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C4-Alkylthiols with activity against *Moraxella catarrhalis* and *Mycobacterium tuberculosis*

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ABSTRACT

Antimicrobial resistance represents a global threat to healthcare. The ability to adequately treat infectious diseases is increasingly under siege due to the emergence of drug-resistant microorganisms. New approaches to drug development are especially needed to target organisms that exhibit broad antibiotic resistance due to expression of β-lactamases which is the most common mechanism by which bacteria become resistant to β-lactam antibiotics. We designed and synthesized 20 novel monocyclic β-lactams with alkyl- and aryl-thio moieties at C4, and subsequently tested these for antibacterial activity. These compounds demonstrated intrinsic activity against serine β-lactamase producing *Mycobacterium tuberculosis* wild type strain (Mtb) and multiple ($n = 6$) β-lactamase producing *Moraxella catarrhalis* clinical isolates.

1. Introduction

Discovered in 1928 by Fleming, penicillin was the first of the β-lactam class of antibiotics which now includes members that are natural, semi-synthetic and synthetic. Mechanistically, these compounds act by inhibiting cell wall growth, thus cell division. This occurs via inhibition of transpeptidases, otherwise referred to as penicillin-binding proteins, which have been shown to catalyze several reactions in the cross-linking of peptidoglycan strands.[1] Because humans lack peptidoglycan, the selective toxicity of this class of antibiotics is high and the compounds are generally safe for humans. Unfortunately, bacteria now have several mechanisms of resistance, including modification of penicillin-binding proteins and expression of β-lactamases, which are enzymes that hydrolytically cleave the β-lactam ring thus inactivating the drug.[1] Production of β-lactamase is the most common mechanism by which bacteria become resistant to β-lactam antibiotics. This is why the development of new β-lactamase-resistant antimicrobials is essential.

To address the growing need for more antimicrobials, new types of β-lactam pharmacophores consisting of a series of C4-substituted monocyclic β-lactam compounds, were prepared in our laboratories and tested against a range of bacteria including species that elaborate β-lactamase. The design, synthesis and antibacterial activities of these novel lactams are reported here.

2. Results and discussion

2.1. Design and synthesis

β-Lactams have long been known to be effective bactericidal agents. In general, β-lactams are thought to exert their antibacterial activity by specifically acylating enzymes containing serine or cysteine as active-site nucleophiles. Until recently, it was generally accepted that for β-lactams to exert bactericidal activity they must contain a scaffold which specifically has an ionizable group at the lactam nitrogen within 3.6 Å of the β-lactam carbonyl carbon. However, there now appear to be exceptions to this scaffold requirement since N-methylthiolated β-lactams possess inhibitory, although not cidal, antimicrobial activity.[2] In addition, it has been demonstrated that essential bacterial serine or cysteine enzymes can be inhibited by the positioning of leaving groups at C4, such as those found in monocyclic β-lactams.[3–6] Still others have shown that hydrolysis of the β-lactam ring of the cephalosporins generate

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thiol intermediates that appear to specifically inhibit *Bacillus cereus* metallo-β-lactamase.[7] In addition, compounds carrying a free thiol have been prepared as both serine and metallo-β-lactamase inhibitors.[8]

The focus of this study was to design, synthesize and test novel thio-β-lactams incorporating the knowledge gained by these previous studies. Towards this end, we prepared and determined the antibacterial activity of monocyclic β-lactams having alkylthioether-, and arylthioether groups, thioacetate, and a variety of different disulfide groups attached to the lactam's C4.

2.2. β-Lactams with alkyl sulfides/thiols at C4

The C3 unsubstituted β-lactams were prepared from commercially available β-lactam **1** following the procedures of Clauss et al.[9] and Wasserman et al.,[10] where the nucleophile (Nu) is a thiol group (Scheme 1). β-Lactams unsubstituted at C3 were first synthesized to test the hypothesis that a lack of substituent at C3 would generate a compound capable of inhibiting bacterial growth.

We then hypothesized that the thioacetate group could be transformed into a free thiol, upon enzymatic hydrolysis by the bacteria, thus, converting the pro-drug into an active antibacterial compound. Thus the synthesis of β-lactam **3** (Scheme 2) was performed.

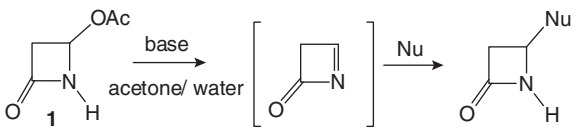
The synthesis of *S*-acetyl β-lactam **2** has been previously described by Clauss et al.[9] using different multistep synthetic routes. For our synthesis, commercially available 4-acetoxy-2-azetidinone **1** was used as the starting material to produce β-lactam **2** in a single step. In the presence of thiolacetic acid the acetate group is replaced by a thioacetate to yield lactam **2** using the methods described previously.[10] β-Lactam **3** (Scheme 2) was obtained from azetidinone **2** using a $PySO_3$ complex in pyridine.

Next unbranched C4 alkylthio-β-lactams were synthesized from lactam **1** and commercially available ethyl and propyl alkylthiols (Scheme 3).

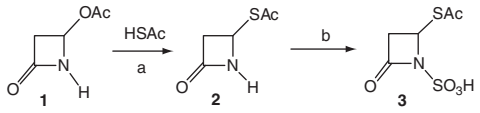
To determine whether the branching of the sulfur substituent at C4 had an effect on biological activity, we also synthesized branched C4 alkylthio-β-lactams **9** and thiolalcohol **8** (Scheme 4). The latter was prepared as described previously.[14] Dialdehyde **7** was prepared from commercially available 2-ethylbutanal **6** following the procedure suggested by Roy et al.,[15] where S_2Cl_2 was added dropwise at 55 °C to a solution of the aldehyde **6** (Scheme 4). Reduction of the latter with $LiAlH_4$ in tetrahydrofuran (THF) led to formation of thioalcohol **8**. β-Lactam **9** (Scheme 4) was prepared from **1**, using the methods of Ghannoum et al.[16]

In addition to production of β-lactamases, many bacterial species produce extracellular polysaccharide matrices (biofilm) which protect the bacterial population from environmental assaults including antibiotics.[11] Compounds with bismuth-coordinated thiols have been reported to possess potent antibacterial activity against biofilm-producing bacteria.[11–14] Synthesis of bismuth-coordinated thiols is straightforward, requiring addition of bismuth nitrate to the thiol of interest in 1,2-propanediol.[14,17] Based on published studies of model compounds, the following structures are suggested for the investigated bismuth-coordinated C4 thio-β-lactams (Fig. 1).[18,19]

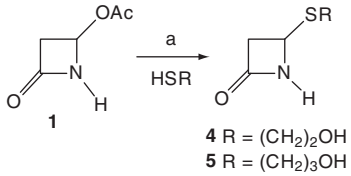
We next synthesized C3-substituted lactams. C3-Methoxy-substituted β-lactams having branched alkylthio-substituents



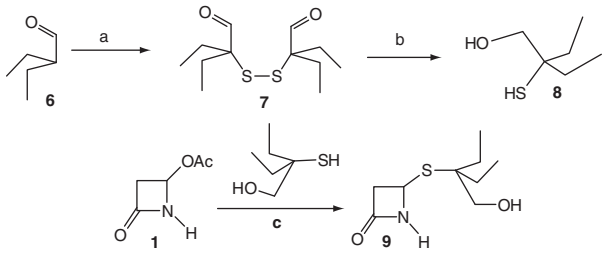
Scheme 1. Synthesis of C3 unsubstituted alkylthio-β-lactams.



Scheme 2. Synthesis of C4 thioacetyl β-lactams. Reagents and conditions: (a) $NaHCO_3$ in acetone/water, rt, 12 h, (**2**) 60%; (b) $PySO_3$/Py, inert atmosphere, 80 °C, 2 h, (**3**) 26%.



Scheme 3. Synthesis of unbranched alkylthio β-lactams. Reagents and conditions: (a) $NaHCO_3$ in acetone/water, rt, 12 h, (**4**, **5**) 25%.



Scheme 4. Synthesis of branched alkyl-thio β-lactams. Reagents and conditions: (a) S_2Cl_2/CCl_4, 55 °C, (**7**) 50%; (b) LAH, THF, (**8**) 70%; (c) $NaHCO_3$ in acetone/water, rt, 12 h, (**9**) 60%.

