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Syntheses and Studies of New Forms of *N*-Sulfonyloxy β -Lactams as Potential Antibacterial Agents and β -Lactamase Inhibitors

Serena Carosso and Marvin J. Miller*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, 46556

Abstract

The synthesis of a small library of *N*-sulfonyloxy-2-azetidinones is reported and the preliminary results of the investigation of the biological activity of these molecules are discussed. These new multi-electrophilic β -lactams (“electrophilic bombs”) display unexpected selectivity in their antibacterial activity and β -lactamase inhibitory activity.

1. Introduction

The production of β -lactamase enzymes is an important mechanism of bacterial resistance to β -lactam antibiotics.^{1–5} These enzymes acylate the β -lactam generating an acyl-enzyme species that undergoes subsequent hydrolysis with concomitant destruction of the essential β -lactam ring. The enzyme is regenerated and the inactivated antibiotic molecule is released. A temporarily successful approach to overcome the adverse action of these enzymes has been the use of β -lactamase inhibitors in combination with classical β -lactam antibiotics, such as penicillins.^{6–8} However, the development of variants of β -lactamases is rendering the current inhibitors less effective. Three β -lactam derivatives are used clinically for the inhibition of class A β -lactamases: clavulanic acid, sulbactam and tazobactam.⁹ Since these important compounds have a similar mode of action, mutations in the targeted β -lactamase enzymes may result in cross-resistance to all three compounds. No effective inhibitors have been developed for metallo β -lactamase enzymes which are also of growing prevalence and

*Corresponding author mmiller1@nd.edu.

§Author Contributions

The authors contributed equally to the manuscript.

Supporting information

The NMR spectra of the compounds are available free of charge via the Internet at <http://pubs.acs.org>.

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threaten the utility of our still essential β -lactam antibiotic arsenal. The development of new β -lactamase inhibitors with different modes of action is therefore needed.

Our group previously reported a novel class of *N*-sulfonyloxy monocyclic β -lactams, with the generalized structure **1** (Figure 1) and determined that many, including derivatives **2-4**, were efficient inhibitors of class A β -lactamases.¹⁰⁻¹⁴

The mechanism of action of these *N*-tosyloxy-2-azetidinones is related to the ability of the constituent sulfonyloxy group to act as a good leaving group (Scheme 1). Rapid acylation of the active site of the serine β -lactamase is followed by an elimination step which generates an acyl-enzyme species that is resilient to hydrolysis.

Syntheses of additional *N*-sulfonyloxy β -lactams **10-13** (Figure 2) with β -lactamase inhibitory activity were reported in 1995.¹¹ Studies on a model of the acyl-enzyme intermediate in the active site of the crystal structure of the enzyme revealed that the sulfonate group was a surrogate for the carboxylate group found in penicillin and other β -lactam antibiotics and also indicated that aryl groups at the C4 position and in the sulfonyl group interacted with specific hydrophobic pockets of the enzymes. However, recovery of the β -lactamase enzyme activity was observed within 2–3 h. Further studies reported in 1999,¹³ led to the design, synthesis and study of 3-hydroxyethyl substituted *N*-tosyloxy β -lactam **4** which was found to be an efficient inhibitor of the TEM-1 enzyme from *Escherichia Coli*. The hydroxyethyl group was introduced in order to improve the acyl-enzyme stability and, in fact, the compound resists deacylation from the active site of the enzyme for several days. The relatively simple 4-methyl-*N*-tosyloxy- β -lactam **10** was also reported to inactivate the NMC-A β -lactamase from *Enterobacter cloacae* rapidly, efficiently and irreversibly.¹² This was of particular importance because NMC-A β -lactamases display a broad substrate profile and are also able to hydrolyze carbapenems, which are now considered to be antibiotics of last resort. In 2000, our group also reported the synthesis of a variety of *N*-sulfonyloxy β -lactams of type **3** with different aryl groups (as in **11**) other than tosyl on the sulfonate moiety.¹⁴

As also illustrated in Scheme 1, the β -lactamase inhibitory activity of *N*-sulfonyloxy β -lactams is initiated by the inherent electrophilic character of the *N*-sulfonyloxy β -lactam carbonyl, followed by ring opening, loss of the sulfonate and subsequent generation of additional electrophilic species (**7-9**) at or near the enzyme active site. Additional chemical reactivity studies of *N*-sulfonyloxy β -lactams revealed that they also are electrophilic at the C3 position (α -carbon) as shown in Scheme 2 (**14** $\rightarrow$ **15**). This is in stark contrast to classical *N*-unsubstituted or *N*-alkyl β -lactams, which can be deprotonated at the α -carbon, thus making it nucleophilic (Scheme 2, **16** $\rightarrow$ **17**).

Our early studies related to the syntheses of monocyclic β -lactams focused on developing practical methods for making the β -lactam ring by N-C₄ cyclization. Two processes relied on the N-H acidity of hydroxamates to facilitate cyclization. The most widely used version relies on Mitsunobu-mediated cyclization of α -hydroxy amino acid hydroxamates **18** to the *N*-alkoxy β -lactams, **19**, which were versatile intermediates for syntheses of oxamazins **20**, monosulfactams **21** and monobactams **22**, with a modified route still being used to

synthesize the only clinically used monobactam, aztreonam, **23** (Scheme 3). A second alternative relied on bromine-induced cyclization of γ,δ -unsaturated hydroxamates (**24** $\rightarrow$ **25**).^{15–16} 4-Halomethyl substituted β -lactams, **26** (Scheme 4), are versatile intermediates for syntheses of a variety of substituted monocyclic and bicyclic β -lactams, including the highly functionalized monobactams (**27**) recently disclosed by the Pfizer group.¹⁷ While the Mitsunobu process was used to make the *N*-sulfonyloxy β -lactams described earlier,^{10–14} the halocyclization intermediates were never converted to *N*-sulfonyloxy derivatives **28** (Scheme 4). These compounds possess multiple electrophilic sites and therefore are extremely reactive species (“electrophilic bombs”).

Based on the growing need for new β -lactamase inhibitors and new forms of β -lactam antibiotics, we decided to specifically prepare new *N*-sulfonyloxy β -lactams from the *N*-hydroxy- 4-bromomethyl derivatives with two initial focused goals: 1. Determination of fundamental chemistry and potential bioactivity of the new multi-electrophilic β -lactams and 2. Utilization of these reactive moieties as versatile intermediates for the syntheses of new monobactam derivatives that would extend structure-activity relationship (SAR) studies of heteroatom activated monocyclic β -lactams.^{18–23} Herein, we focus on the chemistry related to the generation of and reactivity of 4-bromomethyl-*N*-sulfonyloxy β -lactams **28**. The chemistry of these compounds was expected to be especially interesting, since, based on the precedent already noted, it could be anticipated that all the carbons of the core structure (**28**) would be electrophilic (δ^+). Not only did we find that the chemistry can be controlled and localized to carry out extended syntheses but the initially anticipated random reactivity of these “electrophilic bombs” was instead determined to induce remarkably selective, albeit moderate, antibiotic and β -lactamase inhibitory activity.

2. Results and Discussion

The synthesis of the 4-halomethyl-*N*-sulfonyloxy β -lactams is summarized in Scheme 5 below. Similar to our previously reported method for halogen-induced β -lactam cyclization, we first converted the simple γ,δ -unsaturated acid, vinyl acetic acid (**29**) to the corresponding hydroxamic acid **31** via the intermediate acid chloride (**30**).^{15–16} Subsequent treatment of **31** with CbzCl gave the *O*-protected hydroxamate **32** with the appropriately acidified N-H bond needed for the halolactamization (**32** $\rightarrow$ **33**). Indeed, reaction of the protected hydroxamate with bromine and aqueous base provided the desired 4-bromomethyl β -lactam **33** cleanly. Hydrogenolytic removal of the Cbz group produced the free *N*-hydroxy β -lactam (**34**) that was immediately reacted with a series of sulfonyl chlorides to generate a small library of *N*-sulfonyloxy- 4-bromomethyl β -lactams (**28a-j**) in moderate to good overall yields. Only compounds **28g** and **28h**, with strong electron-withdrawing groups on the aromatic ring, were obtained in low yields because of their high reactivity and instability during chromatographic purification on silica gel.

