Supporting Information

*N-Aryl-3-mercaptopussuccinimides as Antivirulence Agents Targeting* *Pseudomonas aeruginosa* *Elastase* and *Clostridium Collagenases*

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Zebrafish-embryo toxicity.

Table S1. Results of zebrafish-embryo toxicity for compounds 15, 25, 5 and 6

| Compound | Conc. [µM] | Observations after 1 day of incubation | Observations after 2 days of incubation | Observations after 3 days of incubation | Observations after 4 days of incubation | Final survival rate [%] |
|----------|-----------|---------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|------------------------|
| 15       | 100       | no pigmentation, cpd precipitation     | impaired pigmentation cpd precipitation | impaired pigmentation cpd precipitation | impaired pigmentation cpd precipitation | 90                     |
|          | 30        | impaired pigmentation                  | -                                      | -                                      | -                                      | 100                    |
|          | 10        | impaired pigmentation                  | -                                      | -                                      | -                                      | 100                    |
|          | 2         | -                                     | -                                      | -                                      | -                                      | 90                     |
| 25       | 100       | no pigmentation, cpd precipitation     | impaired pigmentation cpd precipitation | impaired pigmentation cpd precipitation | impaired pigmentation cpd precipitation | 100                    |
|          | 30        | -                                     | -                                      | -                                      | -                                      | 100                    |
|          | 10        | -                                     | -                                      | -                                      | -                                      | 100                    |
|          | 2         | -                                     | -                                      | -                                      | -                                      | 90                     |
| 5        | 100       | all embryos dead, cpd precipitation    | all embryos dead cpd precipitation     | all embryos dead cpd precipitation     | all embryos dead cpd precipitation     | 0                      |
|          | 30        | all embryos dead, cpd precipitation    | all embryos dead cpd precipitation     | all embryos dead cpd precipitation     | all embryos dead cpd precipitation     | 0                      |
|          | 10        | no pigmentation                        | impaired pigmentation                  | impaired pigmentation                  | impaired pigmentation                  | 90                     |
|          | 2         | -                                     | -                                      | -                                      | -                                      | 80                     |
| 6        | 100       | no pigmentation, cpd precipitation     | impaired pigmentation cpd precipitation | impaired pigmentation cpd precipitation | malformation: 5 embryos cpd precipitation | 70 (however, malformation in 50% of embryos) |
|          | 30        | slightly impaired pigmentation         | -                                      | -                                      | -                                      | 90                     |
|          | 10        | -                                     | -                                      | -                                      | -                                      | 100                    |
|          | 2         | -                                     | -                                      | -                                      | -                                      | 100                    |

Precipitation of compound 5 was observed by eye at 30 µM and 100 µM, while for compounds 6, 15 and 25 it was observed only at the highest concentration tested (100 µM).
Ex vivo pig-skin experiment.

Figure S1. Stability of ColQ1 after different time points and under different buffer conditions.

Figure S2. Quantification of hydroxyproline as a product of ColQ1-induced degradation of collagen in small pieces of pig ear skin. Different concentrations (0 – 500 nM) ColQ1 were used; hydroxyproline formation was determined after different time points. Mean ± SD of three independent measurements are presented.
Figure S3. Calibration curve of hydroxyproline. Mean ± SD of three independent measurements are depicted.
Chemistry.

**General procedure A: Synthesis of succinimides 7–27 and 50–52**
Mercaptosuccinic acid (1.0 eq) and the corresponding aniline (1.0 eq) were mixed in a crimp vial under Ar atmosphere and heated at 120–160 °C from 3.5 h to overnight. The crude product was purified using column chromatography. In case of 3-mercaptomethyl-N-arylsuccinimides, 4-(aminoaryl)-2-(mercaptomethyl)-4-oxobutanoic acid was heated at 120 °C overnight.

**General procedure B: Synthesis of thioacetates 28–30 by acetylation of free thiol**
Succinimide (1.0 eq) was dissolved in DCM and the solution was cooled in an ice bath. Pyridine (2.0 eq) and DMAP (0.1 eq) were added, followed by dropwise addition of Ac₂O (2.0 eq). After 30 minutes at 0 °C, the reaction mixture was allowed to warm up to r.t. and stirred overnight. Volatiles were evaporated under reduced pressure and crude product was purified using column chromatography.

**General procedure C: Synthesis of α-itaconamic acids 31–35**
α-Itaconamic acids were synthesized following the procedure described in the literature.¹ Itaconic anhydride (1.0 eq) was dissolved in CHCl₃. The corresponding aniline (1.0 eq) was added to the vigorously stirring solution. After 2 h, the product was collected by filtration and washed with a small amount of chloroform. The product was used in the next step without further purification.

**General procedure D: Synthesis of itaconimides 36–39**
Itaconimides were synthesized following the procedure described in the literature from intermediate α-itaconamic acids described in general procedure C.¹ α-Itaconamic acid (1.0 eq) was mixed with NaOAc (0.5 eq) and Ac₂O (3.5 eq) and heated at 100 °C for 1–2 h. The dark reaction mixture was cooled to r.t., poured into ice-cold water, and extracted 3 times with EtOAc. Combined organic layers were washed with brine and dried over anh. Na₂SO₄, filtered and the solvent removed under reduced pressure. The crude product was purified using column chromatography. In all cases except in the case of the 4-OMe derivative, the corresponding citraconimides were isolated as a side product and therefore the yield of obtained itaconimides was low to moderate.

**General procedure E: Synthesis of thioacetates 40–46 using Michael addition**
Corresponding itaconimide/α-itaconamic acid (1.0 eq) was dissolved in DME/DCM/THF under Ar atmosphere. Thioacetic acid (1.1–1.5 eq) was added, followed by Et₃N (0.01–0.1 eq). The reaction mixture was stirred at r.t. overnight. Crude product was purified using column chromatography or used in the next step without further purification.
**General procedure F: Thioacetate hydrolysis to obtain compounds 47–49**

Thioacetate (1.0 eq) was dissolved in methanol under Ar atmosphere, and 2 M aqueous solution of NaOH (2.0–3.0 eq) was added. The reaction was stirred 1–2 h at r.t. After quenching with 1M HCl, the reaction was extracted 3 times with EtOAc. Combined organic extracts were washed with brine and dried over anh. Na₂SO₄, filtered and the solvent was removed under reduced pressure. The crude product was purified using column chromatography or used in the next step without further purification.

**3-mercapto-1-phenylpyrrolidine-2,5-dione (7).**

Compound 7 was synthesized according to the general procedure A, using aniline (93 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (105 mg, 51%, M.p. 146 °C).

**1H NMR (500 MHz, DMSO-d6) δ ppm:** 7.51 (t, \( J = 8.0 \) Hz, 2H), 7.43 (t, \( J = 7.5 \) Hz, 1H), 7.27 (t, \( J = 8.0 \) Hz, 2H), 4.11 (dd, \( J = 4.5, 9.0 \) Hz, 1H), 3.87 (s, 1H), 3.37 (dd, \( J = 9.5, 18.0 \) Hz, 1H), 2.74 (dd, \( J = 4.5, 18.0 \) Hz, 1H). **13C NMR (126 MHz, DMSO-d6) δ ppm:** 176.6, 174.2, 132.5, 128.9, 128.4, 127.0, 39.3, 34.8. **HRMS (ESI⁺) m/z calcd. for C₁₀H₁₀NO₂S [M+H]⁺ 208.04322, found 208.06846.**