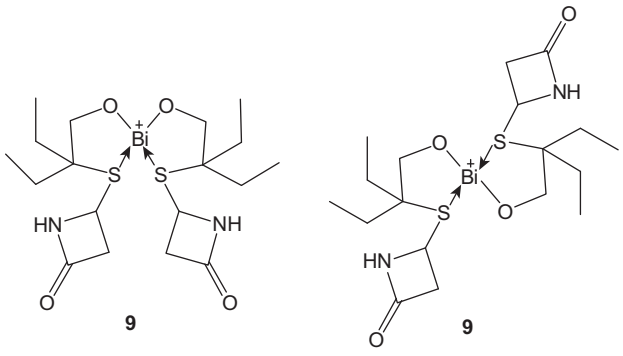
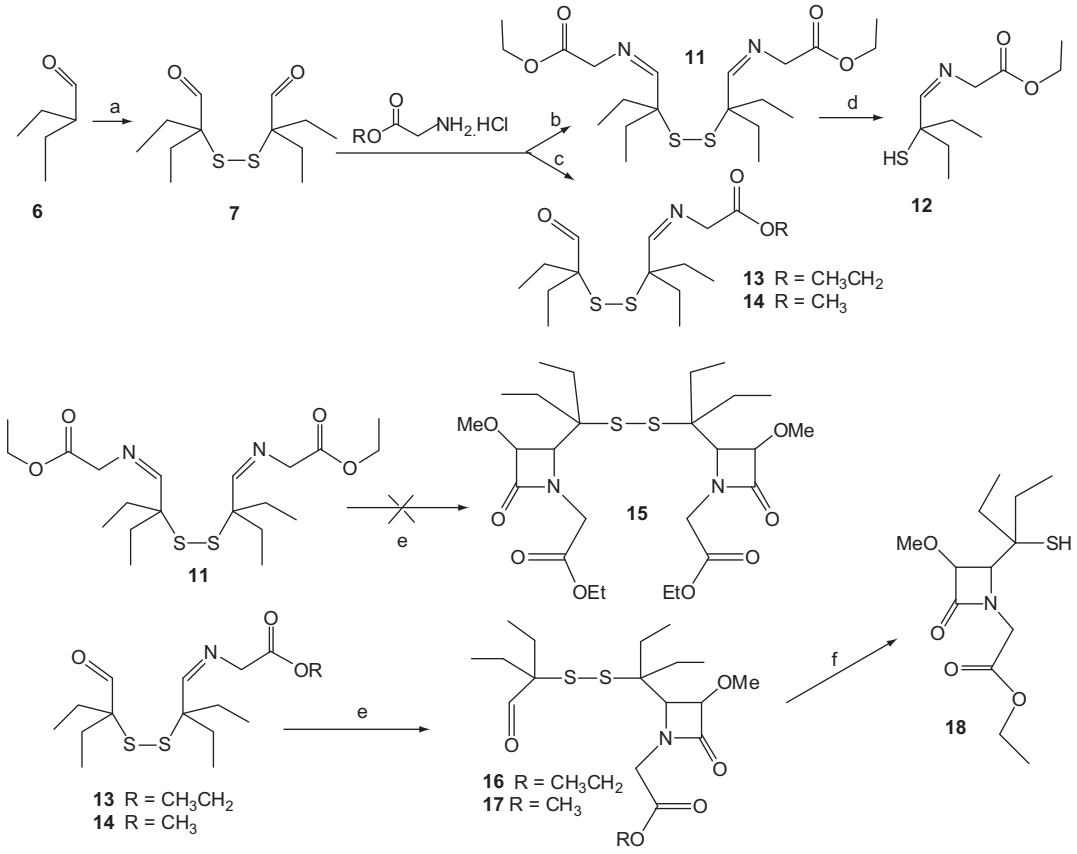


Figure 1. Suggested structures of bismuth-coordinated C4 thio-β-lactam **10**.

attached at C4 through a one-carbon spacer were prepared. It has been reported that a sulbactam analog possessing a $-CH_2CO_2H$ group at C3 exhibited a 10-fold improved inhibitory activity against class C β-lactamases when compared to the parent compound sulbactam.[3] Following the synthetic procedure depicted below, the synthesis of compounds **16**, **17** and **18** was accomplished.

The overall design of the substituted β-lactams with sterically hindered thiols is shown in Scheme 5. Aliphatic aldehyde **6** was reacted with sulfur monochloride to give disulfide-dialdehyde **7**.[15] For the synthesis of the imines **13** and **14**, glycine ethyl and methyl esters as hydrochloride salts were used. A procedure for preparation of the imines directly from the commercially available hydrochloride salts of the glycine esters was developed. This resulted in successful synthesis of bis-imine **11** and monoimines **13** and **14**



Scheme 5. Synthesis of β-lactams, where the sterically hindered thiol is attached through a carbon spacer to the β-lactam ring. Reagents and conditions: (a) S_2Cl_2/CCl_4, 55 °C, (**7**) 50%; (b) C_6H_6, Et_3N, reflux, 2 days, (**11**) 89%; (c) CH_2Cl_2, Et_3N, $MgSO_4$, rt, 3 days, (**13**, **14**) 85–90%; (d) $MeOCH_2COOH$ in C_6H_6, reflux, 12 h, (**12**) 27%; (e) $MeOCH_2COCl$, Et_3N, in CH_2Cl_2, reflux, 12 h, (**16**, **17**) 40–46%, (f) Zn/HCl in EtOH, reflux, inert atmosphere, (**18**) 70%.

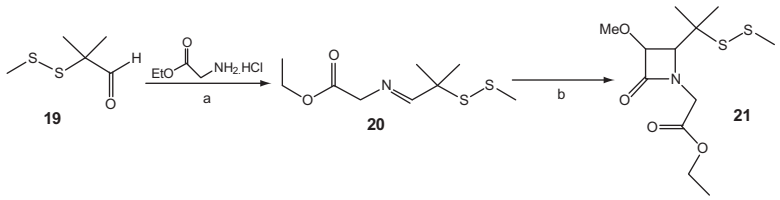
(Scheme 5). Imines **13** and **14** were prepared in methylene chloride at room temperature for 3 days. $MgSO_4$ was used to absorb the water released as a by-product of the reaction. Production of bis-imine **11** was accomplished using benzene. In this case a Dean–Stark apparatus was used to remove water formed during the reaction. The bis-imine **11** was obtained after reflux when maximum yield was obtained (2 days). Samples of the reflux were taken periodically (every 4 h) and analyzed for product by NMR over the course of 2 days. Stability of these imines was also monitored by NMR. At RT, imines synthesized were stable for 48 h (i.e., no more than 10% was degraded). Thus, these compounds were used within two days. Thioimine **12** was also prepared and characterized (Scheme 5); this compound was stable and unreactive toward [2+2] cycloaddition. We tested thioimine **12** to determine whether building blocks of the final β-lactam structure would possess antibacterial activity. Monomeric β-lactams **16** and **17** (Scheme 5) were prepared using the corresponding monoimines **13** and **14**. Using the same experimental conditions, we were unable to synthesize β-lactam **15** from bis-imine **11**. The configuration of the main products **16**, **17** and **18** from the [2+2] reaction based on the coupling constant is *cis*, as expected for the Staudinger reaction taking place in a nonpolar solvent.[20]

Several procedures were explored in an attempt to produce β-lactam **18**. Successful synthesis of compound **18** required reduction of the disulfide group of **16** with Zn/HCl in an inert atmosphere (Scheme 5). Conversion of disulfides to the corresponding mercaptans was attempted with propanethiol, DTT, Ph_3P–dioxane–water,[21] polymer-bond Ph_3P,[22,23] Bu_3P,[24] $ZnCl_4/NaBH_4$,[25] Zn/glacial acidic acid,[16] and Zn/HCl.[26] Changes in the conditions and the solvents of these reactions did not generate the desired product **18**. Reduction of the disulfide bond of compound **16** was achieved using Zn/HCl in ethanol under reflux and argon atmosphere. In all cases the solvents used for the reduction were deoxygenated. All attempts to neutralize the acid and bring the pH to 6 with sodium bicarbonate, as described in the literature,[26] led to opening of the ring system. Therefore, the work-up procedure was simply done by dilution with water and extraction with ethyl acetate. Flash chromatography was used to purify the final product and afforded a 70% yield of **18** as colorless oil.

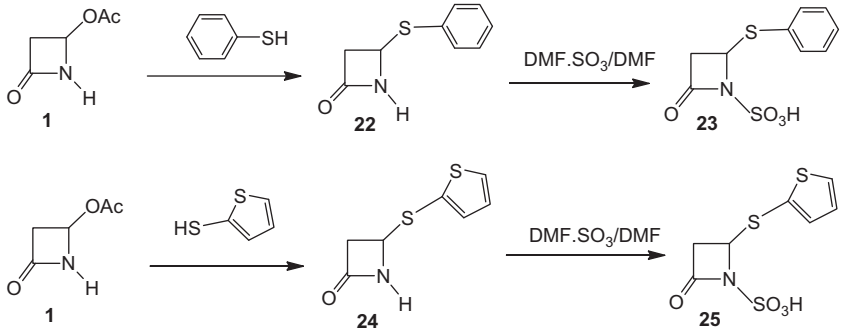
A similar reaction scheme was used for the synthesis of β-lactam **21** (Scheme 6). Commercially available aldehyde **19** was reacted with glycine ethyl ester–HCl in the presence of triethyl amine at room temperature to give imine **20**. Stirring the mixture for 18 h resulted in a high yield (95%). The product was sufficiently pure to be used for the preparation of lactam **21**.

Syntheses of a variety of β-lactams with arylthio groups were also accomplished. The choice of the arylthiol was based on the reported monocyclic lactams as cysteine inhibitors.[3] In order to determine the optimal C4 substituent, lactams containing an unsubstituted thiophenyl- (**23**, Scheme 7), thiophene- (**25**, Scheme 7) group as well as those prepared by addition of 4-mercaptophenol, benzene-1,4-dithiol and 4-aminobenzenethiol at C4 (Scheme 8), were also prepared. β-Lactams **22** and **24** (Scheme 7), **26**–**28** (Scheme 8) were synthesized from 4-acetoxy-2-azetidone **1**, using procedures described previously.[10,27]

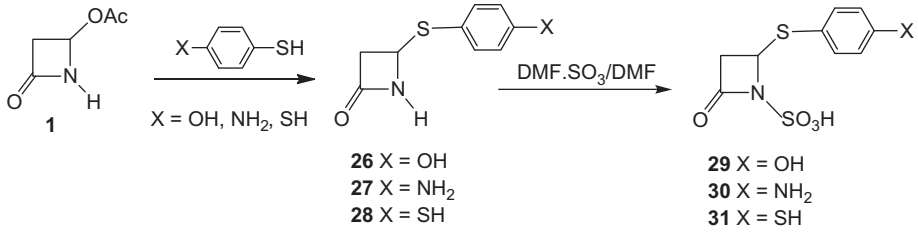
Addition of the commercially available aromatic thiols to β-lactam **1** led to the compounds of interest, that is, the arylthio-β-lactams **26**–**28**, **29**–**31**, in two steps. Sulfur trioxide dimethylformamide complex was successfully used for the sulfonation reaction of the lactam nitrogen when aromatic thiols were present



Scheme 6. Synthesis of β-lactams containing alkyl disulfide at C4. Reagents and conditions: (a) CH_2Cl_2, Et_3N, $MgSO_4$, rt, overnight, (**20**) 95%; (b) $MeOCH_2COCl$, Et_3N, in CH_2Cl_2, reflux, 2 h, (**21**) 58%.



