Syntheses and Biological Evaluations of Highly Functionalized Hydroxamate Containing and N-Methylthio Monobactams as Anti-Tuberculosis and β-Lactamase Inhibitory Agents.

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SUPPORTING INFORMATION

Commercial grade reagents and solvents were used without further purification except as indicated. All reactions were carried out in anhydrous solvents, unless otherwise stated. Tetrahydrofuran (THF) was distilled from benzophenone and sodium and both DCM and MeCN were distilled from calcium hydride. All reactions were carried out in oven or flame-dried glassware under an atmosphere of dry argon only when specified in the experimental details. For reactions in which a hydroxamic acid was made, all glassware used for both reaction and workup were washed with 6 M HCl and dried prior to use. All reactions were magnetically stirred and monitored by analytical thin-layer chromatography using aluminum-backed 0.2 mm silica gel 60 F-254 plates. Visualization was accomplished by UV light (254 nm), potassium permanganate, and vanillin spray. Column chromatography was performed with silica gel 60 (230–400 mesh). Both 1H NMR and 13C NMR spectra were recorded at ambient temperature with the residual solvent peaks as internal standards. IR spectra were recorded on a Jasco FT-IR 6300. High-
resonance mass spectra (HRMS) data were obtained as specified. Melting points were
performed on a Thomas Hoover capillary melting point apparatus and are uncorrected.

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**S-1 Agar Diffusion Procedure**

Overnight cultures of test organisms were grown in LB broth for 18-24 h and standard
suspending of ~1.5 x 10⁸ cfu/mL were prepared in sterile saline solution (0.9% NaCl) according
to a BaSO₄ 0.5 McFarland Standard. Of this standardized suspension, 0.1 mL was added to 34
mL of sterile, melted, and tempered (47-50 °C) Mueller-Hinton No. 2 agar. After gentle mixing,
the inoculated melted agar was poured into a sterile petri dish (145 mm x 20 mm, Greiner Bio-
One) and allowed to solidify next to the flame with lids slightly ajar. Wells of 9 mm diameter
were cut from the petri dish agar and filled with 50 μL of the test sample solution. Solutions were
made @ 20 mM in DMSO and diluted in MeOH to 2 mM. The petri dish was incubated at 37 °C
for 18-24 h and the inhibition zone diameters were measured (mm) with an electronic caliper
after 24-48 h.
S-2 Microplate Alamar Blue Assay

Procedure and details for this assay may be found in the following references:


S-3 In Vitro β-Lactamase Screening Procedure

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 Bovine Serum Albumin (BSA). 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 mL 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 μM to 3 nM. The master block was then used to add 50 μL to each corresponding well in the 96-well flat-bottom plate. An additional 50 μL of buffer was added to each well. Once the plates were prepared, 50 μL of the enzyme to be tested against was added in buffer to each well. The plates were incubated at rt for 10 min before 50 μL of an appropriate indicator was 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 is appropriate because when cleaved by a β-lactamase, the color changes from yellow to red. For NDM-1, imipenem was the indicator of choice. Rather than observe the growing presence of optical density at 495 nm, as done with nitrocefin, the disappearance of imipenem was monitored. The inhibition curves were monitored and used to
perform enzyme kinetics according to Waley\textsuperscript{1} to determine the $K_i$ ($\mu$M) of each compound against the $\beta$-lactamases screened.

**S-4 Synthetic Procedures**

$N$-(Benzyloxy)-$N$-(oxoazetidin-2-yl)benzamide (4).

To a flame dried RB flask under argon was added $N$-(benzyloxy)benzamide (1.19 g, 5.23 mmol). The flask was cooled to 0 °C and a solution of 0.8 M NaOEt in EtOH (10 mL, 8.0 mmol) was slowly added. The reaction was stirred for 5 min, 4-acetoxy-2-azetidinone (Sigma-Aldrich, 1.00 g, 7.74 mmol) in anhydrous EtOH (5 mL) was added dropwise and the reaction was stirred overnight, warming to rt. The reaction was diluted with EtOAc (75 mL), washed with $H_2O$, brine, dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give crude product that was triturated with hexanes and then purified with column chromatography (90/10 ether/hexanes) to give 4 (1.10 g, 3.71 mmol) as a white powder in 71% yield.

**mp** 124-126 °C

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ ppm 3.14 - 3.23 (m, 1 H) 3.49 (dd, $J$=14.98, 1.91 Hz, 1 H) 4.70 (d, $J$=9.39 Hz, 1 H) 4.87 (d, $J$=9.48 Hz, 1 H) 5.99 - 6.05 (fine m, 1 H) 6.48-6.52 (br. s., 1 H) 7.11 (dd, $J$= 7.22, 1.84 Hz, 2 H) 7.28 - 7.73 (m, 8 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.73, 166.64, 133.93, 133.65, 131.79, 130.06, 129.49, 128.89, 128.70, 128.65, 80.57, 60.51, 42.41

IR (FT-IR) $\nu$ 1772, 1650 cm$^{-1}$

HRMS calcd. for C$_{17}$H$_{16}$N$_2$O$_3$ (M + H$^+$) 297.1234, found 297.1223.
**N-(Benzyloxy)-N-(1-(methylthio)-4-oxoazetidin-2-yl)benzamide (5).**

To a flame dried pressure tube under argon was added 4 (75 mg, 0.25 mmol) and anhydrous DCM (5 mL). To the resulting solution was added 2-(methylthio)isoindoline-1,3-dione (53 mg, 0.27 mmol) and Et₃N (39 µL, 0.28 mmol). The tube was sealed with a teflon screw cap, heated to 40 ºC (bath temperature) and stirred overnight. The reaction was concentrated under reduced pressure and then purified with column chromatography (40/60 EtOAc/hexanes) to give 5 (45 mg, 0.13 mmol) as a white solid in 52% yield

mp = >250 ºC (Color change from white to orange noted in range of 170-175ºC)

¹H NMR (300 MHz, CDCl₃) δ = 7.79 - 7.14 (m, 10 H), 5.84 (br. s., 1 H), 4.82 (br. s, 2 H), 3.32 (dd, J = 2.2, 12.7 Hz, 1 H), 3.18 (dd, J = 5.0, 10.0 Hz, 1 H), 2.48 (s, 3 H)

¹³C NMR (125 MHz, CDCl₃) δ 171.2, 168.6, 133.8, 133.5, 131.6, 129.6, 129.1, 128.6, 128.5, 128.4, 79.0, 67.4, 42.1, 22.2

HRMS calcd. for C₁₈H₁₈N₂O₃S (M + H⁺) 342.1111, found 342.1146.

**N-(benzyloxy)-N-(1-(tert-butyldimethylsilyl)-4-oxoazetidin-2-yl)benzamide (6).**

To a flame dried RB flask under argon was added 4 (250 mg, 0.844 mmol) and anhydrous DMF (15 mL). The RB flask was cooled in an ice bath, TBS-Cl (159 mg, 1.05 mmol) and Et₃N (0.18 mL, 1.3 mmol) were added, and the reaction was stirred overnight, warming to rt. The solvent was removed under reduced pressure and the crude material was dissolved in DCM, washed with
brine, dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give a residue that was purified with column chromatography (75/25 hexanes/EtOAc) to give 6 (206 mg, 0.501 mmol) as a white solid in 60% yield.

mp = 113-114 °C

$^1$H NMR (300 MHz, CDCl$_3$) δ = 7.80 - 6.96 (m, 10 H), 6.15 (br. s., 1 H), 4.87 (d, $J$ = 7.7 Hz, 1 H), 4.58 (d, $J$ = 8.8 Hz, 1 H), 3.49 (d, $J$ = 14.1 Hz, 1 H), 3.34 (dt, $J$ = 5.0, 10.5 Hz, 1 H), 0.98 (s, 9 H), 0.36 (s, 3 H), 0.27 (s, 3 H)

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 171.5, 170.0, 133.9, 133.5, 131.6, 129.7, 129.4, 128.9, 128.7, 128.5, 80.5, 61.5, 43.0, 26.4, 19.2, -5.17

IR (FT-IR) ν 1750, 1660 cm$^{-1}$

HRMS calcd. for C$_{23}$H$_{31}$N$_2$O$_3$Si (M + H$^+$) 411.2123, found 411.2098.

