Discovery of Lead Compounds Targeting the Bacterial Sliding Clamp using a Fragment-Based Approach

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SUPPORTING INFORMATION

Supplementary Data

Table S1. Data collection and refinement statistics for X-ray co-crystal structures of the *E. coli* SC in complex with fragment hits **1**–**4** (i.e. complexes SC^1 – SC^4, respectively).

Name	**SC^1**	**SC^2**	**SC^3**	**SC^4**
PDB Code	4N94	4N95	4N96	4N97
Data collection				
Space group	P1	P1	P1	P1
Cell dimensions	a, b, c (Å) / α, β, γ (°) 40.74, 64.51, 71.72 / 74.01, 83.21, 84.54	a, b, c (Å) / α, β, γ (°) 40.99, 65.51, 73.44/ 73.03, 85.29, 85.65	a, b, c (Å) / α, β, γ (°) 40.88, 64.67, 72.02 / 73.97, 83.55, 84.36	a, b, c (Å) / α, β, γ (°) 40.88, 64.78, 72.59 / 73.91, 84.51, 84.89
Resolution (Å)	30.38–1.73(1.82–1.73)	28.86–1.80 (1.90–1.80)	30.51–1.70 (1.79–1.70)	28.9–1.97 (2.08–1.97)
R_{merge} (%)	6.7 (55.1)	6.4 (39.9)	4.8 (32.8)	5.6 (19.1)
No. of Reflections	242483	235693	242187	153662
Unique	66608 (8933)	61187 (8833)	64167 (9074)	42650 (6264)
Mean I/σ(I)	9.9 (2.1)	14.3 (3.2)	15.2 (3.4)	12.7 (6.4)
Completeness (%)	91.7 (84.2)	95.9 (94.7)	83.0 (80.4)	84.8 (85.2)
Multiplicity	3.6 (3.6)	3.9 (3.8)	3.8 (3.7)	3.6 (3.3)
Refinement				
Resolution (Å)	28.91–1.73 (1.78–1.73)	28.32–1.80 (1.85–1.80)	28.72–1.70 (1.74–1.70)	28.9–1.97 (2.02–1.97)
R_{work}/R_{free} (%)	25.1 (35.5)/29.2 (44)	18.6 (26.4)/22.4 (30.3)	22.7 (33.2)/27.5 (42.0)	23.2 (30.9)/30.6 (42.7)
R.m.s deviations				
Bond lengths (Å)	0.0098	0.0068	0.0083	0.0077
Bond angles (º)	1.4687	1.2819	1.3235	1.3011
B-factors				
M. Chain	20.6	18.5	19.3	20.25
S. Chain & Water	24.2	23.3	22.6	22.89
Ligands*	41.5	49.7	26.6	35.1
Ramachandran Plot Outliers	0.57%	0.44 %	0.58 %	0.58 %

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

Diffraction data were collected at Beamline MX1, Australian Synchrotron, at a wavelength of 0.95 Å. Diffraction data were processed with MOSFLM and SCALA.

*Ligands refer to the fragment compounds bound to Chain A.

Table S2. Data collection and refinement statistics for X-ray co-crystal structures of the *E. coli* SC in complex with compounds **5**, **7** and (*R*)-**8** (i.e. complexes SC^5 – SC^7 and SC^8, respectively).

Name	SC^5	SC^7	SC^8
PDB Code	4N98	4N99	4N9A
Data collection			
Space group	$P2_1$	P1	$P2_1$
Cell dimensions	a, b, c (Å) / α, β, γ (°) 79.90, 67.16, 81.06 / 90.00, 114.11, 90.00	a, b, c (Å) / α, β, γ (°) 41.01, 65.13, 73.24 / 72.95, 85.06, 85.24	a, b, c (Å) / α, β, γ (°) 79.78, 67.37, 81.12 / 90.00, 113.92, 90.00
Resolution (Å)	30.50–1.70 (1.79–1.70)	40.95–2.30 (2.42–2.30)	43.86–1.90 (2.00–1.90)
R_{merge} (%)	9.7 (68.3)	14.7 (64.3)	5.2(39.7)
No. of Reflections	603665	112766	137425
Unique Reflections	86092 (12490)	29744 (4227)	57937 (7647)
Mean I/σ(I)	9.2 (2.4)	8.5 (2.2)	9.8 (2.4)
Completeness (%)	99.9 (99.8)	92.9 (89.9)	93.7 (85.6)
Multiplicity	7.0 (6.9)	3.8 (3.7)	2.4 (2.3)
Refinement			
Resolution (Å)	30.17–1.70 (1.74–1.70)	40.95–2.30 (2.36–2.30)	36.46–1.90 (1.95–1.90)
R_{work}/R_{free} (%)	25.6 (30.0)/29.6 (35.5)	26.1 (32.1)/33.7 (42.9)	21.4 (38.8)/26.1(40.7)
R.m.s. deviations			
Bond lengths (Å)	0.0079	0.0042	0.0090
Bond angles (°)	1.2817	0.8770	1.3791
B-factors			
M. Chain	22.3	21.0	28.1
S. Chain & Water	26.4	23.1	33.2
Ligands*	39.8	51.7	42.1
Ramachandran Plot Outliers	0.58%	0.56 %	0.72 %

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

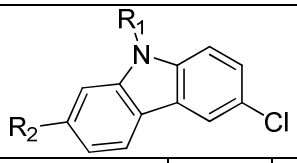
Diffraction data for SC^5 were collected at Beamline MX1, Australian Synchrotron, at a wavelength of 0.95 Å.

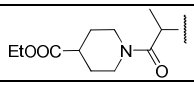
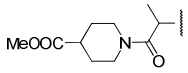
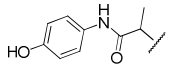
Diffraction data for SC^7 and SC^8 were collected using an in-house X-ray generator (University of Wollongong) at a wavelength of 1.5418 Å.

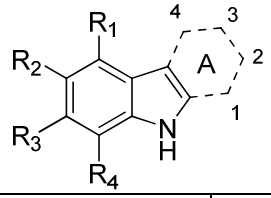
Diffraction data were processed with MOSFLM and SCALA.

*Ligands refer to the compounds bound to Chain A.

