Bacterial Sliding Clamp Inhibitors that Mimic the Sequential Binding Mechanism of Endogenous Linear Motifs

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Supplementary Results

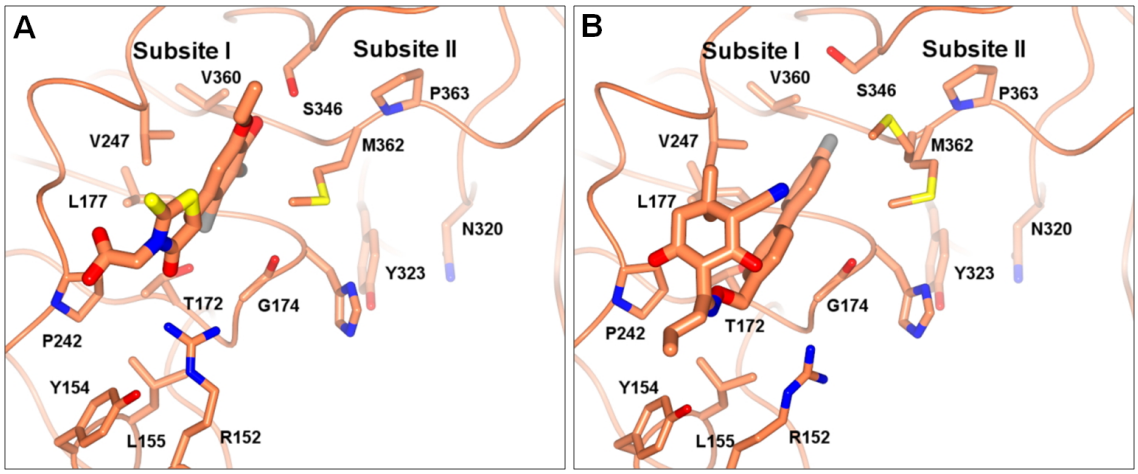


Figure S1. X-ray co-crystal structures of the *E. coli* SC in complex with (A) a thioxothiazolinine derivative (PDB entry 3D1G)[1] and (B) a biphenyloxime ether derivative (PDB entry 3QSB).[2] Carbon atoms are colored orange and non-carbon atoms follow CPK convention. Both inhibitors occupy subsite I.

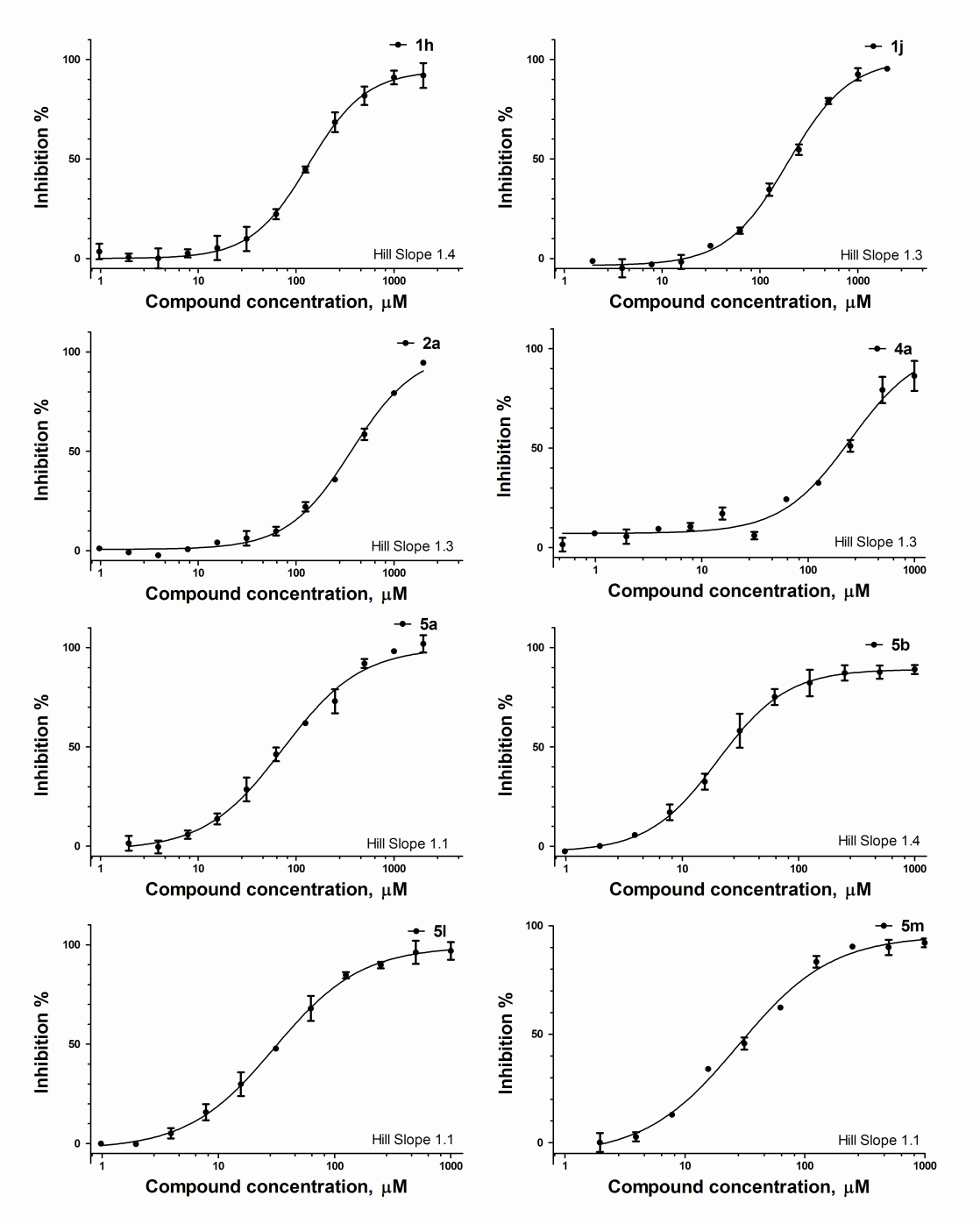


Figure S2. Dose-response curves of representative THC analogs inhibiting the *E. coli* SC, as measured by fluorescence polarization.

Table S1. Data collection and refinement statistics for X-ray co-crystal structures of the *E. coli* SC in complex with compounds 1e, 2a, 5b and 5l

Name	SC^{1e}	SC^{2a}	SC^{5b}	SC^{5l}
PDB Code	4OVF	4OVG	4OVH	4PNU
Data collection				
Space group	$P2_1$			
Cell dimensions	a, b, c (Å) / α, β, γ (°) 79.87, 67.26, 81.19 / 90.00, 114.27, 90.00	a, b, c (Å) / α, β, γ (°) 80.08, 66.20, 80.62 / 90.00, 114.89, 90.00	a, b, c (Å) / α, β, γ (°) 80.03, 66.42, 80.79 / 90.00, 114.76, 90.00	a, b, c (Å) / α, β, γ (°) 79.89, 67.19, 81.02 / 90.00, 114.54, 90.00
Resolution (Å)	50.00–2.05 (2.12–2.05)	40.00–1.90 (1.97–1.90)	30.00–2.25 (1.79–1.70)	30.00–1.90 (1.97–1.90)
R_{merge} (%)	4.3 (26.1)	2.8 (17.1)	7.1 (45.6)	3.2 (38.0)
No. of Reflections	169349	219352	116359	217379
Unique Reflections	49386 (4716)	60330 (6013)	37099 (3029)	61732 (5986)
Mean $I/\sigma(I)$	27.4 (3.5)	41.5 (7.0)	16.6 (2.2)	36.1 (2.7)
Completeness (%)	96.4 (95.9)	99.9 (100.0)	96.4 (82.4)	99.7 (97.4)
Multiplicity	3.6 (3.5)	3.7 (3.5)	3.1 (2.6)	3.5 (3.1)
Refinement				
Resolution (Å)	34.61–2.05 (2.10–2.05)	21.62–1.90 (1.95–1.90)	29.82–2.24 (2.29–2.24)	28.27–1.90 (1.95–1.90)
R_{work} / R_{free} (%)	21.0 (19.7) / 27.0 (29.9)	18.0 (21.9) / 22.6 (27.3)	19.2 (20.5) / 25.0 (32.1)	18.2 (25.0) / 23.0 (28.9)
RMS deviations				
Bond lengths (Å)	0.0056	0.0108	0.0070	0.0071
Bond angles (º)	1.0960	1.5211	1.2173	1.2654
B-factors				
main chain	22.6	17.1	24.8	17.8
sidechain & water	26.4	21.7	26.8	22.1
ligand*	32.7	22.1	33.1	20.3
Ramachandran Plot Outliers	0.43%	0.29%	0.42%	0.29%

Values for data in the highest resolution shell are given in parentheses.

Diffraction data were collected using a Rigaku 007HF X-ray generator producing Cu Kα X-rays (wavelength of 1.5418 Å) and Mar345dtb area detector. Diffraction data were processed using HKL2000.[3]

*Ligand refers to the compounds bound to SC chain A.

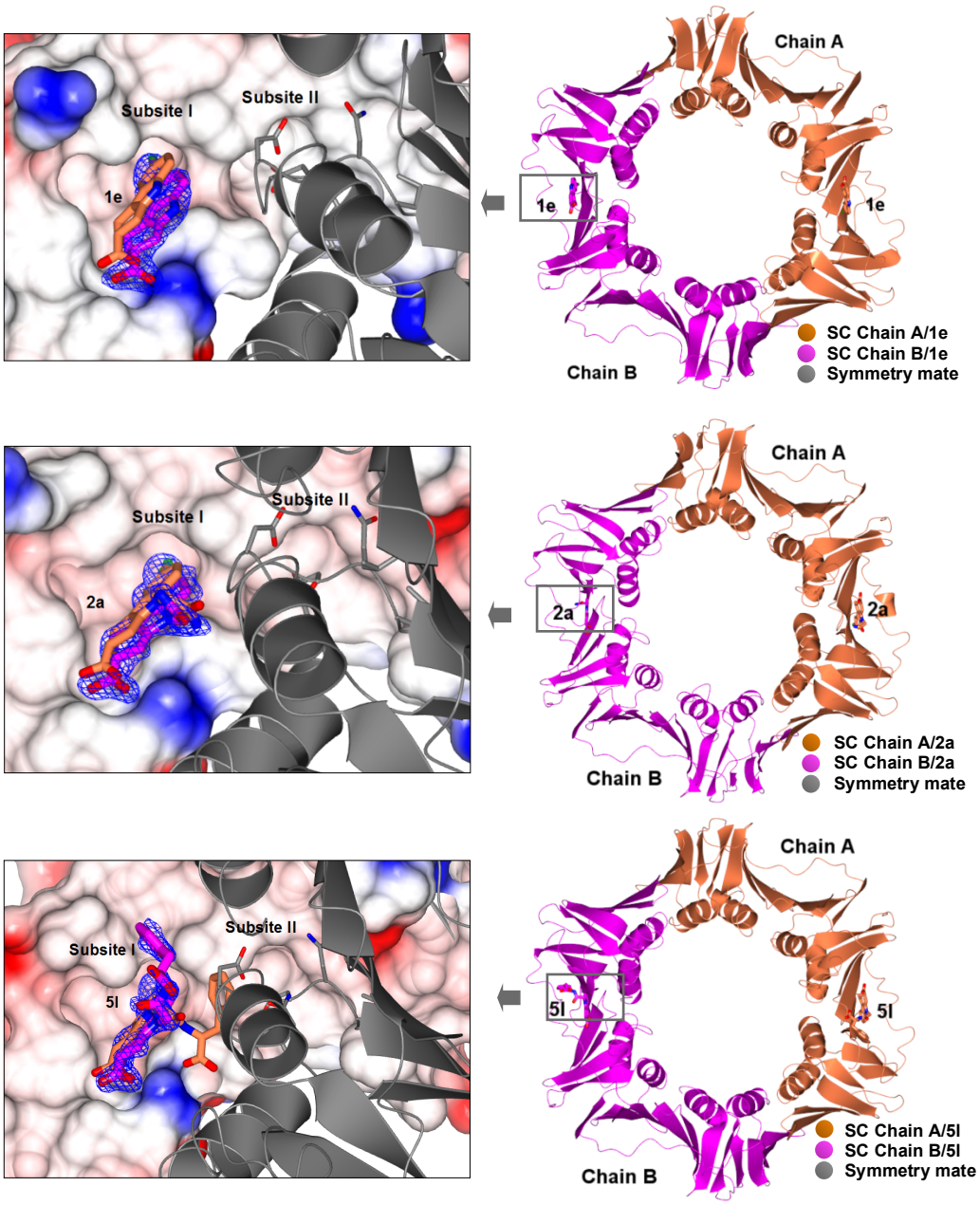


Figure S3. X-ray co-crystal structures of the *E. coli* SC showing chain B in complex with compounds **1e**, **2a** and **5l**. Carbon atoms of SC chain A/B and the bound compounds **1e**, **2a** and **5l** are colored orange/magenta respectively. All other atoms are colored according to the CPK convention. The SC symmetry partner is shown in gray. SC chain A is superimposed with chain B in the enlarged views and the electrostatic potential surfaces of chain B are shown with blue = positive and red = negative. Electron density maps ($2mF_o–DF_c$) contoured at 1σ are shown in blue wire-basket form.

