MECHANISTIC AND BIOLOGICAL CHARACTERIZATION OF NOVEL N^5-SUBSTITUTED PAULLONES TARGETING THE BIOSYNTHESIS OF TRYPANOTHIONE IN LEISHMANIA

Andrea Medeiros, Diego Benítez, Ricarda S. Korn, Vinicius C. Ferreira, Exequiel Barrera, Federico Carrión, Otto Pritsch, Sergio Pantano, Conrad Kunick, Camila I. de Oliveira, Oliver C. F. Orban, Marcelo A. Comini

Supplemental Materials and Methods

Table of contents

Heterologous expression and purification of recombinant CfGspS

E. coli strain BL21 (DE3) cells were transformed with the plasmid *Cf*GspSpET15b (kindly provided by Dr. Krauth-Siegel, Biochemie Zentrum Heidelberg, Heidelberg University, Germany). A starter culture was prepared with freshly transformed bacteria inoculated in LB medium with 100 µg/mL ampicillin and grown overnight under aerobic conditions at 37 °C and 180 rpm. Cells were inoculated 1:100 in Terrific Broth medium supplemented with 10 g/L glucose and 100 µg/mL ampicillin and grown at 37 °C and 180 rpm until an optical density of ~1.0 at 600 nm. The cultures were then chilled at 4 °C for 15 min and expression of recombinant protein was induced with 0.5 mM isopropyl 1-thio-β-D-galactopyranoside (IPTG) for 5 h at 25 °C and 180 rpm.

Cells harvested by centrifugation at 4,000 *g* for 15 min at 4 °C (Sorvall RC-6, Thermo Fisher Scientific) were resuspended at a ratio of 1 g wet weight pellet per 5 ml buffer A (50 mM NaH_2PO_4 pH 7.2, 500 mM NaCl) with 10 mM imidazole. Cell lysis was achieved by gently shaking with lysozyme (30 mg % w/v) for 1 h at 4 °C followed by sonication on ice (4 pulses during 30 sec interspersed by pauses of 60 sec) at amplitude of 40 % using a Digital Sonifier 450 (Branson). Ten mM $MgSO_4$ and 2 U/mL DNase were added 30 min after the lysis was initiated. The lysate was centrifuged at 40,000 *g* for 30 min at 4 °C to remove debris and the supernatant was cleared by filtration (0.45 µM filter) and then loaded onto one 1 mL HisTrap Fast Flow column (GE Healthcare) pre-equilibrated in buffer A with 10 mM imidazole. The column was washed with buffer A supplemented with 25 mM imidazole and the recombinant protein was eluted in an isocratic fashion with 5 mL of buffer B (buffer A with 500 mM imidazole). This chromatography was performed at 4 ºC and at a flow rate of 1 mL/min using a peristaltic pump (TRIS Teledyne ISCO). The elution fractions containing the protein of interest were pooled and subjected to diafiltration with reaction buffer (5 mM DTT, 10 mM $MgSO_4$, 0.5 mM EDTA, 100 mM HEPES) containing 150 mM NaCl, pH

7.4 using a 10 kDa cut-off Amicon filter (Millipore). The sample was centrifuged at 5,000 *g* at 4 ºC (Thermo-Sorvall centrifuge) until a volume of 0.5 mL was achieved. The diafiltrated sample was loaded onto a Superdex^{TM} 200 10/300 GL column (GE Healthcare) equilibrated in reaction buffer containing 150 mM NaCl and run at 0.75 mL/min at room temperature (RT, 20-25 ºC) using an Äkta-FPLC device (GE Healthcare). GspS protein eluted with a retention volume compatible with a monomeric specie of about 85 kDa. After the final chromatography step, enzyme purity and activity were assessed by SDS-PAGE (10 %) under reducing conditions and the end-point GspS activity assay described below, respectively. Fractions with a GspS homogeneity > 90 % were pooled, concentrated (~1 mg/mL) and stored at 4 °C until use.

GspS activity assay

Similar to TryS activity, GspS activity was determined using the end-point assay based on the BIOMOL GREEN^{TM} reagent (Enzo Life Sciencies; Benítez et al. 2016).

For the GspS assay, ATP was used at 150 µM (1.11-fold the K_m value), SP to 1 mM (1.96-fold the K_m value) and GSH to 1 mM (1.41-fold the K_m value), while the enzyme concentration was adjusted to obtain an activity of 8.7 x 10^{-7} µmol/min.mL.

A master mix (MM) solution containing all the substrates at 1.25-fold their end concentration in assay was prepared in the screening reaction buffer (5 mM DTT, 10 mM $MgSO_4$, 0.5 mM EDTA, 100 mM HEPES pH 7.4). Ninety-six wells microtiter plates were loaded with 5 µL of MOL2008 (stock concentration prepared in DMSO at 6 mM, evaluated at 300 µM, 30 µM and 0.15 µM), DMSO (reaction control) and 40 µL of MM. The reactions were then started by adding 5 µL of *Cf*GspS (with an activity of 8.7 x 10^{-7} µmol/min.mL) and stopped after 15 min with 200 µL BIOMOL GREEN^{TM} reagent. The plates were incubated 20 min at room temperature and then $A_{650\ nm}$ was measured with a MultiScan EX plate reader (Thermo Fisher SCIENTIFIC).

Blanks were prepared for each condition by adding 5 µL of screening reaction buffer instead of enzyme. Interference with the colorimetric reaction was evaluated in a sample containing 20 µM K_2HPO_4 dissolved in MM.

Synthetic chemistry

The synthesis of 3-chlorokenpaullone (**1**), **3** and **6** was previously reported (**1**: Orban, 2015; **3** and **6**: Orban, 2016). The synthesis of compound **MOL2008** is described in (Stuhlmann, 2007). All other paullones tested in this work were synthesized by the following procedures.

General procedure A

The reaction was carried out under nitrogen. To a suspension of 9-bromo-3-chloro-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (**1**, 362 mg, 1.00 mmol) in dry THF (25 mL), potassium *tert*-butoxide (112 mg, 1.00 mmol) was added and the mixture was stirred for 1 h at room temperature. After addition of the alkyl halide (1.30 mmol) stirring was continued overnight at room temperature. The mixture was evaporated and the residue was dissolved in CH_2Cl_2 (100 mL). After filtration, the organic layer was washed with water (100 mL) and evaporated. For the purification of the residue column chromatography or crystallization were used.

*General procedure **B***

To an ice-cooled solution of 2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-*b*]indol-5-yl) acetic acid (**3**, 294 mg, 700 µmol) in DMF (1 mL), PyBOP (442 mg, 850 µmol) and DIPEA (521 µL, 3.00 mmol) were added and

the solution was stirred for 20 min under nitrogen. The amine (830 µmol) was added drop wise. The solution was covered with an argon layer and stirred for 20 h at room temperature and then 100 mL of ethyl acetate was added. The precipitate was filtered off and set aside and the organic solution was washed with 0.1 M hydrochloric acid (100 mL), 0.1 M sodium hydroxide (100 mL) and water (100 mL). The washed organic solution was dried with Na_2SO_4, filtered and evaporated. For the purification of the combined precipitate, column chromatography and crystallization were used.

*General procedure **C***

To a stirred solution of the Boc-protected amine (350 µmol) in dry CH_2Cl_2 (50 mL), TFA (9 mL) was added and stirring was continued for 24 h under nitrogen. The organic solvent was evaporated and the solid or oil was dissolved in propan-2-ol (3 mL). 10 drops of 5 M propan-2-olic HCl (4.2 mL of 36% HCl and propan-2-ol ad 10 mL) were added. Diethyl ether (50 mL) was added to the solution and the suspension was refluxed for 1 h. The precipitate was filtered off, washed with diethyl ether and dried.

*Synthesis of compounds **2**-**36***

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(2-hydroxyethyl)acetamide (**13**)*

Synthesized according to general procedure **B** from **3** (212 mg, 504 µmol), DMF (1 mL), DIPEA (370 µL, 2.12 mmol), PyBOP (310 mg, 594 µmol) and 2-aminoethan-1-ol (36.0 µL, 595 µmol). Crystallized from ethanol to yield a colorless solid (20 mg, 19%). Dec. starting at 285 °C; IR (KBr): 3323 cm^{-1} (NH), 1660 cm^{-1} (C=O), 1641 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) =11.99 (s, 1H, indole-NH), 8.12 (t, *J* =

5.6 Hz, 1H, NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.67 (d, *J* = 2.1 Hz, 1H, ArH), 7.49 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.43 (d, *J* = 8.7 Hz, 1H, ArH), 7.31 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.70 (t, *J* = 5.4 Hz, 1H, -OH), 4.41 and 4.10 (bs, 2H, -N^5-CH_2), 3.98 and 3.11 (bs, 2H, azepine-CH_2), 3.42 (q, *J* = 5.9 Hz, 2H, O-CH_2), 3.18 (bq, 2H, N-CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 30.0, 41.3, 53.2, 59.5 (CH_2); 113.4, 120.5, 123.8, 124.7, 124.9, 128.3 (CH), 109.4, 111.9, 124.0, 127.8, 132.4, 133.0, 136.0, 141.2, 168.3, 170.1 (C); CHN: calculated C 51.91, H 3.70, N 9.08, found C 51.67, H 3.59, N 8.98; $C_{20}H_{17}BrClN_3O_3$ (462.73); MS (EI): m/z (%): 463.0 $[M]^{+\bullet}$ (6), 402.0 $[M^{+\bullet}–61]$ (29), 361.0 $[M^{+\bullet}–102]$ (100); HPLC (isocratic): 99.5% at 254 nm, 99.6% at 280 nm, t_{M+S} = 5.14 min, t_M (DMSO) = 1.06 min (ACN/water = 40:60); λ_{max}: 231 nm, 319 nm, 382 nm; HPLC (gradient method A): 98.5% at 254 nm, t_{M+S} = 10.12 min, t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(tert-butyl)acetamide (**15**)*