Table S3: Binding data for carbazole and tetrahydrocarbazole derivatives against the *E. coli* SC.



cpd	R_1	R_2	IC_{50} (μM)	K_i (μM)	LE (kcal/ mol)	LogD (pH 7.2)	LLE_{AT}
6a	H		> 1000	n/a	n/a	n/a	n/a
6b	H		885	492	0.16	4.41	0.06
6c	H		> 1000	n/a	n/a	n/a	n/a



cpd	R_1	R_2	R_3	R_4	ring A	IC_{50} (μM)	K_i (μM)	LE (kcal/ mol)	LogD (pH 7.2)	LLE_{AT}
11	H	H	H	Cl	3-CO_2Me cyclohexyl	851	473	0.25	3.91	0.07
12	H	Cl	CO_2H	H	cycloheptyl	877	487	0.25	–0.16	0.38
13	H	CO_2H	Cl	H	cyclohexyl	> 1000	n/a	n/a	n/a	n/a
14	Cl	CO_2H	H	H	cyclohexyl	> 1000	n/a	n/a	n/a	n/a
15	H	Cl	H	H	3-CO_2Et cyclohexyl	> 1000	n/a	n/a	n/a	n/a
16	H	Cl	H	H	3-CO_2H cyclohexyl	> 1000	n/a	n/a	n/a	n/a
17	H	Cl	H	H	3-CO_2Me cyclohexyl	> 1000	n/a	n/a	n/a	n/a
18	H	Cl	H	H	cyclopentyl	> 1000	n/a	n/a	n/a	n/a
19	H	Cl	H	H	3-piperidinyl	> 1000	n/a	n/a	n/a	n/a
20	H	Cl	H	H	cycloheptyl	> 1000	n/a	n/a	n/a	n/a
21	Cl	H	H	H	1-CO_2Me cyclohexyl	> 1000	n/a	n/a	n/a	n/a
22	H	H	H	Cl	cyclohexyl	> 1000	n/a	n/a	n/a	n/a
23	H	H	H	Cl	3-CO_2Et cyclohexyl	> 1000	n/a	n/a	n/a	n/a
24	H	H	H	Cl	3-CO_2H cyclohexyl	> 1000	n/a	n/a	n/a	n/a
25	H	Cl	OH	H	cyclohexyl	> 1000	n/a	n/a	n/a	n/a
26	CO_2H	Cl	H	H	2-CO_2H cyclohexyl	> 1000	n/a	n/a	n/a	n/a
27	CO_2H	Cl	H	H	cyclopentyl	> 1000	n/a	n/a	n/a	n/a
28	CO_2H	Cl	H	H	cycloheptly	> 1000	n/a	n/a	n/a	n/a
29	H	Cl	CO_2Me	H	cyclohexyl	> 1000	n/a	n/a	n/a	n/a

n/a: not applicable

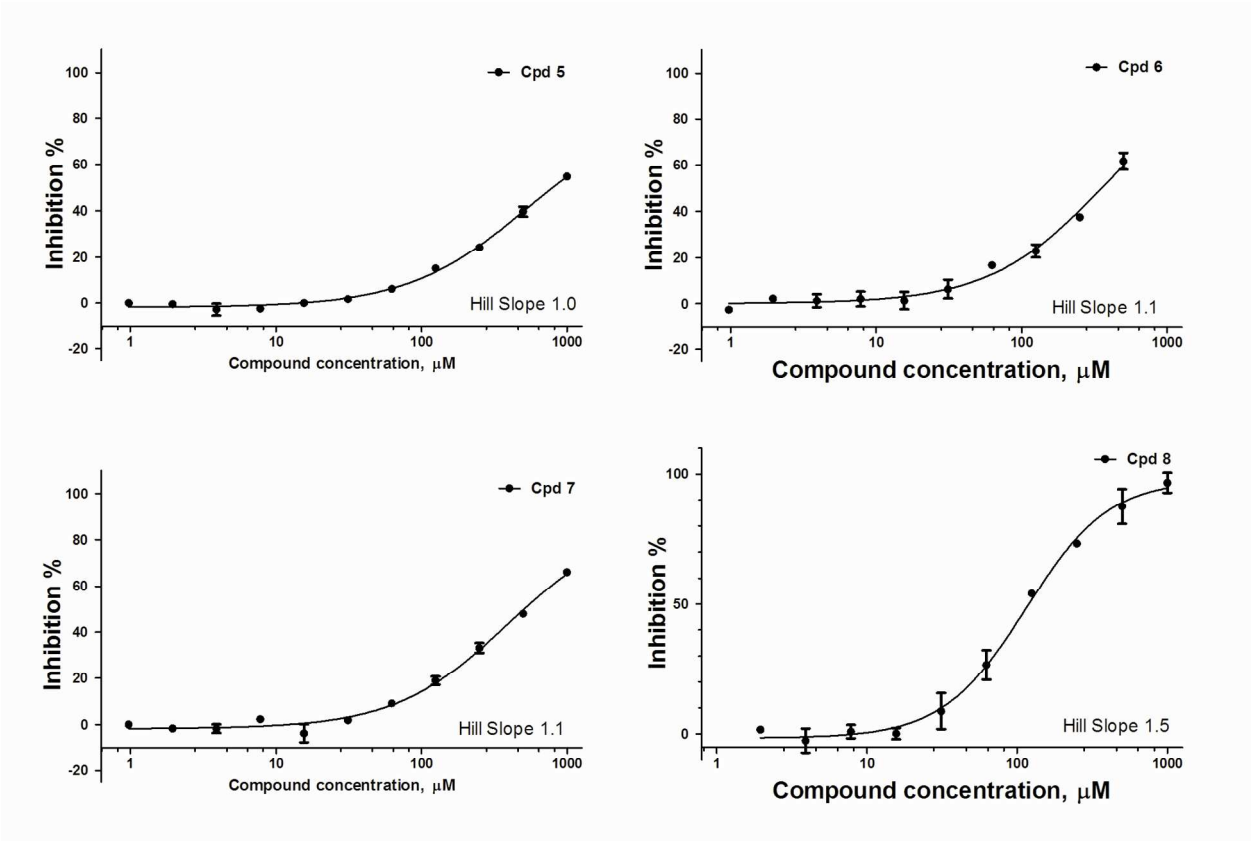
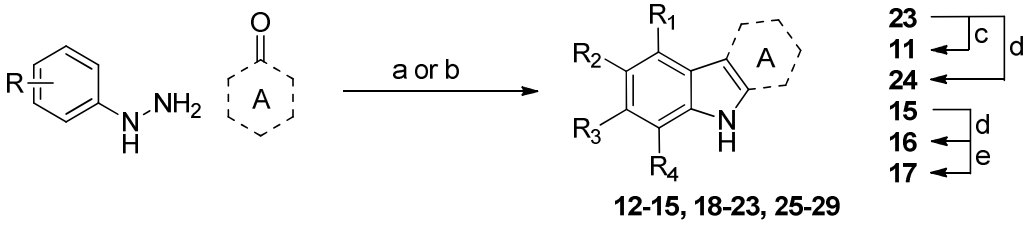


Figure S1. Inhibition of binding of a fluorescently labelled tracer peptide (5FAM-QLDLF) to the *E. coli* SC. Data are standardized and shown as inhibition (%, decreased polarization divided by background subtracted total polarization). Error bars represent standard deviation (n=2).