**3-mercapto-1-(p-tolyl)pyrrolidine-2,5-dione (8).**

Compound 8 was synthesized according to the general procedure A, using p-toluidine (107 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (103 mg, 47%, M.p. 166 °C). **1H NMR (500 MHz, DMSO-d6) δ ppm:** 7.30 (d, \( J = 8.0 \) Hz, 2H), 7.15 (d, \( J = 8.5 \) Hz, 2H), 4.09 (dd, \( J = 4.5, 9.0 \) Hz, 1H), 3.85 (s, 1H), 3.35 (dd, \( J = 9.5, 18.5 \) Hz, 1H), 2.72 (dd, \( J = 4.5, 18.5 \) Hz, 1H), 2.35 (s, 3H). **13C NMR (126 MHz, DMSO-d6) δ ppm:** 176.7, 174.3, 137.9, 129.9, 129.4, 126.8, 39.2, 34.7, 20.7. **HRMS (ESI⁺) m/z calcd. for C₁₁H₁₂NO₂S [M+H]⁺ 222.05887, found 222.05806.**

**1-(3,4-dimethylphenyl)-3-mercaptopyrrolidine-2,5-dione (9).**

Compound 9 was synthesized according to the general procedure A, using 3,4-dimethylaniline (121 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (80 mg, 34%, M.p. 122 °C). **1H NMR (500 MHz, DMSO-d6) δ ppm:** 7.24 (d, \( J = 8.0 \) Hz, 1H), 7.02 (br s, 1H), 6.97 (dd,
J = 1.5 , 8.0 Hz, 1H), 4.08 (dd, J = 4.5, 9.5 Hz, 1H), 3.84 (s, 1H), 3.34 (dd, J = 9.5, 18.5 Hz, 1H), 2.71 (dd, J = 4.5, 18.5 Hz, 1H), 2.25 (s, 3H), 2.24 (s, 3H). $^{13}$C NMR (126 MHz, DMSO-d6) δ ppm: 176.7, 174.3, 136.9, 136.7, 130.1, 129.8, 127.8, 124.3, 39.2, 34.7, 19.3, 19.1. HRMS (ESI$^+$) m/z calcd. for C$_{12}$H$_{14}$NO$_2$S [M+H]$^+$: 236.07452, found 236.07348.

1-(2-chlorophenyl)-3-mercaptopyrrolidine-2,5-dione (10).

Compound 10 was synthesized according to the general procedure A, using 2-chloroaniline (128 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (160 mg, 66%, M.p. 112 °C).

$^1$H NMR (500 MHz, DMSO-d6) δ ppm: 7.59–7.48 (m, 2H), 7.41 (br s, 1H), 7.29 (br d, J = 7.5 Hz, 1H), 4.10 (dd, J = 4.5, 9.0 Hz, 1H), 3.87 (s, 1H), 3.37 (dd, J = 9.5, 18.5 Hz, 1H), 2.73 (dd, J = 4.5, 18.0 Hz, 1H).

$^{13}$C NMR (126 MHz, DMSO-d6) δ ppm: 176.4, 174.0, 133.9, 132.9, 130.6, 128.4, 127.0, 125.9, 39.5, 34.8.

HRMS (ESI$^-$) m/z calcd. for C$_{10}$H$_7$ClNO$_2$S [M-H]$^-$: 239.98860, found 239.98863.

1-(3-chlorophenyl)-3-mercaptopyrrolidine-2,5-dione (11).

Compound 11 was synthesized according to the general procedure A, using 3-chloroaniline (128 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (133 mg, 55%, M.p. 111 °C).

$^1$H NMR (500 MHz, DMSO-d6) δ ppm: 7.55 (t, J = 8.0 Hz, 1H), 7.52 (dt, J = 2.0, 8.0 Hz, 1H), 7.41 (t, J = 2.0 Hz, 1H), 7.29 (dt, J = 9.5, 18.5 Hz, 1H), 2.74 (dd, J = 4.5, 18.0 Hz, 1H).

$^{13}$C NMR (126 MHz, DMSO-d6) δ ppm: 176.4, 174.0, 133.9, 132.9, 130.6, 128.5, 127.0, 125.9, 39.5, 34.8. HRMS (ESI$^+$) m/z calcd. for C$_{10}$H$_7$ClNO$_2$S [M-H]$^+$: 239.98860, found 239.98867.

1-(4-chlorophenyl)-3-mercaptopyrrolidine-2,5-dione (12).

Compound 12 was synthesized according to the general procedure A, using 4-chloroaniline (150 mg, 1.176 mmol) and mercaptosuccinic acid (177 mg, 1.176 mmol), at 120 °C overnight. The product was purified using column chromatography (DCM → DCM/MeOH=95/5). Final product was obtained as white solid (165 mg, 58%, M.p. 146 °C). $^1$H NMR (500 MHz, DMSO-d6) δ ppm: 7.58 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 8.5 Hz, 2H), 4.09 (dd, J = 4.5, 9.5 Hz, 1H), 3.87 (s, 1H), 3.36 (dd, J = 9.0, 18.0 Hz, 1H), 2.73 (dd, J = 4.5, 18.0 Hz, 1H).

$^{13}$C NMR (126 MHz, DMSO-d6) δ ppm: 176.4, 174.1, 132.9, 131.4, 129.0, 128.8, 39.4, 34.8. HRMS (ESI$^+$) m/z calcd. for C$_{10}$H$_7$ClNO$_2$S [M-H]$^+$: 239.98860, found 239.98872.
1-(3,4-dichlorophenyl)-3-mercaptopyrrolidine-2,5-dione (13).

Compound 13 was synthesized according to the general procedure A, using 3,4-dichloroaniline (162 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (155 mg, 56%, M.p. 141 °C). $^1$H NMR (500 MHz, DMSO-d$_6$) δ ppm: 7.81 (d, $J = 8.5$ Hz, 1H), 7.62 (d, $J = 2.0$ Hz, 1H), 7.34 (dd, $J = 2.0$, 8.5 Hz, 1H), 4.10 (dd, $J = 4.5$, 9.0 Hz, 1H), 3.87 (s, 1H), 3.37 (dd, $J = 9.0$, 18.0 Hz, 1H), 2.73 (dd, $J = 4.5$, 18.5 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO-d$_6$) δ ppm: 176.2, 173.8, 132.4, 131.2, 131.1, 131.0, 128.9, 127.5, 39.2, 34.9.

HRMS (ESI) m/z calcd. for C$_{10}$H$_6$Cl$_2$NO$_2$S [M-H] - 273.94963, found 273.94931.

1-(2,6-dichlorophenyl)-3-mercaptopyrrolidine-2,5-dione (14).

Compound 14 was synthesized according to the general procedure A, using 2,6-dichloroaniline (162 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 160 °C for 3.5 h. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (84 mg, 30%, M.p. 111 °C). $^1$H NMR (500 MHz, DMSO-d$_6$) δ ppm: 7.75–7.66 (m, 2H), 7.59 (t, $J = 8.0$ Hz, 1H), 4.42 (dd, $J = 4.0$, 9.0 Hz, 1H), 3.97 (s, 1H), 3.62 (dd, $J = 9.0$, 18.5 Hz, 1H), 2.94 (dd, $J = 4.0$, 18.5 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO-d$_6$) δ ppm: 175.2, 172.7, 133.7, 133.5, 132.5, 129.1, 129.0, 127.8, 38.7, 34.6. HRMS (ESI$^+$) m/z calcd. for C$_{10}$H$_8$Cl$_2$NO$_2$S [M+H]$^+$ 275.96528, found 275.96398.

1-(3,4-difluorophenyl)-3-mercaptopyrrolidine-2,5-dione (15).

