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Penicillin-derived inhibitors that simultaneously target both metallo- and serine-β-lactamases

John D. Buynak,[a,*] Hansong Chen,[a] Lakshminaryana Vogeti,[a] Venkat Rao Gadhachanda,[a] Christine A. Buchanan,[b] Timothy Palzkill,[c] Robert W. Shaw,[d] James Spencer[e] and Timothy R. Walsh[e]

[a]*Department of Chemistry, Southern Methodist University, Dallas, TX 75275-0314,USA*
[b]*Department of Biological Sciences, Southern Methodist University, Dallas, TX 75275-0376, USA*
[c]*Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA*
[d]*Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409-1061, USA*
[e]*Department of Pathology and Microbiology, University of Bristol School of Medical Sciences, University Walk, Bristol BS8 1TD, UK*

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Abstract—The synthesis and β-lactamase inhibitory activity of four 6-(mercaptomethyl)penicillinates and the four corresponding 6-(hydroxymethyl)penicillinates are described. These penicillins include both C6 stereoisomers as well as the sulfide and sulfone oxidation states of the penam thiazolidine sulfur. All compounds were evaluated as inhibitors of representative metallo- and serine-β-lactamases enzymes. Selected (mercaptomethyl)penicillinates are shown to inactivate both metallo- and serine-β-lactamases and to display synergism with piperacillin against β-lactamase producing strains.

1. Introduction

The most common form of bacterial resistance to the β-lactam antibiotics involves the ability to produce one or more types of β-lactamase.[1] More than 300 β-lactamases have been characterized[2] and divided into four molecular classes, A–D. Classes A, C, and D are serine enzymes, while class B are zinc metalloenzymes. Although strains expressing class A serine β-lactamases are the most clinically relevant, increasing numbers of strains are developing resistance through classes B, C, and D enzymes. Class B metalloenzymes have a broad substrate profile[3] and metallo-β-lactamase producing strains may also possess a serine enzyme, thus combining the protective effects of both a metallo- and a serine-β-lactamase. While metallo-β-lactamase inhibitors are known,[4] none are clinically useful. Current commercial β-lactamase inhibitors are only useful against strains producing class A enzymes. The Buynak Group has reported new inhibitors that simultaneously inactivate class A, C, and D β-lactamases.[5] We now report the synthesis and evaluation of inhibitors which inactivate both metallo- and serine-β-lactamases.[6] Such dual inhibitors needed to overcome several differences between the metallo and serine β-lactamases.[7] Serine β-lactamase inhibitors usually involve formation of a stable acyl-enzyme. The metallo-β-lactamases utilize active site zinc as a Lewis acid to catalyze the hydrolysis of the β-lactam in a single step.[8] Inhibitors of the zinc β-lactamases are tight binding reversible inhibitors, often with a strongly coordinative zinc ligand. Commercial serine-β-lactamase inhibitors are substrates of the metallo-β-lactamases.[9] Additionally, the general architecture of the active sites of serine and metallo-β-lactamases are dissimilar. However, these two classes of hydrolase share the property of being able to specifically hydrolyze β-lactam antibiotics. Thus, we employed a penicillin scaffold to ensure recognition of both classes of β-lactamase. These inhibitors are designed to form a stabilized acyl-enzyme to inhibit the serine β-lactamases, but also incorporate a ligand to inhibit the metallo-β-lactamases.

Several antibiotics and β-lactamase inhibitors possess a hydroxyalkyl side chain α to the β-lactam carbonyl. These include the carbapenems (**1**), penems (**2**), oxapenems (**3**), and the 6-hydroxyalkylpenicillins (**4**, $n=0$ or 2) shown in Figure 1. Penams **4** are reported to have the ability to inhibit serine β-lactamases.[10] We hypothesized that substituting the hydroxyl group with a thiol would

* Corresponding author. Tel.: +1-214-768-2484; fax: +1-214-768-4089; e-mail: jbuynak@mail.smu.edu

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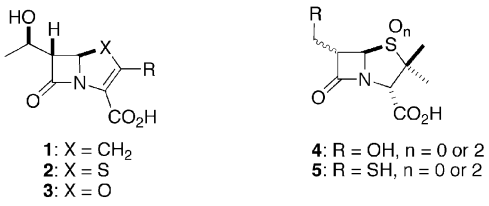


Figure 1. Representative hydroxyalkyl and mercaptoalkyl antibiotics and β-lactamase inhibitors.

