Piperidine Carbamate Peptidomimetic Inhibitors of the Serine Proteases HGFA, Matriptase and Hepsin

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Supplementary material

Fluorescent Inhibitor Assays of HGFA, matriptase, and hepsin:

Inhibitors (0-20 μM final concentration in reaction) were diluted in DMSO (2% DMSO final concentration in reaction) and then mixed with either recombinant HGFA serine protease domain, Matriptase (#3946-SE-010, R&D Systems, Minneapolis, Minnesota), or activated Hepsin (#4776-SE-010, R&D Systems, Minneapolis, Minnesota) in black 384 well plates (Corning # 3575. Corning, NY). The final assay concentration for HGFA, Matriptase, and Hepsin were 6.25 nM, 0.2 nM, and 0.3 nM, respectively in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Triton X-100, pH 8). After thirty minutes incubation at room temperature, Boc-QLR-AMC substrate was added to the HGFA assays and Boc-QAR-AMC substrate was added to the Matriptase and Hepsin assays. Changes in fluorescence (excitation at 380 nm and emission at 460 nm) were measured at room temperature over time in a Biotek Synergy 2 plate reader (Winnoski,

VT). From a plot of the mean reaction velocity versus the inhibitor concentration, a non-linear four parameter curve fit was performed using GraphPad Prism version 6.04 for Windows (GraphPad Software, San Diego, CA, www.graphpad.com) to determine inhibitor IC_{50}s. The IC_{50} values that were determined from the average of three separate experimental determinations.

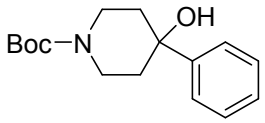
Hepsin Activation: Based upon the manufacturer's recommendations, recombinant Hepsin (#4776-SE-010, R&D Systems, Minneapolis, Minnesota) was diluted 5.5 fold in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Triton X-100, pH 8) and incubated at 37°C. After twenty-four hours, the Hepsin was diluted in glycerol to 50%. This stock Hepsin (1.2 µM) was stored in a -20°C freezer and diluted in TNC buffer for use in assays.

Chromogenic Kinetic Enzyme Assay of Thrombin and Factor Xa: Inhibitors (0-20 µM final concentration) were serially diluted in DMSO (2% DMSO final concentration) and then mixed with recombinant thrombin (0.15 nM final concentration) or Factor Xa (0.35 nM final concentration) in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Triton X-100, pH 8) using clear 384 well plates. After incubating for 30 minutes at 25° C, the chromogenic substrate (S2238; D-Phe-Pip-Arg-pNA) for thrombin (K_m = 14.5 µM) or (S2222; Bz-Ile-Glu-Gly-Arg-pNA) for Factor Xa (K_m = 200 µM) was added to a final concentration of K_m (4 x K_m (50 µM) for thrombin) in a final reaction volume of 40 microliters. Changes in absorbance at 405 nm were measured over time in a Biotek Synergy 2 plate (Winnoski, VT). Using GraphPad Prism version 6.04 software program, (GraphPad Software, San Diego, CA, www.graphpad.com), a four parameter curve fit was used to determine the inhibitor IC_{50}s from a plot of the mean reaction

velocity versus the inhibitor concentration. The IC_{50} values represent the average of three separate experimental determinations.

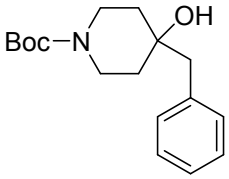
Spectral data

***t*-Butyl 4-hydroxy-4-phenylpiperidine-1-carboxylate (3a).**



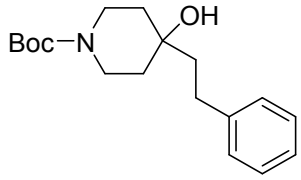
Sticky oil, yield (0.47 g), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.49 (d, *J* = 7.8 Hz, 2 H), 7.38 (t, *J* = 7.6 Hz, 2 H), 7.30 (d, *J* = 7.4 Hz, 1 H), 4.04 (d, *J* = 12.9 Hz, 2 H), 3.25 (t, *J* = 12.5 Hz, 2 H), 2.01 (dt, *J* = 4.5, 13.2 Hz, 2 H), 1.74 (d, *J* = 13.7 Hz, 2 H), 1.62 (s, 1 H), 1.49 (s, 9 H). LCMS (ESI+) expected m/z 277.17, found 278.30 ($M+H^+$).

***t*-Butyl 4-benzyl-4-hydroxypiperidine-1-carboxylate (3b).**



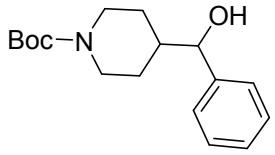
Oil, yield (0.43 g), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.38-7.20 (m, 4 H), 7.10 (t, *J* = 7.5 Hz, 1 H), 3.94 (d, *J* = 12.9 Hz, 2 H), 3.15 (t, *J* = 12.5 Hz, 2 H), 2.65 (s, 2 H), 1.64-1.50 (m, 4 H), 1.51 (s, 9 H). LCMS (ESI+) expected m/z 291.18, found 292.30 ($M+H^+$).

***t*-Butyl 4-hydroxy-4-phenethylpiperidine-1-carboxylate (3c).**



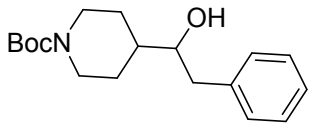
Oil, yield (0.24 g), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.32 - 7.27 (m, 2 H), 7.20 (d, *J* = 7.0 Hz, 3 H), 3.83 (d, *J* = 10.2 Hz, 3 H), 3.26 - 3.13 (m, 3 H), 2.76 -2.68 (m, 3 H), 1.85 - 1.76 (m, 3 H), 1.61 (d, *J* = 4.3 Hz, 5 H), 1.47 (s, 9 H). LCMS (ESI+) expected m/z 305.20, found 306.40 ($M+H^+$).

t-Butyl 4-(hydroxy(phenyl)methyl)piperidine-1-carboxylate (3d).