Compound 15 was synthesized according to the general procedure A, using 3,4-difluoroaniline (129 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (121 mg, 50%, M.p. 99 °C). $^1$H NMR (500 MHz, DMSO-d$_6$) δ ppm: 7.60 (dt, $J = 9.0$, 10.5 Hz, 1H), 7.43 (ddd, $J = 2.5$, 7.5, 11.5 Hz, 1H), 7.24–7.13 (m, 1H), 4.10 (dd, $J = 4.5$, 9.0 Hz, 1H), 3.87 (s, 1H), 3.37 (dd, $J = 9.0$, 18.0 Hz, 1H), 2.73 (dd, $J = 4.5$, 18.0 Hz, 1H). $^{13}$C NMR (126 MHz, DMSO-d$_6$) δ ppm: 176.3, 174.0, 149.1 ($J_{C,F} = 12.5$, 245 Hz), 149.0 ($J_{C,F} = 12.5$, 245 Hz), 129.1 ($J_{C,F} = 2.5$, 8.8 Hz), 124.5 ($J_{C,F} = 2.5$, 7.5 Hz), 117.8 ($J_{C,F} = 17.5$ Hz), 116.7 ($J_{C,F} = 18.8$ Hz), 39.4, 34.8. HRMS (ESI) m/z calcd. for C$_{10}$H$_6$F$_2$NO$_2$S [M-H]$^-$ 242.00873, found 242.00894.
1-(5-chloro-2-methylphenyl)-3-mercapto pyrrolidine-2,5-dione (16).

Compound 16 was synthesized according to the general procedure A, using 5-chloro-2-methylaniline (142 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (90 mg, 35%, MP= 104 °C). ¹H NMR (500 MHz, DMSO-d6) δ ppm: 7.45 (dd, J = 2.0, 8.0 Hz, 1H+1H*), 7.40 (dd, J = 4.0, 8.0 Hz, 1H+1H*), 7.35 (d, J = 2.0 Hz, 1H*), 7.32 (d, J = 2.5 Hz, 1H), 4.22 (dd, J = 4.5, 9.0 Hz, 1H*), 4.10 (dd, J = 4.0, 9.0 Hz, 1H), 3.92 (s, 1H+1H*), 3.47 (dd, J = 9.0, 18.0 Hz, 1H*), 3.38 (dd, J = 9.0, 18.5 Hz, 1H), 2.81 (dd, J = 4.0, 18.5 Hz, 1H), 2.74 (dd, J = 4.5, 18.0 Hz, 1H*), 2.09 (s, 3H), 2.06 (s, 3H*). ¹³C NMR (126 MHz, DMSO-d6) δ ppm: 176.5, 176.2*, 174.0, 173.9*, 135.2, 135.1*, 132.9, 132.7*, 132.28, 132.26*, 130.3, 130.2*, 129.1, 128.19, 128.16*, 39.9, 39.0*, 35.0, 34.8*, 16.6, 16.4*. Compound 16 was obtained as a mixture of atropisomers (55:45). The values labeled with * belong to the second atropisomer. HRMS (ESI⁻) m/z calcd. for C₁₁H₉ClNO₂S [M-H]⁻ 254.00425, found 254.00454.

1-(3-chloro-2,6-dimethylphenyl)-3-mercapto pyrrolidine-2,5-dione (17).

Compound 17 was synthesized according to the general procedure A, using 3-chloro-2,6-dimethylaniline hydrochloride (192 mg, 1 mmol) and mercaptosuccinic acid (150 mg, 1 mmol), at 160 °C for 3.5 h. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (145 mg, 54%, M.p. 104 °C). ¹H NMR (500 MHz, DMSO-d6) δ ppm: 7.48 (d, J = 8.5 Hz, 1H+1H*), 7.24 (d, J = 8.0 Hz, 1H+1H*), 4.35–4.24 (m, 1H+1H*), 4.01 (s, 1H+1H*), 3.56 (dd, J = 3.0, 9.0 Hz, 1H*), 3.53 (dd, J = 3.0, 9.0 Hz, 1H), 2.89 (dd, J = 4.0, 13.5 Hz, 1H), 2.86 (dd, J = 3.5, 13.0Hz, 1H*), 2.12 (s, 3H*), 2.09 (s, 3H), 2.07 (s, 3H*), 2.04 (s, 3H). ¹³C NMR (126 MHz, DMSO-d6) δ ppm: 176.31, 176.28*, 173.84, 173.78*, 135.5, 135.4*, 134.2, 134.0*, 131.6, 131.4, 131.3*, 129.79, 129.78*, 129.18, 129.16*, 38.7, 34.7, 17.3, 17.1*, 15.1, 14.8*. Compound 17 was obtained as a mixture of atropisomers (56:44). The values labeled with * belong to the second atropisomer. HRMS (ESI⁺) m/z calcd. for C₁₂H₁₃ClNO₂S [M+H]⁺ 270.03555, found 270.03422.

3-mercapto-1-(2-methoxyphenyl)pyrrolidine-2,5-dione (18).

Compound 18 was synthesized according to the general procedure A, using o-anisidine (328 mg, 2.66 mmol) and mercaptosuccinic acid (400 mg, 2.66 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as pale yellow oil (268.7 mg, 42%). ¹H
NMR (500 MHz, CDCl$_3$) δ ppm: 7.44–7.39 (m, 1H+1H*), 7.17–7.10 (m, 1H+1H*), 7.07–6.99 (m, 2H+2H*), 4.14–4.08 (m, 1H), 4.05–4.00 (m, 1H*), 3.82 (s, 3H), 3.78 (s, 3H*), 3.40–3.29 (m, 1H+1H*), 2.80 (dd, 1H, $J = 4.1, 18.5$ Hz), 2.72 (dd, 1H*, $J = 4.4, 18.6$ Hz), 2.67 (d, 1H, $J = 4.2$ Hz), 2.65 (d, 1H*, $J = 5.5$ Hz). $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm: 176.1, 175.9*, 173.3, 173.1*, 154.7, 154.6*, 131.1, 131.1*, 129.0, 129.0*, 121.0, 120.9*, 120.3, 120.2*, 112.2, 112.1*, 55.9, 55.8*, 38.2, 37.7*, 34.6*, 34.4. Compound 18 was obtained as a mixture of atropisomers (54:46). The values labeled with * belong to the second atropisomer. HRMS (ESI$^+$) m/z calcd. for C$_{11}$H$_{12}$NO$_3$S [M+H]$^+$ 238.05324, found 238.05164.

3-mercapto-1-(3-methoxyphenyl)pyrrolidine-2,5-dione (19).

Compound 19 was synthesized according to the general procedure A, using m-anisidine (328 mg, 2.66 mmol) and mercaptosuccinic acid (400 mg, 2.66 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (189.1 mg, 30%, M.p. 105 °C). $^1$H NMR (500 MHz, CDCl$_3$) δ ppm: 7.40 (t, 1H, $J = 8.2$ Hz), 7.00–6.95 (m, 1H), 6.91–6.87 (m, 1H), 6.85–6.82 (m, 1H), 4.11–4.05 (m, 1H), 3.83 (s, 3H), 3.34 (dd, 1H, $J = 9.2, 18.8$ Hz), 2.74 (dd, 1H, $J = 4.2, 18.7$ Hz), 2.71 (d, 1H, $J = 4.3$ Hz). $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm: 176.0, 173.1, 160.1, 132.5, 130.0, 118.5, 114.9, 112.1, 55.4, 37.4, 34.2. HRMS (ESI$^+$) m/z calcd. for C$_{11}$H$_{12}$NO$_3$S [M+H]$^+$ 238.05324, found 238.05217.

3-mercapto-1-(4-methoxyphenyl)pyrrolidine-2,5-dione (20).