In addition to determining the biological activity (described later in this paper) of the multi-electrophilic *N*-sulfonyloxy-4-bromomethyl β -lactams, we also wanted to determine if the library of *N*-sulfonyloxy β -lactams could be expanded by reaction of intermediate 4-bromomethyl derivatives like **33** (the OCbz compound) with nucleophiles to displace the halogen without destruction of the β -lactam. With future goals of elaborating monobactams

with peripheral thiol, triazol and amine substitution, we reacted **33** with a representative thiol and azide but found it impossible to isolate the expected substitution products. Suspecting that the O-Cbz group of **33** was too labile or electronically activated the β -lactam to induce destruction, we decided to replace it with a less activating allyl protecting group (Scheme 6). To this end, we again hydrogenolytically removed the Cbz group of **33** to generate the intermediate *N*-hydroxy-4-bromomethyl β -lactam (**34**) and, after solvent exchange, treated it directly with allyl bromide. Interestingly, the allylation reaction proceeded in high yield to give **35** with no competitive reaction of the free *N*-hydroxy group of the intermediate (**34**) with the bromomethyl group of another molecule of *N*-hydroxy-4-bromomethyl β -lactam (**34**). This suggested that reactions of the 4-bromomethyl group with other nucleophiles might not be facile. However, reaction of the *O*-allyl-4-bromomethyl β -lactam (**35**) with a representative thiol (**36**, 2-mercapto-thiazoline) proceeded to give the desired substitution product **37** in good yield. Catalytic Pd-mediated deallylation produced the new free *N*-hydroxy β -lactam intermediate **38** which was immediately reacted with *p*-toluenesulfonyl chloride to give the targeted thiosubstituted *N*-tosyloxy β -lactam **39**.

Similar reactions of 4-bromomethyl β -lactams with azide were anticipated to give the corresponding azide derivatives that could serve as precursors to amine and triazole-substituted β -lactams for extended structure-activity-relationship (SAR) studies. Tazobactam (**40**) is one of the three clinically used β -lactam-based β -lactamase inhibitors. It contains a triazol substituent and, similar to many of the *N*-sulfonyloxy β -lactams described in earlier schemes, it does not have a substituent at the α -carbon of the β -lactam. The mechanism of action of tazobactam (Scheme 7) involves ring opening by attack of a serine hydroxyl to generate an acyl enzyme intermediate that, similar to the inhibitory mechanisms determined for our previously described *N*-tosyloxy β -lactams (Scheme 1), undergoes further elimination chemistry to generate additional electrophilic sites (an imine, **42**, and enamines **43** and **44**) that contribute to the enzyme inhibitory chemistry.⁵ Furthermore, although a recent Pfizer patent disclosed alternative routes to β -lactam azides and triazoles with antibacterial activity,¹⁷ there have been no reports related to syntheses and studies of correspondingly substituted *N*-sulfonyloxy β -lactam derivatives.

Again, reaction of sodium azide with the O-Cbz β -lactam derivative **33** resulted in decomposition. However, hydrogenolytic removal of the Cbz group followed by alkylation of the free *N*-hydroxy β -lactam with benzyl bromide gave the *N*-benzyloxy derivative **46** in 80% yield, again with no competitive alkylation with the β -lactam bromomethyl group to give dimeric-like structures (**45**, Scheme 8). Displacement of the bromide of the *N*-benzyloxy β -lactam (**46**) with azide proceeded cleanly to give the targeted azide substituted β -lactam **47**. Click reactions^{24–26} of the azide with representative alkynes (**48a-c**) gave the triazoles (**49a-c**) as expected. Reductive removal of the benzyl group gave the free *N*-hydroxy triazol-containing β -lactams (**50a-c**), which, upon reaction with *p*-toluenesulfonyl chloride under standard conditions produced a set of targeted *N*-tosyloxy-triazole containing β -lactams (**51a-c**) for study.

3. Biological activity

N-Sulfonyloxy β -lactams **28a-j**, **39** and **51a-c** were tested for antimicrobial activity using agar diffusion assays. As displayed in Table 1, compounds **28a-i** showed weak antibacterial activity against Gram-positive bacteria but they were not active against Gram-negative bacteria. Compound **28j**, which contains a thiophene group instead of a phenyl ring on the sulfonyl moiety, was not active. It is also worth noting the remarkable growth inhibition zone for compound **28h** against *M. Vaccae*. These results appeared to be in contrast with the electrophilic properties of these molecules that present multiple electrophilic sites (“electrophilic bombs”) and were anticipated to be extremely reactive and unselective. The reason for this selectivity is unknown and, while classical β -lactam antibiotics work by PBP (penicillin binding protein) interactions, details will be the subject of further investigations that will include consideration of other targets. As also displayed in Table 1 compounds **39** and **51a-c**, in which the bromine atom at C4 had been displaced by a different functionality, were not active.

Compounds **28c,e,f,i** were also tested for β -lactamase inhibitory activity against selected enzymes from classes A, B, C and D (Ambler classification). Also in this case an unusual selectivity was observed (Table 2): while compound **28e** was active only against the class A enzyme SHV-12, compounds **28c,f** inhibited enzymes from both classes A and D. Although there are two β -lactamase inactivators that show activity against both classes A and C of β -lactamases (the penem BRL 42715 and tazobactam), there are no examples of clinically used inhibitors of both classes A and D. Also worth noting is the activity of **28c,f** against the class D enzyme Oxa-48, which is particularly relevant for its notable carbapenem-hydrolyzing activity and its resistance to the clinically used β -lactamase inhibitors clavulanic acid, sulbactam and tazobactam.

4. Summary and Conclusion

The 4-bromomethyl substituted β -lactam **33** was synthesized using a bromine-induced cyclization on a γ,δ -unsaturated hydroxamate as the key step. Studies on the reactivity of this compound were performed in order to evaluate the possibility to use it as an intermediate for the syntheses of novel monocyclic and bicyclic β -lactams. The halocyclization intermediate was also converted to *N*-sulfonyloxy derivatives **28a-j** which displayed weak antibacterial activity against Gram-positive bacteria. Compounds **28c,e,f** also displayed selective β -lactamase inhibition activity against enzymes from classes A and D. Further studies on the mode of action and selectivity of these compounds are under consideration.

5. Experimental section

5.1. General experimental section

Unless otherwise indicated, chemicals and solvents were from commercial suppliers and were used as received. All of the solvents were of analytical grade or were distilled prior to use. Dichloromethane (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled from CaH_2 . Tetrahydrofuran (THF) was distilled from a mixture of sodium metal and benzophenone ketyl. Dimethylformamide (DMF) and diisopropylethylamine (DIPEA) were used from

Acros Seal ® anhydrous bottles. ^1H NMR and ^{13}C NMR spectra were obtained on a 500 MHz Bruker spectrometer and FIDs were processed using ACD/SpecManager version 11. Chemical shifts (δ) are given in parts per million (ppm) and are referenced to residual solvent peaks as internal standards. Coupling constants (J) are reported in Hertz (Hz). High resolution, accurate mass measurements were obtained with a Bruker micrOTOF II electrospray ionization time-of-flight mass spectrometer in positive mode. Infrared spectra were recorded with a ThermoNicolet IR 200 Spectrometer and reported as cm^{-1} . All reactions were conducted under Argon atmosphere unless otherwise noted. Solvents were removed in vacuo on a rotary evaporator. Reactions were monitored by thin layer chromatography (TLC) performed with aluminum-backed Merck 60-F254 silica gel plates using a 254 nm lamp, ceric ammonium molybdate (CAM) stain, FeCl_3 stain, KMnO_4 stain or ninhydrin stain for visualization. Silica gel chromatography was performed using Sorbent Technologies silica gel 60 (32–63 μm). Melting points were determined in capillary tubes using a Thomas Hoover melting point apparatus and are uncorrected.

