

## SUPPORTING INFORMATION TO THE PUBLICATION

### **Binding Mode Characterization and Early *in Vivo* Evaluation of Fragment-Like Thiols as Inhibitors of the Virulence Factor LasB from *Pseudomonas aeruginosa***

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# 1 Supporting Tables and Figures

Table S1. Structures of compounds showing no activity in the *in vitro* LasB inhibition assay.

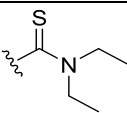

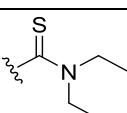

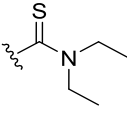

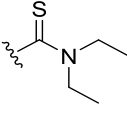

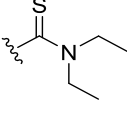





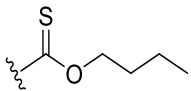
Compound	R <sub>1</sub>	R <sub>2</sub>
75		
76		
77		
78		
79		
80		
81		
82		

Table S2. Data collection and refinement statistics.

<b>LasB-27</b>	
Resolution range	38.22 - 1.3 (1.346 - 1.3)
Space group	P 1 21 1
Unit cell	40.3 90.0 41.9 90 114.1 90
Total reflections	128214 (12304)
Unique reflections	64739 (6304)
Multiplicity	2.0 (2.0)
Completeness (%)	96.96 (94.56)
Mean I/sigma(I)	17.78 (3.97)
R-merge	0.02876 (0.1924)
R-work	0.1421 (0.1982)
R-free	0.1557 (0.2238)
Number of non-hydrogen atoms	2736
macromolecules	2285
ligands	28
solvent	423
Protein residues	298
RMS(bonds)	0.008
RMS(angles)	0.99
Ramachandran favored (%)	96.28
Ramachandran allowed (%)	3.38
Ramachandran outliers (%)	0.34
Rotamer outliers (%)	0.43
Clashscore	2.50
Average B-factor	13.78
macromolecules	11.34
ligands	16.93
solvent	26.79

Statistics for the highest-resolution shell are shown in parentheses.

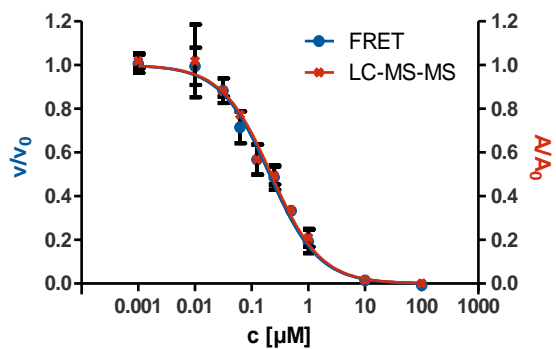


Figure S1. Comparison of FRET-assay results (blue,  $v/v_0$ ) with results from the LC-MS-based counterassay (red,  $A/A_0$ ) using the positive control phosphoramidon.  $IC_{50}$  values:  $207 \pm 13$  nM (FRET);  $223 \pm 15$  nM (LC-MS)

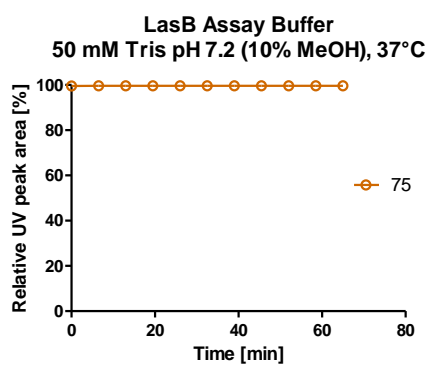


Figure S2. LC-MS stability assay of dithiocarbamate **75** in 50 mM Hepes pH 7.2 (10% methanol) at 37°C, confirming dithiocarbamates to be stable toward hydrolysis within the experimental timeframe of the assay.

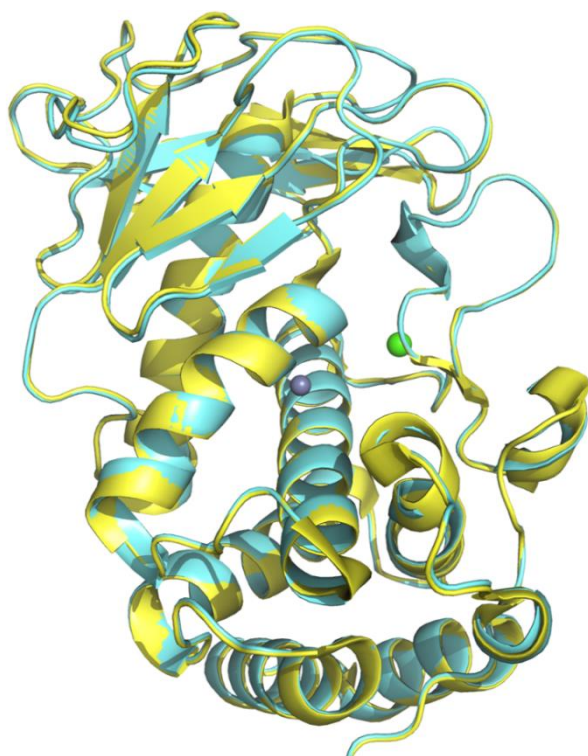


Figure S3. Superposition of LasB apo (yellow) with LasB-**27** (cyan). Binding of the inhibitor does not trigger LasB to adopt a closed conformation.