Compound 20 was synthesized according to the general procedure A, using p-anisidine (500 mg, 4.06 mmol) and mercaptosuccinic acid (610 mg, 4.06 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (105.2 mg, 11%, M.p. 133 °C). $^1$H NMR (500 MHz, CDCl$_3$) δ ppm: 7.25–7.20 (m, 2H), 7.02–6.98 (m, 2H), 4.10–4.04 (m, 1H), 3.84 (s, 3H), 3.33 (dd, 1H, $J = 9.2, 18.6$ Hz), 2.73 (dd, 1H, $J = 4.3, 18.8$ Hz), 2.70 (d, 1H, $J = 4.3$ Hz). $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm: 176.3, 173.5, 159.7, 127.5, 124.1, 114.6, 55.5, 37.4, 34.1. HRMS (ESI$^+$) m/z calcd. for C$_{11}$H$_{12}$NO$_3$S [M+H]$^+$ 238.05324, found 238.05228.

1-(2,4-dimethoxyphenyl)-3-mercaptopopyrrolidine-2,5-dione (21).

Compound 21 was synthesized according to the general procedure A, using 2,4-dimethoxyaniline (400 mg, 2.61 mmol) and mercaptosuccinic acid (392 mg, 2.61 mmol), at 120 °C overnight. The product was purified using column chromatography (Hex → Hex/EtOAc=6/4). Final product was obtained as transparent sticky solid (176.6 mg, 25%). $^1$H NMR (500 MHz, CDCl$_3$) δ ppm:
7.10–7.06 (m, 1H), 7.05–7.01 (m, 1H*), 6.60–6.54 (m, 2H+2H*), 4.13–4.08 (m, 1H), 4.04–3.99 (m, 1H*), 3.83 (s, 3H+3H*), 3.81 (s, 3H), 3.77 (s, 3H*), 3.39–3.28 (m, 1H+1H*), 2.79 (dd, 1H, J = 4.1, 18.6 Hz), 2.71 (dd, 1H*, J = 4.3, 18.5 Hz), 2.68 (d, 1H, J = 4.4 Hz), 2.65 (d, 1H*, J = 5.3 Hz). 13C NMR (126 MHz, CDCl3) δ ppm: 176.4, 176.3*, 173.7, 173.5*, 161.8, 161.8*, 155.7, 155.5*, 129.4, 129.4*, 113.1, 113.0*, 104.9, 104.9*, 99.7, 99.7*, 55.9, 55.8*, 55.6, 55.6*, 38.1, 37.6*, 34.5*, 34.3. Compound 21 was obtained as a mixture of atropisomers (55:45). The values labeled with * belong to the second atropisomer. HRMS (ESI+) m/z calcd. for C12H14NO4S [M+H]+ 268.063804, found 268.06201.

1-(3,4-dimethoxyphenyl)-3-mercaptopyrrolidine-2,5-dione (22).

Compound 22 was synthesized according to the general procedure A, using 3,4-dimethoxyaniline (450 mg, 2.94 mmol) and mercaptosuccinic acid (441 mg, 2.94 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as colorless oil (152.9 mg, 20%). 1H NMR (500 MHz, CDCl3) δ ppm: 6.97–6.62 (m, 1H), 6.88–6.83 (m, 1H), 6.80–6.75 (m, 1H), 4.10–4.03 (m, 1H), 3.91 (s, 4H), 3.88 (s, 3H), 3.33 (dd, 1H, J = 9.2, 18.6 Hz), 2.77–2.67 (m, 2H). 13C NMR (126 MHz, CDCl3) δ ppm: 176.3, 173.5, 149.4, 149.3, 124.2, 118.9, 111.1, 109.7, 56.0, 37.4, 34.1. HRMS (ESI+) m/z calcd. for C12H14NO4S [M+H]+ 268.063804, found 268.06240.

1-(3-chloro-4-(trifluoromethoxy)phenyl)-3-mercaptopyrrolidine-2,5-dione (23).

Compound 23 was synthesized according to the general procedure A, using 3-chloro-4-trifluoromethoxyaniline (300 mg, 1.42 mmol) and mercaptosuccinic acid (213 mg, 1.42 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (267.6 mg, 58%, M.p. 102 °C). 1H NMR (500 MHz, CDCl3) δ ppm: 7.55 (d, 1H, J = 2.4 Hz), 7.47–7.43 (m, 1H), 7.34 (dd, 1H, J = 2.4, 8.9 Hz), 4.13–4.08 (m, 1H), 3.37 (dd, 1H, J = 9.2, 18.8 Hz), 2.77 (dd, 1H, J = 4.2, 18.8 Hz), 2.73 (d, 1H, J = 4.3 Hz). 13C NMR (126 MHz, CDCl3) δ ppm: 175.5, 172.5, 145.1 (d, J = 1.8 Hz), 130.6, 128.7, 128.1, 125.7, 122.8, 120.4 (q, JCF = 260.8 Hz), 37.3, 34.1. HRMS (ESI+) m/z calcd. for C11H8ClF3NO3S [M+H]+ 325.986002, found 325.98434.

3-mercapto-1-(3-nitrophenyl)pyrrolidine-2,5-dione (24).

Compound 24 was synthesized according to the general procedure A, using 3-nitroaniline (414 mg, 2.99 mmol) and mercaptosuccinic acid (450 mg, 2.99 mmol), at 120 °C overnight. The product was purified using column chromatography (Hex → Hex/EtOAc=1/1). Final product was obtained as pale
yellow solid (54 mg, 6%, M.p. 135 °C). $^1$H NMR (500 MHz, CDCl$_3$) δ ppm: 8.34–8.25 (m, 2H), 7.77–7.66 (m, 2H), 4.18–4.11 (m, 1H), 3.41 (dd, 1H, $J = 9.2, 18.8$ Hz), 2.81 (dd, 1H, $J = 4.2, 18.8$ Hz), 2.75 (d, 1H, $J = 4.4$ Hz). $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm: 175.4, 172.4, 148.5, 132.6, 132.1, 130.1, 123.5, 121.6, 37.3, 34.1. HRMS (ESI) m/z calcd. for C$_{10}$H$_7$N$_2$O$_4$S [M-H]$^-$ 251.0132, found 251.01134.

1-(4-acetylphenyl)-3-mercaptopyrrolidine-2,5-dione (25).

1-(4-phenylaniline (150 mg, 0.89 mmol) and mercaptosuccinic acid (133.1 mg, 0.89 mmol), at 120 °C overnight. The product was purified using column chromatography (Hex/EtOAc=7/3). Final product was obtained as pale yellow solid (117.1 mg, 32%, M.p. 104 °C). $^1$H NMR (500 MHz, CDCl$_3$) δ ppm: 8.11–8.04 (m, 2H), 7.50–7.44 (m, 2H), 4.14–4.08 (m, 1H), 3.38 (dd, 1H, $J = 9.2, 18.8$ Hz), 2.77 (dd, 1H, $J = 4.3, 18.8$ Hz), 2.73 (d, 1H, $J = 4.4$ Hz). $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm: 196.9, 175.6, 172.7, 136.9, 135.5, 129.2, 126.2, 37.4, 34.2. HRMS (ESI) m/z calcd. for C$_{12}$H$_{10}$NO$_3$S [M-H]$^-$ 248.03867, found 248.03867.

1-([1,1'-biphenyl]-3-yl)-3-mercaptopyrrolidine-2,5-dione (26).

Compound 26 was synthesized according to the general procedure A, using 3-phenylaniline (150 mg, 0.89 mmol) and mercaptosuccinic acid (133.1 mg, 0.89 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (130 mg, 52%, M.p. 127 °C). $^1$H NMR (500 MHz, CDCl$_3$) δ ppm: 7.67–7.63 (m, 1H), 7.54–7.51 (m, 1H), 7.49–7.44 (m, 2H), 7.41–7.35 (m, 1H), 7.31–7.28 (m, 1H), 4.15–4.08 (m, 1H), 3.38 (dd, 1H, $J = 9.2, 18.8$ Hz), 2.78 (dd, 1H, $J = 4.2, 18.7$ Hz), 2.73 (d, 1H, $J = 4.3$ Hz). $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm: 176.1, 173.2, 142.6, 139.9, 132.0, 129.6, 128.8, 127.8, 127.7, 127.2, 125.2, 125.0, 37.4, 34.2. HRMS (ESI) m/z calcd. for C$_{16}$H$_{12}$NO$_3$S [M-H]$^-$ 282.05960, found 282.05960.