Oil, yield (0.56 g), ^{1}H NMR (400MHz, $CDCl_3$) δ = .49 (d, *J* = 7.8 Hz, 2 H), 7.38 (t, *J* = 7.6 Hz, 2 H), 7.30 (d, *J* = 7.4 Hz, 1 H), 4.04 (d, *J* = 12.9 Hz, 2 H), 3.25 (t, *J* = 12.5 Hz, 2 H), 2.01 (dt, *J* = 4.5, 13.2 Hz, 2 H), 1.74 (d, *J* = 13.7 Hz, 2 H), 1.62 (s, 1 H), 1.58 - 1.47 (m, 2 H), 1.45 (s, 9 H). LCMS (ESI+) expected m/z 291.18, found 292.30 ($M+H^+$).

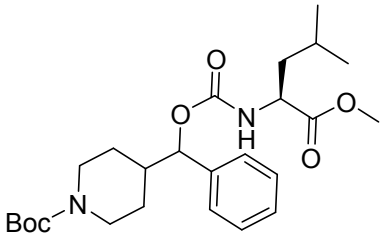
t-Butyl 4-(1-hydroxy-2-phenylethyl)piperidine-1-carboxylate (3e).



Oil, yield (0.50 g), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.29 (d, *J* = 0.8 Hz, 1 H), 7.25 (s, 1 H), 7.21 (s, 1 H), 7.20 - 7.14 (m, 2 H), 4.53 (d, *J* = 5.5 Hz, 1 H), 4.10 - 4.03 (m,1 H), 3.55 (br. s., 1 H), 2.84 (dd, *J* = 3.1, 13.7 Hz, 1 H), 2.69 - 2.50 (m, 2 H), 1.99 (s, 2 H), 1.82 (d, *J* = 12.9 Hz, 1 H), 1.72 -

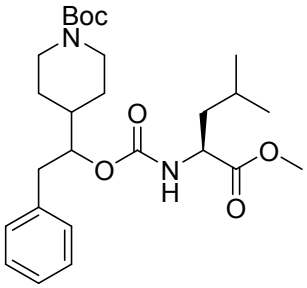
1.60 (m, 2 H), 1.58 - 1.47 (m, 2 H), 1.40 (d, *J* = 3.5 Hz, 9 H). LCMS (ESI+) expected m/z 305.20, found 306.40 ($M+H^+$).

_t_-Butyl4-(((((S)-1-methoxy-4-methyl-1-oxopentan-2-yl)carbamoyl)oxy) (phenyl)methyl)piperidine-1-carboxylate (9a).



Oil, yield (124 mg),^{1}H NMR (400MHz, $CDCl_3$) δ = 7.38 - 7.29 (m, 5 H), 4.80 (br. s., 1 H), 4.47 (br. s., 1 H), 4.39 (d, *J* = 7.4 Hz, 1 H), 4.17 - 4.10 (m, 1 H),3.74 (s, 3 H), 2.71 - 2.54 (m, 2 H), 2.05 (s, 1 H), 1.66 - 1.55 (m, 5 H), 1.46 (br. s., 1 H), 1.45 (s, 9 H), 1.31 - 1.25 (m, 2 H), 1.21 - 1.12 (m, 1 H), 0.95 (td, *J* = 3.1, 6.3 Hz, 6 H). LCMS (ESI+) expected m/z 462.27, found 463.40 ($M+H^+$).

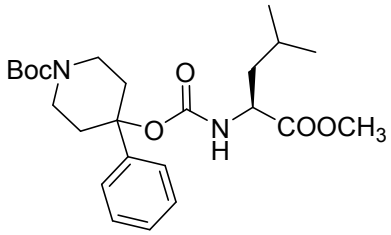
_t_-Butyl 4-(1-((((S)-1-methoxy-4-methyl-1-oxopentan-2-yl)carbamoyl)oxy)-2-phenylethyl)piperidine-1-carboxylate (9b).



Oil, yield (152 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.29 - 7.26 (m, 1 H), 7.22 - 7.18 (m, 1 H), 7.16 (d, *J* = 7.8 Hz, 2 H), 4.94 - 4.86 (m, 1 H), 4.53 (d, *J* = 5.1 Hz, 1 H), 4.06 (q, *J* = 7.2 Hz, 2 H), 5.13 - 3.83 (m, 2 H), 3.66 (d, *J* = 3.1 Hz, 1 H), 3.59 - 3.52 (m, 1 H), 3.26 - 3.19 (m, 1 H), 2.84 (dd, *J* = 3.3, 13.5 Hz, 1 H), 2.62 (d, *J* = 9.4 Hz, 2 H), 1.99 (s, 1 H), 1.82 (d, *J* = 13.3 Hz, 1 H), 1.70 -

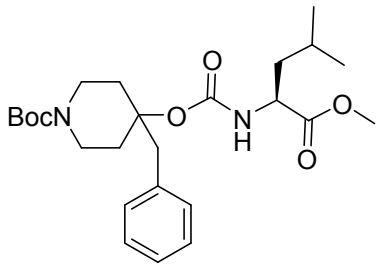
1.62 (m, 3 H), 1.53 - 1.50 (m, 1 H), 1.41 (s, 9 H), 1.20 (t, *J* = 7.2 Hz, 2 H), 0.89 - 0.87 (m, 6 H). LCMS (ESI+) expected m/z 476.29, found 476.40 ($M+H^+$).