Table S2. Data collection and refinement statistics for the X-ray crystal structures *of apo-E. coli* SC (SC^{apo}) and *E. coli* SC in complex with compound 5m (SC^{5m}), respectively

Structure Name	**SC^{apo}**	**SC^{5m}**
PDB Code	4PNV	4PNW
Data collection		
Space group	$P2_1$	
Cell dimensions	a, b, c (Å) / α, β, γ (°) 80.14, 70.26, 84.47 / 90.00, 114.67, 90.00	a, b, c (Å) / α, β, γ (°) 79.81, 68.71, 83.06 / 90.00, 115.72, 90.00
Resolution (Å)	30.00–1.86 (1.93–1.86)	30.00–2.00 (2.07–2.00)
R_{merge} (%)	2.8 (31.9)	5.1 (45.6)
No. of Reflections	251660	188410
Unique Reflections	71719 (6779)	55049 (5267)
Mean $I/\sigma(I)$	41.1 (3.2)	24.0 (2.6)
Completeness (%)	99.3 (95.3)	98.0 (96.2)
Multiplicity	3.5 (3.1)	3.4 (3.2)
Refinement		
Resolution (Å)	27.55–1.86 (1.91–1.86)	28.01–2.00 (2.05–2.00)
R_{work} / R_{free} (%)	18.3(26.0) / 23.3(33.8)	18.4(20.4) / 23.3(28.2)
R.M.S. deviations		
Bond lengths (Å)	0.0067	0.0079
Bond angles (°)	1.2017	1.3198
B-factors		
mainchain	19.4	23.1
sidechain & water	23.8	26.8
ligand*	N/a	39.4
Ramachandran Plot Outliers	0.29%	0.57 %

Values for data in the highest resolution shell are given in parentheses.

Diffraction data were collected using a Rigaku 007HF X-ray generator producing Cu Kα X-rays (wavelength of 1.5418 Å) and Mar345dtb area detector. Diffraction data were processed with HKL2000.[3]

*Ligand refers to compounds bound to SC Chain B.

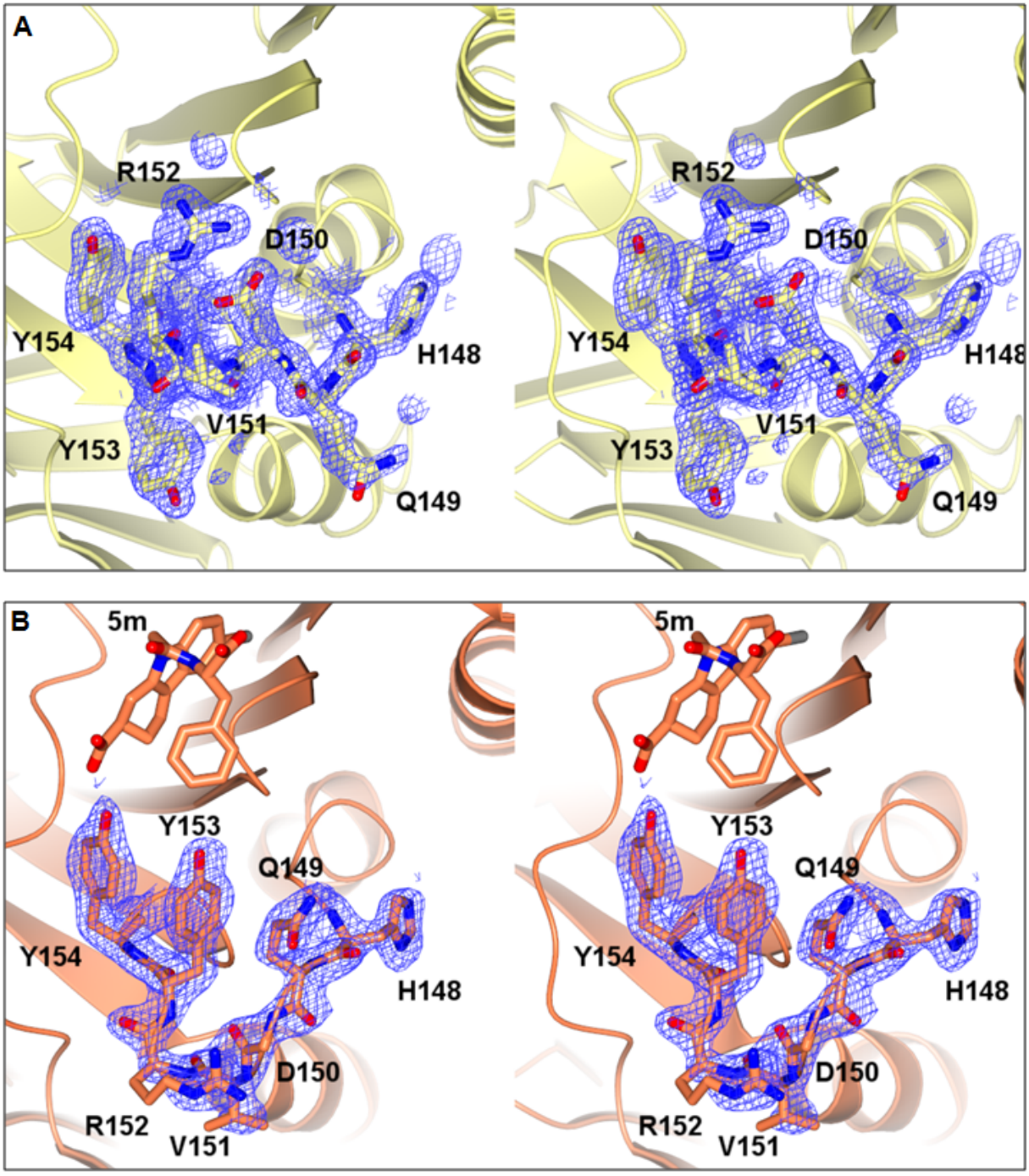


Figure S4. Stereo view of the loop rearrangement induced upon binding of compound **5m** to the *E. coli* SC. (A) The H148–Y154 loop region of SC^{apo}. Carbon atoms are shown in yellow. All other atoms are colored according to CPK convention. (B) The H148–Y154 loop region of $SC^{\mathbf{5m}}$. Carbon atoms are colored orange. All other atoms are colored according to CPK convention. Electron density maps ($2mF_o–DF_c$) contoured at 1σ are shown in blue wire-basket form.

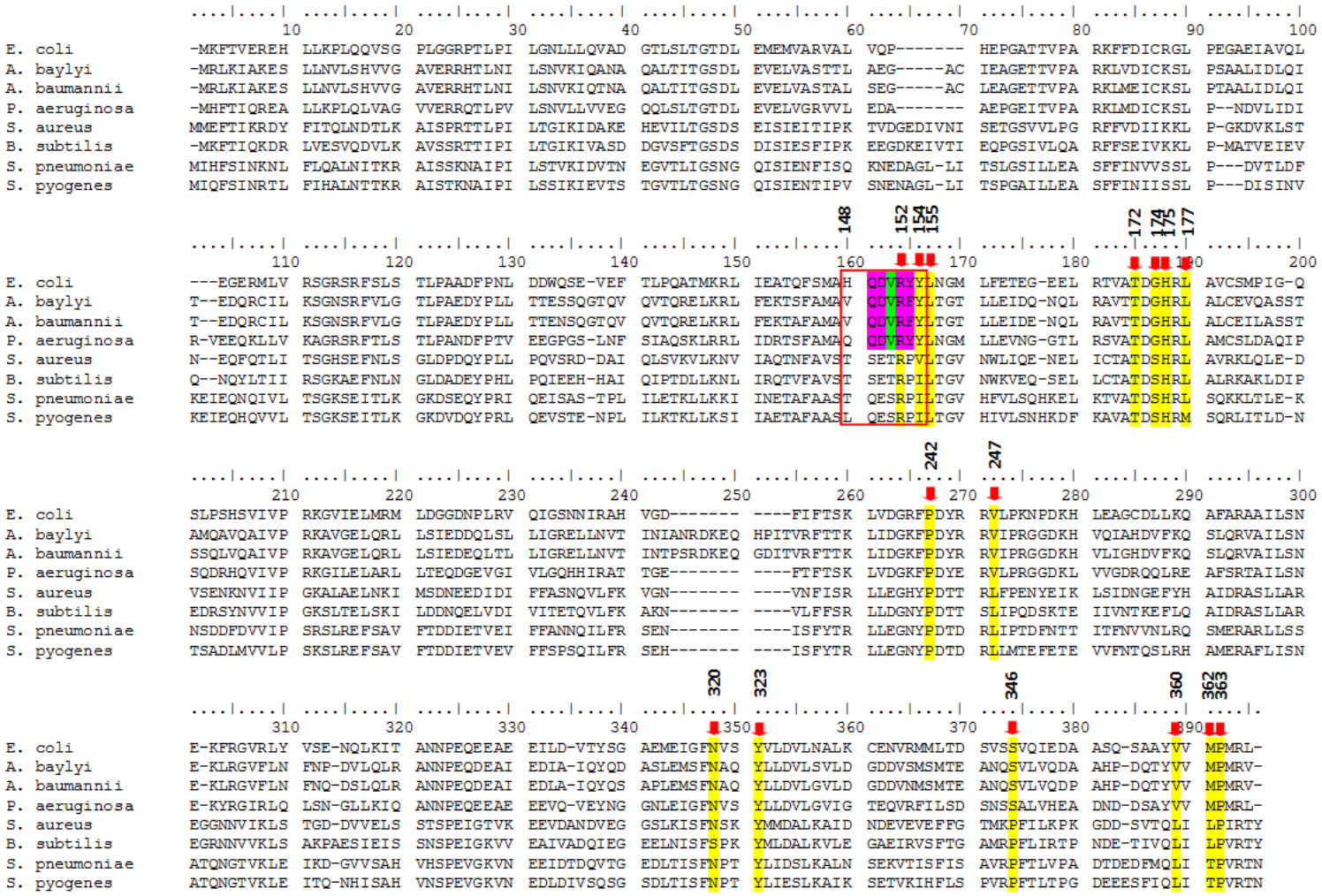


Figure S5. Sequence Alignment of SCs from four representative Gram-negative (*Escherichia coli*, *Acinetobacter baylyi*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) and four Gram-positive bacterial species (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*). Red box indicates the H148–Y154 loop region. The switching pairs of residues, i.e., Q149–D150 and R152–Y153, are highlighted in purple and V151 in green for the Gram-negative species. Residues comprising subsites I and II are highlighted in yellow. Numbering is based on the *E. coli* SC sequence.