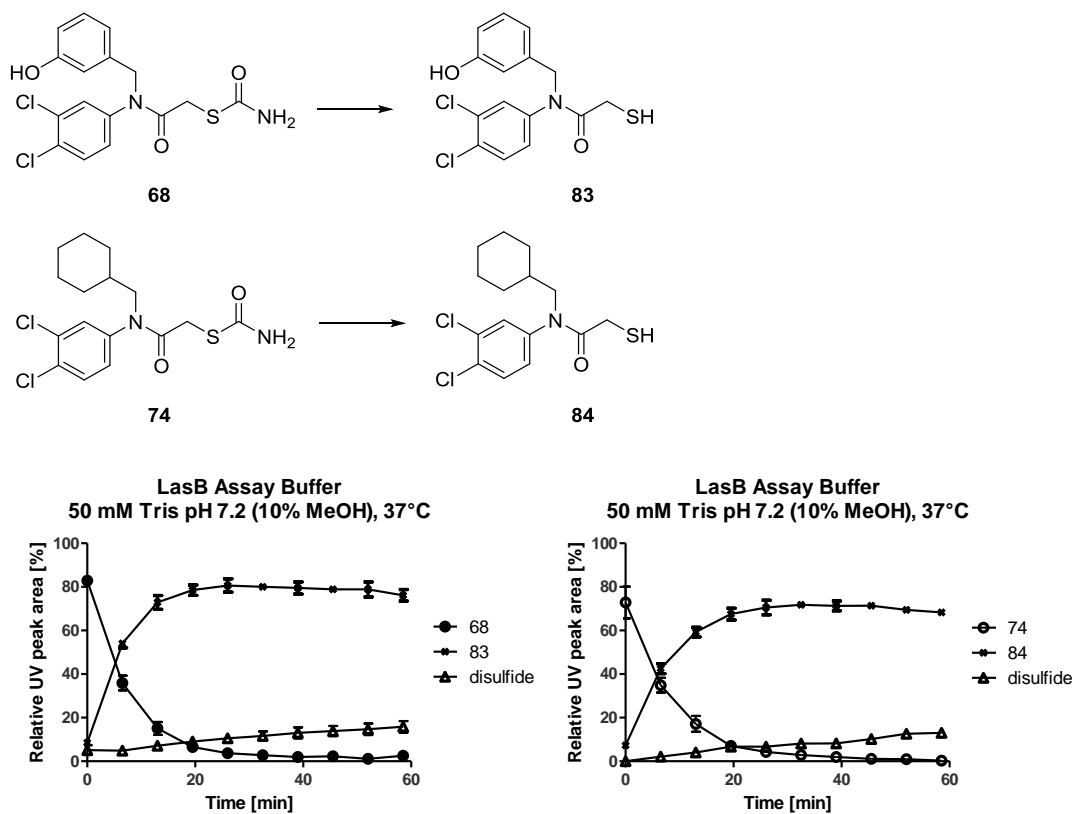


Figure S4. LC-MS stability assay of compounds **68** and **74**, showing *N*-substitution not to affect thiocarbamate half-life.

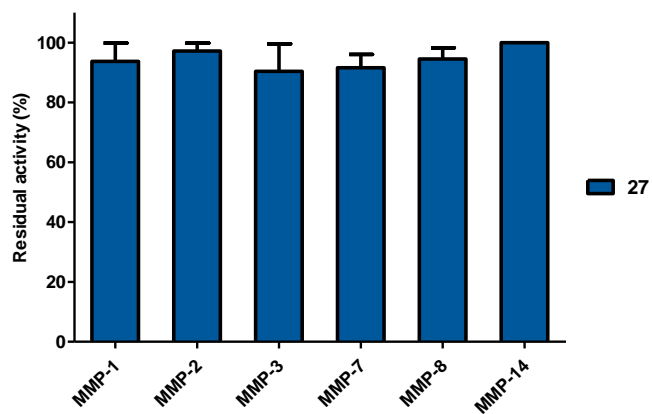
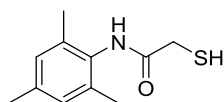


Figure S5. Inhibition of selected MMPs by **27**. Values are means of at least three independent determinations at 100  $\mu$ M (50  $\mu$ M on MMP-8).