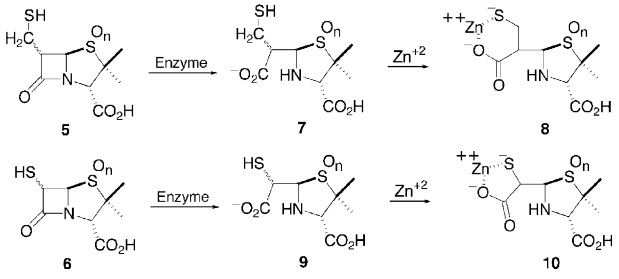
produce a compound **5** with the dual ability to inhibit both serine and metallo-β-lactamases.

As shown in Scheme 1, either compounds **5**, or compounds **6**, having one less carbon, would also be capable of bidentate chelation of zinc subsequent to enzymatic hydrolysis of the β-lactam. We thus targeted four compounds of structure **5** and four compounds of structure **6** as shown in Figure 2. For comparison, we also prepared the corresponding 6-(hydroxymethyl)penicillinates, **4**, using procedures of Kellogg[10a] and of Mobashery.[10e,j]

2. Results

2.1. Synthesis

As shown in Scheme 2 and 3, **5a** and **5b** were prepared from 6-APA. 6,6-Dibromopenicillanic acid (**12**) was prepared using the procedure of Volkmann[11] and esterified. This dibromide, **13**, was converted into bromoalcohols **14** and stereoselectively reduced to 6β-(hydroxymethyl)penicillinate **15** using the procedure of Kellogg.[10] Conversion to mesylate **16**, followed by



Scheme 1.

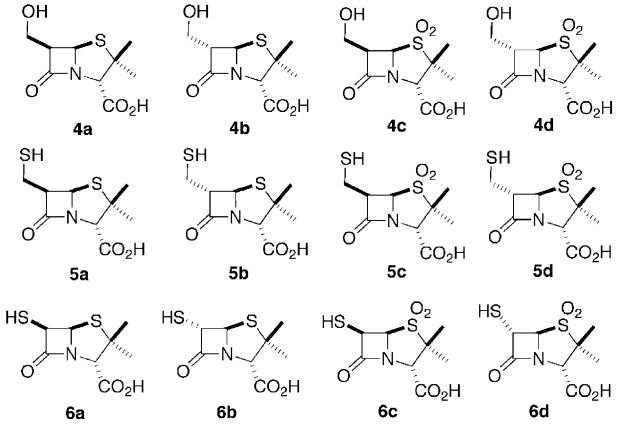


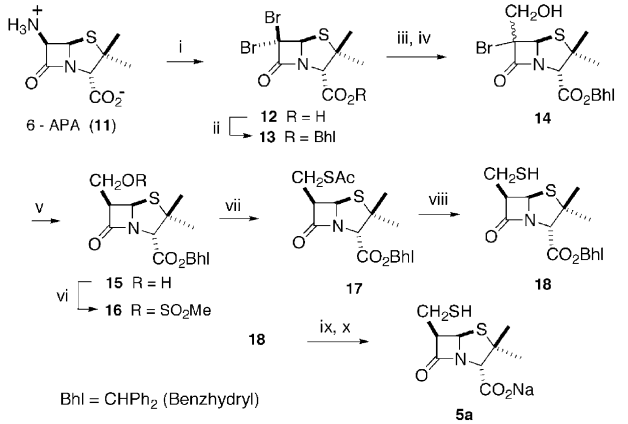
Figure 2. β-Lactamase inhibitors prepared and tested.

treatment with cesium thioacetate produced thioester **17**. Selective cleavage of thioacetate **17**, deprotection with *m*-cresol,[12] and neutralization produced **5a**.