$\text{N-(Benzyloxy)-4-nitro-N-(4-oxoazetidin-2-yl)benzamide (7a).}$

To a flame dried round bottom flask under argon was added 2a (4.00 g, 14.6 mmol). The RB flask was cooled to 0° C and a solution of 0.8 M NaOEt in anhydrous EtOH (18.3 mL, 14.6 mmol) was added dropwise. The reaction was stirred for 5 min and 4-acetoxy-2-azetidinone (1.80 g, 14.6 mmol), dissolved in anhydrous EtOH, was slowly added. The reaction was left to stir overnight, warming to rt. The solvent was removed under reduced pressure and the crude material was dissolved with EtOAc (75 mL), washed with H$_2$O, brine, dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give an off white solid that was purified with
column chromatography (50/50 EtOAc/hexanes to 100% EtOAc) to give 7a as a white solid (2.11 g, 6.18 mmol) in 44% yield.

\[ \text{mp} = 180-182 \, ^\circ\text{C} \]

\( ^1\text{H NMR} \) (300 MHz, DMSO-d\(_6\)) \( \delta \) 8.62 (s, 1 H), 8.29 (d, \( J = 8.8 \, \text{Hz}, 2 \, \text{H} \)), 7.80 (d, \( J = 8.8 \, \text{Hz}, 2 \, \text{H} \)), 7.42 - 6.99 (m, 5 H), 5.86 (br. s, 1 H), 4.90 (d, \( J = 9.6 \, \text{Hz}, 1 \, \text{H} \)), 4.79 (d, \( J = 9.6 \, \text{Hz}, 1 \, \text{H} \)), 3.28 - 3.13 (br. s., 2 H)

\( ^{13}\text{C NMR} \) (75 MHz, DMSO-d\(_6\)) \( \delta \) 168.7, 166.5, 149.1, 140.7, 134.6, 129.9, 129.8, 129.5, 129.1, 124.1, 80.2, 60.5, 42.8

IR (FT-IR) \( \nu \) 1723, 1656 cm\(^{-1}\)

HRMS calcd. for C\(_{17}\)H\(_{15}\)N\(_3\)O\(_5\) (M + H\(^+\)) 342.1084, found 342.1074.

\[ \text{N-(Benzyloxy)-4-ethoxy-N-(4-oxoazetidin-2-yl)benzamide (7b).} \]

To a flame dried RB flask under argon and at 0 \(^\circ\text{C} \) was added 2b (5.25 g, 19.4 mmol) and 24.15 mL (19.32 mmol) of a 0.8 M NaOEt solution. The reaction was stirred for 5 min and 4-acetox-2-azetidinone (2.24 g, 17.35 mmol), already dissolved in 20 mL of anhydrous EtOH, was added slowly. The reaction was left to stir overnight, warming to rt. The solvent was removed under reduced pressure and the crude material was dissolved with EtOAc (80 mL), washed with H\(_2\)O, brine, dried with MgSO\(_4\), filtered, and concentrated under reduced pressure to give an off white solid that was purified with column chromatography (100% diethyl ether to 100% EtOAc) to give 7b as a white solid (3.08 g, 9.05 mmol) in 47% yield.

\[ \text{mp} = 114-115 \, ^\circ\text{C} \]
$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.71 (d, $J = 8.8$ Hz, 2 H), 7.39 - 7.10 (m, 5 H), 6.89 (d, $J = 8.8$ Hz, 2 H), 6.68 (s, 1 H), 6.00 (dd, $J = 1.8$, 2.9, 4.7 Hz, 1 H), 4.85 (d, $J = 9.3$ Hz, 1 H), 4.66 (d, $J = 9.3$ Hz, 1 H), 4.07 (q, $J = 7.0$ Hz, 2 H), 3.41 (dd, $J = 1.7$, 15.1 Hz, 1 H), 3.13 (dq, $J = 1.9$, 2.9, 7.4 Hz, 1 H), 1.44 (t, $J = 6.9$ Hz, 3 H)

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 170.0, 166.8, 161.9, 134.0, 131.2, 130.0, 129.4, 128.8, 125.2, 114.2, 80.4, 63.9, 60.5, 42.3, 14.9

IR (FT-IR) $\nu$ 1727, 1658 cm$^{-1}$

HRMS calcd. for C$_{19}$H$_{20}$N$_2$O$_4$ (M + Na$^+$) 363.1315, found 363.1332.

![Chemical Structure](image)

N-(Allyloxy)-4-nitro-N-(4-oxoazetidin-2-yl)benzamide (7c).

To a flame dried RB flask under argon was added 2c (500 mg, 2.25 mmol). The RB flask was cooled to 0 °C and a solution of 0.8 M NaOEt in anhydrous EtOH (2.8 mL, 2.2 mmol) was added dropwise. The reaction was stirred for 5 min and then 4-acetoxy-2-azetidinone (319 mg, 2.48 mmol) already dissolved in anhydrous EtOH was added dropwise. The reaction was left to stir overnight under argon, warming to room temperature. The solvent was removed under reduced pressure and the crude material was dissolved with EtOAc (50 mL), washed with H$_2$O, brine, dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give a yellow oil which was purified with column chromatography (100% ether to 100% EtOAc) to give 7c (367 mg, 1.26 mmol) as a white solid in 56% yield.

mp = 140-141 °C

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.29 (d, $J = 9.0$ Hz, 2 H), 7.90 (d, $J = 9.0$ Hz, 2 H), 6.36 (br. s., 1 H), 6.12 (br. s., 1 H), 5.72 - 5.49 (m, $J = 6.4$, 6.4, 10.4, 17.0 Hz, 1 H), 5.23 (d, $J = 10.4$ Hz, 1
H), 5.18 (dd, J = 1.2, 17.1 Hz, 1 H), 4.41 (dd, J = 6.6, 10.4 Hz, 1 H), 4.16 (dd, J = 7.0, 10.6 Hz, 1 H), 3.53 (dd, J = 2.0, 15.2 Hz, 1 H), 3.42 - 3.21 (m, 1 H)

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 168.8, 166.2, 149.5, 139.2, 130.2, 129.9, 123.6, 122.1, 80.5, 59.8, 42.4

IR (FT-IR) ν 1763, 1649 cm$^{-1}$

HRMS calcd. for C$_{13}$H$_{14}$N$_3$O$_5$ (M + H$^+$) 292.0928, found 292.0908.

\[
\begin{array}{c}
\text{BnO} \\
\text{N} \\
\text{O} \\
\text{N} \\
\text{O} \\
\text{SMe} \\
\text{Me} \\
\text{N}-(\text{Benzyloxy})-\text{N}-(1-(\text{methylthio})-4-\text{oxazetidin-2-yl})-4-\text{nitrobenzamide (8a).}
\end{array}
\]

To a flame dried pressure tube under argon was added 7a (100 mg, 0.293 mmol) and anhydrous DCM (7 mL). To this solution was added 2-(Methylthio)isoindoline-1,3-dione (63 mg, 0.32 mmol) and Et$_3$N (0.045 mL, 0.322 mmol). The tube was capped tightly, heated to 40°C, and stirred overnight. Upon completion (TLC: 80/20 EtOAc/hexanes), the solvent was removed under reduced pressure and the residue was purified with column chromatography (60/40 hexanes/EtOAc) to afford 8a (65 mg, 0.17 mmol) as a colorless oil in 57% yield.