5.3. Procedures for Biological Assays

5.3.1. General Comments—All liquids and media were sterilized by autoclaving (121 $^{\circ}\text{C}$, 15 min) before use. All aqueous solutions and media were prepared using distilled, deionized and filtered water (Millipore Milli-Q Advantage A10 Water Purification System). Luria broth (LB) was purchased from VWR. Mueller-Hinton No. 2 broth (MHII broth; cation adjusted) was purchased from Sigma-Aldrich. McFarland BaSO_4 turbidity standards were purchased from bioMérieux, Inc. Sterile plastic petri dishes (145 mm $\times$ 20 mm; Greiner Bio-One) were purchased from VWR. Ciprofloxacin was purchased from Sigma-Aldrich. The enzymes KPC-2, Oxa-48, AmpC and NDM-1 were purchased from Emerald Bioscience and the enzymes CTX-14 and SHV-12 were expressed and purified from *E. coli* at Rempex pharmaceuticals (San Diego, CA).

5.3.2. Procedures for Agar-diffusion Assays—Antibacterial activity of the compounds was determined by a modified Kirby-Bauer^{27–28} agar-diffusion assay. Overnight cultures of test organisms were grown in LB broth for 18–24 h and standard suspensions of 1.5×10^6 CFU/mL were prepared in saline solution (0.9% NaCl) according to a 0.5 BaSO_4 McFarland Standard. This standardized suspension (0.1 mL) was added to 34 mL of sterile, Mueller Hinton No. 2 agar tempered to 47–50 $^{\circ}\text{C}$. After gentle mixing, the inoculated agar media was poured into a sterile plastic petri dish (145 mm $\times$ 20 mm) and allowed to solidify near a flame with the lid cracked for ~30 min. Wells of 9.0 mm diameter were cut from the petri dish agar and filled with exactly 50 μL of the test sample solution. For studies using mixtures of compounds, both test compounds were diluted to equal concentrations and 50 μL of the mixed compound solution was added to the cut well. The petri dish was incubated at 37 $^{\circ}\text{C}$ for 18–24 h and the inhibition zone diameters were measured (mm) with an electronic caliper after 24–48 h.

5.3.3. Procedures for Enzyme Inhibition Assays—To the first row of a 2-mL deep 96-well block was added 1 mL of 50 mM sodium phosphate buffer at pH 7 with 0.1 mg/mL BSA (bovine serum albumin). To the first row was added an appropriate amount of a 10 mg/mL solution of the compound to be tested to make a 640 μM (4X) solution. Each of the

other wells in the 96-well block were charged with 750 μ L of the aforementioned buffer. The rows were then repeatedly diluted 1:3 so that the final concentrations of the individual 96-well plates would range in concentrations from 160 μ M to 3 nM (8 dilutions). The master block was then used to add 50 μ L to each corresponding well in the 96-well flat-bottom plate. An additional 50 μ L of buffer was added to each well. Once the plates were prepared, 50 μ L of the enzyme to be tested against were added in buffer to each well [KPC-2 (0.147 mg/mL), CTX-M-14 (0.102 mg/mL), Oxa-14 (0.331 mg/mL), AmpC (0.699 mg/mL), SHV-12 (0.111 mg/mL), NDM-1 (0.110 mg/mL)]. The plates were incubated at rt for 10 min before 50 μ L of an appropriate indicator were added to each well and the optical density at 495 nm was measured over time. For each enzyme, other than NDM-1, nitrocefin was used. Nitrocefin (10 μ M for SHV-12, 50 μ M for the other enzymes), was appropriate because when cleaved by a β -lactamase, the color changes from yellow to red. For NDM-1, imipenem (10 μ M) was the indicator of choice. Rather than observe the growing presence of optical density at 495 nm, as with nitrocefin, the disappearance of imipenem was monitored. The inhibition curves were monitored and used to perform enzyme kinetics according to Waley²⁹ to determine the K_i (μ M) of each compound against the β -lactamases screened.

5.4. Chemistry: Protocols and analytical data

5.4.1. General procedure for the synthesis of compounds 28a-j from precursor 33—Cbz protected β -lactam **33** (500 mg, 1.59 mmol)^{15–16} was dissolved in 14 mL of MeOH, at rt under an argon atmosphere. Pd/C 10% (20 mg) was added and the mixture was flushed with hydrogen gas. The reaction mixture was left to stir at rt under a hydrogen atmosphere for 2 h then it was purged with argon and filtered through celite. The filtrate was evaporated under vacuum to give crude *N*-hydroxy β -lactam **34** as a pink oil.

Compound **34** was dissolved in dry CH₃CN (20 mL) and the solution was cooled to 0 °C. DIPEA (415 μ L, 2.39 mmol) was added, followed by the sulfonyl chloride (2.39 mmol). The reaction mixture was left to stir at 0 °C for 10 min then the solvent was evaporated under vacuum. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexanes).

5.4.1.1. 4-(Bromomethyl)-2-oxoazetidin-1-yl-4-methylbenzenesulfonate (28a): yield 67% (1.06 mmol, 356 mg); white solid; IR (KBr) 1792, 1649, 1596, 1380, 1178; m.p. 65–68 °C; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 7.90 (d, J = 8.0 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 4.29–4.25 (m, 1H), 3.75 (dd, J = 11.5, 3.1 Hz, 1H), 3.63 (dd, J = 11.5, 6.6 Hz, 1H), 2.95 (dd, J = 14.6, 6.1 Hz, 1H), 2.75 (dd, J = 14.6, 3.3 Hz, 1H), 2.47 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 164.5, 146.9, 130.3, 129.6, 129.5, 58.5, 38.5, 31.5, 22.1. HRMS (ESI) m/z calcd for C₁₁H₁₂BrNNaO₄S [M+Na]⁺ 355.9568, found 355.9576.

5.4.1.2. 4-(Bromomethyl)-2-oxoazetidin-1-yl-4-methoxybenzenesulfonate (28b): yield 70% (1.11 mmol, 390 mg); white solid; IR (KBr) 2844, 1797, 1595, 1497, 1460, 1379, 1266; m.p. 80–83 °C; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 7.93 (dt, J = 9.5, 2.5 Hz, 2H), 7.02 (dt, J = 9.5, 2.5 Hz, 2H), 4.26–4.23 (m, 1H), 3.90 (s, 3H), 5.72 (dd, J = 11.5, 3.0 Hz, 1H), 3.62 (dd, J = 11.5, 6.5 Hz, 1H), 2.94 (dd, J = 14.5, 6.0 Hz, 1H), 2.74 (dd, J = 14.5, 3.5 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 165.2, 164.5, 131.9, 124.3, 114.9, 58.4,

56.0, 38.4, 31.6. HRMS (ESI) m/z calcd for $C_{11}H_{12}BrNNaO_5S$ $[M+Na]^+$ 371.9517, found 371.9526.