Scheme S1. Synthesis of substituted tetrahydrocarbazoles **11**–**29**. Reagents: (a) AcOH or EtOH and HCl (cat)), ketone, reflux, 3–64%; (b) i) AcOH, ketone, reflux, ii) MeOH, H_2SO_4 (cat.) reflux, 18%; (c) i) NaOH, EtOH, rt., ii) MeOH, H_2SO_4 (cat.) reflux, 44%; (d) NaOH, EtOH, rt, 31–47%; (e) MeOH, H_2SO_4 (cat.) reflux, 65%.

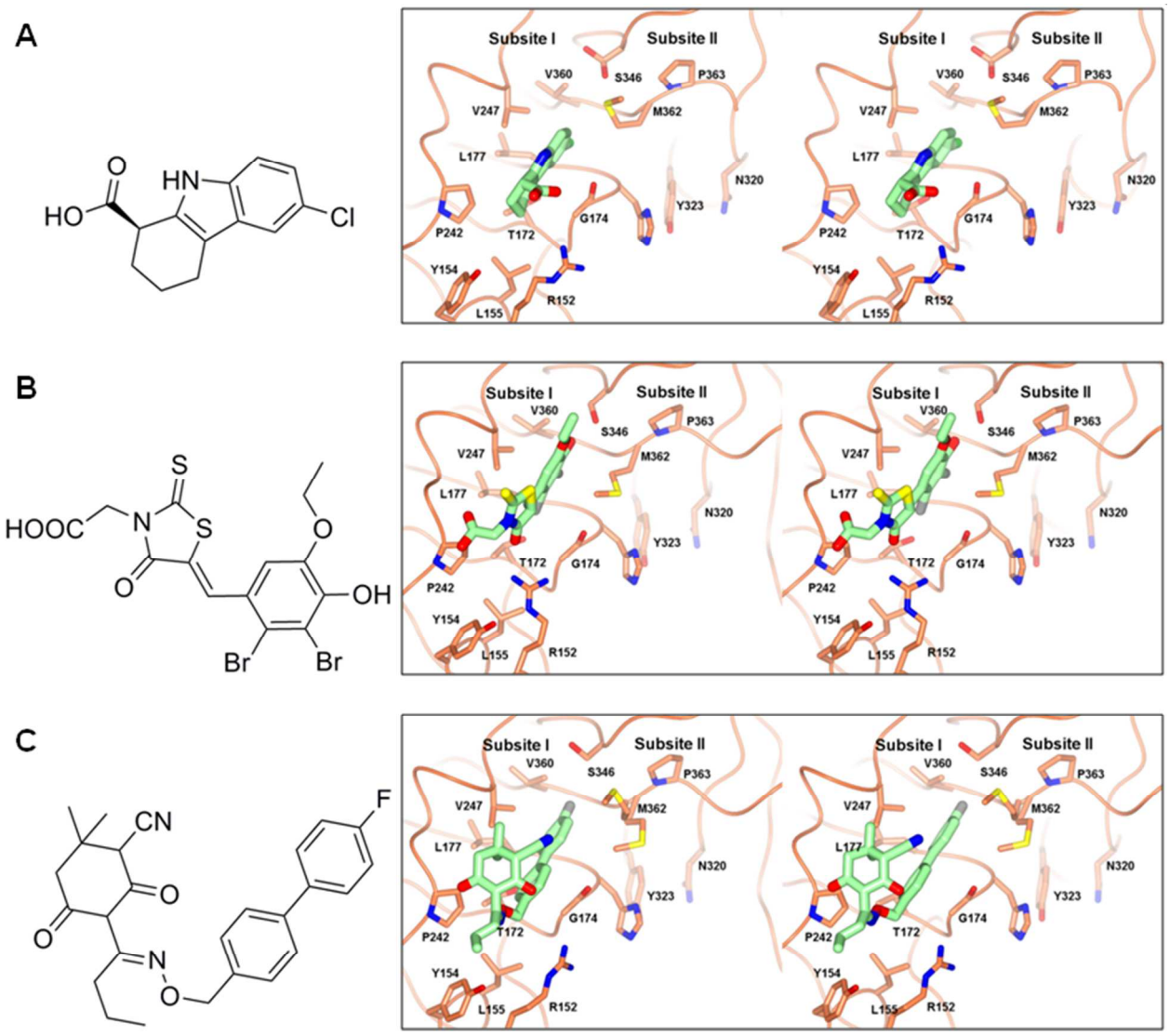


Figure S2. Stereo diagrams of (**A**) (*R*)-**8**, (**B**) RU7 reported by Georgescu *et al.*[1] and (**C**) a biphenyloxime ether derivative reported by Wijffels *et al.*[2] binding to subsite I of the *E. coli* SC LM-binding pocket. Carbon atoms of the inhibitors are colored light green and the SC carbon atoms are colored orange. Other atoms are in CPK colors.

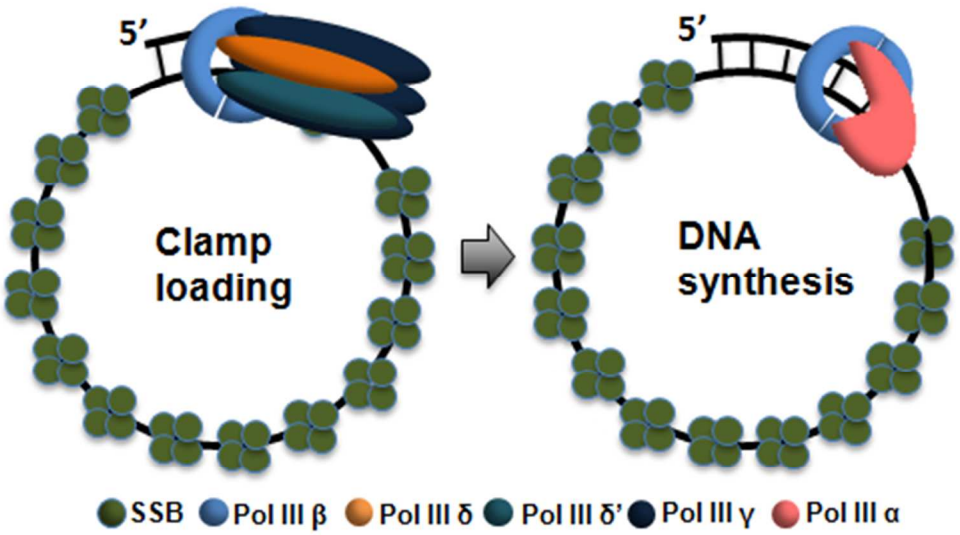


Figure S3. Schematic representation of the *in vitro* DNA replication assay.