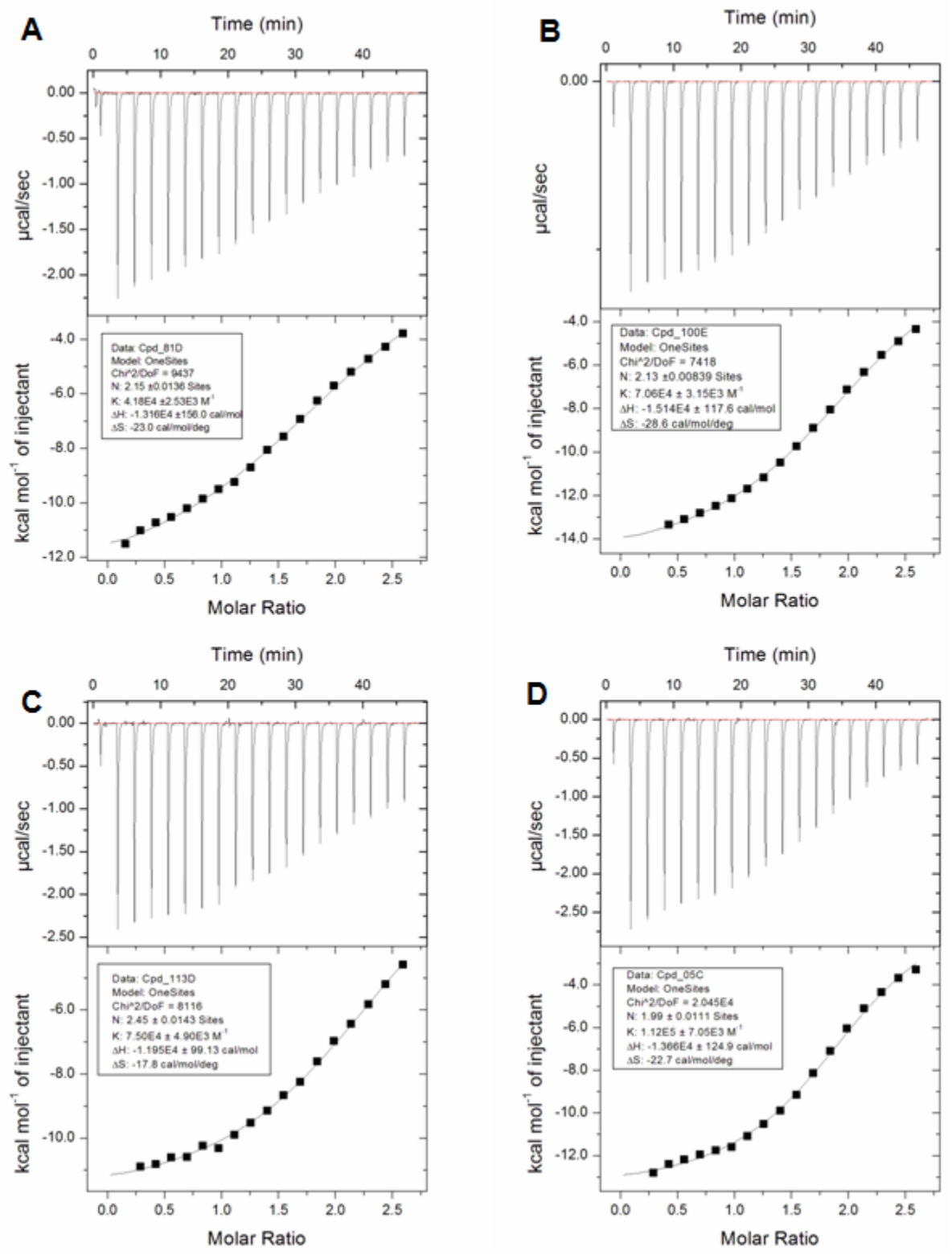


Figure S6. Isothermal titration calorimetry data for the binding of (A) **5a**, (B) **5b**, (C) **5l** and (D) **5m** to the *E. coli* SC.

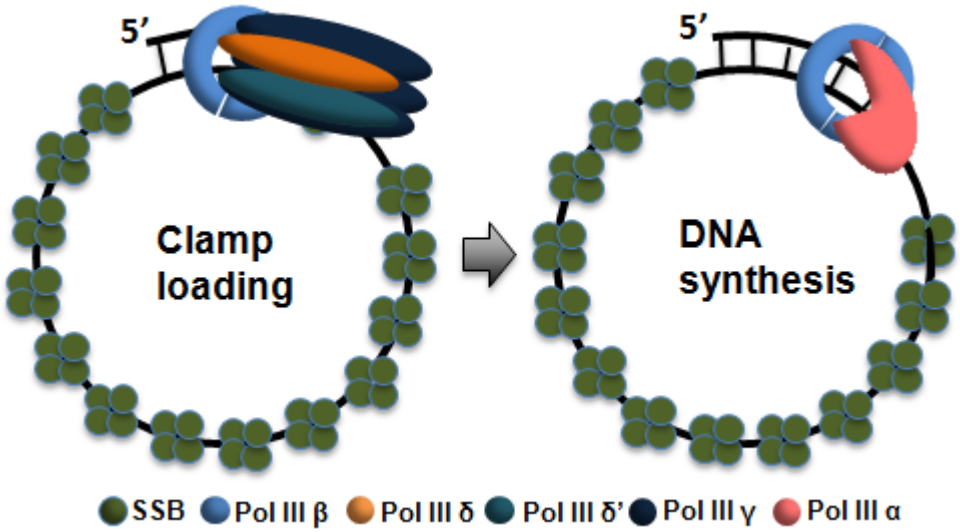


Figure S7. Schematic representation of the *in vitro* DNA replication assay.

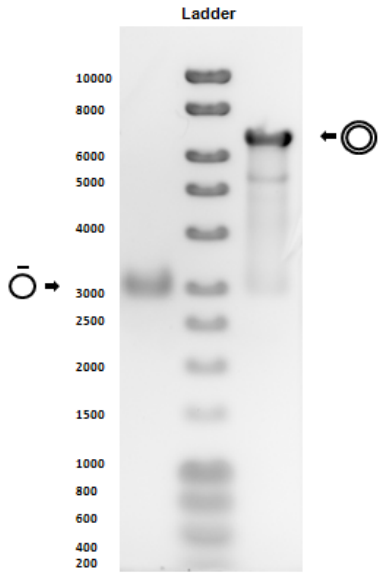


Figure S8. Control *in vitro* DNA replication assay. Molecular sizes (in bp) corresponding to bands in the DNA ladder are shown. The circle with dashed line above represents primed ssDNA template. Two concentric circles represent completed dsDNA replication products.

Supplementary Methods

Chemo-informatics. LogD values at *p*H 7.2 were calculated using Accord for Excel 6.2 (Accelrys). Calculations of Ligand Lipophilicity Efficiency (LLE_{AT}) followed the published methods[4] and are summarized below:

$\Delta G^* = \Delta G - \Delta G_{lipo}$

$= RT\ln(K_i) + RT\text{LogD}$

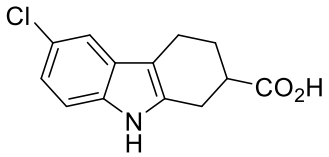
$LLE_{AT} = 0.11 - \Delta G^*/HAC$

ΔG: difference in Gibbs free energy; K_i: inhibition constant; HAC: heavy atom count; LogD: distribution coefficient at *p*H 7.2.

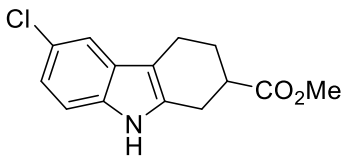
Bioinformatics. Sequence alignments of bacterial sliding clamps (NCBI IDs: YP_859300.1, WP_004930066.1, ZP_08441263.1, NP_064722.1, NP_373240.1, WP_003242509.1, YP_815419.1 and AAF98349.2) were carried out with COBALT.[5]

Chemistry – General. ^{1}H and ^{13}C NMR spectra were acquired on a Varian Mercury 300 MHz, Varian Inova 500 MHz or VNMRS 500 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to the solvent and coupling constants (*J*) are in Hz. Electrospray ionisation (EI) low resolution mass spectra (LRMS) were recorded on a Waters Micromass Platform LCZ spectrometer. High resolution mass spectra (HRMS) were recorded on a Waters Xevo spectrometer using either an EI source or atmospheric solids analysis probe (ASAP). Melting points were recorded using a Gallenkamp (Griffin) melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco P-2000 polarimeter. TLC analysis was performed using pre-coated Merck silica gel 60 PF_{254} aluminium sheets. Flash column chromatography was performed using Davisil silica gel (40-63 μm). Petrol refers to petroleum spirits of bp 40–60°C. All compounds examined showed ≥ 95% purity by ^{1}H NMR and HPLC-MS.

(±)-6-Chloro-2,3,4,9-tetrahydrocarbazole-2-carboxylic acid (1a).[6,7] To a solution of 4-chlorophenylhydrazine hydrochloride (305 mg, 1.70 mmol) in glacial acetic acid (2 mL) was added 3-oxo-cyclohexanecarboxylic acid (237 mg, 1.67 mmol) in glacial acetic acid (2 mL) and the mixture heated at reflux overnight. The reaction mixture was cooled and the resulting precipitate collected by vacuum filtration and washed with cold water. The resultant solid was purified by silica gel column chromatography (25:75:0.5 to 50:50:0.5 Et_2O/petrol/AcOH) followed by recrystallization from EtOH/H_2O to give **1a** (42 mg, 10% yield) as a yellow powder: mp 242–244°C (lit.[6] 249–250°C); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.80–183 (1H, m), 2.14–2.16 (1H, m), 2.56–2.92 (5H, m), 6.97 (1H, d, *J* = 8.0 Hz), 7.24 (1H, d, *J* = 8.5 Hz), 7.34 (1H, s), 10.90 (1H, s), 12.32 (1H, s.); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.4, 25.1, 25.7, 39.3, 107.6, 112.0, 116.5, 119.9, 122.8, 128.1, 134.3, 135.2, 175.97; LRMS (ES^+) *m/z*: 272.2 $[M+Na]^+$; HRMS ($ASAP^+$) calcd. for $C_{13}H_{13}NO_2Cl$ $[M+H]^+$ 250.0635, found 250.0628.

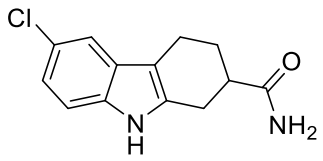


(±)-Methyl 6-chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylate (1b).[6] To a solution of **1a** (50 mg, 0.20 mmol) in MeOH (1.5 mL) was added concentrated H_2SO_4 (200 μL) and the mixture heated at reflux overnight. The resulting solution was cooled, concentrated to dryness, treated with saturated aqueous $NaHCO_3$ and extracted with EtOAc (3 x 10 mL). The combined organic extracts were

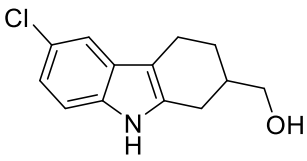


washed with water (10 mL) and brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated. The crude residue was recrystallized from MeOH/H_2O to give **1b** (16 mg, 31% yield) as a beige powder: mp 164–166 °C (lit.[6] 175–176 °C); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.81–1.86 (1H, m), 2.15–2.17 (1H, m), 2.59–2.71 (2H, m), 2.89–2.96 (3H, m), 3.65 (3H, s), 6.98 (1H, dd, *J* = 18.5, 2.0 Hz), 7.26 (1H, d, *J* = 9.0 Hz), 7.35 (1H, d, *J* = 1.0 Hz), 10.94 (1H, brs); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.4, 25.1, 25.7, 39.1, 51.6, 107.6, 112.0, 116.6, 120.0, 122.8, 128.0, 134.3, 134.8, 174.7; LRMS (ES^+) *m/z*: 264.1 $[M+H]^+$; HRMS (ES^+) calcd. for $C_{14}H_{15}NO_2Cl$ $[M+H]^+$ 264.0791, found 264.0791.