$^1$H NMR (300 MHz, CDCl$_3$) δ 8.27 (d, J = 8.4 Hz, 2 H), 7.85 (d, J = 8.4 Hz, 2 H), 7.39 - 6.98 (m, 5 H), 6.02-5.96 (br s, 1 H), 4.75 (dd, J = 10.0, 13.9 Hz, 2 H), 3.42 (d, J = 14.1 Hz, 1 H), 3.29 (dd, J = 5.0, 10.0 Hz, 1 H), 2.51 (s, 3 H)

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 169.8, 168.5, 149.4, 139.4, 133.3, 129.9, 129.8, 129.7, 129.0, 123.7, 80.4, (broad) 66.7 (broad), 42.5, 22.4

IR (FT-IR) ν 1778, 1678 cm$^{-1}$

HRMS calcd. for C$_{18}$H$_{17}$N$_3$O$_5$S (M + Na$^+$) 410.0781, found 410.0785.
**N-(Benzyloxy)-4-ethoxy-N-(1-(methylthio)-4-oxoazetidin-2-yl)benzamide (8b).**

To a flame dried pressure tube under argon was added 7b (75 mg, 0.22 mmol) and anhydrous DCM (7 mL). To this solution was added 2-(Methylthio)isoindoline-1,3-dione (47 mg, 0.24 mmol) and Et$_3$N (0.034 mL, 0.243 mmol). The tube was capped tightly, heated to 40 °C, and stirred overnight. Upon completion (TLC: 80/20 EtOAc/hexanes), the solvent was removed under reduced pressure and the residue was purified with column chromatography (60/40 hexanes/EtOAc) to afford 8b (72 mg, 0.19 mmol) as a clear oil in 85% yield.

$^1$H NMR (300 MHz, CDCl$_3$) δ 7.75 (d, $J = 7.7$ Hz, 2 H), 7.39 - 7.18 (m, 5 H), 6.93 (d, $J = 8.6$ Hz, 2 H), 5.88 (d, $J = 2.6$ Hz, 1 H), 4.80 (s, 2 H), 4.09 (q, $J = 7.2$ Hz, 2 H), 3.34 (d, $J = 14.8$ Hz, 1 H), 3.13 (dd, $J = 5.3, 9.8$ Hz, 1 H), 2.47 (overlapping s, restricted rotation isomerism, 3 H), 1.44 (t, $J = 5.7$, 3 H)

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 171.0, 169.0, 162.0, 134.2, 131.1, 129.8, 129.3, 128.8, 125.3, 114.4, 79.1, 67.8, 63.9, 42.3, 22.5, 14.9

IR (FT-IR) ν 1772, 1673 cm$^{-1}$

HRMS calcd. for C$_{20}$H$_{22}$N$_2$O$_4$S (M + Na$^+$) 409.1192, found 409.1205.

**N-(Allyloxy)-N-(1-(methylthio)-4-oxoazetidin-2-yl)-4-nitrobenzamide (8c).**

To a flame dried pressure tube was added 7c (100 mg, 0.343 mmol) and 7 mL of dry DCM. To this solution was added 2-(Methylthio)isoindoline-1,3-dione (73 mg, 0.38 mmol) and Et$_3$N
(0.053 mL, 0.377 mmol). The tube was capped tightly, heated to 40 °C, and stirred overnight. Upon completion (TLC: 80/20 EtOAc/hexanes), the solvent was removed under reduced pressure and the residue was purified with column chromatography (60/40 hexanes/EtOAc) to give 8c (105 mg, 0.311 mmol) as a clear oil in 91% yield.

$^1$H NMR (300 MHz, CDCl$_3$) δ = 8.31 (dt, $J = 2.2$, 5.3 Hz, 2 H), 7.92 (dt, $J = 1.4$, 2.4, 5.3 Hz, 2 H), 6.01 - 5.82 (br. s., 1 H), 5.74 - 5.56 (m, 1 H), 5.24 (overlapping m, 2 H), 4.28 (ddt, $J = 5.0$, 6.0, 10.8 Hz, 2 H), 3.48 (dd, $J = 2.4$, 12.7 Hz, 1 H), 3.33 (dd, $J = 5.0$, 9.8 Hz, 1 H), 2.49 (s, 3 H)

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 169.7, 168.4, 149.5 139.4, 130.4, 129.9 123.8, 121.9, 79.2, 66.6, 42.4, 22.3

IR (FT-IR) ν 1781, 1681 cm$^{-1}$

HRMS calcd. for C$_{14}$H$_{15}$N$_3$NaO$_5$S (M + Na$^+$) 360.0625, found 360.0633.

$N$-(Benzyloxy)-3-nitro-$N$-(4-oxoazetidin-2-yl)-5-(trifluoromethyl)benzamide (9a).

To a flame dried RB flask under argon was added 3a (300 mg, 0.882 mmol) and 15 mL of acetone. The solution was cooled to 0 °C in an ice bath and 1M NaOH (0.882 mL, 0.880 mmol) was added dropwise, and the reaction was stirred for 10 min. 4-Acetoxy-2-azetidinone (140 mg, 1.1 mmol), dissolved in a minimal amount of acetone, was then added dropwise at 0 °C and the reaction was stirred overnight, warming to rt. The reaction was diluted with H$_2$O and extracted with EtOAc (x 2). The combined organic extracts were then washed with brine, dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give the crude material that was then purified twice with column chromatography (50/50 hexanes/EtOAc) to give 9a (176 mg, 0.429 mmol) as a white solid in 49% yield.
mp = 151-152 °C

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ = 8.58 - 8.49 (overlapping s, 2 H), 8.12 (s, 1 H), 7.32 - 6.89 (m, 5 H), 6.31 (br. s., 1 H), 6.21 (fine d, $J$ = 2.4 Hz, 1 H), 4.96 (d, $J$ = 10.2 Hz, 1 H), 4.74 (d, $J$ = 10.6 Hz, 1 H), 3.66 (dd, $J$ = 2.1, 15.3 Hz, 1 H), 3.42 - 3.34 (m, 1 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ = 167.06, 166.14, 148.05, 136.12, 132.74, 131.55, 131.53, 130.11, 129.94, 129.05, 127.36, 123.01, 122.98, 82.14, 60.24, 42.71, 30.57

IR (FT-IR) $\nu$ 1790, 1651 cm$^{-1}$

HRMS calcd. for C$_{18}$H$_{15}$F$_3$N$_5$O$_5$ (M + H$^+$) 410.0958, found 410.0936.

\[
\text{N-(Allyloxy)-3-nitro-N-(4-oxoazetidin-2-yl)-5-(trifluoromethyl)benzamide (9b).}
\]

To a flame dried RB flask under argon was added 3b (150 mg, 0.517 mmol) and 20 mL of acetone. The solution was cooled to 0 °C in an ice bath and 1M NaOH (0.540 mL, 0.540 mmol) was added dropwise, and the reaction was stirred for 10 min. 4-Acetoxy-2-azetidinone (70 mg, 0.54 mmol), dissolved in a minimal amount of acetone, was then added dropwise at 0 °C and the reaction was stirred overnight, warming to rt. The reaction was diluted with H$_2$O and extracted with EtOAc (x 2). The combined organic extracts were then washed with brine, dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give the crude material that was then purified with column chromatography (60/40 EtOAc/hexanes) to give 9b (99 mg, 0.28 mmol) as a colorless film in 53% yield.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ = 8.85 (s, 1 H), 8.64 (s, 1 H), 8.40 (s, 1 H), 6.39 (br. s., 1 H), 6.18 (fine d, $J$ = 2.8 Hz, 1 H), 5.68 - 5.52 (m, $J$ = 6.4, 6.4, 10.5, 17.0 Hz, 1 H), 5.31 - 5.12
(overlapping m, 2 H), 4.49 (dd, J = 6.4, 10.4 Hz, 1 H), 4.17 (dd, J = 6.7, 10.9 Hz, 1 H), 3.58 (dd, J = 2.1, 15.3 Hz, 1 H), 3.44 - 3.24 (m, 1 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ = 166.6, 165.7, 148.3, 136.0, 132.9, 132.6, 131.7, 129.7, 127.4, 123.4, 122, 5, 80.9, 59.9, 42.5