Scheme 7. Synthesis of unsubstituted arylthio-β-lactams, overall yield 50%.



Scheme 8. Synthesis of substituted phenylthio β-lactams, overall yield 30–66%.

in the structure; for the aliphatic thiols, pyridine SO_3^- complex resulted in better yields (50%), as compared to the DMF·SO_3 complex (30%). The alternative sulfonation of the functional groups of the thiophenol at C4 as in compounds **29**, **30** and **31** was not observed, which was determined by NMR and X-ray crystallography.

While most synthetic products were sufficiently pure to be used directly for the next step, some were first recrystallized. Purification of lactam **28** using column chromatography led to formation of a disulfide dimer. For a single -SH 'monomer' a molecular ion peak $[M-H]^-$ with m/z 210 was present while a $[M-H]^-$ peak with m/z 421 was detected for the dimer. The dimer/monomer ratio after purification was observed to be 1:10.

All of the compounds were prepared and subsequently tested for antibacterial activity as racemates.

2.3. Antibacterial activity

Kirby-Bauer disc diffusion assays were initially used to screen compounds for antibacterial activity.[28] Clinical isolates of *Moraxella catarrhalis*, (n = 5 or 6) a major cause of otitis media, sinusitis and acute exacerbation of chronic obstructive pulmonary disease (COPD), were screened. All *M. catarrhalis* strains produced β-lactamase as documented by nitrocefin cleavage studies.[29]

Initially we screened precursors of the C4-thio-β-lactams compounds for antibiotic activity (Table 1). Several of the C4-thio-β-lactams compound precursors (**7**, **8**, **12** and **16**, Scheme 5) exhibited activity against various *M. catarrhalis* clinical strains.

Of the C4-thio-β-lactams compounds screened for activity compounds **2**, **3** and **29** demonstrated activity against at least five of the six clinical β-lactam-producing *M. catarrhalis* isolates. In contrast, compounds **21**–**25** (Schemes 6 and 7) exhibited no inhibitory activity. Since these compounds lack peripheral structural similarities, we have no rationale for their lack of activity. The mechanism of action of compounds **2**, **3** and **29** may be different from the 'classical' β-lactams, since these compounds lack an ionizable group at N1. Since glutathione could inactivate the thio group of the compounds in vivo, we tested the C4-thio-β-lactams in the presence of glutathione (1 mol equiv) which was added to each lactam-containing disc just prior to testing. Overall, reduced glutathione did not affect the biological activity of the C4-thio-β-lactams tested, with the exception of compound **9**, which was inactivated by the presence of glutathione. This might be due to the formation of an adduct of glutathione with lactam **9**, whose 2-ethylbutanol group could have been oxidized to 2-ethylbutanal in vitro. This adduct might then be further modified to products, for example, *S*-(hydroxymethyl)-glutathione, by an enzymatic reaction.[30] In addition, the C4-thiol-β-lactams were tested in the presence of bismuth nitrate, using an equimolar amount to that used in compound synthesis. This was done because compounds with bismuth-coordinated thiols have been reported to possess potent antibacterial biofilm activity.[11,14] The presence of bismuth had no effect on the activity of any of the C4-thiol-β-lactams tested.

After initial screening of the C4-alkyl- and C4-aryl-thio β-lactams using the Kirby Bauer disc diffusion assay (Tables 1 and 2),

Table 1
Susceptibility of β-lactamase producing *Moraxella catarrhalis* clinical isolates to C4-thio-β-lactam and precursers

Compounds (10 μg/disc)	*Moraxella catarrhalis* clinical isolates (n = 5) zone of inhibition[a] (mm)				
	1	2	3	4	5
7	10	10	NE[b]	5	10
8	5	3	NE	3	12
12	NE	2	NE	NE	11
16	20	NE	NE	NE	3

[a] Kirby-Bauer disc diffusion assay. Zone size indicates area of bacterial growth inhibition beyond the edge of the disc.
[b] NE = no effect.

Table 3
Minimum bactericidal concentrations (μg/ml) of novel compounds against *Moraxella catarrhalis* β-lactamase producing clinical isolates and *Mycobacterium tuberculosis* (Mtb) H37Rv[32,34]

Compounds	*Moraxella catarrhalis*						Mtb
	1[a]	2	3	4	5	6	H37Rv
17	>500	>500	<15.6	<15.6	<15.6	<15.6	200
24	>500	>500	500	500	500	>500	100
26	>500	<15.6	<15.6	<15.6	<15.6	<15.6	>200
28	250	125	125	500	125	125	25
29	>500	<15.6	<15.6	<15.6	<15.6	<15.6	>200

[a] Clinical isolates of *M. catarrhalis* (n = 6).

the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the compounds was determined. MIC and MBC levels for all compounds were congruent. Organisms tested were the same as those used for the screening tests with the addition of *Mycobacterium tuberculosis* (Mtb) which was tested as previously described.[29] The data in Table 3 indicates an improved sensitivity of the microdilution method vs. the disc screening method. Compounds were considered to have potential for therapeutic usage if their MBC was ⩽100 μg/mL. Compounds **4**, **5**, **18**, and **22** were inactive against all organisms tested while compounds **9**, **16**, **21**, **23**, **25**, **27**, **30**, and **31** exhibited limited activity (within 4-fold serial dilution of maximum concentration tested; 500 μg/mL for *M. catarrhalis* and 200 μg/mL for Mtb). The compounds with the best activity against *M. catarrhalis* were **17** (four of six strains had an MBC of <15.6 μg/mL), and **26** and **29** (five of six strains had an MBC of <15.6 μg/mL). Some activity was also measured for compounds **30** and **23** (125 μg/mL; n = 4 and 6, respectively). For Mtb, the compounds that showed the most potent antimicrobial activity, were **2** (MBC = 25 μg/mL) and **28** (MBC = 25 μg/mL).

Compound **26**, one of the most active β-lactams against *M. catarrhalis*, possesses an aromatic ring with a hydroxyl group at the *p*-position (see Scheme 8). The presence of a sulfonyl group at the lactam nitrogen did not appear to improve the activity of the compounds, for example, the activities of lactam **26** and lactam **29** are the same. Addition of the $-SO_3$ group to lactam **2**, leading to lactam **3**, which displayed activity in the agar diffusion assay, reduced biological activity by almost 50%. The fact that presence of the $-SO_3$ group in these β-lactams does not have any effect, or even decreases β-lactam bioactivity in certain cases, clearly indicates that the $-SO_3$ group at the lactam nitrogen is not necessary for the biological activity of these lactams.

Analysis of the effects various substituents on the aromatic ring have on antimicrobial activity led to the conclusion that the hydroxyl group (**26** and **29**) is a better choice of substituent than the sulfhydryl and amino groups for bioactivity against *M. catarrhalis*. [33]

With respect to Mtb, compound **28**, with a *p*-dithiophenyl substituent (MBC of 25 μg/mL), is significantly more active against Mtb than its phenol-containing counterpart **26** (MBC >200 μg/mL). This activity may be attributed to the highly lipophilic character of the cell wall of Mtb or to a specific molecular target, interacting with the thiol group of compounds **2** and **28**.

Both Kirby-Bauer disc diffusion assays and MIC/MBC were used to screen compounds for antibacterial activity against non-β-lactamase producing *Escherichia coli*, ATCC 25922, *Pseudomonas aeruginosa*, ATCC27853, *Staphylococcus aureus*, ATCC25923, *Enterococcus faecalis*, ATCC29212 which are highly stable quality control strains routinely used for antimicrobial testing. They also represent a range of organisms, both Gram positive and Gram negative, that cause clinically important infections. None of the synthesized compounds affected the growth of *E. coli*, *P. aeruginosa*, *S. aureus*, or *E. faecalis*.

3. Conclusion

We have designed, synthesized and tested a variety of novel monocyclic β-lactams having alkyl- and aryl-thio-groups at C4. Several compounds have shown specific antibacterial activity against β-lactamase-producing *Moraxella catarrhalis* and *Mycobacterium tuberculosis*, while having no activity against other

Table 2
Effect of reduced glutathione on C4 thiol-β-lactam anti-*Moraxella catarrhalis* activity

Compounds (10 μg/disc)	*Moraxella catarrhalis* clinical isolates (n = 6) zone of inhibition (mm)[a]					
	1[b]	2	3	4	5	6
2[d]	19	20	24	26	28	20
+GSH[e]	16	19	19	22	24	19
3	NE[c]	12	17	12	17	11
+GSH	NE	11	12	14	18	12
9	NE	NE	NE	NE	NE	7[d]
+GSH	NE	NE	NE	NE	NE	NE
17	NE	10	NE	NE	7	NE
+GSH	NE	7	NE	NE	7	NE
28	NE	8	NE	7	8	NE
+GSH	7	7	NE	7	7	NE
29	13	9	7	8	9	8
+GSH	9	7	7	7	8	8
30	NE	NE	NE	8	NE	NE
+GSH	NE	NE	NE	8	NE	NE

[a] Kirby-Bauer disc diffusion assay. Zone size indicates area of bacterial growth inhibition beyond the edge of the disc.
[b] 1–6 = clinical isolates of *Moraxella catarrhalis*.
[c] NE = no effect.
[d] The data on the first line (numbers) is for the corresponding compound.
[e] (GSH)—the corresponding compound in the presence of reduced glutathione.

representative non-β-lactamase-producing gram positive and gram negative bacteria. The bioactivity of our β-lactams appears to be a function of the type of group, for example, aromatic attached to the S-atom at C4, even though pronounced changes in the lactam-C–S bond lengths and angles are not observed when compared to similar compounds described in the literature. Interestingly, the presence of a polar, charged group (–SO_3), necessary to secure binding in the enzyme active site in the 'classical' β-lactams, is not required for the bioactivity of our compounds. In fact, in certain cases the β-lactam thioethers unsubstituted at the lactam nitrogen have higher bioactivity than those sulfonated at N1. In addition, it is promising that these novel compounds have activity against β-lactamase producing bacteria. Current studies in our laboratories are directed towards the synthesis of additional compounds with increased activity against important human pathogens and a better understanding of the structure–activity relationships of these promising new antibacterial β-lactam compounds.