Synthesized according to general procedure **B** from **3** (305 mg, 727 µmol), DMF (1 mL), DIPEA (511 µL, 2.94 mmol), PyBOP (450 mg, 865 µmol) and 2-methylpropan-2-amine (87 µL, 0.83 mmol). Crystallized from ethanol to yield a colorless solid (118 mg, 34%). Dec. starting at 291 °C; IR (KBr): 3336 cm^{-1} (NH), 3284 cm^{-1} (NH), 1681 cm^{-1} (C=O), 1647 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 12.00 (s, 1H, indole-NH), 7.95 (d, *J* = 2.0 Hz, 1H, ArH), 7.71 (d, *J* = 8.5 Hz, 1H, ArH), 7.61 (bs, 2H, ArH and -NH), 7.47 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, ArH), 4.36 and 4.07 (bs, 2H, -N^5-CH_2), 4.00 and 3.07 (bs, 2H, azepine-CH_2), 1.23 (s, 9H, -CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 28.5 (3C) (CH_3); 31.2, 53.3 (CH_2); 113.6, 120.7, 123.9, 124.9, 125.0, 128.6 (CH); 50.3, 109.5, 111.9, 124.0, 127.8, 132.4, 133.0, 136.0, 141.0, 167.2, 170.1 (C); CHN:

calculated C 55.66, H 4.46, N 8.85, found C 55.32, H 4.43, N 8.61; $C_{22}H_{21}BrClN_3O_2$ (474.78); MS (EI): m/z (%): 475.1 $[M]^{+\bullet}$ (31), 402.0 [$M^{+\bullet}$–72.1] (100), 361 [$M^{+\bullet}$–114.1] (20); HPLC (isocratic) 98.1% at 254 nm, 97.8% at 280 nm, t_{M+S} = 4.46 min, t_M (DMSO) = 1.06 min (ACN/water = 60:40); λ_{max}: 223 nm, 219 nm, 384 nm; HPLC (gradient method A) 98.0% at 254 nm, t_{M+S} = 12.68 min, t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N,N-diethylacetamide (**12**)*

Synthesized according to general procedure **B** from **3** (291 mg, 693 µmol), DMF (1 mL), DIPEA (1.40 mL, 8.05 mmol), PyBOP (442 mg, 849 µmol) and diethylamine (860 µL, 830 µmol). Crystallized successively from ethanol and ethanol/diethyl ether 1:1 to yield a colorless solid (23 mg, 8%). Dec. 279–281 °C; IR (KBr): 3293 cm^{-1} (NH), 1665 cm^{-1} (C=O), 1642 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.94 (d, *J* = 1.8 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.58 (d, *J* = 2.1 Hz, 1H, ArH), 7.47 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.68 and 4.36 (bs, 2H, -N^5-CH_2), 3.29 (q, *J* = 7.2 Hz, 4H, -CH_2), 3.97 and 3.07 (bs, 1H, azepine-CH_2), 1.11 (t, *J* = 7.0 Hz, 3H, -CH_3), 1.02 (t, *J* = 7.0 Hz, 3H, -CH_3). ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 13.0, 13.9 (CH_3); 31.2, 40.0, 40.7, 52.0 (CH_2); 113.6, 120.7, 123.9, 124.9, 125.0, 128.6 (CH); 109.4, 111.8, 124.1, 127.8, 132.4, 133.1, 136.0, 141.1, 166.7, 169.9 (C); CHN: calculated C 55.66, H 4.46, N 8.85, found C 55.45, H 4.42, N 8.64; $C_{22}H_{21}BrClN_3O_2$ (474.78); MS (EI): m/z (%): 475.1 $[M]^{+\bullet}$ (31), 402 [$M^{+\bullet}$–72.1] (100); HPLC (isocratic) 96.0% at 254 nm, 96.1% at 280 nm, t_{M+S} = 4.08 min, t_M (DMSO) = 1.06 min (ACN/water = 60:40); λ_{max}: 223 nm, 232 nm, 319 nm; HPLC (gradient method A) 95.1% at 254 nm, t_{M+S} = 12.48 min, t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)acetamide (**14**)*

Synthesized according to general procedure **B** from **3** (301 mg, 717 µmol), DMF (1 mL), DIPEA (511 µL, 2.93 mmol), PyBOP (444 mg, 853 µmol) and 2-amino-2-(hydroxymethyl)propane-1,3-diol (101 mg, 834 µmol). Crystallized from ethanol/ethyl acetate 1:1 to yield a colorless solid (28 mg, 7%). Dec. 261–262 °C; IR (KBr): 3270 cm^{-1} (OH), 1655 cm^{-1} (C=O), 1633 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.95 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.66 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.35 (bs, 1H, -NH), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, ArH), 4.59 (t, *J* = 5.8 Hz, 3H, -OH), 4.49 and 4.16 (bs, 2H, -N^5-CH_2), 3.56 (d, *J* = 5.8 Hz, 6H, -CH_2), 4.02 and 3.07 (bs, 2H, azepine-CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 31.2, 53.6, 60.3 (3C) (CH_2); 113.7, 120.7, 124.0, 125.0, 125.1, 128.6 (CH); 62.4, 109.4, 111.9, 124.0, 127.8, 132.5, 133.0, 136.0, 140.9, 168.9, 170.2 (C); $C_{22}H_{21}BrClN_3O_5$ (522.78); MS (EI): m/z (%): 523.0 $[M]^{+\bullet}$ (9), 402.0 [$M^{+\bullet}$–121.0] (37), 361.0 [$M^{+\bullet}$–162.0] (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 521.03476 found 521.03512; HPLC (isocratic) 99.5% at 254 nm, 99.8% at 280 nm, t_{M+S} = 4.27 min, t_M (DMSO) = 1.06 min (ACN/water = 40:60); λ_{max}: 232 nm, 319 nm; HPLC (gradient method A) 96.7% at 254 nm, t_{M+S} = 12.68 min; t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-methylacetamide (**9**)*

Synthesized according to general procedure **B** from **3** (294 mg, 701 µmol), DMF (1 mL), DIPEA (511 µL, 2.94 mmol), PyBOP (441 mg, 847 µmol) and methylamine in THF (c = 2 M, 415 µL, 830 µmol). Crystallized from ethanol to yield a colorless solid (132 mg, 37%). Dec. starting at 351 °C; IR (KBr): 3312 cm^{-1} (NH), 1658 cm^{-1} (C=O),

1639 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 8.07 (q, *J* = 4.6 Hz, 1H, -NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.68 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.36 and 4.07 (bs, 2H, -N^5-CH_2), 3.99 and 3.10 (bs, 2H, azepine-CH_2), 2.64 (d, *J* = 4.6 Hz, 3H, -CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 25.6 (CH_3); 31.2, 53.7 (CH_2); 113.6, 120.7, 124.1, 124.9, 125.1, 128.6 (CH); 109.4, 111.9, 123.9, 127.8, 132.4, 133.0, 136.0, 141.2, 168.7, 170.1 (C); CHN: calculated C 52.74, H 3.49, N 9.71, found C 52.48, H 3.40, N 9.43; $C_{19}H_{15}BrClN_3O_2$ (432.70); MS (EI): m/z (%): 433.0 $[M]^{+\bullet}$ (31), 402.0 $[M^{+\bullet}–31.0]$ (100), 361.0 $[M^{+\bullet}–72]$ (11); HPLC (isocratic) 99.2% at 254 nm, 99.3% at 280 nm, t_{M+S} = 3.77 min, t_M (DMSO) = 1.06 min (ACN/water = 50:50); λ_{max}: 232 nm, 319 nm, 395 nm; HPLC (gradient method A) 98.8% at 254 nm, t_{M+S} = 10.93 min, t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(1,3,4-thiadiazol-2-yl)acetamide (**16**)*

Synthesized according to general procedure **B** from **3** (296 mg, 705 µmol), DMF (1 mL), DIPEA (510 µL, 2.93 mmol), PyBOP (442 mg, 849 µmol) and 2-amino-1,3,4-thiadiazole (84.0 mg, 831 µmol). Crystallized from ethanol to yield a colorless solid (99 mg, 28%). Dec. starting at 304 °C; IR (KBr): 3330 cm^{-1} (NH), 1712 cm^{-1} (C=O), 1655 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 12.86 (s, 1H, -NH), 12.05 (s, 1H, indole-NH), 9.20 (s, 1H, ArH), 7.97 (d, *J* = 1.9 Hz 1H, ArH), 7.75 (d, *J* = 8.4 Hz, 1H, ArH), 7.68 (d, *J* = 2.1 Hz, 1H, ArH), 7.51 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.44 (d, *J* = 8.6 Hz, 1H, ArH), 7.31 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.66 (bs, 2H, -N^5-CH_2), 4.00 and 3.15 (bs, 1H, azepine-CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 30.9, 53.8 (CH_2); 113.7, 120.8, 124.2, 125.0, 125.5, 128.7, 148.8 (CH); 109.2, 111.9, 124.1, 127.8, 132.6, 133.0, 136.0, 140.6, 158.5, 168.2, 170.6 (C); CHN: calculated C 47.78, H

2.61 N 13.93, found C 47.60, H 2.59, N 13.51; $C_{20}H_{13}BrClN_5O_2S$ (502.77); MS (EI): m/z (%): 502.9 $[M]^{+\bullet}$ (22), 401.9 [$M^{+\bullet}$–101] (100); HPLC (isocratic) 98.6% at 254 nm, 98.8% at 280 nm, t_{M+S} = 4.33 min, t_M (DMSO) = 1.06 min (ACN/buffer = 50:50); λ_{max}: 234 nm, 319 nm, 385 nm.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-ethylacetamide (**11**)*