## 2 Synthetic Procedures

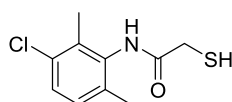
### 2.1 Thiol synthesis

#### 2-mercapto-*N*-mesitylacetamide (**33**)



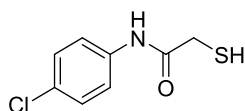
2,4,6-trimethylaniline (208  $\mu$ L, 1.48 mmol) was placed in a crimp vial. The vial was evacuated and flushed with nitrogen three times, followed by addition of thioglycolic acid (114  $\mu$ L, 1.62 mmol). The vial was flushed with argon and heated to 120°C for 6 hours. The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 5:95 to 100:0) to yield the title compound as a white powder (43 mg, 14%).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.10 (s, 6 H), 2.21 (s, 3 H), 2.93 (br. s, 1 H), 3.28 (s, 2 H), 6.86 (s, 2 H), 9.30 (s, 1 H);  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  ppm 17.9, 18.1, 20.5, 27.2, 128.2, 132.1, 134.8, 135.4, 168.5; MS (ESI $^+$ )  $m/z$  210 (M+H) $^+$

#### *N*-(3-chloro-2,6-dimethylphenyl)-2-mercaptoacetamide (**34**)



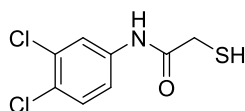
3-chloro-2,6-dimethylaniline (170 mg, 1.09 mmol) was placed in a crimp vial. The vial was evacuated and flushed with nitrogen three times, followed by addition of thioglycolic acid (91  $\mu$ L, 1.30 mmol). The vial was flushed with argon and heated to 120°C for 40 hours. The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 30:70 to 80:20) to yield the title compound as a white powder (33 mg, 13%).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.14 (s, 3 H), 2.18 (s, 3 H), 2.99 (br. s., 1 H), 3.32 (s, 2 H), 7.11 (d,  $J$  = 8.2 Hz, 1 H), 7.27 (d,  $J$  = 8.3 Hz, 1 H), 9.61 (s, 1 H);  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  ppm 15.3, 17.8, 27.1, 127.1, 128.4, 130.8, 133.3, 134.5, 136.2, 168.7; MS (ESI $^+$ )  $m/z$  230 (M+H) $^+$

*N*-(4-chlorophenyl)-2-mercaptoacetamide (**35**)



4-chloroaniline (200 mg, 1.57 mmol) was placed in a crimp vial. The vial was evacuated and flushed with nitrogen three times, followed by addition of thioglycolic acid (120  $\mu$ L, 1.72 mmol). The vial was flushed with argon and heated to 120°C for 5 hours. The crude was purified by automated flash chromatography (petroleum ether:ethyl acetate 90:10 to 70:30) to yield the title compound as a white powder (187 mg, 59%).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.97 (br. s., 1 H) 3.29 (s, 2 H) 7.30 - 7.42 (m, 2 H) 7.53 - 7.68 (m, 2 H) 10.21 (s, 1 H);  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  ppm 28.2, 120.6, 126.9, 128.7, 137.9, 168.7; MS (ESI $^+$ )  $m/z$  202 (M+H) $^+$

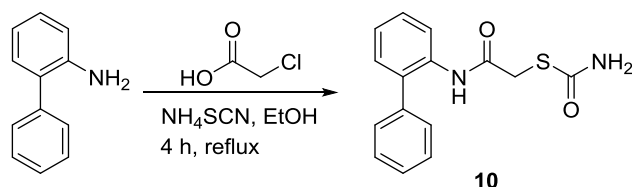
*N*-(3,4-dichlorophenyl)-2-mercaptoacetamide (**36**)



3,4-dichloroaniline (800 mg, 4.94 mmol) was placed in a crimp vial. The vial was evacuated and flushed with nitrogen three times, followed by addition of thioglycolic acid (350  $\mu$ L, 5.02 mmol). The vial was flushed with argon and heated to 120°C for 5 hours. The crude was purified by automated flash chromatography (petroleum ether:ethyl acetate 90:10 to 55:45) to yield the title compound as a white powder (792 mg, 68%).  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  ppm 3.00 (s, 1 H), 3.31 (s, 2 H), 7.47 (dd,  $J$  = 8.9, 2.4 Hz, 1 H), 7.58 (d,  $J$  = 8.8 Hz, 1 H), 7.97 (d,  $J$  = 2.4 Hz, 1 H), 10.38 (s, 1 H);  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  ppm 28.3, 119.1, 120.2, 124.8, 130.7, 131.0, 139.0, 169.1; MS (ESI $^+$ )  $m/z$  236 (M+H) $^+$

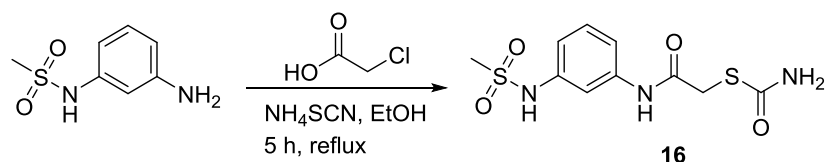
## 2.2 Thiocarbamate synthesis

*S*-2-([1,1'-biphenyl]-2-ylamino)-2-oxoethyl carbamothioate (**10**)