4. Experimental

4.1. Equipment and materials

All air- or moisture-sensitive reactions were performed under argon or nitrogen atmosphere using glassware that was pre-dried in an oven at 120 °C overnight. All reactions were monitored by thin-layer chromatography (TLC) using EM Reagent plates with fluorescence indicator (SiO_2-60, F_{254}) and layer thickness: 250 μm; purchased from EMD Chemicals, Inc. (Gibbstown, NJ). Unless otherwise noted, the compounds were detected under UV light and iodine vapors.

Flash chromatography was performed by gradient elution from silica gel columns (60 Å, particle size 40–75 μm, Sorbent Technologies, Inc., Atlanta, GA).

Melt-Temp II melting point apparatus was used to determine the melting points of the synthesized compounds. NMR spectra (25 °C) were obtained at 400 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR with a Bruker 400 spectrometer (Billerica, MA) in $CDCl_3$ or acetone-d_6. In most cases, signals due to exchangeable protons have been omitted. IR spectra were obtained as a thin film on NaCl plates or in solid form (KBr standard) on a Shimadzu FT-IR-8300 (Columbia, MD). Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. High Resolution Mass Spectrometry (HRMS) was carried out by University of Iowa, (Iowa City, IA).

All other solvents, chemicals and enzymes were purchased from Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Pittsburgh, PA) and Acros Organics (Geel, Belgium). Unless stated otherwise, solutions in organic solvents were dried with anhydrous magnesium sulfate, and concentrated under vacuum conditions using rotatory evaporation.

4.2. LC–MS

Mass spectra were measured on LC/MS 2010 Shimadzu mass spectrometer (Columbia, MD), equipped with electrospray ionization source. ESI/MS spectra were recorded mostly in the negative-ion setting. LC/MS grade methanol was purchased from Fisher and used as received. Electrospray mass spectrometric analysis was performed using high purity nitrogen as the nebulizing gas at a pressure of 80/80 kgf/cm^2, with a gas-flow rate of 4.5 L/min and the temperature of the probe at 250 °C. LC/MS analysis was carried out without any backflow or drying gas, with a scan speed of 2000 amu/s in the range of m/z 20–800. Each sample was introduced into the system at 3 μL/injection.

For the β-lactam-glutathione test, stock solutions (6.5×10^{-6} mol in 400 μL) of each β-lactam and glutathione were prepared. Stock solutions (100 μL) were further diluted to produce a total volume of 400 μL and a final concentration of 8 mM; a sample with the latter concentration was injected directly into the instrument. The β-lactams and glutathione were dissolved in methanol and in water, respectively. Methanol: water (60:40; vol/vol) provided the optimal mobile phase for the study. Prior to injection, a one to one molar ratio of the β-lactam and the glutathione were mixed and incubated for varying periods of time (0 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 24 h).

4.3. Synthesis

4.3.1. 4-Acetylthio-2-azetidinone (2)

4-Acetoxy-2-azetidinone (0.5 g, 4.0 mmol) was dissolved in 25 mL acetone/water (3:2). Thioacetic acid (0.3 mL, 4 mmol) and sodium bicarbonate (1.3 g, 15 mmol) were added, and the resulting solution was allowed to stir overnight. Sodium chloride was then added until two layers formed. The solution was filtered and the layers separated. The aqueous layer was extracted with AcOEt (3×50 mL), the combined organic layers were dried with $MgSO_4$, filtered and evaporated under pressure. The residue was purified on silica gel (CH_2Cl_2/hexanes, 9:1; v/v) to afford the desired product as colorless oil (60%). The structure of the compound was spectroscopically characterized as described by Ernest et al.[12] and Clauss et al.[8] ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.37 (1H, br s), 5.24 (1H, dd, ^1J = 5.2, ^2J = 2.4 Hz), 3.46 (1H, ddd, ^1J = 15.4, ^2J = 5.3, ^3J = 2.0 Hz), 2.97 (1H, ddd, ^1J = 15.3, ^2J = 2.3, ^3J = 1.3 Hz), 2.39 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.20, 165.10, 49.90, 44.20, 30.95.

4.3.2. 2-Acetylsulfanyl-4-oxo-azetidine-1-sulfonic acid tetra-*n*-butylammonium salt (3)

4-Acetylthio-2-azetidinone (**2**) (1 g, 6.9 mmol) was dissolved in 15 mL of anhydrous pyridine and heated to 85 °C under argon. Two equivalents of pyridine-sulfur trioxide (2.2 g, 14 mmol) were added and the solution was stirred for 1.5 h to give a homogeneous mixture that was then poured into 150 mL of 0.5 N KH_2PO_4. The resulting solution was extracted with CH_2Cl_2 (1×100 mL) and the same organic layer was back-washed with another 50 mL of phosphate solution. The combined aqueous layers were treated with 1 equiv (1.67 g) of TBAHS and extracted with AcOEt (3×60 mL). The organic extracts were dried over $MgSO_4$ and concentrated under low pressure. Flash chromatography (CH_2Cl_2/AcOEt, 3:2 and CH_2Cl_2/AcOEt, 1:1; v/v) resulted in colorless oil (26%). All attempts to crystallize the product were unsuccessful. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.50 (1H, dd, ^1J = 5.0, ^2J = 2.3 Hz), 3.54 (1H, dd, ^1J = 15.5, ^2J = 5.2 Hz), 3.26 (8H, m), 2.29 (1H, dd, ^1J = 15.4, ^2J = 2.3 Hz), 2.33 (3H, s), 1.64 (8H, pentet, J = 7.6 Hz), 1.44 (8H, sextet, J = 7.3 Hz), 1.00 (12H, t, J = 7.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.36, 162.44, 58.67, 54.46, 46.36, 30.96, 23.94, 19.70, 13.72. IR (film) ν_{max}: 1770, 1691 cm^{-1}. Anal. Calcd for $C_{21}H_{42}N_2O_5S_2$: C, 54.04; H, 9.07; N, 6.00. Exact mass Calcd for $C_5H_6NO_5S_2^-$: 223.9687 and $C_{16}H_{36}N^+$: 242.2848; found by HRMS: m/z (M^-) 223.9679.

4.3.3. 4-(3-Mercapto-propoxy)-azetidin-2-one (4)

4-Acetoxy-2-azetidinone (2 g, 15.49 mmol) was dissolved in 100 mL acetone/water (3:2). 2-Mercaptoethanol (1.18 mL, 17.04 mmol) and sodium bicarbonate (5.20 g, 49.6 mmol) were added, and the resulting solution was allowed to stir overnight. Sodium chloride was then added until two layers formed. The solution was filtered and the layers separated. The aqueous layer was extracted with AcOEt (3×50 mL), and the combined organic layers were dried with $MgSO_4$, filtered and evaporated under pressure to afford the desired product as colorless oil (48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (1H, br s), 4.90–4.92 (1H, dd, ^1J = 3.7, ^2J = 2.4 Hz),

4.06–4.08 (2H, t, J = 5.5 Hz), 3.7 (1H, m), 3.3 (1H, m), 2.96 (1H, s), 2.77–2.78 (2H, t, J = 3.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.65, 33.82, 61.98, 52.75, 45.17.

4.3.4. 4-(3-Hydroxy-propylsulfanyl)-azetidin-2-one (5)

4-Acetoxy-2-azetidinone (2 g, 15.49 mmol) was dissolved in 100 mL acetone/water (3:2). 3-Mercapto-1-propanol (1.47 mL, 17.04 mmol) and sodium bicarbonate (5.20 g, 49.6 mmol) were added, and the resulting solution was stirred overnight. Sodium chloride was then added until two layers formed. The solution was filtered and the layers separated. The aqueous layer was extracted with AcOEt (3 × 50 mL), and the combined organic layers were dried with $MgSO_4$, filtered and evaporated under pressure to afford the title compound as colorless oil (70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (1H, br s), 4.81–4.83 (1H, dd, ^1J = 2.5, ^2J = 2.2 Hz), 3.67–3.73 (2H, t, J = 5.2 Hz), 3.35–3.41 (1H, m), 2.88–2.92 (1H, m), 2.69–2.80 (2H, m), 1.80–1.91 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.63, 60.49, 52.35, 45.26, 32.49, 27.04.

4.3.5. Ethyl-2-(1-ethyl-1-formyl-propyldisulfanyl)-butyraldehyde (7)

2-Ethylbutanal (1.0 mol, 100 g) was dissolved in 70 mL of CCl_4. The solution was kept at 50–55 °C when fresh S_2Cl_2 (70 g, 0.5 mol) was added dropwise. The reaction mixture was heated to 65 °C for 2 h and stirred overnight at 50 °C. The released HCl gas was displaced by continuous air flow into the flask during the overnight period. Crystallization from absolute ethanol resulted in white crystals (50%, lit.[19] 70%). The product was further recrystallized from acetone/ethanol, 1:1, v/v. The determined melting point of 71–72 °C was in agreement with previously published data (lit.[20] mp: 71–72 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.03 (2H, s), 1.76 and 1.68 (8H, m), 0.89 (12H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 194.94, 65.63, 21.80, 8.15.

