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N-Thiolated β-lactam antibacterials: Effects of the *N*-organothio substituent on anti-MRSA activity

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Abstract—A study on the structure–activity profiles of *N*-thiolated β-lactams **1** is reported which demonstrates the importance of the *N*-organothio moiety on antibacterial activity. Our results indicate that elongation of the *N*-alkylthio residue beyond two carbons, or extensive branching within the organothio substituent, diminishes antibacterial effects. Of the derivatives we examined, the *N*-*sec*-butylthio β-lactam derivative **5g** possesses the strongest growth inhibitory activity against methicillin-resistant *Staphylococcus aureus* strains. Sulfur oxidation state is important, as the *N*-sulfenyl and *N*-sulfinyl groups provide for the best antibacterial activity, while lactams bearing the *N*-sulfonyl or *N*-sulfonic acid functionalities have much weaker or no anti-MRSA properties. Stereochemistry within the organothio chain does not seem to be a significant factor, although for *N*-*sec*-butylthio β-lactams **15a–d**, the 3*R*,4*S*-lactams **15c**, **d** are more active than the 3*S*,4*R*-stereoisomers **15a**, **b** in agar diffusion experiments. The *N*-methylthio lactams are the most sensitive to the presence of glutathione, followed by *N*-ethylthio and *N*-*sec*-butylthio lactams, which indicates that bioactivity and perhaps bacterial selectivity of the lactams may be related to the amount of organothiols in the bacterial cell. These results support the empirical model for the mechanism of action of the compounds in which the lactam transverses the bacterial membrane to deliver the organothio moiety to its cellular target.

1. Introduction

N-Thiolated β-lactams **1** are a new family of antibacterial agents active against *Staphylococcus* bacteria, including methicillin-resistant strains of *Staphylococcus aureus* (MRSA).[1] Although these compounds exhibit antigrowth properties against other *Staphylococcus* species, including *S. epidermidis*, *S. simulans*, *S. saprophyticus*, and a few other genera such as *Micrococcus luteus* and *Neisseria gonorrhoeae*, a wide range of other Gram-positive and Gram-negative microbes appear to be unaffected.[1b] This observed selectivity for certain bacteria is rather interesting and undoubtedly relates to the mode of action, which has not yet been completely defined. Moreover, the ability of lactams **1** to retain their full antibacterial activity against drug-resistant microbes such as MRSA is attributable to the stability these compounds have toward bacterial penicillinases.[1b] The compounds are inert to hydrolytic degradation by these enzymes, a property highly desirable for a β-lactam drug, and do not inhibit the hydrolytic cleavage of penicillin G by β-lactamases. Additionally, the lactams possess promising anticancer properties, yet are not cytotoxic to normal mammalian (human fibroblast) cells at concentrations well beyond that needed to inhibit bacterial growth.[2] Our studies also indicate that these *N*-thiolated lactams display different structure–activity profiles to those of traditional β-lactam antibacterials and exert their growth inhibitory effects in a manner which is unique to those of other β-lactams. For instance, the C_3 and C_4 ring substituents (R_1–R_4) have only a marginal effect on anti-MRSA activity, and neither relative nor absolute chirality at these two centers seems to significantly perturb activity.[3]These preliminary structure–activity studies also indicate that the *N*-organothio group may play a more important role, however, since

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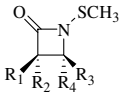
replacement of the *N*-methylthio moiety for other *N*-substituents such as hydrogen, alkyl or alkoxy results in complete loss of bioactivity.



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2. Results and discussion

2.1. Preliminary model studies

A hypothetical model for the mode of action of these lactams, as depicted in Figure 1, serves as a starting point for these investigations. We have evidence that the antibacterial properties of these *N*-thiolated lactams come from the transfer of the organothio moiety onto its target with consequential loss of the *N*-protio lactam. We have been able to isolate this by-product by extraction of the cultured broth media with ethyl acetate. ^{1}H NMR studies of the extract indicate the clean formation of the *N*-protio β-lactam, as represented by the appearance of the broad N–H signal and the simultaneous loss of the *N*-methylthio substituent (Fig. 2). Antimicrobial testing of the resulting N–H lactam shows that this N–S cleavage product possesses no antibacterial properties. The identity of the cellular target has not yet been determined but is most likely a cytoplasmic thiol needed for bacterial survival.

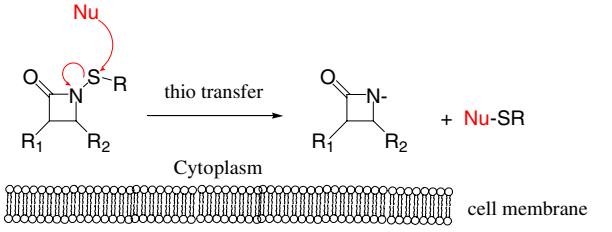


Figure 1. Model depicting the proposed mode of action of *N*-thiolated β-lactams.

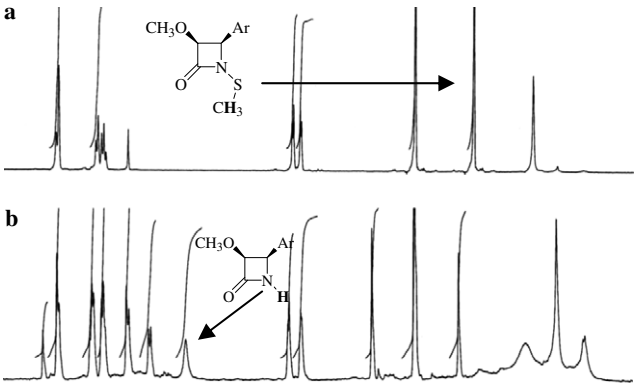


Figure 2. Proton NMR spectra of lactam **5a** (a) prior to inoculation with *S. aureus* and (b) after inoculation with *S. aureus* and ethyl acetate extraction of the growth media.

Given the requirement for a thio transfer from the lactam to its target, we speculated whether the anti-MRSA properties of the compounds could be enhanced by altering the structure of the organothio side chain. In this report, we investigate this in more detail by examining *N*-thiolated lactams in which the *N*-organothio substituent is systematically varied either in length or in its degree of chain branching. We also examine the effect of sulfur oxidation state on anti-MRSA properties. The results we obtained from this study, in conjunction with those of our previous findings, enable us to clarify the role of the ring substituents and the process by which these compounds may function as antibacterials.