IR (FT-IR) $\nu$ 1779, 1660 cm$^{-1}$

HRMS calcd. for C$_{14}$H$_{13}$F$_3$N$_3$O$_5$ (M + H$^+$) 360.0802, found 360.0823.

\[ \text{N-(Benzylxy)-N-(1-(methylthio)-4-oxoazetidin-2-yl)-3-nitro-5-(trifluoromethyl)benzamide (10a).} \]

To a flame dried pressure tube was added 9a (75 mg, 0.18 mmol) and 10 mL of dry DCM. To this solution was added 2-(Methylthio)isoindoline-1,3-dione (46 mg, 0.24 mmol) and Et$_3$N (0.034 mL, 0.238 mmol). The tube was capped tightly, heated to 40 °C, and stirred overnight. The solvent was removed under reduced pressure and the residue was purified with column chromatography (60/40 hexanes/EtOAc) to give 10a (73 mg, 0.16 mmol) as a sticky white gel in 87% yield.

$^1$H NMR (600 MHz, CD$_3$OD) $\delta$ = 8.61 (s, 1 H), 8.59 (s, 1 H), 8.22 (s, 1 H), 7.28 - 7.18 (m, 3 H), 7.14 (br. s., 2 H), 6.12 - 6.01 (br. s., 1 H), 4.91 - 4.83 (s, overlap with CD$_3$OD, 2 H), 3.54 (d, J = 15.3 Hz, 1 H), 3.40 (dd, J = 5.0, 10.0 Hz, 1 H), 2.51 (s, 3 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ = 169.6, 167.9, 148.1, 136.3, 133.6, 130.84, 130.82, 130.80, 130.7, 129.6, 128.8, 128.2, 126.8, 123.5, 122.38, 122.36, 121.7, 79.9, 67.4, 41.8, 20.4

IR (FT-IR) $\nu$ 1770, 1667 cm$^{-1}$

HRMS calcd. for C$_{19}$H$_{16}$F$_3$N$_3$NaO$_5$S (M + Na$^+$) 478.0655, found 478.0658.
N-(Allyloxy)-N-(1-(methylthio)-4-oxazetidin-2-yl)-3-nitro-5-(trifluoromethyl)benzamide (10b).

To a flame dried pressure tube under argon was added 9b (40 mg, 0.11 mmol) and 10 mL of dry DCM. To this solution was added 2-(methylthio)isoindoline-1,3-dione (30 mg, 0.15 mmol) and Et$_3$N (0.017 mL, 0.122 mmol). The tube was capped tightly, heated to 40 °C, and stirred overnight. The solvent was removed under reduced pressure and the residue was purified with column chromatography (60/40 hexanes/EtOAc) to give 10b (28 mg, 0.07 mmol) as a clear oil in 62% yield.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ = 8.86 (s, 1 H), 8.64 (s, 1 H), 8.41 (s, 1 H), 6.04 (br. s., 1 H), 5.63 (br. s., 1 H), 5.30 - 5.15 (m, 2 H), 4.32 (br. s., 1 H), 4.23 (br. s., 1 H), 3.57 (dd, $J$ = 1.8, 15.0 Hz, 1 H), 3.37 (dd, $J$ = 5.0, 15.0 Hz, 1 H), 2.52 (s, 3 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ = 168.3, 167.5, 148.3, 136.2, 131.78, 131.78, 129.8, 127.3, 123.53, 123.50, 79.9, 66.3, 42.5, 22.2

IR (FT-IR) $\nu$ 1777, 1670 cm$^{-1}$

HRMS calcd. for C$_{15}$H$_{15}$F$_3$N$_3$O$_5$S (M + H$^+$) 406.0679, found 406.0649.

N-(Benzyloxy)-N-((2R,3S)-3-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-oxazetidin-2-yl)benzamide (11).
To a flame dried round bottom flask under argon was added N-(benzyloxy)benzamide (4.25 g, 18.7 mmol). The RB flask was cooled to 0° C and a solution of 0.8 M NaOEt in anhydrous EtOH (23.5 mL, 18.8 mmol) was added slowly. The reaction was stirred for 5 min and (2R,3R)-3-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-oxoazetidin-2-yl acetate (5.67 g, 19.8 mmol), dissolved in anhydrous EtOH was added slowly. The reaction was left to stir overnight, warming to rt. The solvent was removed under reduced pressure and the crude material was dissolved in EtOAc (50 mL), washed with H2O, brine, dried with MgSO4, filtered, and concentrated under reduced pressure to give a yellow oil was purified with column chromatography (85/15 DCM/EtOAc) to give 11 (5.17 g, 11.4 mmol) as a clear oil that slowly solidified into a white waxy solid at 0 °C in 61% yield.

mp = 79-80 °C

1H NMR (600 MHz, CDCl3) δ = 7.69 - 6.96 (m, 5 H), 6.05 - 5.95 (fine m, 1 H), 5.90 - 5.83 (fine m, 1 H), 4.88 (d, J = 9.3 Hz, 1 H), 4.75 (d, J = 9.6 Hz, 1 H), 4.22 (dq, J = 2.3, 3.8, 6.2 Hz, 1 H) 3.61 - 3.53 (dd, 2.2, 1.2 Hz, 1 H), 1.08 (d, J = 6.2 Hz, 1 H), 0.72 (s, 9 H), -0.04 (s, 6 H)

13C NMR (125 MHz, CDCl3) δ 170.3, 167.0, 133.9, 133.8, 131.2, 129.7, 129.0, 128.5, 128.3, 128.2, 79.9, 64.1, 62.8, 62.3, 25.6, 22.3, 17.7, -4.47 -5.15

IR (FT-IR) ν 1751, 1669 cm⁻¹

HRMS calcd. for C25H35N2O4Si (M + H⁺) 455.2361, found 455.2351.

\[ \text{N-(Benzyloxy)-N-((2R,3S)-3-((R)-1-hydroxyethyl)-4-oxoazetidin-2-yl)benzamide (12).} \]

To a RB flask was added 11 (40 mg, 0.11 mmol) and MeCN (5 mL). To the resulting solution was added 0.4 mL of 1 M HCl and the reaction was stirred until complete by TLC (60/40
EtOAC/hexanes). After 7 h, the reaction was diluted with EtOAc/H2O, added to a separatory funnel, and washed with saturated solution of NaHCO3. The organic layer was dried with MgSO4, filtered, and concentrated under reduced pressure. The crude material was purified with column chromatography to give 12 (14 mg, 0.04 mmol) as colorless residue in 47% yield.