4.3.6. 2-Ethyl-2-mercapto-butan-1-ol (8)

The compound was synthesized using protocol reported by Bandarage et al.[16] $LiAlH_4$ (1 M, 38.2 mL) in THF (freshly distilled over sodium) was added dropwise to a stirred solution of dialdehyde **7** (10 g, 38.17 mmol) in THF (70 mL). Additional portions of $LiAlH_4$ (3 × 100 mg) were added and the reaction mixture was stirred at room temperature under argon for 2 h. The mixture was poured onto ice, treated with 3 M HCl (100 mL), and extracted with AcOEt (3 × 100 mL). The combined organic layers were then dried over $MgSO_4$, filtered, and evaporated to yield colorless oil (70%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.50 (2H, s), 2.22 (1H, br s), 1.63 (4H, m), 1.32 (1H, s), 0.95 (6H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 69.06, 55.03, 29.51, 8.42.

4.3.7. 4-(2-Ethyl-2-mercapto-butoxy)-azetidin-2-one (9)

4-Acetoxy-2-azetidinone (1 g, 8 mmol) was dissolved in 50 mL acetone/water (3:2). Thioalcohol **8** (1.05 g, 8 mmol) and sodium bicarbonate (4 equiv) were added, and the resulting solution was allowed to stir overnight. Sodium chloride was then added until two layers formed. The solution was filtered and the layers separated. The aqueous layer was extracted with AcOEt (3 × 50 mL), and the combined organic layers were dried with $MgSO_4$, filtered and evaporated under pressure. The residue was purified on silica gel (CH_2Cl_2/AcOEt, 3:2; v/v) to yield the title compound as colorless oil or white crystalline mass (60%) with mp 52–53 °C. Two different products were isolated and identified by NMR and GC/MS. The thiol and the alcohol were not products of the same reaction mixture; they were isolated from different batches made under the same reaction conditions. The reaction was repeated multiple times with the intent to identify the reason for the formation of two products. However, we were unable to explain this phenomenon. ^{1}H NMR (400 MHz, $CDCl_3$): (**9**-desired thiol-conjugate) δ 6.75 (1H, br s), 4.89 (1H, dd, ^1J = 5.2, ^2J = 2.6 Hz), 3.63 (2H, m), 3.41 (1H, ddd, ^1J = 15.2, ^2J = 5.2, ^3J = 1.6 Hz), 2.86 (1H, ddd, ^1J = 15.4, ^2J = 2.3, ^3J = 1.5 Hz), 2.45 (1H, br s), 1.66 (2H, q, J = 7.5 Hz), 1.64–1.44 (2H, m), 3.36 (1H, ddd, ^1J = 15.3, ^2J = 5.1, ^3J = 1.6 Hz), 0.95 (6H, dt, ^1J = 7.4, ^2J = 3.0 Hz); (**9***) δ 6.44 (1H, br s), 4,77 (1H, dd, ^1J = 5.1, ^2J = 2.5 Hz), 2.88 (1H, dt, ^1J = 15.3, ^2J = 2.0 Hz), 2.72 (2H, q, J = 13.1 Hz), 1.74 (1H, br s), 1.66–1.52 (4H, m), 0.92 (6H, td, ^1J = 18.0, ^2J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): (**9**) δ 166.97, 67.64, 56.62, 48.32, 45.50, 26.40, 25.11, 7.83, 7.72; (**9***) 165.93, 74.55, 53.75, 45.36, 40.96, 31.01, 30.04, 8.04, 7.86. IR (**9**) (film) ν_{max}: 3393, 1751, 1257 cm^{-1}; IR (**9***) (film) ν_{max}: 1736, 1699, 1078 cm^{-1}. Anal. Calcd for $C_9H_{17}NO_2S$: C, 53.17; H, 8.43; N, 6.89. Found: C, 53.03; H, 8.47; N, 6.82.

4.3.8. {2-[1-(Ethoxycarbonylmethylimino-methyl)-1-ethyl-propyldisulfanyl]-2-ethyl-butylideneamino}-acetic acid ethyl ester (11)

Dialdehyde **7** (5 g, 19 mmol) and glycine ethyl ester hydrochloride (5.9 g, 42 mmol) were stirred in 80 mL of benzene. Triethylamine (5.8 mL, 42 mmol) was added and reaction was set up with Dean–Stark apparatus and left to reflux for 2 days. The progress of the reaction was monitored by NMR, by taking small aliquots, evaporating the solvent and re-dissolving in $CDCl_3$. The mixture was filtered and the resulting solution was evaporated under low pressure to result in (89%) amber viscous oil that was used within 48 h without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (2H, s), 4.24 (4H, s), 4.20 (4H, q, J = 7.1 Hz), 1.79 and 1.69 (8H, m), 1.28 (6H, t, J = 7.2 Hz), 0.90 (12H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.24, 169.83, 61.30, 60.98, 60.45, 25.16, 14.15, 8.26. IR (film) ν_{max}: 1736, 1653, 1186 cm^{-1}.

4.3.9. (2-Ethyl-2-mercapto-butylideneamino)-acetic acid ethyl ester (12)

Methoxyacetic acid (2 equiv) was added to the freshly prepared and filtered solution of diimine **11**. The mixture was refluxed overnight and evaporated under reduced pressure. The resulting brown viscous oil was purified by flash chromatography using hexanes/CH_2Cl_2 (1:1) to yield the desired product as yellow oil (1.8 g, 27%). ^{1}H (400 MHz, $CDCl_3$): δ 7.72 (1H, br s), 4.44 (2H, d, J = 4.5 Hz), 4.28 (2H, q, J = 7.1 Hz), 2.31 (1H, m, J = 4.7 Hz), 1.77 (2H, m), 1.62 (2H, m), 1.32 (3H, t, J = 7.2 Hz), 0.87 (6H, t, J = 7.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 210.54, 169.10, 61.97, 60.13, 46.94, 28.78, 14.13, 11.93. IR (film) ν_{max}: 1736, 1204 cm^{-1}. Anal. Calcd for $C_{10}H_{19}NO_2S$: C, 55.26; H, 8.81; N, 6.44. Found: C, 55.41; H, 8.90, N, 6.43.

4.3.10. [2-Ethyl-2-(1-ethyl-1-formyl-propyldisulfanyl)-butylideneamino]-acetic acid ethyl ester (13)

Dialdehyde **7** (5 g, 19 mmol) and glycine ethyl ester hydrochloride (6.5 g, 47 mmol) were suspended in 50 mL of CH_2Cl_2. Triethylamine (6 mL, 43 mmol) and $MgSO_4$ (10 g) were added and the mixture was stirred at room temperature for 3 days. The suspension was filtered and the solvent was evaporated to dryness. The obtained product was a mixture of white crystalline mass (unreacted amine and unwanted salts) and imine oil. A small portion of acetone was used to dissolve the oil and separate it from the crystals. Solvent evaporation resulted in yellow viscous oil (90%), which was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (1H, s), 7.44 (1H, s), 4.22 (2H, s), 4.20 (2H, q, J = 7.1 Hz), 1.79 and 1.68 (8H, m), 1.28 (3H, t, J = 7.1 Hz), 0.90 (6H, t, J = 7.4 Hz), 0.89 (6H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.57, 171.27, 169.87, 65.16, 61.22, 60.92, 60.48, 25.06, 21.56, 14.18, 8.30, 8.13. IR (film): ν_{max} 1745, 1716, 1660 cm^{-1}.

4.3.11. [2-Ethyl-2-(1-ethyl-1-formyl-propyldisulfanyl)-butylideneamino]-acetic acid methyl ester (14)

Imine **14** was synthesized using glycine methyl ester hydrochloride following the procedure described for imine **13**. The reaction mixture was stirred for 3 days at room temperature resulting in light yellow viscous oil (85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (1H, s), 7.44 (1H, s), 4.24 (2H, s), 3.74 (3H, s), 1.79 and 1.68 (8H, m), 1.28 (3H, t, J = 7.1 Hz), 0.90 (6H, t, J = 7.4 Hz), 0.89 (6H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.56, 171.33, 170.49, 65.15, 61.12, 60.48, 51.98, 25.15, 21.54, 8.28, 8.12. IR (film): ν_{max} 1751, 1713, 1659 cm^{-1}.

4.3.12. {2-[1-Ethyl-1-(1-ethyl-1-formyl-propyldisulfanyl)-propyl]-3-methoxy-4-oxo-azetidin-1-yl}-acetic acid ethyl ester (16)

Glycine mono-imine **13** (6 g, 17 mmol) and triethylamine (4.8 mL, 35 mmol) were stirred into 100 mL of CH_2Cl_2. A second solution, of methoxy-acetyl chloride (2.2 mL, 24 mmol) in 10 mL of CH_2Cl_2, was added dropwise to the mixture. The reaction mixture was refluxed overnight and then washed with a 5% aqueous solution of NH_4Cl (3 × 50 mL). The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The [2+2] reaction yielded 5.7 g of crude mono-β-lactam. Flash chromatography of the product (2:3 hexanes/CH_2Cl_2; 3:7 hexanes/CH_2Cl_2; v/v) afforded the product as clear yellow oil (46%). ^{1}H (400 MHz, $CDCl_3$): δ 9.23 (1H, s), 4.64 (1H, d, J = 5.3 Hz), 4.42 (1H, d, J = 18.1 Hz), 4.2 (2H, q, J = 7.1 Hz), 3.93 (2H, d, J = 17.9 Hz), 3.57 (3H, s), 1.76 and 1.61 (8H, m), 1.3 (3H, t, J = 7.1 Hz), 0.97 (6H, t, J = 7.6 Hz), 0.91 (6H, t, J = 7.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.36, 169.21, 168.11, 85.42, 64.99, 64.13, 61.61, 59.87, 42.97, 27.26, 26.57, 22.23, 21.33, 14.19, 9.05, 8.92, 8.36, 7.96. IR (film) ν_{max}: 1767, 1736, 1713, 1204 cm^{-1}. Anal. Calcd for $C_{19}H_{33}NO_5S_2$: C, 54.39; H, 7.93; N, 3.34. Found: C, 54.32; H, 7.93; N, 3.52.