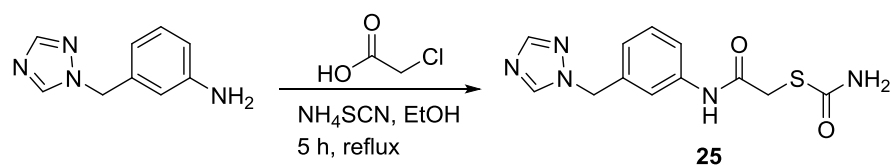
2-Aminobiphenyl (100 mg, 0.59 mmol) was added to a solution of chloroacetic acid (56 mg, 0.59 mmol) and ammonium thiocyanate (45 mg, 0.59 mmol) in 5 mL absolute ethanol. The reaction was refluxed for 4 hours. Water was added and the mixture extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to give the crude which was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 20:80 to 80:20) to yield the title compound as a white powder (44 mg, 26%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ ppm 3.53 (s, 2 H), 7.20 - 8.00 (br. s., 2 H), 7.22 - 7.50 (m, 8 H), 7.65 (d, *J* = 7.8 Hz, 2 H), 9.25 (s, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ ppm 33.4, 125.4, 125.6, 127.4, 127.8, 128.6, 128.8, 130.3, 134.5, 135.2, 138.3, 166.0, 167.3; MS (ESI<sup>+</sup>) *m/z* 287 (M+H)<sup>+</sup>

*S*-2-((3-(methylsulfonamido)phenyl)amino)-2-oxoethyl carbamothioate (**16**)



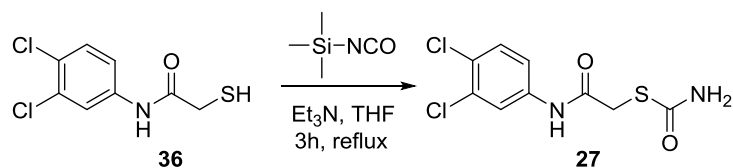
*N*-(3-Aminophenyl)methanesulfonamide (100 mg, 0.54 mmol) was added to a solution of chloroacetic acid (51 mg, 0.54 mmol) and ammonium thiocyanate (41 mg, 0.54 mmol) in 5 mL absolute ethanol. The reaction was refluxed for 5 hours. Water was added and the mixture extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to give the crude which was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 5:95 to 30:70) to yield the title compound as a white powder (33 mg, 20%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ ppm 2.97 (s, 3 H), 3.68 (s, 2 H), 6.88 (m, 1 H), 7.24 (t, *J* = 8.1 Hz, 1 H), 7.34 (m, 1 H), 7.40 - 7.89 (br. s., 2 H), 7.50 (t, *J* = 2.0 Hz, 1 H), 9.75 (s, 1 H), 10.17 (s, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ ppm 34.1, 110.3, 114.5, 114.6, 129.5, 138.8, 139.8, 166.2, 167.0; MS (ESI<sup>+</sup>) *m/z* 304 (M+H)<sup>+</sup>

*S*-2-((3-((1*H*-1,2,4-triazol-1-yl)methyl)phenyl)amino)-2-oxoethyl carbamothioate (**25**)



4-((1*H*-1,2,4-triazol-1-yl)methyl)aniline (100 mg, 0.57 mmol) was added to a solution of chloroacetic acid (54 mg, 0.57 mmol) and ammonium thiocyanate (44 mg, 0.57 mmol) in 7.5 mL absolute ethanol. The reaction was refluxed for 5 hours. Water was added and the mixture extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to give the crude which was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 10:90 to 30:70) to yield the title compound as a white powder (10 mg, 6%).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ )  $\delta$  ppm 3.68 (s, 2 H), 5.34 (s, 2 H), 7.23 (m, 2 H), 7.54 (m, 2 H), 7.39 - 7.89 (br. s., 2 H), 7.96 (s, 1 H), 8.61 (s, 1 H), 10.15 (s, 1 H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-d}_6$ )  $\delta$  ppm 34.0, 48.6, 51.7, 119.1, 128.5, 131.0, 138.7, 144.0, 151.6, 166.2, 166.9; MS (ESI<sup>+</sup>)  $m/z$  292 (M+H)<sup>+</sup>

*S*-2-((3,4-dichlorophenyl)amino)-2-oxoethyl carbamothioate (**27**)



**36** was prepared according to the procedure described above and used without further purification starting from 200 mg 3,4-dichloroaniline (1.23 mmol). The crude was dissolved in 4 mL dry THF and triethylamine (170  $\mu\text{L}$ , 1.23 mmol) and trimethylsilyl isocyanate (170  $\mu\text{L}$ , 1.26 mmol) were added. The reaction mixture was refluxed under argon atmosphere for 3 hours. The reaction was quenched for 30 min with methanol. The crude was purified using automated flash chromatography (petroleum ether:ethyl acetate 80:20 to 90:10) to yield the title compound as a white powder (160 mg, 47% over two steps).  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ )  $\delta$  ppm 3.70 (s, 2 H), 7.47 (dd,  $J = 8.9, 2.4$  Hz, 1 H), 7.55 (d,  $J = 8.8$  Hz, 1 H), 7.39-7.87, (br. s., 2 H), 7.97 (d,  $J = 2.3$  Hz, 1 H), 10.42 (s, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-d}_6$ )  $\delta$  ppm 34.1, 119.1, 120.2, 124.7, 130.7, 131.0, 139.1, 166.0, 167.5; MS (ESI<sup>+</sup>)  $m/z$  279 (M+H)<sup>+</sup>