2.2. Synthesis and evaluation of β-lactam analogues

2.2.1. Linear *N*-alkylthio side-chain derivatives. The first series of compounds we investigated are *N*-alkylthio lactams **5a–e**, in which the alkylthio side chain is linear and contains from one to eight carbons (Fig. 3). These derivatives were obtained through synthesis as shown in Scheme 1. Staudinger coupling of *N*-(4-methoxyphenyl)-imine **2** with methoxyacetyl chloride afforded exclusively the *cis*-disubstituted β-lactam **3**.[4] Following oxidative cleavage of the *N*-aryl ring with aqueous ceric ammonium nitrate, the resulting lactam **4** was *N*-alkylthiolated using readily prepared sulfenylating reagents[5]

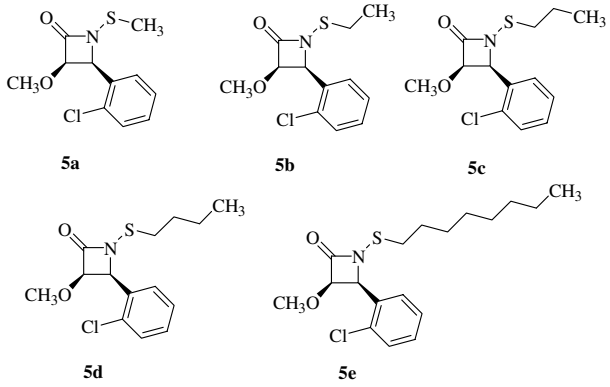
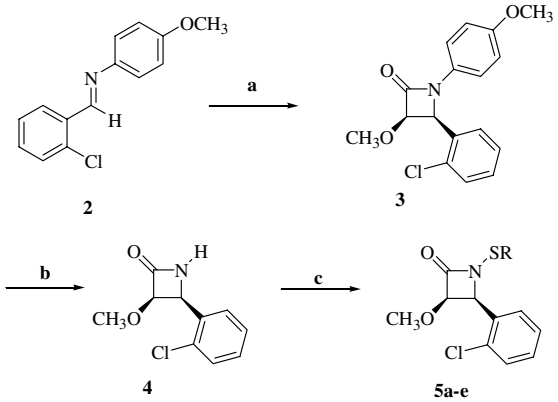


Figure 3. Linear *N*-alkylthio-substituted β-lactams **5a–e**.



Scheme 1. Reagents and conditions: (a) AcCl, NEt^{i}Pr$_2$, PhMe, 0 °C to rt; (b) $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O, 0 °C; (c) *N*-alkylthiophthalimide, NEt^{i}Pr$_2$, C_6H_6, reflux.

to give *N*-thiolated lactams **5a**–**e**. These five analogues were tested for antibacterial activity against MRSA isolates using the Kirby–Bauer method of disk diffusion on agar plates (Table 1). Previously, we have demonstrated that the growth inhibition zone sizes for *N*-methylthio lactams done in this manner correlate well with their minimum inhibitory concentrations (MICs) from broth dilution experiments, and thus represent a reliable way to gauge bioactivity within a closely related series of analogues.[6] For each compound, the microbiological data from these agar plate assays are plotted out graphically in Figure 4, with the vertical bars indicating the average diameter (from triplicate trials) of the growth inhibition zones observed. Nine clinical strains of MRSA were tested against each derivative, including eight clinical isolates and one ATCC strain. All MRSA's were β-lactamase-producing strains.

While the lead compound, *N*-methylthio lactam **5a**, shows inhibition zones of around 28 mm, the *N*-ethylthio analogue **5b** is about 15% more active. Bioactivity then drops off steadily as the *N*-alkylthio chain length is further increased, with *N*-octylthio lactam **5e** retaining less than half the activity of **5a**. This suggests that the increase of lipophilicity of the *N*-alkylthio chain through elongation of the alkyl residue beyond two carbons causes a systematic drop in bioactivity.

We considered the possibility that the reason why antibacterial activity of lactams **5a**–**e** diminishes as a function of the *N*-alkylthio chain length could be artifactual, due to the decreasing ability of the different analogues to diffuse through the agar media. Thus, smaller zone sizes could simply be the result of diminished water solubility.

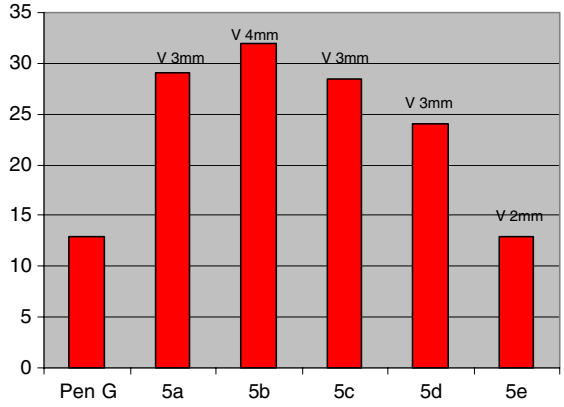
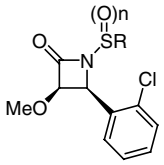


Figure 4. Antimicrobial activities of lactams **5a**–**e** against MRSA. Penicillin G (Pen G) is used as a control. The vertical bars measured along the *y* axis indicate the average diameter in mm of the growth inhibition zones produced against nine different MRSA strains (in triplicate). Margin of error for these triplicate measurements is ±1 mm. The number (V) above each vertical bar indicates the range of variation observed among the nine MRSA strains, taken from Table 1.