4.3.13. {2-[1-Ethyl-1-(1-ethyl-1-formyl-propyldisulfanyl)-propyl]-3-methoxy-4-oxo-azetidin-1-yl}-acetic acid methyl ester (17)

Mono-β-lactam **17** was synthesized using the procedure described for **16** using the corresponding mono-imine. The product was purified on silica gel (CH_2Cl_2/AcOEt, 9:1; v/v) to afford the title compound as clear yellow oil (40%). ^{1}H (400 MHz, $CDCl_3$): δ 9.22 (1H, s), 4.64 (1H, d, J = 5.3 Hz), 4.50 (1H, d, J = 17.9 Hz), 4.17(1H, d, J = 5.3 Hz), 3.96 (1H, d, J = 17.9 Hz), 3.76 (3H, s), 3.57 (3H, s), 1.76 and 1.61 (8H, m), 0.97 (6H, t, J = 7.6 Hz), 0.91 (6H, t, J = 7.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.37, 169.18, 168.56, 85.43, 65.00, 64.19, 59.89, 52.46, 42.82, 27.24, 26.57, 22.21, 21.35, 9.05, 8.93, 8.36, 7.98. IR (film) ν_{max}: 1773, 1745, 1716, 1207 cm^{-1}. Anal. Calcd for $C_{18}H_{31}NO_5S_2$: C, 53.31; H, 7.70; N, 3.45. Found: C, 53.39; H, 7.73; N, 3.57.

4.3.14. [2-(1-Ethyl-1-mercapto-propyl)-3-methoxy-4-oxo-azetidin-1-yl]-acetic acid ethyl ester (18)

β-Lactam (2.5 g) **16** were dissolved in EtOH/6 M HCl (120 mL, 5:1; v/v) in a three neck round bottom flask. Zn dust (10 g) was added to the solution and the reaction mixture was refluxed overnight under argon. The reaction mixture was left to cool to room temperature and the solids were filtered out. The filtrate was concentrated under reduced pressure and diluted with water. The acidic solution was extracted with AcOEt (3 × 60 mL). The combined organic layers were dried with $MgSO_4$, filtered, and evaporated. Flash chromatography (hexanes/CH_2Cl_2 3:7; v/v) resulted in colorless oil (70%). ^{1}H (400 MHz, $CDCl_3$): δ 4.2 (2H, dq, ^1J = 7.1, ^2J = 2.7 Hz), 3.95 (2H, d, J = 10.2 Hz), 3.61 (3H, s), 3.56 (2H, d, J = 4.5 Hz), 3.25 (1H, d, J = 10.2 Hz), 1.93 (1H, m), 1.8 (4H, m), 1.29 (3H, t, J = 7.2 Hz), 1.07 (3H, t, J = 7.3 Hz), 1.01 (3H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.08, 169.79, 152.00, 118.06, 78.30, 61.55, 56.47, 40.88, 28.95, 24.21, 14.16, 13.39, 12.31. IR (film) ν_{max}: 1736, 1703, 1205 cm^{-1}. Anal. Calcd for $C_{13}H_{23}NO_4S$: C, 53.95; H, 8.01; N, 4.84. Found: C, 53.90; H, 8.02; N, 4.75.

4.3.15. (2-Methyl-2-methyldisulfanyl-propylideneamino)-acetic acid ethyl ester (20)

2-Methyldithiol-isobutyraldehyde **19** (0.5 g, 3 mmol) and glycine ethyl ester hydrochloride (0.7 g, 5 mmol) were dissolved in 10 mL of CH_2Cl_2. Triethylamine (0.7 mL, 5 mmol) and 1.0 g of $MgSO_4$ were added and the reaction mixture was stirred overnight at room temperature. The resulting solution was evaporated under low pressure. Acetone (2 mL) was used to dissolve the oil and separate it from the crystals. Solvent evaporation resulted in viscous oil (95%), which was used without further purification. ^{1}H (400 MHz, $CDCl_3$): δ 7.55 (1H, s), 4.21 (4H, overlapping q (J = 3.7 Hz) and s), 2.38 (3H, s), 1.50 (6H, s), 1.29 (3H, t, J = 7.2 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.78, 169.81, 61.33, 61.03, 53.17, 25.22, 24.06, 14.19. IR (film) ν_{max}: 1744, 1663, 1186 cm^{-1}.

4.3.16. [3-Methoxy-2-(1-methyl-1-methyldisulfanyl-ethyl)-4-oxoazetidin-1-yl]-acetic acid ethyl ester (21)

Imine **20** (0.75 g, 3 mmol) and triethylamine (0.8 mL, 5 mmol) were stirred in 20 mL of CH_2Cl_2. Methoxy-acetyl chloride (0.4 mL, 4 mmol) was added dropwise and the reaction mixture was refluxed for 2 h. The mixture was washed with 5% aqueous NH_4Cl (3 × 20 mL). The organic layer was dried with $MgSO_4$ and concentrated to dryness. The [2+2] reaction yielded 0.78 g of crude mono-β-lactam. Flash chromatography of the product (hexanes/CH_2Cl_2,1:1 and 2:3; v/v) afforded the product as colorless oil (58%). ^{1}H (400 MHz, $CDCl_3$): δ 4.62 (1H, d, J = 5.3 Hz), 4.33 (1H, d, J = 5.3 Hz), 4.19 (2H, dq, ^1J = 7.1, ^2J = 1.0 Hz), 4.40–4.05 (2H, dd, ^1J = 37.4, ^2J = 17.9 Hz), 3.58 (3H, s), 2.42 (3H, s), 1.45 (3H, s), 1.4 (3H, s), 1.28 (3H, t, J = 7.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.45, 166.36, 83.28, 61.48, 59.96, 58.10, 50.92, 41.00, 23.71, 23.06, 20.11, 12.59. IR (film) ν_{max}: 1759, 1738, 1207 cm^{-1}. Anal. Calcd for $C_{12}H_{21}NO_4S_2$: C, 46.88; H, 6.89; N, 4.56. Found: C, 46.73; H, 6.84; N, 4.62.

4.3.17. General procedure for synthesis of β-lactams containing aromatic thiols, phenol and thiophene

Lactams **22** and **24**, as well as **26–28** were prepared in a manner similar to the synthesis of lactam **9**. 4-Acetoxy-2-azetidone (1 g, 8 mmol) was stirred in 50 mL acetone/water (3:2) and 1.05 equiv of the corresponding substituents (4-mercaptophenol, 4-aminothiophenol, 1,4-benzenedithiol, thiophenol, or 2-thiophene thiol, respectively) was added. Sodium bicarbonate (4 equiv) was added to the mixture, which was then stirred vigorously for 12 h in a closed round bottom flask. Sodium chloride was subsequently added to the solution and after the formation of two layers, the mixture was filtered out and extracted with AcOEt (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by flash chromatography or crystallized to give white crystals for all five products in good yield (30–66%).

4.3.18. 4-Phenylsulfanyl-azetidin-2-one (22)

The crude product was crystallized from CH_2Cl_2 and hexanes to afford white crystals (62%) with mp. 66–68 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 and 7.37 (4H, m), 6.1 (1H, br s), 5.02 (1H, dd, ^1J = 4.9, ^2J = 2.3 Hz), 3.38 (1H, ddd, ^1J = 15.2, ^2J = 4.9, ^3J = 1.9 Hz), 2.9 (1H, ddd, ^1J = 15.2, ^2J = 2.3, ^3J = 1.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.56, 133.60, 131.21, 129.43, 128.76, 54.20, 45.40. IR (KBr) ν_{max}: 1766.7, 742.5, 690.5 cm^{-1}. Anal. Calcd for C_9H_9NOS: C, 60.31; H, 5.06; N, 7.81. Found: C, 60.26; H, 5.04; N, 7.81.

4.3.19. 4-(Thiophen-2-ylsulfanyl)-azetidin-2-one (24)

The crude product was crystallized from CH_2Cl_2 and hexanes to afford white crystals (90%) with mp 57–58 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (1H, dd, ^1J = 5.4, ^2J = 1.2 Hz), 7.23 (1H, dd, ^1J = 3.6, ^2J = 1.2 Hz), 7.07 (1H, dd, ^1J = 5.4, ^2J = 3.6 Hz), 6.19 (1H, br s), 4.86 (1H, dd, ^1J = 4.9, ^2J = 2.3 Hz), 3.31 (1H, ddd, ^1J = 15.3, ^2J = 4.9, ^3J = 1.9 Hz), 2.91 (1H, ddd, ^1J = 15.2, ^2J = 2.0, ^3J = 1.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.52, 136.75, 131.82, 128.12, 127.49, 55.38, 44.72. IR (KBr) ν_{max}: 1739.7, 968.2, 700.1 cm^{-1}. Anal. Calcd for $C_7H_7NOS_2$: C, 45.38; H, 3.81; N, 7.56. Found: C, 45.50; H, 3.76; N, 7.43.