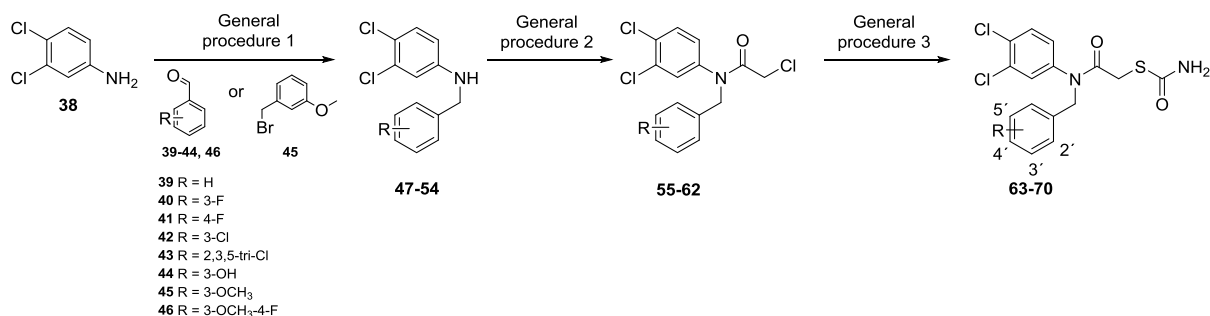
### 2.3 Synthesis of *N*-benzyl and *N*-alkyl mercaptoacetamides

*Preparation of N-benzylanilines 47-54 and N-alkylanilines 85-88 (General procedure 1).* To a solution of 3,4-dichloroaniline **38** (1 equiv.) in DCM (10 mL) in a crimp reaction vial was added the appropriate aldehyde (1.0 – 1.2 equiv.), followed by acetic acid (0.1 equiv). Sodium triacetoxyborohydride (1.5 – 2.0 equiv) was added stepwise. The vial was flushed with argon and the reaction was stirred for 20 hours at room temperature. The mixture was quenched 10% NaOH (5 mL) under cooling and extracted using DCM. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to afford crude product.

*Preparation of N-benzyl-N-(3,4-dichlorophenyl)acetamides 55-62 and N-alkyl-N-(3,4-dichlorophenyl)-acetamides 89-92 (General procedure 2).* The appropriate *N*-benzylaniline or *N*-alkylaniline was dissolved in 10 mL of acetone. Chloroacetyl chloride (1.0 – 1.4 equiv.) was added dropwise under cooling on ice. After 30 min the reaction was allowed to warm at room temperature for 1 hour. The reaction was quenched by addition of saturated NaHCO<sub>3</sub> solution (5 mL) and stirring for 10 min. The mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to afford crude product.

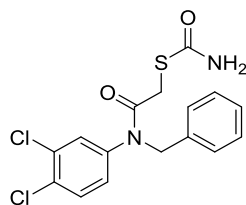
*Preparation of S-(2-((3,4-dichlorophenyl)(arylamino)-2-oxoethyl) carbamothioates 63-70 and S-(2-((3,4-dichlorophenyl)(alkylamino)-2-oxoethyl) carbamothioates 71-74 (General procedure 3).* The appropriate *N*-benzyl-*N*-(3,4-dichlorophenyl)acetamide or *N*-alkyl-*N*-(3,4-dichlorophenyl)-acetamide was dissolved in 4 mL ethanol and placed in a crimp vial, followed by addition ammonium thiocyanate (1 – 1.1 equiv.). The reaction was heated to 80°C for 2 hours. Water was added and the mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure to give crude intermediate. The crude was dissolved in 2 mL ethanol and added dropwise to a pre-cooled mixture of 3 mL 90% sulfuric acid and 10% acetic acid. After stirring on ice for 30 min, the reaction mixture was diluted in water and extracted with ethyl acetate. Washing with brine, followed by drying over sodium sulfate and concentration under reduced pressure gave the crude.

## Synthesis of *S*-2-((3,4-dichlorophenyl)(arylamino)-2-oxoethyl) carbamothioates **63-70**



Scheme S1. Synthesis of *N*-benzyl mercaptoacetamides.

