Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Rhodanine carboxylic acids as novel inhibitors of histone acetyltransferases

Electronic supplementary information

Silviya D. Furdas[a], Suhaib Shekfeh[b], Srinivasaraghavan Kannan[b], Wolfgang Sippl[b], Manfred Jung[a*]

[a]Institute of Pharmaceutical Sciences, Albert-Ludwigs-Unversity of Freiburg, Albertstr. 23, 79104 Freiburg, Germany. E-mail: manfred.jung@pharmazie.uni-freiburg.de; Fax: +49-761-203-6321; Tel: +49-761-203-4896

[b]Department of Pharmaceutical Chemistry, Martin-Luther University of Halle-Wittenberg, Germany

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1. Computational methods and results

All calculations were performed on a Pentium IV 2.2 GHz based Linux cluster (20 CPUs). The GOLD software[1] (Cambridge Crystallographic Data Centre) was used for docking, whereas the calculation of all molecular descriptors and the analysis of the docking results were carried out in MOE2008.10 (Chemical Computing Group).[2]

1.1. Virtual Screening

MACCS Structural Keys (feature list version) were used to filter compounds from 21 commercial chemical databese (Acb-eurochem, Ambinter, Asinex, Chembridge, ChemDiv, Com-Genex, Enamine, Ibscreen, Interchem, Keyorganics, Life Chemicals, Maybridge, Nanosyn, NCI, Otava, PeakDale, PHARMEKS, PUBCHEM, Ryan-Scientific, Sigma-Aldrich, Spec, UkrOrgSynth). MACCS fingerprints generated from published rhodanine-indolinone AANAT inhibitors were used as a search query to identify chemically similar compounds. A tanimoto coefficient of 0.85 was applied. The similarity based screen identified 6423 compounds by taking into consideration the Lipinsky rule of five.[3]

1.2. Ligand docking

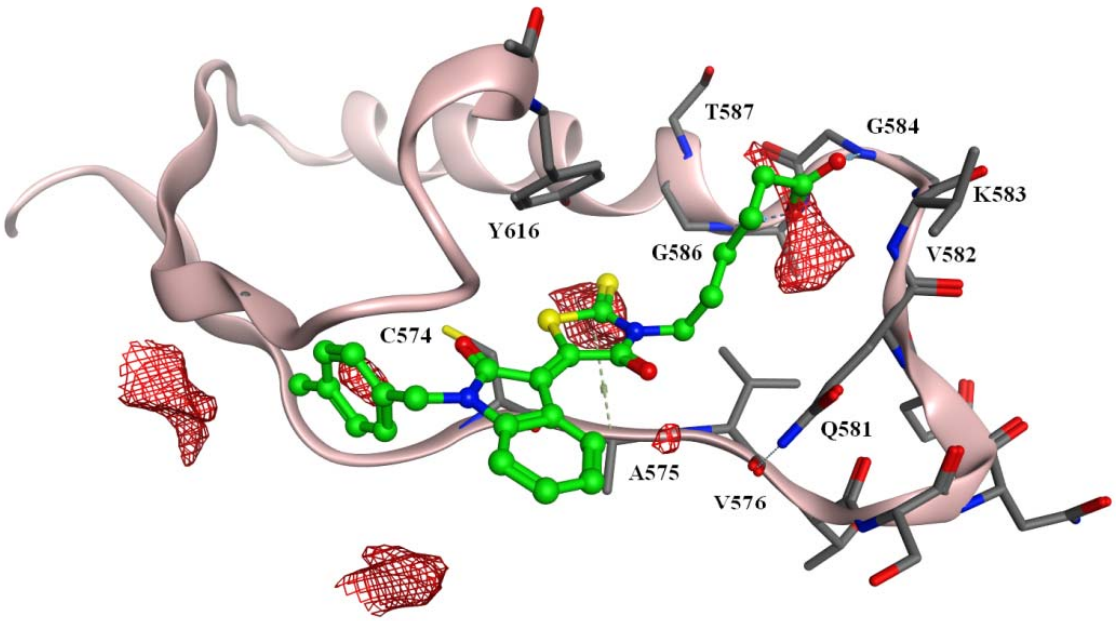
The crystal structure of human PCAF in complex with acetylCoA (pdb code: 1CM0, chain B) resolved at 2.30 Å was taken from the Protein Data Bank. The cofactor and the water molecules were removed, hydrogen and missing heavy atoms were added using MOE 2008.10. The pK_a prediction was made using the H++ server[4] to determine the tautomeric state of histidine residues. Docking of the ligands was carried out using the GOLD 4.0 program with default settings. A sphere of 20 Å around the oxygen atom of Tyr616 was defined for ligand docking. For each ligand a maximum number of 10 conformations was allowed. To test the applicability of the docking tool, a control docking was carried out with the cofactor acetylCoA. Using Goldscore as scoring function an RMSD value of 1.45 was

derived for the top-ranked conformation of acetylCoA (data not shown). For all compounds under study the Goldscore was calculated and analyzed.

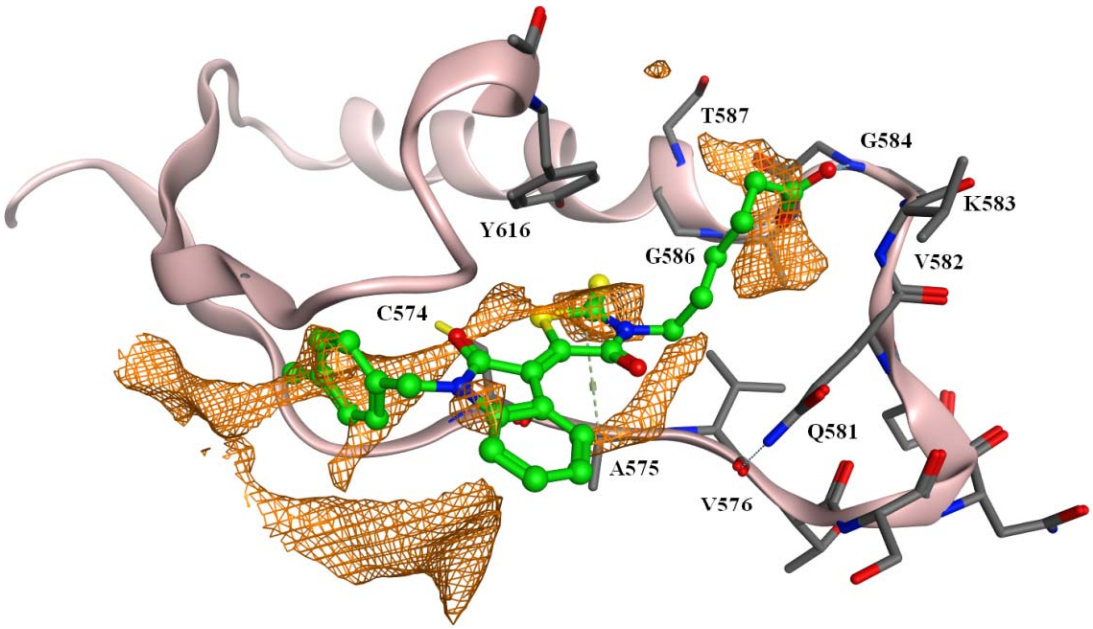
To support the obtained docking poses we calculated the molecular interaction fields for the PCAF binding pocket. The calculations were carried out using program MOE2008.10. The favourable interaction field of a carboxylate probe (Fig. S1a) as well as of a hydrophobic methyl (C3) probe was calculated. The results agree well with the location of the carboxylic head group as well as the hydrophobic parts of the active inhibitors (exemplarily the docking pose of compound **12e** is shown in Figure S1).

Figure S1. Molecular interaction fields calculated for the PCAF binding pocket. In comparison the predicted binding mode of the active inhibitor **12e** (colored green) is shown. Molecular interaction fields were calculated for the binding pocket using ***a)*** a carboxylate probe (contour level -4.5 kcal/mol, colored red), ***b)*** a hydrophobic methyl (C3) probe (contour level -2.5 kcal/mol, colored orange).

a)



b)

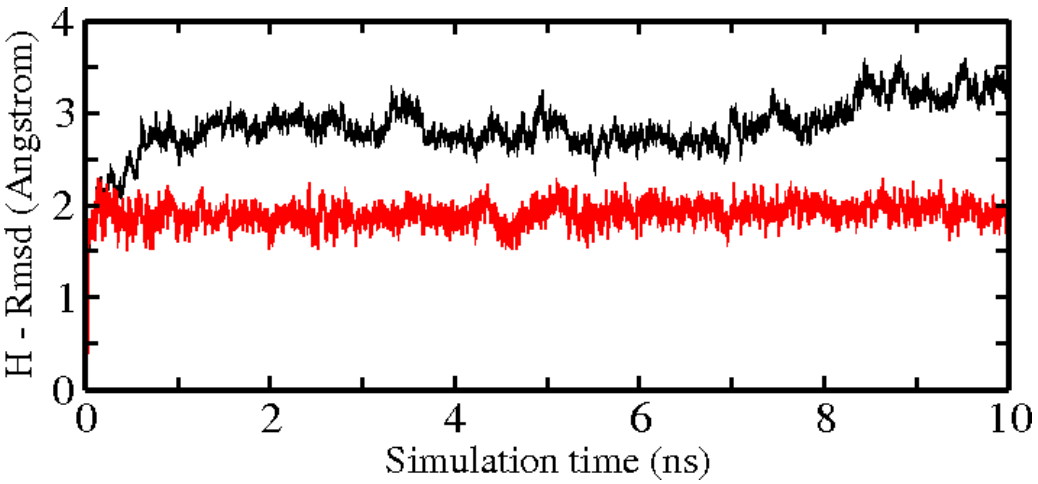


1.3. MD simulations and results

Molecular dynamics was carried out using AMBER 9.0[5] program. Protein and ligand molecule were parameterized using AMBER ff033[6] and general AMBER force field (GAFF)[7] respectively. The initial structures of the PCAF–inhibitor complexes were taken from the GOLD docking study. Complex systems were neutralized with 8 Cl^- counterions by using the xleap module of AMBER 9.0. The structures were solvated in an octahedral box with TIP3P[8] water molecules leaving at least 10 Å between the solute atoms and the borders of the box. The fully solvated and neutralized systems were subjected to energy minimization with the sander module of the AMBER 9.0 package. Following minimization the systems were gradually heated from 50 to 300 K with positional restraints (force constant: 50 kcal/mol/Å) on protein-ligand complex over a period of 0.25 ns allowing water molecules and ions to move freely. A 9 Å cutoff for the short-range nonbonded interactions was used in combination with the particle mesh Ewald option[9] using a grid spacing of ~0.9 Å to account for long-range electrostatic interactions. The Settle algorithm[10] was used to constrain bond