4.3.20. 4-(4-Mercapto-phenoxy)-azetidin-2-one (26)

The crude product was crystallized from AcOEt/hexanes to afford white crystals (66%) with mp 143–144 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.72 (1H, s), 7.66 (1H, br s), 7.39 (2H, d, J = 8.6 Hz), 6.86 (2H, d, J = 8.6 Hz), 4.94 (1H, dd, ^1J = 4.9, ^2J = 2.3 Hz), 3.25 (1H, ddd, ^1J = 14.9, ^2J = 4.9, ^3J = 1.9 Hz), 2.7 (1H, ddd, ^1J = 14.9, ^2J = 2.2, ^3J = 1.2 Hz). ^{13}C NMR (125 MHz, acetone-d_6): δ 165.68, 139.27, 137.59, 120.95, 117.04, 55.05, 45.63. IR (KBr) ν_{max}: 1732, 1695, 1580 cm^{-1}. Anal. Calcd for $C_9H_9NO_5S$: C, 55.37; H, 4.65; N, 7.17. Found: C, 55.35; H, 4.57; N, 7.20.

4.3.21. 4-(4-Mercapto-phenylamino)-azetidin-2-one (27)

The crude product was purified on a silica gel column (CH_2Cl_2/AcOEt, 1:1; v/v). The resulting yellow solid (51%) was a mixture of two forms. The two forms were observed by NMR, where a single compound as well as a formation of hydrogen bond between the two structures was observed. The data reported here corresponds to the single compound, mp 106–108 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.6 (1H, br s), 7.24 (2H, d, J = 8.6 Hz), 6.66 (2H, d, J = 8.6 Hz), 4.98 (1H, br s), 4.85 (1H, dd, ^1J = 4.9, ^2J = 2.3 Hz), 3.22 (1H, ddd, ^1J = 14.9, ^2J = 4.9, ^3J = 1.9 Hz), 2.67 (1H, ddd, ^1J = 14.9, ^2J = 2.2, ^3J = 1.4 Hz). ^{13}C NMR (125 MHz, acetone-d_6): δ 165.76, 150.57, 135.68, 116.36, 115.57, 55.22, 45.41. IR (film) ν_{max}: 3462, 1747.4, 1627.8, 1595 cm^{-1}. Anal. Calcd for $C_9H_{10}N_2OS$: C, 55.65; H, 5.19; N, 14.42. Found: C, 55.43; H, 5.15; N, 14.28.

4.3.22. 4-(4-Mercapto-phenylsulfanyl)-azetidin-2-one (28)

The crude product was sufficiently pure to be used for the next step of the synthesis. When purification was required column chromatography (AcOEt/MeOH, 4:1; v/v) was performed to afford the desired product as white amorphous solid (30%) with a mp 124–125 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.92 (1H, br s), 7.51 (4H, m), 5.19 (1H, dd, ^1J = 5.0, ^2J = 2.3 Hz), 3.43 (1H, ddd, ^1J = 15.1, ^2J = 5.1, ^3J = 2.2 Hz), 2.8 (1H, ddd, ^1J = 15.1, ^2J = 2.3, ^3J = 1.2 Hz). ^{13}C NMR (125 MHz, acetone-d_6): δ 165.75, 136.88, 133.61, 129.07, 129.16, 54.06, 46.24. IR (film) ν_{max}: 1745.4, 1712.7, 1571.9 cm^{-1}. Anal. Calcd for $C_9H_9NOS_2$: C, 51.16; H, 4.29; N, 6.63. Found: C, 51.37; H, 3.08; N, 6.54.

4.3.23. General procedure for preparation of mercapto-phenyl-azetidine sulfonates

β-Lactam (1.0 equiv) **22**, **24** or **26**–**28** and sulfur trioxide–DMF (5 equiv) were dissolved in 12 mL of anhydrous DMF. The mixture was stirred for 2 h and poured into 0.5 N KH_2PO_4 (80 mL), followed by addition of 1 equiv of TBAHS. The aqueous layer was extracted with AcOEt (3 × 20 mL) and dried over $MgSO_4$. The solvent was evaporated under low pressure to afford solid or viscous oil that was further purified.

4.3.24. 2-Oxo-4-phenylsulfanyl-azetidine-1-sulfonic acid tetra-*n*-butylammonium salt (23)

The product was purified using column chromatography (AcOEt/MeOH, 9:1; v/v) to afford the title compound as colorless oil (46%). All attempts to crystallize this product were unsuccessful. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (2H, m), 7.31 (2H, m), 5.22 (1H, dd, ^1J = 5.4, ^2J = 2.6 Hz), 3.27 (8H, m), 3.15 (1H, dd, ^1J = 15.2, ^2J = 5.4 Hz), 2.62 (1H, dd, ^1J = 15.2, ^2J = 2.6 Hz), 1.64 (8H, m), 1.43 (8H, s, J = 7.3 Hz), 0.99 (12H, t, J = 7.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.80, 134.96, 130.63, 128.88, 128.28, 58.68, 58.41, 43.12, 23.97, 19.70, 13.71. IR (film) ν_{max}: 1766.7, 1247.8, 1049.2 cm^{-1}. Anal. Calcd for $C_{25}H_{44}N_2O_4S_2$: C, 59.96; H, 8.86; N, 5.59. Exact mass Calcd for $C_9H_8NO_4S_2^-$: 257.9895 and $C_{16}H_{36}N^+$: 242.2848; found by HRMS: m/z (M^-) 257.9903.

4.3.25. 2-Oxo-4-(thiophen-2-ylsulfanyl)-azetidine-1-sulfonic acid tetra-*n*-butylammonium salt (25)

The product was crystallized from AcOEt/hexane to afford the title compound as white crystals (55%) with mp 99–102 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (1H, dd, ^1J = 7.5, ^2J = 3.6 Hz), 7.40 (1H, dd, ^1J = 5.3, ^2J = 1.2 Hz), 7.02 (1H, dd, ^1J = 5.3, ^2J = 3.6 Hz), 5.06 (1H, dd, ^1J = 5.3, ^2J = 2.5 Hz), 3.27 (8H, m), 3.08 (1H, dd, ^1J = 15.2, ^2J = 5.3 Hz), 2.7 (1H, dd, ^1J = 15.2, ^2J = 2.7 Hz), 1.64 (8H, m), 1.43 (8H, s, J = 7.4 Hz), 0.99 (12H, t, J = 7.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.56, 137.97, 130.84, 128.06, 126.71, 58.96, 58.62, 42.16, 23.96, 19.69, 13.72. IR (KBr) ν_{max}: 1766.7, 1051.1 cm^{-1}. Anal. Calcd for $C_{23}H_{42}N_2O_4S_3$: C, 54.51; H, 8.35; N, 5.53. Found: C, 54.76; H, 8.44; N, 5.50.

4.3.26. 2-(4-Mercapto-phenoxy)-4-oxo-azetidine-1-sulfonic acid tetra-*n*-butylammonium salt (29)

The product was purified on silica gel (AcOEt/MeOH, 9:1; v/v) to afford the compound as white crystals (50%) with mp 148–150 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.72 (1H, s), 7.64 (2H, d, J = 8.6 Hz), 6.83 (2H d, J = 8.6 Hz), 4.93 (1H, dd, ^1J = 5.4, ^2J = 2.5 Hz), 3.45 (8H, m), 3.25 (1H, dd, ^1J = 14.8, ^2J = 5.5 Hz), 2.4 (1H, dd, ^1J = 14.7, ^2J = 2.5 Hz), 1.82 (8H, m), 1.43 (8H, s, J = 7.4 Hz), 0.98 (12H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, acetone-d_6): δ 162.83, 159.42, 138.99, 119.40, 116.72, 59.29, 58.98, 42.39, 24.41, 20.34, 13.86. IR (KBr) ν_{max}: 3205.5, 1776.3, 1043.4, 842.8 cm^{-1}. Anal. Calcd for $C_{25}H_{44}N_2O_5S_2$: C, 58.11; H, 8.58; N, 5.42. Found: C, 57.84; H, 8.56; N, 5.39.

4.3.27. 2-(4-Mercapto-phenylamino)-4-oxo-azetidine-1-sulfonic acid tetra-*n*-butylammonium salt (30)

The product was purified on silica gel column (CH_2Cl_2/AcOEt, 1:9; v/v) to afford the title compound as white crystals (47%) with mp 111–113 °C. This β-lactam was observed in two different forms in the NMR: free compound and dimer structure formed as a result of hydrogen bonding. The two forms were interchangeable when the product was left in solvent for a few days. The data reported here is for the dimer, as this was the prevalent form. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.71 (2H, d, J = 8.5 Hz), 7.48 (2H, d, J = 8.6 Hz), 6.64 (4H, dd, ^1J = 5.9, ^2J = 8.5 Hz), 4.99 (1H, dd, ^1J = 2.6, ^2J = 5.4 Hz), 4.86 (1H, dd, ^1J = 2.6, ^2J = 5.4 Hz), 3.44 (16H, m), 3.08 (1H, dd, ^1J = 14.8, ^2J = 5.5 Hz), 2.95 (1H, dd, ^1J = 14.7, ^2J = 5.5 Hz), 2.86 (2H, br s), 2.43 (1H, dd, ^1J = 14.6, ^2J = 2.6 Hz), 2.41 (1H, dd, ^1J = 14.6, ^2J = 2.6 Hz), 1.81 (16H, m), 1.43 (16H, s, J = 7.5 Hz), 0.98 (24H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, acetone-d_6): δ 162.35, 162.14, 153.05, 150.21, 138.83, 137.36, 124.91, 120.69, 115.69, 115.28, 59.33, 59.28, 58.91, 58.83, 42.97, 42.19, 24.46, 20.37, 13.93. IR (KBr) ν_{max}: 3440.8, 1776.7, 1049.2 cm^{-1}. Anal. Calcd for $C_{25}H_{45}N_3O_4S_2$: C, 58.22; H, 8.79; N, 8.15. Found: C, 58.01; H, 8.72; N, 8.06.

4.3.28. 2-(4-Mercapto-phenylsulfanyl)-4-oxo-azetidine-1-sulfonic acid tetra-*n*-butylammonium salt (31)

The product was purified using column chromatography (AcOEt/MeOH, 8:2 and AcOEt/MeOH 7:3; v/v) to afford the title compound as thick colorless oil (28%). The purification step was challenging due to polymerization of the product on the silica gel column and as a result the final yield was lower then expected.