Therefore, broth minimum inhibitory concentration (MIC) values were determined for lactams **5a**, **b**, and **e** against *S. aureus* (ATCC 25923) and MRSA (ATCC 43300) (Table 2). The MICs further validate the trend observed in the Kirby–Bauer tests, with anti-MRSA activity of the lactams increasing in the order **5e** < **5a** < **5b**. The MIC data also show that these

Table 1. Kirby–Bauer well-diffusion assays of *N*-thiolated β-lactams against nine different MRSA strains



Lactam	*n*	R	MRSA 652	MRSA 653	MRSA 654	MRSA 655	MRSA 656	MRSA 657	MRSA 658	MRSA 659	ATCC 43300
5a	0	CH_3	30	29	28	27	27	28	27	27	28
5b	0	CH_2CH_3	33	33	29	29	30	30	31	29	31
5c	0	$CH_2CH_2CH_3$	30	30	28	28	27	27	28	27	28
5d	0	$CH_2(CH_2)_2CH_3$	25	24	24	22	23	24	25	24	25
5e	0	$CH_2(CH_2)_6CH_3$	13	12	13	12	12	14	13	13	14
5f	0	$CH(CH_3)_2$	33	32	31	29	30	31	32	30	31
5g	0	$CH(CH_3)CH_2CH_3$	44	41	39	39	39	40	41	39	40
5h	0	$C(CH_3)_3$	10	10	10	10	10	10	10	10	10
5i	0	c-C_6H_{11}	24	24	23	23	22	23	26	24	24
5j	0	Ph	27	24	25	22	23	24	25	24	25
5k	0	CH_2Ph	28	27	28	27	26	27	29	27	28
6i	1	c-C_6H_{11}	22	21	21	19	19	20	21	19	20
7i	2	c-C_6H_{11}	11	10	9	9	8	10	9	8	10
9a	3	H	0	0	0	0	0	0	0	0	0

The values indicate the average diameters (of three trials) in mm for the zone of growth inhibition. For each test, 20 μg of the lactam in DMSO solution (1 mg/mL) was applied to a 6-mm well prior to inoculation. Incubation was for 24 h at 37 °C. All of the microbes are β-lactamase-producing strains. MRSAs 652–659 are clinical isolates obtained from Lakeland Regional Medical Center (Lakeland, Florida). Error values for each measurement are typically within ±1 mm.

Table 2. Minimum inhibitory concentrations, MIC (μg/mL), of lactams **5a**, **b**, **e**, and **g** versus penicillin G (Pen G)

Lactam	*S. aureus* (ATCC 25923)	MRSA (ATCC 43300)
PenG	0.025	64
5a	16	16
5b	8	8
5e	64	64
5g	0.25	0.25

N-thiolated lactams have the same activity against drug-susceptible *S. aureus* and β-lactamase-producing MRSA, in contrast to penicillin G (PenG) whose inhibitory activity is markedly diminished for MRSA.

2.2.2. Branched *N*-alkylthio β-lactams. Investigating the effects of branching within the alkylthio chain should provide some evidence for, or against, a steric effect on bioactivity. We therefore conducted a study to ascertain this by examining the anti-MRSA activity of branched *N*-organothio lactams **5f–k** (Fig. 5). Each of these lactams was prepared analogously to that shown in Scheme 1. As the plot of bioactivities in Figure 6 shows, replacement of the *N*-ethylthio substituent of lactam **5b** with an *N*-isopropylthio group (lactam **5f**) led to a 7% increase in anti-MRSA activity based on agar diffusion testing. Thus, enhancement of steric hindrance around the sulfur atom does not necessarily diminish activity, but in this case, seems to enhance bacterial growth inhibition. Further lengthening of the alkyl chain from this branch point, as shown for *sec*-butyl analogue **5g**, continues to increase potency. MIC testing concurred with these results, yielding a value of less than 0.25 μg/mL for 100% growth inhibition of the MRSA (ATCC 43300) compared to 8 μg/mL for *N*-methylthio compound **5b** (Table 1).

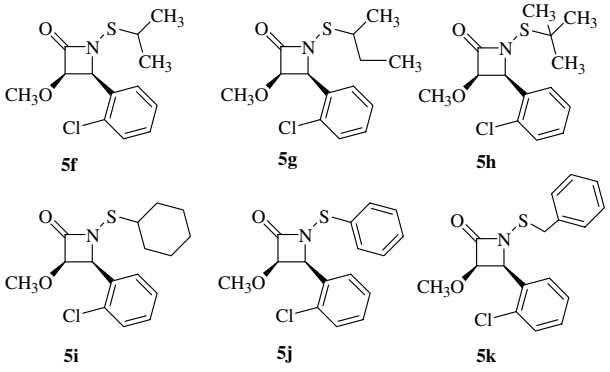


Figure 5. Branched *N*-alkylthio β-lactams **5f–k**.

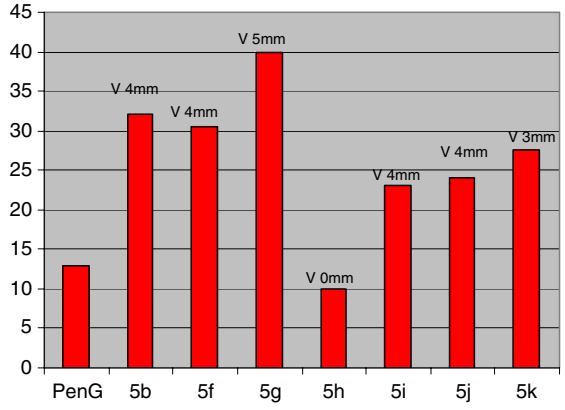
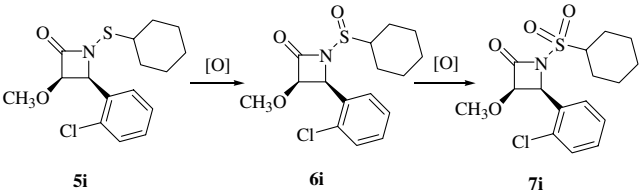


Figure 6. Antimicrobial activities of *N*-ethylthio compound **5b** and lactams **5f–k** against MRSA. Penicillin G (Pen G) is used as a control. The vertical bars along the *y* axis indicate the average diameter in mm of the growth inhibition zones produced on agar plates against nine different MRSA strains (in triplicate). Margin of error for these triplicate measurements is ±1 mm. The number (V) above each vertical bar indicates the range of variation observed among the nine MRSA strains, taken from Table 1.

We then extended these studies to *N*-thiolated lactams having alicyclic or aromatic rings as branched residues within the organothio chain. To this end, *N*-cyclohexylthio, *N*-phenylthio, and *N*-benzylthio β-lactams **5i–k** were synthesized accordingly and tested against the MRSAs. All three of these lactams were found to be roughly equal to each other in activity, but showed lower effectiveness than the *sec*-butylthio (**5g**) and isopropylthio (**5f**) analogues. This suggests that the presence of bulkier cyclic side chains on the sulfur atom diminishes anti-MRSA activity to some degree.