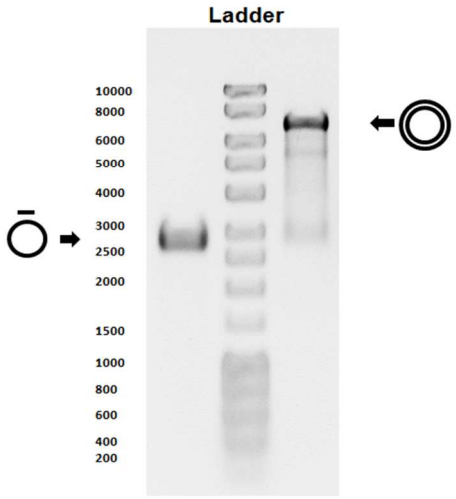


Figure S4. *In vitro* DNA replication control assay carried out in the presence of 3.5% DMSO (no inhibitor). Molecular sizes (in bp for dsDNA) corresponding to bands in the DNA ladder are shown. Circles with a dash represent primed ssDNA template. Concentric circles represent dsDNA replication products.

Table S4: Optical density (absorbance at 595 nM) measured after 24h of bacterial growth with varying compound concentrations.*

E. coli												
Compd (μM)	**5000**	**2500**	**1250**	**625**	**312.5**	**156.25**	**78.13**	**39.06**	**19.53**	**9.77**	**4.88**	**2.44**
5	-0.11	0.05	0.12	0.15	0.20	0.13	0.20	0.17	0.13	0.15	0.14	0.14
7	0.01	0.07	0.20	0.17	0.18	0.16	0.14	0.11	0.14	0.14	0.12	0.17
8	-0.57	-0.29	-0.08	-0.04	-0.01	0.16	0.15	0.24	0.12	0.14	0.15	0.18
9	-0.02	-0.03	-0.08	-0.06	-0.04	-0.02	0.16	0.15	0.15	0.13	0.12	0.17
10	-0.35	-0.15	-0.14	-0.11	-0.09	0.02	0.03	0.13	0.12	0.15	0.15	0.17
A. baylyi												
Compd (μM)	**5000**	**2500**	**1250**	**625**	**312.5**	**156.25**	**78.13**	**39.06**	**19.53**	**9.77**	**4.88**	**2.44**
5	-0.20	0.31	0.42	0.44	0.49	0.39	0.39	0.38	0.46	0.45	0.46	0.39
7	0.01	0.19	0.37	0.46	0.52	0.48	0.53	0.46	0.38	0.47	0.45	0.49
8	-0.57	-0.28	-0.10	-0.03	0.07	0.34	0.39	0.39	0.38	0.39	0.48	0.45
9	-0.07	-0.04	-0.01	0.07	0.03	0.04	0.04	0.39	0.40	0.41	0.41	0.45
10	-0.28	-0.09	-0.09	0.01	0.01	0.03	0.37	0.39	0.39	0.43	0.39	0.45
B. subtilis												
Compd (μM)	**5000**	**2500**	**1250**	**625**	**312.5**	**156.25**	**78.13**	**39.06**	**19.53**	**9.77**	**4.88**	**2.44**
5	-0.08	0.01	0.15	0.21	0.21	0.23	0.18	0.18	0.16	0.15	0.19	0.27
7	0.00	0.08	0.11	0.13	0.16	0.20	0.23	0.24	0.24	0.24	0.22	0.21
8	-0.33	-0.17	-0.04	-0.02	0.00	0.00	0.13	0.19	0.26	0.23	0.23	0.23
9	0.01	-0.01	-0.05	-0.05	-0.03	-0.01	0.08	0.20	0.22	0.22	0.20	0.23
10	-0.33	-0.10	-0.03	-0.04	-0.06	-0.04	0.07	0.15	0.18	0.19	0.22	0.21
S. aureus												
Compd (μM)	**5000**	**2500**	**1250**	**625**	**312.5**	**156.25**	**78.13**	**39.06**	**19.53**	**9.77**	**4.88**	**2.44**
5	-0.09	0.13	0.20	0.17	0.18	0.19	0.19	0.22	0.21	0.17	0.22	0.23
7	0.00	0.07	0.12	0.16	0.19	0.15	0.18	0.17	0.18	0.14	0.17	0.21
8	-0.35	-0.17	-0.01	-0.02	0.00	0.00	0.10	0.15	0.17	0.22	0.18	0.21
9	0.00	-0.01	-0.05	-0.05	-0.03	0.05	0.18	0.21	0.27	0.23	0.21	0.23
10	-0.32	-0.09	-0.02	-0.04	-0.06	-0.04	0.04	0.19	0.17	0.22	0.23	0.22

*Optical density values are subtracted from the background level.

Supplementary Methods

Molecular Docking and Chemo-Informatics. Fragment scaffolds were used for similarity and/ or substructure searches in the UCSF ZINC library.[3] A number of larger scaffolds were used for substructure search of commercially available compounds for molecular docking using UCSF DOCK 6.5.[4] The receptor was prepared by removing all crystallographic water molecules from the sidling clamp except for a structurally conserved water molecule near residue M362. Residues greater than 15 Å away from the binding pocket were deleted. Hydrogen atoms were added and partial charges were assigned. Ligands were prepared using UCSF Chimera or downloaded from UCSF ZINC library. Spheres for ligand orientations were generated using the identified fragment binders. A grid map for scoring was generated encompassing residues within 15 Å of the spheres. Docking was carried out using Grid score as the primary score and GB/SA Hawkins score as a secondary scoring method. The top 50 poses were clustered and the top 10 cluster heads assessed using the secondary scoring method. Additionally, Amber scoring[5] was performed with 100 steps of minimization, 3000 md cycles and another 100 minimization steps, each step corresponded to 2 fs. In all cases ligand flexibility was allowed and the receptor kept rigid. Selected molecules were acquired for testing.

Calculation of *Log*D values at pH 7.2 was performed using Accord for Excel 6.2 (Accelrys).

The equations used to determine LE and LLE_{AT} followed the published methods[6] and are detailed below:

$$LE = -\Delta G/HAC$$

$$= -RTln(K_i)/HAC$$

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

$$= RTln(K_i) + RTLogD$$

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

LE: ligand efficiency; ΔG: delta Gibbs energy; K_i: inhibition constant; HAC: heavy atom count; *log*D: distribution coefficient at pH 7.2; LLE_{AT}: ligand lipophilicity efficiency.

Organic Synthesis. ^1H 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 given in Hz. Electrospray (EI) LRMS were recorded on a Waters Micromass Platform LCZ spectrometer and HRMS were recorded on a Waters Xevo spectrometer, using an EI or ASAP source. Melting points were recorded using a Gallenkamp (Griffin) melting point apparatus and are uncorrected. Optical rotations for chiral compounds were measured on a Jasco P-2000 polarimeter. TLC was performed on precoated Merck silica gel 60 PF_{254} aluminium sheets and flash column chromatography (FCC) performed with Davisil silica gel (40–63 μm). Petrol refers to petroleum spirits of bp 40–60°C. 2-chloro-4-hydrazinobenzoic acid hydrochloride (**31**) and 2-chloro-5-hydrazinobenzoic acid hydrochloride (**32**) were prepared as previoulsy reported by Green *et al.*[7] (see general hydrazine formation procedure) from 2-chloro-4-aminobenzoic acid and 2-chloro-5-amino-benzoic acid respectively. All final compounds have a purity of ≥ 95% by proton NMR.