5.4.1.3. 4-(Bromomethyl)-2-oxoazetidin-1-yl-naphthalene-1-sulfonate (28c): yield 74% (1.18 mmol, 435 mg); thick colorless oil; IR (neat) 2969, 1803, 1731, 1593, 1507, 1379, 1183; 1H NMR (500 MHz, $CDCl_3$) δ (ppm) = 8.67 (dd, J = 8.5, 0.5 Hz, 1H), 8.37 (dd, J = 8.5, 1.5 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 7.98 (dt, J = 8.0, 0.5 Hz, 1H), 7.77 (ddd, J = 8.5, 7.0, 1.5 Hz, 1H), 7.67 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 7.59 (dd, J = 8.0, 7.5 Hz, 1H), 4.16-4.14 (m, 1H), 3.59 (dd, J = 11.0, 3.0 Hz, 1H), 3.44 (dd, J = 11.0, 7.0 Hz, 1H), 2.87 (dd, J = 14.7, 6.0 Hz, 1H), 2.65 (dd, J = 14.7, 3.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) = 164.1, 137.2, 134.3, 132.8, 129.5, 129.2, 129.0, 127.8, 125.0, 125.1, 124.2, 58.4, 38.5, 31.2. HRMS (ESI) m/z calcd for $C_{14}H_{12}BrNNaO_4S$ $[M+Na]^+$ 391.9568, found 391.9570

5.4.1.4. 4-(Bromoethyl)-2-oxoazetidin-1-yl 5-(dimethylamino)naphthalene-1-sulfonate (28d): yield 72% (1.14 mmol, 473 mg); yellow solid; IR (KBr) 2834, 1804, 1571, 1455, 1377, 1183; m.p. 89–92 °C; 1H NMR (500 MHz, $CDCl_3$) δ (ppm) = 8.69 (d, J = 8.5 Hz, 1H), 8.35 (dd, J = 7.5, 1.5 Hz, 1H), 8.30 (d, J = 8.5 Hz, 1H), 7.66 (dd, J = 8.5, 7.5 Hz, 1H), 7.56 (dd, J = 8.5, 7.5 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 4.14-4.10 (m, 1H), 3.54 (dd, J = 11.3, 3.0 Hz, 1H), 3.38 (dd, J = 11.3, 7.5 Hz, 1H), 2.91-2.87 (m, 1H), 2.90 (s, 6H), 2.67 (dd, J = 14.5, 3.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) = 164.1, 152.0, 133.4, 132.8, 130.5, 130.0, 129.6, 129.1, 123.2, 119.3, 116.1, 58.4, 45.7, 38.5, 31.1; HRMS (ESI) m/z calcd for $C_{16}H_{17}BrN_2NaO_4S$ $[M+Na]^+$ 434.9990, found 434.9949.

5.4.1.5. 4-(Bromomethyl)-2-oxoazetidin-1-yl-naphthalene-2-sulfonate (28e): yield 67% (1.06 mmol, 394 mg); colorless oil; IR (neat) 2965, 1803, 1625, 1589, 1383, 1184; 1H NMR (500 MHz, $CDCl_3$) δ (ppm) = 8.61 (d, J = 2.0 Hz, 1H), 8.01 (dd, J = 12.7, 8.5 Hz, 2H), 7.98-7.94 (m, 2H), 7.74-7.70 (m, 1H), 7.68-7.64 (m, 1H), 4.32-4.28 (m, 1H), 3.73 (dd, J = 11.5, 3.0 Hz, 1H), 3.66 (dd, J = 11.5, 6.5 Hz, 1H), 2.95 (dd, J = 15.0, 6.0 Hz, 1H), 2.74 (dd, J = 15.0, 3.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) = 164.6, 136.1, 132.1, 132.0, 130.3, 130.2, 130.0, 129.8, 128.4, 128.3, 123.2, 58.5, 38.5, 31.6. HRMS (ESI) m/z calcd for $C_{14}H_{12}BrNNaO_4S$ $[M+Na]^+$ 391.9568, found 391.9564.

5.4.1.6. 4-(Bromomethyl)-2-oxoazetidin-1-yl (E)-2-phenylethene-1-sulfonate (28f): yield 61% (0.97 mmol, 336 mg); white solid; IR (KBr) 1798, 1613, 1375, 1172; m.p. 70–73 °C; 1H NMR (500 MHz, $CDCl_3$) δ (ppm) = 7.75 (d, J = 15.0 Hz, 1H), 7.55-7.41 (m, 5H), 6.87 (d, J = 15.0 Hz, 1H), 4.34-4.32 (m, 1H), 3.76 (dd, J = 11.0, 3.5 Hz, 1H), 5.18 (dd, J = 11.0, 6.0 Hz, 1H), 3.01 (dd, J = 14.5, 6.0 Hz, 1H), 2.80 (dd, J = 14.5, 3.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) = 164.8, 149.3, 132.5, 131.7, 129.5, 129.3, 118.3, 58.7, 38.5, 31.7; HRMS (ESI) m/z calcd for $C_{12}H_{13}BrNO_4S$ $[M+H]^+$ 345.9749, found 345.9759.

5.4.1.7. 4-(Bromomethyl)-2-oxoazetidin-1-yl 4-nitrobenzenesulfonate (28g): yield 51% (0.81 mmol, 296 mg); white solid; IR (KBr) 1802, 1696, 1526, 1351, 1186; m.p. 50–55 °C; 1H NMR (500 MHz, $CDCl_3$) δ (ppm) = 8.42 (dt, J = 9.1, 2.3 Hz, 2H), 8.24 (dt, J = 9.1, 2.3 Hz, 2H), 4.34-4.32 (m, 1H), 3.78 (dd, J = 11.5, 3.4 Hz, 1H), 3.76 (dd, J = 11.5, 5.4 Hz,

1H), 3.49 (dd, $J = 14.9, 6.4$ Hz, 1H), 2.81 (dd, $J = 14.9, 3.5$ Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ (ppm) = 164.9, 151.7, 139.2, 131.0, 124.7, 58.6, 38.3, 31.5.

5.4.1.8. 4-(Bromomethyl)-2-oxoazetidin-1-yl 2,4-dinitrobenzenesulfonate (28h): yield 35% (0.56 mmol, 228 mg); yellow solid; IR (KBr) 1804, 1638, 1529, 1348, 1225; m.p. 104.2–105.7 °C; ^1H NMR (500 MHz, CDCl_3) δ (ppm) = 8.85 (d, $J = 3.0$ Hz, 1H), 8.48 (dd, $J = 9.5, 3.0$ Hz, 1H), 7.81 (d, $J = 9.5$ Hz, 1H), 4.49–4.45 (m, 1H), 3.72 (dd, $J = 11.5, 4.5$ Hz, 1H), 3.68 (dd, $J = 11.5, 4.5$ Hz, 1H), 3.21 (dd, $J = 15.0, 3.5$ Hz, 1H), 3.00 (dd, $J = 15.0, 3.5$ Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ (ppm) = 167.0, 156.8, 142.8, 137.0, 129.7, 122.3, 117.7, 58.1, 38.1, 31.4.

5.4.1.9. 4-Bromomethyl-2-oxoazetidin-1-yl 2,4,6-triisopropylbenzenesulfonate (28i): yield 69% (1.10 mmol, 490 mg); white solid; IR (KBr) 2870, 1799, 1660, 1426, 1193; m.p. 96.6–97.9 °C; ^1H NMR (500 MHz, CDCl_3) δ (ppm) = 7.10 (s, 2H), 4.31–4.27 (m, 1H), 4.08 (sept., $J = 6.5$ Hz, 2H), 3.72 (dd, $J = 11.0, 3.2$ Hz, 1H), 3.49 (dd, $J = 11.0, 7.6$ Hz, 1H), 2.98 (dd, $J = 14.5, 5.9$ Hz, 1H), 2.93 (sept., $J = 6.9$ Hz, 1H), 2.73 (dd, $J = 14.5, 3.1$ Hz, 1H), 1.29 (dd, $J = 6.5, 4.0$ Hz, 12H), 1.26 (d, $J = 6.9$ Hz, 6H); ^{13}C NMR (125 MHz, CDCl_3) δ (ppm) = 164.1, 155.6, 152.6, 127.4, 124.3, 58.7, 38.8, 34.5, 31.1, 30.4, 24.9, 23.7; HRMS (ESI) m/z calcd for $\text{C}_{19}\text{H}_{29}\text{BrNO}_4\text{S}$ $[\text{M}+\text{H}]^+$ 446.1001, found 446.0999.