2.2.3. *N*-Sulfinyl and *N*-sulfonyl derivatives. While prior studies in our laboratory[1] suggested that the antibacterial properties of *N*-thiolated β-lactams could be modulated by changing the sulfur oxidation state, we decided to reinvestigate this in more detail using *N*-cyclohexylthio lactam **5i** as a point of reference. Thus, *N*-sulfinyl lactam **6i** and *N*-sulfonyl analogue **7i** were prepared as shown in Scheme 2. Oxidation of **5i** with 30% hydrogen peroxide in glacial acetic acid gave the *N*-sulfinyl product **6i** as a 1:1 diastereomeric mixture, whereas the use of *m*CPBA in diethyl ether led to the *N*-protio lactam through cleavage of the sulfur–nitrogen bond. Re-subjection of *N*-sulfinyl compounds **6i** to the hydrogen peroxide conditions afforded *N*-sulfonyl product **7i** in nearly quantitative yield. Antimicrobial screening of **6i** and **7i** against MRSA indicated that the *N*-sulfinyl lactam showed similar but slightly lower anti-MRSA activities to that of the *N*-sulfenyl precursor **5i**, but the *N*-sulfonyl analogue **7i** was largely devoid of activity (Fig. 7). This suggests that the availability of an electron pair on sulfur may be needed for bioactivity.

2.2.4. *N*-Sulfonate and *N*-sulfonic acid lactams. In follow-up to the previous pair of *S*-oxidized lactams **6i** and **7i**, we next investigated *N*-sulfonate salts **8a–c** and *N*-sulfonic acids **9a–c**. These derivatives were formed



Scheme 2.

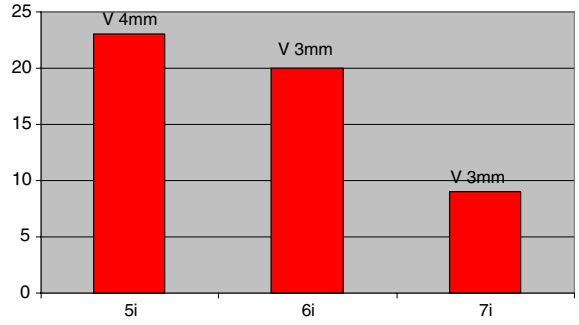
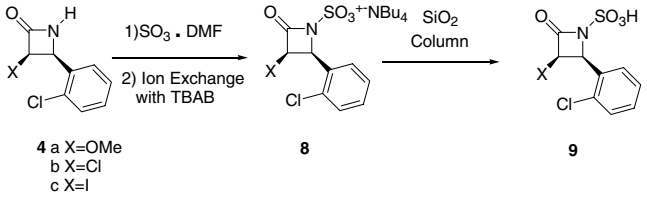


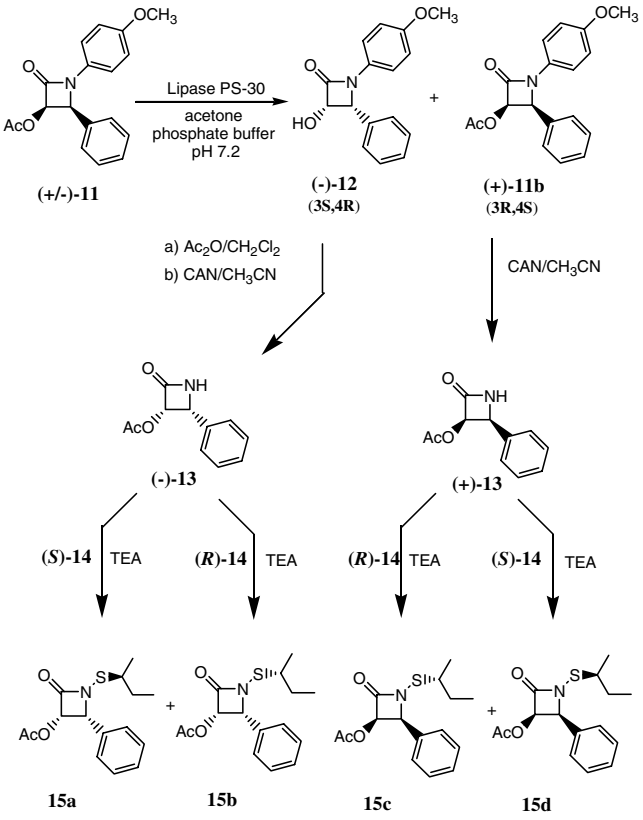
Figure 7. Antimicrobial activities of lactams **5i**, **6i**, and **7i.** Penicillin G (Pen G) is used as a control. The vertical bars along the *y* axis indicate the average diameter in mm of the growth inhibition zones produced against nine different MRSA strains (in triplicate). Margin of error for these triplicate measurements is ±1 mm. The number (V) above each vertical bar indicates the range of variation observed among the nine MRSA strains, taken from Table 1.

from the respective *N*-protio lactams **4a**–**c** using sulfur trioxide/pyridine/DMF conditions (Scheme 3).[7] This provides the *N*-sulfonate potassium salts which can be converted by ion exchange to the tetrabutylammonium salts **8**. Eluting the tetrabutylammonium salt through a silica gel chromatography column gave the *N*-sulfonic acid derivatives **9a**–**c** in good overall yield. Testing of lactams **8** and **9** revealed that both forms of the *N*-sulfonic acid were devoid of anti-MRSA activity. Neither compound produced growth inhibition zones against MRSA on agar plates, nor inhibited bacterial growth in broth at 128 μg/mL concentration levels.

2.2.5. Effect of chirality in the organothio chain on anti-MRSA activity. Recently, we reported that the absolute stereochemistry of 3,4-disubstituted *N*-methylthio β-lactams has no discernible effect on anti-MRSA activity. This was demonstrated with the enantiomeric lactams **10a** and **b** (Fig. 8).[3] Both antipodes displayed the same potencies, suggesting that absolute chirality of the lactam has no influence on bioactivity. Since biological activity of the lactams seems to be more dependent on the sulfur side chain, we thought it would be informative to examine the effect of absolute stereochemistry within the organothio side chain. We therefore focused on our most potent compound, *N*-*sec*-butylthio β-lactam **5g**, which was initially tested above as a 1:1 mixture of diastereomeric racemates (four stereoisomers). For comparison, we decided to independently examine each of the four stereoisomeric forms of **5g**, compounds **15a**–**d**, to look for possible differences in anti-MRSA activity. The syntheses of these compounds are shown in Scheme 4. To prepare these four lactams in enantiomerically pure form, we were able to take advantage of a method we reported earlier for the enzymatic resolution of racemic 3-acetoxy lactams **11** using lipase PS-30.[8] This led to the production of 3-hydroxy lactam **12** and unreacted 3-acetoxy lactam **11b**, each in better than 95% ee. These intermediates could then be taken on to **15a**, **b** using enantiomeric *sec*-butylthio phthalimide transfer reagents, (*R*)-**14** and (*S*)-**14** (Fig. 9).[9] The four lactam stereoisomers were independently obtained with better



Scheme 3.