### *S*-2-(benzyl(3,4-dichlorophenyl)amino)-2-oxoethyl) carbamothioate (**63**)

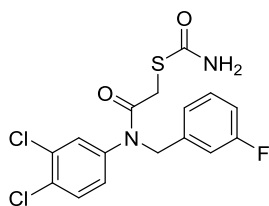


**47** was prepared according to general procedure 1 using 3,4-dichloroaniline (250 mg, 1.54 mmol), benzaldehyde (156  $\mu$ L, 1.54 mmol), acetic acid (8.8  $\mu$ L, 0.15 mmol) and sodium triacetoxyborohydride (491 mg, 2.32 mmol). The obtained brown oil (352 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  252 (M+H)<sup>+</sup>

**55** was prepared according to general procedure 2 using **47** (352 mg, 1.40 mmol) and chloroacetyl chloride (111  $\mu$ L, 1.54 mmol). After 5 hours at room temperature, additional chloroacetyl chloride was added (37  $\mu$ L, 0.51 mmol) and the reaction stirred at room temperature for 17 hours. The obtained crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 30:70 to 50:50) to yield the title compound as a white powder (282 mg, 56% over two steps). MS (ESI<sup>+</sup>)  $m/z$  328 (M+H)<sup>+</sup>

**63** was prepared according to general procedure 3 using **55** (44 mg, 0.13 mmol) and ammonium thiocyanate (10 mg, 0.13 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 50:50 to 100:0) to yield the title compound as a white powder after freeze-drying (20 mg, 40%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.56 (br. s, 2 H), 4.89 (s, 2 H), 7.14 - 7.34 (m, 6 H), 7.39 - 7.84 (br. s, 2 H), 7.61 (d,  $J$  = 2.2 Hz, 1 H), 7.64 (d,  $J$  = 8.6 Hz, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 32.7, 52.2, 127.3, 127.8, 128.4, 128.7, 130.3, 130.6, 131.1, 131.5, 136.8, 141.5, 165.9, 167.8; MS (ESI<sup>+</sup>)  $m/z$  369 (M+H)<sup>+</sup>

*S*-2-((3,4-dichlorophenyl)(3-fluorobenzyl)amino)-2-oxoethyl carbamothioate (**64**)

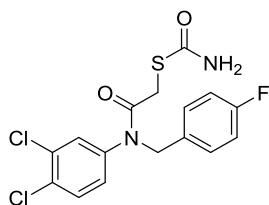


**48** was prepared according to general procedure 1 using 3,4-dichloroaniline (250 mg, 1.54 mmol), 3-fluorobenzaldehyde (196  $\mu$ L, 1.85 mmol), acetic acid (8.8  $\mu$ L, 0.15 mmol) and sodium triacetoxyborohydride (654 mg, 3.09 mmol). The obtained brown oil (401 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  270 (M+H)<sup>+</sup>

**56** was prepared according to general procedure 2 using **48** (200 mg, 0.74 mmol) and chloroacetyl chloride (82  $\mu$ L, 1.04 mmol). The obtained crude (258 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  346 (M+H)<sup>+</sup>

**64** was prepared according to general procedure 3 using **56** (258 mg, 0.74 mmol) and ammonium thiocyanate (56 mg, 0.74 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 30:70 to 100:0) to yield the title compound as a white powder after freeze-drying (117 mg, 39% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.57 (br. s, 2 H), 4.90 (s, 2 H), 7.01- 7.11 (m, 3 H), 7.28 (dd,  $J$  = 8.6, 1 H), 7.30- 7.38 (m, 1 H), 7.66 (m, 1 H), 7.40- 7.80 (br. s, 2 H), 7.67 (d,  $J$  = 1.0, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 32.8, 51.9, 114.2 (d,  $J_{C-F}$  = 20.9 Hz), 114.6 (d,  $J_{C-F}$  = 21.6 Hz), 123.9 (d,  $J_{C-F}$  = 2.24 Hz), 128.7, 130.4, 130.5 (d,  $J_{C-F}$  = 8.2 Hz), 130.8, 131.3, 131.7, 139.9 (d,  $J_{C-F}$  = 7.5 Hz), 141.6, 162.3 (d,  $J_{C-F}$  = 243.6 Hz), 166.1, 168.1; MS (ESI<sup>+</sup>)  $m/z$  387 (M+H)<sup>+</sup>

*S*-2-((3,4-dichlorophenyl)(4-fluorobenzyl)amino)-2-oxoethyl carbamothioate (**65**)



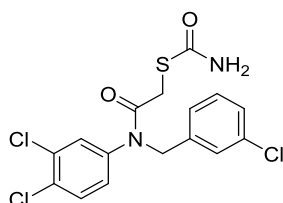
**49** was prepared according to general procedure 1 using 3,4-dichloroaniline (250 mg, 1.54 mmol), 4-fluorobenzaldehyde (199  $\mu$ L, 1.85 mmol), acetic acid (8.8  $\mu$ L, 0.15 mmol) and sodium triacetoxyborohydride (653 mg, 3.09 mmol). The obtained brown oil (398 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  270 (M+H)<sup>+</sup>

**57** was prepared according to general procedure 2 using **49** (185 mg, 0.68 mmol) and chloroacetyl chloride (54  $\mu$ L, 0.68 mmol). After 3 hours at room temperature, additional chloroacetyl chloride was

added (27  $\mu\text{L}$ , 0.34 mmol) and the reaction stirred at room temperature for 2 hours. The obtained crude (226 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  346 (M+H)<sup>+</sup>