General Fischer Indole Procedure. To a solution of the phenylhydrazine in glacial acetic acid (or absolute ethanol with HCl (cat.)) was added the ketone, and the resulting mixture refluxed for 2–17 h. The reaction mixture was cooled, concentrated and extracted with EtOAc (or CH_2Cl_2). The combined extracts were washed with water, brine, dried ($MgSO_4$), concentrated and the residue purified by either recrystallisation or FCC.

General Esterification Procedure. To a solution of the carboxylic acid in methanol (or ethanol), concentrated H_2SO_4 (cat.) was added and the solution refluxed for 2–23 h. The mixture was cooled, concentrated, neutralised with saturated $NaHCO_3$ (aq) solution and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried ($MgSO_4$), concentrated and the residue purified by recrystallisation or trituration.

General Deesterification Procedure. To a solution of the ester in absolute ethanol, sodium hydroxide (2 M) was added and the reaction mixture stirred at room temperature for 2–17 h. The reaction mixture was concentrated, diluted with water and washed with CH_2Cl_2. The aqueous layer was subsequently acidified with 1 M HCl and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried ($MgSO_4$) and concentrated. The residue was either used crude in subsequent reactions or purified by recrystallisation or trituration.

General Hydrazine Formation Procedure. A suspension of the amine in concentrated HCl was stirred for 30 min in an ice/salt bath and then a cooled solution of $NaNO_2$ in water added drop wise. The solution was stirred for 1 h and a cooled solution of $SnCl.2H_2O$ in concentrated HCl added drop wise. The suspension was allowed to warm to room temperature overnight and the resulting precipitate collected by vacuum filtration (washing with cold water then Et_2O).

Methyl 8-chloro-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylate (11)

General Fischer indole procedure with 2-chlorophenylhydrazine hydrochloride (310.3 mg, 1.73 mmol) and ethyl-4-oxocyclohexane-1-carboxylate (268.1 mg, 1.58 mmol) provided ethyl 8-chloro-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylate (**23;** 240.5 mg, 0.87 mmol, 55% yield) as a pale yellow crystalline solid following recrystallisation from methanol, mp 152 – 154 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): 1.29 (3H, t, *J* = 7.5 Hz), 2.00-2.07 (1H, m), 2.30-2.32 (1H, m), 2.76-2.92 (4H, m), 3.00 (1H, dd, *J* = 15.5 , 4.8 Hz), 4.20 (2H, q, *J* = 6.7 Hz), 7.00 (1H, t, *J* = 8.0 Hz), 7.11 (1H, d, *J* = 8.0 Hz), 7.35 (1H, d, *J* = 7.5 Hz), 7.98 (1H, s). ^{13}C NMR ($CDCl_3$, 125MHz): 14.4, 22.4, 24.1, 25.8, 40.4, 60.6, 110.0, 116.1, 116.5, 120.2, 120.9, 129.2, 133.4, 134.0, 175.3. LRMS (ES^+) *m/z*: 316.23 $[M+K]^+$. HRMS (ES^+) calcd. for $C_{15}H_{17}NO_2Cl$ $[M+H]^+$ 278.0948, found. 278.0948.

General deesterification procedure with **23** (109.9 mg, 0.40 mmol), followed by general esterification procedure on the crude product in methanol provided **11** (46.0 mg, 44% yield) as an off-white crystalline solid following recrystallisation from methanol, mp 164 - 166°C. ^{1}H NMR ($CDCl_3$, 500 MHz): 2.01-2.08 (1H, m), 2.30-2.33 (1H, m), 2.80-2.93 (4H, m), 3.06-3.07 (1H, m), 3.74 (3H, s), 7.00 (1H, t, *J* = 7.8 Hz), 7.12 (1H, d, *J* = 7.5 Hz), 7.35 (1H, d, *J* = 7.5 Hz), 7.84 (1H, br s). ^{13}C NMR ($CDCl_3$, 125 MHz): 22.4, 24.0, 25.7, 40.2, 52.0, 109.8, 116.1, 116.5, 120.3, 120.9, 129.1, 133.3, 134.0, 175.9. LRMS (ES^-) *m/z*: 261.75 $[M-H]^-$. HRMS (ES^-) calcd. for $C_{14}H_{13}NO_2Cl$ $[M-H]^-$ 262.0635, found 262.0627.

9-chloro-1,2,3,4,5,6-hexahydrocyclohepta[b]indole-8-carboxylic acid (12) and 9-chloro-1,2,3,4,5,6-hexahydrocyclohepta[b]indole-10-carboxylic acid (28)

General Fischer indole procedure with 2-chloro-5-hydrazinobenzoic acid hydrochloride (**32**; 487.1 mg, 2.18 mmol) and cycloheptanone (206.7 mg, 1.84 mmol) in glacial acetic acid (4 mL) produced a mixture of two isomers. The two products were obtained following FCC on silica gel (gradient elution; 30:70:0.5-70:30:0.5 Et_2O/petrol/acetic acid) and recrystallisation of each isomer from methanol/water. **12** (32.0 mg, 7% yield) was obtained as a beige powder, mp >250 °C (dec.). ^{1}H NMR (d_6-DMSO, 300 MHz): 1.65-1.70 (4H, m,), 1.84 (2H, m), 2.70-2.74 (2H, m), 2.82-2.86 (2H, m), 7.48 (1H, s,), 7.79 (1H, s), 11.21 (1H, s), 12.78 (1H, s). ^{13}C NMR (d_6-DMSO, 75 MHz): 24.1, 26.9, 28.4, 28.7, 31.4, 112.6, 114.3, 18.7, 120.4, 122.2, 131.6, 131.8, 143.9, 167.3. LRMS (ES^-) *m/z*: 262.0 $[M-H]^-$. HRMS ($ASAP^-$) calcd. for $C_{14}H_{13}NO_2Cl$ $[M-H]^-$262.0635, found 262.0626. **28** (12.3 mg, 3% yield) was obtained as a red/brown powder, mp 118 -120 °C. ^{1}H NMR (d_6-DMSO, 500 MHz): 1.61-1.68 (2H, m), 1.67-1.68 (2H, m), 1.79-1.80 (2H, m), 2.66 (2H, t like, *J* = 5.25 Hz), 2.80 (2H, *t* like, *J* = 5.5 Hz), 6.97 (1H, d, *J* = 8.5 Hz), 7.25 (1H, d, *J* = 8.5 Hz), 11.12 (1H, s), 13.25 (1H, br s). ^{13}C NMR (d_6-DMSO, 75 MHz): 24.2, 26.7, 28.1, 28.2, 31.2, 111.5, 112.4, 118.6, 119.6, 124.7, 124.8, 133.0, 141.6, 168.9. LRMS (ES^-) *m/z*: 262.0 $[M-H]^-$. HRMS (ES^-) calcd. for $C_{14}H_{13}NO_2Cl$ $[M-H]^-$ 262.0635, found 262.0642.