5.4.1.10. 4-(Bromomethyl)-2-oxoazetidin-1-yl thiophene-2-sulfonate (28j): yield 75% (1.19 mmol, 389 mg); colorless oil; IR (neat) 2965, 1798, 1698, 1505, 1384, 1183; ^1H NMR (500 MHz, CDCl_3) δ (ppm) = 7.89 (dd, $J = 4.0, 1.5$ Hz, 1H), 7.85 (dd, $J = 5.0, 1.5$ Hz, 1H), 7.20 (dd, $J = 5.0, 4.0$ Hz, 1H), 4.31–4.28 (m, 1H), 3.73 (dd, $J = 11.5, 3.0$ Hz, 1H), 3.63 (dd, $J = 11.5, 6.5$ Hz, 1H), 2.99 (dd, $J = 14.7, 6.5$ Hz, 1H), 2.79 (dd, $J = 14.7, 3.0$ Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ (ppm) = 164.6, 137.6, 137.0, 128.4, 58.6, 38.5, 31.5; HRMS (ESI) m/z calcd for $\text{C}_8\text{H}_8\text{BrNNaO}_4\text{S}_2$ $[\text{M}+\text{Na}]^+$ 347.8976, found 347.8976.

5.4.2. 1-(Allyloxy)-4-(bromomethyl)azetidin-2-one (35)—Compound **33** (4.00 g, 12.7 mmol) was dissolved in MeOH (30 mL) at rt under an argon atmosphere. Pd/C 10% (40 mg) was added and the mixture was flushed with hydrogen gas. The reaction mixture was left to stir under an atmosphere of hydrogen, at rt, for 2.5 h then it was purged with argon and filtered through celite. The solvent was evaporated under vacuum to give crude *N*-hydroxy β -lactam **34** as a yellow oil.

Crude *N*-hydroxy β -lactam **34** was dissolved in dry CH_3CN (40 mL) at rt, under an argon atmosphere. K_2CO_3 (3.50 g, 25.5 mmol) was added, followed by allyl bromide (1.40 mL, 16.5 mmol). The reaction mixture was left to stir at rt for 15 h then water (20 mL) and Et_2O (30 mL) were added. The layers were separated and the aqueous layer was extracted with Et_2O (20 mL). The combined organic extracts were dried, filtered and evaporated under vacuum. Column chromatography on silica gel (hexanes/ EtOAc 7:3) afforded compound **35** in 80% yield (2.23 g, 10.2 mmol) as a yellow oil. IR (neat) 3082, 2955, 1777, 1644, 1420, 1343, 974; ^1H NMR (500 MHz, CDCl_3) δ (ppm) = 5.98 (dddd, $J = 17.0, 10.0, 6.5, 6.5$ Hz, 1H), 5.37 (ddd, $J = 17.0, 2.8, 1.4$ Hz, 1H), 5.32 (dddd, $J = 10.0, 1.4, 1.0, 1.0$ Hz, 1H), 4.48–4.39 (m, 2H), 4.08–4.06 (m, 1H), 3.63 (dd, $J = 11.0, 4.2$ Hz, 1H), 3.51 (dd, $J = 11.0, 6.0$ Hz, 1H), 2.83 (dd, $J = 14.0, 5.3$ Hz, 1H), 2.56 (dd, $J = 14.0, 2.4$ Hz, 1H); ^{13}C NMR (125

MHz, CDCl₃) δ (ppm) = 164.0, 132.3, 121.3, 77.8, 57.3, 37.9, 32.1; HRMS (ESI) m/z calcd for C₇H₁₁BrNO₂ [M+H]⁺ 219.9973, found 219.9983.

5.4.3. 1-(Allyloxy)-4-(((4,5-dihydrothiazol-2-yl)thio)methyl)azetidin-2-one (37)—

Compound **35** (2.27 g, 10.31 mmol) was dissolved in dry CH₃CN (50 mL) at rt under an argon atmosphere. K₂CO₃ (2.85 g, 20.6 mmol) was added, followed by 2-thiazoline-2-thiol **36** (3.07 g, 25.8 mmol). The resulting mixture was left to stir at rt for 24 h then the solvent was evaporated under vacuum. The residue was purified by column chromatography on silica gel (hexanes/EtOAc 7:3) to give compound **37** as a colorless oil in 68% yield (1.81 g, 7.01 mmol). IR (neat) 2932, 2852, 1768, 1644, 1572, 1418, 1349, 1305, 1202; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 5.96-5.90 (m, 1H), 5.35-5.27 (m, 2H), 4.41-4.33 (m, 2H), 4.17-4.09 (m, 3H), 3.53 (dd, J = 5.0, 1.5 Hz, 1H), 3.38 (dd, J = 8.0, 1.5 Hz, 2H), 3.27 (dd, J = 6.5, 1.5 Hz, 1H), 2.78 (dd, J = 5.5, 1.5 Hz, 1H), 2.49 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 164.6, 164.5, 132.5, 121.2, 77.5, 64.2, 56.5, 37.7, 36.1, 34.0; HRMS (ESI) m/z calcd for C₁₀H₁₅N₂O₂S₂ [M+H]⁺ 259.0575, found 259.0586.

5.4.4. 4-(((4,5-Dihydrothiazol-2-yl)thio)methyl)-2-oxoazetidin-1-yl 4-methylbenzenesulfonate (39)—

PPh₃ (487 mg, 1.86 mmol) was added to a solution of Pd(OAc)₂ (104 mg, 0.46 mmol) in dry MeOH (7 mL) at rt, under an argon atmosphere. The mixture was left to stir at rt for 30 min then 1,3-dimethylbarbituric acid (726 mg, 4.64 mmol) was added followed by a solution of compound **37** (1.20 g, 4.64 mmol) in dry MeOH (10 mL). The reaction mixture was left to stir at rt for 2.5 h then it was filtered through celite. The filtrate was evaporated under vacuum to afford crude *N*-hydroxy- β -lactam **38** as an orange oil.

Crude **38** was dissolved in dry CH₃CN (25 mL) and the resulting orange solution was cooled to 0°C. DIPEA (1.20 mL, 6.96 mmol) and TsCl (1.32 g, 6.96 mmol) were added sequentially and the reaction mixture was left to stir at 0 °C for 15 min. The solvent was evaporated under vacuum and the resulting residue was purified by column chromatography on silica gel (hexanes/EtOAc 7:3 to 1:1). Compound **39** was obtained as a white solid in 65% yield (1.12 g, 3.02 mmol). IR (neat) 2952, 1803, 1688, 1572, 1435, 1382, 1193; m.p. 72.9–75.0 °C; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 7.87 (d, J = 10.5 Hz, 2H), 7.37 (d, J = 10.5 Hz, 2H), 4.34-4.31 (m, 1H), 4.19-4.15 (m, 2H), 3.74 (dd, J = 14.5, 3.6 Hz, 1H), 3.42 (dd, J = 8.0, 1.1 Hz, 1H), 3.40 (dd, J = 8.0, 2.0 Hz, 1H), 3.35 (dd, J = 14.5, 6.2 Hz, 1H), 2.87 (dd, J = 14.8, 6.2 Hz, 1H), 2.70 (dd, J = 14.8, 3.5 Hz, 1H), 2.46 (3H, s); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 165.6, 165.0, 146.7, 130.6, 129.5, 64.1, 58.5, 37.6, 36.2, 33.4, 22.1; HRMS (ESI) m/z calcd for C₁₄H₁₇N₂O₄S₃ [M+H]⁺ 373.0350, found 373.0353.