Scheme 4.

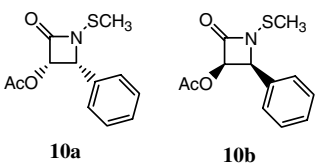


Figure 8. Enantiomeric β-lactams **10a** and **b**.

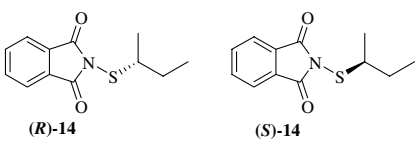


Figure 9. Enantiomeric *sec*-butylthio transfer reagents (*R*)-**14** and (*S*)-**14**.

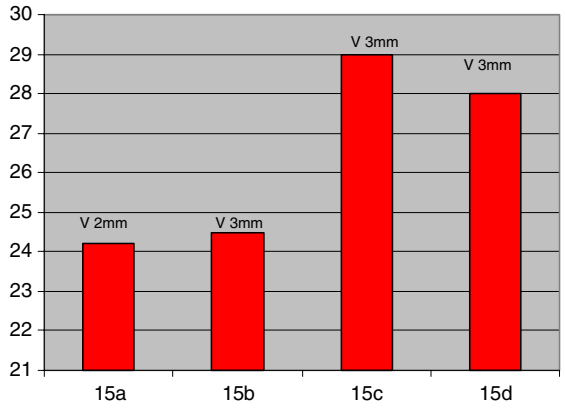


Figure 10. Comparison of antimicrobial activities of the four stereoisomers **15a–d**. The vertical bars along the *y* axis indicate the average diameter in mm of the growth inhibition zones produced against nine different MRSA strains (in triplicate). Margin of error for these triplicate measurements is ±1 mm. The number (V) above each vertical bar indicates the range of variation observed among the nine MRSA strains, taken from Table 1.

than 95% de and % ee, and tested individually for antibacterial activity (Fig. 10).

To our surprise, there was a clear difference in bioactivity between these four stereoisomers. In particular, we noted that for each of the nine clinical strains of MRSA, two of the compounds, lactams **15a** and **b**, were consistently equal in activity but also considerably less potent than their enantiomers, compounds **15c** and **d**. This was unexpected, since it appears in this case that activity is not dependent on the chirality of the organothio moiety, but instead is related to the stereochemistry at the C3 and C4 centers of the lactam. This contrasts with our previous observations for enantiomeric *N*-methylthio lactams **10a** and **b**, which carry a non-stereogenic thio side chain. It is rather curious that absolute stereochemistry of the lactam ring could have an effect on bioactivity when the *N*-organothio group is chiral, but not when the *N*-moiety is achiral (*N*-methylthio). We do not have a reasonable explanation for this yet.

Previously, we described experiments which showed that the *N*-thiolated lactams are stable to penicillinases, thus accounting for their ability to retain full antibacterial activity against β-lactamase-producing strains of *S. aureus.* Our studies indicate that compounds **5a**, **b**, and **g** are likewise impervious to the hydrolytic destruction of the β-lactam ring by pencillinases, as evidenced by the fact that they can be recovered completely unchanged after 24 h of standing in pH 7.4 buffer solution containing penicillinase protein. Under identical conditions, penicillin G is completely destroyed within 20 min.

While work is in progress to identify the biological target of the lactams and to determine the effects these lactams have on the primary cellular processes in bacteria, one of the more immediate issues we are examining is the effect of cellular thiols on bioactivity and microbial selectivity of the lactams. Among the more ubiquitous of these organothiols in bacteria is glutathione, a natural reductant which helps maintain proper redox conditions in cells. We therefore decided to examine the sensitivity of the lactams to glutathione by bioassaying *N*-methylthio lactam **5a**, ethylthio lactam **5b**, and *N*-*sec*-butylthio lactam **5g** against MRSA in the presence of different amounts of glutathione. In these experiments, shown in Table 3, the growth inhibition zones of the three lactams produced after 24 h of incubation with MRSA were compared first in the absence of additional glutathione versus when 20 μg of glutathione was added to the wells along with the drug, and then when 50 μg of glutathione was added. Using 20 μg of glutathione, *N*-methylthio lactam **5a** loses all of it activity, while *N*-ethylthio lactam **5b** and *N*-*sec*-butylthio compound **5g** retain most of their activity. Similarly, using 50 μg of glutathione, the activity of **5b** is completely sacrificed, while **5g** is still quite active. Thus, these experiments indicate an order in relative stabilities of the different *N*-alkylthio lactams to glutathione, with *N*-methylthio < *N*-ethylthio < *N*-*sec*-butylthio, which coincides with the observed trend in anti-MRSA activity. MIC testing of the most active of these three compounds, lactam **5g**, in broth media gives an MIC value of 0.25 μg/mL against both MSSA and MRSA, which increases to 2 μg/mL in the presence of 20 μg of glutathione. To study the interaction of glutathione with lactams **5a**, **b**, and **g**, we followed the reaction of glutathione with equimolar amounts of the individual lactams in buffer solution, finding that all three lactams reacted rapidly with glutathione to produce the *N*-protio lactam and the *S*-alkylthio glutathione disulfide adduct. Thus, transfer of the *N*-alkylthio substituent from the lactam to the thiol occurs spontaneously. To further substantiate the neutralizing effect of glutathione, a Kirby–Bauer plate was prepared with a 1 mg reservoir of glutathione located at the center and three surrounding wells containing 20 μg of different *N*-methylthio β-lactams (Fig. 11). The ability of glutathione to consume the lactams was again clearly evident from the presence of concaved regions within the growth inhibition zones, between the wells containing glutathione and the lactam, where the concentration of glutathione was sufficient to deactivate the lactams.