***t*-Butyl (S)-4-(((1-methoxy-4-methyl-1-oxopentan-2-yl)carbamoyl)oxy)-4-phenylpiperidine-1-carboxylate (4a).**



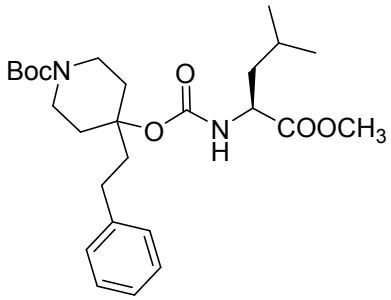
Oil, yield (125 mg), ^1H NMR (400MHz, $CDCl_3$) δ = 7.42 - 7.38 (m, 2 H), 7.32 - 7.28 (m, 1 H), 7.23 - 7.17 (m, 2 H), 4.07 - 3.82 (m, 2 H), 3.64 (s, 3 H), 3.25 - 3.10 (m, 2 H), 1.96 (s, 2 H), 1.70 - 1.61 (m, 3 H), 1.40 (s, 9 H), 1.17 (t, *J* = 7.6 Hz, 1 H), 0.90 - 0.82 (m, 9 H). LCMS (ESI+) expected m/z 448.26, found 449.40 ($M+H^+$).

***t*-Butyl (S)-4-benzyl-4-(((1-methoxy-4-methyl-1-oxopentan-2-yl)carbamoyl)oxy)piperidine-1-carboxylate (4b).**



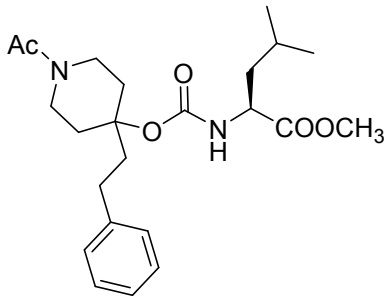
Oil, yield (200 mg),^1H NMR (400MHz, $CDCl_3$) δ = 7.36 - 7.28 (m, 3 H), 7.20 (d, *J* = 7.4 Hz, 2 H), 5.03 (dd, *J* = 8.2, 18.4 Hz, 2 H), 4.48 (dt, *J* = 4.5, 8.9Hz, 2 H), 3.73 (s, 3 H), 3.10 (br. s., 1 H), 2.76 (s, 1 H), 1.64 - 1.55 (m, 4 H), 1.53 - 1.49 (m, 2 H), 1.46 (s, 9 H), 0.96 - 0.93 (m, 6 H). LCMS (ESI+) expected m/z 462.27, found 463.40 ($M+H^+$).

***t*-Butyl (S)-4-(((1-methoxy-4-methyl-1-oxopentan-2-yl)carbamoyl)oxy)-4-phenethylpiperidine-1-carboxylate (4c).**



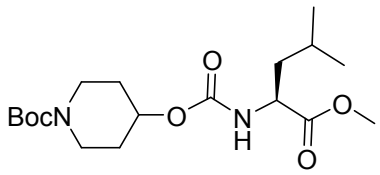
Oil, yield (215 mg),^{1}H NMR (400MHz ,$CDCl_3$) δ = 7.32 - 7.27 (m, 3 H), 7.20 (d, *J* = 6.7 Hz, 2 H), 4.92 - 4.84 (m, 1 H), 4.48 (br. s., 1 H), 3.95 - 3.79 (m, 2 H), 3.73 (s, 1 H), 3.26 - 3.13 (m, 2 H), 2.76 - 2.69 (m, 2 H), 1.84 - 1.77 (m, 2 H), 1.61 (d, *J* = 4.3 Hz, 5 H), 1.47 (s, 9 H), 0.94 (dd, *J* = 3.3, 6.5 Hz, 6 H). LCMS (ESI+) expected m/z 476.29, found 477.50 ($M+H^+$).

Methyl (((1-acetyl-4-phenethylpiperidin-4-yl)oxy)carbonyl)-L-leucinate (4d).



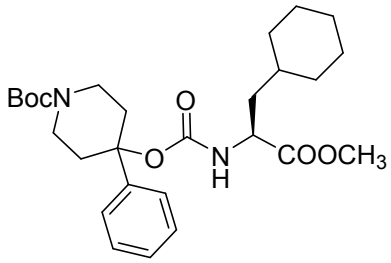
Oil, yield (150 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.33 - 7.27 (m, 2 H), 7.20 (d, *J* = 7.4 Hz, 3 H), 5.00 - 4.90 (m, 1 H), 4.52 - 4.43 (m, 1 H), 4.36 (d, *J* = 12.9 Hz, 1 H), 3.73 (s, 3 H), 3.61 (d, *J* = 13.3 Hz, 1 H), 3.53 - 3.43 (m, 1 H), 3.11 - 3.02 (m, 1 H), 2.76 - 2.69 (m, 2 H), 2.11 (s, 3 H), 1.85 - 1.78 (m, 2 H), 1.74 - 1.57 (m, 9 H), 1.53 - 1.46 (m, 2 H), 0.94 (dd, *J* = 2.9, 6.5 Hz, 7 H). LCMS (ESI+) expected m/z 418.25, found 419.50 ($M+H^+$).

***t*-Butyl (S)-4-(((1-methoxy-4-methyl-1-oxopentan-2-yl)carbamoyl)oxy)piperidine-1-carboxylate (4e).**



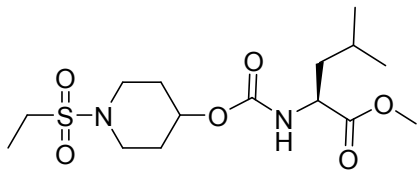
Oil, yield (2.4 g), ^{1}H NMR (400MHz, $CDCl_3$) δ = 5.06 (d, *J* = 8.6 Hz, 1 H), 4.80 (br. s., 2 H), 4.52 - 4.44 (m, 1 H), 4.37 (d, *J* = 5.1 Hz, 1 H), 3.74 (s, 3H), 3.24 - 3.14 (m, 2 H), 1.85 (br. s., 3 H), 1.60 (dd, *J* = 7.4, 12.5 Hz, 4 H), 1.46 (s, 9 H), 0.97 - 0.92 (m, 8 H). LCMS (ESI+) expected m/z 372.23, found 373.40 ($M+H^+$).

t-Butyl (S)-4-(((3-cyclohexyl-1-methoxy-1-oxopropan-2-yl)carbamoyl)oxy)-4-phenylpiperidine-1-carboxylate (4f).