vibrations involving hydrogen atoms. During additional 0.25 ns the positional restraints were gradually reduced to allow finally unrestrained MD simulation of all atoms over a subsequent equilibration time of 1 ns. Further 10 ns free MD simulations were carried using these equilibrated structures as a start structures. VMD[11] was used for visualization of trajectories and preparation of figures. The derived RMSD plot (calculated for heavy atoms of PCAF protein and ligand **12e**, respectively) in Figure S2 shows that the complex as well as the interaction of the inhibitor with the protein is stable over the whole simulation time of 10 ns. Similar results were obtained for compounds **14** and **15** (data not shown).

Figure S2. Heavy atom root mean square deviation (H-Rmsd) of PCAF protein (black) and ligand (**12e**, red) conformations sampled during 10 ns MD simulation with respect to the initial structure *versus* simulation time.



2. *In vitro* screening

In-vitro screening is performed using antibody-based assay with time-resolved detection mode described in **2.1.** with the following four members of the histone acetyltransferase family: $PCAF_{aa493-658}$ *(KAT2B)* (enzyme preparation obtained in our lab according to the protocol, described in **2.2.**), $Gcn5_{aa362-837}$ *(KAT2A)* (BPS Bioscience, #50070, Lot: 100329), $CBP_{aa1319-1710}$ *(KAT3A)* (Biomol, #SE-452, Lot: T6533), and $p300_{aa965-1810}$ *(KAT3B)* (Active Motif , #31205, Lot: 30910007).

2.1. *In vitro* time-resolved fluorescence immunosorbent histone acetyltransferase assay

The heterogeneous assay is performed in streptavidin-coated 96-well plates (Nunc, #436022). After each incubation step (1 hour at 30 °C), six washing steps as well as a prewash step are necessary to remove the non bound fraction using wash buffer pH 7.5 (0.1% (v/v) Tween 20, 150 mM NaCl, 20 mM Tris base, 80 mM Tris-HCl) with 300 µL/step in an overflow mode (Tecan Columbus Plate washer). In the first incubation step, 20 pmol/well biotinylated histone peptide residues 1-21 of human histone H3 (Millipore, #12-403) diluted in the same buffer as mentioned above, is bound to the wells. In the second step, the bound substrate is turned over in an enzymatic reaction. For this, preincubation of 10 µL/well histone acetyltransferase (equal to 57 ng PCAF enzyme preparation (see below), triplicate, 50 ng Gcn5 *(BPS Bioscience, Lot: 100329)*, duplicate, 100 ng CBP *(Biomol, Lot: T6533)*, duplicate, or 10 ng p300 *(Active Motif, Lot: 30910007)*, duplicate) diluted in HAT-buffer pH 7.5 (0.1% (w/v) BSA (protease-free), 0.8% (v/v) Triton X-100, 100 mM HEPES), 2 µL of inhibitor DMSO-solution, and 18 µL of the HAT-buffer is performed for 10 minutes at 30 °C. Controls are treated in the same manner, using DMSO instead of the inhibitor solution. Then, each mixture is transferred to the plate. To start the enzymatic reaction, 10 µL/well of a 400 µM acetyl-CoA solution in bidest. water is added to each well (final assay concentration 100 µM acetyl-CoA). The amount of the enzymatic turnover is detected by a primary rabbit IgG antibody

against the modification. 100 µL/well of anti-acetyl-histone H3 (Millipore, #06-599) is added in appropriate dilutions in Tris-buffer pH 7.5 (0.1% (v/v) Tween 20, 0.5% (w/v) BSA (protease-free), 150 mM NaCl, 20 mM Tris base, 80 mM Tris-HCl). In the next step incubation with 100 µL/well of a N1-Eu-labelled anti-rabbit secondary antibody (Perkin-Elmer, #AD0105), diluted in the same Tris-buffer as the first antibody, is followed. The europium label is cleaved off by adding of 100 µL/well of enhancement solution (Perkin-Elmer, #1244-105). After incubation for 10 minutes at room temperature, time-resolved fluorescence measurement at $\lambda_{ex}/\lambda_{em}$: 340/615 nm (BMG Polarstar) is performed for the quantification. Readout data (RFU relative fluorescence units) were used to determine HAT inhibition of the tested compound according to the equation shown below. Computed inhibition was plotted against logarithm of compound concentration using GraphPad Prism 4.00 Software in order to obtain IC_{50} values. DMSO alone has no significant HAT inhibition and thus calculated values are not DMSO-corrected.

$$HAT\ inhibition\,[\%] = \frac{(RFU_{inhibitor} - RFU_{positive\ control})}{(RFU_{buffer\ control} - RFU_{positive\ control})}$$

2.2. Enzyme preparation for PCAF

The plasmid construct pQE80_P/CAF1.16 (5.2 kb, PCAF residues 1923-2420 (a gift of Prof. John M. Denu, University of Wisconsin, Madison) was transformed for propagation and maintenance in competent *Escherichia coli (TG1)*. The recombinant HAT domain of PCAF (amino acid residues 493-657) was overexpressed as *N*-terminal His-tagged fusion protein in ampicillin-sensitive *Escherichia coli (BL21 D3)* and 1 L LB medium. After IPTG induction at 18 ºC overnight,[12-14] bacterial cells were centrifuged for 15 minutes (8000 rcf, 4 ºC) and the supernatant was removed. The cell pellet was resuspended in 40 mL of lysis buffer pH 8.0 (1 mg/mL lysozyme, 0.1 mM phenylmethanesulfonyl fluoride, 1 mM β-mercaptoethanol, 300 mM NaCl, 50 mM Tris base). After 30 minutes incubation and following sonification (5fold

for 15 seconds, 60% output), the cell debris were removed by centrifugation (9000 rcf, 4 ºC) in order to obtain clear supernatant. Protein purification was performed using Ni-NTA superflow resin (Qiagen) according to the supplier´s instructions. Prewashing of the beads with lysis buffer pH 8.0 (300 mM NaCl, 50 mM Tris base) was repeated twice and subsequently the collected supernatant was placed on the resin. After washing with 40 mL of wash buffer pH 8.0 (1 mM β-mercaptoethanol, 10 mM imidazole, 300 mM NaCl, 50 mM Tris base), the enzyme elution was performed according to a replacement from the resin with elution buffer pH 8.0 (1 mM β-mercaptoethanol, 500 mM imidazole, 300 mM NaCl, 50 mM Tris base). Sephadex™ G-25 PD-10 Desalting column (Amershem Biosciences) was used according to the technical instructions for buffer exchange against storage buffer pH 6.0 (1 mg/mL BSA, 10% (v/v) glycerol, 20 mM trisodium citrate, 250 mM NaCl). In order to verify the purity and the presence of a 6xHis-tagged protein, SDS-PAGE and immunodetection with HRP mouse anti-6xHis antibody (BD Pharmingen, #552565) respectively were performed under standard conditions. Protein quantification (12.6 mg enzyme from 1 L LB medium) was determined using Pierce® BCA Protein Assay Kit (Pierce Biotechnology, #22237). Samples with high specific histone acetyltransferase activity were stored in aliquots at -80 ºC and used for the enzyme inhibition assay described above.

3. Synthetic procedures

All reactions were carried out employing standard chemical techniques. All chemicals were purchased from Acros, Sigma Aldrich, or Fluka at the highest available quality and were used without further purification. Solvents in technical grade were purified by distillation and dried before use according to standard procedures. Other solvents used for synthesis were HPLC grade. Merck silica gel 60 was used for flash chromatography with the given eluents. All products were dried in high vacuum with a liquid nitrogen cold trap.

3.1. Synthetic procedure 1: *N*-alkylation of isatin[15]

Isatin (1*H*-indole-2,3-dione) (30 mmol, 4.4 g) was suspended in 100 mL of acetonitrile. After addition of potassium carbonate (60 mmol, 8.3 g) and an appropriately substituted alkyl iodide (33 mmol) the preparation was stirred at room temperature overnight. Starting material consumption was monitored by TLC and the reaction was quenched with deionised water after certain reaction time. The aqueous phase was extracted with ethyl acetate. The organic layer was washed consecutive three times with 5% (w/v) sodium bicarbonate solution, deionised water and brine, dried then after over anhydrous sodium sulphate and concentrated *in vacuo*. The crude product was recrystallized from ethyl acetate with petrol ether (80-100 ºC).

3.2. Synthetic procedure 2: *N*-benzylation of isatin[15]

Isatin (1*H*-indole-2,3-dione) (30 mmol, 4.4 g) was suspended in 100 mL of acetonitrile. After addition of potassium carbonate (60 mmol, 8.3 g), potassium iodide (3 mmol, 0.5 g), and an appropriately substituted benzyl chloride (33 mmol), the preparation was stirred at room temperature overnight. Starting material consumption was monitored by TLC and the reaction was quenched with deionised water after the specified reaction time. The workup was performed in the analogy of the synthetic procedure 1. The crude product was recrystallized from ethyl acetate with petrol ether (80-100 ºC).