Table 3. Effect of glutathione on the growth inhibition zones of lactams **5a**, **b**, and **g** against MRSA (ATCC 43300) by Kirby–Bauer diffusion

Compound	No glutathione (mm)	20 μg glutathione (mm)	100 μg glutathione (mm)
5a	29	0	0
5b	33	27	0
5g	40	38	25

In each case, 20 μg of the lactam (applied as a 1 mg/mL solution in DMSO) was added to the well, along with the indicated amount of glutathione. Zone diameters were then measured after 24 h of incubation at 37 °C.

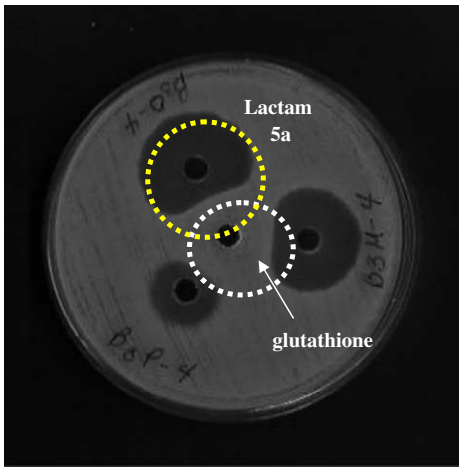


Figure 11. Effect of glutathione on the anti-MSSA activity of *N*-thiolated β-lactams. The center well was loaded with 1 mg of glutathione in aqueous solution, and the three peripheral wells each contain 20 μg of test compound. The dotted circles indicate the regions where the lactam (yellow) and glutathione (white) are present. Where the circles meet is where bacterial growth occurs, indicating that glutathione blocks the anti-MRSA activity of lactam **5a** in this region.

It is likely, then, that the in vitro anti-MRSA activity, and the selectivity of the lactams to *S. aureus* over many other microbes, could be directly attributed to cellular glutathione levels. Additional studies are underway in our laboratory to investigate this in more detail and will be reported in due course.

3. Conclusions

The results of this study, in concert with our previously acquired data, support the empirical model presented in Figure 1 for the mode of antibacterial activity of *N*-thiolated β-lactams. While the lipophilic nature of the C3/C4 ring substituents may help the molecule transverse the lipid bilayer of the bacterial cell membrane, having too much lipophilicity in the organothio side chain appears to have a detrimental affect on antibacterial properties. This suggests that the organothio moiety must be available for presentation to the thiol target in the cell, rather than sequestered within the membrane. Therefore, the distribution of lipophilicity on the periphery of the molecule must be balanced among the substituents. Ongoing work in our laboratories is attempting to delineate the full mechanistic basis for the bioactivity of these compounds and to develop suitable candidates for clinical development.

4. Experimental

All reagents were purchased from Sigma–Aldrich Chemical Company and used without further purification. Solvents were obtained from Fisher Scientific Company. Thin layer chromatography (TLC) was carried out using EM Reagent plates with a fluorescence indicator (SiO_2-60, F-254). Products were purified by flash chromatography using J.T. Baker flash chromatography silica gel (40 mm). NMR spectra were recorded in $CDCl_3$unless otherwise noted. ^{13}C NMR spectra were proton broad-band decoupled.

4.1. Procedure for the preparation of *N*-alkylthio-phthalimide reagents

In dry benzene, chlorine gas was bubbled until the increase in weight equaled 0.93 g (13 mmol). This solution was then added to a round-bottomed flask containing 1.00 g (13 mmol) of 2-propanethiol in a minimal quantity of benzene. The mixture was then sealed at 0 °C with stirring for 1 h, then added dropwise to a second round-bottomed flask containing a slurry of 1.93 g (13 mmol) of phthalimide and 2.89 g (22 mmol) of Hunig's base. The mixture was allowed to reach room temperature over 2 h with stirring. The solution was then poured into water, extracted three times with benzene, dried over magnesium sulfate and concentrated via rotary evaporation. Any unreacted phthalimide was removed by trituration with chloroform. Further purification was done by recrystallization with methanol or column chromatography.

4.2. 2-Propylthioisoindoline-1,3-dione

White solid, mp 77–78 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.87 (4H, m), 2.86 (2H, t, $J = 7.3$ Hz), 1.62 (2H, m) 1.04 (3H, t, $J = 7.3$ Hz).

4.3. 2-Methylthioisoindoline-1,3-dione

White solid; mp 178–180 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.82 (4H, m), 3.77 (3H, s).

4.4. 2-Ethylthioisoindoline-1,3-dione

White solid; mp 158–160 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.86 (4H, m), 2.92 (2H, q, $J = 7.3$ Hz), 1.56 (3H, t, $J = 8.1$ Hz).

4.5. 2-Butylthioisoindoline-1,3-dione

White solid; mp 189–190 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.8 (4H, m), 2.85 (2H, t, $J = 7.4$ Hz), 1.61 (2H, m), 1.42 (2H, m), 0.91 (3H, t, $J = 5.0$ Hz).

4.6. 2-Isopropylthioisoindoline-1,3-dione

White solid, mp 61–62 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.8–7.5 (4H, m), 4.9 (1H, m), 2.17 (6H, d, $J = 6.9$ Hz).

4.7. (±)-2-*sec*-Butylthioisoindoline-1,3-dione

White solid, mp 43–45 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.83 (4H, m), 3.21 (1H, m), 1.55 (2H, m), 1.24 (3H, d, $J = 6.8$ Hz), 1.04 (3H, t, $J = 7.3$ Hz).

4.8. 2-Cyclohexylthioisoindoline-1,3-dione

White solid, mp 92–94 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.80 (4H, m), 3.05 (1H, m), 1.90–1.25 (1H, m).

4.9. 2-Phenylthioisoindoline-1,3-dione

White solid, mp 149–155 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.0–6.8 (9H, m).

4.10. 2-Benzylthioisoindoline-1,3-dione

White solid, mp 165–168 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.9–7.5 (4H, m), 7.31 (5H, m), 1.75 (2H, s).

4.11. Procedure for the preparation of *N*-thiolated azetidin-2-ones

In a round-bottomed flask were added 5.0 mg (0.24 mmol) of *N*-protio β-lactam **4**, 6.7 mg (0.24 mmol) of *N*-phenylthiophthalimide, and 11.9 mg (0.12 mmol) of triethylamine, in a minimal quantity of dichloromethane. The solution was refluxed and monitored by TLC until all of the starting lactam was consumed. Reaction was generally complete within 12 h. After cooling, the solution was poured into an equal volume of water, washed with aqueous solution of 5% sodium bicarbonate, 1% sodium bisulfate, and saturated sodium chloride. The extracts were then dried over magnesium sulfate and concentrated via rotary evaporation. Phthalimide was removed via trituration with chloroform and the remaining impurities were removed by column chromatography, eluting with dichloromethane.