Synthesized according to general procedure **B** from **3** (297 mg, 708 µmol), DMF (1 mL), DIPEA (511 µL, 2.93 mmol), PyBOP (445 mg, 855 µmol) and ethylamine in THF (c = 2 M, 415 µL, 830 µmol). Crystallized from ethanol to yield a colorless solid (200 mg, 70%). Dec. starting at 327 °C; IR (KBr): 3324 cm^{-1} (NH), 1641 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 8.09 (t, *J* = 5.5 Hz, 1H, -NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.5 Hz, 1H, ArH), 7.67 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.7 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.34 and 4.10 (bs, 2H, -N^5-CH_2), 4.01 and 3.12 (bs, 2H, azepine-CH_2), 3.12 (qd, *J* = 7.2, 5.4 Hz, 2H, -CH_2), 1.03 (t, *J* = 7.2 Hz, 3H, -CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 15.1 (CH_3); 31.7, 34.0, 54.0 (CH_2); 114.1, 121.2, 124.6, 125.4, 125.6, 129.1 (CH); 109.4, 111.8, 124.0, 127.8, 132.4, 133.0, 136.0, 141.1, 167.8, 170.1 (C); CHN: calculated C 53.77, H 3.84, N 9.41, found C 53.84, H 3.76, N 9.26; $C_{20}H_{17}BrClN_3O_2$ (446.73); MS (EI): m/z (%): 447.0 $[M]^{+\bullet}$ (24), 402.0 [$M^{+\bullet}$–45.0] (100), 361 [$M^{+\bullet}$–86] (11); HPLC (isocratic) 97.7% at 254 nm, 98.0% at 280 nm, t_{M+S} = 4.67 min, t_M (DMSO) = 1.06 min (ACN/water = 50:50); λ_{max}: 232 nm, 319 nm, 389 nm; HPLC (gradient method A) 95.4% at 254 nm, t_{M+S} = 11.45 min, t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)acetamide (**7**)*

Synthesized according to general procedure **A** from **1** (371 mg, 1.03 mmol), potassium *tert*-butoxide (126 mg, 1.12 mmol) and 2-bromoacetamide (248 mg, 1.80 mmol). Purified by column chromatography (ethyl acetate/petroleum ether 1:1) to yield a colorless solid (15 mg, 3%): Dec. starting at 297 °C; IR (KBr): 3466 cm^{-1} (NH), 3306 cm^{-1} (NH), 1678 cm^{-1} (C=O), 1666 cm^{-1} (C=O), 1638 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.61 (d, *J* = 2.1 Hz, 1H, ArH), 7.58 (s, 1H, -NH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, ArH), 7.18 (s, 1H, -NH), 4.36 (bs, 1H, -N^5-CH_2), 4.01 (bs, 2H, -N^5-CH_2 and azepine-CH_2), 3.11 (bs, 1H, azepine-CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 30.9, 53.2 (CH_2); 113.4, 120.5, 123.6, 124.7, 124.8, 128.3 (CH); 109.4, 111.9, 123.9, 127.8, 132.4, 133.0, 136.0, 141.2, 170.2, 170.3 (C); $C_{18}H_{13}BrClN_3O_2$ (418.68); MS (EI): m/z (%): 419.0 $[M]^{+\bullet}$ (56), 402.0 $[M^{+\bullet}–17]$ (100), 361 $[M^{+\bullet}–58]$ (32); HRMS (ESI): m/z calculated ($C_{18}H_{13}BrClN_3O_2Na$) 441.97494, found 441.97507; HPLC (isocratic) 97.7% at 254 nm, 97.8% at 280 nm, t_{M+S} = 5.29 min, t_M (DMSO) = 1.06 min (ACN/water = 40:60); λ_{max}: 231 nm, 319 nm, 384 nm; HPLC (gradient method A) 97.3% at 254 nm, t_{M+S} = 10.52 min, t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(4,5-dihydrothiazol-2-yl)acetamide (**17**)*

Synthesized according to general procedure **B** from **3** (294 mg, 701 µmol), DMF (1 mL), DIPEA (1.02 mL, 5.86 mmol), PyBOP (441 mg, 847 µmol) and 2-amino-2-thiazoline (61.0 mg, 597 µmol). Purified by column chromatography (ethyl acetate) followed by crystallization from ethyl acetate/toluene 1:1 to yield a colorless solid

(40 mg, 13%). Dec. starting at 266 °C; IR (KBr): 3289 cm^{-1} (NH), 1722 cm^{-1} (C=O), 1650 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 12.01 (s, 1H, indole-NH), 9.91 (bs, 1H, -NH), 7.96 (d, *J* = 2.0 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.54 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (dd, *J* = 8.6, 0.5 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.52 and 4.32 (bs, 1H, -N^5-CH_2), 3.92 and 3.08 (bs, 2H, azepine-CH_2), 3.67 (bs, 2H, -CH_2), 3.22 (t, *J* = 8.0 Hz, 2H, -CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 30.3 (bs), 31.1, 55.6 (bs) (CH_2); 113.6, 120.7, 123.8, 124.9, 125.1, 128.6 (CH); 109.4, 111.9, 124.0, 127.8, 132.5, 133.0, 136.0, 141.0, 170.1 (C); $C_{21}H_{16}BrClN_4O_2S$ (503.79); MS (EI): m/z (%): 504.0 $[M]^{+\bullet}$ (2), 402.0 $[M^{+\bullet}–102]$ (48), 129.0 $[M^{+\bullet}–375.0]$ (100), HRMS (ESI): m/z $[M]^{+\bullet}$ calculated ($C_{21}H_{16}BrClN_4O_2SNa$) 526.97367, found 526.97349; HPLC (isocratic) 96.2% at 254 nm, 96.4% at 280 nm, t_{M+S} = 3.66 min, t_M (DMSO) = 1.06 min (ACN/buffer = 50:50); λ_{max}: 230 nm, 319 nm, 384 nm

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(1,3-oxazol-2-yl)acetamide (**18**)*

Synthesized according to general procedure **B** from **3** (301 mg, 711 µmol), DMF (1 mL), DIPEA (1.02 mL, 5.86 mmol), PyBOP (444 mg, 853 µmol) and 2-amino-1,3-oxazole (51.0 mg, 607 µmol). Purified by column chromatography (ethyl acetate) to yield a colorless solid (25 mg, 7%). Dec. starting at 288 °C; IR (KBr): 3429 cm^{-1} (NH), 1694 cm^{-1} (C=O), 1643 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 12.02 (s, 1H, indole-NH), 11.53 (s, 1H, -NH), 7.97 (d, *J* = 1.9 Hz, 1H, ArH), 7.87 (d, *J* = 1.0 Hz, 1H, ArH), 7.73 (d, *J* = 8.4 Hz, 1H, ArH), 7.63 (d, *J* = 2.1 Hz, 1H, ArH), 7.50 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.43 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 7.10 (d, *J* = 1.0 Hz, 1H, ArH), 4.51 (bs, 2H, -N^5-CH_2), 4.00 and 3.13 (bs, 2H, azepine-CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 30.7, 53.7 (CH_2); 113.4, 120.5, 123.9,

124.7, 125.1, 126.4, 128.4, 135.8 (CH); 109.2, 111.9, 124.1, 127.8, 132.6, 133.0, 136.0, 140.7, 152.9, 167.0 (bs), 170.4 (C); $C_{21}H_{14}BrClN_4O_3$ (485.72); MS (EI): m/z (%): 486.0 $[M]^{+\bullet}$ (16), 402.0 $[M^{+\bullet}–84]$ (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 483.99323, found 483.99294; HPLC (isocratic) 96.7% at 254 nm, 96.8% at 280 nm, t_{M+S} = 3.93 min, t_M (DMSO) = 1.06 min (ACN/buffer = 50:50); λ_{max}: 232 nm, 319 nm.

*9-Bromo-3-chloro-5-(2-hydroxyethyl)-7,12-dihydroindolo[3,2-d][1]benzazepin6(5H)-one (**2**)*

To a stirred suspension of $LiAlH_4$ (16.0 mg, 422 µmol) in dry THF (10 mL) a solution of **5** (160 mg, 357 µmol) in THF (30 mL) was added drop wise. After the addition was done, the reaction was refluxed for 10 h and each hour $LiAlH_4$ (20 mg, 527 µmol) was added. After the reaction was cooled down to room temperature, water was added drop wise (caution!) till hydrogen production ceased. The precipitate was solved by adding 10% H_2SO_4 drop wise. The solution was washed with ethyl acetate (3x50 mL). The organic solution was dried with Na_2SO_4 and evaporated. The solid was purified by column chromatography (CH_2Cl_2/ethyl acetate 10:1) to yield a colorless solid (12 mg, 7%). Dec. 255–256 °C; IR (KBr): 3280 cm^{-1} (NH), 1638 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.96 (s, 1H, indole-NH), 8.09 (d, *J* = 2.1 Hz, 1H, ArH), 7.95 (dd, *J* = 1.9, 0.6 Hz, 1H, ArH), 7.70 (d, *J* = 8.4 Hz, 1H, ArH), 7.47 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.41 (dd, *J* = 8.6, 0.5 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, ArH), 4.91 (t, *J* = 5.3 Hz, 1H, -OH), 3.95 (bs, 1H), 3.77 (bs, 2H), 3.60 (bs, 1H), 3.40 (bs, 1H), 3.01 (bs, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 31.6, 53.6, 58.2 (CH_2); 113.6, 120.8, 124.9, 125.1, 125.5, 128.6 (CH); 109.8, 111.8, 124.3, 127.7, 132.4, 133.0, 136.1, 141.2, 169.3 (C); $C_{18}H_{14}BrClN_2O_2$ (405.68); CHN: calculated C 53.29, H 3.48, N 6.91, found C 53.36, H 3.42, N 6.74; MS (EI): m/z (%): 406.0 $[M]^{+\bullet}$ (82), 362.0 $[M^{+\bullet}–44]$ (100); HPLC (isocratic) 97.6% at 254 nm, 96.9% at 280 nm, t_{M+S} = 4.61 min, t_M (DMSO)