3.3. Synthetic procedure 3: preparation of rhodanine-carboxylic acids with open chain aliphatic spacer[16]

An ω-amino acid (40 mmol) with appropriate chain length was dissolved in 30 mL of 22% (w/v) potassium hydroxide (6.2 g) solution in deionised water. To the stirred solution carbon disulfide (43 mmol, 3.2 g, 2.6 mL) was added slowly, while the temperature was monitored to not exceed over 25 ºC. Stirring was then continued for 1.5 hours at room temperature.

Subsequently, monochloracetic acid (40 mmol, 3.8 g) was added in portions over 20 minutes. After 30 minutes of further stirring, the mixture was acidified with concentrated sulphuric acid (3.6 mL). The precipitation of the crude product was induced at room temperature overnight by gentle stirring. The precipitate was then filtered and washed with deionised water until the filtrate was not acidic anymore. The obtained solid material was dried in high vacuum.

3.4. Synthetic procedure 4: preparation of rhodanine-carboxylic acids with rigid cyclic spacer[17]

para-Aminomethyl benzene- or *trans*-aminomethyl cyclohexane-carboxylic acid (20 mmol) was dissolved in a solution of sodium carbonate (0.2 g) in 60 mL deionised water. The mixture was then heated on water bath (~100 ºC). Then, bis(carboxymethyl)trithiocarbonate (20 mmol, 4.5 g) was added and the batch was refluxed overnight. The preparation was cooled to room temperature and acidified with dilute sulphuric acid at pH 1-2, while precipitation of a solid material was observed. The precipitate was filtered, washed with deionised water, and dried.

3.5. Synthetic procedure 5: Knoevenagel-type condensation[18]

A reaction mixture consisting of equimolar amounts (2 mmol) of a rhodanine carboxylic acid or the corresponding methyl ester and an *N*-substituted indolinone derivative in 40 mL of absolute ethanol (10 mL per 1 mmol reagent) was refluxed at 120-150 ºC. The reaction progress was monitored by TLC and, if necessary, vaporized absolute ethanol was supplemented. After complete consumption of the starting materials (given reaction time), the solvent was concentrated *in vacuo*. The crude product was suspended in ethyl acetate and subsequently evaporated to dryness. The condensation product was obtained by recrystallization from the reported solvents.

4. Inhibitor synthesis and characterisation

Melting points were obtained after measurement with a SMP2 (Stuart Scientific) and are uncorrected. NMR spectra were recorded on an Avance DRX 400 MHz spectrometer (Bruker) or a Unity 300 MHz spectrometer (Varian) respectively. NMR spectra interpretation was performed by means of the SpinWorks 2.4 Software. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent peaks for ^{1}H and ^{13}C NMR spectra ($(CD_3)_2SO$: ^{1}H δ = 2.50, ^{13}C δ = 39.92; $CDCl_3$: ^{1}H δ = 7.29, ^{13}C δ = 76.98). Multiplicity is declared as follows: s = singlet, d = doublet, dd = double doublet, pd = pseudo-doublet, t = triplet, pt = pseudo-triplet, q = quartet, m = multiplet, b = broad, *a* = axial, *e* = equatorial. The coupling constants (*J*) are reported in Hertz. Clear signal assignment was performed with the aid of two-dimensional NMR experiments. EI- and CI-mass spectra were measured with a TSQ700 mass spectrometer (Thermoelectron), ESI- and APCI-mass spectra were recorded with a LCQ-Advantage mass spectrometer. Microanalyses were performed with Vario-EL (Elementaranalysensysteme). HPLC data for purity verification were obtained with Alliance Equipment (Waters, 2695 Separations Module, 2487 Dual λ Absorbance Detector). Quantification was performed by determination of the peak area using the Millenium[32] Software (Waters). HPLC analysis was performed under following conditions: i) Synergi^{TM}, 4 µm, Polar-RP, 80Å LC column, 250 x 4.6 mm (Phenomenex) or ii) Synergi^{TM}, 4 µm, Hydro-RP, 80Å LC column, 150 x 4.6 mm (Phenomenex); gradient mode elution (CH_3CN (0.05% (v/v) TFA) and H_2O (0.05% (v/v) TFA)); flow rate: 1 mL/min; detection mode: UV absorption: λ = 254 nm). The samples for the HPLC analysis were solved in suitable solvent (acetonitrile, methanol, or dimethyl sulphoxide, concentration: 1 mg/mL).

4.1. 1-Methylindoline-2,3-dione (6a)

Starting out from isatin (30 mmol, 4.4 g) and iodomethane (33 mmol, 4.7 g, 2.1 mL) according to synthetic procedure 1, **6a** (1-methylindoline-2,3-dione) was obtained after ~44 hours reaction time as red, needle-like crystals. Yield: 27 mmol, 4.4 g, 91%; $C_9H_7NO_2$, *MW* 161.16; mp 136 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.70-7.66 (m, $1H_{ind}$, 4-H), 7.55-7.53 (m, $1H_{ind}$, 6-H), 7.15-7.11 (m, $2H_{ind}$, 5/7-H), 3.14 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 183.87 (C=O, 3-C_{ind}), 158.59 (C=O, 2-C_{ind}), 151.78 (C_q, 7'-C_{ind}), 138.58 (CH_{ar}, 6-C_{ind}), 124.65 (CH_{ar}, 4-C_{ind}), 123.60 (CH_{ar}, 5-C_{ind}), 117.80 (C_q, 3'-C_{ind}), 110.98 (CH_{ar}, 7-C_{ind}), 26.43 (CH_3); MS (EI direct mode): *m/z (%)* 161.0 $[M]^+$ (100).

4.2. 1-Ethylindoline-2,3-dione (6b)

Starting out from isatin (30 mmol, 4.4 g) and iodoethane (33 mmol, 5.2 g, 2.7 mL) according to synthetic procedure 1, **6b** (1-ethylindoline-2,3-dione) was obtained after ~48 hours reaction time as red, cubic crystals. Yield: 4.8 mmol, 0.9 g, 16%; $C_{10}H_9NO_2$, *MW* 175.19; mp 96 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.67 (dt, ^3J = 7.8 Hz, ^4J = 1.3 Hz, $1H_{ind}$, 4-H), 7.55 (dd, ^3J = 7.4 Hz, ^4J = 0.8 Hz, $1H_{ind}$, 6-H), 7.20 (d, ^3J = 8.0 Hz, $1H_{ind}$, 7-H), 7.13 (dt, ^3J = 7.5 Hz, ^4J = 0.7 Hz, $1H_{ind}$, 5-H), 3.71 (q, ^3J = 7.2 Hz, 2H, CH_2), 1.19 (t, ^3J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 184.06 (C=O, 3-C_{ind}), 158.17 (C=O, 2-C_{ind}), 150.80 (C_q, 7'-C_{ind}), 138.59 (CH_{ar}, 6-C_{ind}), 124.91 (CH_{ar}, 4-C_{ind}), 123.49 (CH_{ar}, 5-C_{ind}), 117.92 (C_q, 3'-C_{ind}), 111.02 (CH_{ar}, 7-C_{ind}), 34.70 (CH_2), 12.70 (CH_3); MS (EI direct mode): *m/z (%)* 175.1 $[M]^+$ (100).

4.3. 1-Benzylindoline-2,3-dione (7a)

Starting out from isatin (30 mmol, 4.4 g) and benzyl chloride (33 mmol, 4.2 g, 3.8 mL) according to synthetic procedure 2, **7a** (1-benzylindoline-2,3-dione) was after ~60 hours reaction time obtained as orange, needle-like crystals. Yield: 12 mmol, 2.9 g, 40%;

$C_{15}H_{11}NO_2$, *MW* 237.26; mp 138 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.60-7.56 (m, $2H_{ind}$, 4/6-H), 7.45-7.42 (m, $2H_{bn}$, 2"/6"-H), 7.37-7.33 (m, $2H_{bn}$, 3"/5"-H), 7.31-7.27 (m, $1H_{bn}$, 4"), 7.12 (dt, ^3J = 7.6 Hz, ^4J = 0.8 Hz, $1H_{ind}$, 5-H), 6.99-6.97 (m, $1H_{ind}$, 7-H), 4.92 (s, 2H, *Bn*-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 183.50 (C=O, 3-C_{ind}), 158.73 (C=O, 2-C_{ind}), 150.78 (C_q, 7'-C_{ind}), 138.39 (CH_{ar}, 6-C_{ind}), 135.95 (C_q, 1"-C_{bn}), 129.07 (2 x CH_{ar}, 3"/5"-C_{bn}), 127.96 (C_q, 4"-C_{bn}), 127.78 (2 x CH_{ar}, 2"/6"-C_{bn}), 124.90 (CH_{ar}, 4-C_{ind}), 123.75 (CH_{ar}, 5-C_{ind}), 118.14 (C_q, 3'-C_{ind}), 111.49 (CH_{ar}, 7-C_{ind}), 43.37 (*Bn*-CH_2); MS (EI direct mode): *m/z (%)* 237.1 $[M]^+$ (100).