**65** was prepared according to general procedure **3** using **57** (266 mg, 0.65 mmol) and ammonium thiocyanate (50 mg, 0.66 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 40:60 to 100:0) to yield the title compound as a white powder after freeze-drying (109 mg, 37% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.54 (br. s, 2 H), 4.86 (s, 2 H), 7.06 - 7.16 (m, 2 H), 7.17 - 7.27 (m, 3 H), 7.37-7.96 (br. s, 2 H), 7.61 (d,  $J = 2.4$  Hz, 1 H), 7.65 (d,  $J = 8.6$  Hz, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 32.7, 51.5, 115.2 (d,  $J_{\text{C-F}} = 20.9$  Hz), 128.8, 130.0 (d,  $J_{\text{C-F}} = 8.2$  Hz), 130.4, 130.6, 131.2, 131.6, 133.0 (d,  $J_{\text{C-F}} = 3.0$  Hz), 141.4, 161.4 (d,  $J_{\text{C-F}} = 243.6$  Hz), 165.9, 167.8; MS (ESI<sup>+</sup>)  $m/z$  387 (M+H)<sup>+</sup>

*S*-(2-((3,4-dichlorophenyl)(3-chlorobenzyl)amino)-2-oxoethyl) carbamothioate (**66**)

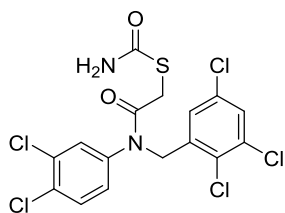


**50** was prepared according to general procedure 1 using 3,4-dichloroaniline (250 mg, 1.54 mmol), 3-chlorobenzaldehyde (210  $\mu\text{L}$ , 1.85 mmol), acetic acid (8.8  $\mu\text{L}$ , 0.15 mmol) and sodium triacetoxyborohydride (654 mg, 3.09 mmol). The obtained brown oil (458 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  286 (M+H)<sup>+</sup>

**58** was prepared according to general procedure 2 using **50** (229 mg, 0.80 mmol) and chloroacetyl chloride (70  $\mu\text{L}$ , 0.88 mmol). After 1 hour at room temperature, additional chloroacetyl chloride was added (35  $\mu\text{L}$ , 0.44 mmol) and the reaction stirred at room temperature for 2 hours. The obtained crude (255 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  362 (M+H)<sup>+</sup>

**66** was prepared according to general procedure **3** using **58** (255 mg, 0.70 mmol) and ammonium thiocyanate (53 mg, 0.70 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 30:70 to 100:0) to yield the title compound as a white powder after freeze-drying (150 mg, 49% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.57 (br. s, 2 H), 4.89 (s, 2 H), 7.13- 7.21 (m, 1 H), 7.23- 7.36 (m, 4 H), 7.45- 7.85 (br. s, 2 H), 7.66 (dd,  $J = 8.6$ , 2 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 32.7, 51.7, 126.5, 127.3, 127.7, 128.7, 130.2, 130.7, 131.2, 131.6, 133.1, 139.4, 141.4, 165.9, 168.0; MS (ESI<sup>+</sup>)  $m/z$  403 (M+H)<sup>+</sup>

*S*-2-((3,4-dichlorophenyl)(3-chlorobenzyl)amino)-2-oxoethyl carbamothioate (**67**)

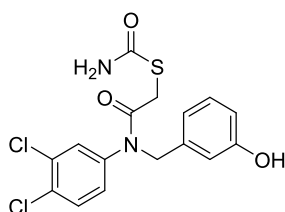


**51** was prepared according to general procedure 1 using 3,4-dichloroaniline (250 mg, 1.54 mmol), 2,3,5-trichlorobenzaldehyde (386 mg, 1.85 mmol), acetic acid (8.8  $\mu$ L, 0.15 mmol) and sodium triacetoxyborohydride (654 mg, 3.09 mmol). The obtained crude was purified using automated flash chromatography (petroleum ether:ethyl acetate 100:0 to 30:70) to yield the title compound as a white powder (300 mg, 55%). MS (ESI<sup>+</sup>) *m/z* 403 (M+H)<sup>+</sup>

**59** was prepared according to general procedure 2 using **51** (150 mg, 0.42 mmol) and chloroacetyl chloride (48  $\mu$ L, 0.59 mmol). After 1 hour at room temperature, additional chloroacetyl chloride was added (48  $\mu$ L, 0.59 mmol) and the reaction stirred at room temperature for 2 hours. The obtained crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 30:70 to 100:0) to yield the title compound as a white powder (116 mg, 64%). MS (ESI<sup>+</sup>) *m/z* 430 (M+H)<sup>+</sup>

**67** was prepared according to general procedure 3 using **59** (116 mg, 0.27 mmol) and ammonium thiocyanate (21 mg, 0.27 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 40:60 to 100:0) to yield the title compound as a white powder after freeze-drying (88 mg, 43% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.60 (br. s, 2 H), 4.97 (br. s, 2 H), 7.33- 7.90 (br. s, 2 H) 7.40 (d, *J* = 8.4, 1 H), 7.50 (br. s, 1 H), 7.69 (d, *J* = 8.6, 1 H), 7.72- 7.78 (m, 1 H), 7.81 (br. s, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 32.7, 51.0, 127.9, 128.5, 128.9, 129.2, 130.2, 130.9, 131.3, 131.7, 132.3, 132.9, 138.3, 141.3, 165.9, 168.3; MS (ESI<sup>+</sup>) *m/z* 473 (M+H)<sup>+</sup>