5.4.5. 1-(Benzyloxy)-4-(bromomethyl)azetidin-2-one (46)—

Compound **33** (3.0 g, 9.5 mmol) was dissolved in 25 mL of MeOH at rt, under an argon atmosphere. Pd/C 10% (50 mg) was added and the reaction mixture was flushed with hydrogen. The mixture was left to stir at rt, under hydrogen atmosphere for 2.5 h then it was purged with argon and filtered through celite. The filtrate was evaporated under vacuum to give the crude *N*-hydroxy β -lactam **34** as a pink oil.

Crude compound **34** was dissolved in dry CH₃CN (40 mL) at rt, under an argon atmosphere. K₂CO₃ (2.64 g, 19.1 mmol) was added, followed by benzyl bromide (1.48 mL, 12.4 mmol). The reaction mixture was left to stir at rt for 12 h then the solvent was evaporated under vacuum. The resulting residue was purified by column chromatography on silica gel (hexanes/EtOAc 7:3) to give the produce in 80% yield (2.0 g, 7.6 mmol) as a colorless oil. IR (neat) 3063, 3031, 2957, 2882, 1772, 1648, 1497, 1454, 1360, 1214; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 7.41-7.37 (m, 5H), 4.98 (d, *J* = 11.0 Hz, 1H), 4.94 (d, *J* = 11.0 Hz, 1H), 3.77-3.74 (m, 1H), 3.33 (dd, *J* = 11.0, 4.0 Hz, 1H), 3.22 (dd, *J* = 11.0, 6.5 Hz, 1H), 2.77 (dd, *J* = 13.7, 5.5 Hz, 1H), 2.50 (dd, *J* = 13.7, 2.5 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 164.0, 135.4, 129.5, 129.4, 128.9, 78.8, 57.5, 38.1, 31.9; HRMS (ESI) *m/z* calcd for C₁₁H₁₃BrNO₂ [M+H]⁺ 270.0130, found 270.0101.

5.4.6. 4-(Azidomethyl)-1-(benzyloxy)azetidin-2-one (47)—Compound **46** (3.0 g, 11.1 mmol) was dissolved in 40 mL of dry DMF at rt, under an argon atmosphere. NaN₃ (2.90g, 44.4 mmol) was added, followed by NaI (167 mg, 1.11 mmol). The reaction mixture was left to stir at rt for 48 h then it was partitioned between water (30 mL) and EtOAc (40 mL). The aqueous layer was extracted with EtOAc (30 mL) and the combined organic extracts were dried (Na₂SO₄), filtered and evaporated under vacuum. The residue was purified by column chromatography on silica gel (hexanes/EtOAc 7:3) to give the product in 70% yield (1.80 g, 7.77 mmol) as a colorless oil; IR (neat) 2928, 2104, 1774, 1673, 1498, 1386; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 7.41-7.37 (m, 5H), 4.98 (d, *J* = 11.0 Hz, 1H), 4.94 (d, *J* = 11.0 Hz, 1H), 3.77-3.74 (m, 1H), 3.33 (dd, *J* = 11.0, 4.0 Hz, 1H), 3.22 (dd, *J* = 11.0, 6.5 Hz, 1H), 2.77 (dd, *J* = 13.7, 5.5 Hz, 1H), 2.50 (dd, *J* = 13.7, 2.5 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 164.4, 135.5, 129.5, 129.3, 128.9, 78.6, 56.1, 51.2, 35.6. HRMS (ESI) *m/z* calcd for C₁₁H₁₂N₄NaO₂ [M+Na]⁺ 255.0858, found 255.0828.

5.4.7. Ethyl 1-((1-(benzyloxy)-4-oxoazetidin-2-yl)methyl)-1H-1,2,3-triazole-4-carboxylate (49a)—Compound **47** (350 mg, 1.50 mmol) was dissolved in a *t*BuOH/H₂O 4:1 mixture (10 mL). Ethyl propiolate (152 μL, 1.50 mmol) was added, followed by CuSO₄·5H₂O (7.49 mg, 0.03 mmol) and sodium ascorbate (30 mg, 0.15 mmol). The reaction mixture was left to stir at rt for 48 h then it was partitioned between EtOAc (6 mL) and water (8 mL). The aqueous layer was extracted with EtOAc (3 × 8 mL) and the combined organic extracts were dried (Na₂SO₄), filtered and evaporated under vacuum. The residue was purified by column chromatography on silica gel (from hexanes/EtOAc 1:1 to EtOAc) to give the product in 67% yield (0.33 g, 1.01 mmol) as a white solid; IR (KBr) 1776, 1732, 1654, 1543, 1370; m.p. 93–95 °C; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 8.01 (bs, 1H), 7.41-7.34 (m, 5H), 4.93 (s, 2H), 4.48 (dd, *J* = 14.6, 4.7 Hz, 1H), 4.44-4.38 (m, 3H), 3.83-3.80 (m, 1H), 2.78 (dd, *J* = 14.0, 5.3 Hz, 1H), 2.43(dd, *J* = 14.0, 2.4 Hz, 1H), 1.40 (t, *J* = 7.1 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 163.6, 160.6, 140.8, 135.1, 129.7, 129.6, 129.2, 128.7, 78.8, 61.6, 56.1, 50.5, 36.2, 14.5; HRMS (ESI) *m/z* calcd for C₁₆H₁₉N₄O₄ [M+H]⁺ 331.1406, found 331.1381.

5.4.8. Ethyl 1-((4-oxo-1-(toxyloxy)azetidin-2-yl)methyl)-1H-1,2,3-triazole-4-carboxylate (51a)—Compound **49a** (136 mg, 0.41 mmol) was dissolved in MeOH (10 mL) at rt, under an argon atmosphere. Pd/C 10% (35 mg) was added and the mixture was

flushed with hydrogen and left to stir at rt for 4 h. The reaction mixture was purged with argon and filtered through celite and the solvent was evaporated under vacuum to give *N*-hydroxy β -lactam **50a** as a colorless oil.

Crude **50a** was dissolved in dry CH₃CN (10 mL) at rt, under an argon atmosphere. The solution was cooled to 0 °C and DIPEA (100 μ L, 0.61 mmol) was added, followed by TsCl (117 mg, 0.61 mmol). The reaction mixture was left to stir at 0 °C for 10 min then the solvent was evaporated under vacuum. The resulting residue was purified by column chromatography on silica gel (hexanes/EtOAc 1:1 to EtOAc) to give the product in 65% yield (105 mg, 0.27 mmol) as a white solid; IR (KBr) 2983, 1807, 1727, 1596, 1544, 1379, 1194; m.p. 117–120 °C; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 8.55 (s, 1H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 2H), 4.94 (dd, *J* = 15.0, 3.1 Hz, 1H), 4.87 (dd, *J* = 15.0, 3.8 Hz, 1H), 4.45 (dd, *J* = 14.2, 7.1 Hz, 2H), 4.47–4.43 (m, 1H), 2.99 (dd, *J* = 15.5, 6.9 Hz, 1H), 2.64 (dd, *J* = 14.5, 4.0 Hz, 1H), 2.44 (s, 3H), 1.43 (dd, *J* = 7.1, 7.1 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 165.7, 160.7, 147.2, 140.6, 138.8, 130.4, 129.9, 129.3, 61.7, 57.5, 49.6, 36.1, 22.1, 14.6; HRMS (ESI) *m/z* calcd for C₁₆H₁₉N₄O₆S [M+H]⁺, 395.1025, found 395.1037.