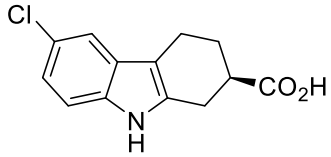
(±)-6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxamide (1c).[7] To a solution of **1a** (75 mg, 0.30 mmol) in dry CH_2Cl_2 (2 mL) containing a few drops of DMF was added *N*-hydroxysuccinimide (59 mg, 0.51 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (97 mg, 0.51 mmol) and the mixture stirred for 2.5 h at room temperature. The resulting suspension was diluted with water and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated. The resultant residue was redissolved in THF (1 mL), NH_4OH (28%; 1 mL) added and the mixture stirred for 2 h. The resulting orange solution was diluted with water and extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated. Trituration of the residue with CH_2Cl_2 gave **1c** (23 mg, 31% yield) as an off-white powder: mp 194–196°C (lit.[6] 203–204 °C); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.69–1.75 (1H, m), 2.05–2.09 (1H, m), 2.53–2.64 (2H, m), 2.71–2.87 (3H, m), 6.87 (1H, br s), 6.97 (1H, dd, *J* = 8.0, 1.3 Hz), 7.24 (1H, d, *J* = 8.5 Hz), 7.35 (1H, s), 7.42 (1H, br s), 10.90 (1H, s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.9, 25.5, 26.7, 40.4, 107.6, 112.0, 116.5, 119.9, 122.8, 128.1, 134.3, 135.7, 176.5; LRMS (ES^+) *m/z*: 271.11 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{13}H_{13}N_2OClNa$ $[M+Na]^+$ 271.0614, found 271.0616.



(±)-(6-Chloro-2,3,4,9-tetrahydro-1H-carbazol-2-yl)methanol (1d).[6] To a solution of **1a** (75 mg, 0.30 mmol) in dry THF (2 mL) was added portion-wise $LiAlH_4$ (35 mg, 0.93 mmol) and the mixture stirred at room temperature for 3 h. A saturated solution of sodium potassium tartrate in water was then added and the mixture stirred for 30 min before being extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated. The resulting residue was triturated with CH_2Cl_2 to give **1d** (30 mg, 20% yield) as a yellow powder: mp 152–154°C (lit.[6] 168–169 °C); ^{1}H NMR (CD_3OD, 500 MHz): δ 1.50–1.52 (1H, m), 2.04–2.05 (2H, m), 2.43–2.48 (1H, m), 2.59–2.61 (1H, m), 2.71–2.74 (1H, m), 2.81–2.85 (1H, m), 3.55–3.62 (2H, m), 6.94 (1H, d, *J* = 8.0 Hz), 7.17 (1H, d, *J* = 8.5 Hz), 7.29 (1H, s); ^{13}C NMR (CD_3OD, 125 MHz): δ 21.0, 27.2, 27.4, 38.4, 67.5, 109.8, 112.4, 117.6, 121.2, 125.0, 130.0, 136.2, 136.8; LRMS (ES^+) *m/z*: 258.0 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{13}H_{13}NO_2Cl$ $[M+H]^+$ 236.0842, found 236.0831.

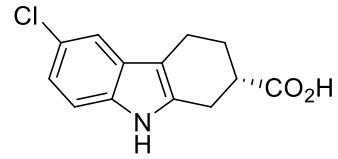


(*R*)-6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (1e). To a solution of (*R*)-3-oxo-cyclohexane-1-carboxylic acid (104 mg, 0.12 mmol) in glacial acetic acid (0.5 mL), 4-chlorophenylhydrazine hydrochloride (136 mg, 0.76 mmol) was added in glacial acetic acid (1.0 mL) and the resulting suspension heated at reflux overnight. The reaction mixture was cooled, diluted with water (15 mL) and extracted with EtOAc (3 x 15 mL). The combined organic extracts were washed with water (10 mL), brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated. The resulting residue was purified by silica gel column chromatography (20:80:0.5 to 50:50:0.5 Et_2O/petrol/acetic acid) followed by trituration with petrol to give **1e** (31 mg, 17% yield) as a yellow crystalline solid: mp 242–244°C (lit.[6] 249–251°C); ^{1}H NMR (CD_3OD, 500 MHz): δ 1.86–1.94 (1H, m),

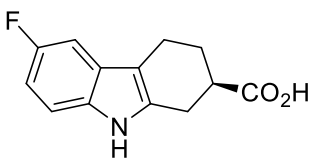


2.25–2.27 (1H, m), 2.60–2.66 (1H, m), 2.73–2.78 (1H, m), 2.80–2.86 (1H, m), 2.91 (2H, br d, *J* = 7.5 Hz), 6.95 (1H, dd, *J* = 8.5, 1.8 Hz), 7.17 (1H, d, *J* = 8.5 Hz), 7.29 (1H, s), 10.2 (1H, br s); ^{13}C NMR (CD_3OD, 125 MHz): δ 20.9, 26.5, 27.5, 41.3, 109.2, 112.5, 117.7, 121.5, 125.1, 129.8, 135.7, 136.2, 178.9; LRMS (ES^-) *m/z*: 248.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{13}H_{11}NO_2Cl$ $[M-H]^-$ 248.0478, found 248.0486; $[\alpha]_{589}^{25}$ +47.7 (c 1.02, MeOH).

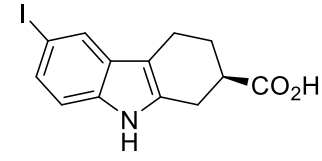
(*S*)-6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (1f). The compound was prepared according to the method described for **1e** from (*S*)-3-oxo-cyclohexane-1-carboxylic acid (101 mg, 0.71 mmol) and 4-chlorophenylhydrazine hydrochloride (133.0 mg, 0.74mmol). **1f** (59 mg, 33% yield) was obtained as a yellow crystalline solid: mp 244–246°C (lit[6] 249–250°C); ^{1}H NMR (CD_3OD, 500 MHz): δ 1.87–1.95 (1H, m), 2.25–2.28 (1H, m), 2.61–2.67 (1H, m), 2.74–2.79 (1H. m), 2.81–2.86 (1H, m), 2.95 (2H, d, *J* = 7.0Hz), 6.95 (1H, dd, *J* = 8.5, 1.5 Hz), 7.17 (1H, d, *J* = 9.0 Hz), 7.29 (1H, d, *J* = 1.5 Hz), 10.19 (1H, br s); ^{13}C NMR (CD_3OD, 125 MHz): δ 20.9, 26.6, 27.5, 41.4, 109.3, 112.6, 117.7, 121.5, 125.2, 129.8, 135.8, 136.3, 178.9; LRMS (ES^+) *m/z*: 250.0 $[M+H]^+$; HRMS ($ASAP^+$) calcd. for $C_{13}H_{13}NO_2Cl$ $[M+H]^+$ 250.0635, found 250.0643; $[\alpha]_{589}^{25}$ –45.2 (c 1.02, MeOH).



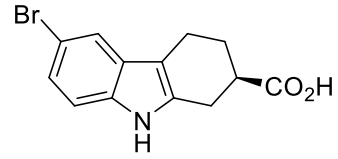
(*R*)-6-Fluoro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (1g). The compound was prepared according to the method described for **1e** from (*R*)-3-oxo-cyclohexane-1-carboxylic acid (127 mg, 0.89 mmol) and 4-fluorophenylhydrazine hydrochloride (122 mg, 0.75 mmol). **1g** (51 mg, 29% yield) was obtained as a yellow powder: mp 244–246°C; ^{1}H NMR (CD_3OD, 300 MHz): δ 1.87–1.96 (1H, m), 2.24–2.28 (1H, m), 2.58–2.86 (3H, m), 2.95 (2H, d, *J* = 7.2 Hz), 6.75 (1H, t, *J* = 9.0 Hz), 6.98 (1H, d, *J* = 9.9 Hz), 7.14–7.18 (1H, m), 10.09 (1H, br s); ^{13}C NMR (CD_3OD, 75 MHz): δ 21.0, 26.6, 27.6, 41.4, 103.1 (d, *J* = 24.0 Hz), 109.1 (d, *J* = 26.3 Hz), 109.5 (d, *J* = 4.6 Hz), 112.0 (d, *J* = 10.3 Hz), 128.9 (d, *J* = 10.3 Hz), 134.3, 136.0, 158.8 (d, *J* = 230.0 Hz), 179.0; LRMS (ES^-) *m/z*: 232.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{13}H_{11}NO_2F$ $[M-H]^-$ 232.0774, found 232.0769; $[\alpha]_{589}^{25}$ +16.2 (c 0.52, MeOH).



(*R*)-6-Iodo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (1h). The compound was prepared according to the method described for **1e** from (*R*)-3-oxo-cyclohexane-1-carboxylic acid (84 mg, 0.59 mmol) and 4-iodophenylhydrazine (107 mg, 0.46 mmol). **1h** (24 mg, 15% yield) was obtained as a brown powder: mp 202–204°C; ^{1}H NMR (CD_3OD, 500 MHz): δ 1.88–1.95 (1H, m), 2.25–2.28 (1H, m), 2.60–2.67 (1H, m), 2.73–277 (1H, m), 2.81–2.86 (1H, m), 2.95 (2H, d, *J* = 7.0 Hz), 7.05 (1H, d, *J* = 9.0 Hz), 7.25 (1H, d, *J* = 8.0 Hz), 7.65 (1H, s), 10.22 (1H, br s); ^{13}C NMR (CD_3OD, 125 MHz): δ 20.9, 26.5, 27.5, 41.4, 82.2, 108.8, 113.6, 127.3, 129.8, 131.3, 135.2, 136.9, 178.9; LRMS (ES^-) *m/z*: 340.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{13}H_{11}NO_2I$ $[M-H]^-$ 339.9835, found 339.9830; $[\alpha]_{589}^{25}$ –27.7 (c 0.28, MeOH).

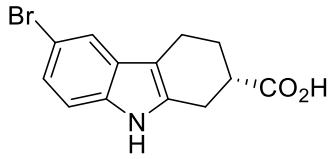


(*R*)-6-Bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (1i). The compound was prepared according to the method described for **1e** from (*R*)-3-oxo-cyclohexane-1-carboxylic acid (568 mg, 3.99 mmol) and 4-bromophenylhydrazine hydrochloride (908 mg, 4.06 mmol). **1i** (943 mg, 80% yield) was obtained as a yellow powder: mp 230–232°C;^{1}H NMR (CD_3OD, 500 MHz): δ 1.87–1.95 (1H, m), 2.25–2.28 (1H, m), 2.61–2.67 (1H, m), 2.74–2.87 (2H, m), 2.96 (2H, d, *J* =

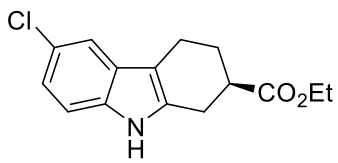


7.0 Hz), 7.08 (1H, d, *J* = 8.5 Hz), 7.14 (1H, d, *J* = 8.5 Hz), 7.45 (1H, s); ^{13}C NMR (CD_3OD, 125 MHz): δ 20.9, 26.5, 27.5, 41.4, 109.1, 112.6, 113.0, 120.9, 124.1, 130.5, 135.6, 136.4, 178.9; LRMS (ES^-) *m/z*: 294.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{13}H_{11}NO_2Br$ $[M-H]^-$ 291.9973, found 291.9964; $[\alpha]_{589}^{25}$+49.0 (c 0.51, MeOH).