1H NMR (500 MHz, CDCl3) δ = 7.79 - 7.04 (m, 10 H), 6.62 (br. s, 1 H), 5.97 (br. s, 1 H), 4.86 (d, J = 9.4 Hz, 1 H), 4.80 - 4.65 (m, 1 H), 4.21 (q, J = 6.2 Hz, 3 H), 3.66 (d, J = 3.2 Hz, 2 H), 3.01 (br. s, 1 H), 1.23 (d, J = 6.2 Hz, 3 H)

13C NMR (125 MHz, CDCl3) δ 170.7, 167.7, 133.7, 133.4, 131.5, 129.8, 129.1, 128.6, 128.4, 128.3, 80.1, 63.6, 62.9, 62.2, 21.2

IR (FT-IR) ν 1779, 1660 cm⁻¹

HRMS calcd. for C19H21N2O4 (M + H⁺) 341.1496, found 341.1483.

\[ \text{N-(Benzyloxy)-N-((2S,3S)-3-((S)-1-((tert-butyldimethylsilyl)oxy)ethyl)-1-(methylthio)-4-oxoazetidin-2-yl)benzamide (13).} \]

To a flame dried pressure tube under argon was added 11 (100 mg, 0.220 mmol) and anhydrous DCM (12 mL). To this solution was added 2-(methylthio)isoindoline-1,3-dione (55 mg, 0.29 mmol), Et3N (0.037 mL, 0.264 mmol), the reaction was capped, and stirred overnight at 50 °C. The solvent was removed under reduced pressure and the crude material was purified with column chromatography (80/20 hexanes/EtOAc) to give 13 (85 mg, 0.17 mmol) as an oil in 77% yield.
$^{1}$H NMR (300 MHz, CDCl$_3$) $\delta$ = 7.77 - 7.15 (m, 10 H), 5.81 (br. s., 1 H), 4.88 (br. s., 2 H), 4.14 (m, 1 H), 3.49 (br. s., 1 H), 2.43 (s, 3 H), 0.97 (d, $J$ = 5.7 Hz, 3 H), 0.69 (s, 9 H), -0.03 (d, $J$ = 5.7 Hz, 6 H)

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 171.5, 170.1, 134.4, 134.1, 131.6, 130.0, 129.3, 128.9, 128.8, 128.4, 78.8, 64.0, 62.3, 25.7, 22.7, 22.6, 17.9, -4.55, -4.70

IR (FT-IR) $\nu$ 1767, 1672 cm$^{-1}$

HRMS calcd. for C$_{26}$H$_{37}$N$_2$O$_4$Si (M + H$^+$) 501.2238, found 501.2245.

\[
\begin{align*}
\text{NH} & \quad \text{O} \\
\text{O} & \quad \text{Ph} \\
\text{O} & \quad \text{NH} \\
\end{align*}
\]

$N$-((2R,3S)-3-((R)-1-((tert-Butyldimethylsilyl)oxy)ethyl)-4-oxoazetidin-2-yl)-$N$-hydroxybenzamide (14).

To a flame dried RB flask was added 11 (3.58 g, 7.87 mmol) and EtOAc (25 mL). The resulting solution was purged with argon and then palladium on carbon (10%) was added (840 mg). The contents were once again purged with argon by sticking the needle directly into the solution. Once purged, the argon line was removed and a vacuum line was inserted through the septum and held above the solution to remove the argon. Hydrogen gas was then bubbled into the reaction momentarily and then held above the solution. Upon completion (TLC: 100% EtOAc) the reaction was filtered through 2 filter papers. The filter papers were washed with EtOAc and the filtrate was concentrated under reduced pressure to give 14 (2.77 g, 7.59 mmol) as an off-white sticky paste in 97% yield.

$^{1}$H NMR (300 MHz, CDCl$_3$) $\delta$ = 7.66 - 7.30 (m, 5 H), 7.00 - 6.89 (br. s., 1 H), 5.89 (br. s., 1 H), 4.26 - 4.08 (m, 1 H), 3.60 (br. s., 1 H), 1.12 (d, $J$ = 6.2 Hz, 3 H), 0.73 (s, 9 H), -0.03 (d, $J$ = 5.5 Hz, 6 H)
(2S,3S)-4-Oxo-3-(2-phenylacetamido)azetidin-2-yl acetate (16).

To a RB flask was added penicillin G Na salt (1.00 g, 2.87 mmol) and 18 mL of glacial AcOH. To this suspension was slowly added Hg(OAc)$_2$ (1.83 g, 5.74 mmol) and the reaction was vigorously stirred for 2 h at 70 °C. The precipitate was filtered off and the solvent was removed under reduced pressure to give crude material that was dissolved in 80 mL of DCM/H$_2$O (1:1). The layers were separated and the aqueous layer was extracted a second time with DCM (20 mL). The pooled organic layers were dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give the crude material that was purified with column chromatography (60/40 EtOAc/hexanes) to give (2S,3S)-1-(2-Methylprop-1-en-1-yl)-4-oxo-3-(2-phenylacetamido)azetidin-2-yl acetate (201 mg, 0.636 mmol) as a pasty oil in 23% yield.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta =$ 7.35 - 7.18 (m, 5 H), 6.76 (d, $J = 7.6$ Hz, 1 H), 6.03 (d, $J = 1.4$ Hz, 1 H), 5.58 (s, 1 H), 4.62 (d, $J = 7.6$ Hz, 1 H), 3.55 (s, 2 H), 2.08 (s, 13 H), 1.72 (s, 3 H), 1.69 (s, 3 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta =$ 172.1, 170.4, 162.8, 134.4, 133.1, 129.7, 129.2, 127.6, 115.5, 82.5, 63.1, 43.2, 22.9, 21.1, 19.0

To a RB flask was added (2S,3S)-1-(2-methylprop-1-en-1-yl)-4-oxo-3-(2-phenylacetamido)azetidin-2-yl acetate (100 mg, 0.316 mmol) and 20 mL of DCM/MeOH (1:1).
The solution was cooled to -78 °C (bath temperature) and O₃ was passed through the solution until complete by TLC. After 15 min, the reaction was purged with argon and dimethyl disulfide (0.225 mL, 3.16 mmol) was added at -78°C to quench the reaction. The reaction was stirred overnight, warming to rt. The solvent was removed under reduced pressure and the crude material was purified twice with column chromatography (60/40 EtOAc/hexanes) to give known compound 16² (31 mg, 0.12 mmol) as a white solid in 37% yield.

mp = 98-104 °C

¹H NMR (500 MHz, CDCl₃) δ = 7.41 - 7.18 (m, 5 H), 7.02 (br. s, 1 H), 6.42 (d, J = 7.6 Hz, 1 H), 5.80 (s, 1 H), 4.66 (dd, J = 1.3, 7.5 Hz, 1 H), 3.60 (s, 2 H), 2.10 (s, 3 H)

¹³C NMR (125 MHz, CDCl₃) δ 172.2, 171.2, 165.0, 134.1, 129.8, 129.4, 127.9, 78.9, 63.9, 43.3, 21.0

(2S,3S)-1-(Methylthio)-4-oxo-3-(2-phenylacetamido)azetidin-2-yl acetate (17).

To a flame dried pressure tube under argon was added 16 (75 mg, 0.29 mmol) and anhydrous DCM (10 mL). To this solution was added 2-(methylthio)isoindoline-1,3-dione (72 mg, 0.37 mmol) and pyridine (0.025 mL, 0.31 mmol). The tube was capped tightly, heated to 40 °C (bath temperature), and stirred overnight. The solvent was removed under reduced pressure and the residue was purified with column chromatography (65/35 EtOAc/hexanes) to give 17 (39 mg, 0.16 mmol) as a white solid in 44% yield.

mp 117-119 °C

¹H NMR (500 MHz, CDCl₃) δ = 7.40 - 7.19 (m, 5 H), 6.18 (d, J = 1.4 Hz, 1 H), 6.11 (d, J = 7.0 Hz, 1 H), 4.45 (dd, J = 1.6, 7.2 Hz, 1 H), 3.61 (s, 2 H), 2.53 (s, 3 H), 2.14 (s, 3 H)
$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ = 172.1, 170.2, 167.2, 133.9, 129.7, 129.4, 127.9, 83.2, 65.4, 43.2, 21.8, 21.0

IR (FT-IR) $\nu$ 1787, 1656 cm$^{-1}$

HRMS calcd. for C$_{14}$H$_{16}$N$_2$NaO$_4$S (M + Na$^+$) 331.0723, found 331.0722.