1-([1,1'-biphenyl]-4-yl)-3-mercaptopyrrolidine-2,5-dione (27).

Compound 27 was synthesized according to the general procedure A, using 4-phenylaniline (150 mg, 0.89 mmol) and mercaptosuccinic acid (133.1 mg, 0.89 mmol), at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (100 mg, 40%, M.p. 187 °C). $^1$H NMR (500 MHz, CDCl$_3$) δ ppm: 7.74–7.68 (m, 2H), 7.62–7.57 (m, 2H), 7.49–7.44 (m, 2H), 7.42–7.37 (m, 3H), 4.15–4.09 (m, 1H), 3.38 (dd, 1H, $J = 9.2, 18.8$ Hz), 2.78 (dd, 1H, $J = 4.2, 18.7$ Hz), 2.74 (d, 1H, $J = 4.4$ Hz). $^{13}$C NMR (126 MHz, CDCl$_3$) δ ppm:
δ ppm: 176.1, 173.3, 142.0, 140.1, 130.6, 128.9, 128.0, 127.8, 127.2, 126.5, 37.4, 34.2. HRMS (ESI+) m/z calcd. for C₁₆H₁₂NO₂S [M-H]- 282.059422, found 282.05773.

S-(1-(4-methoxyphenyl)-2,5-dioxopyrrolidin-3-yl) ethanethioate (28).

Compound 28 was synthesized according to the general procedure B, using compound 20 (66.7 mg, 0.28 mmol), pyridine (45 μL, 0.56 mmol), Ac₂O (53 μL, 0.56 mmol) and DMAP (3.4 mg, 0.028 mmol). The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (51 mg, 65%, M.p. 129 °C). Product observed to suffer from degradation in MeOH. ¹H NMR (500 MHz, CDCl₃) δ ppm: 7.27–7.22 (m, 2H), 7.02–6.98 (m, 2H), 4.25 (dd, 1H, J = 5.6, 9.8 Hz), 3.84 (s, 3H), 3.40 (dd, 1H, J = 9.8, 18.6 Hz), 2.90 (dd, 1H, J = 5.7, 18.5 Hz), 2.42 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ ppm: 194.4, 174.2, 173.8, 159.7, 127.8, 124.5, 114.6, 55.5, 40.4, 36.9, 30.1. HRMS (ESI-) m/z calcd. for C₁₆H₁₂NO₂S [M-H]- 278.04779.

S-(1-(3,4-dichlorophenyl)-2,5-dioxopyrrolidin-3-yl) ethanethioate (29).

Compound 29 was synthesized according to the general procedure B, using compound 13 (50 mg, 0.18 mmol), pyridine (30 μL, 0.36 mmol), Ac₂O (35 μL, 0.36 mmol) and DMAP (2.2 mg, 0.018 mmol). The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (44.8 mg, 78%, M.p. 137 °C). Product observed to suffer from degradation in MeOH. ¹H NMR (500 MHz, CDCl₃) δ ppm: 7.57 (d, 1H, J = 8.5 Hz), 7.51 (d, 1H, J = 2.3 Hz), 7.25 (dd, 1H, J = 2.4, 8.6 Hz), 4.19 (dd, 1H, J = 5.6, 9.9 Hz), 3.41 (dd, 1H, J = 9.8, 18.7 Hz), 2.92 (dd, 1H, J = 5.8, 18.6 Hz), 2.43 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ ppm: 194.6, 173.6, 172.8, 133.2, 131.1, 130.8, 128.5, 125.8, 40.4, 36.8, 30.1. HRMS (ESI+) m/z calcd. for C₁₂H₁₀Cl₂NO₃S [M+H]+ 317.97457, found 317.97529.

S-(1-(3-chloro-4-(trifluoromethoxy)phenyl)-2,5-dioxopyrrolidin-3-yl) ethanethioate (30).

Compound 30 was synthesized according to the general procedure B, using compound 23 (54.6 mg, 0.17 mmol), pyridine (27 μL, 0.34 mmol), Ac₂O (32 μL, 0.34 mmol) and DMAP (2 mg, 0.017 mmol). The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (45.2 mg, 73%, M.p. 120 °C). Product observed to suffer from degradation in MeOH. ¹H NMR (500 MHz, CDCl₃) δ ppm: 7.55 (d, 1H, J = 2.4 Hz), 7.46–7.43 (m, 1H), 7.35 (dd, 1H, J = 8.9 Hz), 4.19 (dd, 1H, J = 5.6, 9.9 Hz), 3.43 (dd, 1H, J = 9.9, 18.6 Hz), 2.93 (dd, 1H, J = 5.6, 18.6 Hz), 2.43 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ ppm: 194.7, 173.6, 172.8, 145.1 (d, J = 1.8 Hz), 131.0, 129.1, 128.1, 126.1, 122.8, 120.4 (q, J_C-F = 259.8 Hz), 40.4,
36.9, 30.1. HRMS (ESI⁺) m/z calcd. for C₁₃H₁₀ClF₃NO₄S [M+H]⁺ 367.996567, found 367.99408.

4-((3,4-dichlorophenyl)amino)-2-methylene-4-oxobutanoic acid (32).

Compound 32 was synthesized according to the general procedure C, using itaconic anhydride (500 mg, 4.46 mmol) and 3,4-dichloroaniline (723 mg, 4.46 mmol). Compound 32 (with 7% of β-itaconamic acid) was obtained as white solid (1.07 g, 88%) and was used in the next step without further purification. ¹H NMR (500 MHz, DMSO-d₆) δ ppm: 12.58 (br s, 1H), 10.33 (s, 1H), 7.98 (d, 1H, J = 2.4 Hz), 7.56 (d, 1H, J = 8.9 Hz), 7.46 (dd, 1H, J = 2.4, 8.9 Hz), 6.20–6.16 (m, 1H), 5.78–5.74 (m, 1H), 3.35 (s, 2H).

¹³C NMR (126 MHz, DMSO-d₆) δ ppm: 169.1, 167.6, 139.4, 135.4, 131.0, 130.8, 128.1, 124.4, 120.1, 119.0, 39.5.

4-((3,4-difluorophenyl)amino)-2-methylene-4-oxobutanoic acid (33).

Compound 33 was synthesized according to the general procedure C, using itaconic anhydride (400 mg, 3.57 mmol) and 3,4-difluoroaniline (354 μL, 3.57 mmol). Compound 33 (with 8% of β-itaconamic acid) was obtained as pale purple solid (774 mg, 90%) and was used in the next step without further purification. ¹H NMR (500 MHz, DMSO-d₆) δ ppm: 12.53 (br s, 1H), 10.24 (s, 1H), 7.76 (ddd, 1H, J = 2.5, 7.5, 13.3 Hz), 7.37 (d, 1H, J = 9.2, 10.5 Hz), 7.29–7.24 (m, 1H), 6.19–6.16 (m, 1H), 5.76–5.73 (m, 1H), 3.34 (s, 2H).

¹³C NMR (126 MHz, DMSO-d₆) δ ppm: 168.8, 167.5, 148.9 (J_C-F = 13.2, 243.7 Hz), 145.0 (J_C-F = 12.9, 241.4 Hz), 136.3 (J_C-F = 2.8, 9.2 Hz), 135.5, 127.8, 117.4 (J_C-F = 18.4 Hz), 115.1 (J_C-F = 3.7, 5.5 Hz), 107.8 (J_C-F = 22.1 Hz), 39.3.