7-chloro-2,3,4,9-tetrahydrocarbazole-6-carboxylic acid (13) and 5-chloro-2,3,4,9-tetrahydrocarbazole-6-carboxylic acid (14)[8]

General Fischer indole procedure with 2-chloro-4-hydrazinobenzoic acid hydrochloride (**31**; 549.8 mg, 2.46 mmol) and cyclohexanone (300.6 mg, 3.06 mmol) in glacial acetic acid (3 mL) produced a mixture of two isomers. The two products were obtained following FCC on silica gel (gradient elution; 2:8–6:46 Et_2O/petrol) and recrystallisation of each isomer from methanol/water. **13** (16.9 mg, 3% yield) was obtained as a beige powder, mp 248 – 250 °C. ^{1}H NMR (MeOD, 500 MHz): 1.87-1.92 (4H, m), 2.68 (2H, d, *J* = 5 Hz), 2.72 (2H, d, *J*= 5.5 Hz), 7.31 (1H, s), 7.97 (1H, s). ^{13}C NMR ($CDCl_3$+d_6-DMSO, 125 MHz): 20.4, 22.6, 22.7, 22.9, 110.3, 112.4, 119.5, 122.0, 125.6, 125.8, 136.5, 137.6, 168.5 (CO_2H). LRMS (ES^+) *m/z*: 272.2 $[M+23]^+$. HRMS ($ASAP^+$) calcd. for $C_{13}H_{13}NO_2Cl$ $[M+H]^+$ 250.0635, found 250.0699. **14** (19.9mg, 3% yield) was obtained as a pink powder, mp 242 -244 °C. ^{1}H NMR ($CDCl_3$+d_6-DMSO, 500 MHz): 1.86 (4H, br s), 2.72 (2H, s), 3.11 (2H, s), 7.15 (1H, d, *J* = 8.5 Hz), 7.63 (1H, d, *J* = 8.5 Hz), 9.78 (1H, br s). ^{13}C NMR ($CDCl_3$+d_6-DMSO, 125 MHz): 22.3, 23.3, 23.4, 108.6, 111.1, 120.3, 123.8, 125.9, 136.6, 138.3, 168.7. LRMS (ES^-) *m/z*: 247.9 $[M-H]^-$. HRMS (ES^-) calcd for $C_{13}H_{11}NO_2Cl$ $[M-H]^-$ 248.0478, found. 248.0467.

Ethyl 6-chloro-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylate (15)[9]

General Fischer indole procedure with 4-chlorophenylhydrazine hydrochloride (395.1 mg, 2.21 mmol) and ethyl-4-oxocyclohexane-1-carboxylate (363.3 mg, 2.13 mmol) in absolute ethanol (5.5 mL) provided **15** (378.3 mg, 64% yield) as an off-white crystalline solid following recrystallisation from methanol, mp 134 -136 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): 1.29 (3H, t, *J* = 7Hz), 1.99-2.04 (1H, m), 2.28-2.30 (1H, m), 2.75-2.88 (4H, m), 3.00 (1H, dd, *J* = 15, 5.0 Hz), 4.20 (2H, q, *J* = 7.2 Hz), 7.05 (1H, d, *J* = 8.5 Hz), 7.15 (1H, d, *J* = 8.5 Hz), 7.41 (1H, s), 7.81 (1H, s). ^{13}C NMR ($CDCl_3$, 125MHz): 14.4, 22.4, 23.8, 25.7, 40.3, 60.7, 108.6, 111.5, 117.5,

121.5, 125.1, 128.7, 134.4, 134.8, 175.5. LRMS (ES^+) *m/z*: 316.15 $[M+K]^+$. HRMS (ES^+) calcd. for $C_{15}H_{17}NO_2Cl$ $[M+H]_+$ 278.0948, found 278.0951.

6-chloro-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (16)

General deesterification procedure with **15** (111.8 mg, 0.40 mmol) provided **16** (47.7 mg, 47% yield) as a beige powder following trituration with CH_2Cl_2, mp 178 -180 °C. ^1H NMR (MeOD, 500 MHz): 1.97-1.99 (1H, m), 2.26-2.29 (1H, m), 2.78-2.82 (4H, m,), 2.96 (1H, d, *J* = 11 Hz), 6.96 (1H, d, *J* = 8 Hz), 7.17 (1H, d, *J* = 8.5 Hz), 7.31 (1H, s), 10.21 (1H, br s). ^{13}C NMR (MeOD, 125 MHz): 23.1, 24.8, 27.0, 41.5, 108.3, 112.5, 117.6, 121.5, 125.2, 129.9, 136.2, 136.6, 179.4 (C). LRMS (ES^-) *m/z*: 247.99 $[M-H]^-$. HRMS (ES^-) calcd. for $C_{13}H_{11}NO_2Cl$ $[M-H]^-$ 248.0478, found 248.0474.

Methyl 6-chloro-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylate (17)

General esterification procedure with **16** (43.7 mg, 0.18 mmol) in methanol provided **17** (30.7 mg, 65% yield) as a pale yellow powder following trituration with petrol, mp 104 - 106 °C. ^1H NMR ($CDCl_3$, 300 MHz): 1.99-2.05 (1H, m), 2.27-2.31 (1H, m), 2.79-3.01 (5H, m), 3.74 (3H, s), 7.05 (1H, dd, *J* = 8.7, 1.5 Hz), 7.14 (1H, d, *J* = 8.4 Hz), 7.40 (1H, s), 7.84 (1H, br s). ^{13}C NMR ($CDCl_3$, 125 MHz): 22.4, 22.8, 25.6, 40.1, 52.0, 108.4, 111.5, 117.5, 121.5, 125.1, 128.7, 134.4, 134.8, 175.9 (C). LRMS (ES^-) *m/z*: 262.04 $[M-H]^-$. HRMS (ES^-) calcd. for $C_{14}H_{13}NO_2Cl$ $[M-H]^-$ 262.0635, found 262.0630.

7-chloro-1,2,3,4-tetrahydrocyclopenta[b]indole (18)[10]

General Fischer indole procedure with 4-chlorophenylhydrazine hydrochloride (429.9 mg, 2.40 mmol) and cyclopentanone (248.4 mg, 2.95 mmol) in glacial acetic acid (4 mL) provided **18** (46.9 mg, 10 % yield) as beige crystalline solid following FCC on silica (gradient elution; 30:70:0.5 - 50:50:0.5 Et_2O/petrol/acetic acid), mp 124 – 126 °C. ^1H NMR (d_6-DMSO, 300 MHz): 2.42-2.47 (2H, m), 2.68-2.73 (2H, m), 2.79-2.84 (2H, m), 6.95 (1H, dd, *J* = 8.4, 2.1 Hz), 7.26 (1H, d, *J* = 8.4 Hz), 7.32 (1H, d, *J* = 2.1 Hz), 11.05 (1H, s). ^{13}C NMR (d_6-DMSO, 125MHz): 23.8, 25.2, 28.1, 112.8, 117.0, 117.4, 119.2, 123.0, 125.1, 139.3, 146.3. LRMS (ES^+) *m/z*: 192.0 $[M+H]^+$. HRMS (ES^+) calcd. for $C_{11}H_{11}NCl$ $[M+H]^+$ 192.0580, found 192.0587.