4.4. 1-(4-Methylbenzyl)indoline-2,3-dione (7b)

Starting out from isatin (30 mmol, 4.4 g) and 4-methylbenzyl chloride (33 mmol, 4.6 g, 4.4 mL) according to synthetic procedure 2, **7b** (1-(4-methylbenzyl)indoline-2,3-dione) was obtained after ~36 hours reaction time as orange, needle-like crystals. Yield: 26 mmol, 6.6 g, 88%; $C_{16}H_{13}NO_2$, *MW* 251.29; mp 147 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.60-7.56 (m, $2H_{ind}$, 4/6-H), 7.32-7.30 (pd, ^3J = 8.0 Hz, $2H_{bn}$, 2"/6"-H), 7.16-7.09 (m, $3H_{bn/ind}$, 5/3"/5"-H), 6.96 (dd, ^3J = 8.4 Hz, ^4J = 0.7 Hz, $1H_{ind}$, 7-H), 4.86 (s, 2H, *Bn*-CH_2), 2.67 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 183.57 (C=O, 3-C_{ind}), 158.68 (C=O, 2-C_{ind}), 150.76 (C_q, 7'-C_{ind}), 138.38 (CH_{ar}, 6-C_{ind}), 137.18 (C_q, 1"-C_{bn}), 132.84 (C_q, 4"-C_{bn}), 129.63 (2 x CH_{ar}, 3"/5"-C_{bn}), 127.80 (2 x CH_{ar}, 2"/6"-C_{bn}), 124.88 (CH_{ar}, 4-C_{ind}), 123.72 (CH_{ar}, 5-C_{ind}), 118.09 (C_q, 3'-C_{ind}), 111.54 (CH_{ar}, 7-C_{ind}), 43.09 (*Bn*-CH_2), 21.08 (CH_3); MS (EI direct mode): *m/z (%)* 251.2 $[M]^+$ (94), 146.1 $[C_8H_4NO_2]^+$ (100).

4.5. 1-(4-Methoxybenzyl)indoline-2,3-dione (7c)

Starting out from isatin (30 mmol, 4.4 g) and 4-methoxybenzyl chloride (33 mmol, 4.6 g, 4.4 mL) according to synthetic procedure 2, **7c** (1-(4-methoxybenzyl)indoline-2,3-dione) was obtained after ~24 hours reaction time as orange, needle-like crystals. Yield: 27 mmol, 7.1 g,

89%; $C_{16}H_{13}NO_3$, *MW* 267.29; mp 173 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 7.60-7.56 (m, $2H_{ind}$, 4/6-H), 7.38-7.35 (m, $2H_{bn}$, 2"/6"-H), 7.11 (t, ^3J = 7.5 Hz, $1H_{ind}$, 5-H), 7.00 (d, ^3J = 7.9 Hz, $1H_{ind}$, 7-H), 6.91-6.89 (m, $2H_{bn}$, 3"/5"-H), 4.84 (s, 2H, *Bn*-CH_2), 3.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 183.62 (C=O, 3-C_{ind}), 159.13 (C_q, 4"-C_{bn}), 158.64 (C=O, 2-C_{ind}), 150.75 (C_q, 7'-C_{ind}), 138.35 (CH_{ar}, 6-C_{ind}), 129.30 (2 x CH_{ar}, 2"/6"-C_{bn}), 127.73 (C_q, 1"-C_{bn}), 124.87 (CH_{ar}, 4-C_{ind}), 123.69 (CH_{ar}, 5-C_{ind}), 118.09 (C_q, 3'-C_{ind}), 114.46 (2 x CH_{ar}, 3"/5"-C_{bn}), 111.56 (CH_{ar}, 7-C_{ind}), 55.48 (CH_3), 43.37 (*Bn*-CH_2); MS (EI direct mode): *m/z (%)* 267.1 $[M]^+$ (100).

4.6. 1-(4-Nitrobenzyl)indoline-2,3-dione (7d)

Starting out from isatin (30 mmol, 4.4 g) and 4-nitrobenzyl chloride (33 mmol, 5.7 g) according to synthetic procedure 2, **7d** (1-(4-nitrobenzyl)indoline-2,3-dione) was obtained after ~60 hours reaction time as orange powder. Yield: 5.7 mmol, 1.6 g, 19%; $C_{15}H_{10}N_2O_4$, *MW* 282.26; mp 192 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 8.20 (d, ^3J = 8.4 Hz, $2H_{bn}$, 3"/5"-H), 7.74 (d, ^3J = 8.4 Hz, $2H_{bn}$, 2"/6"-H), 7.61-7.56 (m, $2H_{ind}$, 4/6-H), 7.14 (t, ^3J = 7.3 Hz, $1H_{ind}$, 5-H), 6.95 (dd, ^3J = 7.9 Hz, ^4J = 0.4 Hz, $1H_{ind}$, 7-H), 5.70 (s, 2H, *Bn*-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 183.15 (C=O, 3-C_{ind}), 158.91 (C=O, 2-C_{ind}), 150.38 (C_q, 1"-C_{bn}), 147.42 (C_q, 7'-C_{ind}), 143.96 (C_q, 4"-C_{bn}), 138.31 (CH_{ar}, 6-C_{ind}), 128.94 (2 x CH_{ar}, 2"/6"-C_{bn}), 124.95 (CH_{ar}, 4-C_{ind}), 124.11 (2 x CH_{ar}, 3"/5"-C_{bn}), 123.88 (CH_{ar}, 5-C_{ind}), 118.39 (C_q, 3'-C_{ind}), 111.28 (CH_{ar}, 7-C_{ind}), 42.91 (*Bn*-CH_2); MS (EI direct mode): *m/z (%)* 282.1 $[M]^+$ (100).

4.7. 5-(4-Oxo-2-thioxothiazolidin-3-yl)pentanoic acid (8a)

Starting out from 5-aminovaleric acid (40 mmol, 4.7 g) according to synthetic procedure 3, **8a** (5-(4-oxo-2-thioxothiazolidin-3-yl)pentanioc acid) was obtained as yellowish crystalline solid. Yield: 21 mmol, 5.0 g, 53%; $C_8H_{11}NO_3S_2$, *MW* 233.31; mp 122 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 12.06 (bs, 1H, COOH), 4.25 (s, $2H_{rh}$, Rh-CH_2), 3.86 (t, ^3J = 7.1 Hz,

$2H_{aliph}$, 5-CH_2), 2.24 (t, ^3J = 7.1 Hz, $2H_{aliph}$, 2-CH_2), 1.59-1.47 (m, $4H_{aliph}$, 3/4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 203.67 (C=S, 2-C_{rh}), 174.88 (COOH, 1-C_{aliph}), 174.54 (C=O, 4-C_{rh}), 44.04 (CH_2, 5-C_{aliph}), 36.22 (CH_2, 5-C_{rh}), 33.55 (CH_2, 2-C_{aliph}), 26.23 (CH_2, 4-C_{aliph}), 22.10 (CH_2, 3-C_{aliph}); MS (EI direct mode): *m/z (%)* 233.0 $[M]^+$ (100).

4.8. 6-(4-Oxo-2-thioxothiazolidin-3-yl)hexanoic acid (8b)

Starting out from 6-aminocaproic acid (40 mmol, 5.3 g) according to synthetic procedure 3, **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) was obtained as yellowish crystalline solid. Yield: 25 mmol, 6.2 g, 63%; $C_9H_{13}NO_3S_2$, *MW* 247.34; mp 86 ºC; ^1H NMR (400 MHz, $(CD_3)_2SO$): δ = 11.87 (bs, 1H, COOH), 4.25 (s, $2H_{rh}$, Rh-CH_2), 3.84 (t, ^3J = 7.5 Hz, $2H_{aliph}$, 6-CH_2), 2.19 (t, ^3J = 7.3 Hz, $2H_{aliph}$, 2-CH_2), 1.58-1.47 (m, $4H_{aliph}$, 3/5-CH_2), 1.31-1.23 (m, $2H_{aliph}$, 4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 203.63 (C=S, 2-C_{rh}), 174.85 (COOH, 1-C_{aliph}), 174.77 (C=O, 4-C_{rh}), 44.18 (CH_2, 6-C_{aliph}), 36.22 (CH_2, 5-C_{rh}), 33.80 (CH_2, 2-C_{aliph}), 26.33 (CH_2, 5-C_{aliph}), 26.03 (CH_2, 4-C_{aliph}), 24.43 (CH_2, 3-C_{aliph}); MS (EI direct mode): *m/z (%)* 247.1 $[M]^+$ (56), 134.0 $[C_3H_4NOS_2]^+$ (100); MS (CI NH_3 direct mode): *m/z (%)* 248.1 $[M + H]^+$ (100).

4.9. Methyl 6-(4-Oxo-2-thioxothiazolidin-3-yl)hexanoat (9)

Methanol (250 mL) was cooled down to -20 ºC. Then, thionyl chloride (87.5 mmol, 10.4 g, 6.4 mL; i.e. 3.5 mmol $SOCl_2$ per 1 mmol acid), was added dropwise over 15 minutes and then the addition of compound **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) followed. The mixture was then stirred at room temperature over night. The solvent was removed *in vacuo*. The oily residue was dissolved in ethyl acetate and washed subsequently three times with diethyl ether, ethyl acetate, saturated sodium bicarbonate solution and deionised water. The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness. The corresponding methyl ester of compound **8b,** (methyl 6-(4-oxo-2-thioxothiazolidin-3-

yl)hexanoat) **9**) was obtained as red-brownish oil. Yield: 23 mmol, 6.1 g, 93%; $C_{10}H_{15}NO_3S_2$, *MW* 261.36; density 1.369 g/mL; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 4.25 (s, $2H_{rh}$, Rh-CH_2), 3.84 (t, ^3J = 7.5 Hz, $2H_{aliph}$, 6-CH_2), 3.58 (s, CH_3), 2.29 (t, ^3J = 7.4 Hz, $2H_{aliph}$, 2-CH_2), 1.58-1.50 (m, $4H_{aliph}$, 3/5-CH_2), 1.31-1.25 (m, $2H_{aliph}$, 4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 203.65 (C=S, 2-C_{rh}), 174.85 (C=O, 1-C_{aliph}), 174.65 (C=O, 4-C_{rh}), 51.62 (OCH_3), 44.12 (CH_2, 6-C_{aliph}), 36.24 (CH_2, 5-C_{rh}), 33.43 (CH_2, 2-C_{aliph}), 26.26 (CH_2, 5-C_{aliph}), 25.93 (CH_2, 4-C_{aliph}), 24.37 (CH_2, 3-C_{aliph}); MS (EI direct mode): *m/z (%)* 261.2 $[M]^+$ (100).

