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Non-transpeptidase binding arylthioether β-lactams active against *Mycobacterium tuberculosis* and *Moraxella catarrhalis*

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ABSTRACT

The prevalence of drug resistance in both clinical and community settings as a consequence of alterations of biosynthetic pathways, enzymes or cell wall architecture is a persistent threat to human health. We have designed, synthesized, and tested a novel class of non-transpeptidase, β-lactamase resistant monocyclic β-lactams that carry an arylthio group at C4. These thioethers exhibit inhibitory and cidal activity against serine β-lactamase producing *Mycobacterium tuberculosis* wild type strain (Mtb) and multiple ($n = 8$) β-lactamase producing *Moraxella catarrhalis* clinical isolates.

1. Introduction

Traditional β-lactam ring containing antibiotics have as an essential requirement for activity, that is, the presence of an ionizable group either in the proximity (carboxylic acid, bicyclic, penicillin-like structures) or onto (sulfonic acid, monobactams) the lactam nitrogen of the β-lactam ring.[1–3] This is required for traditional β-lactam compounds to interact with the bacterial transpeptidases and inhibit peptidoglycan strand cross-linking.[4–6] These compounds are losing their efficacy due to the production of β-lactamases by resistant bacteria.[7,8]

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.[4–6] However, there now appear to be exceptions to this scaffold requirement, since *N*-alkylthiolated β-lactams possess inhibitory, although not cidal, antimicrobial activity and are stable to the hydrolytic activity of β-lactamases.[9–13] The range of bacterial genera affected by these *N*-alkylthiolated β-lactams is extremely narrow. The afore-mentioned *N*-alkylthiolated β-lactams are highly selective towards *Staphylococcus* spp. and *Bacillus* spp.[9–11] However, unlike penicillins, which inhibit cell wall crosslinking enzymes, *N*-thiolated lactams are characterized by a bacteriostatic activity and act through a different mechanism of action.[13] Reports confirm that once the ionizable group is removed from the lactam nitrogen, a variety of novel molecular targets emerge.[14–18] While the mechanisms of *N*-thiolated β-lactams are still not completely understood, they appear to affect cellular processes associated with coenzyme A and lipid biosynthesis.[13]

We developed, synthesized and tested a library of beta-lactamase resistant, non-transpeptidase binding monocyclic β-lactams, some of which have antimicrobial activity against two phylogenetically distant β-lactamase producing bacterial species—*Moraxella catarrhalis* (*M.cat.*) and *Mycobacterium tuberculosis* (Mtb).[19] The active compounds were substituted at C4 with alkylthio- as well as arylthio-groups, with amino-, hydroxy- and sulfhydryl-substituents at the *para* position on the aromatic ring, and their *N*-sulfonates (Fig. 1). The most active compounds were type **I** azetidinone thioethers with arylthio group at C4, lacking the sulfonate at the lactam nitrogen. *N*-Sulfenation of these lactams did not enhance their antimicrobial activity (type **II**, Fig. 1).[19] Structure–activity relationship (SAR) studies of lactams **I** and **II** indicate that the presence of the C4-arylthio group is essential for antimicrobial activity. In addition, the N1-substituent has an important role. Taken together, these data demonstrate that lactams with sulfenated N1, even with a C4-arylthioether, have either comparable or diminished antibacterial activity against Mtb and *M.cat.*[19]

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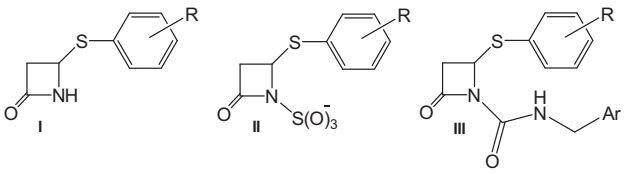


Figure 1. C4-arylthiolactams with antibacterial activity against Mtb and *M.cat*.

The aims of the present study were to: (1) determine the antimicrobial activity of compounds in which the sulfur atom at C4 was replaced with an alternative heteroatom; (2) determine the effects of electron-withdrawing groups (EWGs) or an electron-donating groups (EDGs) at the arylthio—substituent at C4 on antimicrobial activity; and (3) identify the optimal functional group at the lactam nitrogen for maximal antimicrobial activity.

2. Chemistry

The overall procedures for generating the various C4-substituted β-lactams are summarized in Scheme 1. The requirement for an arylthio-group at the C4 was determined by the synthesis and subsequent antimicrobial testing of compounds having phenoxy- and selenophenoxy-substituents at C4. These compounds were either unsubstituted or carbamylated at N1.

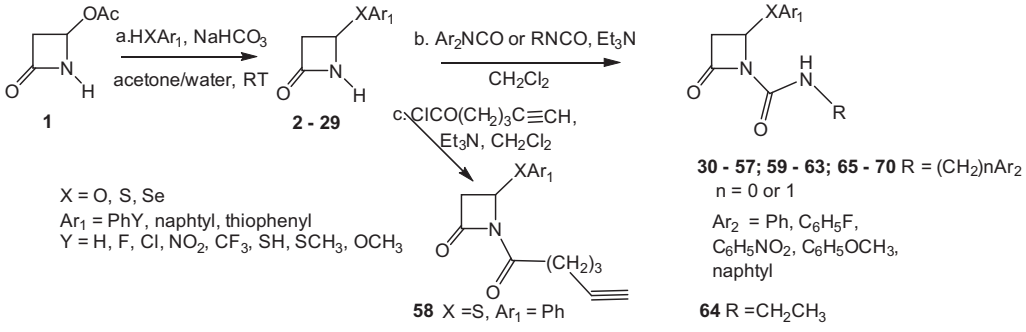
The effect on antimicrobial activity of various EWGs or EDGs at different positions on the aromatic ring was determined. This set of compounds, C4-arylthio-β-lactams **2**–**27** (Scheme 1, Table 1) was prepared from 4-acetoxy-2-azetidinone **1** and the corresponding arylthiols, by a procedure described by Clauss et al.[20] and Wasserman et al.[21] Carbamylation of the lactam nitrogen (lactams **30**–**70**) with a variety of isocyanates was accomplished by adopting a procedure from Mulchande et al.[23] or by a microwave-based method developed in our laboratory. β-Lactams **37**–**39**, **55**, **56**, **59**–**63**, **65**–**68**, **70** were prepared in methylene chloride (5 ml) with 1.2 M equivalents of triethylamine using a CEM microwave apparatus (300 W, 30 °C for 10–45 min). Incubation times varied depending on the nature of isocyanate and the unsubstituted lactams. Lactams **28** and **29** were purchased from Sigma–Aldrich (St. Louis, MO). Lactam **58**, with a terminal triple bond, was prepared by the addition of 5-hexanoic acyl chloride to lactam **2**.[14]

3. Results and discussion

We recently described the synthesis and characterization of a family of C4-arylthio-β-lactams having antimicrobial activity against Mtb and *M.cat*.[19] The initial library of compounds was either unsubstituted at N1 (**I**, Fig. 1) or sulfenated at N1 (**II**, Fig. 1). Antimicrobial activities of the compounds unsubstituted at the N1 lactam were either similar, or increased, when compared to the activities of compounds sulfenated at N1. Treatment of β-lactam **2** with benzylisocyanate produced **30**. β-Lactam **30** was significantly more active against both *M.cat.* and Mtb (MICs of 12.5 μg/ml and 6.25 μg/ml, respectively). The addition of a benzylcarbamyl group to the lactam nitrogen also resulted in an increase in activity for *M.cat.* and Mtb (>16- and 4-fold, respectively; lactams **2** vs **30**; Table 1).

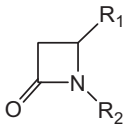
To determine the optimal substituents at C4 and N1, we systematically synthesized and determined the antimicrobial activities of compounds containing a H versus a carbamyl moiety at N1 (Table 1). In addition, β-lactam ethers having arylthio-groups at C4 with both EWGs and EDGs at *o*-, *m*- and *p*-positions were synthesized then tested. β-Lactams **6**, **8**–**13** and **27** demonstrated antimicrobial activity against *M.cat*. (MIC ⩽ 12.5 μg/ml; MBC ⩽ 50 μg/ml). The addition of a carbamyl group at N1 improved the anti-*M.cat.* activity (lactams **30**–**33**, **35**, **42**–**44**, Table 1). Moreover, these lactams exhibit targeted narrow spectrum activity against *M.cat*. and Mtb; they were inactive against β-lactamase-negative Gram positive and Gram negative quality control strains and β-lactamase-positive clinical isolates (Table 5; data not shown).

When considering the effect of the XAr1 group on anti-*M.cat.* and anti-Mtb activity, the most active compounds had halogenated aryl moieties on the thioether (X = S) groups at C4 (Scheme 1). For example, lactams with arylthioether groups at C4 with multiple fluoro- or chloro- groups exhibited the highest levels of activity, regardless of whether the substituent on the lactam nitrogen was a hydrogen or a carbamyl group (Table 1; compounds **6** & **34**; **7** & **35**, **8** & **36**, **9** & **37**, **10** & **38**, **11** & **39**). Interestingly, positioning of the fluorine in di-fluorinated compounds affects antimicrobial activity. Lactam **6** (*ortho* and *para*) exhibits a 16- and 8- fold higher level of cidal activity against *M.cat.* and Mtb, respectively, when compared to compound **7** (*meta* and *para*). We hypothesize that placement of fluorine atoms at the *ortho*- and *para*-positions, relative to the sulfur atom in the thiophenoxy group at C4, allows for a positive electrostatic potential at these regions of the molecule, which could be necessary for formation of a halogen bond to an appropriate base at the binding site. A halogen bond is a non-covalent bond between a halogen atom (X) and a Lewis base (B), similar to the H-bond. This type of bond is attributed to the anisotropic distribution of the charge density on the halogen atom, resulting in the formation of a positive cap (called the σ-hole) centered on the R–X axis. For a given R, the σ-hole potential on the halogen X in RX becomes more positive in the order F << Cl < Br < I (following the trend in the halogens' polarizabilities).[24] The σ-hole



Scheme 1. Synthesis of N-substituted C4 arylthio-, aryloxy- and arylseleno-β-lactams. (a) β-Lactams **2**–**29** were prepared from the commercially available β-lactam **1** in the presence of $NaHCO_3$ in acetone/water.[20,21] Preparation of lactams **2**[20], **17**[22] and **22**[19] have been reported earlier. (b) β-Lactams **30**–**70**, excluding lactam **58** (R_2 = $CO(CH_2)_3CCH$) were prepared from β-lactams **2**–**29** using aryl isocyanates and Et_3N in methylene chloride at rt[23], or by using a CEM microwave apparatus at 300 W, 30 °C for 20–45 min. (c) Lactam **58** can be prepared from lactam **2** as described earlier.[14]

Table 1
Antimicrobial activity of C4-arylthio-, C4-arylseleno- and C4-aryloxy-, unsubstituted at N1, and N1-carbamylated β-lactams against Mtb and *M.cat.* ($n = 6$)



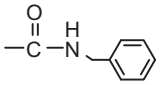
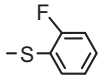
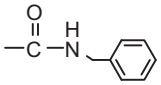
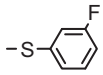
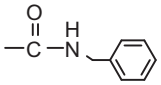
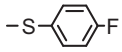
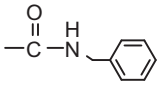
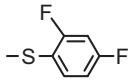
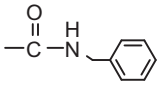
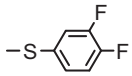
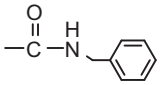
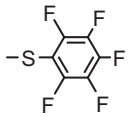
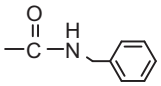
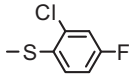
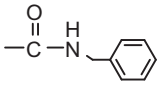
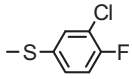
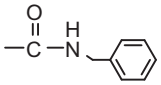
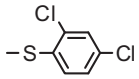
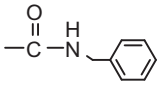
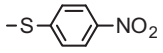
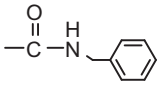
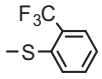
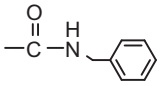
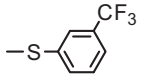
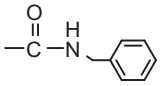
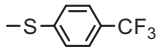
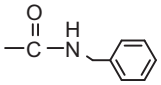
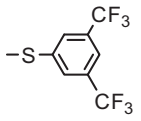
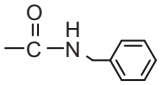
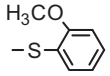
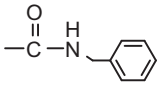
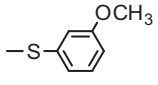
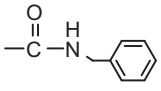
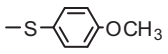
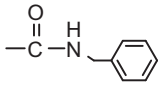
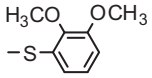
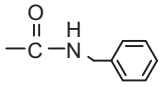
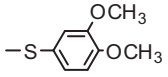
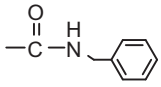
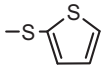
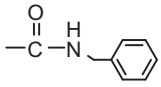
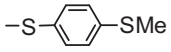
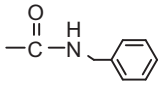
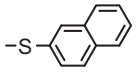
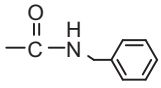
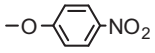
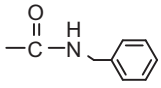
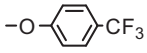
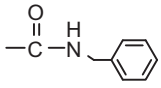
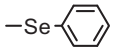
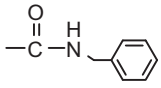
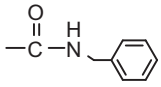
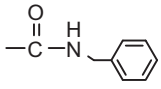
R1	#	R2	*M.cat.*[a] MIC/MBC (μg/ml)	Mtb MIC (μg/ml)	#	R2	*M.cat.* MIC/MBC (μg/ml)	Mtb MIC (μg/ml)
	**2**	H	>200	25	**30**		12.5/12.5	6.25
	**3**	H	200	25	**31**		25/50	3.13
	**4**	H	>200	100	**32**		25/100	NT[c]
	**5**	H	>200	>100	**33**		25/25	6.25
	**6**	H	1.5/3.13	3.13	**34**		1.63/1.63	100
	**7**	H	50/50	50	**35**		12.5/25	6.25
	**8**	H	1.5/1.5	>100	**36**		3.1/6.25	>100
	**9**	H	12.5/50	25	**37**		12.5/25	25–50
	**10**	H	12.5/25	25	**38**		6.25/6.25	25
	**11**	H	6.25/6.25	25	**39**		3.13/6.25	25
	**12**	H	6.25/12.5	25	**40**		3.125/25	25/
	**13**	H	1.5/1.5	⩾100	**41**		25/25	125
	**14**	H	200	6.25	**42**		3.125/6.25	25
	**15**	H	100/100	12.5	**43**		1.625/12.5	12.5
	**16**	H	12.5/100	>100	**44**		6.25/6.25	6.25
	**17**	H	100/>200	250	**45**		200/200	125–250
	**18**	H	100/>200	>100	**46**		200/200	100

Table 1 (*continued*)

R1	#	R2	*M.cat.*[a] MIC/MBC (μg/ml)	Mtb MIC (μg/ml)	#	R2	*M.cat.* MIC/MBC (μg/ml)	Mtb MIC (μg/ml)
	**19**	H	200/>200	100	**47**		200/200	125
	**20**	H	100/>200	250	**48**		100/200	50
	**21**	H	200/200	250	**49**		200/>200	250
	**22**	H	25/100	6.25/	**50**		50/100	6.25/
	**23**	H	100/100	>100	**51**		NT	>100
	**24**	H	100/>200	>250	**52**		200/>200	>100
	**25**	H	50/50	25	**53**		6.25/6.25	250
	**26**	H	200/200	250	**54**		50/>200	125
	**27**	H	6.25/6.25	25	**55**		50/50	25
H	**28**	H	NT	NT	**56**		>100	>100
	**29**	H	>200	>200	**57**		NT/chemically unstable	NT/chemically unstable

[b] All compounds are tested as racemates.