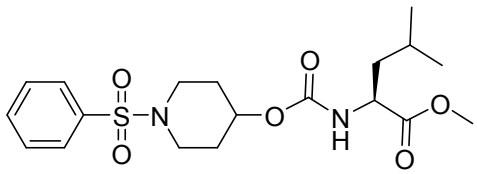
Oil, yield (0.37 g),^{1}H NMR (400MHz, $CDCl_3$) δ = 7.48 (d, *J* = 7.4 Hz, 2 H), 7.38 (t, *J* = 7.6 Hz, 2 H), 7.29 (d, *J* = 7.4 Hz, 1 H), 4.83 (d, *J* = 8.6 Hz, 1 H), 4.49 (d, *J* = 5.5 Hz, 1 H), 4.09 - 3.99 (m, 2 H), 3.73 (s, 3 H), 3.26 (br. s., 2 H), 2.01 (br. s., 2 H), 1.83 - 1.58 (m, 13 H), 1.49 (s, 9 H), 1.41 - 1.30 (m,2 H), 1.30 - 1.10 (m, 6 H), 0.99 - 0.83 (m, 4 H). LCMS (ESI+) expected m/z 488.29, found 489.40 ($M+H^+$).

Methyl (((1-(ethylsulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucinate (5a).



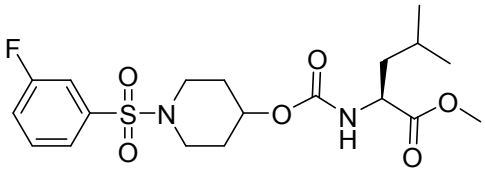
Oil, yield (150 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 5.94 (br. s., 2 H), 5.12 (br. s., 1 H), 4.84 (br. s., 1 H), 4.37 (d, *J* = 3.1 Hz, 1 H), 3.75 (s, 1 H), 3.56 - 3.44 (m, 2 H), 3.23 (dd, *J* = 3.5, 7.8 Hz, 2 H), 3.01 - 2.93 (m, 2 H), 1.96 (d, *J* = 3.1 Hz, 2 H), 1.86 - 1.64 (m, 4 H), 1.64 - 1.49 (m, 2 H), 1.37 (t, *J* = 7.2 Hz, 3 H), 0.97 (d, *J* = 5.9 Hz, 6 H). LCMS (ESI+) expected m/z 364.17, found 365.30 ($M+H^+$).

Methyl (((1-(phenylsulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucinate (5b).



Oil, yield (159 mg),^{1}H NMR (400MHz, $CDCl_3$) δ = 7.78 (d, *J* = 7.8 Hz, 2 H), 7.66 - 7.60 (m, 1 H), 7.59 - 7.53 (m, 2 H), 5.00 (d, *J* = 8.6 Hz, 1 H), 4.92 - 4.85 (m, 1 H), 4.69 (br. s., 1 H), 4.53 - 4.43 (m, 1 H), 4.38 - 4.28 (m, 2 H), 3.72 (s, 3 H), 3.26 (br. s., 2 H), 2.96 (br. s., 2 H), 1.95 (d, *J* = 3.9 Hz, 2 H), 1.78 (br. s., 2 H), 1.70 - 1.55 (m, 3 H), 1.53 - 1.43 (m, 2 H), 0.97 - 0.88 (m, 6 H). LCMS (ESI+) expected m/z 412.17, found 413.30 ($M+H^+$).

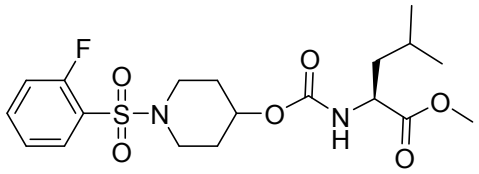
Methyl (((1-((3-fluorophenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucinate (5c).



Oil, yield (148 mg),^{1}H NMR (400MHz, $CDCl_3$) δ = 7.61 - 7.50 (m, 2 H), 7.48 (d, *J* = 7.8 Hz, 1 H), 7.39 - 7.30 (m, 1 H), 5.01 (d, *J* = 8.2 Hz, 1 H), 4.91 - 4.80 (m, 1 H), 4.72 (br. s., 1 H), 4.48 (d, *J* = 5.1 Hz, 1 H), 4.37 - 4.28 (m, 1 H), 3.73 (s, 3 H), 3.23 (br. s., 2 H), 3.03 (br. s., 2 H), 1.96 (br.

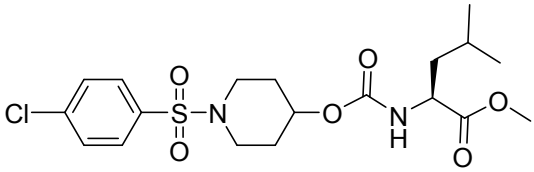
s., 2 H), 1.80 (br. s., 2 H), 1.62 (s, 2 H), 1.54 - 1.44 (m, 2 H), 0.93 (br. s., 6 H). LCMS (ESI+) expected m/z 430.16, found 431.30 ($M+H^+$).

Methyl (((1-((2-fluorophenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucinate (5d).