4-((4-acetylphenyl)amino)-2-methylene-4-oxobutanoic acid (34).

Compound 34 was synthesized according to the general procedure C, using itaconic anhydride (500 mg, 4.46 mmol) and 4-aminoacetoephone (670 mg, 4.46 mmol). Compound 34 (with 14% of β-itaconamic acid) was obtained as pale yellow solid (760 mg, 69%) and was used in the next step without further purification. ¹H NMR (500 MHz, DMSO-d₆) δ ppm: 12.53 (br s, 1H), 10.36 (s, 1H), 7.92 (d, 2H, J = 8.7 Hz), 7.71 (d, 2H, J = 8.7 Hz), 6.21–6.16 (m, 1H), 5.76 (s, 1H), 3.39 (s, 2H), 2.52 (s, 3H). ¹³C NMR (126 MHz, DMSO-d₆) δ ppm: 196.5, 169.2, 167.6, 143.6, 135.5, 131.5, 129.5, 127.9, 118.2, 26.4.
3-methylene-1-phenylpyrrolidine-2,5-dione (36).

Compound 36 was synthesized according to the combined general procedures C and D, using itaconic anhydride (301 mg, 2.68 mmol) and aniline (249.6 mg, 2.68 mmol). Corresponding α-itaconamic acid was converted to final compound using Ac₂O (0.9 mL, 9.38 mmol) and NaOAc (110 mg, 1.34 mmol). The final compound was separated from isomeric citraconimide using column chromatography (Hex/EtOAc=75/25). Compound 36 was obtained as off white solid (163.7 mg, 33%). ¹H NMR and ¹³C NMR are in accordance with the spectra from the literature.¹

1-(3,4-dichlorophenyl)-3-methylenepyrrolidine-2,5-dione (37).

Compound 37 was synthesized according to the combined general procedures C and D, using itaconic anhydride (350 mg, 3.12 mmol) and 3,4-dichloroaniline (506 mg, 3.12 mmol). Corresponding α-itaconamic acid 32 was converted to the final compound using Ac₂O (1 mL, 10.9 mmol) and NaOAc (128.1 mg, 1.56 mmol). The final compound was separated from isomeric citraconimide using column chromatography (Hex/EtOAc=7/3). Compound 37 was obtained as yellow solid (64.4 mg, 8%).

¹H NMR (500 MHz, CDCl₃) δ ppm: 7.57 (d, 1H, J = 8.7 Hz), 7.54 (d, 1H, J = 2.3 Hz), 7.27 (dd, 1H, J = 2.4, 8.6 Hz), 6.51 (t, 1H, J = 2.5 Hz), 5.80 (t, 1H, J = 2.1 Hz), 3.53 (t, 2H, J = 2.4 Hz). ¹³C NMR (126 MHz, CDCl₃) δ ppm: 172.1, 167.8, 133.1, 132.8, 132.3, 131.0, 130.7, 128.2, 125.5, 122.6, 33.9.

1-(4-acetylphenyl)-3-methylenepyrrolidine-2,5-dione (38).

Compound 38 was synthesized according to the combined general procedures C and D, using itaconic anhydride (300 mg, 2.68 mmol) and 4-aminoacetophenone (402 mg, 2.68 mmol). Corresponding α-itaconamic acid 34 was converted to the final compound using Ac₂O (0.9 mL, 9.38 mmol) and NaOAc (109.9 mg, 1.34 mmol). The final compound was separated from isomeric citraconimide using column chromatography (Hex/EtOAc=8/2 → Hex/EtOAc=6/4). Compound 38 was obtained as white solid (64.1 mg, 10%). ¹H NMR (500 MHz, CDCl₃) δ ppm: 8.11–8.06 (m, 2H), 7.54–7.50 (m, 2H), 6.52 (t, 1H, J = 2.5 Hz), 5.80 (t, 1H, J = 2.1 Hz), 3.55 (t, 2H, J = 2.3 Hz), 2.64 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ ppm: 197.0, 172.3, 168.0, 136.6, 135.8, 132.5, 129.1, 126.3, 122.4, 34.0, 26.7.
1-(4-methoxyphenyl)-3-methylenepyrrolidine-2,5-dione (39).

Compound 39 was synthesized according to the combined general procedures C and D, using itaconic anhydride (400 mg, 3.57 mmol) and p-anisidine (439.5 mg, 3.57 mmol). Corresponding α-itaconamic acid was converted to the final compound using Ac₂O (1.2 mL, 12.5 mmol) and NaOAc (146.4 mg, 1.78 mmol). Final compound was purified using column chromatography (Hex/EtOAc=7/3).

Compound 39 was obtained as yellow solid (301.2 mg, 39%), while corresponding citraconimide was not isolated in this case. ¹H NMR and ¹³C NMR are in accordance with the spectra from the literature.²

S-((2,5-dioxo-1-phenylpyrrolidin-3-yl)methyl) ethanethioate (40).

Compound 40 was synthesized according to the general procedure E, using compound 36 (50 mg, 0.27 mmol), thioacetic acid (21 μL, 0.29 mmol) and Et₃N (5 μL, 0.027 mmol) in DME. The product was purified using column chromatography (Hex/EtOAc=6/4). Final product was obtained as yellow solid (66 mg, 94%, M.p. 94 °C). ¹H NMR (500 MHz, CDCl₃) δ ppm: 7.51–7.46 (m, 2H), 7.44–7.39 (m, 1H), 7.31–7.27 (m, 2H), 3.58–3.50 (m, 1H), 3.38–3.30 (m, 2H), 3.01 (dd, 1H, J = 8.6, 18.7 Hz), 2.64 (dd, 1H, J = 4.9, 18.6 Hz), 2.41 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ ppm: 194.8, 176.8, 174.9, 131.6, 129.2, 128.8, 126.4, 40.0, 33.1, 30.7, 29.2. HRMS (ESI⁺) m/z calcd. for C₁₃H₁₄NO₃S [M+H]⁺ 264.06889, found 264.06775.

S-((1-(3,4-dichlorophenyl)-2,5-dioxopyrrolidin-3-yl)methyl) ethanethioate (41).

Compound 41 was synthesized according to the general procedure E, using compound 37 (60 mg, 0.23 mmol), thioacetic acid (18 μL, 0.26 mmol) and Et₃N (3 μL, 0.023 mmol) in DME. The product was purified using column chromatography (Hex/EtOAc=7/3). Final product was obtained as pale yellow solid (47.7 mg, 61%, M.p. 108 °C). ¹H NMR (500 MHz, CDCl₃) δ ppm: 7.55 (d, 1H, J = 8.7 Hz), 7.48 (d, 1H, J = 2.3 Hz), 7.21 (dd, 1H, J = 2.4, 8.6 Hz), 3.56–3.49 (m, 1H), 3.37–3.30 (m, 2H), 3.01 (dd, 1H, J = 8.9, 18.7 Hz), 2.64 (dd, 1H, J = 5.0, 18.7 Hz), 2.42 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ ppm: 194.7, 176.2, 174.1, 133.1, 132.9, 130.8, 130.8, 128.2, 125.5, 40.0, 33.0, 30.7, 29.1. HRMS (ESI⁺) m/z calcd. for C₁₃H₁₀Cl₂NO₃S [M-H]⁻ 329.976392, found 329.97640.
Compound 42 was synthesized according to the general procedure E, using compound 38 (54 mg, 0.24 mmol), thioacetic acid (18 μL, 0.26 mmol) and Et₃N (3 μL, 0.023 mmol) in DCM. The product was purified using column chromatography (DCM/MeOH=95/5). Final product was obtained as pale yellow solid (71 mg, 99%, M.p. 94 °C).