[a] The values for *M.cat.* are the results from the six isolates tested. The MIC/MBCs reported were within 2-fold dilutions for all isolates tested regardless of compound. The concentrations reported are the highest MIC/MBC measured for that compound.

[c] NT = not tested.

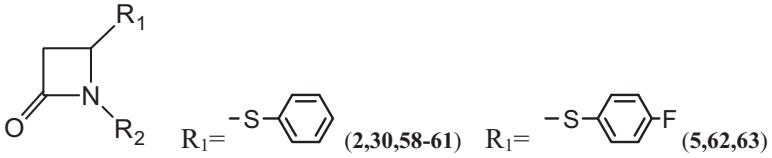
potential is also more positive as R is more electron-withdrawing. The more positive the potential σ-hole of X becomes, the more the binding energy of the complex R–X. . .B increases for a given B.[24] The probability that the electrostatic potential of the halogens at the aromatic ring of our compounds may play a role for their antimicrobial activity is supported by the findings that the di-fluorinated lactams **6** and **7** have better antimicrobial activity than their mono-fluorinated analogs (lactams **3**–**5**). In contrast, the fluorine at the *meta* position appears to have decreased capability to form an appropriate halogen bond, based on its location in the aromatic ring. This might explain observed differences in antimicrobial activity (Table 1, compounds **6** and **7**).

Monofluorinated thiophenoxy groups at C4 alone were insufficient to make the compounds active within the accepted therapeutic range for screening of novel antimicrobials (MBC <35 μg/ml; Table 1, compounds **3**–**5**).[25,26] However, the replacement of the proton with a carbamyl group at N1 resulted in significantly improved antimicrobial activity (Table 1, compounds **31**–**33** vs **3**–**5**). This improvement in antimicrobial activity could be explained by several different mechanisms. It may be that the carbamyl group 'regenerated' the isocyanate within the given microorganism (Scheme 3, mechanism b). Alternatively, it is plausible that the weak electron-withdrawing effect of the benzyl carbamyl group increased the electrophilicity of the lactam ring, relative to its counterpart lacking the carbamyl-group at N1, and/or allowed for somewhat increased electrostatic potential on the halogen atom of the aromatic ring.

To further explore the mechanism by which halogens affect antimicrobial activity, lactams with chlorine-substituted thiophenols were synthesized and tested for antimicrobial activity. When one or both fluorines in the thiophenoxy group at C4 were replaced by chlorine atoms, the lactams demonstrated antimicrobial activity, regardless of the R_2 group (Table 1, compounds with hydrogen **9**–**11** vs compounds with carbamyl groups **37**–**39**). However, substitution of a chlorine for a fluorine, in general, decreased antimicrobial activity of the di-halogenated compounds. Of all di-halogenated compounds tested compound **6** demonstrated the most potent antimicrobial activity. The small but measurable difference in activity between lactams **7** and **10** may be due to the higher positive electrostatic potential of Cl as compared to F.

The effects of CF_3 at various positions relative to the S atom indicate the differences between *M.cat.* and Mtb in sensitivity to these compounds (Table 1). When considering the antimicrobial activity of compounds **13**–**16**, lactam **13** demonstrated the best activity against *M.cat.* (MIC 1.5 μg/ml), while lactams **14** and **15** were more effective against Mtb (MIC 6.25–12.5 μg/ml). When comparing lactams **4** and **14**, the presence of a CF_3 group at the *meta*-position increased the anti-Mtb activity. This was likely due

Table 2
Antimicrobial activity of N1-carbamylated β-lactams against Mtb and *M.cat.* ($n = 6$)



#	R2	*M.cat.* MIC/MBC (μg/ml)	Mtb MIC (μg/ml)
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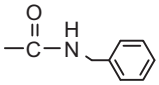
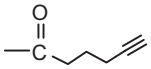
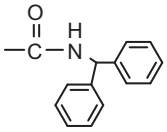
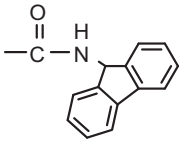
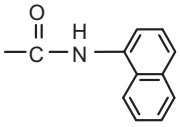
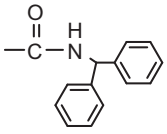
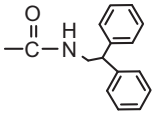
30		12.5/12.5	6.25
58		100/100	45
59		NT[c]	50
60		NT	>100
61		NT	25
5	H	>200	>100
62		>200/>200	>100
63		>200/>200	>100

[a]The values for *M.cat* are the results from the six isolates tested. The MIC/MBCs reported were within 2-fold dilutions for all isolates tested regardless of compound. The concentrations reported are the highest MIC/MBC measured for that compound.
[b]All compounds are tested as racemates.
[c] NT = not tested.

to the higher electron-withdrawing power of the CF_3. Hence, the induction of the CF_3 group may decrease even further the electron density of the atom (in this case S) at C4. The presence of one versus two CF_3 groups at the *meta*-positions affects the antimicrobial activity against *M.cat*. and Mtb but in opposing directions (Table 1, lactam **14** vs **16**). Interestingly, carbamylated **14** and **16**, that is, lactams **42** and **44** (Table 1), demonstrated potent antimicrobial activity against *M.cat*. and Mtb (MIC 3.13–6.25 μg/ml and 6.25–25 μg/ml, respectively). Substitution of another EWG, *para*-nitro lactam, at the thioether moiety at C4, had activity within therapeutic range (<35 μg/ml)[25,26] for both Mtb and *M.cat*., regardless of the presence or absence of a carbamyl group at N1 (Table 1, compounds **12** and **40**).

Similar in size to the CF_3 group the methoxy group, in contrast, is an electron donor. The antimicrobial effect of one or more methoxy groups at all positions on the thioaryl moiety at C4 was determined (Table 1, compounds **17**–**21** and **45**–**49**). However, this group of compounds lacked significant activity, even after carbamylation at N1 (compounds **45**–**49**). In addition, a lactam with a thionaphthyl group at C4 lacked antimicrobial activity, even after carbamylation (compound **24** vs **52**). This lack of activity may be due to steric limitations for the bulkier thionaphthyl group, or to its weak electron-withdrawing capacity. Compound **23** lacked antimicrobial activity as well, even after carbamylation (**51**). Interestingly, compound **22**, with a 2-thiophenethio-moiety at C4, had modest (*M.cat.*) to good (Mtb) antimicrobial activity which was relatively unaffected by carbamylation (lactam **50**).

The antimicrobial activities of lactams with aryl groups substituted at C4, and linked to the lactam ring via an O or Se, were assessed (Scheme 1; Table 1, compounds **25**–**27**, **53**–**55**). With the exception of lactams **27** and **53**, none of these aryloxy lactams, even after carbamylation, possessed improved antimicrobial activity relative to their C4 arylthiol counterparts (Table 1, compounds **25** vs **12**; **26** vs **15**; **54** vs **43**; **55** vs **30**). It is likely that the absence of a sulfur or selenium atom at the phenyl substituent at C4 accounts for the lack of activity. Compound **53** has a nitro-substituted phenoxide at C4, while compound **27** has an arylseleno group at C4, and is unsubstituted at N1. Compared to oxygen, sulfur and seleniums are larger and have greater polarizability. This may explain why sulfur and selenium, in thiophenoxy and selenophenoxy—substituted at C4

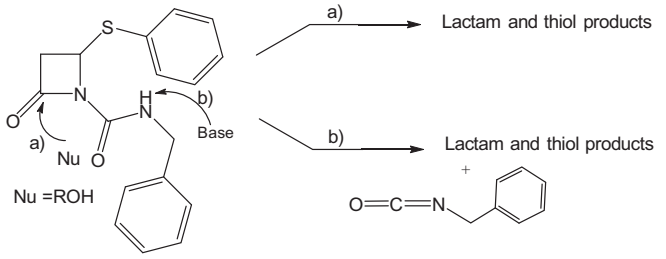
lactams, respectively, carry a larger share of the charge in the transition state, than oxygen carries in analogous oxygen isosters.[27] Thus, this size/polarizability differential appears to improve antimicrobial activity. This hypothesis is supported by the charges at X and C4 and bond lengths of C4-X (Table 4, X = O, S). When compared to the bond lengths and angles of all non-cyclic thioethers in the Cambridge Structural Database, the bond lengths and angles of the C4-thiophenyl β-lactams described here showed good agreement with the rest of the known non-cyclic thioethers (Table 4). A similar analysis showed that ethers and thioethers have bond angles of 116.71° and 102.37°, respectively.

Diminished antibacterial activity resulted from replacement of the *N*-carbamyl moiety with a carbonyl group (Table 2, lactam **30** vs **58**). The intrinsically high chemical reactivity of the lactam ring in this structure due to the presence of the carbonyl group at N1 may explain these findings. Consistent with this rationale, purification of lactam **58** proved difficult due to instability of the lactam ring.

The majority of the lactams having phenoxy-substituents at C4 lacked antimicrobial activity at therapeutic levels. However, the selenoxy-substituted lactams were bactericidal, even when lacking an EWG on the aromatic ring (Table 1, **27** and **55**). This suggests that the transition states for loss of thiophenoxide and especially for selenophenoxide ions from the thiophenoxy- and selenophenoxy-substituted at C4 lactams most likely occured later than those of comparable phenoxy derivatives. This may be a factor determining the antimicrobial activity of these compounds (Scheme 1).

The findings herein support two possible mechanisms for reaction of nucleophiles with arylthio lactams, unsubstituted at N1 (Scheme 2). In the first possible mechanism, an enzyme having a serine as a nucleophile hydrolyses the lactam ring; this is a well documented process (Scheme 2, mechanism a).[28,29] Alternatively, the thiophenol may be the microbicidal part of the molecule with the lactam moiety acting as the thiophenol carrier (Scheme 2, mechanism b). Due to the unsubstituted lactam nitrogen the first mechanism is unlikely to involve the active site serine of the transpeptidases, since these lactams lack the necessary prerequisites for binding to transpeptidases or β-lactamases, that is, a chargeable polar group at the lactam nitrogen.[1–6] Currently, only one monocyclic β-lactam having a phenylthioether at C4 and carbamylated at N1 has been reported to have activity against a pathogen (human cytomegalovirus).[30] As expected, none of the compounds herein were hydrolysed by penicillinase (data not shown). However, our compounds were hydrolyzed by sodium hydroxide with departure of the thiophenoxide from the lactam.[27]

Regardless of the mechanism, the presence of a EWG would be expected to increase the reactivity of the lactam. By increasing the

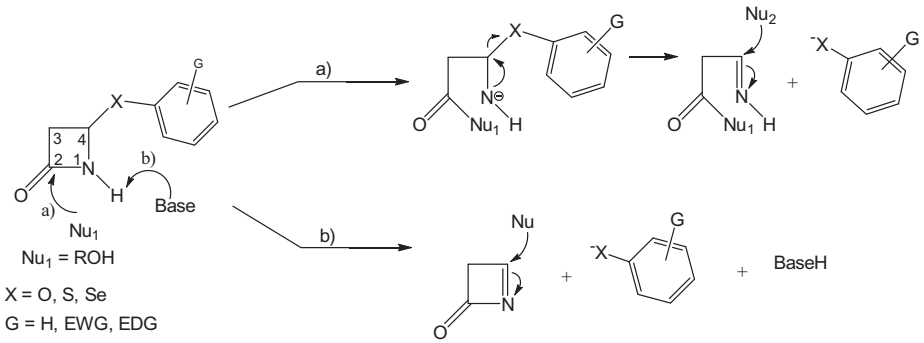


Scheme 3. Probable reaction pathways of the carbamylated at N1 arylthio-lactams. In addition to mechanisms (a) described in Scheme 2; mechanism (b) leads to generation of isocyanate.

electron deficiency on sulfur prior to departure, the EWG will cause the thioether at C4 to act as a leaving group upon deprotonation of the lactam hydrogen (Scheme 2, mechanism a). In the case of the lactam's 'classical' enzymatic hydrolysis (Scheme 2 mechanism b), the presence of the electron withdrawing group(s) will accelerate these reactions. When N1 is carbamylated mechanism b in Scheme 3 should also be considered in addition to the mechanisms described earlier (Scheme 2, mechanism a). As shown in Scheme 3, an isocyanate molecule could also be released, which would generate an electrophile; this in turn can bind to a second nucleophile (Scheme 2, similarly to mechanism b).