4.12. (±)-(3*S*,4*R*)-4-(2-Chlorophenyl)-3-methoxy-*N*-phenylthioazetidin-2-one (5j)

Light yellow solid, mp 60–62 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.34 (9H, m), 5.35 (1H, d, J = 5.0 Hz), 4.91 (1H, d, J = 5.0 Hz), 3.25 (3H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.0, 137.0, 135.0, 134.0, 132.0, 130.0, 129.0, 128.0, 127.0, 125.0, 82.0, 59.0, 48.0.

4.13. (±)-(3*S*,4*R*)-4-(2-Chlorophenyl)-3-methoxy-*N*-(methylthio)azetidin-2-one (5a)

White crystal, mp 71–73 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.35 (1H, d, J = 7.4 Hz), 7.24 (3H, m), 5.29 (1H, d, J = 4.9 Hz), 4.80 (1H, d, J = 4.9 Hz), 3.16 (3H, s), 2.40 (3H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ 170.4, 133.8, 131.4, 129.6, 128.9, 126.8, 86.7, 62.7, 58.9, 21.8.

4.14. (±)-(3*S*,4*R*)-4-(2-Chlorophenyl)-*N*-ethylthio-3-methoxyazetidin-2-one (5b)

White solid, mp 68–70 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.34 (4H, m), 5.33 (1H, d, J = 5.0 Hz), 4.88 (1H, d, J = 5.0 Hz), 3.20 (3H, s), 1.35 (2H, q, J = 10.0 Hz), 0.92 (3H, t, J = 6.8 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.5, 142.1, 137.5, 130.8, 128.8, 128.4, 87.5, 70.5, 46.7, 34.4, 22.0.

4.15. (±)-(3*S*,4*R*)-4-(2-Chlorophenyl)-3-methoxy-*N*-(propylthio)azetidin-2-one (5c)

Colorless oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.6–7.2 (4H, m), 5.45 (1H, d, J = 4.9 Hz), 5.09 (1H, d, J = 4.9 Hz), 3.21 (3H, s), 2.49 (2H, m), 1.55 (2H, m), 0.82 (3H, t, J = 6.9 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.0, 133.6, 128.8, 128.3, 86.3, 66.9, 58.3, 38.2, 30.8, 21.5, 13.6.

4.16. (±)-(3*S*,4*R*)-*N*-Butylthio-4-(2-chlorophenyl)-3-methoxyazetidin-2-one (5d)

Light yellow viscous oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.30 (4H, m), 5.35 (1H, d, J = 4.9 Hz), 4.89 (1H, d, J = 4.9 Hz), 3.23 (3H, s), 2.85 (2H, t, J = 7.0), 1.60 (4H, m), 0.91 (3H, t, J = 7.3 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 176.5, 134.0, 132.1, 129.0, 126.9, 86.8, 64.8, 58.1, 48.0, 12.3.

4.17. (±)-(3*S*,4*R*)-4-(2-Chlorophenyl)-3-methoxy-*N*-octylthio azetidin-2-one (5e)

White amorphous powder; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.35 (4H, m), 5.35 (1H, d, J = 4.9 Hz), 4.89 (1H, d, J = 4.9 Hz), 3.23 (3H, s), 2.77 (2H, m), 1.64–1.26 (12H, m), 0.88 (t, 3H, J = 6.2 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 167.8, 132.5, 131.1, 127.5, 127.0, 80.3, 52.7, 43.7, 28.0, 26.0, 24.5, 22.3, 18.9.

4.18. (±)-(3*S*,4*R*)-4-(2-Chlorophenyl)-*N*-isopropylthio-3-methoxyazetidin-2-one (5f)

Light yellow solid, mp 30–38 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.34 (4H, m), 5.39 (1H, d, J = 4.9 Hz), 4.95 (1H, d, J = 4.9 Hz), 3.27 (1H, m), 3.25 (3H, s), 1.27 (6H, d, J = 5.7 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.3, 133.2, 128.9, 127.0, 87.1, 65.2, 59.7, 49.4, 34.0, 30.1, 28.5, 25.5, 21.5.

4.19. (±)-(3*S*,4*R*)-*N*-*sec*-Butylthio-4-(2-chlorophenyl)-3-methoxyazetidin-2-one (5g)

Light yellow viscous oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.4 (1H, d, J = 7.4 Hz), 7.3 (3H, m), 5.3 (1H, d, J = 4.7 Hz), 4.9 (1H, d, J = 4.8 Hz), 3.2 (3H, s), 3.0 (1H, m), 1.48 (1H, m), 1.2 (3H, dd, J = 6.8, 4.9 Hz), 0.94 (3H, q, J = 6.0 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.0, 133.8, 131.4, 129.5, 128.9, 126.8, 86.3, 64.1, 58.8, 48.1, 28.1, 19.0, 18.6, 11.1.

4.20. (±)-(3*S*,4*R*)-*N*-*tert*-Butylthio-4-(2-chlorophenyl)-3-methoxyazetidin-2-one (5h)

White amorphous powder; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.34 (4H, m), 5.50 (1H, d, J = 4.8 Hz), 4.99 (1H, d, J = 4.8 Hz), 3.25 (3H, s), 1.35 (9H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.0, 133.0, 129.0, 88.0, 65.0, 60.0, 44.0, 34.0, 31.0, 27.0, 19.0.

4.21. (±)-(3*S*,4*R*)-4-(2-Chlorophenyl)-*N*-cyclohexylthio-3-methoxyazetidin-2-one (5i)

Brown solid; mp 78–79 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.34 (4H, m), 5.35 (1H, d, J = 4.9 Hz), 4.93 (1H, d, J = 4.9 Hz), 3.24 (3H, s), 3.05 (1H, m), 2.01–1.41 (10H, m); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.3, 133.5, 128.9, 128.7, 128.3, 85.2, 67.6, 58.3, 49.5, 32.2, 30.9, 25.6, 25.4.