4.10. 4-((4-Oxo-2-thioxothiazolidin-3-yl)methyl)benzoic acid (10)

Starting out from 4-(aminomethyl)benzoic acid (PAMBA) (20 mmol, 3.0 g) according to synthetic procedure 4, **10** (4-(4-oxo-2-thioxothiazolidin-3-yl)methyl)benzoic acid) was obtained as light yellow powder. Yield: 5.6 mmol, 1.5 g, 28%; $C_{11}H_9NO_3S_2$, *MW* 267.33; mp 253 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 12.95 (bs, 1H, COOH), 7.89 (d, ^3J = 8.3 Hz, $2H_{pamba}$, 2/6-H), 7.39 (d, ^3J = 8.3 Hz, $2H_{pamba}$, 3/5-H), 5.14 (s, $2H_{pamba}$, CH_2), 4.38 (s, $2H_{rh}$, Rh-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 203.71 (C=S, 2-C_{rh}), 174.93 (C=O, 4-C_{rh}), 167.38 (COOH), 140.36 (C_q, 4-C_{pamba}), 130.38 (C_q, 1-C_{pamba}), 129.86 (2 x CH_{ar}, 2/6-C_{pamba}), 127.95 (2 x CH_{ar}, 3/5-C_{pamba}), 47.12 (*PAMBA*-CH_2), 36.63 (CH_2, 5-C_{rh}); MS (EI direct mode): *m/z (%)* 267.0 $[M]^+$ (100).

4.11. *trans*-4-((4-Oxo-2-thioxothiazolidin-3-yl)methyl)cyclohexanecarboxylic acid (11)

Starting out from *trans*-4-(aminomethyl)cyclohexane carboxylic acid (TAMCHA) (20 mmol, 3.1 g) according to synthetic procedure 4, **11** (*trans*-4-(4-oxo-2-thioxothiazolidin-3-yl)methyl)cyclohexanecarboxylic acid) was obtained as yellow powder. Yield: 2.2 mmol, 0.6 g, 11%; $C_{11}H_{15}NO_3S_2$, *MW* 273.38; mp 201 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 12.02 (bs, 1H, COOH), 4.28 (s, $2H_{rh}$, Rh-CH_2), 3.73 (d, ^3J = 7.2 Hz, $2H_{tamcha}$, CH_2), 2.17-2.09 (m, $1H_{tamcha}$, 1_a-H), 1.90-1.86 (m, $2H_{tamcha}$, 2_e/6_e-H), 1.82-1.72 (m, $1H_{tamcha}$, 4_a-H), 1.67-1.63 (m,

$2H_{tamcha}$, $3_e/5_e$-H), 1.25-1.15 (m, $2H_{tamcha}$, $2_a/6_a$-H), 1.07-0.97 (m, $2H_{tamcha}$, $3_a/5_a$-H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 204.13 (C=S, 2-C_{rh}), 176.94 (COOH), 175.23 (C=O, 4-C_{rh}), 49.89 (*TAMCHA*-CH_2), 42.54 (CH, 1-C_{tamcha}), 36.10 (CH_2, 5-C_{rh}), 35.33 (CH, 4-C_{tamcha}), 29.62 (2 x CH_2, 3/5-C_{tamcha}), 28.46 (2 x CH_2, 2/6-C_{tamcha}); MS (EI direct mode): *m/z (%)* 174.0 $[C_6H_8NOS_2]^+$ (100); MS (CI NH_3 direct mode): *m/z (%)* 314.1 $[M + Na + NH_4]^+$ (100).

4.12. *(Z)*-6-(4-Oxo-5-((1*H*)-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-3-yl)hexanoic acid (12a)

Starting out from isatin (1*H*-indole-2,3-dione) as indolinone moiety (2 mmol, 294 mg) and compound **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) as rhodanine moiety (2 mmol, 495 mg) according to synthetic procedure 5, **12a** (*(Z)*-6-(4-oxo-5-(2-oxoindolin-3-ylidene)-2-thioxothiazolidin-3-yl)hexanoic acid) was obtained after ~24 hours reaction time and recrystallisation from dichloromethane/tetrahydrofuran mixture 1:1 (v/v) with petrol ether (40-60 ºC) as scarlet red amorphous powder. Yield: 0.54 mmol, 203 mg, 17%; $C_{17}H_{16}N_2O_4S_2$, *MW* 376.43; mp 248 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 11.78 (bs, 1H, COOH), 11.24 (s, 1H, NH), 8.80 (d, ^3J = 7.9 Hz, $1H_{ind}$, 4-H), 7.42 (t, ^3J = 7.7 Hz, $1H_{ind}$, 6-H), 7.08 (t, ^3J = 7.7 Hz, $1H_{ind}$, 5-H), 6.95 (d, ^3J = 7.8 Hz, $1H_{ind}$, 7-H), 4.03 (t, ^3J = 6.9 Hz, $2H_{aliph}$, 6-CH_2), 2.22 (t, ^3J = 7.3 Hz, $2H_{aliph}$, 2-CH_2), 1.68-1.59 (m, $2H_{aliph}$, 5-CH_2), 1.56-1.50 (m, $2H_{aliph}$, 3-CH_2), 1.37-1.30 (m, $2H_{aliph}$, 4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 197.09 (C=S, 2-C_{rh}), 174.75 (COOH, 1-C_{aliph}), 168.40 (C=O, 2-C_{ind}), 167.19 (C=O, 4-C_{rh}), 145.13 (C_q, 7'-C_{ind}), 133.52 (CH_{ar}, 6-C_{ind}), 131.17 (C_q, 3-C_{ind}), 128.34 (CH_{ar}, 4-C_{ind}), 125.68 (C_q, 5-C_{rh}), 122.61 (CH_{ar}, 5-C_{ind}), 120.29 (C_q, 3'-C_{ind}), 111.15 (CH_{ar}, 7-C_{ind}), 44.24 (CH_2, 6-C_{aliph}), 33.80 (CH_2, 2-C_{aliph}), 26.59 (CH_2, 5-C_{aliph}), 26.11 (CH_2, 4-C_{aliph}), 24.46 (CH_2, 3-C_{aliph}); MS (ESI direct negative mode): *m/z (%)* 375.0 $[M - H]^-$ (100); microanalysis $[C_{17}H_{16}N_2O_4S_2]$: *found/calculated (%)* C (54.24/54.23), H (4.28/4.59), N (7.44/7.24), S (17.04/16.89).

4.13. *(Z)*-6-(5-(1-Methyl-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid (12b)

Starting out from **6a** (1-methylindoline-2,3-dione) as indolinone moiety (2 mmol, 322 mg) and compound **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) as rhodanine moiety (2 mmol, 495 mg) according to synthetic procedure 5, **12b** (*(Z)*-6-(5-(1-methyl-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) was obtained after ~12 hours reaction time and recrystallisation from dichloromethane with petrol ether (40-60 ºC) as dark red amorphous powder. Yield: 0.36 mmol, 141 mg, 18%; $C_{18}H_{18}N_2O_4S_2$, *MW* 390.48; mp 237 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 12.01 (bs, 1H, COOH), 8.83 (d, ^3J = 7.8 Hz, $1H_{ind}$, 4-H), 7.51 (dt, ^3J = 7.7 Hz, ^4J = 1.4 Hz, $1H_{ind}$, 6-H), 7.17-7.13 (m, $2H_{ind}$, 5/7-H), 4.04 (t, ^3J = 7.5 Hz, $2H_{aliph}$, 6-CH_2), 3.23 (s, 3H, CH_3), 2.22 (t, ^3J = 7.3 Hz, $2H_{aliph}$, 2-CH_2), 1.69-1.62 (m, $2H_{aliph}$, 5-CH_2), 1.58-1.51 (m, $2H_{aliph}$, 3-CH_2), 1.38-1.28 (m, $2H_{aliph}$, 4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 197.81 (C=S, 2-C_{rh}), 174.75 (COOH, 1-C_{aliph}), 167.06 (C=O, 2-C_{ind}), 166.85 (C=O, 4-C_{rh}), 145.99 (C_q, 7'-C_{ind}), 133.41 (CH_{ar}, 6-C_{ind}), 131.85 (C_q, 3-C_{ind}), 128.03 (CH_{ar}, 4-C_{ind}), 124.71 (C_q, 5-C_{rh}), 123.16 (CH_{ar}, 5-C_{ind}), 119.57 (C_q, 3'-C_{ind}), 109.97 (CH_{ar}, 7-C_{ind}), 44.32 (CH_2, 6-C_{aliph}), 33.80 (CH_2, 2-C_{aliph}), 26.82 (CH_3), 26.59 (CH_2, 5-C_{aliph}), 26.11 (CH_2, 4-C_{aliph}), 24.46 (CH_2, 3-C_{aliph}); MS (EI direct mode): *m/z (%)* 390.1 $[M]^+$ (58), 189.0 $[C_{10}H_7NOS]^+$ (100); MS (ESI direct negative mode): *m/z (%)* 388.9 $[M - H]^-$ (100); HPLC purity analyses: i) 99.1%, retention time 24.71 min; ii) 97.0%, retention time 24.50 min.