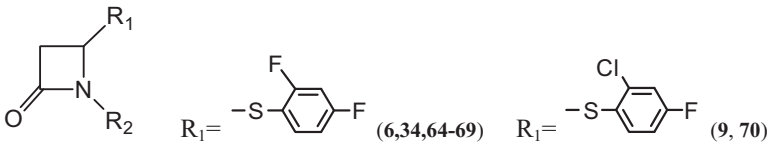
Compounds with various R_2 substituents at the lactam N1 were screened for antimicrobial activity to test the mechanism of action and determine which of our hypotheses were valid. The presence of a benzyl carbamyl group as the R_2 group increased the antimicrobial activity. This increase was most likely because carbamylation makes the lactam ring more electrophilic (Table 1, **2** vs **30**; **3–5** vs **31–33**; **15–16** vs **43–44**). However, the cumulative effect of the EWG at C4 and the benzyl carbonyl group at N1 did not always correlate with increased antimicrobial activity (Table 1, **6** vs **34**; **8–11** vs **36–39**; **27** vs **55**). Data indicate that if the electron-withdrawing ability of the substituent at C4 is sufficient for therapeutic levels of activity, carbamylation at N1, in general, did not increase activity. A comparable pattern occured when an EWG was attached to the arylthio moiety of the benzylcarbamyl group. However, if the substituent at C4 lacked sufficient electron-withdrawing capacity for the compound to be active, then the substituent at N1 contributed to the activity. This change in the antimicrobial activity is most likely the result of increased electrophilicity of the lactam ring.

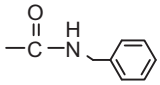
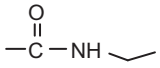
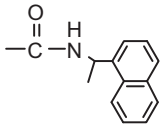
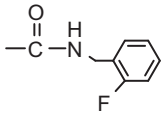
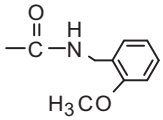
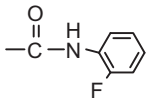
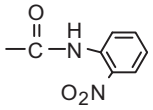
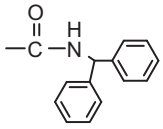
Compounds with alternate EWGs at N1 and minimal electron withdrawing capacity at C4 were subsequently synthesized and tested for antimicrobial activity. *M.cat.* and Mtb responded



Scheme 2. Possible reaction pathways of nucleophiles with the arylthio lactams, unsubstituted at N1. (a) 'Classical' β-lactam nucleophilic attack on the lactam carbonyl carbon; phenoxide, (X = O), or thiophenoxide (X = S) or selenophenoxide (X = Se) as a leaving group. (b) Deprotonation of the lactam nitrogen by a base, with departure of a suitable leaving group, that is, a phenoxide, thiophenoxide or selenophenoxide, followed by protonation to phenol, thiol, or selenol, respectively. The formation of an imine via pathway (a) as well as the formation of an acyliminium species, a possible Michael acceptor, in (b) allows for a 'double hit' mechanism by a second Nu, such as the active site cysteine in the Mtb glutaminase.

Table 3
Antimicrobial activity of fluorinated C4-arylthio-N1-carbamylated β-lactams against Mtb and *M.cat.* ($n = 6$)



#	R_2	*M.cat.* MIC/MBC (μg/ml)	Mtb MIC (μg/ml)
6	H	1.5/3.13	3.13
34		1.63/1.63	100
64		NT[c]	>100
65		>200/>200	>100
66		NT	50
67		NT	⩾100
68		NT	25
69		NT chemically unstable	NT chemically unstable
9	H	12.5/50	25
70		>200/>200	50

[a]The values for *M.cat.* are the results from the six isolates tested. The MIC/MBCs reported were within 2-fold dilutions for all isolates tested regardless of compound. The concentrations reported are the highest MIC/MBC measured for that compound.
[b]All compounds are tested as racemates.
[c] NT = not tested.

Table 4
Comparison of bond lengths and bond angles of β-lactams with C4 thioethers described here, with known non-cyclic thioethers and ethers

Compound	S–C(lac) bond lengths	S–C(R1) bond lengths	C–S–C bond angles
2	1.803	1.773	102.37
22	1.816	1.748	102.24
	1.819	1.750	102.65
	1.819	1.750	102.64
Thio-ethers[a]	1.789	1.777	102.37
Ethers[b]	1.380	1.418	116.71

[a] Average of all thio-ethers in CSD which do not contain a cyclic S-atom ($n = 7154$) from the Cambridge Structural Database.
[b] Average of all ethers in CSD which do not contain a cyclic O-atom ($n > 65{,}000$) from the Cambridge Structural Database.

differently to compounds with EWGs on the N1-carbamyl. In general, the introduction of a benzyl carbamyl group at N1 of the lactam demonstrated better antimicrobial activity than compounds with phenyl- or naphthyl- and even larger carbamyl groups at the N1 (Table 2, **30** vs **58**–**63**). Several of these lactams were chemically unstable (Table 2, compounds **58**; Table 3, **68** and **69**); therefore, the optimal substitution remains the benzyl carbamyl group (Table 1, **30** and **34**). Placing an EDG on the arylthio moiety of the benzyl carbamyl group at N1 does not alter the activity of the compound, as compared to its benzylcarbamylated counterpart (Table 3, **67** vs **34**).

4. Summary

Herein we describe the synthesis and characterization of C4-arylthio-β-lactams carbamylated at N1, a new family of antibacterial agents with specific activity against β-lactamase producing Mtb and *M.cat.* While the presence of a carbamyl substituent at N1 enhances activity, its presence is not necessary if there are EWGs on the arylthioether moiety at C4, or halogen atoms with sufficient electropositive potential for formation of a halogen bond

with a base within the active site. The incorporation of EWGs versus EDGs in the aromatic ring of the arylthio group at C4 led to the observation that F, Cl, and CF_3 substituents at *ortho* and/or *para* positions, in addition to di-substituted compounds at these positions, possess antibacterial activity within therapeutic range for novel compounds. The replacement of the urea moiety at N1 with a carbonyl group, or replacement of the aromatic ring at C4 with an allyl group, demonstrated diminished antibacterial activity. Based on our mechanistic investigations, and the lack of prerequisite groups for binding to the transpeptidase enzymes, these novel lactams likely have a different mechanism of action from the β-lactams in current use. Studies are ongoing to optimize the design and synthesis of the present series of C4-arylthio-, N1-carbamylated β-lactams.

5. Experimental

5.1. General chemistry methods

All air- or moisture-sensitive reactions were performed under argon or nitrogen atmosphere. Irradiation reactions were performed in a Discover LabMate from CEM (Weddington, NC). Reactions were monitored by thin-layer chromatography (TLC) using EM Reagent plates with fluorescence indicator (SiO_2-60, F_{254}; thickness 250 μm; EMD Chemicals, Inc., Gibbstown, NJ). Unless otherwise noted, the compounds were detected under UV light (254 nm) and iodine vapors.

General methods of compound purification involved flash chromatography by gradient elution from silica gel columns (60 Å, particle size 40–75 μm, Sorbent Technologies, Inc., Atlanta, GA), preparatory chromatography plates (silica G prep TLC plates with UV254, 20 × 20 nm glass backed, 1000 μm thickness, Sorbent Technologies, Inc., Atlanta, GA) and/or recrystallization.

Table 5
Organisms tested

Organism	Cell wall type	β-Lactamase production
Mycobacterium tuberculosis H37Rv	Acid fast	BlaC
Moraxella catarrhalis 6 clinical isolates	Gram negative	BRO-1/BRO-2
Staphylococcus aureus ATCC 25923	Gram positive	None
Escherichia coli ATCC 25922	Gram negative	None
Enterococcus faecalis ATCC 29212	Gram positive	None
Pseudomonas aeruginosa ATCC 27853	Gram negative	None
E. coli MISC 206	Gram negative	Broad spectrum β-lactamases
E. coli GB 8	Gram negative	Broad spectrum β-lactamases
E. coli MISC 262	Gram negative	ESBL[a]
Citrobacter freundii Citro 314	Gram negative	ESBL
Klebsiella pneumoniae MISC 304	Gram negative	AmpC β-lactamase
K. pneumoniae KLEB 249	Gram negative	AmpC β-lactamase
C. freundii CITRO 21	Gram negative	AmpC β-lactamase
Stenotrophomonas maltophilia GM24	Gram negative	Metallo β-lactamases
Acinetobacter baumannii L185	Gram negative	ESBL & Carbapenemase
Acinetobacter baumannii L186	Gram negative	ESBL & Carbapenemase
Acinetobacter baumannii L187	Gram negative	ESBL & Carbapenemase
K. pneumoniae/KPC L133	Gram negative	ESBL & Carbapenemase
K. pneumoniae/ESBL L174	Gram negative	ESBL & Carbapenemase
E. coli ESBL L108	Gram negative	ESBL & Carbapenemase
E. coli ESBL L109	Gram negative	ESBL & Carbapenemase

[a] ESBL = extended spectrum β-lactamase.

All compounds were characterized by ^1H and ^{13}C NMR spectra (25 °C). Spectra were obtained at 400 MHz for ^1H NMR and 125 MHz for ^{13}C NMR in $CDCl_3$ or acetone-d_6 (Bruker 400 spectrometer, Billerica, MA). Chemical shifts were reported in ppm (δ) relative to the residual solvent peak in the corresponding spectra; chloroform d 7.26 and d 77.23 and acetone d 2.05 coupling constants (J) are reported in hertz (Hz). Abbreviations used follow: s = singlet, br = broad singlet, d = doublet, dd = double of doublet, bd = broad doublet, ddd = doublet of doublet of doublet, t = triplet, dt = doublet of triplet, q = quartet, p = pentet, m = multiplet. In most cases, signals due to exchangeable protons were omitted.

IR spectra were obtained from thin films (NaCl plates) and from solid samples (KBr standard) using a Shimadzu FT-IR-8300 (Columbia, MD). Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA).

Melting points were determined using TA Instruments Q-2000 Differential Scanning Calorimeter (New Castle, DE connected to a cooling system). Samples (3.0 ± 0.2 mg) were heated or cooled at a scan rate of 10 °C/min while purged with 50 ml/min N_2. For compounds that did not recrystallize, temperatures during the first heating scan were reported.

Unless stated otherwise, solutions in organic solvents were dried with anhydrous magnesium sulfate, 37 °C and concentrated under vacuum conditions using rotator evaporation. Abbreviations: DCM = dichloromethane; DMF = dimethylformamide; ACN = acetonitrile; EtOAc = ethyl acetate. All compounds were >98% pure by elemental analysis. Anhydrous solvents, reagent grade solvents for chromatography and starting materials were obtained from various sources (Sigma–Aldrich, St. Louis, MO; Fisher Scientific, Pittsburgh, PA; Aldrich Chemical Co., Milwaukee, WI; Matrix Scientific, Columbia, SC; Acros Organics, Geel, Belgium).

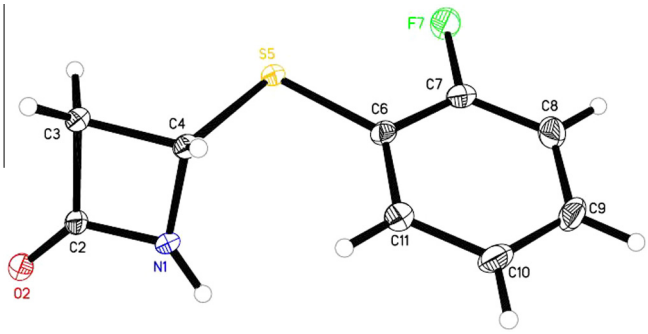
5.1.1. General procedure for synthesis of β-lactams containing aromatic thiols, phenol and benzeneselenol

Synthesis of compounds **2**–**28** was performed using procedures adapted from Grimm and co-workers[20] and Wasserman et al.[21] To a solution of 4-acetoxy-2-azetidinone **1** (1 g; 8 mmol in 50 ml acetone/water, 3:2) 1.05 mol equiv of thiophenols, phenol or benzeneselenol, respectively, were added. Sodium bicarbonate (4 mol equiv) was added and the mixture was stirred vigorously (12 h; closed round bottom flask). Sodium chloride was added to the solution until two layers were formed. The mixture was then filtered and extracted (EtOAc; 3 × 50 ml). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by a variety of methods (preparative HPLC; flash chromatography; and/or recrystallization).

5.1.1.1. 4-Phenylsulfanyl-azetidin-2-one (2). The crude product was crystallized from CH_2Cl_2 and hexanes to afford white crystals (62%) with mp 66–68 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.42 (5H, m), 6.1 (1H, br s), 5.02 (1H, dd, J = 2.3, 4.9), 3.38 (1H, ddd, J = 1.9, 4.9, 15.2), 2.9 (1H, ddd, J = 1.5, 2.3, 15.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.56, 133.60, 131.21, 129.43, 128.76, 54.20, 45.40. IR (neat) ν_{max} (C=O): 1766.7 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.

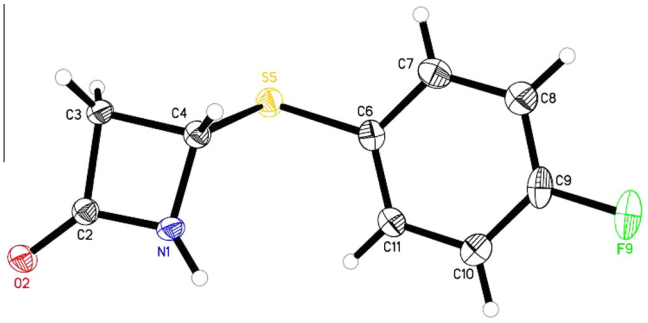
5.1.1.2. 4-(2-Fluoro-phenylsulfanyl)-azetidin-2-one (3). The product was purified by flash chromatography from ethyl acetate/hexanes to give white crystals (65%) yield with mp 101.83 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.30 (5H, m), 6.16 (1H, br s), 4.99 (1H, dd, J = 2.3, 5.0), 3.36 (1H, ddd, J = 2.4, 7.8, 15.7), 2.87 (1H, dd, J = 1.3, 7.8, 15.7).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.75, 164.02, 161.57, 136.30, 131.40, 125.06, 116.62, 114.89, 54.44, 46.04; IR (neat) ν_{max} (C=O) 1750 cm^{-1}; X-ray analyses.



5.1.1.3. 4-(3-Fluoro-phenylsulfanyl)-azetidin-2-one (4). The product was purified by flash chromatography from ethyl acetate/hexanes to give white crystals (65%) with mp 60.94 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (5H, m), 6.16 (1H, br s), 4.99 (1H, dd, J = 2.3, 4.9), 3.36 (1H, ddd, J = 1.8, 7.8, 15.7), 2.87 (1H, dd, J = 1.3, 7.8, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.95, 160.92, 130.93, 128.60, 119.92, 119.70, 115.67, 54.21, 45.81; IR (neat) ν_{max} (C=O) 1750 cm^{-1}. Anal. Calcd for $C_{17}H_{14}F_2N_2O_2S$: C, 58.61; H, 4.05; N, 8.04. Found: C, 58.29; H, 3.99; N, 8.07.