= 1.06 min (ACN/water = 50:50); λ_{max}: 233 nm, 319 nm, 381 nm; HPLC (gradient, method A) 97.7% at 254 nm, t_{M+S} = 11.38 min, t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(2-(diethylamino)ethyl)acetamide (**28**)*

Synthesized according to general procedure **B** from **3** (210 mg, 500 µmol), DMF (1 mL), DIPEA (500 µL, 2.87 mmol), PyBOP (312 mg, 600 µmol) and N^1,N^1-diethylethan-1,2-diamine (58 µL, 0.61 mmol). Crystallized from ethanol to yield a colorless solid (84 mg, 32%). Dec. starting at 267 °C; IR (KBr): 3317 cm^{-1} (NH), 1639 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.98 (t, *J* = 5.7 Hz, 1H, -NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.65 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 1H, ArH), 7.30 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 4.38 (bs, 1H, -N^5-CH_2), 4.05 (bs, 2H, -N^5-CH_2 and azepine-CH_2), 3.14 (q and bs, *J* = 6.2 Hz, 3H, azepine-CH_2 and -CH_2), 2.47 (q, *J* = 7.1 Hz, 4H, -CH_2), 2.43 (t, *J* = 7.0 Hz, 2H, -CH_2), 0.94 (t, *J* = 7.1 Hz, 6H, -CH_3); ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm) = 11.6 (2C) (CH_3); 30.9, 36.9, 46.4 (2C), 51.2 , 53.3 (CH_2); 113.4, 120.5, 123.7, 124.7, 124.9, 128.4 (CH); 109.4, 111.9, 124.0, 127.8, 132.4, 133.0, 136.0, 141.1, 168.0, 170.1 (C); CHN: calculated C 55.67, H 5.06, N 10.82, found C 55.40, H 4.81, N 10.61; $C_{24}H_{25}BrClN_4O_2$ (516.86); MS (EI): m/z (%): 518.0 $[M]^{+\bullet}$ (14), 402.0 [$M^{+\bullet}$–116] (29), 361.0 [$M^{+\bullet}$–157] (24), 99.1 [$M^{+\bullet}$–419] (100); HPLC (isocratic): 98.9% at 254 nm, 99.7% at 280 nm, t_{M+S} = 4.34 min, t_M (DMSO) = 1.06 min (ACN/buffer = 40:60); λ_{max}: 231 nm, 319 nm, 391 nm.

*9-Bromo-3-chloro-5-(2-oxo-2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethyl)-7,12-dihydrobenzo[2,3]azepino[4,5-b]indol-6(5H)-one (**23**)*

Synthesized according to general procedure **B** from **3** (211 mg, 502 µmol), DMF (1 mL), DIPEA (450 µL, 3.34 mmol), PyBOP (316 mg, 607 µmol) and 2-(piperazin-1-yl)pyrimidine (75 µL, 0.73 mmol). Crystallized from ethanol to yield a yellow solid (107 mg, 38%). Dec. starting at 275 °C; IR (KBr): 3277 cm^{-1} (NH), 1644 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 12.00 (s, 1H, indole -NH), 8.38 (d, *J* = 4.7 Hz, 2H, ArH), 7.95 (d, *J* = 1.9 Hz, 1H, ArH), 7.73 (d, *J* = 8.4 Hz, 1H, ArH), 7.58 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.7 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, ArH), 6.67 (t, *J* = 4.7 Hz, 1H, ArH), 4.79 and 4.50 (bs, 2H, -N^5-CH_2), 3.99 and 3.09 (bs, 2H, azepine-CH_2), 3.80–3.62 (m, 4H, piperazine-CH_2), 3.59–3.46 (m, 4H, piperazine-CH_2);^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 30.9, 41.0, 42.9, 43.0, 43.7, 51.6 (CH_2); 110.2, 113.4, 120.4, 123.8, 124.6, 124.9, 128.4, 157.7 (2C) (CH); 109.4, 111.8, 124.2, 127.8, 132.4, 133.2, 136.0, 140.9, 161.0, 166.6, 169.9 (C); $C_{26}H_{22}BrClN_6O_2$ (565.86); CHN: calculated C 55.19, H 3.92, N 14.85, found C 55.09, H 3.51, N 14.52; MS (EI): m/z (%): 566.0 $[M]^{+\bullet}$ (22), 402.0 $[M^{+\bullet}–164]$ (100); HPLC (isocratic): 98.8% at 254 nm, 99.6% at 280 nm, t_{M+S} = 6.85 min, t_M (DMSO) = 1.06 min (ACN/buffer = 50:50); λ_{max}: 237 nm, 319 nm, 386 nm.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(2-morpholinoethyl)acetamide (**30**)*

Synthesized according to general procedure **B** from **3** (110 mg, 261 µmol), DMF (2 mL), DIPEA (400 µL, 2.35 mmol), PyBOP (170 mg, 327 µmol) and 2-morpholinoethan-1-amine (480 mg, 3.69 mmol). Crystallized from ethanol to yield a colorless solid (66 mg, 23%). Dec. starting at 260 °C; IR (KBr): 3298 cm^{-1} (NH), 1660 cm^{-1} (C=O), 1639 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s,

1H, indole-NH), 8.04 (t, *J* = 5.6 Hz, 1H, -NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.65 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.38 and 4.11 (bs, 2H, -N^5-CH_2), 3.98 and 3.10 (bs, 2H, azepine-CH_2), 3.57 (t, *J* = 4.6 Hz, 4H, -CH_2), 3.22 (q, *J* = 6.4 Hz, 2H, -CH_2), 2.37 (bs, 4H, -CH_2), 2.35 (t, *J* = 6.7 Hz, 2H, -CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 31.2, 36.0, 53.3 (2C), 53.5, 57.4, 66.2 (2C) (CH_2); 113.4, 120.5, 123.8, 124.6, 124.9, 128.4 (CH); 109.4, 111.9, 113.6, 127.8, 132.4, 133.0, 136.0, 141.1, 168.1, 170.1 (C); $C_{24}H_{24}BrClN_4O_3$ (531.84); CHN: calculated C 54.20, H 4.55, N 10.53, found C 53.96, H 4.35, N 10.33; MS (EI): m/z (%): 532.0 $[M]^{+\bullet}$ (24), 402.0 $[M^{+\bullet}–130]$ (23), 361.0 $[M^{+\bullet}–171]$ (14), 113.1 $[M^{+\bullet}–419]$ (100); HPLC (isocratic): 98.0% at 254 nm, 99.4% at 280 nm, t_{M+S} = 7.41 min, t_M (DMSO) = 1.06 min (ACN/buffer = 40:60); λ_{max}: 232 nm, 319 nm, 391 nm.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-[2-(piperidin-1-yl)ethyl]acetamide (**31**)*

Synthesized according to general procedure **B** from **3** (108 mg, 257 µmol), DMF (1 mL), DIPEA (511 µL, 2.94 mmol), PyBOP (168 mg, 323 µmol) and 2-(piperidin-1-yl)ethan-1-amine (300 mg, 2.34 mmol). Purified by column chromatography (ethyl acetate/TEA/petroleum ether 10:1:1) followed by crystallization from ethanol/petroleum ether 1:1 to yield a colorless solid (8 mg, 6%). Dec. starting at 272 °C; IR (KBr): 3300 cm^{-1} (NH), 1654 cm^{-1} (C=O); 1638 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 8.00 (t, *J* = 5.7 Hz, 1H, -NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.64 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, ArH), 4.37 and 4.07 (bs, 2H, -N^5-CH_2), 3.99 and 3.11 (bs, 2H, azepine-CH_2), 3.19 (q, *J* = 6.5 Hz, 2H, -CH_2), 2.33 (bs, 4H, -CH_2), 2.31 (t, *J* = 7.0 Hz, 2H -CH_2), 1.48 (p, *J* = 5.7 Hz, 4H, -

CH_2), 1.42–1.31 (m, 2H, -CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 24.1, 25.5 (2C), 31.2, 36.4, 53.5, 54.1 (2C), 57.7 (CH_2); 113.7, 120.8, 124.0, 125.0, 125.2, 128.6 (CH); 109.4, 111.9, 124.0, 127.8, 132.4, 133.0, 136.0, 141.1, 168.1, 170.1 (C); $C_{25}H_{26}BrClN_4O_2$ (526.08); MS (EI): m/z (%): 530.0 $[M]^{+\bullet}$ (4), 402.0 $[M^{+\bullet}–128.0]$ (3), 361.0 $[M^{+\bullet}–169.0]$ (7), 98.1 $[M^{+\bullet}–431.9]$ (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 528.09222 found 528.09032; HPLC (isocratic): 98.8% at 254 nm, 99.8% at 280 nm, t_{M+S} = 7.46 min, t_M (DMSO) = 1.06 min (ACN/buffer = 40:60); λ_{max}: 231 nm, 319 nm, 391 nm.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-(2-(4-methylpiperazin-1-yl)ethyl)acetamide (**33**)*

Synthesized according to general procedure **B** from **3** (210 mg, 500 µmol), DMF (1.4 mL), DIPEA (450 µL, 2.58 mmol), PyBOP (370 mg, 711 µmol) and 2-(4-methylpiperazin-1-yl)ethan-1-amine (86.0 mg, 600 µmol). Crystallized from ethanol to yield a colorless solid (110 mg, 40%). Dec. starting at 266 °C; IR (KBr): 3311 cm^{-1} (NH), 1654 cm^{-1} (C=O), 1639 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 8.01 (t, *J* = 5.7 Hz, 1H, -NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.65 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, ArH), 4.36 and 4.08 (bs, 2H, -N^5-CH_2), 3.98 and 3.10 (bs, 2H, azepine-CH_2), 3.20 (q, *J* = 6.5 Hz, 2H, -CH_2), 2.33 (t and bs, *J* = 6.8 Hz, 10H, -CH_2), 2.14 (s, 3H, -CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 45.5 (CH_3); 30.9, 36.1 (2C), 52.4, 53.3, 54.4, 56.7 (2C) (CH_2); 113.4, 120.5, 123.8, 124.7, 124.9, 128.4 (CH); 109.4, 111.9, 124.0, 127.8, 132.4, 133.0, 136.0, 141.1, 168.1, 170.1 (C); $C_{25}H_{27}BrClN_5O_2$ (544.88); CHN: calculated C 55.11, H 4.99, N 12.85, found C 54.82, H 4.75, N 12.53; MS (EI): m/z (%): 545.0 $[M]^{+\bullet}$ (70), 361 $[M^{+\bullet}–184]$ (74), 402.0 $[M^{+\bullet}–143]$ (47); 126.1 $[M^{+\bullet}–419]$ (100); HPLC