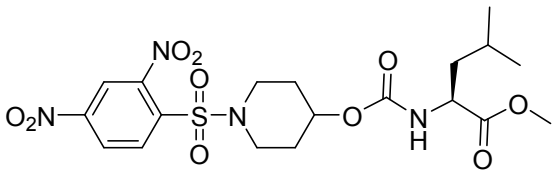
Oil, yield (126 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 8.00 (t, *J* = 7.4 Hz, 1 H), 7.86 (t, *J* = 7.4 Hz, 1 H), 7.81 - 7.71 (m, 1 H), 7.63 - 7.56 (m, 1 H), 7.43 -7.35 (m, 1 H), 5.31 (s, 1 H), 5.03 (d, *J* = 9.4 Hz, 1 H), 4.83 - 4.73 (m, 1 H), 4.53 - 4.42 (m, 1 H), 4.34 (d, *J* = 4.7 Hz, 1 H), 3.73 (s, 3 H), 3.41 (br. s., 2 H), 3.17 (d, *J* = 7.8 Hz, 2 H), 1.95 (br. s., 2 H), 1.79 (br. s., 2 H), 1.71 - 1.57 (m, 6 H), 1.54 - 1.43 (m, 2 H), 0.93 (d, *J* = 4.3 Hz, 6 H). LCMS (ESI+) expected m/z 430.16, found 431.30 ($M+H^+$).

Methyl (((1-((4-chlorophenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucinate (5e).



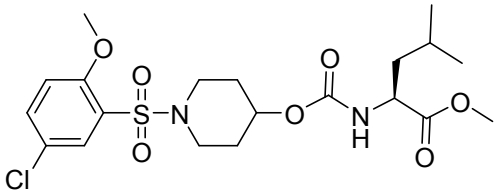
Oil, yield (150 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.71 (d, *J* = 7.4 Hz, 2 H), 7.53 (d, *J* = 7.8 Hz, 2 H), 5.01 (d, *J* = 8.2 Hz, 1 H), 4.72 (br. s., 1 H), 4.52 - 4.43 (m, 1 H), 4.38 - 4.28 (m, 1 H), 3.73 (s, 3 H), 3.19 (br. s., 2 H), 3.02 (br. s., 2 H), 1.95 (br. s., 2 H), 1.80 (br. s., 2 H), 1.62 (br. s., 4 H), 1.54 - 1.42 (m, 2 H), 0.94 (br. s., 6 H). LCMS (ESI+) expected m/z 446.13, found 447.20 ($M+H^+$).

Methyl (((1-((2,4-dinitrophenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucinate (5f).



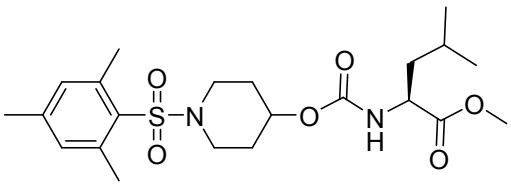
Yellow solid, yield (136 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 8.54 - 8.47 (m, 2 H), 8.23 (d, *J* = 8.6 Hz, 1H), 5.08 (d, *J* = 8.2 Hz, 1 H), 4.86 (br. s., 1 H), 4.40 - 4.31 (m, 1 H), 3.74 (s, 3 H), 3.56 - 3.31 (m, 4 H), 1.97 (d, *J* = 4.3 Hz, 2 H), 1.84 (d, *J* = 5.9 Hz, 2 H), 1.74 - 1.56 (m, 2 H), 1.55 - 1.45 (m, 2 H), 1.32 -1.21 (m, 2 H), 0.94 (t, *J* = 4.9 Hz, 6 H). LCMS (ESI+) expected m/z 502.10, found 503.20 ($M+H^+$).

Methyl (((1-((5-chloro-2-methoxyphenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucinate (5g).



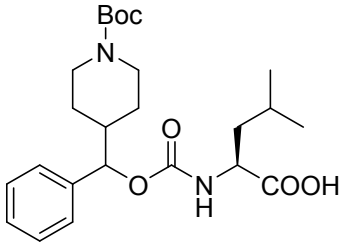
Oil, yield (167 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.88 (d, *J* = 2.3 Hz, 1 H), 7.51 - 7.44 (m, 1 H), 6.97 (d, *J* = 9.0 Hz, 1 H), 5.05 (d, *J* = 8.6 Hz, 1 H), 4.77 (br. s., 1 H), 4.40 - 4.31 (m, 1 H), 3.93 (s, 3 H), 3.74 (s, 3 H), 3.48 (d, *J* = 5.9 Hz, 2 H), 3.22 - 3.11 (m, 2 H), 1.93 (br. s., 2 H), 1.80 - 1.68 (m, 2 H), 1.62 (s, 2 H), 1.55 - 1.46 (m, 2 H), 0.94 (d, *J* = 5.5 Hz, 6 H). LCMS (ESI+) expected m/z 476.14, found 477.30 ($M+H^+$).

Methyl (((1-(mesitylsulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucinate (5h).



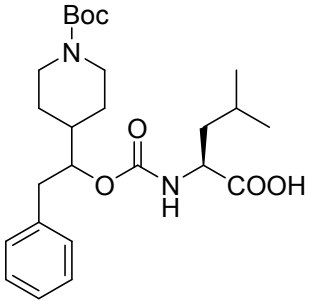
Oil, yield (162 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 6.96 (s, 2 H), 5.05 (d, *J* = 8.6 Hz, 1 H), 4.89 - 4.78 (m, 1 H), 4.36 (d, *J* = 5.1 Hz, 1 H), 3.74 (s, 3 H), 3.36 (br. s., 2 H), 3.11 (d, *J* = 7.8 Hz, 2 H), 2.62 (s, 6 H), 2.31 (s, 3 H), 1.90 (br. s., 3 H), 1.80 - 1.65 (m, 4 H), 1.56 - 1.45 (m, 2 H), 0.95 (d, *J* = 5.5Hz, 6 H). LCMS (ESI+) expected m/z 454.21, found 455.40 ($M+H^+$).