5.1.1.4. 4-(4-Fluoro-phenylsulfanyl)-azetidin-2-one (5). The product was purified by flash chromatography from ethyl acetate/hexanes to give white crystals (70%) with mp 76.67 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (2H, ddd, J = 0.5, 2.0, 8.9), 6.99 (2H, ddd, J = 0.5. 5.1, 8.9), 6.11 (1H, br s), 4.96 (1H, dd, J = 2.4, 4.8), 3.37 (1H, ddd, J = 1.8, 7.9, 15.7), 2.87 (1H, ddd, J = 1.6, 7.8, 15.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.73, 162.21, 136.63, 126.13, 116.59, 54.78, 45.28; IR (neat) ν_{max} (C=O) 1750 cm^{-1}. X-ray analysis.



5.1.1.5. 4-(2,4-Difluoro-phenylsulfanyl)-azetidin-2-one (6). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (70%) with mp 70–73 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (1H, dd, J = 0.5, 8.8), 6.85 (1H, ddd, J = 2.7, 5.1, 8.8), 6.78 (1H, ddd, J = 0.5, 2.7, 5.1), 6.13 (1H, br s), 4.96 (1H, dd, J = 2.3, 4.9), 3.42 (1H, ddd, J = 1.8, 8.5, 15.7), 2.29 (1H, ddd, J = 1.8, 7.8, 15.6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.43, 165.01, 161.90, 139.36, 112.31, 11.34, 104.88, 54.38, 45.55; IR (neat) ν_{max} (C=O) 1750 cm^{-1}. Anal. Calcd for $C_9H_7F_2NOS$: C, 50.23; H, 3.28; N, 6.51. Found: C, 50.32; H, 3.30; N, 6.35.

5.1.1.6. 4-(3,4-Difluoro-phenylsulfanyl)-azetidin-2-one (7). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (47.1%) with mp 74–77 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11–7.37 (3H, m), 6.17 (1H, br s), 5.00 (1H, dd, J = 2.3, 4.9), 3.64 (1H, ddd, J = 1.7, 7.8, 15.7), 2.90 (1H, ddd, J = 1.6, 7.8, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.53, 151.87, 149.89, 130.77, 127.39, 123.23, 118.33, 54.70, 45.45; IR (neat) ν_{max} (C=O) 1750 cm^{-1}. Anal. Calcd for $C_9H_7F_2NOS$: C, 50.23; H, 3.28; N, 6.51. Found: C, 50.15; H, 3.26; N, 6.45.

5.1.1.7. 4-Pentafluorophenylsulfanyl-azetidin-2-one (8). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (46%) with mp 80–82 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75 (1H, s), 4.91 (1H, dd, J = 2.3, 4.9), 3.40 (1H, ddd, J = 2.4, 7.9, 15.2), 2.92 (1H, ddd, J = 1.6, 7.9, 15.3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.81, 150.54, 148.98, 140.59, 136.05, 112.26, 51.63, 46.26; IR (neat) ν_{max} (C=O) 1755 cm^{-1}. Anal. Calcd for $C_9H_4F_5$ NOS: C, 40.16; H, 1.50; N, 5.20. Found: C, 40.36; H, 1.79; N, 4.97.

5.1.1.8. 4-(2-Chloro-4-fluoro-phenylsulfanyl)-azetidin-2-one (9). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (66%) mp 84–85 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (1H, dd, J = 0.5, 8.8), 7.20 (ddd, J = 0.5, 2.6, 5.5), 7.04 (1H, ddd, J = 2.6, 5.5, 8.8), 6.46 (1H, br s), 5.02 (1H, dd, J = 2.4, 4.8), 3.45 (1H, ddd, J = 1.8, 3.0, 8.5), 2.97 (1H, td, J = 1.8, 11.6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.65, 164.32, 161.78, 137.58, 126.45, 118.63, 115.09, 54.36, 45.81; IR (neat) ν_{max} (C=O) 1756.93 cm^{-1}. Anal. Calcd for $C_9H_7ClFNOS$: C, 46.66; H, 3.05; Cl, 15.30; F, 8.20; N, 6.05; O, 6.91; S, 13.84. Found: C, 46.97; H, 3.23; Cl, 15.51; F, 8.13; N, 6.22; O, 6.71; S, 13.64.

5.1.1.9. 4-(3-Chloro-4-fluoro-phenylsulfanyl)-azetidin-2-one (10) . The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (56%) with mp 97–98 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (1H, dd, J = 0.5, 2.5), 7.39 (1H, dd, J = 2.2, 8.9), 7.20 (1H, ddd, J = 0.5, 5.1, 8.6), 6.28 (1H, br s), 4.99 (1H, dd, J = 2.3, 4.9), 3.42 (1H, ddd, J = 2.3, 7.8, 15.4), 2.90 (1H, ddd, J = 1.2, 7.8, 15.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.54, 159.99, 136.29, 134.31, 130.82, 127.34, 121.99, 117.46, 54.58, 45.47; IR (neat) ν_{max} (C=O) 1761.81 cm^{-1} Anal. Calcd for C_9H_7ClF-NOS: C, 46.66; H, 3.05; Cl, 15.30; F, 8.20; N, 6.05; O, 6.91; S, 13.84. Found: C, 46.57; H, 3.28; Cl, 15.41; F, 8.27; N, 6.28; O, 6.86; S, 13.76.

5.1.1.10. 4-(2,4-Dichloro-phenylsulfanyl)-azetidin-2-one (11). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (50%) yield with mp 143–144 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (1H, dd, J = 0.5, 2.2), 7.43 (1H, dd, J = 2.2, 8.3), 7.29 (2H, dd, J = 0.5, 8.5), 6.32 (1H, br s), 5.70 (1H, dd, J = 2.3, 4.9), 3.50 (1H, ddd, J = 1.9, 8.5, 15.7), 3.02 (1H, ddd, J = 1.5, 7.9, 15.5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.32, 138.25, 135.49, 135.20, 130.35, 129.65, 127.97, 53.90, 46.02; IR (neat) ν_{max} (C=O) 1683.76 cm^{-1}. Anal. Calcd for C_9H_7 Cl_2NOS: C, 43.57; H, 2.84; Cl, 28.57; N, 5.65; O, 6.45; S, 12.92. Found: C, 43.77; H, 2.98; Cl, 28.73; N, 5.57; O, 6.37; S, 12.84.

5.1.1.11. 4-(4-Nitro-phenylsulfanyl)-azetidin-2-one (12). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (81%) with mp 124.03 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (2H, dd, J = 2.0, 8.4), 7.48 (2H, dd, J = 3.1, 8.5), 6.24 (1H, br s), 5.22 (1H, dd, J = 2.3, 4.9), 3.58 (1H, ddd, J = 1.7, 8.7, 15.7), 3.05 (1H, ddd, J = 1.5, 7.9, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.95, 130.93, 128.60, 119.92, 119.70, 115.88, 115.67, 54.21, 45.81; IR (neat) ν_{max} (C=O) 1700 cm^{-1}. Anal. Calcd for $C_9H_8N_2O_3S$: C, 48.21; H, 3.60; N, 12.49. Found: C, 48.16; H, 3.50; N, 12.39.

5.1.1.12. 4-((2-(Trifluoromethyl)phenyl)thio)azetidin-2-one (13). The product was purified by recrystallization in ethyl acetate/hexanes to give a yellow-white powder (90%) with mp 81–82 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, d, J = 7.6), 7.62 (1H, dd, J = 1.3, 7.5), 7.56 (1H, dd, J = 1.5, 7.8), 7.47 (1H, d, J = 7.1), 6.91 (1H, s), 5.03 (1H, dd, J = 2.3, 4.9), 3.44 (1H, ddd, J = 1.8, 7.9, 15.7), 2.95 (1H, ddd, J = 1.6, 7.9, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.26, 135.88, 132.63, 131.42, 128.80, 127.47, 55.05, 45.99; IR (neat) ν_{max} (C=O) 1766 cm^{-1}. Anal. Calcd for $C_{10}H_8F_3NOS$: C, 48.58; H, 3.26; N, 5.67. Found: C, 48.73; H, 3.39; N, 5.83.

5.1.1.13. 4-((3-(Trifluoromethyl)phenyl)thio)azetidin-2-one (14). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (83%) with mp 73–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (1H, d, J = 1.9), 7.67 (1H, dd, J = 1.8, 9.2), 7.62 (1H, dd, J = 7.7, 7.9), 7.51 (1H, dd, J = 1.9, 7.7), 6.80 (1H, s), 5.07 (1H, dd, J = 2.3, 4.9), 3.45 (1H, ddd, J = 1.9, 8.5, 15.7), 2.92 (1H, ddd, J = 1,6, 7.9, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.39, 136.32, 133.41, 131.95 (1C, q, J = 0.33), 130.03, 129.75 (1C, q, J = 0.03), 125.43 (1C, q, J = 0.03), 54.38, 45.83; IR (neat) ν_{max} (C=O) 1766 cm^{-1}. Anal. Calcd for $C_{10}H_8F_3NOS$: C, 48.58; H, 3.26; N, 5.67. Found: C, 48.65; H, 3.09; N, 5.63.

5.1.1.14. 4-((4-(Trifluoromethyl)phenyl)thio)azetidin-2-one (15). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (81%) with mp 95–96.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (2H, doublet, J = 8.2), 7.51 (2H, doublet, J = 8.1), 7.01 (1H, singlet), 5.11 (1H, dd, J = 2.3, 4.9), 3.47 (1H, ddd, J = 1.9, 3.0, 8.4), 2.95 (1H, ddd, J = 0.8, 1.4, 11.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.43, 137.57, 131.75, 130.13 (1C, q, J = 0.33), 126.34, 123.79, 53.78, 45.91; IR (neat) ν_{max} (C=O) 1764 cm^{-1}. Anal. Calcd for $C_{10}H_8F_3NOS$: C, 48.58; H, 3.26; N, 5.67. Found: C, 48.83; H, 3.29; N, 5.53.

5.1.1.15. 4-((3,5-Bis(trifluoromethyl)phenyl)thio)azetidin-2-one (16). The product was purified by recrystallization in ethyl acetate/hexanes to give white crystals (79%) with mp 77–79 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (2H, dd, J = 2.0, 8.3), 7.86 (2H, dd, J = 1.9, 8.3), 6.46 (1H, s), 5.17 (1H, dd, J = 2.3, 4.9), 3.55 (1H, ddd, J = 2.4, 8.3, 15.7), 3.00 (1H, ddd, J = 1.6, 7.9, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.04, 135.93, 132.77 (1C, q, J = 0.33), 132.11, 124.30, 122.11, 121.59, 54.22, 46.04; IR (neat) ν_{max} (C=O) 1771 cm^{-1}. Anal. Calcd for $C_{11}H_7F_6NOS$: C, 41.91; H, 2.24; N, 4.44. Found: C, 41.83; H, 2.08; N, 4.43.

5.1.1.16. 4-(2-Methoxy-phenylsulfanyl)-azetidin-2-one (17). The product was purified by recrystallization in hexane/ethyl acetate to give a white solid (87%) with mp 106–107 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43–6.88 (4H, m), 6.75 (1H, br s), 4.86 (1H, dd, J = 2.3, 4.9), 3.80 (3H, s), 3.28 (1H, ddd, J = 1.8, 8.5, 15.7), 2.93 (1H, ddd, J = 1.4, 8.5, 15.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.56, 160.74, 136.93, 120.82, 114.96, 55.54, 54.90, 44.99; IR (neat) ν_{max} (C=O) 1760 cm^{-1}. Anal. Calcd for $C_{10}H_{11}NO_2S$: C, 57.39; H, 5.30; N, 6.69. Found: C, 57.37; H, 5.19; N, 6.67.

5.1.1.17. 4-(3-Methoxy-phenylsulfanyl)-azetidin-2-one (18). The product was purified on a silica gel column (hexanes/ethyl acetate, 1:1; v/v) to give yellow oil in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29–6.87 (4H, m), 6.07 (1H, br s), 5.03 (1H, dd, J = 2.3, 4.9), 3.81 (3H, s), 3.39 (1H, ddd, J = 2.4, 7.9, 15.7), 2.91 (1H, dd, J = 1.4, 7.9, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.99, 160.01, 133.17, 130.30, 125.02, 118.24, 114.22, 55.46, 54.22, 45.41; IR (neat) ν_{max} (C=O) 1756 cm^{-1}. Anal. Calcd for $C_{10}H_{11}NO_2S$: C, 57.39; H, 5.30; N, 6.69. Found: C, 57.25; H, 5.34; N, 6.52.

5.1.1.18. 4-(4-Methoxy-phenylsulfanyl)-azetidin-2-one (19). The product was purified by recrystallization in hexanes/ethyl acetate to give a white solid (57%) with mp 83–85 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–6.86 (4H, m), 6.81 (1H, br s), 4.97 (1H, dd, J = 2.3, 4.9), 3.89 (3H, s), 3.37 (1H, ddd, J = 2.4, 8.8, 15.7), 2.9 (1H, dd, J = 1.9, 7.9, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.69, 159.16, 134.93, 130.47, 121.44, 111.47, 56.06, 54.06, 45.87; IR (neat) ν_{max} (C=O) 1756 cm^{-1}. Anal. Calcd for $C_{10}H_{11}NO_2S$: C, 57.39; H, 5.30; N, 6.69. Found: C, 57.20; H, 5.22; N, 6.58.

5.1.1.19. 4-(2,5-Dimethoxy-phenylsulfanyl)-azetidin-2-one (20). The product was purified by recrystallization in hexanes/ethyl acetate to give a white solid (51%) with mp 116–118 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–6.87 (4H, m), 6.46 (1H, br s), 5.01 (1H, dd, J = 2.3, 4.9), 3.78 (3H, s), 3.41 (1H, ddd, J = 2.4, 8.8, 15.7), 3.86 (3H, s), 2.98 (1H, ddd, J = 2.0, 8.8, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.26, 153.59, 153.19, 120.93, 120.13, 114.78, 112.22, 56.44, 55.83, 53.88, 45.73; IR (neat) ν_{max} (C=O) 1760 cm^{-1}. Anal. Calcd for $C_{11}H_{13}NO_3S$: C, 55.21; H, 5.48; N, 5.85. Found: C, 54.92; H, 5.36; N, 5.81.13.