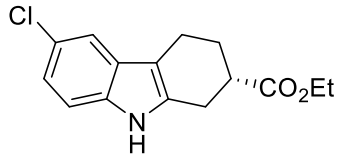
(*S*)-6-Bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (1j). The compound was prepared according to the method described for **1e** from (*S*)-3-oxo-cyclohexane-1-carboxylic acid (241 mg, 1.70 mmol) and 4-bromophenylhydrazine hydrochloride (405 mg, 1.81 mmol). **1j** (212 mg, 42% yield) was obtained as a yellow powder: mp 236–238°C; ^{1}H NMR (CD_3OD, 500 MHz): δ 1.89–1.90 (1H, m), 2.24 –2.26 (1H, m), 2.61–2.67 (1H, m), 2.72–2.95 (4H, m), 7.07 (1H, br d, *J* = 8.0 Hz), 7.13 (1H, d, *J* = 7.5 Hz), 7.44 (1H, s); ^{13}C NMR (CD_3OD, 125MHz): δ 20.9, 26.5, 27.5, 41.4, 109.1, 112.5, 113.0, 120.9, 124.1, 130.4, 135.6, 136.4, 178.9; LRMS (ES^-) *m/z*: 292.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{13}H_{11}NO_2Br$ $[M-H]^-$ 291.9973, found 291.9980; $[\alpha]_{589}^{25}$ –39.6 (c 0.52, MeOH).



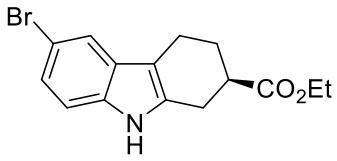
Ethyl (*R*)-6-chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylate (1k). To a solution of 4-chlorophenylhydrazine hydrochloride (974 mg, 6.85 mmol) in glacial acetic acid (8 mL) was added (*R*)-3-oxo-1-cyclohexane carboxylic acid (702 mg, 4.94 mmol) and the suspension heated at reflux for 6 h. The reaction mixture was cooled, diluted with water and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL), dried over anhydrous $MgSO_4$ and concentrated. The resulting crude residue was redissolved in absolute ethanol (5 mL) containing concentrated H_2SO_4 (100 µL) and the reaction heated at reflux overnight. The solution was cooled, concentrated, made alkaline with saturated $NaHCO_{3(aq)}$ and the mixture extracted with EtOAc (3 x 40 mL). The combined organic fractions were washed with brine (40 mL), dried over anhydrous $MgSO_4$ and concentrated. The resulting residue was purified by silica gel column chromatography (10:0–8:2 Et_2O/petrol) to give **1k** (877 mg, 64% yield) as a yellow powder: mp 130–132°C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.29 (3H, t, *J* = 7.3 Hz), 1.91–1.98 (1H, m), 2.28–2.31 (1H, m), 2.64–2.70 (1H, m), 2.77–3.05 (4H, m), 4.16–4.21 (2H, m), 7.06 (1H, d, *J* = 8.0 Hz), 7.16 (1H, d, *J* = 8.5 Hz), 7.39 (1H, s), 7.83 (1H, br s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.4, 20.1, 25.7, 26.3, 40.3, 60.9, 109.5, 111.5, 117.6, 121.5, 125.1, 128.6, 134.0, 134.4, 175.1; LRMS (ES^+) *m/z*: 278.0 $[M+H]^+$; HRMS (ES^+) calcd. for $C_{15}H_{17}NO_2Cl$ $[M+H]^+$ 278.0948, found 278.0938; $[\alpha]_{589}^{25}$ +48.4 (c 1.05, MeOH).



Ethyl (*S*)-6-chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylate (1l). The compound was prepared according to the method described for **1k** from (*S*)-3-oxocyclohexane-1-carboxylic acid (272 mg, 1.91 mmol) and 4-chlorophenylhydrazine hydrochloride (340 mg, 1.89 mmol). **1l** was obtained (184 mg, 35% yield) as a pale yellow solid: mp 130–132°C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.29 (3H, t, *J* = 7.3 Hz), 1.92–1.98 (1H, m), 2.29–2.31 (1H, m), 2.65–2.71 (1H, m), 2.77–3.05 (4H, m), 4.17–4.23 (2H, m), 7.06 (1H, dd, *J* = 8.0, 1.5 Hz), 7.17 (1H, d, *J* = 9.0 Hz), 7.40 (1H, s), 7.81 (1H, br s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.4, 20.1, 25.7, 26.3, 40.3, 60.9, 109.5, 111.5, 117.6, 121.6, 125.1, 128.6, 134.0, 134.4, 175.1; LRMS (ES^-) *m/z*: 276.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{15}H_{15}NO_2Cl$ $[M-H]^-$ 276.0791, found 276.0780; $[\alpha]_{589}^{25}$ –42.8 (c 1.03, MeOH).



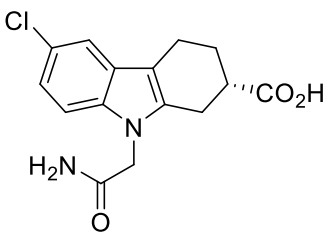
Ethyl (*R*)-6-bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylate (1m). The compound was prepared according to the method described for **1k** from (*R*)-3-oxocyclohexane-1-carboxylic acid (2.28 g, 10.18



mmol) and 4-bromophenylhydrazine hydrochloride (1.45 g, 10.17 mmol). **1m** (1.99 g, 58% yield) was obtained as a yellow powder: mp 144–146°C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.29 (3H, t, *J* = 7.0 Hz), 1.92–1.98 (1H, m), 2.28–2.31 (1H, m), 2.65–2.70 (1H, m), 2.77–3.05 (4H, m), 4.17–4.23 (2H, m, CH_2), 7.11 (1H, d, *J* = 8.5 Hz), 7.18 (1H, d, *J* = 8.5 Hz), 7.55 (1H, s), 7.84 (1H, br s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.5, 20.1, 25.7, 26.4, 40.3, 61.0, 109.5, 112.1, 112.7, 120.7, 124.2, 129.3, 133.9, 134.8, 175.2; LRMS (ES^+) *m/z*: 362.0 $[M+K]^+$; HRMS (ES^+) calcd. for $C_{15}H_{17}NO_2Br$ $[M+H]^+$ 322.0443, found 322.0443; $[\alpha]_{589}^{25}$ +49.5 (c 1.04, MeOH).

Ethyl (*S*)-6-bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylate (1n). The compound was prepared according to the method described for **1k** from (*S*)-3-oxocyclohexane-1-carboxylic acid (227 mg, 1.60 mmol) and 4-bromophenylhydrazine hydrochloride (387 mg, 1.73 mmol). **1n** (183 mg, 35% yield) was obtained as a yellow powder: mp 136–138°C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.29 (3H, d, *J* = 7.3 Hz), 1.90–1.98 (1H, m), 2.28–2.31 (1H, m), 2.64–2.70 (1H, m), 2.76–3.04 (4H, m), 4.16–4.23 (2H, m, CH_2), 7.11 (1H, d, *J* = 8.5 Hz), 7.18 (1H, dd, *J* = 8.0, 1.5 Hz), 7.55 (1H, s), 7.84 (1H, br s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.4, 20.1, 25.6, 26.3, 40.3, 60.9, 109.4, 112.0, 112.6, 120.7, 124.1, 129.3, 133.8, 134.7, 175.1; LRMS (ES^-) *m/z*: 320.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{15}H_{15}NO_2Br$ $[M-H]^-$ 320.0286, found 320.0288; $[\alpha]_{589}^{25}$ –39.3 (c 0.98, MeOH).

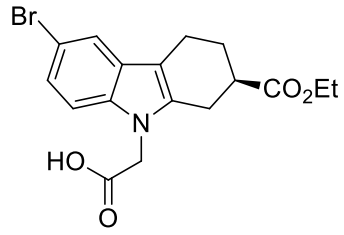
(*R*)-9-(2-Amino-2-oxoethyl)-6-chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (2a). To a solution of **1k** (163 mg, 0.59 mmol) in dry DMF (1.0 mL) was added Cs_2CO_3 (304 mg, 0.93 mmol) and the suspension stirred at room temperature for 15 min. A solution of iodoacetamide (152 mg, 0.82 mmol) in dry DMF (0.5 mL) was added drop wise and the mixture stirred at 60°C under N_2 overnight. The reaction was quenched with 1 M HCl (20 mL) and extracted with EtOAc (3 x 20 mL). The combined extracts were washed with 0.5 M HCl (2 x 20 mL), brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated. The crude residue was purified by silica gel column chromatography (6:4:0–9:0:1 Et_2O/petrol/MeOH) to give ethyl (*R*)-9-(2-amino-2-oxoethyl)-6-chloro-2,3,4,9-tetrahydro-*1*H-carbazole-2-carboxylate as a yellow powder. The compound was dissolved in absolute EtOH (1 mL) and an aqueous solution of NaOH (2 M, 400 µL) added. The resulting suspension was stirred at room temperature overnight. The reaction mixture was concentrated, diluted with water and washed with CH_2Cl_2 (10 mL). The aqueous layer was acidified with 1 M HCl and extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with water (10 mL), brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated. The resulting residue was triturated with Et_2O to give **2a** (11 mg, 6% yield) as a white solid: mp > 260°C (dec.); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.74–1.76 (1H, m), 2.17–2.20 (1H, m), 2.60–2.63 (1H, m), 2.73–2.92 (4H, m), 4.68 (2H, s), 7.05 (1H, d, *J* = 9.0 Hz), 7.23 (1H, br s), 7.33 (1H, d, *J* = 8.5 Hz), 7.41 (1H, s), 7.53 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.7, 24.0, 25.6, 39.40, 45.2, 107.9, 110.5, 116.7, 120.1, 123.3, 127.7, 135.4, 136.7, 169.4, 176.0; LRMS (ES^+) *m/z*: 329.0 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{15}H_{16}N_2O_3Cl$ $[M+H]^+$ 307.0849, found 307.0847; $[\alpha]_{589}^{25}$ +10.4 (c 0.6, DMSO).



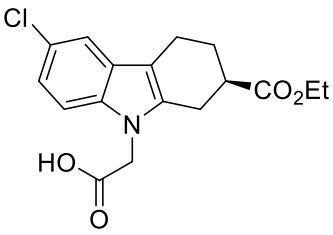
(*S*)-9-(2-Amino-2-oxoethyl)-6-chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (2b). The compound was prepared according to the method described for **2a** from **1l** (170 mg, 0.61 mmol), iodoacetamide (104 mg, 0.56 mmol) and Cs_2CO_3 (260 mg, 0.80 mmol) in dry DMF (1.5 mL). Following ethyl ester hydrolysis, **2b** (7.4 mg, 4% yield) was obtained as a white powder: mp >

260°C (dec.);^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.74–1.76 (1H, m), 2.18–2.20 (1H, m), 2.61–2.62 (1H, m), 2.77–2.91 (4H, m), 4.68 (2H, s, CH_2), 7.05 (1H, d, J = 8.5 Hz), 7.23 (1H, br s), 7.34 (1H, d, J = 8.0 Hz), 7.40 (1H, s), 7.53 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.7, 24.0, 25.7, 39.5, 45.2, 107.9, 110.6, 116.7, 120.1, 123.3, 127.7, 135.4, 136.7, 169.5, 176.0; LRMS (ES^+) *m/z*: 329.0 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{15}H_{15}N_2O_3ClNa$ $[M+Na]^+$ 329.0669, found 329.0683; $[\alpha]_{589}^{25}$ –9.2 (c 0.29, DMSO).