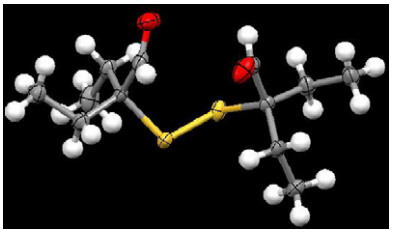
^{1}H NMR (400 MHz, acetone-d_6): δ 7.67 (4H, m), 5.14 (1H, m), 3.42 (16H, m), 3.27 (1H, m), 2.54 (1H, m), 1.78 (16H, m), 1.43 (16H, m), 0.97 (24H, t, J = 7.4 Hz). ^{13}C NMR (125 MHz, acetone-d_6): δ 162.29, 134.62, 132.80, 132.47, 59.10, 58.49, 44.02, 24.59, 20.47, 15.14. IR (film) ν_{max}: 1774.4, 1245.9, 1049.2 cm^{-1}. Anal. Calcd for $C_{25}H_{44}N_2O_4S_3$: C, 56.35; H, 8.32; N, 5.26. Exact mass Calcd for $C_{25}H_{44}N_2O_4S_3^-$: 289.9615 and $C_{16}H_{36}N^+$: 242.2848; found by HRMS: m/z (M^-) 289.9630.

4.4. X-ray crystal structure determination

Single-crystal X-ray diffraction data on compounds **7**, **22**, **24**, **26**, **29**, and **30** were collected at 113 K using Mo Kα radiation and a Bruker APEX 2 CCD area detector. Crystals were prepared for data collection by coating with high viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was mounted on a Micro Mesh mount (MiteGen Inc.) and transferred immediately to the cold stream (−160 °C) on the diffractometer. Corrections were applied for Lorentz, polarization, and absorption effects. All structures were solved by direct methods and refined by full-matrix least squares on F^2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.10, 2000, Bruker AXS Inc., Madison, WI). Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C–H distance set at 0.96 Å. Atomic coordinates for compounds **7**, **22**, **24**, **26**, **29**, and **30** have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers from 803445 to 803450). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

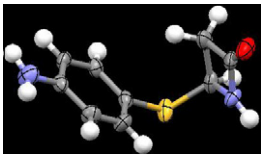
4.4.1. Crystallographic details for compound 15

The crystal of the dialdehyde (2,2′-disulfanediylbis(2-ethylbutanal)), **7**, was monoclinic in space group $P2_1/c$ with unit cell dimensions a = 10.221(4) Å, b = 10.239(4) Å, c = 13.839(5) Å, and β = 97.906(6)°. Data were 97.8% complete to 29.18°θ (approximately 0.73 Å) with an average redundancy of 2.8.



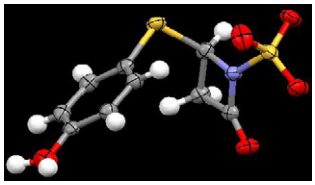
4.4.2. Crystallographic details for compound 27

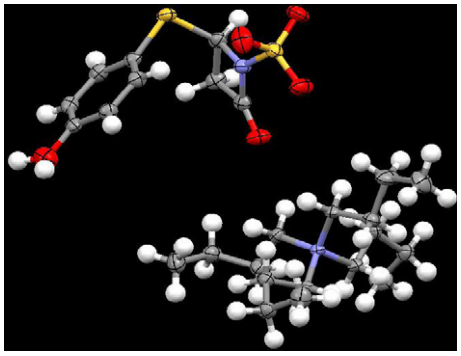
The crystal of 4-(4-aminophenylthio)azetidin-2-one, **27**, was monoclinic in space group $P2_1/c$ with unit cell dimensions a = 5.7085(12) Å, b = 8.0637(17) Å, c = 20.094(4) Å, and β = 95.954(6)°. Data were 98.2% complete to 25.73° θ (approximately 0.82 Å) with an average redundancy of 4.09.



4.4.3. Crystallographic details for compound 29

The crystal of 2-(4-hydroxyphenylthio)-4-oxoazetidine-1-sulfonate, **29**, was triclinic in space group $P\bar{1}$ with unit cell dimensions a = 9.8191(13) Å, b = 10.5484(14) Å, c = 13.6158(18) Å, α = 75.954(2)°, β = 89.389(2)°, and γ = 81.793(3)°. Data were 98.4% complete to 25.79° θ (approximately 0.82 Å) with an average redundancy of 2.19.



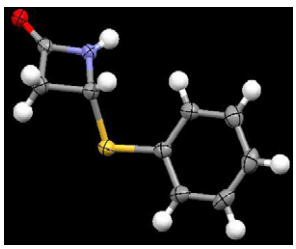


4.4.4. Crystallographic details for compound 30

The crystal of 2-(4-aminophenylthio)-4-oxoazetidine-1-sulfonate, **30**, was triclinic in space group $P\bar{1}$ with unit cell dimensions a = 9.8030(4) Å, b = 10.5558(4) Å, c = 13.7240(6) Å, α = 76.2140(10)°, β = 89.6720(10)°, and γ = 83.076(2)°. Data were 97.7% complete to 29.19° θ (approximately 0.73 Å) with an average redundancy of 2.07.

4.4.5. Crystallographic details for compound 22

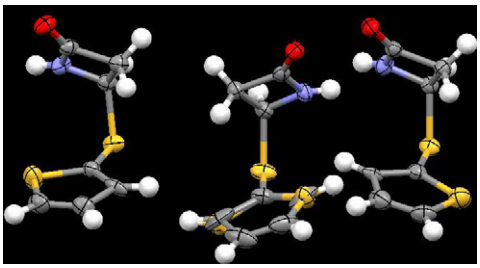
The crystal of 4-(phenylthio)azetidin-2-one, **22**, was monoclinic in space group $P2_1/c$ with unit cell dimensions a = 12.3179(4) Å, b = 7.8615(3) Å, c = 9.1767(3) Å, and β = 101.7610(10)°. Data were 98.0% complete to 29.61° θ (approximately 0.73 Å) with an average redundancy of 3.9.



4.4.6. Crystallographic details for compound 25

The crystal of 4-(thiophen-2-ylthio)azetidin-2-one, **25**, was monoclinic in space group $P2_1/c$ with unit cell dimensions a = 15.1939(9) Å, b = 5.4702(3) Å, c = 29.9729(17) Å, and

$\beta = 97.2650(10)^\circ$. Data were 99.4% complete to 28.17° θ (approximately 0.75 Å) with an average redundancy of 3.87.



4.5. Biological assays

Moraxella catarrhalis clinical isolates ($n = 6$) used were β-lactamase (nitrocefin-cleavage assay positive) producing.[29] The clinical isolates used were kindly provided by J. Tjhio (Loyola University Stritch School of Medicine, Maywood, IL). *Moraxella catarrhalis* antimicrobial activity was evaluated by B. J. Plotkin and J. M. Green (Midwestern University, Downers Grove, IL). *Mycobacterium tuberculosis* (Mtb) H37Rv was used for all Mtb susceptibility determinations. These determinations were done by Helena Boshoff and Clifton Barry, III (Tuberculosis Research Section, NIAID, NIH Bethesda, MD). In addition, non-β-lactamase producing quality control strains of *Escherichia coli*, ATCC 25922; *Pseudomonas aeruginosa*, ATCC 27853; *Staphylococcus aureus*, ATCC 25923; *Enterococcus faecalis*, ATCC 29212 were tested for anti-microbial susceptibility as described below. Inability to cleave nitrocefin was confirmed for all ATCC quality control strains. All isolates were maintained at −80 °C until use.

Two types of assays (qualitative screening and quantitative) were performed to assess the antibacterial properties of these compounds: Kirby-Bauer disc diffusion assays and minimal inhibitory concentration/minimum bactericidal concentration (MIC/MBC) assays, respectively. All assays were performed in triplicate. Testing was done in a blinded manner. For Kirby-Bauer disc diffusion assays, five colonies of each bacterial strain were suspended in Mueller-Hinton (MH) broth to a density equivalent to a 0.5 McFarland standard and spread onto an MH agar plates (cotton swab) according to the standard protocol.[28] Kirby-Bauer discs, each containing compound (10 μg compound tested/disc) were placed on plates inoculated with bacteria. Discs were prepared by dissolving compounds in methanol, applying the solution to the disc then drying discs at room temperature. Each agar plate with discs was incubated at 37 °C overnight and the zone of inhibition measured (mm of no growth from edge of disc). Controls consisted of discs with diluent (methanol) alone and penicillin (positive control).

MIC/MBC assays were performed using the microdilution broth method in accordance with the guidelines of the Clinical and Laboratory Standards Institute.[28] Compounds were prepared in 95% ethanol then diluted at least 10-fold into Mueller-Hinton (MH) broth. Each diluted compound solution (100 μL/well) was then serially diluted in MH (100 μL/well; series of two-fold dilutions, between 500 μg/mL and 15.6 μg/mL). Controls consisted of ethanol without compound and penicillin similarly diluted. To each well 100 μL/well, 5×10^5 cells/mL of an actively growing culture MH broth was added. Plates were incubated at 37 °C for 48 h, and scored for growth at 24 and 48 h for visible growth. The MIC was defined as the lowest concentration of drug at which no growth was observed. Minimal bactericidal growth was also measured in these experiments; in wells where no growth was observed, a sample (10 μL) was taken and applied to a MH plate, to test whether cells were dead (MBC) or simple not growing (MIC). The MBC was defined as the lowest concentration of drug in which no growth occured. All tests were performed in triplicate.

The antimycobacterial effects of the compounds against *M. tuberculosis* were tested by the Alamar blue assay as detailed in Kim et al.[31]

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