(isocratic): 97.0% at 254 nm, 98.4% at 280 nm, t_{M+S} = 5.77 min, t_M (DMSO) = 1.06 min (ACN/buffer = 40:60); λ_{max}: 232 nm, 319 nm, 382 nm.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N,N-dimethylacetamide (**10**)*

Synthesized according to general procedure **B** from **3** (160 mg, 381 µmol), DMF (1 mL), DIPEA (800 µL, 4.59 mmol), PyBOP (254 mg, 488 µmol) and dimethylamine hydrochloride (42 mg, 0.52 mmol). Crystallized twice from ethanol and ethanol/petroleum ether 1:1 to yield a yellow solid (23 mg, 14%). Dec. starting at 301 °C; IR (KBr): 3432 cm^{-1} (NH), 3286 cm^{-1} (NH), 1664 cm^{-1} (C=O), 1643 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.95 (d, *J* = 2.1 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.54 (d, *J* = 2.1 Hz, 1H, ArH), 7.47 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.70 and 4.35 (bs, 2H, -N^5-CH_2), 3.97 and 3.07 (bs, 2H, azepine-CH_2), 2.95 (s, 3H, -CH_3), 2.85 (s, 3H, -CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 35.2, 35.9 (CH_3); 31.1, 52.2 (CH_2); 109.4, 111.8, 124.1, 127.8, 132.4, 133.1, 136.0, 141.1, 167.5, 169.9 (C); $C_{20}H_{17}BrClN_3O_2$ (446.73); CHN: calculated C 53.77, H 3.84, N 9.41, found C 54.17, H 3.62, N 9.11; MS (EI): m/z (%): 447.0 $[M]^{+\bullet}$ (16), 401.9 [$M^{+\bullet}$–270.1] (100), HPLC (isocratic): 98.1% at 254 nm, 99.5% at 280 nm, t_{M+S} = 4.83 min, t_M (DMSO) = 1.06 min (ACN/water = 50:50); λ_{max}: 232 nm, 319 nm, 391 nm; HPLC (gradient method A) 98.3% at 254 nm, t_{M+S} = 11.46 min, t_M (DMSO) = 1.25 min.

*tert-Butyl 4-{2-[2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)acetamido]ethyl}piperazine-1-carboxylate (**34**)*

Synthesized according to general procedure **B** from **3** (171 mg, 407 µmol), DMF (1 mL), DIPEA (400 µL, 2.30 mmol), PyBOP (254 mg, 488 µmol) and *tert*-butyl 4-(2-

aminoethyl)piperazine-1-carboxylate (112 mg, 488 µmol). Crystallized from ethanol to yield a colorless solid (130 mg, 51%). Dec. starting at 256 °C; IR (KBr): 3311 cm^{-1} (NH), 1696 cm^{-1} (C=O), 1641 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 8.03 (t, *J* = 5.7 Hz, 1H, -NH), 7.96 (d, *J* = 1.9 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, ArH), 7.65 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, ArH), 4.37 and 4.09 (bs, 2H, -N^5-CH_2), 3.98 and 3.10 (bs, 2H, azepine-CH_2), 3.32–3.26 (m, 4H, -CH_2), 3.22 (q, *J* = 6.4 Hz, 2H, -CH_2), 2.36 (t, *J* = 6.7 Hz, 2H, -CH_2), 2.35–2.32 (m, 4H), -CH_2), 1.40 (s, 9H, -CH_3); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 28.1 (3C) (CH_3); 31.2, 36.2 (2C), 52.4 (2C), 53.5, 56.9 (2C) (CH_2); 113.6, 120.8, 124.0, 124.9, 125.1, 128.6 (CH); 78.7, 109.4, 111.9, 124.0, 127.8, 132.4, 133.0, 136.0, 141.1, 153.8, 168.1, 170.1 (C); $C_{29}H_{33}BrClN_5O_4$ (630.97); CHN: calculated C 55.20, H 5.27, N 11.10, found C 55.19, H 5.19, N 10.89; MS (EI): m/z (%): 631.1 $[M]^{+\bullet}$ (7), 402.0 [$M^{+\bullet}$–229.1] (28), 361.0 [$M^{+\bullet}$–270.1] (71), 143.1 [$M^{+\bullet}$–488] (100); HPLC (isocratic): 95.7% at 254 nm, 95.8% at 280 nm, t_{M+S} = 4.31 min, t_M (DMSO) = 1.06 min (ACN/buffer = 60:40); λ_{max}: 232 nm, 319 nm, 395 nm.

*tert-Butyl {4-[2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)acetamido]butyl}carbamate (**36**)*

Synthesized according to general procedure **B** from **3** (110 mg, 262 µmol), DMF (1 mL), DIPEA (250 µL, 1.44 mmol), PyBOP (187 mg, 359 µmol) and *tert*-butyl (4-aminobutyl)carbamate (68.0 mg, 361 µmol). Crystallized from ethanol to yield a colorless solid (20 mg, 13%). Dec. 270–271 °C; IR (KBr): 3344 cm^{-1} (NH), 1679 cm^{-1} (C=O), 1657 cm^{-1} (C=O), 1642 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 11.98 (s, 1H, indole-NH), 8.08 (t, *J* = 5.6 Hz, 1H, -NH), 7.95 (d, *J* = 1.9 Hz, 1H, ArH), 7.71 (d, *J* = 8.4 Hz, 1H, ArH), 7.66 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz,

1H, ArH), 7.42 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 6.79 (t, *J* = 5.7 Hz, 1H; -NH-BOC), 4.35 and 4.10 (bs, 2H, -N^5-CH_2), 3.97 and 3.07 (bs and bq, 4H, azepine-CH_2 and -CH_2), 2.91 (bq, 2H, -CH_2), 1.37 (bs, 13H, -CH_3 and -CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 28.0 (3C) (CH_3); 26.2, 26.6, 30.9, 38.2, 39.3, 53.2 (CH_2); 113.4, 120.5, 123.8, 124.7, 124.8, 128.3 (CH); 77.3, 109.4, 111.8, 123.9, 127.8, 132.4, 133.0, 136.0, 141.1, 155.6, 168.0, 170.1 (C); CHN: calculated C 54.97, H 5.13, N 9.50, found C 55.00, H 4.93, N 9.22; $C_{27}H_{30}BrClN_4O_4$ (589.92); MS (EI): m/z (%): 590.0 $[M]^{+\bullet}$ (4), 401.9 [$M^{+\bullet}$–188.1] (100); HPLC (isocratic) 95.5% at 254 nm, 95.8% at 280 nm, t_{M+S} = 4.35 min, t_M (DMSO) = 1.06 min (ACN/water = 60:40); λ_{max}: 232 nm, 319 nm, 395 nm; HPLC (gradient method A) 95.8% at 254 nm, t_{M+S} = 12.52 min, t_M (DMSO) = 1.25 min.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-[2-(piperazin-1-yl)ethyl]acetamide dihydrochloride (**32**)*

Synthesized according to general procedure **C** from **34** (102 mg, 162 µmol), CH_2Cl_2 (14 mL), TFA (2.6 mL), propan-2-ol (3 mL), 10 drops propan-2-olic HCl and diethyl ether (30 mL) to yield a beige solid (43 mg, 47%). Dec. starting at 239 °C; IR (KBr): 3423 cm^{-1} (NH), 1661 cm^{-1} (C=O), 1638 cm^{-1} (C=O); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 12.07 (s, 1H, indole-NH), 11.63 (bs, 1H, -NH), 9.47 (bs, 2H, -NH_2^+), 8.45 (bs, 1H, -NH^+), 7.94 (d, *J* = 1.9 Hz, 1H, ArH), 7.73 (d, *J* = 8.4 Hz, 1H, ArH), 7.70 (d, *J* = 2.1 Hz, 1H, ArH), 7.49 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.43 (d, *J* = 8.6 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.33 (bs, 2H, -N^5-CH_2), 3.37 (bs, 14H, azepine-CH_2, and -CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 31.2, 33.8 (bs), 48.2 (bs, 2C), 54.0, 55.0 (bs) (CH_2); 113.7, 120.7, 124.3, 124.9, 125.2, 128.6 (CH); 109.3, 111.9, 127.8, 129.9, 132.5, 133.0, 136.0, 141.1, 169.1, 170.3 (C); MS (EI): m/z (%): 531.1 [$M^{+\bullet}$–72] (3), 402 [$M^{+\bullet}$–201] (2), 361.0 [$M^{+\bullet}$–242] (6), 99.1 [$M^{+\bullet}$–432.0] (100); $C_{24}H_{27}BrCl_3N_5O_2$

(603.77); HRMS (ESI): m/z $[M]^{+\bullet}$ ($C_{24}H_{26}BrClN_5O_2$) calculated 532.09419 found 532.09293; HPLC (isocratic): 98.9% at 254 nm, 99.3% at 280 nm, t_{M+S} = 3.72 min, t_M (DMSO) = 1.06 min (ACN/buffer = 30:70); λ_{max}: 231 nm, 319 nm, 393 nm.