5.1.1.20. 4-(3,4-Dimethoxy-phenylsulfanyl)-azetidin-2-one (21). The product was purified by recrystallization in hexanes/ethyl acetate to give a white powder (63%) with mp 79–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07–6.34 (4H, m), 6.36 (1H, br s), 4.71 (1H, dd, J = 2.3, 4.9), 3.68 (3H, s), 3.67 (3H, s), 3.10 (1H, ddd, J = 1.9, 8.4, 15.7), 2.64 (1H, ddd, J = 1.6, 8.3, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.20, 166.21, 150.12, 149.15, 128.05, 121.20, 117.74, 111.59, 60.41, 56.02, 55.91, 54.78, 44.89, 21.06, 14.19; IR (neat) ν_{max} (C=O) 1760 cm^{-1}. Anal. Calcd for $C_{11}H_{13}NO_3S$: C, 55.21; H, 5.48; N, 5.85. Found: C, 55.03; H, 5.40; N, 5.78.

5.1.1.21. 4-(Thiophen-2-ylsulfanyl)-azetidin-2-one (22). The crude product was crystallized from hexanes/methylene chloride to afford white crystals (90%) with mp 57–58 °C. ^{1}H NMR (400 MHz,$CDCl_3$): δ 7.48 (1H, dd, J = 1.2, 5.4), 7.23 (1H, dd, J = 1.2, 3.6), 7.07 (1H, dd, J = 3.6, 5.4), 6.19 (1H, br s), 4.86 (1H, dd, J = 2.3, 4.9), 3.31 (1H, ddd, J = 1.9, 4.9, 15.3), 2.91 (1H, ddd, J = 1.6, 2.0, 15.2); ^{13}C NMR (100 MHz, CDCl3) δ 165.52, 136.75, 131.82, 128.12, 127.49, 55.38, 44.72; IR (neat) ν_{max} (C=O) 1739.7.1 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.

5.1.1.22. 4-(4-Methylsulfanyl-phenylsulfanyl)-azetidin-2-one (23). The product was recrystallized from hexanes/ethyl acetate to give white fluffy powder (49%) with mp 60.16 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.27 (2H, dd, J = 2.0, 8.1), 7.0 (2H, dd, J = 2.0, 8.1), 6.07 (1H, br s), 4.97 (1H, dd, J = 2.6, 5.3), 3.36 (1H, ddd, J = 2.4, 7.6, 15.7), 2.88 (1H, ddd, J = 1.6, 7.8, 15.5), 2.50 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.12, 140.67, 134.80, 126.93, 125.89, 54.63, 45.36, 15.52; IR (neat) ν_{max} (C=O) 1700 cm^{-1}. Anal. Calcd for $C_{10}H_{11}NOS_2$: C, 53.30; H, 4.92; N, 6.22. Found: C, 53.33; H, 4.90; N, 6.16.

5.1.1.23. 4-(Naphthalen-1-ylsulfanyl)-azetidin-2-one (24). The product was purified by recrystallization in hexanes/ethyl acetate to give white crystals (73%) with mp 124.12 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (3H, m), 7.87–7.55 (4H, m), 6.175 (1H, br s), 5.14 (1H, dd, J = 2.5, 5.0), 3.45 (1H, ddd, J = 1.81, 7.9, 15.7), 3.00 (1H, ddd, J = 1.7, 7.8, 15.4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.42, 149.70, 133.63, 132.90, 132.79, 130.16, 129.14, 128.72, 127.81, 127.62, 126.97, 126.94, 54.29, 45.49; IR (neat) ν_{max} (C=O) 1755 cm^{-1}. Anal. Calcd for $C_{13}H_{11}NOS$: C, 68.10; H, 4.84; N, 6.11; O, 6.98; S, 13.98. Found: C, 68.35; H, 4.97; N, 6.19; O, 6.79; S, 13.78.

5.1.1.24. 4-(4-Nitro-phenoxy)-azetidin-2-one (25). Purified by recrystallization in hexanes/ethyl acetate to give a beige powder (68%) with mp 124–127 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (2H, dd, J = 2.8, 9.4), 6.97 (2H, dd, J = 3.1, 9.3), 6.56 (1H, br s), 5.81 (1H, dd, J = 2.3, 4.9), 3.49 (1H, ddd, J = 1.3, 2.0, 15.0), 3.2 (1H, ddd, J = 1.5, 3.9, 15.1); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 166.14, 162.45, 143.17, 126.74, 116.62, 77.40, 46.69; IR (neat) ν_{max} (C=O) 1775 cm^{-1}. Anal. Calcd for $C_9H_8N_2O_4$: C, 51.93; H, 3.87; N, 13.46. Found: C, 51.64; H, 4.02; N, 13.46.

5.1.1.25. 4-(4-Trifluoromethyl-phenoxy)-azetidin-2-one (26). The product was purified by recrystallization in hexanes/ethyl acetate to give a white powder (78%) with mp 95–98 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (2H, dd, J = 1.9, 8.7), 6.87 (2H, dd, J = 2.5, 8.6), 6.53 (1H, br s), 5.67 (1H, dd, J = 2.3, 5.0), 3.35 (1H, ddd, J = 1.4, 1.6, 15.0), 3.07 (1H, d, J = 1.6, 3.9, 15.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.46, 158.37, 127.36, 125.11, 124.78, 115.43, 76.23, 46.18; IR (neat) ν_{max} (C=O) 1780 cm^{-1}. Anal. Calcd for $C_{10}H_8F_3NO_2$: C, 51.96; H, 3.49; N, 6.06. Found: C, 51.91; H, 3.41; N, 5.97.

5.1.1.26. 4-Phenylselanyl-azetidin-2-one (27). The product was purified on silica gel (hexanes/ethyl acetate 1:1; v/v) to afford the compound as white crystals (50%) with mp 78–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.89 (5H, m), 6.33 (1H, br s), 5.14 (1H, dd, J = 2.3, 4.9), 3.45 (1H, ddd, J = 1.70, 7.8, 14.8), 2.97 (1H, ddd, J = 1.6, 7.8, 15.6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.91, 135.79, 131.52, 129.52, 129.23, 127.76, 47.29, 46.46; IR (neat) ν_{max} (C=O) 1749.95 cm^{-1}. Anal. Calcd for C_9H_9NOSe: C, 47.80; H, 4.01; N, 6.19; O, 7.07; Se, 34.92. Found: C, 47.92; H, 4.21; N, 6.39; O, 7.27; Se, 34.89

Lactams **28** (azetidin-2-one) and **29** (4-allylsulfanyl-azetidin-2-one) were purchased from Sigma–Aldrich (**28**, **29**).

5.1.2. General procedure for the synthesis of *N*-carbamoylazetidin-2-one derivatives (30–36, 40–54, 57, 58, 64, 69 from 2 to 29, respectively)

The synthetic procedure was adapted from Mulchande et al.[23]

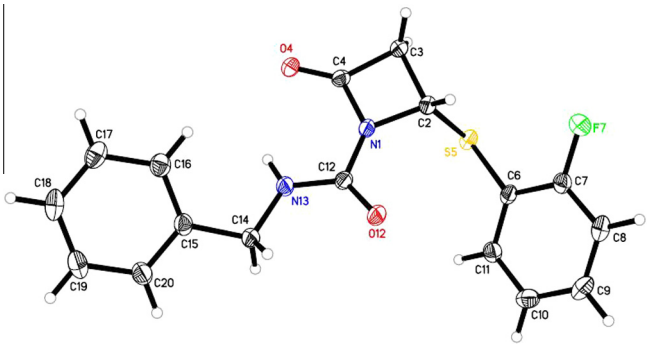
To a solution of appropriate azetidin-2-one 5 (1.7 g, 5.4 mmol) in DCM (5 ml) was added to 1.2 mol equiv of triethylamine and 1.2 mol equiv of the corresponding isocyanates: benzyl isocyanate, ethyl isocyanate, 2-nitrophenyl isocyanate. The reaction was stirred at room temperature and monitored by TLC. After completion of the reaction, the solution was evaporated under reduced pressure.

5.1.3. General procedure for the synthesis of *N*-carbamoylazetidin-2-one derivatives via irradiation (37–39, 55, 56, 59–63, 65–68, 70)

The synthetic procedure developed in our laboratory using a CEM microwave apparatus was applied to the aforementioned compounds: to a solution of an appropriate azetidin-2-one 5 (0.1 g, 0.46 mmol) in dichloromethane (4 ml), 2 mol equiv of triethylamine was added followed by addition of 1.1 mol equiv of the corresponding isocyanates: benzyl isocyanate, diphenylmethyl isocyanate, 9H-fluoren-9-yl isocyanate, 1-naphthyl isocyanate, 2-fluorobenzyl isocyanate, 2-fluorophenyl isocyanate, 2-methoxybenzyl isocyanate, diphenylethyl isocyanate, and 1-(1-naphthyl)ethyl isocyanate. The reaction was irradiated under-pressure in the microwave for 10–60 min at 300 W, and 35 °C and monitored by TLC and NMR.

5.1.3.1. 2-Oxo-4-phenylsulfanyl-azetidine-1-carboxylic acid benzylamide (30). The product was purified on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (73%) with mp 88.69 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (6H, m), 7.37 (1H, m), 7.13 (3H, m), 6.85 (1H, br s), 5.32 (1H, dd, J = 2.3, 4.9), 4.53 (1H, dd, J = 1.0, 17.0), 3.43 (1H, dd, J = 7.5, 15.7), 2.85 (1H, dd, J = 7.6, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.44, 149.63, 137.82, 135.27, 129.32, 129.30, 128.78, 127.73, 127.69, 127.70, 100.00, 56.69, 43.99, 43.69; IR (neat) ν_{max} (C=O) 1755 cm^{-1}. Anal. Calcd for $C_{17}H_{16}N_2O_2S$: C, 65.36; H, 5.16; N, 8.97; Found: C, 65.58; H, 5.45; N, 8.89.

5.1.3.2. 2-(2-Fluoro-phenylsulfanyl)-4-oxo-azetidine-1-carboxylic acid benzylamide (31). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (67%) with mp 89.79 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61–7.30 (3H, m), 7.18–7.12 (5H, m), 6.99 (1H, ddd, J = 1.4, 5.1, 8.3), 6.77 (1H, br s), 5.35 (1H, dd, J = 2.3, 4.9), 4.50 (2H, dd, J = 1.0, 17.0), 3.47 (1H, dd, J = 7.5, 16.1); 2.94 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.46, 162.00, 160.39, 149.64, 137.95, 137.77, 132.04, 128.82, 127.63, 116.49, 116.27, 56.29, 43.85, 40.74; IR (neat) ν_{max} (C=O) 1774, 1709 cm^{-1}. Anal. Calcd for $C_{17}H_{14}F_2N_2O_2S$: C, 61.80; H, 4.85; N, 8.45. Found: C, 61.95; H, 4.62; N, 8.45.

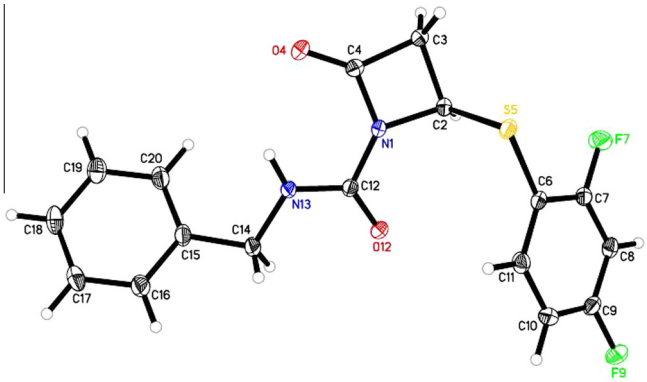


5.1.3.3. 2-(3-Fluoro-phenylsulfanyl)-4-oxo-azetidine-1-carboxylic acid benzylamide (32). The product is a clear yellow oil purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) in 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.32 (2H, m),7.16–7.10 (5H, m), 7.1 (1H, dd, J = 7.7, 8.3), 6.84 (1H, ddd, J = 1.8, 5.1, 8.3), 6.78 (1H br s), 5.33 (1H, dd, J = 2.3, 4.9), 4.52 (2H, dd, J = 1.0, 17.0), 3.46 (1H, dd, J = 7.5, 16.0), 2.94 (1H, dd, J = 7.5, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.44, 156.15, 149.77, 138.04, 132.60, 130.70, 130.13, 128.95, 127.84, 121.35, 121.13, 116.39, 116.18, 56.98, 44.47, 43.82; IR (neat) ν_{max} (C=O) 1774, 1709 cm^{-1}. Anal. Calcd for $C_{17}H_{14}F_2N_2O_2S$: C, 61.80; H, 4.58; N, 8.44. Found: C, 61.77; H, 4.49; N, 8.44.

5.1.3.4. 2-(4-Fluoro-phenylsulfanyl)-4-oxo-azetidine-1-carboxylic acid benzylamide (33). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (60%) with mp 89.71 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58–7.30 (5H, m), 7.15–7.10 (3H, m) 7.01–6.95 (2H, ddd, J = 2.68, 5.2, 8.9), 6.84 (1H, br s), 5.26 (1H, dd, J = 2.3, 4.9), 4.52 (2H, dd, J = 1.0, 17.0), 3.44 (1H, dd, J = 7.5, 16.0); 2.89 (1H, dd, J = 7.5, 15.6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.44, 165.01, 162.52, 149.79, 137.83, 128.96, 127.88, 124.72, 116.77, 116.56, 57.22, 48.36, 44.07; IR (neat) ν_{max} (C=O) 1774, 1709 cm^{-1}. Anal. Calcd for $C_{17}H_{14}F_2N_2O_2S$: C, 61.80; H, 4.58; N, 8.44. Found: C, 61.75; H, 4.49; N, 8.47.

5.1.3.5. 2-(2,4-Difluoro-phenylsulfanyl)-4-oxo-azetidine-1-carboxylic acid benzylamide (34). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1;

v/v) to afford the compound as white crystals (97%) with mp 134–136 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (1H, dd, J = 0.7, 8.8), 7.40–7.35 (2H, m) 7.20–7.14 (3H, m), 6.85–6.80 (1H,ddd, J = 2.7, 5.1, 8.8), 6.80–6.69 (1H, ddd, J = 0.7, 2.7, 5.1), 5.15 (1H, dd, J = 2.3, 4.9), 4.34 (1H, dd, J = 1.0, 16.9),3.32 (1H, dd, J = 7.5, 16.0), 2.79 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.24, 163.12, 162.51, 149.64, 139.30, 137.96, 128.84, 127.74, 112.53, 105.05, 56.59, 44.23, 43.84; IR (neat) ν_{max} (C=O) 1774, 1709 cm^{-1}. Anal. Calcd for $C_{17}H_{14}F_2N_2O_2S$: C, 58.61; H, 4.05; N, 8.04. Found: C, 58.53; H, 4.06; N, 8.03.