5.4.9. N-(Prop-2-yn-1-yl)benzamide (48b)—A catalytic amount of dry DMF (one drop) was added to a mixture of benzoic acid (1.0 g, 8.18 mmol) and (COCl)₂ (1.40 mL, 16.4 mmol) in dry CH₂Cl₂ (50 mL) at 0 °C, under an argon atmosphere. The reaction mixture was left to stir at 0 °C for 30 min then the solvent was evaporated under vacuum. The residual HCl was eliminated by co-evaporation with CHCl₃ (10mL) and toluene (10 mL). The crude acid chloride was dried under vacuum and then dissolved in dry CH₂Cl₂ (20 mL). The solution was cooled to –78 °C and DIPEA (3.56 mL, 20.4 mmol) was added, followed by propargyl amine (0.79 mL, 12.3 mmol). The reaction mixture was left to stir overnight being allowed to warm to rt during this period. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel (hexanes/EtOAc 1:1 to EtOAc) to give the product in 89% yield (1.16 g, 7.28 mmol) as a white solid; IR (KBr) 3300, 2928, 2116, 1642, 1538; m.p. 108.5–109.5 °C; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 7.80–7.78 (m, 2H), 7.48–7.45 (m, 1H), 7.40–7.36 (m, 2H), 6.94 (bs, 1H), 4.20 (dd, *J* = 7.6, 2.5 Hz, 2H), 2.24 (dd, *J* = 2.5, 2.5 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 167.6, 133.9, 131.9, 128.8, 127.4, 79.9, 71.9, 29.9; HRMS (ESI) *m/z* calcd for C₁₀H₁₀NO [M+H]⁺ 160.0762, found 160.0756.

5.4.10. N-((1-((1-Benzyloxy)-4-oxoazetidin-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (49b)—Compound **47** (350 mg, 1.50 mmol) was added to a mixture of compound **48b** (239 mg, 1.50 mmol), CuSO₄·5H₂O (7.49 mg, 0.03 mmol) and sodium ascorbate (30.0 mg, 0.15 mmol) in *t*BuOH/H₂O 4:1 (8 mL). The reaction mixture was left to stir at rt for 48 h then it was partitioned between water (15 mL) and EtOAc (15 mL). The aqueous layer was extracted with EtOAc (15 mL) and the combined organic extracts were dried (Na₂SO₄), filtered and evaporated under vacuum. The residue was purified by column chromatography on silica gel (hexanes/EtOAc 1:1 to EtOAc) to give the product in 77% yield (452 mg, 1.16 mmol) as a white solid; IR (KBr) 3061, 1780, 1638, 1523, 1431, 1298; m.p. 124.7–126.8 °C; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 7.78–7.76

(m, 2H), 7.67-7.66 (m, 1H), 7.43-7.40 (m, 1H), 7.34-7.31 (m, 5H), 4.87-4.82 (m, 2H), 4.86 (d, $J = 12.0$ Hz, 1H), 4.84 (d, $J = 12.0$ Hz, 1H), 4.65 (dd, $J = 15.0, 5.7$ Hz, 1H), 4.58 (dd, $J = 15.0, 5.7$ Hz, 1H), 4.38 (dd, $J = 14.5, 4.5$ Hz, 1H), 4.28 (dd, $J = 14.5, 5.0$ Hz, 1H), 3.81-3.78 (m, 1H), 2.68 (dd, $J = 14.1, 5.2$ Hz, 1H), 2.39 (dd, $J = 14.1, 2.2$ Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ (ppm) = 167.7, 163.9, 145.4, 135.2, 134.2, 131.8, 129.6, 129.5, 129.0, 128.7, 127.3, 123.9, 78.7, 56.4, 50.3, 36.4, 35.5; HRMS (ESI) m/z calcd for $\text{C}_{21}\text{H}_{22}\text{N}_5\text{O}_3$ $[\text{M} + \text{H}]^+$ 392.1723, found 392.1738.

5.4.11. 2-((4-(Benzamidomethyl)-1H-1,2,3-triazol-1-yl)methyl)-4-oxoazetidin-1-yl 4-methylbenzenesulfonate (51b)—Compound **49b** (78 mg, 0.2 mmol) was dissolved in MeOH (5 mL) at rt, under an argon atmosphere. Pd/C 10% (10 mg) was added and the mixture was flushed with hydrogen and left to stir at rt for 4 h. The reaction mixture was filtered through celite and the solvent was evaporated under vacuum to give *N*-hydroxy β -lactam **50b** as a colorless oil.

Crude **50b** was dissolved in dry CH_3CN (20 mL) at rt, under an argon atmosphere. The solution was cooled to 0 °C and DIPEA (51 μL , 0.3 mmol) was added, followed by TsCl (56 mg, 0.3 mmol). The reaction mixture was left to stir at 0 °C for 10 min then the solvent was evaporated under vacuum. The resulting residue was purified by column chromatography on silica gel (hexanes/EtOAc 1:1 to EtOAc) to give the product in 70% yield (63.8 mg, 0.14 mmol) as a white solid; IR (KBr) 2120, 1789, 1642, 1488, 1390, 1297; m.p. 71.0–73.0 °C; ^1H NMR (500 MHz, CDCl_3) δ (ppm) = 7.96 (bs, 1H), 7.86-7.83 (m, 2H), 7.81-7.79 (m, 2H), 7.53-7.49 (m, 1H), 7.45-7.41 (m, 2H), 7.35-7.33 (m, 2H), 6.84 (bs, 1H), 4.86 (dd, $J = 14.7, 3.5$ Hz, 1H), 4.79-4.74 (m, 3H), 4.45-4.41 (m, 1H), 2.97 (dd, $J = 15.2, 6.5$ Hz, 1H), 2.70 (dd, $J = 15.2, 3.5$ Hz, 1H), 2.41 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3) δ (ppm) = 167.7, 165.4, 147.0, 134.2, 131.9, 130.4, 130.3, 129.3, 128.8, 127.3, 124.6, 57.8, 49.6, 36.6, 35.6, 22.0; HRMS (ESI) m/z calcd for $\text{C}_{21}\text{H}_{22}\text{N}_5\text{O}_5\text{S}$ $[\text{M} + \text{H}]^+$ 456.1342, found 456.1349.

5.4.12. 2-Chloro-*N*-(prop-2-yn-1-yl)-5-(trifluoromethyl)benzamide (48c)—A catalytic amount of dry DMF (one drop) was added to a mixture of 2-chloro-5-(trifluoromethyl)benzoic acid (1.0 g, 4.45 mmol) and $(\text{COCl})_2$ (0.77 mL, 8.90 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C, under an argon atmosphere. The reaction mixture was left to stir at 0 °C for 30 min then the solvent was evaporated under vacuum. The residual HCl was removed by co-evaporation with CHCl_3 (10 mL) and toluene (10 mL). The crude acid chloride was dried under vacuum then it was dissolved in dry CH_2Cl_2 (20 mL). The solution was cooled to –78 °C and DIPEA (1.86 mL, 10.7 mmol) was added, followed by propargyl amine (0.43 mL, 6.67 mmol). The reaction mixture was left to stir overnight being allowed to warm to rt during this period. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel (hexanes/EtOAc 1:1 to EtOAc) to give the product in 77% yield (897 mg, 3.43 mmol) as a white solid; IR (KBr) 1650, 1546, 1434, 1332, 1266; m.p. 115–117 °C; ^1H NMR (500 MHz, CDCl_3) δ (ppm) = 7.92 (m, 1H), 7.60 (dd, $J = 8.0, 2.5$ Hz, 1H), 7.52 (dd, $J = 8.0, 0.5$ Hz, 1H), 6.63 (bs, 1H), 4.25 (dd, $J = 5.2, 2.5$ Hz, 2H), 2.29 (dd, $J = 2.5, 2.5$ Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ (ppm) = 164.9, 135.1, 134.8, 131.2, 130.1, 129.8, 128.3, 127.6, 78.7, 72.5, 30.1; HRMS (ESI) m/z calcd for $\text{C}_{11}\text{H}_8\text{ClF}_3\text{NO}$ $[\text{M} + \text{H}]^+$ 262.0247, found 262.0216.