**N-(Benzzyloxy)-N-((2S,3S)-4-oxo-3-(2-phenylacetamido)azetidin-2-yl)benzamide (18).**

To a RB flask was added 1 (800 mg, 3.52 mmol) and 50 mL of acetone. The solution was cooled in an ice bath and then 1M NaOH (3.52 mL, 3.52 mmol) was added dropwise and the reaction was left to stir for 10 min. Intermediate 16 (1.00 g, 3.87 mmol), dissolved in a minimal amount of acetone, was then added dropwise at 0$^\circ$ C and the reaction was stirred overnight, warming to rt. The reaction was diluted with H$_2$O and extracted with EtOAc (x 2). The combined organic extracts were then washed with brine, dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give the crude material that was then purified with column chromatography (60/40 EtOAc/hexanes) to give 18 (603 mg, 1.40 mmol) as a white solid in 39% yield.

mp = 179-181 $^\circ$C

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ = 7.69 - 7.04 (m, 15 H), 6.89 (br. s., 1 H), 6.62 (d, $J$ = 7.2 Hz, 1 H), 5.98 (br. s., 1 H), 5.14 (d, $J$ = 7.8 Hz, 1 H), 4.76 (br. s., 2 H), 3.53 (s, 2 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 172.3, 170.9, 166.0, 134.2, 133.9, 133.4, 131.8, 130.1, 129.7, 129.4, 129.2, 128.8, 128.7, 128.6, 127.7, 80.2, 66.8, 60.8, 43.2

IR (FT-IR) $\nu$ 1791, 1654 cm$^{-1}$

HRMS calcd. for C$_{25}$H$_{23}$N$_3$NaO$_4$ (M + Na$^+$) 452.1581, found 452.1560.
N-(Benzyloxy)-N-((2R,3S)-1-(methylthio)-4-oxo-3-(2-phenylacetamido)azetidin-2-yl)benzamide (19).

To a flame dried pressure tube was added 18 (75 mg, 0.17 mmol) and anhydrous DCM (10 mL). To this solution was added 2-(methylthio)isoindoline-1,3-dione (44 mg, 0.23 mmol) and Et₃N (0.027 mL, 0.19 mmol). The tube was capped tightly, heated to 40 °C (bath temperature), and stirred overnight. The solvent was removed under reduced pressure and the residue was purified twice with column chromatography (55/45 hexanes/EtOAc) to give 19 (78 mg, 0.16 mmol) as a white solid in 81% yield.

mp 60-62 °C

¹H NMR (500 MHz, CDCl₃) δ = 7.73 - 7.14 (m, 15 H), 6.29 (d, J = 7.1 Hz, 1 H), 5.86 (br. s., 1 H), 4.92 (d, J = 9.8 Hz, 1 H), 4.81 (d, J = 5.9 Hz, 1 H), 4.77 (d, J = 9.5 Hz, 1 H), 3.53 (dd, J = 4.2, 15.9 Hz, 2 H), 2.44 (s, 3 H)

¹³C NMR (125 MHz, CDCl₃) δ 172.3, 171.1 (broad), 168.1, 134.1, 133.4, 132.0, 130.0, 129.6, 129.43, 129.41, 128.9, 128.8, 128.6, 127.9, 79.4, 72.7 (broad), 61.2, 43.2, 21.2

IR (FT-IR) ν 1768, 1654 cm⁻¹

HRMS calcd. for C₂₆H₂₆N₃O₄S (M + H⁺) 476.1639, found 476.1636.

(2S,3S)-4-Oxo-3-(2-phenoxyacetamido)azetidin-2-yl acetate (20).

To a 250 mL pressure tube was added Hg(OAc)₂ (35 g, 109.8 mmol) and 200 mL of glacial AcOH. The mixture was stirred at 85 °C until all of the solid was dissolved and then penicillin
V Na salt (15 g, 38.6 mmol) was added in portions. The reaction was stirred vigorously for 4 h at 85 °C. The reaction was then cooled to rt, placed in an ice bath for 20 min, and then filtered through a sintered glass funnel. The filtrate was concentrated under reduced pressure and the crude residue was dissolved in DCM and neutralized with 5% NaHCO₃. The neutralized organic layer was then washed with 5% brine, dried with MgSO₄, filtered, and concentrated under reduced pressure to give crude product as a black solid (10 g, 30 mmol) in 82% yield that was then added to a RB flask and 125 mL of DCM. The solution was cooled to -78 °C and O₃ was passed through the solution until the reaction was complete by TLC. After 40 min, the reaction was purged with argon and then dimethyl disulfide (10.6 mL, 150 mmol) was added at -78 °C to quench the reaction. The reaction was stirred overnight, warming to rt. The solvent was removed under reduced pressure and the crude material was purified with column chromatography (50/50 EtOAc/hexanes) to give crude product (4.28 g, 13.9 mmol) as a yellow sticky paste in 46% yield.

Purpald test for aldehydes: positive after 10 sec.

\(^1\)H NMR (500 MHz, CDCl₃) \(\delta = 8.82\) (s, 1 H), 7.48 - 6.85 (m, 6 H), 6.57 (d, \(J = 1.6\) Hz, 1 H), 4.67 (dd, \(J = 2.2, 7.6\) Hz, 1 H), 4.54 (d, \(J = 3.4\) Hz, 2 H), 2.15 (s, 3 H)

The product (4.28 g, 14 mmol) and 125 mL of MeOH was stirred for 72 h at rt. The solvent was removed under reduced pressure and the crude material was purified with column chromatography (50/50 EtOAc/hexanes) to give known compound 20 (2.76 g, 9.92 mmol) as a white solid in 71% yield.

\(^1\)H NMR (500 MHz, CDCl₃) \(\delta = 7.48\) (d, \(J = 8.0\) Hz, 1 H), 7.34 - 7.28 (m, 2 H), 7.11 (s, 1 H), 7.05 - 7.00 (m, 2 H), 6.93 - 6.87 (m, 1 H), 5.91 (s, 1 H), 4.91 (dd, \(J = 1.4, 8.0\) Hz, 1 H), 4.51 (d, \(J = 2.2\) Hz, 2 H), 2.13 (s, 3 H)
$^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.2, 169.5, 164.5, 157.1, 130.1, 122.6, 114.9, 78.8, 67.3, 63.1, 21.0

\[\text{N-}((\text{tert-Butyldimethylsilyl})\text{oxy})\text{-N-}((2S,3S)-4-oxo-3-}(2\text{-phenoxyacetamido})\text{azetidin-2-yl})\text{benzamide (21).}\]

To a flame dried RB flask under argon was added NaH (60% oil dispersion, 12 mg, 0.5 mmol, cleaned with hexanes). The RB flask was cooled to 0 °C and N-((tert-butyldimethylsilyl)oxy)benzamide (75 mg, 0.30 mmol), already dissolved in 10 mL of dry DMF/DCM (1:1), was added dropwise. The reaction was stirred for 10 min and then azetidinone 21 (91 mg, 0.33 mmol), dissolved in 5 mL of anhydrous DCM/DMF (1:1), was added dropwise at 0 °C. The reaction was left to stir overnight, warming to rt. The reaction was diluted with DCM (35 mL), washed with brine (x 3), dried with MgSO$_4$, filtered, and concentrated under reduced pressure to give the crude product that was purified with column chromatography (50/50 EtOAc/hexanes) to give 21 (38 mg, 0.08 mmol) as a colorless film in 27% yield.