5.1.3.6. *N*-Benzyl-2-((3,4-difluorophenyl)thio)-4-oxoazetidine-1-carboxamide (35). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (97%) with mp 89.24 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49–7.35 (2H, m), 7.23 (1H, ddd, J = 0.6, 1.7, 5.1), 7.17–7.12 (4H, m) 6.90–7.10 (1H, ddd, J = 0.6, 5.1, 8.9), 6.75 (1H, br s), 5.17 (1H, dd, J = 2.3, 4.9), 4.43 (2H, dd J = 0.7, 17.0), 3.35 (1H, dd, J = 7.5, 16.0); 2.80 (1H, dd, J = 7.5, 15.7); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.28, 164.85, 162.36, 149.60, 137.76, 128.80, 127.74, 124.47, 116.55, 57.06, 43.70; IR (neat) ν_{max} (C=O) 1775, 1700 cm^{-1}. Anal. Calcd for $C_{17}H_{14}F_2N_2O_2S$: C, 58.61; H, 4.05; N, 8.04. Found: C, 61.77; H, 4.49; N, 8.44.

5.1.3.7. *N*-Benzyl-2-oxo-4-((perfluorophenyl)thio)azetidine-1-carboxamide (36). The product is a cloudy yellow oil purified by preparatory plate on silica gel (3:1 hexanes/ethyl acetate) to give 37.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.46 (2H, m), 7.15–7.10 (3H. m) 6.69 (1H, br s), 5.46 (1H, dd J = 2.3, 4.9), 4.5 (1H, dd, J = 0.7, 17.0), 3.60 (1H, dd, J = 7.6, J = 16.0), 2.99 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.11, 149.21, 128.78, 127.58, 77.36, 55.66, 43.76; IR (neat) ν_{max} (C=O) 1775, 1700 cm^{-1}. Anal. Calcd for $C_{17}H_{11}F_5N_2O_2S$: C, 50.75; H, 2.76; N, 6.96. Found: C, 50.96; H, 2.93; N, 6.61.

5.1.3.8. 2-(2-Chloro-4-fluoro-phenylsulfanyl)-4-oxo-azetidine-1-carboxylic acid benzylamide (37). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (43%) with mp116–119 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.35 (2H, dd, J = 1.4, 7.6), 7.26 (1H, d, J = 8.8),7.15–7.10 (3H, m) 6.97 (1H, dd, J = 2.8, 5.1), 6.87 (1H, dd, J = 5.1, 8.8), 6.82 (1H, br s), 5.36 (1H, dd, J = 2.3, 5.0), 3.48 (1H, dd, J = 0.7, 17.0), 3.51 (1H, dd, J = 7.5, 16.0), 2.98 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.14, 149.51, 138.62, 137.69, 128.78, 127.71, 117.92, 114.98, 56.91, 44.01, 44.73; IR (neat) ν_{max} (C=O) 1772.41, 1700.35 cm^{-1}. Anal. Calcd for $C_{17}H_{14}ClFN_2O_2S$: C, 55.97; H, 3.87; Cl, 9.72; F, 5.21; N, 7.68; O, 8.77; S, 8.79. Found: C, 56.17; H, 4.07; Cl, 9.81; F, 5.19; N, 7.83; O, 8.68; S, 8.67.

5.1.3.9. 2-(3-Chloro-4-fluoro-phenylsulfanyl)-4-oxo-azetidine-1-carboxylic acid benzylamide (38). The product is a yellow oil purified by preparatory plate on silica gel (3:1 hexanes/ethyl acetate) to give 37.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (1H, dd, J = 0.5, 2.6), 7.40–7.35 (2H, m), 7.30 (1H, dd, J = 2.5, 8.9), 7.15–7-10 (2H, m), 7.02 (1H, dd, J = 5.1, 8.7), 6.85 (1H, br s), 5.28 (1H, dd, J = 2.3, 5.0), 4.52 (2H, dd, J = 0.7, 17.0), 3.50 (1H, dd, J = 7.5, 16.0), 2.93 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.06, 160.14, 157.63, 149.54, 137.65, 137.14, 135.20, 128.82, 127.73, 126.51, 121.85, 121.66, 117.42, 57.32, 44.19, 43.74, 30.98; IR (neat) ν_{max} (C=O) 1774.60, 1704.76 cm^{-1}. Anal. Calcd for $C_{17}H_{14}ClFN_2O_2S$: C, 55.97; H, 3.87; Cl, 9.72; F, 5.21; N, 7.68; O, 8.77; S, 8.79. Found: C, 56.08; H, 3.80; Cl, 9.51; F, 5.17; N, 7.61; O, 8.73; S, 8.57.

5.1.3.10. 2-(2,4-Dichloro-phenylsulfanyl)-4-oxo-azetidine-1-carboxylic acid benzylamide (39). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (28%) with mp 95–97 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.40 (1H, d, J = 2.2), 7.25 (1H, dd J = 2.2, 8.5), 7.13 (1H, dd, J = 0.6, 8.5), 7.0 (5H, m), 6.73 (1H, br s), 5.29 (1H, dd, J = 2.5, 4.8), 4.39 (2H, dd, J = 0.7, 15.0), 3.44 (1H, dd, J = 7.5, 16.0), 2.91 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.08, 149.50, 138.63, 137.65, 136.75, 135.77, 130.03, 128.91, 128.79, 127.86, 127.72, 56.62, 44.25, 43.74; IR (neat) ν_{max} (C=O) 1774.53, 1705.07 cm^{-1}. Anal. Calcd for $C_{17}H_{14}Cl_2N_2O_2S$: C, 53.55; H, 3.70; Cl, 18.60; N, 7.35; O, 8.39; S, 8.41. Found: C, 53.78; H, 3.82; Cl, 18.57; N, 7.48; O, 8.31; S, 8.39.

5.1.3.11. *N*-Benzyl-2-((4-nitrophenyl)thio)-4-oxoazetidine-1-carboxamide (40). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (76%) with mp 123.04 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (2H, dd, J = 2.0, 8.4), 7.65 (2H, dd, J = 3.1, 8.5), 7.0 (5H, m), 6.82 (1H, br s), 5.42 (1H, dd, J = 2.3, 4.9), 4.42 (2H, dd, J = 0.7, 15.0), 3.59 (1H, dd J = 5.7, 16.0), 3.0 (1H, dd J = 5.7, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.94, 149.69, 147.22, 141.97, 137.61, 131.59, 129.00, 127.98, 127.84, 124.25, 56.80, 45.12, 43.98; IR (neat) ν_{max} (C=O) 1775, 1700 cm^{-1}. Anal. Calcd for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.43; H, 4.37; N, 12.01.

5.1.3.12. *N*-Benzyl-2-oxo-4-((2-(trifluoromethyl)phenyl)thio)-azetidine-1-carboxamide (41). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as viscous oil in 66% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (1H, d, J = 7.5), 7.67 (1H, d, J = 1.3, 7.6), 7.25 (2H, m); 7.0 (5H, m), 6.76 (1H, s), 5.28 (1H, dd, J = 2.3, 5.0), 4.37 (2H, dd J = 0.7, 15.0), 3.42 (1H, dd, J = 7.5, 16.0), 2.87 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.49, 149.77, 137.92, 137.69, 134.46 (1C, q, J = 0.3), 132.54, 130.49, 129.23, 128.95, 127.86, 127.81, 127.22 (1C, q, J = 0.05), 58.18, 44.62, 43.87; IR (neat) ν_{max} (C=O) 1778, 1706 cm^{-1}. Anal. Calcd for $C_{18}H_{15}F_3N_2O_2S$: C, 56.84; H, 3.97; N, 7.36; Found: C, 56.92; H, 4.03; N, 7.45.

5.1.3.13. *N*-Benzyl-2-oxo-4-((3-(trifluoromethyl)phenyl)thio)-azetidine-1-carboxamide (42). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as viscous oil in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (1H, m), 7.74 (1H, ddd, J = 1.6, 1.8, 7.9), 7.54 (2H, ddd, J = 1.0, 7.7, 7.9), 7.18–7.29 (2H, m), 6.72 (1H, br s), 5.26 (1H, dd, J = 2.3, 4.9), 4.41 (1H, dd, J = 0.7, 15.0), 3.41 (1H, dd, J = 7.5, 16.0), 2.82 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.20, 149.71, 137.79, 137.74, 132.13, 44.57, 131.74 (1C, q, J = 0.32), 131.14, 129.88, 128.95, 127.88, 127.85, 125.90, 57.21,

43.91; IR (neat) ν_{max} (C=O) 1777, 1706 cm^{-1}. Anal. Calcd for $C_{18}H_{15}F_3N_2O_2S$: C, 56.84; H, 3.97; N, 7.36; Found: C, 57.01; H, 4.02; N, 7.46.

5.1.3.14. *N*-Benzyl-2-oxo-4-((4-(trifluoromethyl)phenyl)thio)-azetidine-1-carboxamide (43). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (64%) with mp72–73 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (1H, dd, J = 2.0, 8.2), 7.60 (1H, dd, J = 1.9, 8.3), 7.39 (5H, m), 6.87 (1H,br s), 5.41 (1H, dd, J = 2.3, 4.9), 4.52 (2H, dd, J = 0.7, 15.0), 3.57 (1H, dd, J = 7.5, 16.0), 2.99 (1H, dd, J = 7.9, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.26, 149.75, 137.81, 136.34, 133.38, 30.62 (1C, q, J = 0.33), 127.87, 126.20, 57.01, 44.86, 127.93, 128.98, 143.91; IR (neat) ν_{max} (C=O) 1778, 1708 cm^{-1}. Anal. Calcd for $C_{18}H_{15}F_3N_2O_2S$: C, 56.84; H, 3.97; N, 7.36; Found: C, 56.98; H, 4.01; N, 7.39.

5.1.3.15. *N*-Benzyl-2-((3,5-bis(trifluoromethyl)phenyl)thio)-4-oxoazetidine-1-carboxamide (44). The product was purified by flash chromatography on silica gel (hexanes/ethyl acetate, 1:1; v/v) to afford the compound as white crystals (65%) with mp 61–62 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (1H, m), 7.77 (1H, m), 7.19–7.30 (5H, m), 6.72 (1H, br s), 5.33 (1H, dd, J = 2.3, 4.9), 4.36–4.46 (2H, m), 3.53 (1H, dd, J = 7.5, 16.0), 2.91 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.80, 149.62, 137.61, 135.40, 133.26, 132.52 (2C, q, J = 0.33), 128.97, 127.94, 127.88, 124.40, 122.45, 121.68, 57.49, 44.83, 43.99; IR (neat) ν_{max} (C=O) 1780, 1707 cm^{-1}. Anal. Calcd for $C_{19}H_{14}F_6N_2O_2S$: C, 50.89; H, 3.15; N, 6.25; Found: C, 50.93; H, 3.42; N, 6.41.

5.1.3.16. *N*-Benzyl-2-((2-methoxyphenyl)thio)-4-oxoazetidine-1-carboxamide (45). The product was purified by recrystallization in hexane/ethyl acetate to give a white powder (90%) with mp 105–107 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (1H, dd, J = 1.7, 15.0), 7.20 (1H, ddd, J = 1.7, 7.2, 8.0), 7.0 (5H, m), 6.87 (1H, ddd, J = 1.3, 7.2, 9.1), 6.68 (1H, dd, J = 1.3, 8.0), 5.38 (1H, dd, J = 2.3, 4.9), 4.26 (2H, m), 3.86 (3H, s), 3.40 (1H, dd, J = 7.5, 16.0), 2.94 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.70, 159.93, 149.61, 137.88, 137.28, 131.25, 128.74, 127.75, 127.64, 121.25, 117.37, 111.22, 55.81, 43.94, 43.65; IR (neat) ν_{max} (C=O) 1627, 1576 cm^{-1}. Anal. Calcd for $C_{18}H_{18}N_2O_3S$: C, 63.14; H, 5.30; N, 8.18. Found: C, 63.40; H, 5.28; N, 8.32.

5.1.3.17. *N*-Benzyl-2-((3-methoxyphenyl)thio)-4-oxoazetidine-1-carboxamide (46). The product purified via a flash column chromatography on silica gel with a ratio gradient of 3:1 hexanes and dichloromethane to produce a colorless oil with a 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25–7.33 (3H, m), 7.7 (1H, ddd, J = 1.7, 2.1, 8.1), 7.0 (5H, m), 5.19 (1H, dd, J = 2.3, 4.9), 3.68 (3H, s), 3.22 (1H, dd, J = 7.0, 16.0), 2.78 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.53, 159.87, 149.63, 137.79, 130.47, 130.04, 128.78, 127.74, 127.13, 120.01, 115.34, 56.60, 55.35, 44.01, 43.69; IR (neat) ν_{max} (C=O) 1775, 1700 cm^{-1}. Anal. Calcd for $C_{18}H_{18}N_2O_3S$: C, 63.14; H, 5.30; N, 8.18. Found: C, 63.57; H, 5.51; N, 8.05.

5.1.3.18. *N*-Benzyl-2-((4-methoxyphenyl)thio)-4-oxoazetidine-1-carboxamide (47). The compound was purified by recrystallization in hexanes/ethyl acetate to give a white powder (58%) with mp 105–110 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (2H, d, J = 9.0), 7.2 (2H, d, J = 8.9), 6.98 (5H, m), 5.21 (1H, dd, J = 2.3, 4.9), 4.27 (2H, m), 3.82 (3H, s), 3.36 (1H, dd, J = 7.5, 16.0), 2.86 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.66, 165.53, 160.88, 137.95, 128.76, 127.78, 127.67, 118.74, 114.83, 56.72, 55.35, 43.64, 43.39; IR (neat) ν_{max} (C=O) 1775, 1700 cm^{-1}. Anal. Calcd for $C_{18}H_{18}N_2O_3S$: C, 63.14; H, 5.30; N, 8.18. Found: C, 63.34; H, 5.13; N, 8.18.

5.1.3.19. *N*-Benzyl-2-((2,5-dimethoxyphenyl)thio)-4-oxoazetidine-1-carboxamide (48). The compound was purified by recrystallization in hexanes/ethyl acetate to give a white powder (88%) with mp 87.5–88.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (5H, m), 6.87 (2H, m), 6.74 (1H, d, J = 2.42), 5.21 (1H, dd, J = 2.6, 5.0), 4.27 (2H, m), 3.75 (3H, s), 3.75 (3H, s), 3.36 (1H, dd, J = 7.5, 16.0), 2.86 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.75, 128.63, 127.74, 127.64, 127.49, 121.90, 116.30, 112.16, 56.35, 55.77, 44.04, 43.67; IR (neat) ν_{max} (C=O) 1775, 1705 cm^{-1}. Anal. Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52. Found: C, 61.04; H, 5.39; N, 7.47.