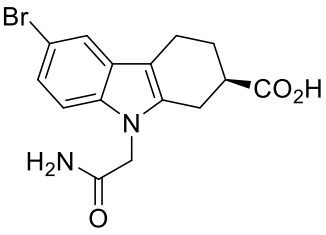
(*R*)-2-(6-Bromo-2-(ethoxycarbonyl)-1,2,3,4-tetrahydro-9H-carbazol-9-yl)acetic acid (3a). To a solution of **1m** (642 mg, 2.31 mmol) in dry DMF (1 mL) was added Cs_2CO_3 (581 mg, 1.78 mmol) and *tert*-butyl bromoacetate (302 mg, 1.55 mmol) and the mixture stirred at 60°C under N_2 for 19 h. The reaction mixture was quenched with 1 M HCl (20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined extracts were washed with 0.5 M HCl (3 x 20 mL), brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated. The resulting crude residue was purified by silica gel column chromatography (2:8–5:5 Et_2O/petrol) to give (*R*)-9-(2-(tert-butoxy)-2-oxoethyl)-6-chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylate, which was treated with TFA (1 mL). Following reaction for 1 h, the TFA was evaporated under a stream of N_2 to afford **3a** (402 mg, 83% yield) as a yellow powder: mp 180–182°C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.22 (3H, t, J = 6.8 Hz, CH_3), 1.77–1.78 (1H, m), 2.17–2.19 (1H, m), 2.63–2.94 (5H, m), 4.11–4.15 (2H, m), 4.92 (2H, s), 7.17 (1H, d, J = 8.0), 7.35 (1H, d, J = 8.5 Hz), 7.55 (1H, br s), 13.09 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 14.1, 19.4, 23.7, 25.6, 39.3, 44.1, 60.2, 108.1, 110.7, 116.8, 120.3, 123.5, 127.7, 135.3, 136.1, 170.3, 174.2; LRMS (ES^-) *m/z*: 380.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{17}H_{17}NO_4Br$ 378.0341 $[M-H]^-$, found. 378.0324; $[\alpha]_{589}^{25}$ +25.7 (c 1.00, MeOH).



2-(6-Chloro-2-(ethoxycarbonyl)-3,4-dihydro-1H-carbazol-9(2H)-yl)acetic acid (3c). The compound was prepared according to the method described for **3a** from **1k** (642 mg, 2.31 mmol), *tert*-butyl bromoacetate (375 µL, 495 mg, 2.54 mmol) and Cs_2CO_3 (1.03 g, 3.15 mmol) in DMF (4 mL). Following *tert*-butyl ester deprotection **3c** (331 mg, 43% yield) was obtained as an pale yellow powder: mp 180–182°C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.22 (3H, t, J = 7.0 Hz), 1.75–1.80 (1H, m), 2.17–2.19 (1H, m), 2.60–2.65 (1H, m), 2.73–2.94 (4H, m), 4.13 (2H, t, J = 6.8 Hz), 4.92 (2H, d, J = 2.0 Hz), 7.05 (1H, dd, J = 8.5, 1.8 Hz), 7.39 (1H, d, J = 8.5 Hz), 7.41 (1H, d, J = 2.0 Hz), 13.00 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 14.1, 19.5, 23.7, 25.6, 39.3, 44.1, 60.2, 108.1, 110.7, 116.8, 120.3, 123.5, 127.7, 135.3, 136.1, 170.3, 174.2; LRMS (ES^+) *m/z*: 358.0 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{17}H_{18}NO_4ClNa$ $[M+Na]^+$ 358.0822, found 358.0815; $[\alpha]_{589}^{25}$ +2.0 (c 0.97, MeOH).

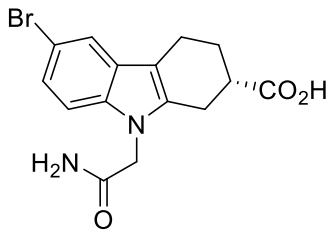


(*R*)-9-(2-Amino-2-oxoethyl)-6-bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (4a). To a solution of **3a** (87.9 mg, 0.23 mmol) in dry CH_2Cl_2 (2 mL) was added NHS (41 mg, 0.36 mmol) and EDC (79 mg, 0.41mmol) and the reaction mixture stirred for 1 h at room temperature. The CH_2Cl_2 was evaporated under nitrogen and the resulting residue redissolved in THF (2 mL). Aqueous NH_4OH solution (28%; 1 mL) was added and the mixture stirred overnight at room temperature. The resulting precipitate was collected by vacuum filtration (washing with water) and then triturated with Et_2O. The resulting ethyl (*R*)-9-(2-amino-2-oxoethyl)-6-bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylate (29 mg, 0.08 mmol) was dissolved in absolute EtOH (2 mL), an aqueous NaOH solution (2 M; 500 µL) added and the suspension stirred at room temperature overnight. The reaction mixture was concentrated, diluted with water, acidified

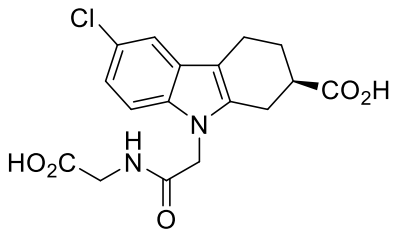


with 1 M HCl and extracted with EtOAc (3 x 10 mL). The combined extracts were washed with water (10 mL), brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated. The resulting crude residue was triturated with Et_2O to give **4a** (12 mg, 14% yield) as a yellow powder: mp > 250°C (dec.); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.70–1.76 (1H, m), 2.16–2.20 (1H, m), 2.60–2.92 (5H, m), 4.69 (2H, s), 7.16 (1H, d, *J* = 8.7 Hz), 7.25 (1H, br s), 7.29 (1H, d, *J* = 8.7 Hz), 7.54 (2H, br s), 12.44 (1H, br s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 19.7, 23.9, 25.6, 39.3, 45.2, 107.8, 111.1, 111.2, 119.7, 122.7, 128.4, 135.6, 136.5, 169.4, 176.0; LRMS (ES^+) *m/z*: 373.0 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{15}H_{15}N_2O_3BrNa$ $[M+Na]^+$ 373.0164, found 373.0182; $[\alpha]_{589}^{25}$ +12.9 (c 0.64, DMSO).

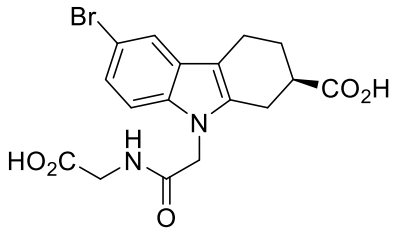
(*S*)-9-(2-amino-2-oxoethyl)-6-bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (4b). (*S*)-2-(6-bromo-2-(ethoxycarbonyl)-1,2,3,4-tetrahydro-9H-carbazol-9-yl)acetic acid (**3b**) was prepared according to the method described for **3a** from **1n** (145 g, 0.45 mmol), Cs_2CO_3 (220 mg, 0.67 mmol) and *tert*-butyl bromoacetate (115 mg, 0.59 mmol). Following TFA deprotection of the *tert*-butyl ester, (*S*)-2-(6-bromo-2-(ethoxycarbonyl)-1,2,3,4-tetrahydro-9H-carbazol-9-yl)acetic acid (92 mg, 0.24 mmol) was subsequently reacted with NHS (40 mg, 0.34 mmol) and EDC (72 mg, 0.38 mmol) according to the method described for **4a.** Ethyl ester hydrolysis in an aqueous solution of NaOH (2 M; 300 µL) in absolute ethanol (4 mL) yielded **4b** (28 mg, 33% yield) as a yellow powder: mp > 250°C (dec.); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.75–1.76 (1H, m), 2.17–2.20 (1H, m), 2.59–2.62 (1H, m), 2.73–2.92 (4H, m), 4.69 (2H, s, CH_2), 7.16 (1H, d, *J* = 8.5 Hz), 7.23 (1H, br s), 7.29 (1H, d, *J* = 8.5 Hz), 7.52 (1H, br s), 7.54 (1H, s), 12.42 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.7, 23.9, 25.6, 39.3, 45.2, 107.8, 111.1, 111.2, 119.7, 122.7, 128.3, 135.6, 136.5, 169.4, 175.9; LRMS (ES^+) *m/z*: 373.0 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{15}H_{15}N_2O_3BrNa$ $[M+Na]^+$ 373.0164, found 373.0174; $[\alpha]_{589}^{25}$ +4.4 (c 0.71, DMSO).



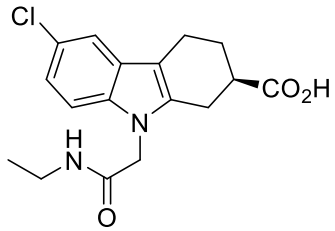
(*R*)-9-(2-((carboxymethyl)amino)-2-oxoethyl)-6-chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5a). To a solution containing **3c** (147 mg, 0.44 mmol), glycine methyl ester hydrochloride (69 mg, 0.55 mmol) and HATU (221 mg, 0.58 mmol) in dry DMF (1 mL) was added DIPEA (250 µL, 1.44 mmol) dropwise and the resulting mixture stirred at room temperature overnight under N_2. The reaction mixture was diluted water and extracted with EtOAc (3 x 15 mL). The combined extracts were washed with 0.5 M HCl (3 x 15 mL), sat. $NaHCO_3$ (15 mL) and brine (15 mL) before being dried over anhydrous $MgSO_4$ and concentrated. The resulting residue containing ethyl (*R*)-6-chloro-9-(2-((2-methoxy-2-oxoethyl)amino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylate was dissolved in absolute EtOH (1 mL), an aqueous solution of NaOH (2 M, 400 µL) added and the suspension stirred at room temperature overnight. The reaction mixture was concentrated, diluted with water and then washed with CH_2Cl_2 (15 mL). The aqueous layer was acidified (1 M HCl) and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with water (15 mL) and brine (15 mL) before being dried over anhydrous $MgSO_4$ and concentrated. The resulting residue was purified by silica gel column chromatography (0:10:0–1:9:0.005 MeOH/CH_2Cl_2/acetic acid) to give **5a** (96 mg, 60% yield) as an off-white powder: mp 220–222°C; ^{1}H NMR (CH_3OD, 500 MHz): δ 1.92–1.99 (1H, m), 2.26–2.29 (1H, m), 2.64–2.70 (1H, m), 2.76–2.79 (1H, m), 2.89–2.98 (3H, m), 3.89 (2H, s), 4.77 (2H, s), 7.04 (1H, d, *J* = 8.5 Hz), 7.23 (1H, d, *J* = 9.0 Hz), 7.35 (1H, s), 7.96 (1H, br s); ^{13}C NMR (CH_3OD, 125 MHz): δ 20.9, 25.2, 27.1, 41.2, 41.8, 46.7, 110.5, 111.0, 118.2, 122.1, 126.1, 129.8, 137.0, 137.2, 171.4, 172.6, 178.6; LRMS (ES^-) *m/z*: 363.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{17}H_{16}N_2O_5Cl$ $[M-H]^-$ 363.0748, found 363.0733; $[\alpha]_{589}^{25}$ –5.7 (c 0.50, MeOH).