$^1$H NMR (500 MHz, CDCl$_3$) δ = 7.62 - 6.71 (m, 12 H), 5.84 (br. s., 1 H), 5.36 - 5.25 (m, 1 H), 4.40 - 4.24 (m, 2 H), 0.97 (s, 9 H), 0.20 (d, $J$ = 20.5 Hz, 6 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 171.2, 169.6, 165.2, 157.0, 133.6, 132.0, 130.0, 129.0, 128.5, 122.5, 114.8, 69.9, 67.0, 59.7, 26.2, -4.37, -4.51

IR (FT-IR) ν 1780, 1663 cm$^{-1}$

HRMS calcd. for C$_{24}$H$_{32}$N$_3$O$_5$Si (M + H$^+$) 470.2106, found 470.2104.
**N-(Benzyloxy)-N-((2S,3S)-4-oxo-3-(2-phenoxyacetamido)azetidin-2-yl)benzamide (23a).**

To a flame dried round bottom flask under argon was added N-(benzyloxy)benzamide (1, 700 mg, 3.08 mmol). The flask was cooled to 0°C and a solution of 0.8 M NaOEt in anhydrous EtOH (3.9 mL, 3.1 mmol) was added dropwise. The reaction was stirred for 5 min and a solution of (2S, 3S)-4-oxo-3-(2-phenoxyacetamido)azetidin-2-yl acetate (900 mg, 3.26 mmol) in anhydrous EtOH was added slowly. The reaction was left to stir overnight under argon, warming to rt. The solvent was removed under reduced pressure and the crude material was dissolved in EtOAc (30 mL), washed with H₂O, brine, dried with MgSO₄, filtered, and concentrated under reduced pressure to give a yellow oil that was purified with column chromatography (70/30 EtOAc/hexanes) to give 23a (414 mg, 0.930 mmol) as a white solid in 30% yield.

mp = 144-145 °C

¹H NMR (300 MHz, CDCl₃) δ = 7.79 - 6.79 (m, 15 H), 6.15 (s, 1 H), 5.39 (d, J = 6.5, 1.6 Hz, 1 H), 4.79 (t, J = 9.6, 13.2 Hz, 2 H), 4.44 (s, 2 H)

¹³C NMR (75 MHz, CDCl₃) δ 170.9, 169.7, 165.7, 157.1, 133.8, 133.3, 131.8, 130.1, 130.0, 129.4, 128.8, 128.7, 128.5, 122.4, 114.9, 80.4, 67.2, 66.5, 60.0

IR (FT-IR) ν 1762, 1652 cm⁻¹

HRMS calcd. for C₂₅H₂₃N₃NaO₅ (M + Na⁺) 468.1530, found 468.1552.
**N-(Benzyloxy)-3-nitro-N-((2S,3S)-4-oxo-3-(2-phenoxyacetamido)azetidin-2-yl)-5-(trifluoromethyl)benzamide (23b).**

To a RB flask was added N-(benzyloxy)-3-nitro-5-(trifluoromethyl)benzamide (22b, 315 mg, 0.920 mmol) and 15 mL of acetone. The solution was cooled in an ice bath and then 1 M NaOH (0.921 mL, 0.921 mmol) was added dropwise and the reaction was left to stir for 10 min. Azetidinone 20 (309 mg, 1.10 mmol), dissolved in 15 mL of acetone, was then added dropwise at 0 °C and the reaction was stirred overnight at rt. The reaction was diluted with H2O and extracted with EtOAc (x 2). The combined organic extracts were then washed with brine, dried with MgSO4, filtered, and concentrated under reduced pressure to give the crude material that was then purified with column chromatography (50/50 hexanes/EtOAc) to give 23b (258 mg, 0.462 mmol) as a colorless film in 50% yield.

1H NMR (500 MHz, CDCl3) δ = 8.57 (s, 1 H), 8.53 (s, 1 H), 8.14 (s, 1 H), 7.42 (d, J = 7.4 Hz, 1 H), 7.37 - 6.84 (m, 10 H), 6.63 (s, 1 H), 6.26 (br. s., 1 H), 5.47 (dd, J = 1.8, 7.2 Hz, 1 H), 4.95 (d, J = 10.6 Hz, 1 H), 4.82 (d, J = 10.4 Hz, 1 H), 4.54 (s, 2 H)

13C NMR (125 MHz, CDCl3) δ 170.2, 167.5, 165.9, 157.1, 147.9, 136.0, 132.8, 132.3, 132.0, 131.5, 130.2, 130.0, 129.8, 128.9, 127.3, 122.8, 122.6, 114.9, 81.9, 67.2, 66.6, 60.5

IR (FT-IR) ν 1775, 1658 cm⁻¹

HRMS calcd. for C26H22F3N4O7 (M + H⁺) 559.1435, found 559.1430.
**N-(Allyloxy)-3-nitro-N-((2S,3S)-4-oxo-3-(2-phenoxyacetamido)azetidin-2-yl)-5-(trifluoromethyl)benzamide (23c).**

To a clean RB flask was added \(N\)-(Allyloxy)-3-nitro-5-(trifluoromethyl)benzamide (22c, 120 mg, 0.413 mmol) and 15 mL of acetone. The solution was cooled in an ice bath and then 1 M NaOH (0.413 mL, 0.413 mmol) was added dropwise and the reaction was left to stir for 10 min. Azetidinone 20 (149 mg, 0.536 mol), dissolved in 15 mL of acetone, was then added dropwise at 0 °C and the reaction was stirred overnight at rt. The reaction was diluted with \(\text{H}_2\text{O}\) and extracted with EtOAc (x 2). The combined organic extracts were then washed with brine, dried with MgSO\(_4\), filtered, and concentrated under reduced pressure to give the crude material that was then purified with column chromatography (50/50 hexanes/EtOAc) to give 23c (69 mg, 0.14 mmol) as a colorless film in 33% yield.

\(^1\text{H} \text{NMR}\) (500 MHz, DMSO-d\(_6\)) \(\delta = 8.87\) (m, 2 H), 8.67 (s, 1 H), 8.40 (s, 1 H), 7.37 - 7.25 (m, 2 H), 7.04 - 6.92 (m, 3 H), 5.92 (br. s., 1 H), 5.68 (br. s., 1 H), 5.25 - 5.15 (m, 3 H), 4.56 (s, 2 H), 4.39 (d, \(J = 22.1 \text{ Hz}\), 2 H)

\(^{13}\text{C} \text{NMR}\) (125 MHz, CD\(_3\)OD) \(\delta = 171.0, 167.1, 166.5, 157.8, 148.5, 136.6, 132.1, 131.0, 130.6, 129.5, 127.0, 122.8, 121.7, 120.6, 114.7, 79.8, 66.9, 66.3, 60.0

IR (FT-IR) \(\nu = 1786, 1670 \text{ cm}^{-1}\)

HRMS calcd. for \(\text{C}_{22}\text{H}_{20}\text{F}_3\text{N}_4\text{O}_7\) (M + H\(^+\)) 509.1279, found 509.1291.
To a flame dried pressure tube under argon was added 23a (55 mg, 0.12 mmol) and anhydrous DCM (10 mL). To this solution was added 2-(methylthio)isoindoline-1,3-dione (29 mg, 0.15 mmol) and Et₃N (0.02 mL, 0.147 mmol). The reaction was capped tightly, heated in an oil bath at 50 °C, and stirred overnight. The solvent was removed under reduced pressure and the crude material was purified with column chromatography (60/40 hexanes/EtOAc) to give 24a (51 mg, 0.10 mmol) as an oil in 84% yield.

¹H NMR (500 MHz, CDCl₃) δ = 7.82 - 6.81 (m, 15 H), 6.02 (br. s., 1 H), 5.02 (d, J = 5.9 Hz, 1 H), 4.92 (d, J = 9.8 Hz, 1 H), 4.81 (d, J = 9.8 Hz, 1 H), 4.47 (q, J = 5.1, 15.2 Hz, 2 H), 2.52 (s, 3 H)

¹³C NMR (125 MHz, CDCl₃) δ 171.1, 169.6, 167.4, 157.0, 134.0, 133.4, 131.9, 130.1, 130.0, 129.4, 129.1, 128.9, 128.7, 122.7, 114.8, 79.4, 72.6, 67.1, 60.6, 21.2

IR (FT-IR) ν 1772, 1644 cm⁻¹

HRMS calcd. for C₂₆H₂₅N₃NaO₅S (M + Na⁺) 514.1403, found 514.1407.
N-(Benzyloxy)-N-((2R,3S)-1-(methylthio)-4-oxo-3-(2-phenoxyacetamido)azetidin-2-yl)-3-nitro-5-(trifluoromethyl)benzamide (24b).