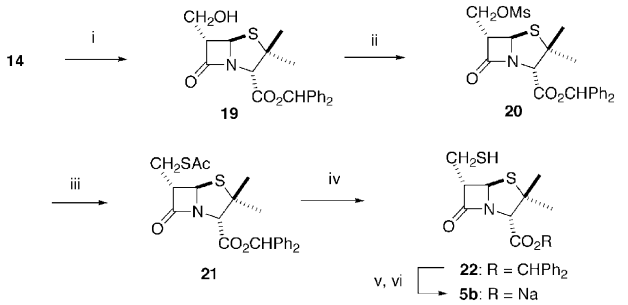
Compound **5b** was prepared from the same mixture **14** by reduction with tributylphosphine[13] as shown in Scheme 3. Conversion of **19** to mesylate **20**, treatment with cesium thioacetate and cleavage of thioester **21** with NaOMe produced **22** which was deprotected with *m*-cresol.

Attempts to oxidize **17** to the corresponding sulfone with excess mCPBA surprisingly produced only a mixture of epimeric sulfoxides **23**. The requisite oxidation to **24** could be achieved using excess $KMnO_4$. However, the subsequent cleavage of the thioester produced an inseparable 2:1 mixture of epimeric 6-(mercaptomethyl)penicillinates **25** and **26** (Scheme 4).

Obviously, sulfone **24** (and/or the corresponding mercaptans **25** and **26**) was less configurationally stable (at C6) under these basic conditions than were sulfides **17** and **18**. Thus we replaced the thioester with a more readily removable protecting group. As shown in Scheme 5, we protected the thiol of **18** with Troc before oxidation of sulfide **27**. Subsequent $KMnO_4$/AcOH



Scheme 2. Synthesis of **5a**. (i) $NaNO_2$, H_2SO_4, Br_2, CH_2Cl_2/H_2O (85%); (ii) $Ph_2C{=}N_2$, acetone, rt, 14 h, (90%); (iii) *t*-BuMgBr, −78 °C; (iv) CH_2O, −78 °C to rt, (36% for steps iii and iv); (v) $(n\text{-}Bu)_3SnH$, cat AIBN, CH_2Cl_2, 89%; (vi) $MeSO_2Cl$, DMAP, $CHCl_3$, 3 h, 74%; (vii) AcSCs, MeCN, rt, 15 h (77%); (viii) NaOMe, THF, MeOH −78 °C to −40 °C (85%); (ix) *m*-cresol, 48 °C, 6 h; (x) $NaHCO_3$ (40% for steps ix and x).

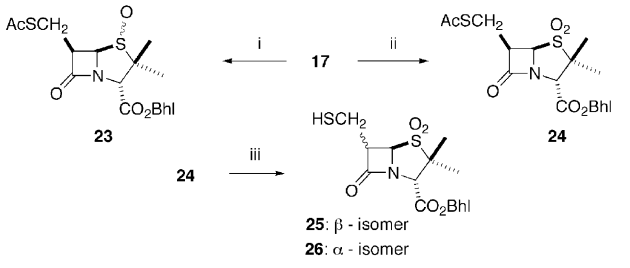


Scheme 3. Synthesis of **5b**. (i) Bu_3P, MeOH, rt, 1.5 h, 85%; (ii) MsCl, DMAP, $CHCl_3$, rt, 1.5 h, 85%; (iii) AcSCs, MeCN, rt, 2.5 h, 85%; (iv) NaOMe, THF–MeOH, −78 °C to −40 °C, 89%; (v) *m*-cresol, 50 °C, 6 h; (vi) $NaHCO_3$ (41% for steps v and vi).

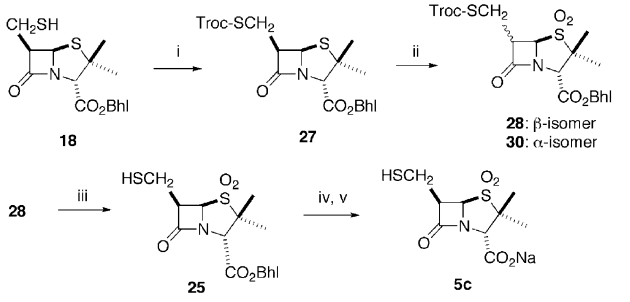
oxidation produced an 85:15 mixture of the 6β and 6α epimers of sulfones **28** in 90% yield. Separation and removal of the Troc group with zinc/copper couple[14] and subsequent removal of the benzhydryl group produced **5c**. A similar strategy produced **5d**, as shown in Scheme 6.