1H NMR (500 MHz, CDCl₃) δ ppm: 8.09–8.04 (m, 2H), 7.48–7.43 (m, 2H), 3.58–3.49 (m, 1H), 3.40–3.31 (m, 2H), 3.08–2.98 (m, 1H), 2.67 (dd, 1H, J = 5.0, 18.6 Hz), 2.63 (s, 3H), 2.42 (s, 3H).

13C NMR (126 MHz, CDCl₃) δ ppm: 196.9, 194.7, 176.3, 174.3, 136.7, 135.7, 129.2, 126.3, 40.1, 33.1, 30.7, 29.1, 26.7. HRMS (ESI⁺) m/z calcd. for C₁₅H₁₆NO₄S [M+H]⁺ 306.079454, found 306.07814.

Compound 43 was synthesized according to the general procedure E, using compound 39 (150 mg, 0.69 mmol), thioacetic acid (55 μL, 0.76 mmol) and Et₃N (10 μL, 0.069 mmol) in DME. The product was purified using column chromatography (Hex/EtOAc=6/4). Final product was obtained as white solid (185.2 mg, 91%, M.p. 118 °C).

1H NMR (500 MHz, CDCl₃) δ ppm: 7.22–7.17 (m, 2H), 7.01–6.97 (m, 2H), 3.83 (s, 3H), 3.56–3.50 (m, 1H), 3.36–3.28 (m, 2H), 3.03–2.94 (m, 1H), 2.62 (dd, 1H, J = 5.0, 18.5 Hz), 2.41 (s, 3H).

13C NMR (126 MHz, CDCl₃) δ ppm: 194.8, 177.0, 175.2, 159.6, 127.6, 124.2, 114.5, 55.5, 39.9, 33.0, 30.7, 29.2. HRMS (ESI⁺) m/z calcd. for C₁₄H₁₆NO₄S [M+H]⁺ 294.079454, found 294.07822.

Compound 44 was synthesized according to the general procedure E, using compound 32 (564 mg, 2.06 mmol), thioacetic acid (220 μL, 3.09 mmol) and Et₃N (3 μL, 0.021 mmol) in THF. Compound 44 (720 mg, 99%) was used in the next step without further purification.

1H NMR (500 MHz, DMSO-d₆) δ ppm: 12.58 (br s, 1H), 10.32 (s, 1H), 7.97 (d, 1H, J = 2.4 Hz), 7.55 (d, 1H, J = 8.9 Hz), 7.43 (dd, 1H, J = 2.4, 8.9 Hz), 3.20–3.09 (m, 2H), 3.05–2.97 (m, 1H), 2.71–2.65 (m, 1H), 2.55–2.52 (m, 1H), 2.34 (s, 3H).

13C NMR (126 MHz, DMSO-d₆) δ ppm: 194.7, 174.0, 169.5, 139.2, 130.9, 130.7, 124.5, 120.1, 119.0, 40.7, 36.8, 30.5, 29.6. MS (ESI⁺) m/z calcd. for C₁₃H₁₆NO₄S [M+H]⁺ 294.079454, found 294.07822.

Compound 45 was synthesized according to the general procedure E, using compound 33 (500 mg, 2.07 mmol), thioacetic acid (220 μL, 3.09 mmol) and Et₃N (3 μL, 0.021 mmol) in THF. Compound 45 (657 mg, 99%) was used in the next step without further purification.
d6) δ ppm: 12.56 (br s, 1H), 10.22 (s, 1H), 7.78–7.72 (m, 1H), 7.40–7.32 (m, 1H), 7.26–7.22 (m, 1H), 3.19–3.10 (m, 2H), 3.04–2.97 (m, 1H), 2.70–2.64 (m, 1H), 2.54–2.51 (m, 1H), 2.34 (s, 3H). 13C NMR (126 MHz, DMSO-d6) δ ppm: 194.7, 174.1, 169.2, 148.9 (JC= 13.3, 243.7 Hz), 145.1 (JC= 12.4, 241.8 Hz), 136.2 (JC= 2.3, 8.7 Hz), 117.4 (JC= 17.5 Hz), 115.2 (JC= 3.2, 6.0 Hz), 107.9 (JC= 22.1 Hz), 40.7, 36.8, 30.5, 29.6. MS (ESI+) m/z 317.98 [M+H]+.

4-((4-acetylphenyl)amino)-2-((acetylthio)methyl)-4-oxobutanoic acid (46). Compound 46 was synthesized according to the general procedure E, using compound 34 (500 mg, 2.02 mmol), thioacetic acid (214 μL, 3.03 mmol) and Et3N (3 μL, 0.02 mmol) in THF. Compound 46 (650 mg, 99%) was used in the next step without further purification. 1H NMR (500 MHz, DMSO-d6) δ ppm: 12.58 (br s, 1H), 10.34 (s, 1H), 7.91 (d, 2H, J = 8.2 Hz), 7.69 (d, 2H, J = 8.4 Hz), 3.21–3.10 (m, 2H), 3.08–2.99 (m, 1H), 2.77–2.69 (m, 1H), 2.58–2.53 (m, 1H), 2.51 (s, 3H), 2.34 (s, 3H). 13C NMR (126 MHz, DMSO-d6) δ ppm: 196.5, 194.7, 174.1, 169.6, 143.5, 131.6, 129.5, 118.2, 40.7, 37.0, 30.5, 29.7, 26.4. MS (ESI+) m/z 324.13 [M+H]+.

4-((3,4-dichlorophenyl)amino)-2-((mercaptomethyl)-4-oxobutanoic acid (47). Compound 47 was synthesized according to the general procedure F, using compound 44 (720 mg, 2.06 mmol), 2 M NaOH (3 mL, 6.17 mmol) in MeOH. Compound 47 (659 mg, 99%) was used in the next step without further purification. 1H NMR (500 MHz, DMSO-d6) δ ppm: 12.50 (br s, 1H), 10.33 (s, 1H), 7.99 (d, 1H, J = 2.4 Hz), 7.55 (d, 1H, J = 8.2 Hz), 7.45 (dd, 1H, J = 2.4, 8.8 Hz), 3.04–2.96 (m, 1H), 2.80–2.71 (m, 3H), 2.65–2.59 (m, 1H), 2.41 (t, 1H, J = 8.3 Hz). 13C NMR (126 MHz, DMSO-d6) δ ppm: 174.1, 169.9, 139.2, 131.0, 130.7, 124.4, 120.1, 119.0, 43.5, 36.4, 25.4. MS (ESI+) m/z 308.0 [M+H]+.

4-((3,4-difluorophenyl)amino)-2-((mercaptometthyl)-4-oxobutanoic acid (48). Compound 48 was synthesized according to the general procedure F, using compound 45 (657 mg, 2.07 mmol), 2 M NaOH (3 mL, 6.21 mmol) in MeOH. Compound 48 (347 mg, 61%) was used in the next step without further purification. 1H NMR (500 MHz, DMSO-d6) δ ppm: 12.43 (br s, 1H), 10.26 (s, 1H), 7.79–7.72 (m, 1H), 7.40–7.32 (m, 1H), 7.28–7.22 (m, 1H), 3.03–2.96 (m, 1H), 2.80–2.70 (m, 3H), 2.63–2.56 (m, 1H), 2.43–2.34 (m, 1H). 13C NMR (126 MHz, DMSO-d6) δ ppm: 174.2, 169.7, 148.9 (JC= 13.4, 243.7 Hz), 145.1 (JC= 12.9, 241.4 Hz), 136.2 (JC= 2.8, 9.2 Hz), 117.5 (JC= 12.8 Hz), 115.2 (JC= 2.8, 5.5 Hz), 108.0 (JC= 22.1 Hz), 43.6, 36.4, 25.5. MS (ESI+) m/z 275.96 [M+H]+.
4-((4-acetylphenyl)amino)-2-(mercaptomethyl)-4-oxobutanoic acid (49).