To a flame dried pressure tube under argon was added 23b (67 mg, 0.12 mmol) and 10 mL of anhydrous DCM. To this solution was added 2-(methylthio)isoindoline-1,3-dione (31 mg, 0.16 mmol) and Et$_3$N (0.021 mL, 0.16 mmol). The tube was capped tightly, heated to 40 °C, and stirred overnight. The solvent was removed under reduced pressure and the residue was purified twice with column chromatography (60/40 hexanes/EtOAc) to give 24b (59 mg, 0.098 mmol) as a white solid in 82% yield.

mp 94-96 °C

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ = 8.58 (s, 1 H), 8.52 (s, 1 H), 8.14 (s, 1 H), 7.38 - 6.85 (m, 8 H), 6.26 (br. s., 1 H), 5.29 (dd, $J$ = 5.2, 1.6 Hz, 1 H), 4.88 (d, $J$ = 10.8 Hz, 1 H), 4.80 (d, $J$ = 10.8 Hz, 1 H), 4.54 (s, 2 H), 2.65 (s, 3 H)

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 169.9, 167.9, 167.2, 156.9, 148.0, 136.0, 132.9, 131.62, 131.60, 130.3, 130.2, 129.8, 129.0, 127.3, 123.0, 122.8, 114.8, 81.3, 71.9, 67.1, 60.9, 21.1

IR (FT-IR) $\nu$ 1785, 1677 cm$^{-1}$

HRMS calcd. for C$_{27}$H$_{24}$F$_3$N$_4$O$_7$S (M + H$^+$) 605.1312, found 605.1325.

28
S-5 H NMR and C NMR Spectra for Select Compounds

\[ \text{BnO} \quad \text{N} \quad \text{O} \quad \text{Ph} \]

\[ \text{O} \quad \text{NH} \]

\[ \text{4} \]

\[ \text{H NMR (500 MHz, CDCl}_3\text{)} \]

\[ \text{of Compound 4} \]
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 4
$^1$H NMR (600 MHz, CDCl$_3$) of Compound 5
$^{13}$C NMR (150 MHz, CDCl$_3$) of Compound 5
$^1$H NMR (300 MHz, CDCl$_3$) of Compound 6
$^{13}$C NMR (75 MHz, CDCl$_3$) of Compound 6
$^1$H NMR (300 MHz, DMSO-$d_6$) of Compound 7a
$^{13}$C NMR (75 MHz, DMSO-d$_6$) of Compound 7a
$^{1}H$ NMR (300 MHz, CDCl$_{3}$) of Compound 7b
$^{13}$C NMR (75 MHz, CDCl$_3$) of Compound 7b
$\text{H NMR (300 MHz, CDCl}_3\text{) of Compound 7c}$
$^{13}$C NMR (75 MHz, CDCl$_3$) of Compound 7c
$^1$H NMR (300 MHz, CDCl$_3$) of Compound 8a
$^{13}$C NMR (75 MHz, CDCl$_3$) of Compound 8a
$\text{EtOAc}$

$\text{EtOAc}$

$^{1}\text{H} \text{NMR (300 MHz, CDCl}_3\text{)} \text{ of Compound 8b}$
13C NMR (75 MHz, CDCl₃) of Compound 8b
$^1$H NMR (300 MHz, CDCl$_3$) of Compound 8c
$^{13}$C NMR (75 MHz, CDCl$_3$) of Compound 8c
$^{1}H$ NMR (500 MHz, CDCl$_3$) of Compound 9a
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 9a
$^1$H NMR (500 MHz, CDCl$_3$) of Compound 9b
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 9b
$^1$H NMR (600 MHz, CD$_3$OD) of Compound 10a
$^{13}$C NMR (150 MHz, CD$_3$OD) of Compound 10a
$\text{H NMR (500 MHz, CDCl}_3\text{) of Compound 10b}$
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 10b
$^1$H NMR (600 MHz, CDCl$_3$) of Compound 11
$^{13}$C NMR (150 MHz, CDCl$_3$) of Compound 11
$^1$H NMR (600 MHz, CDCl$_3$) of Compound 12
$^{13}$C NMR (150 MHz, CDCl$_3$) of Compound 12
$^1$H NMR (300 MHz, CDCl$_3$) of Compound 13
$\text{^{13}C NMR (75 MHz, CDCl}_3\text{) of Compound 13}$
$^1$H NMR (300 MHz, CDCl$_3$) of Compound 14
$^{13}$C NMR (75 MHz, CDCl$_3$) of Compound 14
$^1$H NMR (500 MHz, CDCl$_3$) of Compound 17
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 17
$^1$H NMR (500 MHz, CDCl$_3$) of Compound 18
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 18
$^1$H NMR (500 MHz, CDCl$_3$) of Compound 19
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 19
$^1$H NMR (500 MHz, CDCl$_3$) of Compound 22
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 22
\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) of Compound 23a
$^{13}$C NMR (75 MHz, CDCl$_3$) of Compound 23a
\[ \text{H NMR (500 MHz, CDCl}_3\text{) of Compound 23b} \]
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 23b
\(^1\)H NMR (500 MHz, DMSO-\(d_6\)) of Compound 23c
$^{13}$C NMR (125 MHz, CD$_3$OD) of Compound 23c
\(^1\)H NMR (500 MHz, CDCl\(_3\)) of Compound 24a
\(^{13}\text{C} \text{NMR} \ (125 \text{ MHz, CDCl}_3) \) of Compound 24a
$^1$H NMR (500 MHz, CDCl$_3$) of Compound 24b
$^{13}$C NMR (125 MHz, CDCl$_3$) of Compound 24b
S-6 Homo Decoupling Experiments for Select Compounds

C-3 Proton, now only split by H

C-3 Proton with negligible coupling to C-4

C-4 Proton

Irradiated C-4 Proton

TBSO, H, BnO, O

\( \text{O} \), \( \text{NH} \), \( \text{Ph} \)
C-3 Proton, now a very fine doublet, the result of weak coupling to C-4

Irradiated H Proton

Side Chain H Proton

C-3 Proton
Large Coupling Value of 7.8 MHz Disappears. Only weak coupling with C-4 of 1.6 MHz left.
Coupling no longer present

Irradiated C-3 Proton

Amide Side Chain Proton

C-3 Proton

[Chemical structure and NMR spectra diagram]
C-4 Proton

Coupling of C-3 Proton

Irradiated C-4 Proton

C-3 Proton
86

NHON

BnO

23a

PhO

C-3

C-4

Side Chain Amide Proton

C-3 Proton

Irradiated Side Chain Amide Proton

C-3 Proton

9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 ppm
C-3 Proton Only shows 1.8 MHz coupling, the result of weak coupling with C-4.
# TABLE 1

SPECTRUM OF ACTIVITY IN THE AGAR DIFFUSION ASSAYS FOR SELECT COMPOUNDS

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Zone of Growth Inhibition (mm)</th>
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<tr>
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<td>B. subtilis</td>
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<td>17</td>
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<tr>
<td>2</td>
<td>9a</td>
<td>21V</td>
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<tr>
<td>3</td>
<td>9b</td>
<td>0</td>
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<td>17</td>
<td>18*</td>
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<tr>
<td>5</td>
<td>19</td>
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<tr>
<td>6</td>
<td>23b</td>
<td>15P</td>
</tr>
<tr>
<td>7</td>
<td>23c</td>
<td>18*P</td>
</tr>
<tr>
<td>8</td>
<td>24b</td>
<td>15P</td>
</tr>
<tr>
<td>9</td>
<td>Ciprofloxacin</td>
<td>21/25P</td>
</tr>
</tbody>
</table>

Compounds are dissolved in MeOH/DMSO at concentration of 2 mM. Ciprofloxacin used in water at 1.66 μg/mL.

(*) indicates misshapen zone of inhibition. (P) indicated unclear zone of inhibition. (V) indicated very unclear zone of inhibition.

nt = not tested.
References