6-Mercaptopenicillinates **6a** and **6b** were prepared as shown in Scheme 7. 6-APA was converted to 6α-hydroxypenicillanic acid and esterified to produce the benzhydryl ester **31**. Compound **31** was then converted to triflate, **32**, treated with cesium thioacetate to produce thioacetate **33** and subsequently cleaved with NaOMe to produce thiol **34**, which was deprotected to carboxylate **6a**. The epimer, **6b**, was produced by treating 6-diazopenicillinate **35** with thiolacetic acid in the presence of BF_3–OEt_2. Cleavage of the thioacetate **36** and removal of the benzhydryl group produced **6b**.

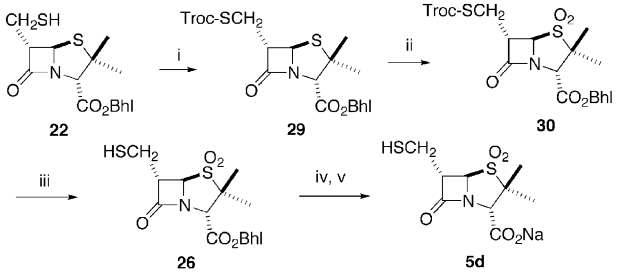
As shown in Scheme 8, the Troc group was again employed in an attempted synthesis of the 6β-mercaptopenicillin sulfone **6c**. Upon treatment of sulfone **39** with zinc/copper couple, the C6 β-isomer was epimerized to the 6α isomer **41**. Given the mild conditions, we decided the 6β-isomer was not isolable. The 6α epimer, **6d**, was prepared without further difficulty. As shown in Scheme 9, the 6-(hydroxymethyl)penicillinates were prepared utilizing reported procedures.[10]



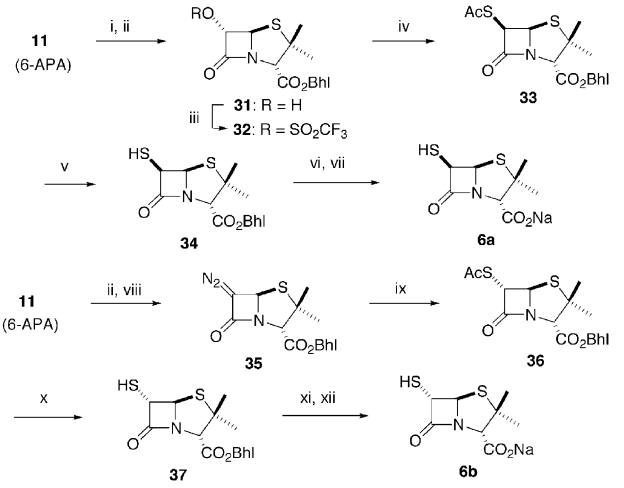
Scheme 4. Initial attempt to prepare **5c**. (i) 4 equiv mCPBA, CH_2Cl_2, pH = 6.4 buffer; (ii) xs $KMnO_4$, AcOH/CH_2Cl_2 = 1/2; (iii) NaOMe/MeOH, −78 °C to −40 °C.



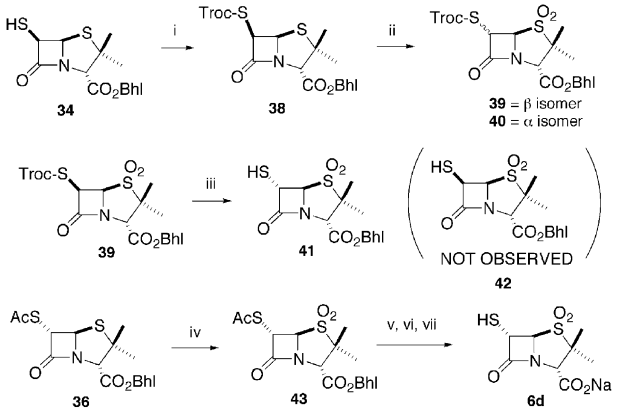
Scheme 5. Synthesis of **5c**. (i) Troc-Cl, DMAP, CH_2Cl_2, 0°C to rt, 3 h, 83%; (ii) $KMnO_4$/HOAc, CH_2Cl_2, rt, 14 h, 90%; (iii) Zn–Cu/HOAc, THF–MeOH, rt, 1 h, 54%; (iv) *m*-cresol, 50 °C, 2.5 h; (v) $NaHCO_3$ (53% for steps iv and v).