5.4.13. N-((1-((1-(Benzyloxy)-4-oxoazetidin-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-2-chloro-5-(trifluoro-methyl) benzamide (49c)—Compound **47** (280 mg, 1.20 mmol) was added to a mixture of compound **48c** (315 mg, 1.20 mmol), CuSO₄·5H₂O (6.0 mg, 0.024 mmol) and sodium ascorbate (23.8 mg, 0.12 mmol) in *t*BuOH/H₂O 4:1 (8 mL). The reaction mixture was left to stir at rt for 48 h then it was partitioned between water (15 mL) and EtOAc (15 mL). The aqueous layer was extracted with EtOAc (15 mL) and the combined organic extracts were dried (Na₂SO₄), filtered and evaporated under vacuum. The residue was purified by column chromatography on silica gel (hexanes/EtOAc 1:1 to EtOAc) to give the product in 73% yield (433 mg, 0.88 mmol) as a white solid; IR (KBr) 1761, 1646, 1536, 1377, 1298, 1128; m.p. 38–41 °C; ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 7.75 (bs, 1H), 7.69 (dd, *J* = 5.5, 5.5 Hz, 1H), 7.65 (s, 1H), 7.52–7.49 (m, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 7.31–7.29 (m, 5H), 4.86 (s, 2H), 4.60 (d, *J* = 6.0 Hz, 2H), 4.40 (dd, *J* = 14.5, 4.5 Hz, 1H), 4.31 (dd, *J* = 14.5, 4.5 Hz, 1H), 3.79–3.76 (m, 1H), 2.65 (dd, *J* = 14.0, 5.0 Hz, 1H), 2.33 (dd, *J* = 14.0, 2.5 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 165.8, 163.8, 144.7, 136.0, 135.1, 135.0, 130.9, 129.5, 129.3, 129.0, 127.8, 126.9, 124.6, 124.1, 122.4, 78.6, 56.3, 50.0, 36.0, 35.5; HRMS (ESI) *m/z* calcd for C₂₂H₂₀ClF₃N₅O₃ [M+H]⁺ 494.1207, found 494.1191.

5.4.14. 2-((4-((2-Chloro-5-(trifluoromethyl)benzamido)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-oxoazetidin-1-yl 4-methyl benzenesulfonate (51c)—

Compound **49c** (340 mg, 0.69 mmol) was dissolved in MeOH (10 mL) at rt, under an argon atmosphere. Pd/C 10% (30 mg) was added and the mixture was flushed with hydrogen and left to stir at rt for 4 h. The reaction mixture was filtered through celite and the solvent was evaporated under vacuum to give *N*-hydroxy β-lactam **50c** as a colorless oil.

Crude **50c** was dissolved in dry CH₃CN (20 mL) at rt, under an argon atmosphere. The solution was cooled to 0 °C and DIPEA (179 μL, 1.04 mmol) was added, followed by TsCl (197 mg, 1.04 mmol). The reaction mixture was left to stir at 0 °C for 10 min then the solvent was evaporated under vacuum. The resulting residue was purified by column chromatography on silica gel (hexanes/EtOAc 1:1 to EtOAc) to give the product in 66% yield (257 mg, 0.46 mmol); white solid; IR (KBr) 2960, 1808, 1655, 1541, 1383, 1194; m.p. 50–54 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm) = 8.03 (s, 1H), 7.91–7.90 (m, 1H), 7.85 (d, *J* = 8.5 Hz, 2H), 7.60–7.58 (m, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.16 (bs, 1H), 4.86 (dd, *J* = 14.5, 3.5 Hz, 1H), 4.80–4.70 (m, 3H), 4.46–4.42 (m, 1H), 2.95 (dd, *J* = 15.3, 6.5 Hz, 1H), 2.62 (dd, *J* = 15.3, 4.0 Hz, 1H), 2.42 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ (ppm) = 165.4, 165.3, 147.1, 144.5, 135.6, 134.9, 131.1, 130.4, 130.1, 129.7, 129.3, 128.2, 128.1, 127.4, 124.5, 57.8, 49.6, 36.4, 35.8, 22.0; HRMS (ESI) *m/z* calcd for C₂₂H₂₀ClF₃N₅O₅S [M+H]⁺ 558.0826, found 558.0831.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Previously reported β -lactamase inhibitors.

Figure 2.
Structures of previously reported β -lactamase inhibitors.

Scheme 1.
Mechanism of action of *N*-tosyloxy-2-azetidinones.

Scheme 2. Reactivity of *N*-sulfonyloxy β -lactams vs *N*-unsubstituted or *N*-alkyl β -lactams.

Scheme 3.
Synthetic strategies toward monocyclic β -lactams.

Scheme 4.

Halomethyl substituted β -lactams as precursors of highly functionalized monobactams.

Scheme 5.
Syntheses of *N*-sulfonyloxy β-lactams **28a-j**.

Scheme 6.
Synthesis of *N*-sulfonyloxy β -lactam **39**.

Scheme 7.
Mechanism of action of tazobactam.

Scheme 8.
Syntheses of *N*-sulfonyloxy β-lactams **51a-c**.

Table 1

Spectrum of antimicrobial activity in the Agar Diffusion Assay

All solutions were prepared by first making a 20 mM solution in DMSO and diluting 10-fold with MeOH to give 2 mM solution. Plates were incubated after inoculation at 37 °C for 20 hours. Plates were photographed after 1 day. *M. vaccae* required a longer incubation time, 48 hours and was photographed after 3 days.

Zone of Growth Inhibition (mm)										
	<i>B. Subtilis</i>	<i>S. Aureus</i>	<i>M. Luteus</i>	<i>M. Vaccae</i>	<i>A. Baumannii</i>	<i>B. Dolosa</i>	<i>P. Aeruginosa</i>		<i>E. Coli</i>	
	ATCC 6633	SG511	ATCC 10240	IMET 10670	ATCC 17961		K799/wt	K799/61	DC0	DC2
28a	11V	h	h	17P	h	0	0	0	0	13V
28b	11V	0	0	17P	0	0	0	0	0	15V
28c	12	14P	13.5P	16V	0	0	0	0	0	0
28d	0	13	12* ppt	16	0ppt	0	0	0	h	14V
28e	13/17P	13	12ppt	17	0	0	0	0	0	15V
28f	13	14V	14V	19P	11V	0	0	0	h	14V
28g	14	h	0	0	11ppt	0	0	0	0	14V
28h	22	14P/23V	16V	32	0	0	0	0	0	15V
28i	0	0	19V	14Pppt	0	0	0	0	0	h ppt
28j	0	0	0	0	0	0	0	0	0	16V
39	0	h	0	H	0	0	0	0	0	14V
51a	15V	0	0	0	0	0	0	0	0	0
51b	0	0	0	0	0	0	0	0	0	0
51c	13P	h	13P	0	13P	0	0	0	0	0

h: Indicates only a hint of growth inhibition detectable. P: Indicates unclear inhibition zone. V: Indicates a very unclear inhibition zone.

* Indicates a slightly misshapen zone, most likely due to solubility issues. ppt: presence of precipitate.

Table 2

Ki values for compounds **28c,e,f,i** against selected β -lactamase enzymes from classes A,B,C and D.

<i>Ki</i> (μ M)											
<i>Enzyme</i>	<i>KPC-2</i>	<i>CTX-M-14</i>	<i>Oxa-48</i>	<i>AmpC</i>	<i>SHV-12</i>	<i>PAM 2035</i>	<i>NDM-1</i>	<i>VIM-1</i>			
Class	A	A	D	C	A	C	B	B			
28c	>160	>160	4.673	>160	0.198	>160	>160	N/A			
28e	>160	>160	>160	>160	0.190	>160	>160	>160			
28f	19,290	0.149	1.481	>160	0.779	>160	>160	>160			
28i	>160	>160	>160	>160	>160	>160	>160	N/A			