4.14. *(Z)*-6-(5-(1-Ethyl-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid (12c)

Starting out from **6b** (1-ethylindoline-2,3-dione) as indolinone moiety (2 mmol, 350 mg) and compound **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) as rhodanine moiety (2 mmol, 495 mg) according to synthetic procedure 5, **12c** (*(Z)*-6-(5-(1-ethyl-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) was obtained after ~20 hours reaction

time and recrystallisation from dichloromethane with petrol ether (40-60 ºC) as dark red crystals. Yield: 0.80 mmol, 324 mg, 40%; $C_{19}H_{20}N_2O_4S_2$, *MW* 404.51; mp 207 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 11.98 (bs, 1H, COOH), 8.83 (d, ^3J = 7.8 Hz, $1H_{ind}$, 4-H), 7.70 (t, ^3J = 7.7 Hz, $1H_{ind}$, 6-H), 7.18 (d, ^3J = 7.8 Hz, $1H_{ind}$, 7-H), 7.14 (t, ^3J = 7.8 Hz, $1H_{ind}$, 5-H), 4.04 (t, ^3J = 7.5 Hz, $2H_{aliph}$, 6-CH_2), 3.80 (q, ^3J = 7.1 Hz, 2H, CH_2), 2.22 (t, ^3J = 7.3 Hz, $2H_{aliph}$, 2-CH_2), 1.70-1.63 (m, $2H_{aliph}$, 5-CH_2), 1.59-1.50 (m, $2H_{aliph}$, 3-CH_2), 1.38-1.32 (m, $2H_{aliph}$, 4-CH_2), 1.20 (t, ^3J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 197.29 (C=S, 2-C_{rh}), 174.33 (COOH, 1-C_{aliph}), 167.55 (C=O, 2-C_{ind}), 167.01 (C=O, 4-C_{rh}), 144.46 (C_q, 7'-C_{ind}), 132.98 (CH_{ar}, 6-C_{ind}), 131.39 (C_q, 3-C_{ind}), 127.80 (CH_{ar}, 4-C_{ind}), 124.26 (C_q, 5-C_{rh}), 122.60 (CH_{ar}, 5-C_{ind}), 119.25 (C_q, 3'-C_{ind}), 109.51 (CH_{ar}, 7-C_{ind}), 43.83 (CH_2, 6-C_{aliph}), 34.63 (CH_2), 33.37 (CH_2, 2-C_{aliph}), 26.14 (CH_2, 5-C_{aliph}), 25.66 (CH_2, 4-C_{aliph}), 24.01 (CH_2, 3-C_{aliph}), 12.56 (CH_3); MS (ESI direct negative mode): *m/z (%)* 402.9 $[M - H]^-$ (100); HPLC purity analyses: i) 97.8%, retention time 25.12 min; ii) 98.4%, retention time 24.50 min.

4.15. *(Z)*-6-(5-(1-Benzyl-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid (12d)

Starting out from **7a** (1-benzylindoline-2,3-dione) as indolinone moiety (2 mmol, 475 mg) and compound **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) as rhodanine moiety (2 mmol, 495 mg) according to synthetic procedure 5, **12d** (*(Z)*-6-(5-(1-benzyl-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) was obtained after ~19 hours reaction time and recrystallisation from dichloromethane with petrol ether (40-60 ºC) as red-brownish amorphous powder. Yield: 0.43 mmol, 159 mg, 17%; $C_{24}H_{22}N_2O_4S_2$, *MW* 466.58; mp 205 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 11.94 (bs, 1H, COOH), 8.89 (d, ^3J = 7.6 Hz, $1H_{ind}$, 4-H), 7.47-7.43 (m, $1H_{ind}$, 6-H), 7.35-7.30 (m, $4H_{bn}$ 2"/3"/5"/6"-H), 7.29-7.28 (m, $1H_{bn}$ 4"-H), 7.16 (t, ^3J = 7.6 Hz, $1H_{ind}$, 5-H), 7.10 (d, ^3J = 8.0 Hz, $1H_{ind}$, 7-H), 5.04 (s, 2H, *Bn*-CH_2), 4.06 (t, ^3J = 7.4 Hz, $2H_{aliph}$, 6-CH_2), 2.22 (t, ^3J = 7.3 Hz, $2H_{aliph}$, 2-CH_2), 1.69-1.65 (m, $2H_{aliph}$, 5-CH_2),

1.56-1.51 (m, $2H_{aliph}$, 3-CH_2), 1.36-1.32 (m, $2H_{aliph}$, 4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 197.66 (C=S, 2-C_{rh}), 174.76 (COOH, 1-C_{aliph}), 167.12 (2 x C=O, 2-C_{ind}/4-C_{rh}), 144.91 (C_q, 7'-C_{ind}), 136.22 (CH_{ar}, 6-C_{ind}), 133.31 (C_q, 4"-C_{bn}), 132.72 (C_q, 3-C_{ind}), 129.16 (2 x CH_{ar}, 3"/5"-C_{bn}), 128.27 (C_q, 1"-C_{bn}), 128.05 (CH_{ar}, 4-C_{ind}), 127.68 (2 x CH_{ar}, 2"/6"-C_{bn}), 124.36 (C_q, 5-C_{rh}), 123.36 (CH_{ar}, 5-C_{ind}), 119.83 (C_q, 3'-C_{ind}), 110.53 (CH_{ar}, 7-C_{ind}), 44.34 (CH_2, 6-C_{aliph}), 43.55 (*Bn*-CH_2), 33.81 (CH_2, 2-C_{aliph}), 26.59 (CH_2, 5-C_{aliph}), 26.09 (CH_2, 4-C_{aliph}), 24.47 (CH_2, 3-C_{aliph}); MS (APCI direct positive mode): *m/z (%)* 467.9 $[M + H]^+$ (26), 466.8 $[M]^+$ (100); MS (APCI direct negative mode): *m/z (%)* 466.1 $[M]^-$ (100), 465.1 $[M - H]^-$ (23); HPLC purity analyses: i) 96.7%, retention time 26.47 min; ii) 97.1%, retention time 27.19 min.

4.16. *(Z)*-6-(5-(1-(4-Methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid (12e)

Starting out from **7b** (1-(4-methylbenzyl)indoline-2,3-dione) as indolinone moiety (2 mmol, 503 mg) and compound **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) as rhodanine moiety (2 mmol, 495 mg) according to synthetic procedure 5, **12e** (*(Z)*-6-(5-(1-(4-methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) was obtained after ~12 hours reaction time and recrystallisation from dichloromethane with petrol ether (40-60 ºC) as red-brownish amorphous powder. Yield: 0.72 mmol, 346 mg, 37%; $C_{25}H_{24}N_2O_4S_2$, *MW* 480.613; mp 211 ºC; ^1H NMR (400 MHz, $(CD_3)_2SO$): δ = 11.92 (bs, 1H, COOH), 8.88 (d, ^3J = 7.8 Hz, $1H_{ind}$, 4-H), 7.46-7.42 (m, $1H_{ind}$, 6-H), 7.22 (d, ^3J = 8.0 Hz, $2H_{bn}$, 2"/6"-H), 7.17-7.13 (m, $3H_{bn/ind}$, 5/3"/5"-H), 7.07 (d, ^3J = 7.9 Hz, $1H_{ind}$, 7-H), 4.98 (s, 2H, *Bn*-CH_2), 4.07 (t, ^3J = 7.4 Hz, $2H_{aliph}$, 6-CH_2), 2.26-2.20 (m, $5H_{aliph}$, 2-CH_2/CH_3), 1.70-1.64 (m, $2H_{aliph}$, 5-CH_2), 1.57-1.52 (m, $2H_{aliph}$, 3-CH_2), 1.37-1.33 (m, $2H_{aliph}$, 4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 197.66 (C=S, 2-C_{rh}), 174.69 (COOH, 1-C_{aliph}), 167.09 (2 x C=O, 2-C_{ind}/4-C_{rh}), 144.95 (C_q, 7'-C_{ind}), 137.29 (C_q, 1"-C_{bn}), 133.27 (C_q, 4"-C_{bn}), 133.13

(CH_{ar}, 6-C_{ind}), 132.58 (C_q, 3-C_{ind}), 129.68 (2 x CH_{ar}, 3"/5"-C_{bn}), 128.24 (CH_{ar}, 4-C_{ind}), 127.69 (2 x CH_{ar}, 2"/6"-C_{bn}), 124.45 (C_q, 5-C_{rh}), 123.29 (CH_{ar}, 5-C_{ind}), 119.84 (C_q, 3'-C_{ind}), 110.53 (CH_{ar}, 7-C_{ind}), 44.34 (CH_2, 6-C_{aliph}), 43.37 (*Bn*-CH_2), 30.84 (CH_2, 2-C_{aliph}), 26.58 (CH_2, 5-C_{aliph}), 26.11 (CH_2, 4-C_{aliph}), 24.47 (CH_2, 3-C_{aliph}), 21.05 (CH_3); MS (ESI direct positive mode): *m/z (%)* 481.1 $[M + H]^+$ (100); MS (ESI direct negative mode): *m/z (%)* 479.0 $[M - H]^-$ (100); HPLC purity analyses: i) 97.0%, retention time 26.89 min; ii) 97.2%, retention time 28.45 min.