(((1-(tert-butoxycarbonyl)piperidin-4-yl)(phenyl)methoxy)carbonyl)-L-leucine (10a).



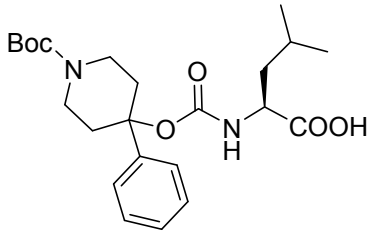
Off white solid, yield (110 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.40 - 7.28 (m, 5 H), 5.56 - 5.39 (m, 1 H), 4.43 - 4.28 (m, 1 H), 4.21 - 3.99 (m, 1 H), 2.63 (br. s., 1 H), 1.90 (br. s., 2 H), 1.84 - 1.63 (m, 4 H), 1.45 (s, 9 H), 1.28 (d, *J* = 9.4 Hz, 3 H), 1.04 - 0.85 (m, 7 H). LCMS (ESI+) expected m/z 448.26, found 449.40 ($M+H^+$).

((1-(1-(tert-butoxycarbonyl)piperidin-4-yl)-2-phenylethoxy)carbonyl)-L-leucine (10b).



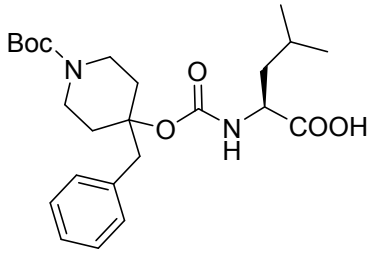
Off white solid, yield (270 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.36 - 7.30 (m, 2 H), 7.26 - 7.20 (m, 3 H), 4.62 - 4.58 (m, 1 H), 4.22 - 4.09 (m, 3 H), 3.62 (s, 2 H), 2.91 (dd, *J* = 3.3, 13.5 Hz, 2 H), 2.75 - 2.57 (m, 4 H), 1.88 (d, *J* = 12.5 Hz, 2 H), 1.72 (t, *J* = 12.7 Hz, 2 H), 1.47 (s, 9 H), 1.34 - 1.23 (m, 2 H), 1.02 - 0.92 (m, 6 H). LCMS (ESI+) expected m/z 462.27, found 463.40 ($M+H^+$).

(((1-(tert-butoxycarbonyl)-4-phenylpiperidin-4-yl)oxy)carbonyl)-L-leucine (6a).



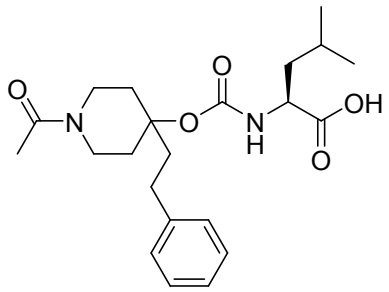
Thick oil, yield (165 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.51 - 7.46 (m, 2 H), 7.38 (t, *J* = 7.6 Hz, 2 H), 7.30 (d, *J* = 7.0 Hz, 1 H), 4.08 - 4.00 (m, 2 H), 3.72 (s, 1 H), 3.26 (dt, *J* = 2.3, 12.9 Hz, 3 H), 2.45 (s, 1 H), 2.00 (dd, *J* = 4.9, 13.1 Hz, 2 H), 1.75 (d, *J* = 12.5 Hz, 2 H), 1.50 (s, 2 H), 1.49 (s, 9 H), 1.01 - 0.93 (m, 6 H). LCMS (ESI+) expected m/z 434.24, found 435.40 ($M+H^+$).

(((4-benzyl-1-(tert-butoxycarbonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6b).



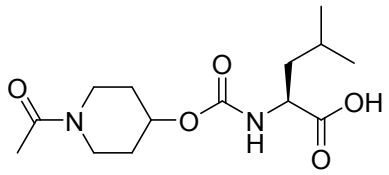
Off white solid, yield (174 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.36 - 7.28 (m, 3 H), 7.20 (d, *J* = 7.8 Hz, 2 H), 6.43 (br. s., 1 H), 4.76 (dd, *J* = 4.3, 11.7 Hz, 1 H), 4.11 (d, *J* = 10.2 Hz, 1 H), 3.87 (br. s., 2 H), 3.10 (br. s., 2 H), 2.77 (s, 2 H), 2.26 (d, *J* = 11.3 Hz, 1 H), 1.93 - 1.76 (m, 4 H), 1.66 - 1.50 (m, 5 H), 1.46 (s, 9 H), 1.28 (t, *J* = 6.5 Hz, 1 H), 0.95 (d, *J* = 6.7 Hz, 6 H). LCMS (ESI+) expected m/z 448.26, found 449.40 ($M+H^+$).

(((1-acetyl-4-phenethylpiperidin-4-yl)oxy)carbonyl)-L-leucine (6c)



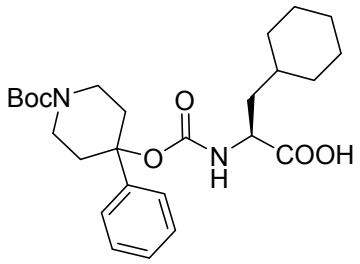
Off white solid, yield (200 mg), 1H NMR (400MHz, $CDCl_3$) δ = 7.33 - 7.28 (m, 2 H), 7.20 (d, *J* = 7.0 Hz, 3 H), 6.45 (br. s., 1 H), 4.75 (dd, *J* = 2.3, 11.3 Hz, 1 H), 4.10 (d, *J* = 9.4 Hz, 1 H), 2.72 (br. s., 2 H), 2.16 (br. s., 3 H), 2.09 (s, 1 H), 1.80 (br. s., 4 H), 1.68 (br. s., 4 H), 0.99 (d, *J* = 8.2 Hz, 2 H), 0.95 (d, *J* = 6.3 Hz, 6 H). LCMS (ESI+) expected m/z 404.23, found 405.40 ($M+H^+$).