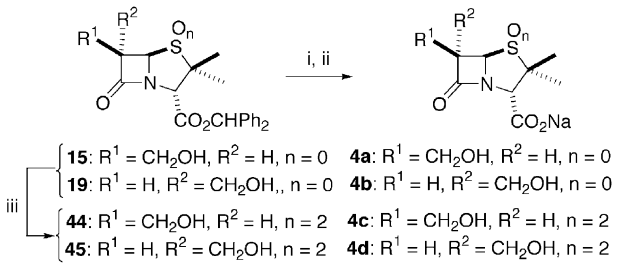
Scheme 6. Synthesis of **5d**. (i) Troc-Cl, DMAP, CH_2Cl_2, 0°C to rt, 3 h, 86%; (ii) $KMnO_4$/HOAc, CH_2Cl_2, rt, 14 h, 90%; (iii) Zn–Cu/HOAc, THF/MeOH, rt, 1 h, 54%; (iv) *m*-cresol, 50 °C, 2.5 h; (v) $NaHCO_3$ (67% for steps iv and v).



Scheme 7. Synthesis of **6a** and **6b**. (i) $NaNO_2$/aq $HClO_4$; (ii) $Ph_2C{=}N_2$; (iii) Tf_2O, Et_3N; (iv) AcSCs, MeCN; (v) NaOMe, THF–MeOH, −78 °C, 8 h, 80%; (vi) *m*-cresol, 50 °C, 6 h; (vii) $NaHCO_3$ (30% for steps vi and vii); (viii) *i*-pr-ONO, cat. TFA; (ix) AcSH, BF_3–OEt_2, rt, 15 min (40%); (x) NaOMe, THF–MeOH, −78 °C to −40 °C, 2.5 h, 71%; (xi) *m*-cresol, 50 °C, 6 h; (xii) $NaHCO_3$ (41% yield for steps xi and xii).



Scheme 8. Synthesis of **6d**. (i) Troc-Cl, DMAP, 0 °C to rt, 3 h, 73%; (ii) $KMnO_4$/HOAc, rt, 15 h, 78%, **39**:**40** = 1:1; (iii) Zn–Cu, HOAc, rt, 30 min; (iv) $KMnO_4$/HOAc, rt, 15 h, 60%; (v) NaOMe, 78%; (vi) *m*-cresol, 50 °C, 4 h; (vii) $NaHCO_3$, (60% for step vi and vii).



Scheme 9. Synthesis of **4a–d**. (i) *m*-cresol; (ii) $NaHCO_3$; (iii) xs $KMnO_4$, AcOH, CH_2Cl_2.

2.2. Inhibitory activity

The activity of the newly prepared molecules as inhibitors of representative serine and metallo-β-lactamases is presented in Table 1. For comparison, the activity of the 6-(hydroxymethyl)-penicillinates (**4a–d**), and the commercial serine β-lactamase inhibitor, tazobactam (**46**), and pyridine 2,6-dicarboxylic acid (**47**) as a standard metallo-β-lactamase inhibitor were also measured (Fig. 3).

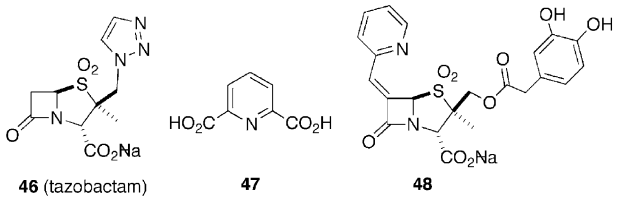


Figure 3. Standard β-lactamase inhibitors.