4.17. *(Z)*-6-(5-(1-(4-Methoxybenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid (12f)

Starting out from **7c** (1-(4-methoxybenzyl)indoline-2,3-dione) as indolinone moiety (2 mmol, 535 mg) and compound **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) as rhodanine moiety (2 mmol, 495 mg) according to synthetic procedure 5, **12f** (*(Z)*-6-(5-(1-(4-methoxybenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) was obtained after ~13 hours reaction time and recrystallisation from dichloromethane with petrol ether (40-60 ºC) as dark red amorphous powder. Yield: 0.42 mmol, 209 mg, 21%; $C_{25}H_{24}N_2O_5S_2$, *MW* 496.61; mp 183 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 12.01 (bs, 1H, COOH), 8.88 (d, ^3J = 8.0 Hz, 1H_{ind}, 4-H), 7.47-7.43 (m, 1H_{ind}, 6-H), 7.28 (d, ^3J = 7.28 Hz, 2H_{bn}, 2"/6"-H), 7.17-7.11 (m, 2H_{ind}, 5/7-H), 6.89 (d, ^3J = 8.7 Hz, 2H_{bn}, 3"/5"-H), 4.95 (s, 2H, *Bn*-CH_2), 4.07-4.03 (m, 2H_{aliph}, 6-CH_2), 3.71 (s, 3H, CH_3), 2.21 (t, ^3J = 7.3 Hz, 2H_{aliph}, 2-CH_2), 1.68-1.63 (m, 2H_{aliph}, 5-CH_2), 1.58-1.48 (m, 2H_{aliph}, 3-CH_2), 1.38-1.30 (m, 2H_{aliph}, 4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 197.70 (C=S, 2-C_{rh}), 174.75 (COOH, 1-C_{aliph}), 167.10 (C=O, 2-C_{ind}), 167.05 (C=O, 4-C_{rh}), 159.15 (C_q, 4"-C_{bn}), 144.89 (C_q, 7'-C_{ind}), 133.27 (CH_{ar}, 6-C_{ind}), 132.64 (C_q, 3-C_{ind}), 129.22 (2 x CH_{ar}, 2"/6"-C_{bn}), 128.42 (C_q, 1"-C_{bn}), 128.07 (CH_{ar}, 4-C_{ind}), 124.41 (C_q, 5-C_{rh}), 123.29 (CH_{ar}, 5-C_{ind}), 119.82 (C_q, 3'-C_{ind}), 114.53 (2 x CH_{ar}, 3"/5"-C_{bn}), 110.58 (CH_{ar}, 7-C_{ind}), 55.49 (CH_3), 44.32 (CH_2, 6-C_{aliph}), 43.01 (*Bn*-CH_2), 33.80

(CH_2, 2-C_{aliph}), 26.58 (CH_2, 5-C_{aliph}), 26.09 (CH_2, 4-C_{aliph}), 24.46 (CH_2, 3-C_{aliph}); MS (APCI direct positive mode): *m/z (%)* 497.8 $[M + H]^+$ (23), 496.7 $[M]^+$ (100); MS (APCI direct negative mode): *m/z (%)* 496.1 $[M]^-$ (100), 495.0 $[M - H]^-$ (23); microanalysis [$C_{25}H_{24}N_2O_5S_2$]: *found/calculated (%)* C (60.47/60.01), H (4.87/5.20), N (5.64/5.49), S (12.91/13.14).

4.18. *(Z)*-6-(5-(1-(4-Nitrobenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)-hexanoic acid (12g)

Starting out from **7d** (1-(4-nitrobenzyl)indoline-2,3-dione) as indolinone moiety (2 mmol, 565 mg) and compound **8b** (6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) as rhodanine moiety (2 mmol, 495 mg) according to synthetic procedure 5, **12g** (*(Z)*-6-(5-(1-(4-nitrobenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid) was obtained after ~24 hours reaction time and recrystallisation from ethanol/tetrahydrofuran mixture 1:1 (v/v) with petrol ether (40-60 ºC) as dark red amorphous powder. Yield: 1.12 mmol, 573 mg, 56%; $C_{24}H_{21}N_3O_6S_2$, *MW* 511.58; mp 219 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 12.05 (bs, 1H, COOH), 8.91 (d, ^3J = 7.8 Hz, 1H_{ind}, 4-H), 8.22-8.20 (m, 2H_{bn}, 3"/5"-H), 7.59 (d, ^3J = 8.7 Hz, 2H_{bn}, 2"/6"-H), 7.46 (t, ^3J = 7.7 Hz, 1H_{ind}, 6-H), 7.18 (t, ^3J = 7.7 Hz, 1H_{ind}, 5-H), 7.09 (d, ^3J = 8.0 Hz, 1H_{ind}, 7-H), 5.02 (s, 2H, *Bn*-CH_2), 4.07-4.03 (t, ^3J = 7.4 Hz, 2H_{aliph}, 6-CH_2), 2.22 (t, ^3J = 7.3 Hz, 2H_{aliph}, 2-CH_2), 1.69-1.63 (m, 2H_{aliph}, 5-CH_2), 1.58-1.51 (m, 2H_{aliph}, 3-CH_2), 1.38-1.32 (m, 2H_{aliph}, 4-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 197.48 (C=S, 2-C_{rh}), 174.76 (COOH, 1-C_{aliph}), 167.22 (C=O, 2-C_{ind}), 167.10 (C=O, 4-C_{rh}), 147.42 (C_q, 4"-C_{bn}), 144.60 (C_q, 7'-C_{ind}), 144.04 (C_q, 1"-C_{bn}), 133.36 (CH_{ar}, 6-C_{ind}), 132.86 (C_q, 3-C_{ind}), 128.81 (2 x CH_{ar}, 2"/6"-C_{bn}), 128.35 (CH_{ar}, 4-C_{ind}), 124.31 (2 x CH_{ar}, 3"/5"-C_{bn}), 124.26 (C_q, 5-C_{rh}), 123.57 (CH_{ar}, 5-C_{ind}), 119.93 (C_q, 3'-C_{ind}), 110.37 (CH_{ar}, 7-C_{ind}), 44.36 (CH_2, 6-C_{aliph}), 43.10 (*Bn*-CH_2), 33.84 (CH_2, 2-C_{aliph}), 26.59 (CH_2, 5-C_{aliph}), 26.10 (CH_2, 4-C_{aliph}), 24.47 (CH_2, 3-

C_{aliph}); MS (ESI direct negative mode): *m/z (%)* 511.0 $[M]^-$ (30), 509.9 $[M - H]^-$ (100); HPLC purity analyses: i) 99.3%, retention time 26.31 min; ii) 99.5%, retention time 26.01 min.

4.19. *(Z)*-5-(5-(1-(4-Methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid (13)

Starting out from **7b** (1-(4-methylbenzyl)indoline-2,3-dione) as indolinone moiety (2 mmol, 503 mg) and compound **8a** (5-(4-oxo-2-thioxothiazolidin-3-yl)pentanioc acid) as rhodanine moiety (2 mmol, 467 mg) according to synthetic procedure 5, **13** (*(Z)*-5-(5-(1-(4-methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)pentanoic acid) was obtained after ~17 hours reaction time and recrystallisation from dichloromethane/tetrahydrofuran mixture 1:1 (v/v) with petrol ether (40-60 ºC) as dark purple amorphous powder. Yield: 0.80 mmol, 373 mg, 40%; $C_{24}H_{22}N_2O_4S_2$, *MW* 466.58; mp 232 ºC; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 12.05 (bs, 1H, COOH), 8.88 (d, ^3J = 7.4 Hz, $1H_{ind}$, 4-H), 7.46-7.42 (m, $1H_{ind}$, 6-H), 7.22 (d, ^3J = 8.0 Hz, $2H_{bn}$, 2"/6"-H), 7.17-7.13 (m, $3H_{bn/ind}$, 5/3"/5"-H), 7.08 (d, ^3J = 7.8 Hz, $1H_{ind}$, 7-H), 4.98 (s, 2H, *Bn*-CH_2), 4.07 (t, ^3J = 7.1 Hz, $2H_{aliph}$, 5-CH_2), 2.29-2.25 (m, $5H_{aliph}$, 2-CH_2/CH_3), 1.73-1.65 (m, $2H_{aliph}$, 4-CH_2), 1.59-1.52 (m, $2H_{aliph}$, 3-CH_2); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ = 197.74 (C=S, 2-C_{rh}), 174.54 (COOH, 1-C_{aliph}), 167.16 (C=O, 2-C_{ind}), 167.09 (C=O, 4-C_{rh}), 144.90 (C_q, 7'-C_{ind}), 137.29 (C_q, 1"-C_{bn}), 133.27 (C_q, 4"-C_{bn}), 133.14 (CH_{ar}, 6-C_{ind}), 132.68 (C_q, 3-C_{ind}), 129.70 (2 x CH_{ar}, 3"/5"-C_{bn}), 128.23 (CH_{ar}, 4-C_{ind}), 127.69 (2 x CH_{ar}, 2"/6"-C_{bn}), 124.37 (C_q, 5-C_{rh}), 123.30 (CH_{ar}, 5-C_{ind}), 119.82 (C_q, 3'-C_{ind}), 110.56 (CH_{ar}, 7-C_{ind}), 44.21 (CH_2, 5-C_{aliph}), 43.31 (*Bn*-CH_2), 33.56 (CH_2, 2-C_{aliph}), 26.48 (CH_2, 4-C_{aliph}), 22.17 (CH_2, 3-C_{aliph}), 21.07 (CH_3); MS (APCI direct positive mode): *m/z (%)* 466.8 $[M + H]^+$ (83), 282.0 $[C_{10}H_8N_2O_4S_2]^+$ (100); MS (APCI direct negative mode): *m/z (%)* 466.1 $[M]^-$ (100), 464.9 $[M - H]^-$ (21); HPLC purity analyses: i) 99.5%, retention time 26.52 min; ii) 98.5%, retention time 26.46 min.