5.1.3.20. *N*-Benzyl-2-((3,4-dimethoxyphenyl)thio)-4-oxoazetidine-1-carboxamide (49). The compound was purified by preparatory plate on silica gel (3:1 hexanes/ethyl acetate) to give a clear oil in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.48–6.84 (4H, m), 5.21 (1H, dd, J = 2.65, 2.91), 3.82 (3H, s), 3.36 (1H, dd, J = 5.61, 10.69), 2.86 (1H, dd, J = 2.67, 13.65); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.59, 150.38, 149.68, 149.03, 137.93, 129.29, 128.73, 127.75, 127.64, 119.13, 118.98, 118.77, 111.37, 56.74, 55.88, 43.58, 43.37; IR (neat) ν_{max} (C=O) 1775, 1705 cm^{-1}. Anal. Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52. Found: C, 61.54; H, 5.56; N, 7.43.

5.1.3.21. *N*-Benzyl-2-oxo-4-(thiophen-3-ylthio)azetidine-1-carboxamide (50). The compound was purified by preparatory plate on silica gel (3:1 hexanes/ethyl acetate) to give a clear oil 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (1H, d, J = 5.2), 7.12 (1H, dd, J = 3.7, 5.2), 6.95 (5H, m), 6.75 (1H, dd, J = 1.3, 3.6), 6.68 (1H, br s), 5.05 (1H, dd, J = 2.3, 4.9), 4.42 (2H, m), 3.25 (1H, dd, J = 7.5, 16.0), 2.85 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.23, 149.48, 138.16, 137.78, 132.52, 128.15, 128.75, 127.77, 127.69, 125.34, 56.49, 43.71, 43.11, 30.98, 29.73; IR (neat) ν_{max} (C=O) 1770, 1700 cm^{-1}. Anal. Calcd for $C_{15}H_{14}N_2O_2S_2$: C, 56.58; H, 4.43; N, 8.80. Found: C, 56.72; H, 4.65; N, 8.92.

5.1.3.22. *N*-Benzyl-2-((4-(methylthio)phenyl)thio)-4-oxoazetidine-1-carboxamide (51). The compound was purified by preparatory plate on silica gel (3:1 hexanes/ethyl acetate) to give a clear oil in 66% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.47 (2H, d, J = 8.1), 7.15 (2H, d, J = 8.1), 7.0 (5H, m), 6.84 (1H, br s), 5.26 (1H, dd, J = 2.3, 5.0), 4.53 (2H, dd, J = 0.7, 15.0), 3.42 (1H, dd, J = 7.5, 16.0), 2.89 (1H, dd, J = 7.5, 16.0), 2.49 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.43, 149.65, 141.11, 137.99, 135.93, 128.77, 127.67, 124.67, 56.76, 43.79, 43.64, 15.20; IR (neat) ν_{max} (C=O) 1700, 1780 cm^{-1}. Anal. Calcd for $C_{18}H_{18}N_2O_2S$: C, 60.31; H, 5.06; N, 7.81. Found: C, 60.50; H, 5.05; N, 7.89.

5.1.3.23. *N*-Benzyl-2-(naphthalen-1-ylthio)-4-oxoazetidine-1-carboxamide (52). The compound was purified by preparatory plate on silica gel (3:1 hexanes/ethyl acetate) to give an opaque oil in 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80–7.50 (7H, m), 7.0 (5H, m), 6.87 (1H, br s), 5.40 (1H, dd, J = 2.3, 4.9), 4.56 (2H, dd, J = 1.0, 15.0), 3.42 (1H, dd, J = 7.5, 16.0), 2.95 (1H, dd, J = 7.5, 15.0); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.42, 149.70, 135.11, 137.92, 133.54, 133.20, 131.55, 128.96, 128.82, 127.86, 127.81, 127.78, 127.71, 127.16, 126.80, 126.63, 56.72, 44.09, 43.71, 29.76; IR (neat) ν_{max} (C=O) 1780, 1700 cm^{-1}. Anal. Calcd for $C_{21}H_{18}N_2O_2S$: C, 69.59; H, 5.01; N, 7.73; Found: C, 69.79; H, 5.30; N, 7.55.

5.1.3.24. *N*-Benzyl-2-(4-nitrophenoxy)-4-oxoazetidine-1-carboxamide (53). The compound was purified by recrystallization in hexanes/ethyl acetate to give yellow crystals in 72% yield with mp 123–124 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (2H, d,

J = 9.2); 7.58 (2H, d, J = 9.3), 7.0–7.1 (5H, m), 6.86 (1H, br s), 6.18 (1H, dd, J = 1.5, 3.6), 4.51 (2H, d, J = 0.7, 15.0), 3.58 (1H, dd, J = 1.5, 16.0), 3.25 (1H, d, J = 1.5, 16.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.73, 161.08, 149.38, 143.04, 137.31, 128.84, 127.84, 127.69, 125.89, 116.65, 78.12, 45.54, 43.84; IR (neat) ν_{max} (C=O) 1715, 1775 cm^{-1}. Anal. Calcd for $C_{17}H_{15}N_3O_5$: C, 59.82; H, 4.43; N, 12.31. Found: C, 59.67; H, 4.53; N, 12.20.

5.1.3.25. *N*-Benzyl-2-oxo-4-(4-(trifluoromethyl)phenoxy)azetidine-1-carboxamide (54). The compound was purified by recrystallization in hexanes/ethyl acetate to give a white powder in 70% yield with mp 103–109 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (2H, d, J = 8.5), 7.17–7.31 (7H, m), 6.79 (1H, br s), 6.04 (1H, dd, J = 1.5, 3.6), 4.43 (2H, d, J = 1.0, 15.0), 3.44 (1H, dd, J = 1.5, 16.0), 3.11 (1H, dd, J = 1.6, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.05, 158.68, 149.47, 137.42, 128.82, 127.78, 127.69, 127.19, 127.08, 125.34, 122.78, 116.76, 78.27, 45.52, 43.80; IR (neat) ν_{max} (C=O) 1690, 1780 cm^{-1}. Anal. Calcd for $C_{18}H_{15}F_3N_2O_3$: C, 59.34; H, 4.15; N, 7.69. Found: C, 59.31; H, 4.37; N, 7.63.

5.1.3.26. *N*-Benzyl-2-oxo-4-(phenylselanyl)azetidine-1-carboxamide (55). The compound was purified by recrystallization in hexanes/ethyl acetate to give a white powder in 30% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.89 (5H, m), 7.0 (5 h, m), 6.33 (1H, br s), 5.14 (1H, dd, J = 2.3, 4.9), 4.27 (2H, m), 3.45 (1H, ddd, J = 1.7, 7.8, 14.8), 2.97 (1H, ddd, J = 1.6, 7.8, 15.6).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.49, 149.60, 137.84, 136.83, 129.35, 128.78, 127.72, 127.32, 126.32, 125.05, 48.15, 44.66, 43.69, 14.24; IR (neat) ν_{max} (C=O) 1771.21, 1701.02 cm^{-1}. Anal. Calcd for $C_{17}H_{16}N_2O_2Se$: C, 56.83; H, 4.49; N, 7.80; O, 8.91; Se, 21.98. Found: C, 56.97; H, 4.69; N, 7.73; O, 8.74; Se, 22.07.

5.1.3.27. *N*-Benzyl-2-oxoazetidine-1-carboxamide (56). The synthesis was performed as developed in our laboratory. To a solution of the appropriate azetidin-2-one **5** (0.1 g, 0.46 mmol in dichloromethane; 4 ml), was added 1.2 mol equiv of triethylamine and 1.1 mol equiv of the corresponding isocyanate (2-fluorobenzyl isocyanate, 2-fluorophenyl isocyanate, or 2-methoxybenzyl isocyanate). The reaction was irradiated under pressure (CEM microwave, 10–60 min, 300 W, 35 °C) and monitored by TLC and NMR. After 10 min of irradiation, the product was confirmed by NMR. Upon evaporation the product proved unstable within 24 h. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.13–7.28 (1H, m), 4.39 (1H, d, J = 6.0), 3.55 (1H, t, J = 4.7), 2.95 (1H, t, J = 4.7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.05, 150.62, 137.94, 128.50, 128.21, 43.68, 37.23, 36.07, 30.07; IR (neat) ν_{max} (C=O) 1760, 1700 cm^{-1}.

5.1.3.28. 2-(Allylthio)-*N*-benzyl-4-oxoazetidine-1-carboxamide (57). The compound yield obtained was 33%; however, it was unstable and appears to undergo a [2+2] cycloreversion. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27–7.36 (9H, m), 6.90 (1H, br s), 5.88 (1H, m), 5.33 (2H, d, J = 16.0), 5.19 (1H, d, J = 9.9), 4.50 (2H, s), 3.92 (1H, dd, J = 4.0, 9.0), 3.42 (2H, ddd, J = 10.1, 5.3, 2.0), 2.92 (1H, d, J = 16.3).

5.1.3.29. 1-Hex-5-ynoyl-4-phenylsulfanyl-azetidin-2-one (58). The compound was purified by extraction with hexanes from the reaction mixture to give yellow oil in 10% yield. However, it was prone to hydrolysis on silicagel.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.56 (6H, m), 5.25 (2H, dd, J = 2.9, 3. 2), 3.43 (2H, dd, J = 6.1, 10.5), 2.93 (2H, dd, J = 13.5, 3.2), 2.85 (2H, dt, J = 4.4, 2.8), 2.60 (1H, t, J = 7.2), 2.54 (2H, t, J = 7.4), 2.19–2.34 (1H, m), 2.25 (1H, dt, J = 2.5, 4.2), 2.01 (2H, t, J = 2.6), 1.92 (2H, dt, J = 1.6, 5.5).

^{13}C NMR (100 MHz, CDCl3) δ 169.78, 163.40, 135.19, 129.40, 127.30, 83.18, 69.34, 55.60, 43.76, 35.29, 31.00, 23.30, 22.49, 22.20, 17.77; IR (neat) ν_{max} (C=O) 1700, 1795 cm^{-1}.

5.1.3.30. 2-Oxo-4-phenylsulfanyl-azetidine-1-carboxylic acid benzhydryl-amide (59). To a solution of **2** (0.107 g, 0.596 mmol) and diphenylmethyl isocyanate (0.116 ml, 0.615 mmol) in methylene chloride (5 ml) was added triethylamine (85.5 μL, 0.614 mmol). The solution was microwave irradiated under pressure at 70 °C for 20 min. The solution was washed with 5% HCl (10 ml aliquots, 3 times) and the product was purified by column chromatography with a linear mobile phase gradient (10:1 methylene chloride/ethyl acetate to 1:10 methylene chloride/ethyl acetate). This procedure yielded product **7** (0.19 g, 86.3%) as a light brown gel. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (2H, dt, J = 1.3, 7.2), 7.35 (5H, m), 7.27 (4H, dt, J = 2.4, 7.4), 7.21 (4H, dd, J = 1.3, 7.4), 7.07 (1H, m), 5.99 (1H, br s), 5.29 (1H, dd, J = 2.3, 4.9), 3.44 (1H, dd, J = 7.5, 15.9), 2.91 (1H, dd, J = 7.5, 16.0).

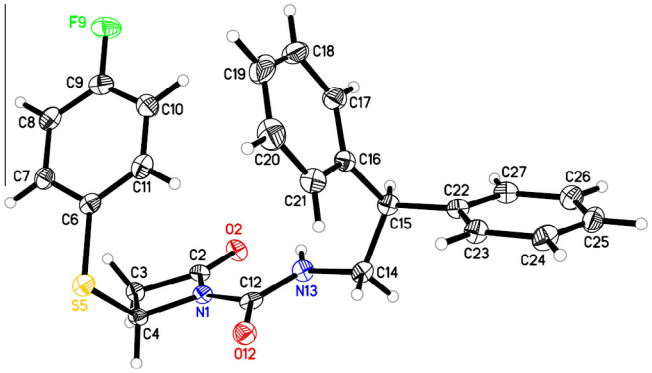
5.1.3.31. 2-Oxo-4-phenylsulfanyl-azetidine-1-carboxylic acid (9*H*-fluoren-9-yl)-amide (60). To a solution of **2** (0.119 g, 0.664 mmol) and 9*H*-fluoren-9-yl isocyanate (0.141 g, 0.798 mmol) in methylene chloride (5 ml) was added triethylamine (95.3 μL, 0.683 mmol). The solution was microwave irradiated under pressure at 70 °C for 15 min. The solution was washed with 5% HCl (10 ml aliquots, 3 times) and the product was purified by column chromatography with a linear mobile phase gradient (10:1 methylene chloride/ethyl acetate to 1:10 methylene chloride/ethyl acetate). This procedure yielded the product as a yellow solid (0.22 g, 89.7%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (2H, dd, J = 1.4, 7.7), 7.36–7.31 (4H, m), 7.30–7.24 (5H, m), 7.18 (1H, m), 7.07 (1H, m), 6.92 (2H, ddd, J = 1.4, 7.3, 7.7), 5.18 (1H, dd, J = 2.3, 4.9), 3.19 (1H, dd, J = 7.5, 16), 2.67 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.16, 141.66, 141.36, 138.31, 133.07, 127.14, 127.05, 126.69, 125.63, 122.86, 117.89, 54.56, 52.55, 41.81. Anal. Calcd for $C_{23}H_{18}N_2O_2S$: C, 71.48; H, 4.69; N, 7.25; O, 8.28; S, 8.30. Found: C, 71.64; H, 4.68; N, 7.43; O, 8.47; S, 8.51.