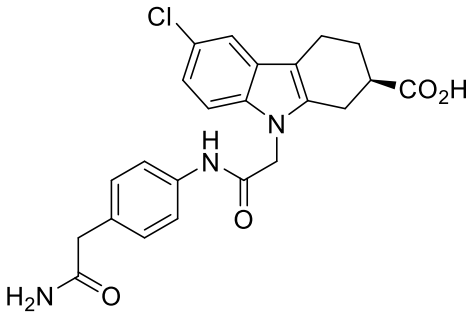
(*R*)-6-bromo-9-(2-((carboxymethyl)amino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5b). The compound was prepared according to the method described for **5a** from **3a** (108 mg, 0.28 mmol), glycine methyl ester hydrochloride (49 mg, 0.39 mmol), HATU (137 mg, 0.36 mmol) and DIPEA (160 µL, 0.92 mmol) in dry DMF (1 mL). Following ester deprotection in an aqueous solution of NaOH (2 M; 300 µL) and absolute ethanol (1 mL), **5b** (46 mg, 40% yield) was obtained as an off white powder: mp 238–240°C; ^{1}H NMR (CH_3OD, 500 MHz): δ 1.92–1.95 (1H, m), 2.26–2.28 (1H, m), 2.64–2.68 (1H, m), 2.76–2.79 (1H, m), 2.89–2.98 (3H, m), 3.89 (2H, s), 4.76 (2H, s), 7.17–7.18 (2H, m), 7.50 (1H, s), 8.01 (1H, br s); ^{13}C NMR (CH_3OD, 125 MHz): δ 20.9, 25.1, 27.2, 41.1, 41.8, 46.6, 110.4, 111.5, 113.5, 121.3, 124.7, 130.4, 137.1, 137.3, 171.3, 172.7, 178.6; LRMS (ES^-) *m/z*: 407.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{17}H_{16}N_2O_5Br$ $[M-H]^-$ 407.0243, found 407.0256; $[\alpha]_{589}^{25}$ +23.1 (c 0.59, MeOH).



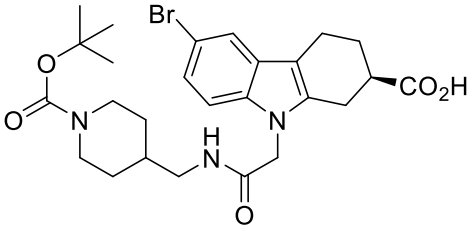
(*R*)-6-chloro-9-(2-(ethylamino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5c). The compound was prepared according to the method described for **5a** from **3c** (140 mg, 0.42 mmol), ethylamine hydrochloride (46 mg, 0.56 mmol), HATU (211 mg, 0.56 mmol) and DIPEA (200 µL, 1.15 mmol) in dry DMF (1 mL). Ester deprotection in an aqueous solution of NaOH (2 M; 400 µL) and absolute ethanol (1 mL) afforded **5c** (54 mg, 38% yield) as an off-white powder: mp 246–248°C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.02 (3H, t, *J* = 7.0 Hz), 1.75–1.76 (1H, m), 2.17–2.20 (1H, m), 2.61–2.62 (1H, m), 2.77–2.93 (4H, m), 3.08–3.12 (2H, m), 4.69 (2H, s), 7.05 (1H, d, *J* = 8.0 Hz), 7.34 (1H, d, *J* = 9.0 Hz), 7.40 (1H, s), 8.12 (1H, s), 12.43 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 14.6, 19.7, 24.1, 25.6, 33.5, 39.6, 45.6, 108.0, 110.6, 116.7, 120.1, 123.3, 127.7, 135.4, 136.7, 167.0, 176.0; LRMS (ES^+) *m/z*: 357.0 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{17}H_{19}N_2O_3ClNa$ $[M+Na]^+$ 357.0982, found 357.0970; $[\alpha]_{589}^{25}$ +2.7 (c 0.52, MeOH).



(*R*)-9-(2-((4-(2-amino-2-oxoethyl)phenyl)amino)-2-oxoethyl)-6-chloro-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5d). The compound was prepared according to the method described for **5a** from **3c** (125 mg, 0.37 mmol), 2-(4-aminophenyl)acetamide (73 mg, 0.48 mmol), HATU (181 mg, 0.48 mmol) and DIPEA (230 µL, 1.32 mmol) in dry DMF (1 mL). Ester deprotection in an aqueous solution of NaOH (2 M; 140 µL) and absolute ethanol (1 mL) gave **5d** (29 mg, 31% yield) as a white powder: mp > 260°C (dec.);^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.76–1.78 (1H, m), 2.18–2.20 (1H, m), 2.63–2.96 (5H, m), 3.31 (2H, s), 4.95 (2H, s), 6.82 (2H, s), 7.06 (1H, d, *J* = 7.5 Hz), 7.19 (2H, d, *J* = 8.0 Hz), 7.39–7.42 (2H, m), 7.49 (2H, d, *J* = 8.0 Hz), 10.34 (1H, s), 12.50 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.7, 24.0, 25.7, 39.3, 41.7, 46.0, 108.1, 110.6, 116.8, 119.1, 120.2, 123.4, 127.7, 129.4, 131.7, 135.5, 136.8, 136.9, 166.1, 172.2, 176.0; LRMS (ES^-) *m/z*: 438.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{23}H_{21}N_3O_4Cl$ $[M-H]^-$ 438.1221, found 438.1215; $[\alpha]_{589}^{25}$ +3.77 (c 0.53, DMSO).

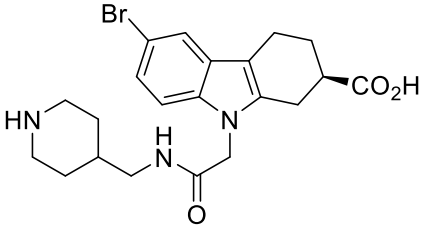


(*R*)-6-bromo-9-(2-(((1-(BOC)piperidin-4-yl)methyl)amino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5e). The compound was prepared according to the method described for **5a** from **3a** (216 mg, 0.57 mmol), *tert*-butyl 4-(aminomethyl)piperidine-1-carboxylate (159 mg, 0.74 mmol), HATU (301 mg, 0.79 mmol) and DIPEA (350 µL, 2.01 mmol) in dry DMF (2 mL). Ester deprotection in an aqueous solution of NaOH (2 M; 300 µL) and absolute ethanol (1 mL) provided **5e** (68 mg, 36% yield) as a



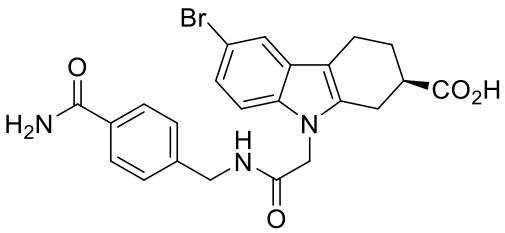
pale yellow powder: mp 158–160°C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 0.95–0.99 (2H, m), 1.39 (9H, s), 1.57–1.59 (3H, m), 1.74–1.77 (1H, m), 2.17–2.20 (1H, m), 2.61–2.64 (3H, m), 2.73–2.91 (3H, m), 2.94–2.97 (3H, m), 3.91 (2H, br d, *J* = 9.5 Hz), 4.72 (2H, s), 7.16 (1H, dd, *J* = 9.0, 1.8 Hz), 7.30 (1H, d, *J* = 9.0 Hz), 7.54 (1H, d, *J* = 1.5 Hz), 8.16 (1H, br t, *J* = 5.8 Hz), 12.44 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.7, 24.0, 25.7, 28.1, 29.4, 35.7, 39.4, 44.0, 45.5, 78.5, 107.9, 111.2, 111.3, 119.8, 122.8, 128.4, 135.6, 136.6, 153.8, 167.5, 176.0; LRMS (ES$^+$) *m/z*: 570.0 [M+Na]$^+$; HRMS (ES$^+$) calcd. for $C_{26}H_{34}N_3O_5BrNa$ [M+Na]$^+$ 570.1580, found 570.1604; $[\alpha]_{589}^{25}$ +8.5 (c 0.52, MeOH).

(*R*)-6-bromo-9-(2-oxo-2-((piperidin-4-ylmethyl)amino)ethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid.TFA salt (5f). Neat TFA (1 mL) was added to **5e** (42 mg, 0.076 mmol) and the mixture stirred at room temperature for 1 h. The TFA was evaporated under a stream of N_2 and the resulting residue triturated with petrol to give **5f** (39 mg, 91% yield) as a yellow powder: mp 212–214°C; ^{1}H NMR (CH_3OD, 500 MHz): δ 1.29–1.37 (2H, m), 1.77–1.84 (3H, m), 1.98–1.99 (1H, m), 2.27–2.30 (1H, m), 2.70–2.81 (2H, m), 2.89–2.94 (5H, m), 3.13 (2H, d, *J* = 6.0 Hz), 3.31–3.36 (2H, m), 4.76 (2H, d, *J* = 3.0 Hz), 7.19 (2H, br s), 7.54 (1H, s); ^{13}C NMR (CH_3OD, 125 MHz): δ 20.8, 25.3, 27.1, 27.6, 35.2, 41.0, 44.8, 45.0, 46.8, 110.4, 111.4, 113.5, 121.4, 124.7, 130.5, 137.1, 137.3, 171.0, 178.5; LRMS (ES$^+$) *m/z*: 448.0 [M+H]$^+$; HRMS (ES$^+$) calcd. for $C_{21}H_{27}N_3O_3Br$ [M+H]$^+$ 448.1236, found 448.1222; $[\alpha]_{589}^{25}$ +4.6 (c 0.51, MeOH).



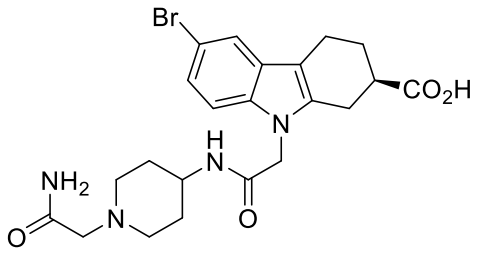
(*R*)-9-(2-((2-amino-2-oxoethyl)amino)-2-oxoethyl)-6-bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5g). The compound was prepared according to the method described for **5a** from **3a** (122 mg, 0.32 mmol), glycinamide hydrochloride (60 mg, 0.55 mmol), HATU (166 mg, 0.44 mmol) and DIPEA (230 µL, 1.32 mmol) in dry DMF (1 mL). Ester hydrolysis in an aqueous solution of NaOH (2 M; 300 µL) and absolute ethanol (1 mL) afforded **5g** (8.5 mg, 6.5% yield) as an off-white powder: mp 232–234°C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.74–1.76 (1H, m), 2.17–2.19 (1H, m), 2.61–2.63 (1H, m), 2.73–2.95 (4H, m), 3.66 (2H, d, *J* = 5.5 Hz, CH_2), 4.80 (2H, s, CH_2), 7.05 (1H, s), 7.15 (1H, d, *J* = 8.5 Hz), 7.31–7.35 (2H, m), 7.54 (1H, s), 8.26 (1H, br s), 12.40 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.6, 23.9, 25.7, 39.8, 41.8, 45.3, 107.9, 111.2, 119.7, 122.7, 128.4, 135.6, 136.6, 167.7, 170.4, 175.9; LRMS (ES$^-$) *m/z*: 408.0 [M-H]$^-$; HRMS (ES$^-$) calcd. for $C_{17}H_{17}N_3O_4Br$ [M-H]$^-$ 406.0402, found 406.0404; $[\alpha]_{589}^{25}$ –9.6 (c 0.19, DMSO).