Table 1. β-Lactamase inhibitory activity (IC_{50}, μM) against representative class A (TEM-1), class C (P99), and class B (metallo) enzymes[15]

Compd	TEM-1 (Class A) Serine	P99 (Class C) Serine	L1 (Class B) Metallo	BCII (Class B) Metallo
tazo	0.122	53.2	>200	>200
4a	752	409	>200	>200
4b	275	96.2	>200	>200
4c	0.65	3.9	72.3	>200
4d	14.6	10.0	>200	>200
5a	601	0.10	32.1	2.9
5b	648	3.75	10.9	1.7
5c	6.8	10.5	0.10	1.4
5d	51.7	7.5	0.30	12.0
6a	>1000	42.3	41.4	17.8
6b	988	7.9	36.9	17.2
6d	69.2	1.4	7.6	106.2
47	NT	NT	7.8	21.8

Table 2. Synergy of **4**, **5**, and **6** with piperacillin against bacteria producing representative serine β-lactamases

	Minimal inhibitory concentration (μg/mL)		
Antibiotic/ Inhibitor	*E. coli* ATCC 35218 (TEM-1) (Class A, Serine)	*E. coli* P99 (AmpC) (Class C, Serine)	*Staphylococcus aureus* ATCC 29213
Piperacillin	>128	4	8
Pip/tazo	1	0.5	1
Pip/**4a**	>128	4	4
Pip/**4b**	>128	4	8
Pip/**4c**	1	0.125	1
Pip/**4d**	8	0.25	2
Pip/**5a**	>128	2	2
Pip/**5b**	>128	2	4
Pip/**5c**	8	0.25	2
Pip/**5d**	32	0.5	4
Pip/**6a**	>128	2	8
Pip/**6b**	>128	2	8
Pip/**6d**	>128	2	8

2.3. Microbiological activity

The new inhibitors were assayed for synergism with piperacillin against β-lactamase-producing strains. Data against serine-β-lactamase producing strains is shown in Table 2. The MIC values shown in Table 2 were obtained while maintaining the inhibitors constant at 4 μg/mL and varying the concentration of piperacillin. In Table 3, 6-(mercaptomethyl)penicillinates **5** were screened as inhibitors of metallo-β-lactamase producing strains, including one *E. coli* strain producing the IMP1 metallo-β-lactamase, and three clinical isolates of *P. aeruginosa* strains carrying either bla_{SPM-1}, bla_{VIM-1} or bla_{VIM-7} as well as an AmpC serine β-lactamases. It is noteworthy that the *P. aeruginosa* carrying bla_{VIM-7} also carries bla_{OXA-45}, an unusual class D enzyme. This initial screen for activity was performed at a concentration of 128 μg/mL while maintaining a 1:1 antibiotic:inhibitor ratio. These inhibitors were tested both in the presence of the serine β-lactamase inhibitors, tazobactam (**46**) and DVR-II-183 (**48**), and, in the case of inhibitor **5c**, without an additional β-lactamase inhibitor. We have previously reported the class A and class C serine β-lactamase inhibition and in vitro synergism (with piperacillin) of **48** against Gram-negative β-lactamase-producing strains.[6d] All metallo-β-lactamase producing strains were resistant to piperacillin and to the co-administration of piperacillin with tazo or with **48**. Table 4 shows the MIC values of **5c** (in combination with piperacillin) against a number of metallo-β-lactamase producing strains. **5c** was tested alone and in combination with tazo and also in combination with **48**.

Table 3. Synergy of compounds **5** with piperacillin and other β-lactamase inhibitors against bacteria producing metallo β-lactamases (S = susceptible; R = resistant)

Antibiotic/ β-Lactamase inhibitors: 1:1:1 at concn of 128 μg/mL	*E. coli* (IMP-1)	*P. aeruginosa* (SPM-1)	*P. aeruginosa* (VIM-1)	*P. aeruginosa* (VIM-7)
pip	R	R	R	R
pip/tazo	R	R	R	R
pip/**48**	R	R	R	R
pip/tazo/**5a**	S	R	R	R
pip/tazo/**5b**	S	R	R	R
pip/tazo/**5c**	S	S	R	R
pip/tazo/**5d**	S	R	R	R
pip/**48**/**5c**	S	S	S	R
pip/**5c**	S	S	R	R

Table 4. Synergy of compound **5c** with piperacillin and other β-lactamase inhibitors against bacteria producing metallo β-lactamases