*N-(4-Aminobutyl)-2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)acetamide hydrochloride (**35**)*

Synthesized according to general procedure **C** from **36** (12.0 mg, 20.3 µmol), CH_2Cl_2 (12 mL), TFA (2.3 mL), propan-2-ol (3 mL), 10 drops propan-2-olic HCl and diethyl ether (30 mL) to yield a yellow solid (10 mg, 94%). Dec. starting at 261 °C; IR (KBr): 3414 cm^{-1} (NH), 3288 cm^{-1} (NH), 1657 cm^{-1} (C=O), 1637 cm^{-1} (C=O);^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 12.06 (s, 1H, indole-NH), 8.19 (t, *J* = 5.7 Hz, 1H, -NH), 7.94 (d, *J* = 1.9 Hz, 1H; ArH), 7.73 (d, *J* = 8.4 Hz, 1H, ArH), 7.73 (bs, 3H, $-NH_3^+$), 7.68 (d, *J* = 2.1 Hz, 1H, ArH), 7.48 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.43 (d, *J* = 8.7 Hz, 1H, ArH), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 4.36 and 4.11 (bs, 2H, -N^5-CH_2), 3.97 (bs, 1H, azepine-CH_2), 3.11 (bs and bq, 3H, azepine-CH_2 and -CH_2), 2.79 (t, *J* = 7.4 Hz, 2H, -CH_2), 1.58–1.52 (m, 2H, -CH_2), 1.52–1.40 (m, 2H, -CH_2); ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 25.5, 27.1, 32.2, 39.1, 39.5, 54.7 (CH_2); 114.7, 121.7, 125.1, 126.0, 126.2, 129.7 (CH); 109.3, 111.8, 124.0, 127.7, 132.4, 133.0, 136.0, 141.2, 168.2, 170.1 (C); $C_{22}H_{23}BrCl_2N_4O_2$ (526.26); MS (EI): m/z (%): 490.0 [$M^{+\bullet}$–36] (16), 402.0 [$M^{+\bullet}$–124] (100); HRMS (ESI): m/z $[M]^{+\bullet}$ ($C_{22}H_{23}BrClN_4O_2$) calculated 491.06761 found 491.06587; HPLC (isocratic) 96.8% at 254 nm, 95.4% at 280 nm, t_{M+S} = 5.86 min, t_M (DMSO) = 1.06 min (ACN/buffer) = 30:70); λ_{max}: 231 nm, 319 nm, 382 nm.

*Methyl-2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)acetate (**4**)*

Synthesized according to general procedure **A** from **1** (325 mg, 898 µmol), potassium *tert*-butoxide (113 mg, 1.00 mmol) and methyl 2-bromoacetate (262 mg, 1.71 mmol). Crystallized from ethanol to yield a yellow solid (161 mg, 41%) Dec.: 300–301 °C; IR (KBr): 3308 cm^{-1} (NH), 1643 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 12.01 (s, 1H, indole-NH), 7.96 (d, 1H, *J* = 1.8 Hz, ArH), 7.74 (d, 1H, *J* = 8.4 Hz, ArH), 7.60 (d, 1H, *J* = 2.0 Hz, ArH), 7.50 (dd, 1H, *J* = 8.4 Hz, *J* = 2.0 Hz, ArH), 7.43 (d, 1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6 Hz, *J* = 1.9 Hz, ArH), 4.48 (s, 2H, -N^5-CH_2), 3.63 (s, 3H, -OCH_3), 3.98 and 3.14 (bs, 2H, azepine-CH_2) ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 51.9 (CH_3); 30.9, 52.2 (CH_2); 113.6, 120.7, 123.8, 124.9, 125.4, 128.6 (CH); 109.1, 111.9, 124.2, 127.8, 132.6, 132.9, 136.0, 140.4, 169.8, 170.4 (C); CHN: calculated C 52.62, H 3.25, N 6.46, found C 52.64, H 3.02, N 6.38; $C_{19}H_{14}BrClN_2O_3$ (433.68); MS (EI): m/z (%) = 434 $[M]^{+\bullet}$ (100), 405 $[M^{+\bullet}–29]$ (29), 361 $[M^{+\bullet}–73]$ (79); HPLC (isocratic): 98.2% at 254 nm, 97.2% at 280 nm, t_{M+S} = 4.00 min, t_M (DMSO) = 1.07 min (ACN/water 60:40); λ_{max}: 234 nm, 317 nm, 384 nm; HPLC (gradient, method B): 94.8% at 254 nm, t_{M+S} = 11.49 min, t_M (DMSO) = 1.07 min.

*Ethyl-2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)acetate (**5**)*

Synthesized according to general procedure **A** from **1** (649 mg, 1.80 mmol), potassium *tert*-butoxide (243 mg, 2.17 mmol) and ethyl 2-bromoacetate (534 mg, 3.20 mmol). Crystallized from ethanol to yield a colorless solid (380 mg, 47%). Mp. 289–294 °C; IR (KBr): 3317 cm^{-1} (NH), 1741 cm^{-1} (C=O), 1652 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 12.01 (s, 1H, indole-NH), 7.96 (d, 1H, *J* = 1.8 Hz, ArH), 7.74 (d, 1H, *J* = 8.4 Hz, ArH), 7.61 (d, 1H, *J* = 2.0 Hz, ArH), 7.50 (dd, 1H, *J* = 8.4, 2.1 Hz, ArH), 7.43 (d,

1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 4.49 (s, 2H, -N^5-CH_2), 4.05 (q, 2H, *J* = 7.1 Hz, -CH_2), 3.98 and 3.14 (bs, 2H, azepine-CH_2), 1.08 (t, 3H, *J* = 7.1 Hz, -CH_3); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 13.8 (CH_3); 30.8, 52.2, 60.6 (CH_2); 113.5, 120.6, 123.9, 124.8, 125.4, 128.6 (CH); 109.1, 111.8, 124.3, 127.8, 132.6, 133.0, 136.0, 140.4, 169.2, 170.3 (C); CHN: calculated C 53.65, H 3.60, N 6.26, found C 53.66, H 3.55, N 6.07; $C_{20}H_{16}BrClN_2O_3$ (447.71); MS (EI): m/z (%) = 448 $[M]^{+\bullet}$ (100), 419 $[M^{+\bullet}–29]$ (12), 361 $[M^{+\bullet}–87]$ (80); HPLC (isocratic): 98.0% at 254 nm, 97.7% at 280 nm, t_{M+S} = 5.16 min, t_M (DMSO) = 1.07 min (ACN/water 60:40), λ_{max}: 234 nm, 318 nm, 380 nm.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)acetohydrazide (**8**)*

A suspension of **5** (277 mg, 618 µmol) and hydrazine hydrate (600 µL, 12.4 mmol) in ethanol (6 mL) was refluxed for 8 h. After the reaction was cooled to room temperature stirring was continued for 12 h. Ice water was added and the precipitate was filtered off and crystallized from ethanol to yield a colorless solid (200 mg, 75%). Dec. starting at 317 °C; IR (KBr): 3315 cm^{-1} (NH), 1664 cm^{-1} (C=O), 1642 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 11.97 (s, 1H, indole-NH), 9.27 (s, 1H, hydrazide-NH), 7.96 (d, 1H, *J* = 1.9 Hz, ArH), 7.70–7.72 (m, 2H, ArH), 7.48 (dd, 1H, *J* = 8.4, 2.0 Hz, ArH), 7.42 (d, 1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, J = 8.6, 1.9 Hz, ArH), 4.29 (b, 5H, azepine-CH_2, -NH_2 and -N^5-CH_2), 3.11 (bs, 1H, azepine-CH_2); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 31.2, 52.3 (CH_2); 113.6, 120.7, 124.0, 124.9, 125.1, 128.5 (CH); 109.3, 111.8, 124.2, 127.7, 132.4, 133.0, 136.0, 141.1, 167.8, 170.0 (C); CHN: calculated C 49.85, H 3.25, N 12.92, found C 49.61, H 3.08, N 12.49; $C_{18}H_{14}BrClN_4O_2$ (433.69); MS (EI): m/z (%) = 434 $[M]^{+\bullet}$ (20), 403 $[M^{+\bullet}–31]$ (83), 361 $[M^{+\bullet}–73\ ]$ (43), 347 $[M^{+\bullet}–87]$ (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 431.99832, found 431.99759; HPLC

(isocratic): 99.4% at 254 nm, 99.9% at 280 nm, t_{M+S} = 5.42 min, t_M (DMSO) = 1.07 min (ACN/water 40:60); λ_{max}: 231 nm, 319 nm, 394 nm.

*tert-Butyl 4-[2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]-indol-5-yl)acetyl]piperazine-1-carboxylate (**22**)*

Synthesized according to general procedure **B** from **3** (410 mg, 977 µmol), DMF (2 mL), DIPEA (740 µL, 4.25 mmol), PyBOP (576 mg, 1.11 mmol) and 1-BOC piperazine (223 mg, 1.20 mmol). Crystallized from ethanol to yield a colorless solid (154 mg, 27%). Mp. 281–282 °C; IR (KBr): 3255 cm^{-1} (NH), 1640 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.95 (d, 1H, *J* = 1.9 Hz, ArH), 7.72 (d, 1H, *J* = 8.4 Hz, ArH), 7.56 (d, 1H, J = 2.1 Hz, ArH), 7.47 (dd, 1H, J = 8.4, 2.1 Hz, ArH), 7.42 (d, 1H, J = 8.7 Hz, ArH), 7.30 (dd, 1H, J = 8.6, 1.9 Hz, ArH), 4.50 and 4.72 (bs, 2H, -N^5-CH_2), 3.96 and 3.08 (bs, 2H, azepine-CH_2), 3.41 (d, 4H, *J* = 5.6 Hz, -CH_2), 3.33–3.20 (m, 4H, -CH_2), 1.40 (s, 9H, -CH_3); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 30.0 (3C) (CH_3); 31.1, 41.3 (2C), 44.0 (2C), 51.7 (CH_2); 113.5, 120.6, 123.9, 124.8, 125.0, 128.6 (CH); 79.1, 109.3, 111.8, 124.2, 127.7, 132.4, 133.1, 136.0, 140.8, 153.7, 166.5, 169.9 (C); CHN: calculated C 55.16, H 4.80, N 9.53, found C 55.24, H 4.78, N 9.43; $C_{27}H_{28}BrClN_4O_4$ (587.89); MS (EI): m/z (%) = 588 $[M]^{+\bullet}$ (65), 402 [$M^{+\bullet}$–186] (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 586.09770, found 586.09783; HPLC (isocratic): 99.0% at 254 nm, 99.2% at 280 nm, t_{M+S} = 4.78 min, t_M (DMSO) = 1.07 min (ACN/water 60:40); λ_{max}: 234 nm, 228 nm, 319 nm; HPLC (gradient method B): 98.6% at 254 nm, t_{M+S} = 12.03 min, t_M (DMSO) = 1.07 min.