(((1-acetylpiperidin-4-yl)oxy)carbonyl)-L-leucine (6d).



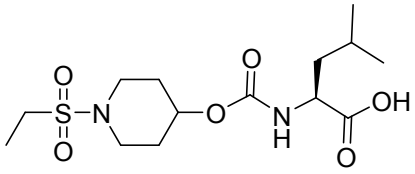
Off white solid, yield (370 mg), ^1H NMR (400MHz ,CHLOROFORM-d) δ = 5.20 (br. s., 1 H), 4.89 (br. s., 1 H), 4.78 - 4.72 (m, 1 H), 4.37 (d, *J* = 3.9 Hz, 2 H), 4.18 - 4.07 (m, 2 H), 3.72 - 3.53 (m, 3 H), 3.40 (br. s., 2 H), 2.14 (s, 3 H), 1.98 - 1.83 (m, 2 H), 1.83 - 1.62 (m, 2 H), 1.57 (ddd, *J* = 5.3, 8.9, 13.6 Hz, 2 H), 0.97 (d, *J* = 6.3 Hz, 6 H). LCMS (ESI+) expected m/z 300.17, found 301.30 ($M+H^+$).

(S)-2-((((1-(tert-butoxycarbonyl)-4-phenylpiperidin-4-yl)oxy)carbonyl)amino)-3-cyclohexylpropanoic acid (6e).



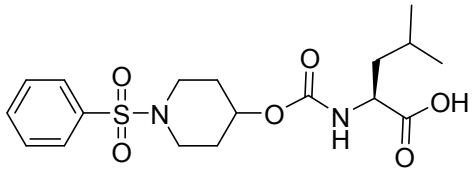
Off white solid, yield (314 mg),[1]H NMR (400MHz, $CDCl_3$) δ = 7.48 (d, *J* = 7.4 Hz, 2 H), 7.38 (t, *J* = 7.6 Hz, 2 H), 7.29 (d, *J* = 7.4 Hz, 1 H), 4.83 (d, *J* = 8.6 Hz, 1 H), 4.49 (d, *J* = 5.5 Hz, 1 H), 4.09 - 3.99 (m, 2 H), 3.73 (s, 3 H), 3.26 (br. s., 2 H), 2.01 (br. s., 2 H), 1.83 - 1.58 (m, 13 H), 1.49 (s, 9 H), 1.41 - 1.30 (m,2 H), 1.30 - 1.10 (m, 6 H), 0.99 - 0.83 (m, 4 H). LCMS (ESI+) expected m/z 474.27, found 475.40 ($M+H^+$).

(((1-(ethylsulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6f).



Oil, yield (120 mg), [1]H NMR (400MHz, $CDCl_3$) δ = 6.40 (br. s., 1 H), 5.62 (br. s., 1 H), 5.07 (d, *J* = 8.2 Hz, 1 H), 4.85 (br. s., 1 H), 4.38 (br. s., 2 H), 3.50 (br. s., 2 H), 3.22 (br. s., 3 H), 2.97 (q, *J* = 7.2 Hz, 2 H), 1.97 (br. s., 3 H), 1.86 - 1.66 (m, 4 H), 1.63 - 1.53 (m, 2 H), 1.37 (t, *J* = 7.2 Hz, 3 H), 1.02 - 0.93 (m, 6 H). LCMS (ESI+) expected m/z 350.15, found 351.20 ($M+H^+$).

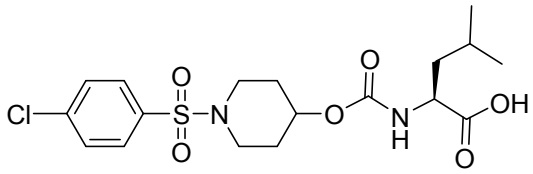
(((1-(phenylsulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6g).



White solid, yield (118 mg), [1]H NMR (400MHz, $CDCl_3$) δ = 7.78 (d, *J* = 7.4 Hz, 2 H), 7.65 - 7.53 (m, 3 H), 5.00 (d, *J* = 8.6 Hz, 1 H), 4.76 (dd, *J* = 4.1, 11.2 Hz, 1 H), 4.69 (br. s., 1 H), 4.38 - 4.28 (m, 1 H), 4.02 (br. s., 1 H), 3.30 (br. s., 2 H), 3.17 (br. s., 1 H), 2.92 (br. s., 2 H), 1.95 (d, *J* = 3.9

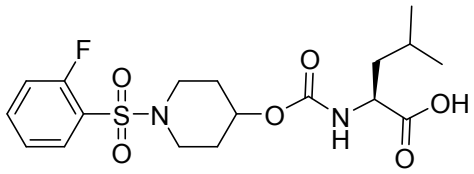
Hz, 2 H), 1.85 - 1.72 (m, 2 H), 1.71 - 1.62 (m, 2H), 1.57 - 1.47 (m, 2 H), 0.97 - 0.90 (m, 6 H). LCMS (ESI+) expected m/z 398.15, found 399.30 ($M+H^+$).

(((1-((4-chlorophenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6h).



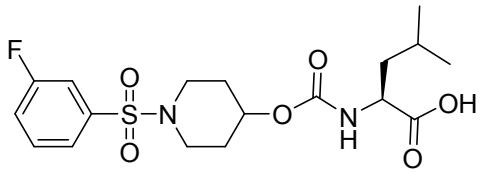
White solid, yield (140 mg), 1H NMR (400MHz, $CDCl_3$) δ = 7.71 (d, *J* = 8.2 Hz, 2 H), 7.53 (d, *J* = 8.2 Hz, 2 H), 6.28 (br. s., 1 H), 4.98 (d, *J* = 8.2 Hz, 1 H), 4.81 -4.68 (m, 1 H), 4.33 (br. s., 1 H), 3.24 (br. s., 2 H), 2.99 (br. s., 2 H), 1.96 (br. s., 2 H), 1.85 - 1.64 (m, 4 H), 1.61 - 1.49 (m, 1 H), 0.95 (d, *J* = 5.5 Hz, 6 H). LCMS (ESI+) expected m/z 432.11, found 432.30 ($M+H^+$).