4.22. (±)-(3*S*,4*R*)-*N*-Benzylthio-4-(2-chlorophenyl)-3-methoxyazetidin-2-one (5k)

Light yellow solid; mp 68–70 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.22 (9H, m), 5.30 (1H, d, $J = 4.7$ Hz), 4.55 (1H, d, $J = 4.8$ Hz), 3.16 (3H, s), 1.25 (3H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ 179.0, 142.0, 138.0, 136.0, 135.0, 134.0, 132.0, 130.0, 128.0, 126.0, 88.0, 59.0, 48.0, 40.0.

4.23. Procedure for the preparation of *N*-sulfoxylated β-lactams 6i and 7i

In a round-bottomed flask was added 135 mg (0.41 mmol) of *N*-cyclohexylthio β-lactam (**5i**) in a minimal amount of glacial acetic acid at 0 °C with stirring. To this, a solution of 80 mg (0.41 mmol) of 30% hydrogen peroxide in several drops of glacial acetic acid was added dropwise. The solution was allowed to come to room temperature over 3 h with stirring. The reaction mixture was then poured into an equivalent portion of water, extracted with three equivalent volumes of benzene, washed with water until the washings were at neutral pH, as observed via pH paper, dried over magnesium sulfate, and concentrated by rotary evaporation.

4.24. (±)-(3*S*,4*R*)-*N*-Cyclohexylsulfinyl-4-(2-chlorophenyl)-3-methoxyazetidin-2-one (6i)

(1:1 mixture of diastereomers); clear oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.41 (m, 8H), 5.85 (1H, d, $J = 6.0$ Hz), 5.71 (1H, d, $J = 5.6$ Hz), 4.94 (1H, d, $J = 6.0$ Hz), 4.91 (1H, d, $J = 6.0$ Hz), 3.75 (2H, m), 3.29 (3H, s), 3.23 (3H, s), 2.72 (2H, m), 2.2–0.80 (20H, m); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.4, 133.6, 130.9, 130.8, 129.3, 123.5, 83.4, 61.6, 53.2, 50.5, 37.4, 29.9, 25.6.

4.25. (±)-(3*S*,4*R*)-*N*-Cyclohexylsulfonyl-4-(2-chlorophenyl)-3-methoxyazetidin-2-one (7i)

White, waxy solid; mp 152–158 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.47 (4H, m), 5.79 (1H, d, $J = 5.0$ Hz), 4.97 (1H, d, $J = 4.6$ Hz), 3.29 (3H, s), 3.12 (1H, m), 2.20–0.86 (10H, m); ^{13}C NMR (63 MHz, $CDCl_3$) δ 174.9, 143.8, 127.9, 125.8, 120.1, 118.5, 84.6, 61.9, 52.0, 45.2, 40.1, 34.9, 33.6.

4.26. Preparation of a β-lactam sulfonic acid

In a round-bottomed flask, a solution of 100 mg (0.47 mmol) of *N*-protio β-lactam (**4**) in 2 mL of freshly distilled dichloromethane and 2 mL of dry DMF was stirred with 140 mg (0.94 mmol) of 50% sulfur trioxide-pyridine under nitrogen at room temperature for 2 h. The solution was concentrated via rotary evaporator and converted to the potassium salt by passing through an ion exchange column. Column chromatography afforded the sulfonic acid in pure form.

4.27. (±)-(3*S*,4*R*)-2-(2-Chlorophenyl)-3-methoxy-4-oxoazetidine-1-sulfonic acid (9a)

Dark brown oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.32 (4H, m), 5.69 (1H, d, $J = 4.9$ Hz), 4.98 (1H, d, $J = 4.9$ Hz), 3.32 (2H, m), 0.89 (1H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ 179.9, 145.2, 137.7, 131.0, 122.7, 83.4, 55.2, 43.3.

4.28. Testing of antimicrobial susceptibilities (Kirby–Bauer well diffusion)

Staphylococcus aureus (ATCC 25923) and MRSA (ATCC 43300) were purchased from ATCC sources. Eight additional strains of MRSA were obtained from Lakeland Regional Medical Center (Lakeland, FL).

4.29. Culture preparation

From a freezer stock in tryptic soy broth (Difco Laboratories, Detroit, MI) and 20% glycerol, a culture of each microorganism was transferred with a sterile Dacron swab to Trypticase® Soy Agar (TSA) plates (Becton-Dickinson Laboratories, Cockeysville, MD), streaked for isolation, and incubated at 37 °C for 24 h. A 10^8 standardized cell count suspension was then made in sterile phosphate-buffered saline (pH 7.2) and swabbed across fresh TSA plates.

4.30. Antimicrobial testing

Prior to swabbing with the culture solution, 20 μL of a 1 mg/mL stock solution of the test lactam compound in dimethylsulfoxide (DMSO) was added to a 6-mm diameter well bored into the agar. The plates were swabbed uniformly with the test microbe compound above and then incubated for 24 h at 37 °C. The antimicrobial susceptibilities were determined by measuring the zones of growth inhibition around each well.

4.31. Determination of minimum inhibitory concentrations

The minimum inhibitory concentration (MIC) values of the lactams were determined for *Staphylococcus aureus* and MRSA by serial dilution according to NCCLS protocols.[10] The test medium was prepared in 24-well plates (Costar 3524, Cambridge, MA) by adding the test drug in DMSO to Mueller–Hinton II agar (Becton-Dickinson Laboratories, Cockeysville, MD) to bring the total volume in each well to 1.0 mL. Starting with an initial well concentration of 256 μg of drug/mL, each sequential dilution contained half the concentration of drug. The medium was allowed to solidify at room temperature for 24 h before inoculation with the bacteria. Using a sterilized inoculating loop, a small amount of each standardized *Staphylococcus* strain cultured on TSA plates for 24 h was transferred into sterile test tubes containing 5 mL of TSA broth and incubated at 37 °C for 24 h. One microliter of each culture was then applied to the appropriate well of Mueller–Hinton agar and incubated at 37 °C overnight. After 24 h, the MICs were determined as being the lowest concentration of drug where bacterial growth was visibly inhibited.

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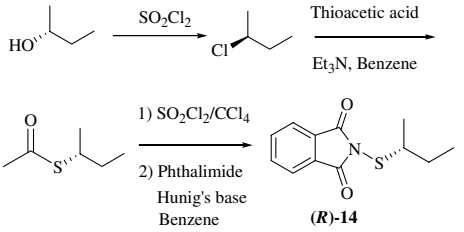

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