*tert-Butyl {2-[2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]-indol-5-yl)acetamido]ethyl}carbamate (**29**)*

Synthesized according to general procedure **B** from **3** (402 mg, 957 µmol), DMF (2 mL), DIPEA (740 µL, 4.25 mmol), PyBOP (587 mg, 1.13 mmol) and BOC ethylenediamine (190 µL, 1.20 mmol). Crystallized from ethanol to yield a yellow solid (403 mg, 75%). Dec. starting at 265 °C; IR (KBr): 3336 cm^{-1} (NH), 1679 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 11.98 (s, 1H, indole-NH), 9.26 (t, 1H, *J* = 5.6 Hz, -NH), 7.99–7.92 (m, 1H, ArH), 7.72 (d, 1H, *J* = 8.4 Hz, ArH), 7.67 (d, 1H, *J* = 2.1 Hz, ArH), 7.48 (dd, 1H, *J* = 8.4, 2.1 Hz, ArH), 7.42 (d, 1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 6.82 (t, 1H, *J* = 5.6 Hz, -NH), 4.51–3.76 (m, 3H, -N^5-CH_2 and azepine-CH_2), 3.22–2.92 (m, 5H, -CH_2 and azepine-CH_2), 1.38 (s, 9H, -CH_3); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 28.8 (3C) (CH_3); 31.7, 39.5, 40.0, 54.1 (CH_2); 114.2, 121.3, 124.7, 125.5, 125.7, 129.2 (CH); 77.7, 109.3, 111.8, 124.1, 127.7, 132.4, 133.0, 136.0, 140.1, 168.3 (2C), 170.1 (C); CHN: calculated C 53.44, H 4.66, N 9.97, found C 53.42, H 4.62, N 9.87; $C_{25}H_{26}BrClN_4O_4$ (561.86); MS (EI): m/z (%) = 562 $[M]^{+\bullet}$ (4), 402 $[M^{+\bullet}–160]$ (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 560.08205, found 560.08265; HPLC (isocratic): 97.3% at 254 nm, 98.1% at 280 nm, t_{M+S} = 3.42 min, t_M (DMSO) = 1.07 min (ACN/water 60:40); λ_{max}: 240 nm, 317 nm; HPLC (gradient method B): 97.7% at 254 nm, t_{M+S} = 12.37 min, t_M (DMSO) = 1.07 min.

*N-(2-Aminoethyl)-2-(9-bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino-[4,5-b]indol-5-yl)acetamide hydrochloride (**27**)*

Synthesized according to general procedure **C** from **29** (350 mg, 623 µmol) CH_2Cl_2 (44.5 mL), TFA (9 mL), propan-2-ol (9 mL), 9 drops of propan-2-olic HCl and diethyl ether (40 mL). Deviating from the general procedure the mixture was kept at 8 °C over night before the precipitate was filtered off and dried. A colorless solid was yielded (285

mg, 99%). Dec. starting at 210 °C; IR (KBr): 3307 cm^{-1} (NH), 1655 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 12.13 (s, 1H, indole-NH), 9.26 (t, 1H, *J* = 5.6 Hz, -NH), 8.03 (m, 3H, $-NH_3^+$), 7.95 (d, 1H, *J* = 2.1 Hz, ArH), 7.75 (d, 1H, *J* = 8.5 Hz, ArH), 7.70 (d, 1H, *J* = 2.1 Hz, ArH), 7.48 (dd, 1H, *J* = 8.4, 2.1 Hz, ArH), 7.44 (d, 1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 4.61 (bs, 2H, -N^5-CH_2), 3.96 and 3.12 (bs, 2H, azepine-CH_2), 2.88 (t, 2H, *J* = 6.2 Hz, -CH_2); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 31.2, 36.4, 38.5, 53.7 (CH_2); 113.7, 120.6, 124.0, 124.2, 125.2, 128.7 (CH); 109.2, 111.8, 124.9, 127.7, 132.4, 133.0, 136.0, 140.1, 168.0, 170.2 (C); $C_{20}H_{19}BrCl_2N_4O_2$ (498.20); MS (EI): m/z (%) = 462 $[M]^{+\bullet}$ (8), 444 $[M^{+\bullet}–18]$ (47), 402 $[M^{+\bullet}–60]$ (32); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 460.02862, found 460.02980; HPLC (isocratic): 95.2% at 254 nm, 98.4% at 280 nm, t_{M+S} = 5.44 min, t_M (DMSO) = 1.07 min (ACN/buffer 30:70); λ_{max}: 236 nm, 228 nm, 318 nm.

*9-Bromo-3-chloro-5-[2-oxo-2-(piperazin-1-yl)ethyl]-7,12-dihydrobenzo[2,3]azepino-[4,5-b]indol-6(5H)-one hydrochloride (**20**)*

Synthesized according to general procedure **C** from **22** (110 mg, 187 µmol) CH_2Cl_2 (13.4 mL), TFA (2.7 mL), propan-2-ole (3 mL), 3 drops of propan-2-olic HCl and diethyl ether (20 mL). Deviating from the general procedure the suspension was kept at 8 °C over night before the precipitate was filtered off and dried. A colorless solid was yield (82 mg, 90%). Dec. starting at 240 °C; IR (KBr): 3410 cm^{-1} (NH), 1655 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 12.09 (s, 1H, indole-NH), 9.26 (bs, 2H, $-NH_2^+$), 7.95 (d, 1H, *J* = 1.9 Hz, ArH), 7.74 (d, 1H, *J* = 8.4 Hz, ArH), 7.56 (d, 1H, *J* = 2.1 Hz, ArH), 7.48 (dd, 1H, *J* = 8.4, 2.1 Hz, ArH), 7.43 (d, 1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 4.48 and 4.78 (bs, 2H, -N^5-CH_2), 3.96 and 3.08 (bs, 2H, azepine-CH_2), 3.68 (bs, 4H, -CH_2), 3.08–3.10 (m, 4H, -CH_2); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 31.1, 38.3, 41.3, 42.6 (2C), 51.7 (CH_2); 113.7, 120.6, 124.0, 124.2, 125.2,

128.7 (CH); 109.3, 111.8, 124.8, 127.7, 132.4, 133.1, 136.0, 140.8, 166.7, 170.0 (C); $C_{22}H_{21}BrCl_2N_4O_2$ (524.24); MS (EI): m/z (%) = 488 $[M]^{+\bullet}$ (19), 402 $[M^{+\bullet}–86]$ (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 486.04527, found 486.04545; HPLC (isocratic): 98.4% at 254 nm, 97.8% at 280 nm, t_{M+S} = 7.13 min, t_M (DMSO) = 1.07 min (ACN/buffer 30:70); λ_{max}: 232 nm, 319 nm, 395 nm.

*2-(9-Bromo-3-chloro-6-oxo-5,6,7,12-tetrahydrobenzo[2,3]azepino[4,5-b]indol-5-yl)-N-phenylacetamide (**19**)*

Synthesized according to general procedure **B** from **3** (218 mg, 519 µmol), DMF (1 mL), DIPEA (370 µL, 2.12 mmol), PyBOP (279 mg, 536 µmol) and aniline (54.8 µL, 598 µmol). Crystallized from ethanol to yield a colorless solid (157 mg, 61%). Dec. starting at 280 °C; IR (KBr): 3311 cm^{-1} (NH), 1672 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 12.03 (s, 1H, indole-NH), 10.22 (s, 1H, -NH), 7.98 (d, 1H, *J* = 2.0 Hz, ArH), 7.76-7.71 (m, 2H, ArH), 7.63–7.58 (m, 2H, ArH), 7.50 (dd, 1H, *J* = 8.4, 2.1 Hz, ArH), 7.44 (d, 1H, J = 8.6 Hz, ArH), 7.36–7.28 (m, 3H, ArH), 7.10–7.02 (m, 1H, ArH), 4.49 (b, 2H, -N^5-CH_2) 4.00 and 3.14 (bs, 2H, azepine-CH_2); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 31.1, 54.2 (CH_2); 113.6, 119.0 (2C), 120.7, 123.3, 124.2, 124.9, 125.2, 128.6, 128.8 (2C) (CH); 109.3, 111.9, 124.0, 127.8, 132.5, 133.0, 136.0, 138.8, 141.0, 167.2, 170.3 (C); CHN: calculated C 58.26, H 3.46, N 8.49, found C 58.08, H 3.58, N 8.30; $C_{24}H_{17}BrClN_3O_2$ (494.77); MS (EI): m/z (%) = 495 $[M]^{+\bullet}$ (42), 402 $[M^{+\bullet}–93]$ (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 495.01669, found 495.01659; HPLC (isocratic): 97.2% at 254 nm, 97.3% at 280 nm, t_{M+S} = 4.47 min, t_M (DMSO) = 1.07 min (ACN/water 60:40); λ_{max}: 255 nm, 316 nm, 393 nm; HPLC (gradient method B): 98.2% at 254 nm, t_{M+S} = 12.94 min, t_M (DMSO) = 1.07 min.