(((1-((2-fluorophenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6i).



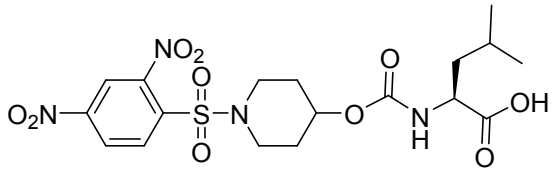
Off white solid, yield (120 mg), ^1H NMR (400MHz, $CDCl_3$) δ = 7.86 (t, *J* = 7.2 Hz, 1 H), 7.60 (q, *J* = 6.7 Hz, 1 H), 7.32 - 7.29 (m, 1 H), 7.25 - 7.20 (m, 1 H), 5.02 (d, *J* = 8.6 Hz, 1 H), 4.78 (br. s., 1 H), 4.38 - 4.31 (m, 1 H), 3.44 (br. s., 2 H), 3.15 (d, *J* = 7.4 Hz, 2 H), 1.96 (br. s., 2 H), 1.83 - 1.64 (m, 4 H), 1.58 - 1.50 (m, 2 H), 1.27 (br. s., 1 H), 0.95 (d, *J* = 6.3 Hz, 6 H). LCMS (ESI+) expected m/z 416.14, found 417.30 ($M+H^+$).

(((1-((3-fluorophenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6j).



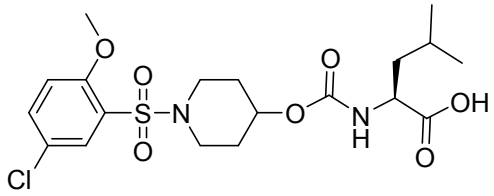
Off white solid, yield (110 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.59 - 7.53 (m, 2 H), 7.48 (d, *J* = 7.4 Hz, 1 H), 7.32 (d, *J* = 7.0 Hz, 1 H), 6.70 (br. s., 1 H), 5.79 (br. s., 1 H), 5.01 (d, *J* = 8.2 Hz, 1 H), 4.82 - 4.68 (m, 1 H), 4.32 (br. s., 1 H), 4.14 - 3.98 (m, 1 H), 3.28 (br. s., 2 H), 2.99 (br. s., 2 H), 1.96 (br. s., 2 H),1.79 (br. s., 2 H), 1.68 (d, *J* = 9.4 Hz, 2 H), 1.53 (br. s., 2 H), 1.26 (br. s., 1 H), 0.94 (d, *J* = 5.1 Hz, 6 H). LCMS (ESI+) expected m/z 416.14, found 417.30 ($M+H^+$).

(((1-((2,4-dinitrophenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6k).



Off yellow solid, yield (90 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 11.08 - 10.99 (m, 1 H), 9.09 (d, *J* = 2.3 Hz, 1 H), 8.53 - 8.39 (m, 1 H), 7.35 (d, *J* = 9.4 Hz, 1 H), 4.11(m, 2 H), 3.94 (m, 2 H), 3.83 - 3.75 (m, 2 H), 3.71 - 3.58 (m, 4 H), 2.18 (m, 3 H), 1.07 - 0.93 (m, 6 H). LCMS (ESI+) expected m/z 488.12, found 489.30 ($M+H^+$).

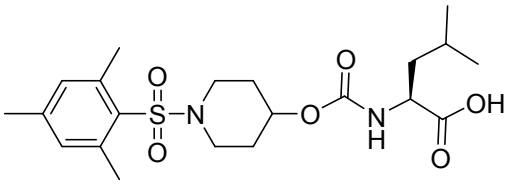
(((1-((5-chloro-2-methoxyphenyl)sulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6l).



Off white solid, yield (100 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.88 (s, 1 H), 7.47 (dd, *J* = 2.3, 8.6 Hz, 1 H), 6.97 (d, *J* = 9.0 Hz, 1 H), 5.02 (d, *J* = 8.6 Hz, 1 H), 4.78 (br. s., 1 H), 4.36 (d, *J* = 3.1 Hz, 1 H), 3.92 (s, 3 H), 3.51 (d, *J* = 6.3 Hz, 2 H), 3.16 (d, *J* = 9.4 Hz, 2 H), 1.94 (br. s., 2 H), 1.79

- 1.66 (m, 4 H), 1.55 (t, J = 8.6 Hz, 2 H), 0.96 (d, J = 5.9 Hz, 6 H). LCMS (ESI+) expected m/z 462.12, found 463.30 ($M+H^+$).

(((1-(mesitylsulfonyl)piperidin-4-yl)oxy)carbonyl)-L-leucine (6m).



Off white solid, yield (130 mg), ^{1}H NMR (400MHz, $CDCl_3$) δ = 6.95 (s, 2 H), 5.15 (d, J = 8.2 Hz, 1 H), 4.82 (br. s., 1 H), 4.35 (br. s., 1 H), 3.36 (br. s., 2 H), 3.11 (br. s., 2 H), 2.61 (s, 6 H), 2.30 (s, 3 H), 2.18 (s, 2 H), 1.88 (d, J = 3.5 Hz, 2 H), 1.82 - 1.69 (m, 3 H), 1.62 - 1.52 (m, 2 H), 0.96 (d, J = 5.9 Hz, 6 H). LCMS (ESI+) expected m/z 440.20, found 441.40 ($M+H^+$).