, 2H, CH=CH_2), 5.99 (m, 1H, CH=CH_2); CI-MS: MH^+ 388.
Compound **10h**[4] (potassium salt): $[\alpha]^{20}_{D}$ +80.9° (c 0.5, H_2O); IR (KBr) 1765 (β-lactam CO), 1620 (carboxylate CO), 1315 and 1135 (sulfone) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.48 (s, 3H, 2α-CH_3), 3.09 (dd, J=16, J'=1.5, 1H, 6-*H*), 3.50 (dd, J=16, J'=4, 1H, 6-*H*), 3.84 and 4.18 (2d, J=14, 2H, 2β-CH_2S), 3.91 (s, 1H, 3-*H*), 3.96 (s, 3H, NCH_3), 4.93 (m, 1H, 5-*H*); ^{13}C NMR (DMSO-d_6) δ 17.02 (2-CH_3), 33.63 (NCH_3), 37.04 (*C*-6), 37.09 (CH_2S), 61.65 (*C*-5), 64.78 (*C*-3), 65.40 (*C*-2), 153.24 (S-*C*=N), 166.75 (*C*-7), 171.17 (3-*C*O).
Compound **12h**: mp 173-174°C (ether); $[\alpha]^{20}_{D}$ -28.6° (c 0.5, $CHCl_3$); IR (KBr) 1795 (β-lactam CO), 1730 (ester CO) cm^{-1}; Anal calcd for $C_{13}H_{15}Br_2N_5O_3S_2$: C 30.42, H 2.95, Br 31.14, N 13.65, S 12.49%; found: C 30.31, H 2.87, Br 31.20, N 13.47, S 12.55%; ^{1}H NMR ($CDCl_3$) δ 1.71 (s, 3H, 3α-CH_3), 2.95 (dd, J=14.8, J'=0.8, 1H, 2-CH_2), 3.81 (d, J=14.8, 1H, 2-CH_2), 4.04 (s, 3H, NCH_3), 4.70 (m, 2H, OCH_2), 5.20 (s, 1H, 4-*H*), 5.38 (m, 2H, CH=CH_2), 5.65 (s, 1H, 6-*H*), 5.92 (m, 1H,CH=CH_2); ^{13}C NMR ($CDCl_3$) δ 25.78 (3-CH_3), 34.16 (NCH_3), 34.67 (*C*-2), 52.70 (*C*-3), 54.40 (*C*-7), 57.93 (*C*-4), 66.24 (*C*-6), 67.09 (OCH_2), 120.83 (CH=CH_2), 130.33 (CH=CH_2), 150.44 (S-*C*=N), 158.80 (*C*-8),166.49 (4-*C*O).