5.1.3.32. 2-Oxo-4-phenylsulfanyl-azetidine-1-carboxylic acid naphthalen-1-ylamide (61). To a solution of **2** (0.139 g, 0.775 mmol) and 1-naphthyl isocyanate (0.115 ml, 0.679 mmol) in methylene chloride (5 ml) was added triethylamine (0.111 ml, 1.09 mmol). The solution was irradiated using CEM microwave at 70° C for 20 min. The solution was then washed with 5% HCl (10 ml aliquots, 3 times). The resulting organic layer was evaporated then purified by column chromatography with a linear mobile phase gradient (10:1 methylene chloride/ethyl acetate to 1:10 methylene chloride/ethyl acetate). This procedure yielded the product as a clear, colorless gel (0.21 g, 82.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.02 (2H, dd, J = 1.1, 8.0), 7.92 (1H, d, J = 1.5, 8.1), 7.71 (2H, dd, J = 1,2, 8.1), 7.54 (1H, dd, J = 1.5, 8.0), 7.51 (2H, dd, J = 1.5, 8.0), 7.34 (2H, dt, J = 1.5, 7.6), 7.25 (2H, m), 7.23 (2H, d, J = 8.0), 5.25 (1H, dd, J = 2.3, 4.9), 3.46 (1H, dd, J = 7.5, 16.0), 2.91 (1H, dd, J = 7.5. 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.30, 147.45, 135.33, 134.01, 131.69, 129.44, 129.34, 128.83, 126.66, 126.18, 125.86, 125.73, 125.20, 120.14, 118.59, 57.24, 44.15. Anal. Calcd for $C_{20}H_{16}N_2O_2S$: C, 68.95; H, 4.63; N, 8.04; O, 9.18; S, 9.20. Found: C, 69.07; H, 4.78; N, 7.94; O, 9.11; S, 9.12.

5.1.3.33. *N*-Benzhydryl-2-((4-fluorophenyl)thio)-4-oxoazetidine-1-carboxamide (62). The compound was purified by preparatory plate on silica gel (3:1 hexanes/ethyl acetate) to give a waxy solid in 28% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (2H, dt, J = 1.3, 7.1), 7.36 (2H, dd, J = 2.0, 8.9), 7.25 (4H, m), 7.23 (4H, d, J = 8.0), 6.87 (1H, m), 6.41 (1H, br s), 5.09 (1H, dd, J = 2.3, 4.9), 3.50 (1H, dd, J = 7.5, 16.0), 3.13 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.48, 164.87, 162.38, 148.82, 141.31, 140.93, 137.91, 128.80, 127.76, 127.28, 124.20, 116.48, 60.44, 57.15, 43.99, 29.75; IR (neat) ν_{max} (C=O) 1770.42, 1708.96 cm^{-1}. Anal. Calcd for

$C_{23}H_{19}FN_2O_2S$: C, 67.96; H, 4.71; F, 4.67; N, 6.89; O, 7.87; S, 7.89. Found: C, 68.16; H, 4.81; F, 4.65; N, 6.95; O, 7.73; S, 7.79.

5.1.3.34. *N*-(2,2-Diphenylethyl)-2-((4-fluorophenyl)thio)-4-oxoazetidine-1-carboxamide (63). The compound was purified by preparatory plate on silica gel (3:1 hexanes/ethyl acetate) to give a solid in 32% yield. mp 155–156 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (6H, m), 7.27–7.23 (2H, m), 7.15 (4H, dt, J = 1.3, 7.3), 6.95 (2H, dd, J = 5.5, 8.9) 6.41 (1H, br s), 5.09 (1H, dd, J = 2.3, 4.9), 3.80 (2H, dd, J = 7.3), 3.76 (1H, dt, J = 0.7, 7.3), 3.45 (1H, dd, J = 7.5, 16.0), 3.15 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.00, 141.54, 141.41, 137.85, 128.80, 128.08, 126.99, 124.11, 116.59, 116.37, 56.61, 50.87, 44.07, 43.45; IR (neat) ν_{max} (C=O) 1772.23, 1705.13 cm^{-1}. Anal. Calcd for $C_{24}H_{21}FN_2O_2S$: C, 68.55; H, 5.03; F, 4.52; N, 6.66; O, 7.61; S, 7.62. Found: C, 68.72; H, 5.24; F, 4.63; N, 6.67; O, 7.52; S, 7.83.



5.1.3.35. 2-((2,4-Difluorophenyl)thio)-*N*-ethyl-4-oxoazetidine-1-carboxamide (64). The compound was purified by flash chromatography on silica gel with 2:1 hexanes/ethyl acetate to give a waxy solid in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (1H, dd, J = 0.5, 8.80), 7.25 (1H, ddd, J = 2.7, 5.1, 8.8), 6.92 (1H, dt, J = 0.5, 2.7, 5.2), 6.39 (1H, br s), 5.26 (1H, dd J = 2.3, 4.9), 3.46 (1H, dd, J = 7.5, 16.0), 3.33 (1H, dd, J = 7.5, 16.0), 2.90 (1H, q, J = 7.35, 14.0), 1.20 (1H, t, J = 7.3);^{13}C NMR (100 MHz, $CDCl_3$) δ 165.10, 149.4, 138.91, 112.45, 104.89, 77.05, 43.99, 34.75, 15.08; IR (neat) ν_{max} (C=O) 1775 cm^{-1}. Anal. Calcd for $C_{12}H_{12}F_2N_2O_2S$: C, 50.34; H, 4.22; F, 13.27; N, 9.78; O, 11.18; S, 11.20. Found: C, 50.61; H, 4.34; F, 13.18; N, 9.87; O, 11.23; S, 11.13.

5.1.3.36. *N*-(1-(Naphthalen-1-yl)ethyl)-2-oxo-4-(phenylthio)-azetidine-1-carboxamide (65). The compound was purified by flash chromatography on silica gel with 2:1 hexanes/ethyl acetate to give a waxy solid in 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (1H, dd, J = 1.4, 8.0), 7.93 (1H, dd, J = 1.5, 8.4), 7.71 (1H, dd, J = 1.5, 7.9), 7.62 (1H, dd, J = 1.4, 7.9), 7.60 (1H, dd, J = 0.8, 7.7), 7.47 (1H, d, J = 8.8), 7.43 (1H, tq, J = 7.3, 7.8), 7.31 (1H, dd, J = 1.4, 7.1), 6.82 (1H, ddd, J = 2.7, 5.2, 8.8), 6.75 (1H, tq, J = 2.7, 5.2), 6.38 (1H, br s), 5.86 (1H, dd, J = 2.3, 4.9), 5.05 (1H, q, J = 6.9), 3.23 (1H, dd, J = 7.5, 16.0), 2.72 (1H, dd, J = 7.5, 16.0),1.57 (3H, d, J = 6.9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.36, 148.71, 138.17, 137.52, 133.95, 130.67, 128.93, 128.37, 126.52, 125.82, 125.38, 124.92, 123.05, 122.46, 116.52, 57.30, 45.49, 44.08, 22.05; IR (neat) ν_{max} (C=O) 1772.15, 1700.24 cm^{-1}. Anal. Calcd for $C_{22}H_{19}FN_2O_2S$: C, 66.99; H, 4.86; F, 4.82; N, 7.10; O, 8.11; S, 8.13. Found: C, 67.19; H, 4.95; F, 4.79; N, 7.22; O, 8.12; S, 8.10.

5.1.3.37. 2-((2,4-Difluorophenyl)thio)-*N*-(2-fluorobenzyl)-4-oxoazetidine-1-carboxamide (66). The compound was synthesized under the conditions as stated above for compound **61** and purified by preparatory place on silica gel (2:1 hexanes/ethyl acetate) of the filtrate to give a waxy solid in 34.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (2H, ddd, J = 1.80, 7.6, 8.4), 7.03 (3H, m), 6.83 (1H, ddd, J = 2.7, 5.1, 8.8), 6.76 (1H, dd, J = 2.7, 5.1), 5.22 (1H, dd, J = 2.3, 4.9), 4.45 (1H, dd, J = 0.7, 15.0), 3.47 (1H, dd, J = 7.5, 16.0), 2.83 (1H, dd, J = 7.5, 15.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.05, 162.37, 159.41, 157.24, 151.09, 131.35, 130.19, 129.60, 128.90, 124.96, 122.07, 115.43, 111.27, 105.02, 63.30, 43.01, 38.95. IR (neat) ν_{max} (C=O) 1774, 1709 cm^{-1}. Anal. Calcd for $C_{17}H_{13}F_3N_2O_2S$: C, 55.73; H, 3.58; F, 15.56; N, 7.65; O, 8.73; S, 8.75. Found: C, 55.93; H, 3.72; F, 15.67; N, 7.78; O, 8.79; S, 8.59.

5.1.3.38. 2-((2,4-Difluorophenyl)thio)-*N*-(2-methoxybenzyl)-4-oxoazetidine-1-carboxamide (67). The compound was synthesized under the conditions as stated above for compound **61** and purified by preparatory place on silica gel (1:1 hexanes/ethyl acetate) of the filtrate to give a waxy solid in 62.6% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47, (1H, d, J = 8.8), 7.39 (1H, ddd, J = 1.7, 7.7, 8.2), 7.17–7.04 (3H, m), 6.82 (1H, ddd, J = 2.7, 5.1, 8.8), 6.74 (1H, dt, J = 2.7, 5.1) 5.15 (1H, dd, J = 2.3, 4.9), 4.38 (1H, dd, J = 0.7, 15.0), 3.78 (1H, s), 3.35 (1H, dd J = 7.5, 16.0), 2.79 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.35, 164.87, 162.89, 162.24, 157.59, 149.35, 139.19, 129.60, 129.10, 125.86, 120.57, 112.43, 112.23, 110.32, 105.06, 104.80, 104.53, 56.30, 55.28, 39.75, 30.07; IR (neat) ν_{max} (C=O) 1775, 1700 cm^{-1}. Anal. Calcd for $C_{18}H_{16}F_2N_2O_3S$: C, 57.14; H, 4.26; F, 10.04; N, 7.40; O, 12.68; S, 8.47. Found: C, 57.34; H, 4.32; F, 10.21; N, 7.51; O, 12.49; S, 8.37.

5.1.3.39. 2-((2,4-Difluorophenyl)thio)-*N*-(2-fluorophenyl)-4-oxoazetidine-1-carboxamide (68). The compound was synthesized under the conditions as stated above for compound **61**.

The product was confirmed by NMR, but the product proved unstable upon evaporation of the solvent.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (1H, ddd, J = 1.4, 5.1, 8.4), 7.79 (1H, dd, J = 1.4, 8.0), 6.99 (1H, ddd, J = 1.4, 7.8, 8.4), 6.82 (1H, ddd, J = 2.7, 5.1, 8.8), 6.75 (1H, dt, J = 2.7, 5.1), 5.28 (1H, dd, J = 2.3, 4.9), 3.47 (1H, dd, J = 7.5, 16.0), 2.90 (1H, dd, J = 7.5, 16.0).

5.1.3.40. 2-((2,4-Difluorophenyl)thio)-*N*-(2-nitrobenzyl)-4-oxoazetidine-1-carboxamide (69). The synthesis was performed as described by Mulchande et al.[23] with the following modifications: 0.04 mmol, 10 mg of lactam **6** were dissolved in $CDCl_3$ (1 ml) with 1.5 mol equiv of 2-nitrophenyl isocyanate. Triethylamine (0.001214 g; 0.3 mol equiv) was then added dropwise. The reaction was stirred, and monitored by NMR. It was confirmed the product was present after 30 min. The product became unstable with removal of the solvent. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.98 (1H, dd, J = 1.4, 7.9), 8.06 (1H, dt, J = 1.4, 7.4), 7.65 (1H, d, J = 8.8), 7.54 (1H, dd, J = 1.4, 7.4), 7.34 (1H, ddd, J = 1.4, 7.5, 8.0), 6.84 (1H, ddd, J = 2.7, 5.1, 8.8), 6.73 (1H, dt, J = 2.7, 5.1), 6.02 (1H, dd, J = 2.3, 4.9), 3.39 (1H, dd, J = 7.5, 16.0), 2.82 (1H, dd, J = 7.5, 16.0).

5.1.3.41. *N*-Benzhydryl-2-((2-chloro-4-fluorophenyl)thio)-4-oxoazetidine-1-carboxamide (70). The compound was synthesized under the conditions as stated above for compound **61** and purified by preparatory place on silica gel (1:1 hexanes/ethyl acetate) of the filtrate to give a waxy solid in 30% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (2H, dt, J = 1.3, 7.2), 7.21 (2H, dt, J = 1.4, 7.1), 7.16 (2H, ddd, J = 1.2, 1.4, 7.4), 6.97 (1H, dd, J = 2.6, 5.1), 6.87 (1H, ddd, J = 2.5, 5.1, 8.8), 6.12 (1H, s), 5.25 (1H, dd, J = 2.3, 4.9), 3.42 (1H, dd, J = 7.5, 16.0), 2.90 (1H, dd, J = 7.5, 16.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.36, 164.36, 161.83, 148.72, 140.97, 140.10, 138.79, 128.80, 127.73, 127.32, 125.03, 117.92, 114.98, 57.29, 56.97, 44.08. IR (neat) ν_{max} (C=O) 1772.09, 1716.36 cm^{-1}.

Anal. Calcd for $C_{23}H_{18}ClFN_2O_2S$: C, 62.65; H, 4.12; Cl, 8.04; F, 4.31; N, 6.35; O, 7.26; S, 7.27. Found: C, 62.87; H, 4.22; Cl, 8.01; F, 4.39; N, 6.56; O, 7.21; S, 7.17.

5.2. Antimicrobial assays

Antimicrobial activity for all isolates, with the exception of Mtb, was evaluated by B. J. Plotkin and J. M. Green (Midwestern University, Downers Grove, IL). All organisms used are listed in Table 5 and were maintained at −80 °C. These bacteria, with the exception of Mtb, were provided by J. Thjio (Loyola University Stritch School of Medicine, Maywood, IL). The microdilution broth method was used to determine the minimal inhibitory concentrations and the minimum bactericidal concentrations (MIC/MBC).[31] All lactams were dissolved in dimethylsufamethoxizone (DMSO), then diluted at least 10-fold into Mueller–Hinton (MH) broth. Each compound was then serially diluted in MH (100 μL/well; series of 2-fold dilutions, between 500 μg/ml and 15.6 μg/ml). Controls consisted of 2-fold dilutions of DMSO alone. Bacteria were added to each well ($5–10^5$ cells/ml; 100 μL MH/well). After incubation (37 °C; 48 h) wells were scored for growth at 24 h and 48 h. The MIC was defined as the lowest concentration of drug at which no growth was observed. The MBC was determined as the lowest concentration of drug at which no growth as measured by standard plating (10 μL, MH agar) of wells where no growth was observed. All tests were performed in quadruplicate.

Mycobacterium tuberculosis (Mtb) H37Rv was used for all Mtb susceptibility determinations. These determinations were done by Kriti Arora (Tuberculosis Research Section, NIAID, NIH Bethesda, MD).

Minimum inhibitory concentrations were done determined by microdilution broth method as described previously.[32]

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