6-chloro-2,3,4,9-tetrahydro-1H-pyrido[2,1-b]indole hydrochloride (19)[11]

General Fischer indole procedure with 4-piperidinone hydrate hydrochloride (200.8 mg, 1.31 mmol) and 4-chlorophenylhydrazine hydrochloride (244.9 mg, 1.37 mmol) in absolute ethanol (2 mL) provided **19** (43.1 mg, 14% yield) as an off white power following recrystallisation from methanol, mp 278 °C (dec.). ^1H NMR (MeOD, 300 MHz): 3.16-3.18 (2H, br t), 3.59-3.63 (2H,t), 4.40 (2H, s), 7.10 (1H, d, *J* = 8.7 Hz), 7.31 (1H, d, *J* = 8.4 Hz), 7.45 (1H, s). ^{13}C NMR (MeOD, 125MHz): 21.4, 42.1, 42.9, 102.6, 113.4, 117.9, 123.1, 126.3, 127.5, 132.8, 136.3. LRMS (ES^+) *m/z*: 207.09 $[M+H]^+$. HRMS ($ASAP^+$) calcd. for $C_{11}H_{12}N_2Cl$ $[M+H]^+$ 207.0689, found 207.0698.

9-chloro-1,2,3,4,5,6-hexahydrocyclohepta[b]indole (20)[12]

General Fischer indole procedure with 4-chlorophenylhydrazine hydrochloride (442.8 mg, 2.47 mmol) and cycloheptanone (300.9 mg, 2.68 mmol) in glacial acetic acid (4 mL) provided **20** (300.9 mg, 55% yield) as an off-white crystalline solid following recrystallisation from ethanol/water, mp 124 – 126 °C. ^1H NMR (d_6-DMSO, 500 MHz): 1.66-1.68 (4H, m), 1.83 (2H,

m), 2.69-2.70 (2H, m), 2.80-2.81 (2H, m), 6.93 (1H, d, *J* = 8.0 Hz), 7.21 (1H, d, *J* = 8.5Hz), 7.38 (1H, s), 10.87 (1H, br s). ^{13}C NMR (d_6-DMSO, 125MHz): 24.1, 27.0, 28.4, 28.5, 31.4, 111.8, 111.9, 116.3, 119.3, 122.7, 129.8, 132.5, 140.1. LRMS (ES^+) *m/z*: 220.1 $[M+H]^+$. HRMS (ES^+) calcd. for $C_{13}H_{15}NCl$ $[M+H]^+$ 220.0893, found 220.0892.

Methyl 5-chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxylate (21)

General Fischer indole procedure with 3-chlorophenylhydrazine hydrochloride (482.3 mg, 2.69 mmol) and methyl-2-oxocyclohexane-1-carboxylate (459.7 mg, 2.94 mmol) in glacial acetic acid (4 mL) provided **21** (32.9 mg, 5% yield) as a pale yellow oil following FCC on silica gel (1:1:98 Et_2O/EtOAc/petrol)**.** ^{1}H NMR ($CDCl_3$, 300 MHz): 1.82-1.88 (1H, m), 2.02-2.07 (1H, m), 2.15-2.23 (3H, m), 3.79 (3H, s), 3.87 (1H, t, *J* = 6.3Hz), 7.00 (1H, t, *J* = 7.8 Hz), 7.15 (1H, d, *J* = 7.5 Hz), 7.37 (1H, d, *J* = 7.8 Hz), 8.50 (1H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): 20.9, 21.7, 26.1, 39.9, 52.5, 113.2, 116.4, 117.0, 120.2, 121.4, 128.9, 130.4, 133.3, 172.9. LRMS (ES^-) *m/z*: 262.05 $[M-H]^-$. HRMS (ESI^-) calcd. for $C_{14}H_{13}NO_2Cl$. 262.0635, found 262.0645.

8-chloro-2,3,4,9-tetrahydro-1H-carbazole (22)[13]

General Fischer indole procedure with 2-chlorophenylhydrazine hydrochloride (258.8 mg, 1.45 mmol) and cyclohexanone (162.8 mg, 1.66 mmol) in glacial acetic acid (4 mL) provided **22** (129.4 mg, 43% yield) as a pale yellow oil following FCC on silica gel (1:9 – 2:8 Et_2O/petrol). ^{1}H NMR (MeOD, 500 MHz): 1.85-1.90 (4H, m) 3.64-2.66 (2H, t like), 2.74-2.76 (2H, t like), 6.89 (1H, t, *J* = 7.8 Hz,), 6.98 (1H, d, *J* = 7.5 Hz), 7.26 (1H, d, *J* = 8.0 Hz). ^{13}C NMR (MeOD, 125MHz): 22.0, 24.0, 24.3, 23.5, 111.0, 116.9, 117.0, 120.0, 120.7, 130.9, 134.4, 136.8. LRMS (ES^-) m/z: 205 $[M-H]^-$. LRMS (ES^-) m/z: 205 $[M-H]^-$. HRMS ($ASAP^+$) calcd. for $C_{12}H_{13}NCl$ $[M+H]^+$ 206.0737, found 206.0739.

8-chloro-2,3,4,9-tetrahydro-1H-carbazole-3-carboxylic acid (24)[14]

General deesterification procedure with **23** (77.0 mg, 0.28 mmol) provided **24** (21.5 mg, 31% yield) as an off-white powder following FCC on silica gel (20:80:0.5 EtOAc/petrol/acetic acid), mp 196 -198 °C. ^{1}H NMR (MeOD, 500 MHz): 1.97-2.00 (1H, m), 2.27-2.30 (1H, m), 2.78-2.87 (4H, m,), 2.97-3.01 (1H, m), 6.91 (1H, t, *J* = 7.8 Hz), 7.01 (1H, d, *J* = 8.0 Hz), 7.29 (1H, d, *J* = 8.0 Hz). ^{13}C NMR (MeOD, 125 MHz): 23.1, 25.0, 27.0, 41.5, 109.3, 117.0, 120.3, 121.0, 130.6, 134.7, 136.1, 179.4. LRMS (ES^-) *m/z*: 248.15 $[M-H]^-$. HRMS (ES^-) calcd. for $C_{13}H_{11}NO_2Cl$ $[M-H]^-$ 248.0478, found 248.0477.