4.20. *(Z)*-4-((5-(1-(4-Methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)methyl)benzoic acid (14)

Starting out from **7b** (1-(4-methylbenzyl)indoline-2,3-dione) as indolinone moiety (2 mmol, 503 mg) and compound **10** (4-(4-oxo-2-thioxothiazolidin-3-yl)methyl)benzoic acid) as rhodanine moiety (2 mmol, 535 mg) according to synthetic procedure 5, **14** (*(Z)*-4-((5-(1-(4-methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)methyl)benzoic acid) was obtained after ~23 hours reaction time and recrystallisation from dichloromethane/tetrahydrofuran mixture 1:1 (v/v) with petrol ether (40-60 ºC) as dark purple crystals. Yield: 1.10 mmol, 501 mg, 55%; $C_{27}H_{20}N_2O_4S_2$, *MW* 500.60; mp 331 ºC; ^{1}H NMR (400 MHz, 60 ºC, $(CD_3)_2SO$): δ = 12.70 (bs, 1H, COOH), 8.85 (d, ^3J = 7.9 Hz, $1H_{ind}$, 4-H), 8.16 (d, ^3J = 8.2 Hz, $2H_{pamba}$, 2/6-H), 7.49-7.42 (m, $3H_{ind/pamba}$, 6/3/5-H), 7.23 (d, ^3J = 7.9 Hz, $2H_{bn}$, 2"/6"-H), 7.15-7.12 (m, $3H_{bn/ind}$, 5/3"/5"-H), 7.07 (d, ^3J = 7.9 Hz, $1H_{ind}$, 7-H), 5.38 (s, $2H_{pamba}$, CH_2), 4.99 (s, 2H, *Bn*-CH_2), 2.67 (s, 3H, CH_3); ^{13}C NMR (100 MHz, 60 ºC, $(CD_3)_2SO$): δ = 197.76 (C=S, 2-C_{rh}), 167.26 (COOH), 167.14 (C=O, 2-C_{ind}), 167.11 (C=O, 4-C_{rh}), 145.17 (C_q, 7'-C_{ind}), 139.99 (C_q, 4-C_{pamba}), 137.31 (C_q, 1"-C_{bn}), 133.42 (CH_{ar}, 6-C_{ind}), 133.08 (C_q, 4"-C_{bn}), 132.15 (C_q, 3-C_{ind}), 130.72 (C_q, 1-C_{pamba}), 129.93 (2 x CH_{ar}, 3/5-C_{pamba}), 129.66 (2 x CH_{ar}, 3"/5"-C_{bn}), 128.29 (CH_{ar}, 4-C_{ind}), 128.92 (2 x CH_{ar}, 2/6-C_{pamba}), 127.67 (2 x CH_{ar}, 2"/6"-C_{bn}), 125.12 (C_q, 5-C_{rh}), 123.29 (CH_{ar}, 5-C_{ind}), 119.93 (C_q, 3'-C_{ind}), 110.55 (CH_{ar}, 7-C_{ind}), 47.31 (*PAMBA*-CH_2), 43.51 (*Bn*-CH_2), 20.99 (CH_3); MS (APCI direct positive mode): *m/z (%)* 502.0 $[M + H]^+$ (29), 500.9 $[M]^+$ (100); HPLC purity analyses: i) 99.8%, retention time 27.42 min; ii) 97.8%, retention time 28.31 min.

4.21. *trans*-4-(((*(Z)*-5-(1-(4-Methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)methyl)cyclohexanecarboxylic acid (15)

Starting out from **7b** (1-(4-methylbenzyl)indoline-2,3-dione) as indolinone moiety (2 mmol, 503 mg) and compound **11** (*trans*-4-(4-oxo-2-thioxothiazolidin-3-yl)methyl)cyclohexanecarboxylic acid) as rhodanine moiety (2 mmol, 547 mg) according to synthetic procedure 5, **14** (*trans*-4-(((*(Z)*-5-(1-(4-methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)methyl)cyclohexanecarboxylic acid) was obtained after ~25 hours reaction time and recrystallisation from dichloromethane/tetrahydrofuran mixture 1:1 (v/v) with petrol ether (40-60 ºC) as dark brown amorphous powder. Yield: 1.02 mmol, 517 mg, 51%; $C_{27}H_{26}N_2O_4S_2$, *MW* 506.65; mp 308 ºC; ^{1}H NMR (400 MHz, 60 ºC, $(CD_3)_2SO$): δ = 11.76 (bs, 1H, COOH), 8.89 (d, ^3J = 8.1 Hz, $1H_{ind}$, 4-H), 7.44 (t, ^3J = 7.3 Hz, $1H_{ind}$, 6-H), 7.22 (d, ^3J = 8.2 Hz, $2H_{bn}$, 2"/6"-H), 7.17-7.13 (m, $3H_{bn/ind}$, 5/3"/5"-H), 7.07 (d, ^3J = 8.0 Hz, $1H_{ind}$, 7-H), 4.99 (s, 2H, *Bn*-CH_2), 3.99 (d, ^3J = 7.0 Hz, $2H_{tamcha}$, CH_2), 2.27 (s, 3H, CH_3), 2.21-2.12 (m, $1H_{tamcha}$, 1_a-H), 1.93-1.90 (m, $3H_{tamcha}$, $2_e/4_a/6_e$-H), 1.78-1.77 (m, $2H_{tamcha}$, $3_e/5_e$-H), 1.28-1.121 (m, $2H_{tamcha}$, $2_a/6_a$-H), 1.17-1.10 (m, $2H_{tamcha}$, $3_a/5_a$-H); ^{13}C NMR (100 MHz, 60 ºC, $(CD_3)_2SO$): δ = 198.21 (C=S, 2-C_{rh}), 176.70 (COOH), 167.49 (C=O, 2-C_{ind}), 167.18 (C=O, 4-C_{rh}), 145.09 (C_q, 7'-C_{ind}), 137.29 (C_q, 1"-C_{bn}), 133.25 (CH_{ar}, 6-C_{ind}), 133.13 (C_q, 4"-C_{bn}), 132.36 (C_q, 3-C_{ind}), 129.66 (2 x CH_{ar}, 3"/5"-C_{bn}), 128.29 (CH_{ar}, 4-C_{ind}), 127.66 (2 x CH_{ar}, 2"/6"-C_{bn}), 124.61 (C_q, 5-C_{rh}), 123.25 (CH_{ar}, 5-C_{ind}), 119.97 (C_q, 3'-C_{ind}), 110.50 (CH_{ar}, 7-C_{ind}), 50.08 (*TAMCHA*-CH_2), 43.49 (*Bn*-CH_2), 42.66 (CH, 1-C_{tamcha}), 35.84 (CH, 4-C_{tamcha}), 29.83 (2 x CH_2, 3/5-C_{tamcha}), 28.52 (2 x CH_2, 2/6-C_{tamcha}), 20.99 (CH_3); MS (ESI direct negative mode): *m/z (%)* 506.9 $[M]^+$ (13), 505.9 $[M - H]^-$ (30), 504.8 $[M – 2H]^-$ (100); HPLC purity analyses: i) 98.4%, retention time 27.48 min; ii) 98.5%, retention time 29.53 min.

4.22. Methyl *(Z)*-6-(5-(1-(4-Methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxo-thiazolidin-3-yl)hexanoat (16)

Starting out from **7b** (1-(4-methylbenzyl)indoline-2,3-dione) as indolinone moiety (2 mmol, 503 mg) and compound **9** (methyl 6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoat) as rhodanine moiety (2 mmol, 547 mg) according to synthetic procedure 5, **16** (methyl *(Z)*-6-(5-(1-(4-methylbenzyl)-2-oxoindolin-3-ylidene)-4-oxo-2-thioxo-thiazolidin-3-yl) hexanoat) was obtained after ~6 hours reaction time, purification using silica gel column chromatography with cyclohexane/ethyl acetate mixture 3:1 as eluent, and recrystallisation from dichloromethane with petrol ether (40-60 ºC) as red-brownish amorphous powder. Yield: 2.4 mmol, 1.15 g, 48%; $C_{26}H_{26}N_2O_4S_2$, *MW* 494.64; mp 185 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.98 (d, ^3J = 7.8 Hz , $1H_{ind}$, 4-H), 7.33 (t, ^3J = 7.7 Hz, $1H_{ind}$, 6-H), 7.20 (d, ^3J = 7.9 Hz, $2H_{bn}$, 2"/6"-H), 7.15-7.09 (m, $3H_{bn/ind}$, 5/3"/5"-H), 6.81 (d, ^3J = 7.8 Hz, $1H_{ind}$, 7-H), 4.96 (s, 2H, *Bn*-CH_2), 4.18 (t, ^3J = 7.6 Hz, $2H_{aliph}$, 6-CH_2), 3.69 (s, 3H, OCH_3), 2.38-2.33 (m, $5H_{aliph}$, 2-CH_2/CH_3), 1.79-1.71 (m, $4H_{aliph}$, 3/5-CH_2), 1.47-1.43 (m, $2H_{aliph}$, 4-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.90 (C=S, 2-C_{rh}), 173.81 (C=O, 1-C_{aliph}), 167.19 (2 x C=O, 2-C_{ind}/4-C_{rh}), 144.91 (C_q, 7'-C_{ind}), 137.68 (C_q, 1"-C_{bn}), 132.57 (CH_{ar}, 6-C_{ind})/(C_q, 4"-C_{bn}), 132.09 (C_q, 3-C_{ind}), 129.56 (2 x CH_{ar}, 3"/5"-C_{bn}), 128.63 (CH_{ar}, 4-C_{ind}), 127.34 (2 x CH_{ar}, 2"/6"-C_{bn}), 124.88 (C_q, 5-C_{rh}), 123.13 (CH_{ar}, 5-C_{ind}), 120.15 (C_q, 3'-C_{ind}), 109.54 (CH_{ar}, 7-C_{ind}), 51.47 (OCH_3), 44.12 (CH_2, 6-C_{aliph}), 43.93 (*Bn*-CH_2), 30.81 (CH_2, 2-C_{aliph}), 26.78 (CH_2, 5-C_{aliph}), 26.29 (CH_2, 4-C_{aliph}), 24.44 (CH_2, 3-C_{aliph}), 21.09 (CH_3); MS (EI direct mode): *m/z (%)* 494.2 $[M]^+$ (99), 105.1 $[C_8H_9]^+$ (100); microanalysis $[C_{26}H_{26}N_2O_4S_2]$: *found/calculated (%)* C (63.13/63.11), H (5.30/5.23), N (5.66/5.49), S (12.97/12.70).

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