Compound 49 was synthesized according to the general procedure F, using compound 46 (652 mg, 2.02 mmol), 2 M NaOH (3 mL, 6.05 mmol) in MeOH. Compound 49 (554.7 mg, 98%) was used in the next step without further purification. $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$ ppm: 12.48 (br s, 1H), 10.36 (s, 1H), 7.91 (d, 2H, $J = 8.6$ Hz), 7.71 (d, 2H, $J = 8.7$ Hz), 3.05–2.98 (m, 1H), 2.84–2.73 (m, 3H), 2.68–2.62 (m, 1H), 2.52 (s, 3H), 2.44–2.37 (m, 1H). $^{13}$C NMR (126 MHz, DMSO-d$_6$) $\delta$ ppm: 196.5, 174.2, 170.0, 143.5, 131.5, 129.5, 118.2, 43.5, 36.6, 26.4, 25.5. MS (ESI$^+$) m/z 282.08 [M+H]+.

1-(3,4-dichlorophenyl)-3-(mercaptomethyl)pyrrolidine-2,5-dione (50).

Compound 50 was synthesized under the conditions described in general procedure A, by heating the compound 47 (260 mg, 0.85 mmol) under Ar atmosphere at 120 °C overnight. The product was purified using column chromatography (100% DCM). Final product was obtained as white solid (60 mg, 25%, M.p. 121 °C). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ ppm: 7.56 (d, 1H, $J = 8.5$ Hz), 7.48 (d, 1H, $J = 2.3$ Hz), 7.22 (dd, 1H, $J = 2.4$, 8.6 Hz), 3.38–3.31 (m, 1H), 3.27–3.19 (m, 1H), 3.08–3.00 (m, 1H), 2.91–2.82 (m, 2H), 1.53–1.47 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm: 176.3, 174.3, 133.1, 133.0, 130.9, 130.8, 128.3, 125.6, 41.8, 32.7, 25.1. HRMS (ESI-) m/z calcd. for C$_{11}$H$_8$Cl$_2$NO$_2$S [M-H]$^-$ 287.965828, found 287.96579.

1-(3,4-difluorophenyl)-3-(mercaptomethyl)pyrrolidine-2,5-dione (51).

Compound 51 was synthesized under the conditions described in general procedure A, by heating the compound 48 (259 mg, 0.94 mmol) under Ar atmosphere at 120 °C overnight. The product was purified using column chromatography (DCM/EtOAc=98/2). Final product was obtained as colorless oil (86 mg, 36%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ ppm: 7.31–7.26 (m, 1H), 7.25–7.20 (m, 1H), 7.13–7.08 (m, 1H), 3.38–3.31 (m, 1H), 3.27–3.19 (m, 1H), 3.08–3.00 (m, 1H), 2.91–2.83 (m, 2H), 1.53–1.46 (m, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm: 176.5, 174.5, 152.2 ($J_{C-F} = 13.8$, 250.6 Hz), 150.1 ($J_{C-F} = 251.5$ Hz), 127.8 ($J_{C-F} = 3.7$, 7.4 Hz), 122.8 ($J_{C-F} = 4.2$, 6.9 Hz), 117.6 ($J_{C-F} = 18.4$ Hz), 116.2 (d, $J_{C-F} = 20.3$ Hz), 41.8, 32.6, 25.1. HRMS (ESI-) m/z calcd. for C$_{11}$H$_8$F$_2$NO$_2$S [M-H]$^-$ 256.024929, found 256.02489.
1-(4-acetylphenyl)-3-(mercaptomethyl)pyrrolidine-2,5-dione (52).

Compound 52 was synthesized under the conditions described in general procedure A, by heating the compound 49 (545 mg, 1.94 mmol) under Ar atmosphere at 120 °C overnight. The product was purified using column chromatography (DCM/EtOAc=95/5). Final product was obtained as white solid (74 mg, 14%, M.p. 97 °C). ¹H NMR (500 MHz, CDCl₃) δ ppm: 8.11–8.03 (m, 2H), 7.49–7.42 (m, 2H), 3.40–3.33 (m, 1H), 3.27–3.19 (m, 1H), 3.10–3.02 (m, 1H), 2.93–2.84 (m, 2H), 2.63 (s, 3H), 1.55–1.48 (m, 1H). ¹³C NMR (126 MHz, CDCl₃) δ ppm: 196.9, 176.5, 174.5, 136.8, 135.7, 129.2, 126.4, 41.9, 32.8, 26.7, 25.1. HRMS (ESI⁺) m/z calcd. for C₁₃H₁₂NO₃S [M-H]⁺ 262.054337, found 262.05423.
Screening of compounds for PAINS and prediction of BBB penetration.

Table S2. Results of the screening using StarDrop software.

Results for PAINS are given as a number of functional groups counted as PAINS. Positive controls: atovaquone (53) and zuclopenthixol (54).

BBB log states for the partition coefficient between brain and blood. BBB category separates compounds into CNS active (+) and inactive (−).

| Compound | Structure | PAINS count | BBB log([brain]/[blood]) | BBB category |
|----------|-----------|-------------|--------------------------|--------------|
| 7        | ![Structure](image1) | 0           | -0.03377                 | +            |
| 8        | ![Structure](image2) | 0           | -0.1721                  | +            |
| 9        | ![Structure](image3) | 0           | -0.1401                  | +            |
| 10       | ![Structure](image4) | 0           | 0.03541                  | +            |
| 11       | ![Structure](image5) | 0           | -0.1665                  | +            |
| 12       | ![Structure](image6) | 0           | -0.1701                  | +            |
| 13       | ![Structure](image7) | 0           | -0.1199                  | +            |
| 14       | ![Structure](image8) | 0           | -0.1108                  | +            |
| 15       | ![Structure](image9) | 0           | -0.2096                  | +            |
| 16       | ![Structure](image10) | 0           | -0.1342                  | +            |
| 17       | ![Structure](image11) | 0           | -0.09312                 | +            |
| 18       | ![Structure](image12) | 0           | -0.1244                  | +            |
| 19       | ![Structure](image13) | 0           | -0.1414                  | +            |
| Compound | Structure | PAINS count | FBB log([brain]/[blood]) | FBB category |
|----------|-----------|-------------|--------------------------|--------------|
| 20       | ![Structure](image) | 0           | -0.1484                  | +            |
| 21       | ![Structure](image) | 0           | -0.09344                 | -            |
| 22       | ![Structure](image) | 0           | -0.09344                 | -            |
| 23       | ![Structure](image) | 0           | 0.09665                  | +            |
| 24       | ![Structure](image) | 0           | -0.4763                  | -            |
| 25       | ![Structure](image) | 0           | -0.1204                  | +            |
| 26       | ![Structure](image) | 0           | -0.2876                  | +            |
| 27       | ![Structure](image) | 0           | -0.2822                  | +            |
| 28       | ![Structure](image) | 0           | -0.3923                  | -            |
| 29       | ![Structure](image) | 0           | -0.3585                  | +            |
| 30       | ![Structure](image) | 0           | -0.12                    | -            |
| 40       | ![Structure](image) | 0           | -0.2314                  | +            |
| 41       | ![Structure](image) | 0           | -0.3531                  | +            |
| Compound | Structure | PAINS count | BBB log[brain]/[blood] | BBB category |
|----------|-----------|-------------|------------------------|--------------|
| 42       |           | 0           | -0.3723                | -            |
| 43       |           | 0           | -0.3902                | -            |
| 50       |           | 0           | -0.1117                | +            |
| 51       |           | 0           | -0.2036                | +            |
| 52       |           | 0           | -0.1239                | +            |
| 53       |           | 2           | 0.04862                | +            |
| 54       |           | 1           | 0.7775                 | +            |

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