*9-Bromo-3-chloro-5-[2-(4-methylpiperazin-1-yl)-2-oxoethyl]-7,12-dihydrobenzo[2,3] azepino[4,5-b]indol-6(5H)one (**21**)*

Synthesized according to general procedure **B** from **3** (205 mg, 488 µmol), DMF (1 mL), DIPEA (370 µL, 2.12 mmol), PyBOP (285 mg, 548 µmol) and 1-methylpiperazine (67 µL, 0.60 mmol). Crystallized from ethanol to yield a yellow solid (33.6 mg, 14%). Dec. starting at 211 °C; IR (KBr): 3289 cm^{-1} (NH), 1643 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.95 (d, 1H, *J* = 1.9 Hz, ArH), 7.72 (d, 1H, *J* = 8.4 Hz, ArH), 7.53 (d, 1H, *J* = 2.1 Hz, ArH), 7.47 (dd, 1H, *J* = 8.4, 2.1 Hz, ArH), 7.42 (d, 1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 4.32 and 4.71 (bs, 2H, -N^5-CH_2), 3.96 and 3.08 (bs, 2H, azepine-CH_2), 3.48–3.35 (m, 4H, -CH_2), 2.30–2.19 (m, 4H, -CH_2), 2.16 (s, 3H, -CH_3); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 45.6 (CH_3); 31.1, 41.5, 44.2, 51.7, 54.3, 54.5 (CH_2); 113.6, 120.6, 123.9, 124.8, 125.0, 128.6 (CH); 109.3, 111.8, 124.2, 127.7, 132.6, 133.1, 136.0, 140.9, 166.1, 169.8 (C); CHN: calculated C 55.05, H 4.42, N 11.17, found C 54.88, H 4.36, N 10.77; $C_{23}H_{22}BrClN_4O_2$ (501.80); MS (EI): m/z (%) = 502 $[M]^{+\bullet}$ (19), 402 $[M^{+\bullet}–100]$ (38); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 500.06092, found 500.06046; HPLC (isocratic): 99.6% at 254 nm, 99.8% at 280 nm, t_{M+S} = 7.64 min, t_M (DMSO) = 1.07 min (ACN/buffer 30:70); λ_{max}: 232 nm, 319 nm.

*9-Bromo-3-chloro-5-[2-oxo-2-(piperidin-1-yl)ethyl]-7,12-dihydrobenzo[2,3]azepino-[4,5-b]indol-6(5H)-one (**24**)*

Synthesized according to general procedure **B** from **3** (211 mg, 503 µmol), DMF (1 mL), DIPEA (370 µL, 2.12 mmol), PyBOP (291 mg, 559 µmol) and piperidine (59.2 µL, 601 µmol). Crystallized from ethanol to yield a colorless solid (123 mg, 50%). Dec. starting at 180 °C; IR (KBr): 3411 cm^{-1} (NH), 1644 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.95 (d, 1H, *J* = 1.9 Hz, ArH), 7.72 (d,

1H, *J* = 8.4 Hz, ArH), 7.52 (d, 1H, *J* = 2.1 Hz, ArH), 7.47 (dd, 1H, *J* = 8.2, 2.1 Hz, ArH), 7.42 (d, 1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 4.32 and 4.79 (bs, 2H, -*N*^{5}-CH_2), 3.41 (m, 4H, -CH_2), 1.57 (m, 2H, -CH_2), 1.44 (m, 4H, -CH_2); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 23.9, 25.3, 25.9, 31.2, 42.6, 45.3, 51.8 (CH_2); 113.5, 120.6, 123.7, 124.8, 124.9, 128.5 (CH); 109.3, 111.8, 124.1, 127.8, 132.4, 133.1, 136.0, 141.0, 165.8, 169.8 (C); $C_{23}H_{21}BrClN_3O_2$ (486.79); MS (EI): m/z (%) = 487 $[M]^{+\bullet}$ (11), 402 [$M^{+\bullet}$–85] (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 485.05002, found 485.05039; HPLC (isocratic): 99.0% at 254 nm, 99.1% at 280 nm, t_{M+S} = 8.58 min, t_M (DMSO) = 1.07 min (ACN/water 60:40); λ_{max}: 232 nm, 319 nm; HPLC (gradient method B): 96.1% at 254 nm, t_{M+S} = 12.39 min, t_M (DMSO) = 1.07 min.

*9-Bromo-3-chloro-5-(2-morpholino-2-oxoethyl)-7,12-dihydrobenzo[2,3]azepino[4,5-b]-indol-6(5H)-one (**26**)*

Synthesized according to general procedure **B** from **3** (202 mg, 481 µmol), DMF (1 mL), DIPEA (370 µL, 2.12 mmol), PyBOP (292 mg, 561 µmol) and morpholine (52.4 µL, 599 µmol). Crystallized from diethyl ether to yield a colorless solid (166 mg, 70%). Mp. 221-223 °C; IR (KBr): 3217 cm^{-1} (NH), 1652 cm^{-1} (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 12.00 (s, 1H, indole-NH), 7.95 (d, 1H, *J* = 1.9 Hz, ArH), 7.72 (d, 1H, *J* = 8.4 Hz, ArH), 7.55 (d, 1H, *J* = 2.1 Hz, ArH), 7.48 (dd, 1H, *J* = 8.4, 2.1 Hz, ArH), 7.42 (d, 1H, *J* = 8.7 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 4.41 and 4.77 (bs, 2H, -*N*^{5}-CH_2), 3.55 (t, 4H, *J* = 4.8 Hz, -CH_2), 3.42 (m, 4H, -CH_2); ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 31.1, 41.9, 44.8, 51.7, 65.9, 66.1 (CH_2); 113.6, 120.7, 124.0, 124.2, 125.1, 128.6 (CH); 109.3, 111.8, 124.8, 127.8, 132.4, 133.1, 136.0, 140.9, 166.5, 169.9 (C); $C_{22}H_{19}BrClN_3O_3$ (488.76); MS (EI): m/z (%) = 489 $[M]^{+\bullet}$ (16), 402 [$M^{+\bullet}$–87] (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 487.02928, found 487.02970; HPLC (isocratic): 98.0% at 254 nm, 97.3% at 280 nm, t_{M+S} = 4.53 min, t_M (DMSO) = 1.07 min

(ACN/water 50:50); λ_{max}: 232 nm, 319 nm, 391 nm; HPLC (gradient method B): 95.9% at 254 nm, t_{M+S} = 11.81 min, t_M (DMSO) = 1.07 min.

*9-Bromo-3-chloro-5-[2-oxo-2-(pyrrolidin-1-yl)ethyl]-7,12-dihydrobenzo[2,3]azepino-[4,5-b]indol-6(5H)-on (**25**)*

Synthesized according to general procedure **B** from **3** (218 mg, 519 µmol), DMF (1 mL), DIPEA (370 µL, 2.12 mmol), PyBOP (284 mg, 546 µmol) and pyrrolidine (49.4 µL, 600 µmol). Crystallized from ethanol/diethyl ether 1:1 to yield a yellow solid (153 mg, 63%). Dec. starting at 320 °C; IR (KBr): 3385 cm^{-1} (NH), 1625 cm^{-1} (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 11.99 (s, 1H, indole-NH), 7.95 (d, 1H, *J* = 2.0 Hz, ArH), 7.72 (d, 1H, *J* = 8.4 Hz, ArH), 7.63 (d, 1H, *J* = 2.1 Hz, ArH), 7.47 (dd, 1H, *J* = 8.4, 2.1 Hz, ArH), 7.42 (d, 1H, *J* = 8.6 Hz, ArH), 7.30 (dd, 1H, *J* = 8.6, 1.9 Hz, ArH), 4.29 and 4.68 (bs, 2H, -N^5-CH_2) 3.40 (t, 2H, *J* = 6.8 Hz, -CH_2), 3.31 (t, 2H, *J* = 6.7 Hz, -CH_2), 1.88–1.92 (m, 2H, -CH_2), 1.75–1.78 (m, 2H, -CH_2); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ (ppm) = 23.7, 25.6, 31.1, 45.0, 45.7, 52.9 (CH_2); 113.6, 120.7, 124.1, 124.8, 125.1, 128.5 (CH); 109.4, 111.8, 124.1, 127.8, 132.4, 133.1, 136.0, 141.1, 166.0, 169.9 (C); $C_{22}H_{19}BrClN_3O_2$ (472.76); MS (EI): m/z (%) = 473 $[M]^{+\bullet}$ (11), 402 $[M^{+\bullet}–71]$ (100); HRMS (EI): m/z $[M]^{+\bullet}$ calculated 471.03437, found 471.03434; HPLC (isocratic): 96.5% at 254 nm, 95.5% at 280 nm, t_{M+S} = 6.18 min, t_M (DMSO) = 1.07 min (ACN/water 50:50); λ_{max}: 232 nm, 319 nm; HPLC (gradient method B): 97.0% at 254 nm, t_{M+S} = 11.17 min, t_M (DMSO) = 1.07 min.

References

Benítez D, Medeiros A, Fiestas L, Panozzo-Zenere E A, Maiwald F, Prousis KC, et al.Identification of Novel Chemical Scaffolds Inhibiting Trypanothione Synthetase from Pathogenic Trypanosomatids. PLoS Negl Trop Dis. 2016; 10: e0004617.

Orban OC, Korn RS, Unger L, Yildiz A, Kunick C. 3-Chlorokenpaullone. Molbank. 2015; M856.

Orban OC, Korn RS, Benítez D, Medeiros A, Preu L, Loaëc N, et al. 5-Substituted 3-chlorokenpaullone derivatives are potent inhibitors of *Trypanosoma brucei* bloodstream forms. Bioorg Med Chem. 2016; 16: 3790-3800.

Stuhlmann F, Jäger T, Flohé L, Schinzer D. N^5-substituted 2-(6-oxo-6,7-dihydro-5H-benzo[2,3]azepino [4,5-b]indol-5-yl)-acetamides for treating tropical diseases. EP 1 757 607 A1. 2007. Feb 28. http://www.google.com/patents/EP1757607A1?cl=en