	Minimal inhibitory concentration (μg/mL)			
Antibiotic/ β-Lactamase inhibitors: 1:1:1	*E. coli* (IMP-1)	*P. aeruginosa* (SPM-1)	*P. aeruginosa* (VIM-1)	*P. aeruginosa* (VIM-7)
pip	>128	>128	>128	>128
pip/tazo	>128	>128	>128	>128
pip/tazo/**5c**	8	64	>128	>128
pip/DVRII183/**5c**	8	64	128	>128
pip/**5c**	8	64	>128	>128

3. Discussion

Table 1 indicates that although nearly all compounds display some inhibition of the two serine β-lactamases, only the thiols have the ability to inhibit the metallo-β-lactamases. Tazobactam displays no inhibition of either of the two metallo-β-lactamases. Data involving the inhibition of the representative class A and class C serine β-lactamases by 6-(hydroxymethyl)penicillinates **4**, parallel results obtained by Wyeth researchers.[10h,i] Both studies observed greater (serine) β-lactamase inhibition by sulfones **4c** and **4d** than by the corresponding sulfides **4a** and **4b**. However, it should be noted that the C6-hydroxyalkyl penicillin sulfides are also known to be β-lactamase inhibitors and have been very effectively utilized to elucidate the direction of approach of the hydrolytic water molecule to the intermediate acyl enzyme.[16,17] Table 1 shows that all of the new 6-(mercaptomethyl)penicillins, **5**, did, in addition to inhibiting serine β-lactamases, also inhibit the two metallo-β-lactamases. Sulfones **5c** and **5d** were good inhibitors of the L1 metallo-β-lactamase, while sulfides **5a** and **5b** and sulfone **5c** were all good inactivators of the BCII hydrolase, implying different chemical mechanisms of inhibition of these penams against the two metalloenzymes. As in the case of the hydroxymethylpenicillinate sulfones (**4c** and **4d**), the corresponding 6-(mercaptomethyl)penicillinate sulfones (**5c** and **5d**) displayed good activity against both serine β-lactamases. By comparison with the (mercaptomethyl)penicillinates (**5**), the 6-mercapto-penicillinates (**6**) were relatively inactive as inhibitors of either the metallo- or serine-β-lactamases. The synergy data against the representative serine-β-lactamase producing strains presented in Table 2 shows a close parallel between the mercaptans and the corresponding alcohols. This involves both the oxidation level of the thiazolidine sulfur as well as the stereochemistry at C6. Thus, in both cases, the 6-(hydroxymethyl)-penicillin sulfones **4c**, **4d** and the corresponding 6-(mercaptomethyl)penicillin sulfones **5c**, **5d** produce the better synergy against these strains, with the α stereochemistry at C6, compounds **4c** and **5c**, being the two most potent inhibitors.

Tables 3 and 4 show that the mercaptomethyl inhibitors **5** also display synergy with piperacillin against strains producing metallo-β-lactamases, including an *E. coli* producing IMP-1, as well as several *Pseudomonas aeruginosa* strains producing either VIM β-lactamases or the recently characterized[8c] SPM-1 metallo-β-lactamase. While a number of the inhibitors displayed synergy against the IMP producing strain, only **5c** showed activity against SPM-1 and the VIM-1 producing strain. None displayed synergy against the VIM-7 producing strain, which also possesses a Class D β-lactamase. An additional serine β-lactamase inhibitor was not needed to support the synergy of **5c** against the SPM-1 producing strain. *P. aeruginosa* represents a particularly challenging microorganism, due to the reduced permeability of its outer membrane and the overexpression of multidrug efflux systems. This is the first report of synergy against a metallo-β-lactamase producing *P. aeruginosa* strain.

4. Conclusion

In conclusion, new compounds, **5**, simultaneously inhibited representative metallo- and serine-β-lactamases and had the ability to differentially inhibit the two metallo-β-lactamases, with the two sulfides (**5a** and **5b**) being more active against the BCII enzyme than against the L1 enzyme and sulfone **5c** displaying inhibition of both metallo-β-lactamases. These new compounds were also active in vitro, displaying synergy with piperacillin against β-lactamase-producing strains, including a strain of *P. aeruginosa*.

Acknowledgements

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