(*R*)-6-bromo-9-(2-((4-carbamoylbenzyl)amino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5h). The compound was prepared according to the method described for **5a** from **3a** (155 mg, 0.41 mmol), 4-(aminomethyl)benzamide (74 mg, 0.49 mmol), HATU (227 mg, 0.60 mmol) and DIPEA (250 µL, 1.44 mmol) in dry DMF (1 mL). Following ester deprotection in an aqueous solution of NaOH (2 M; 300 µL) and absolute ethanol (1 mL), the mixture was acidified and the resulting precipitate collected by vacuum filtration. The precipitate was basified with 1 M NaOH, dissolved in water and filtered through a 1 cm plug

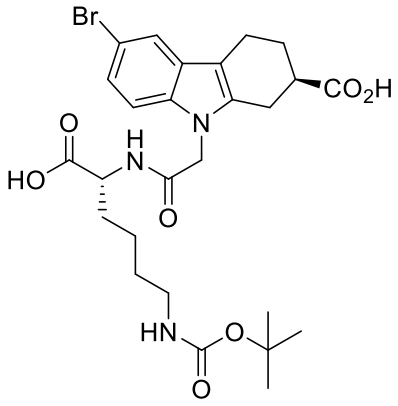


of reverse phase silica, washing with water. The aqueous solution was acidified and the precipitate collected by vacuum filtration, washing with ethanol and petrol to give **5h** (7.0 mg, 4% yield) as an off-white powder: mp > 230°C (dec); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.75–1.77 (1H, m), 2.18–2.20 (1H, m), 2.62–2.63 (1H, m), 2.73–2.97 (4H, m), 4.34 (2H, s), 4.83 (2H, s), 7.17 (1H, br s), 7.28–7.38 (4H, m), 7.55 (1H, s), 7.82 (2H, d, *J* = 7.5 Hz), 7.93 (1H, br s), 8.78 (1H, br s), 12.44 (1H, br s); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 19.7, 24.1, 25.7, 40.2, 42.0, 45.6, 108.1, 111.2, 111.4, 119.8, 122.8, 126.9, 127.6, 128.5, 132.9, 135.7, 136.6, 142.5, 167.7, 176.1; LRMS (ES$^-$) *m/z*: 482.0 [M-H]$^-$; HRMS (ES$^-$) calcd. for $C_{23}H_{21}N_3O_4Br$ [M-H]$^-$ 482.0715, found 482.0717; $[\alpha]_{589}^{25}$ +7.4 (c 0.48, DMSO).

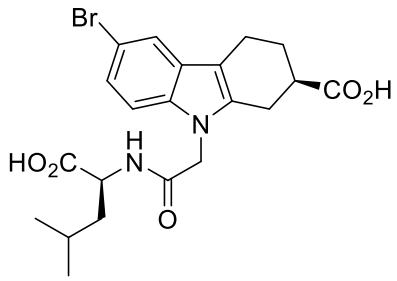
(*R*)-9-(2-((1-(2-amino-2-oxoethyl)piperidin-4-yl)amino)-2-oxoethyl)-6-bromo-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid potassium salt (5i). The compound was prepared according to the method described for **5a** from **3a** (83 mg, 0.22 mmol), 2-(4-amino-1-piperidinyl) acetatmide dihydrochloride (70 mg, 0.25 mmol), HATU (111 mg, 0.29 mmol) and DIPEA (200 µL, 1.15 mmol) in dry DMF (1 mL). Following deprotection in an aqueous solution of KOH (1 M, 38 µL, 0.038 mmol) in THF:EtOH:H_2O (1:1:1; 1 mL) the mixture was concentrated, washed with CH_2Cl_2 (2 x 2 mL) and the aqueous layer lyophilized. The resulting residue was triturated with ether, redissolved in methanol, filtered and concentrated to give the potassium salt of **5i** (13 mg, 64% yield) as an off-white gum: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.47–1.52 (2H, m), 1.68–1.71 (3H, m), 2.06–2.11 (3H, m), 2.27–2.29 (1H, m), 2.48–2.50 (1H, m), 2.65–2.73 (5H, m), 2.81 (2H, s), 3.49–3.51 (1H, m), 4.67 (2H, d, *J* = 3.5 Hz), 7.09–7.11 (2H, m), 7.16 (1H, br s), 7.25 (1H, d, *J* = 8.5 Hz), 7.46 (1H, d, *J* = 2.0 Hz), 8.28 (1H, br d, *J* = 7.0 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 20.4, 25.6, 27.2, 31.2, 43.0, 45.5, 45.8, 52.1, 61.4, 108.1, 110.9, 119.4, 122.0, 128.8, 135.0, 139.2, 166.7, 171.9, 177.4; LRMS (ES$^+$) *m/z*: 491.0 [M-K+H]$^+$; HRMS (ES$^+$) calcd. for $C_{22}H_{28}N_4O_4Br$ [M-K+H]$^+$ 491.1294, found 491.1310; $[\alpha]_{589}^{25}$ –4.3 (c 1.28, MeOH).



(*R*)-6-bromo-9-(2-(((R)-5-((tert-butoxycarbonyl)amino)-1-carboxypentyl)amino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5j). The compound was prepared according to the method described for **5a** with the following modification. Compound **3a** (96 mg, 0.25 mmol) was pre-activated with HATU (136 mg, 0.36 mmol) and DIPEA (160 µL, 0.92 mmol) in dry CH_2Cl_2 (1 mL) containing a few drops of DMF, before a solution of NH_2-(D)-Lys-(BOC)-O^{t}Bu.HCl (107 mg, 0.32 mmol) in dry CH_2Cl_2 (1 mL) was added. Ester deprotection with LiOH.H_2O (27 mg, 0.63 mmol) in THF:EtOH:H_2O (1:1:1; 1 mL) yielded **5j** (98 mg, 57% yield) as a yellow solid: mp 78–80°C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.28 (4H, br s), 1.38 (9H, s), 1.59–1.61 (1H, m), 1.71–1.72 (2H, m), 2.17–2.18 (1H, m), 2.61–2.96 (7H, m), 4.14 (1H, br s), 4.74–4.80 (2H, m), 6.75 (1H, br s), 7.16 (1H, d, *J* = 8.5 Hz), 7.31 (1H, t), 7.54 (1H, s), 8.48 (1H, t), 12.54 (1H, br s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 19.7, 22.7, 24.0, 25.7, 28.3, 29.1, 30.8, 39.4, 39.8, 45.2, 52.1, 77.4, 107.9, 111.2, 111.3, 119.7, 122.7, 128.4, 135.6, 136.6, 155.6, 167.5, 173.4, 176.0; LRMS (ES$^-$) *m/z*: 580.0 [M-H]$^-$; HRMS (ES$^-$) calcd. for $C_{26}H_{33}N_3O_7Br$ [M-H]$^-$ 578.1502, found 578.1504; $[\alpha]_{589}^{25}$ +2.4 (c 2.84, MeOH).

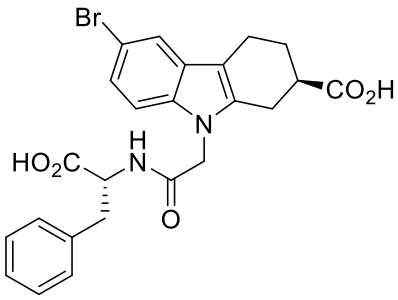


(*R*)-6-bromo-9-(2-(((S)-1-carboxy-3-methylbutyl)amino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5k). The compound was prepared according to the method described for **5a** from **3a**



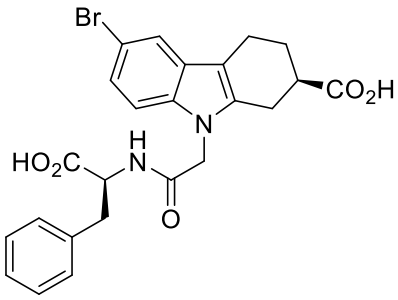
(249 mg, 0.654 mmol), NH_2-(L)-Leu-(O^{t}Bu).HCl (194 mg, 0.87 mmol), HATU (339 mg, 0.891 mmol) and DIPEA (450 µL, 2.58 mmol) in dry DMF (5 mL). Following ethyl ester hydrolysis in an aqueous solution of NaOH (2 M; 500 µL) in EtOH:H_2O (1:1; 3 mL), the resulting residue was treated with TFA (1 mL) and the mixture stirred for 1 h. Removal of the TFA under a stream of N_2 and trituration of the residue with toluene gave **5k** (39 mg, 30% yield); ^{1}H NMR (CD_3OD, 500 MHz): δ 0.82–0.90 (6H, m), 1.25 (1H, br s), 1.52–1.59 (2H, m), 1.86–1.93 (1H, m), 2.25–2.28 (1H, m), 2.63–2.69 (1H, m), 2.74–2.78 (1H, m) 2.86–2.93 (3H, m), 4.23–4.37 (1H, m), 4.76 (2H, s), 7.13 (1H, d, *J* = 9.0 Hz), 7.16 (1H, d, *J* = 8.5 Hz), 7.48 (1H, s); ^{13}C NMR (CD_3OD, 125 MHz): δ 21.0, 23.5, 25.4, 26.1, 27.1, 41.5, 46.6, 49.2, 52.1, 110.3, 111.7, 113.5, 121.5, 124.5, 130.5, 137.2, 137.4, 170.8, 175.6, 178.6; LRMS (ES^-) *m/z*: 465.0 $[M-H]^-$; HRMS (ES^-) calcd. for $C_{22}H_{20}N_6OBr$ $[M–H]^-$ 463.0882, found 463.0879; $[\alpha]_{589}^{25}$+5.22 (c 0.46, MeOH).

(*R*)-6-bromo-9-(2-(((*R*)-1-carboxy-2-phenylethyl)amino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5l).



The compound was prepared according to the method described for **5a** from **3a** (134 mg, 0.35 mmol), NH_2-(D)-Phe-(OMe).HCl (92 mg, 0.43 mmol), HATU (173 mg, 0.46 mmol) and DIPEA (230 µL, 1.32 mmol) in dry DMF (1 mL). Global ester deprotection in an aqueous solution of NaOH (2 M; 300 µL) and absolute ethanol (2 mL) afforded **5l** (69 mg, 39% yield) as an off-white powder: mp 194-196 °C; ^{1}H NMR (DMSO-*d*$_6$, 500 MHz): δ 1.72-1.73 (1H, m), 2.15 (1H, m), 2.58-2.86 (5H, m), 2.86-2.90 (1H, m), 3.06-3.09 (1H, m), 4.41 (1H, br s), 4.65-4.77 (2H, m), 7.11-7.25 (7H, m), 7.52 (1H, s), 8.36 (1H, br s), 12.70 (1H, br s); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ 19.6, 23.8, 25.6, 36.7, 45.1, 53.4, 53.5, 107.9, 111.2, 111.3, 1119.7, 122.7.7, 126.5, 128.2, 128.4, 129.1, 135.5, 136.4, 137.3, 167.3, 172.6, 175.9; LRMS (ES^+) *m/z*: 522.9 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{24}H_{23}N_2O_5BrNa$ $[M+Na]^+$ 521.0688, found 521.0686; $[\alpha]_{589}^{25}$ -22.9 (c 0.52, MeOH).

(*R*)-6-bromo-9-(2-(((*S*)-1-carboxy-2-phenylethyl)amino)-2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-2-carboxylic acid (5m). The compound was prepared according to the method described for **5a** from **3a**



(129 mg, 0.34 mmol), NH_2-(L)-Phe-(OMe).HCl (84 mg, 0.39 mmol), HATU (163 mg, 0.43 mmol) and DIPEA (200 µL, 1.15 mmol) in dry DMF (1 mL). Global ester deprotection in an aqueous solution of NaOH (2 M; 300 µL) and H_2O:EtOH (1:1; 2 mL) gave **5m** (98 mg, 57% yield) as a yellow solid: mp 208–210°C; ^{1}H NMR (DMSO-*d*$_6$, 500 MHz): δ 1.73–1.75 (1H, m), 2.16 (1H, m), 2.59–2.60 (1H, m), 2.70–2.76 (3H, m), 2.87–2.93 (2H, m), 3.08–3.11 (1H, m), 4.47–4.84 (1H, m, CH), 4.66–4.78 (2H, m), 7.12–7.29 (7H, m), 7.53 (1H, s), 8.44–8.46 (1H, m), 12.63 (1H, br s); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ 19.6, 23.8, 25.7, 36.7, 39.4, 45.1, 53.3, 107.9, 111.2, 111.3, 119.7, 122.7, 126.5, 128.2, 128.4, 129.1, 135.5, 136.5, 137.3, 167.3, 172.6, 175.9; LRMS (ES^+) *m/z*: 521.0 $[M+Na]^+$; HRMS (ES^+) calcd. for $C_{24}H_{23}N_2O_5BrNa$ $[M+Na]^+$ 521.0688, found 521.0710; $[\alpha]_{589}^{25}$ +17.8 (c 0.48, MeOH).

Supplementary References

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