6-chloro-2,3,4,9-tetrahydro-1H-carbazol-7-ol (25)

General hydrazine formation procedure with 2-chloro-5-aminophenol (1.93 g, 13.45 mmol) provided 2-chloro-5-hydrazinylphenol hydrochloride (**30**; 1.99 mg, 75% yield) as a pinkish powder, mp >160 °C (dec.). ^{1}H NMR (d_6-DMSO, 500 MHz): 6.42 (1H, dd, *J* = 8.5, 2.5 Hz), 6.60 (1H, d, *J* = 2.0 Hz), 7.20 (1H, d, 8.5 Hz), 8.23 (1H, s), 10.06 (1H, br s), 10.25 (1H, s). LRMS (ES^+) m/z: 159.04 $[M+H]^+$.

General Fischer indole procedure with **30** (528.7 mg, 2.71 mmol) and cyclohexanone (285.3 mg, 2.90 mmol) in absolute ethanol (6 mL) provided **25** (16.7 mg, 8% yield) as a beige powder following FCC on silica gel (gradient elution; 1:9-4:6 Et_2O/petrol), mp 150 – 152 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): 1.84-1.88 (4H, m), 2.60-2.62 (2H, m), 2.65-2.67 (2H, m), 6.90 (1H, s), 7.35

(1H, s), 7.54 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz): 20.9, 23.2, 23.3, 97.3, 109.8, 113.1, 117.3, 123.1, 134.3, 135.5, 146.4. LRMS (ES^+) *m/z*: 221.89 $[M+H]^+$. HRMS ($ASAP^+$) calcd. for $C_{12}H_{13}NOCl$ $[M+H]^+$ 222.0686, found 222.0676.

6-chloro-2,3,4,9-tetrahydrocarbazole-2,5-dicarboxylic acid (26)

General Fischer indole procedure with 2-chloro-5-hydrazinobenzoic acid hydrochloride (**32**; 196.5 mg, 0.88 mmol) and 3-oxocyclohexanecarboxylic acid (117.4 mg, 0.83 mmol) in glacial acetic acid (2 mL) produced a mixture of two isomers. The desired isomer was separated by first methylating the mixture (general esterification procedure), followed by FCC on silica gel (40:60:0.5 EtOAc/petrol/acetic acid) to give the mono-methylated 6-chloro-2,3,4,9-tetrahydrocarbazole-2,5-dicarboxylic acid (48.5 mg), which was demethylated (general deesterification procedure) to give **26** (15.6 mg, 10% yield) as a pale yellow powder, mp 222 – 224 °C. ^{1}H NMR (MeOD, 500 MHz): 1.89-1.94 (1H, m), 2.24-2.66 (1H, m), 2.66-2.72 (1H, m), 2.77-2.87 (2H, m), 2.98-3.00 (2H, m), 7.03 (1H, d, *J* = 8.0 Hz), 7.27 (1H, d, *J* = 9.0 Hz). ^{13}C NMR ($CDCl_3$+MeOD, 125 MHz): 20.4, 25.4, 26.3, 39.6, 107.7, 112.6, 120.4, 121.1, 123.6, 124.4, 134.9, 135.5, 179.0. LRMS (ES^+) *m/z*: 316.15 $[M+Na]^+$. HRMS (ES^+) calcd. for $C_{14}H_{12}NO_4ClNa$ $[M+Na]^+$ 316.0353, found 316.0348.

7-chloro-1,2,3,4-tetrahydrocyclopenta[b]indole-8-carboxylic acid (27)

General Fischer indole procedure with 2-chloro-5-hydrazinobenzoic acid hydrochloride (**32**; 436.2 mg, 1.96 mmol) and cyclopentanone (139.6 mg, 1.66 mmol) in glacial acetic acid (2 mL) provided **27** (11.3 mg, 2% yield) as a yellow powder following FCC on silica gel (30:70:0.5 EtOAc/petrol/acetic acid) and recrystallisation from methanol/water, mp 208 – 210 °C. ^{1}H NMR (d_6-DMSO, 300 MHz): 2.40-2.44 (2H, m), 2.68 (2H, br t, *J* = 6.8 Hz), 2.82 (2H, br t, *J* = 7.2Hz), 7.01 (1H, d, *J* = 9 Hz), 7.34 (1H, dd, *J* = 8.7, 1.2 Hz), 11.26 (1H, s), 13.17 (1H, s). ^{13}C NMR (d_6-DMSO, 75 MHz): 24.9, 25.2, 28.0, 114.1, 117.2, 120.1, 120.2 (C-H), 122.7 (2xC), 139.5, 147.6, 167.8. LRMS (ES^-) m/*z*: 234.0 $[M-H]^-$. HRMS (ES^-) calcd. for $C_{12}H_9NO_2Cl$ $[M-H]^-$ 234.0322, found 234.0320.

Methyl 6-chloro-2,3,4,9-tetrahydrocarbazole-7-carboxylate (29)

General Fischer indole procedure with 2-chloro-5-hydrazinobenzoic acid hydrochloride (**32**; 364.5 mg, 1.63 mmol) and cyclohexanone (113.3 mg, 1.15 mmol) in glacial acetic acid (4 mL) provided 6-chloro-2,3,4,9-tetrahydrocarbazole-7-carboxylic acid (44.4 mg, 16% yield) as a white powder following FCC on silica gel (20:80:0.5 EtOAc/petrol/acetic acid). ^{1}H NMR ($CDCl_3$ + d_6-DMSO, 500 MHz): 1.87-1.91 (4H, m), 2.66-2.67 (2H, m), 2.75-2.76 (2H, m), 7.43 (1H, s), 7.98 (1H, s), 9.79 (1H, s). LRMS (ES^+) *m/z*: 272.2 $[M+Na]^+$. HRMS (ES^+) calcd. for $C_{13}H_{13}NO_2Cl$ $[M+H]^+$ 250.0635, found 250.0657.

General esterification procedure with 6-chloro-2,3,4,9-tetrahydrocarbazole-7-carboxylic acid (36.6 mg, 0.15 mmol) in methanol (1.5 mL) provided **29** (7.0 mg, 18% yield) as a beige powder following trituration with CH_2Cl_2, mp 198 -200 °C. ^{1}H NMR (d_6-DMSO, 500 MHz): 1.77-1.83 (4H, m), 2.59-2.61 (2H, m), 2.72-2.73 (2H, m), 3.82 (3H, s), 7.46 (1H, s), 7.79 (1H, s), 11.19 (1H, br s). ^{13}C NMR (d_6-DMSO, 125 MHz): 20.3, 22.4, 22.6, 22.8, 51.9, 108.8, 114.1, 118.8, 119.7, 121.9, 130.4, 133.2, 140.5, 166.1. LRMS (ES^+) *m/z*: 286.211 $[M+Na]^+$. HRMS (ES^+) calcd. for $C_{14}H_{15}NO_2Cl$ $[M+H]^+$ 264.0791, found 264.0800.

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