*S*-2-((3,4-dichlorophenyl)(3-hydroxybenzyl)amino)-2-oxoethyl carbamothioate (**68**)



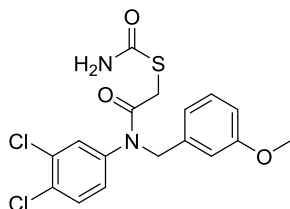
**52** was prepared according to general procedure 1 using 3,4-dichloroaniline (250 mg, 1.54 mmol), 3-hydroxybenzaldehyde (226 mg, 1.85 mmol), acetic acid (8.8  $\mu$ L, 0.15 mmol) and sodium

triacetoxyborohydride (654 mg, 3.09 mmol). The obtained pale oil (344 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  268 (M+H)<sup>+</sup>

**60** was prepared according to general procedure 2 using **52** (172 mg, 0.64 mmol) and chloroacetyl chloride (51  $\mu$ L, 0.64 mmol). After 1 hour at room temperature, additional chloroacetyl chloride was added (26  $\mu$ L, 0.32 mmol) and the reaction stirred at room temperature for 2 hours. The obtained pale oil (220 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  344 (M+H)<sup>+</sup>

**68** was prepared according to general procedure 3 using **60** (220 mg, 0.64 mmol) and ammonium thiocyanate (49 mg, 0.64 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 25:75 to 100:0) to yield the title compound as a white powder after freeze-drying (61 mg, 21% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 2.07 (s, 1 H), 3.55 (br. s, 2 H), 4.79 (br. s, 2 H), 6.50 - 6.66 (m, 3 H), 7.07 (t,  $J$  = 8.0 Hz, 1 H), 7.22 (dd,  $J$  = 8.6, 2.4 Hz, 1 H), 7.32 - 7.88 (br. s, 2 H), 7.59 (d,  $J$  = 2.4 Hz, 1 H), 7.65 (d,  $J$  = 8.6 Hz, 1 H), 9.35 (s, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 32.7, 52.2, 114.3, 114.8, 118.4, 128.7, 129.4, 130.3, 130.4, 131.1, 131.4, 138.2, 141.5, 157.4, 165.8, 167.7; MS (ESI<sup>+</sup>)  $m/z$  385 (M+H)<sup>+</sup>

*S*-2-((3,4-dichlorophenyl)(3-methoxybenzyl)amino)-2-oxoethyl carbamothioate (**69**)

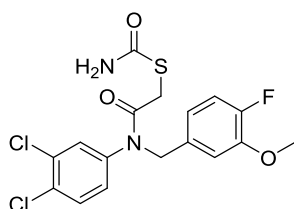


3,4-dichloroaniline (300 mg, 1.85 mmol), 3-methoxybenzyl bromide (260  $\mu$ L, 1.85 mmol) and potassium carbonate (511 mg, 3.7 mmol) were placed in a crimp vial and dissolved in 5 mL DMF. The reaction was heated to 120°C for 20 hours. Water (5 mL) was added and the mixture extracted with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude was purified using automated flash chromatography (petroleum ether:ethyl acetate 100:0 to 80:20) to yield the title compound as a pale oil (342 mg, 66%). MS (ESI<sup>+</sup>)  $m/z$  282 (M+H)<sup>+</sup>

**61** was prepared according to general procedure 2 using **53** (150 mg, 0.53 mmol) and chloroacetyl chloride (44  $\mu$ L, 0.56 mmol). After 1 hour at room temperature, additional chloroacetyl chloride was added (20  $\mu$ L, 0.25 mmol) and the reaction stirred at room temperature for 2 hours. The obtained pale oil (140 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  358 (M+H)<sup>+</sup>

**69** was prepared according to general procedure 3 using **61** (140 mg, 0.39 mmol) and ammonium thiocyanate (30 mg, 0.39 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 40:60 to 100:0) to yield the title compound as a white powder after freeze-drying (45 mg, **21%** over two steps). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ ppm 3.56 (br. s, 2 H), 3.71 (s, 3 H), 4.86 (s, 2 H), 6.71 - 6.84 (m, 3 H), 7.20 (t, *J* = 8.0 Hz, 1 H), 7.25 (dd, *J* = 8.5, 2.5 Hz, 1 H), 7.36-7.85 (br. s, 2 H), 7.61 - 7.68 (m, 2 H); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) δ ppm 32.7, 52.1, 55.0, 112.8, 113.2, 119.9, 128.7, 129.4, 130.2, 130.5, 131.1, 131.5, 138.4, 141.6, 159.3, 165.9, 167.8; MS (ESI<sup>+</sup>) *m/z* 399 (M+H)<sup>+</sup>

*S*-(2-((3,4-dichlorophenyl)(4-fluoro-3-methoxybenzyl)amino)-2-oxoethyl) carbamothioate (**70**)

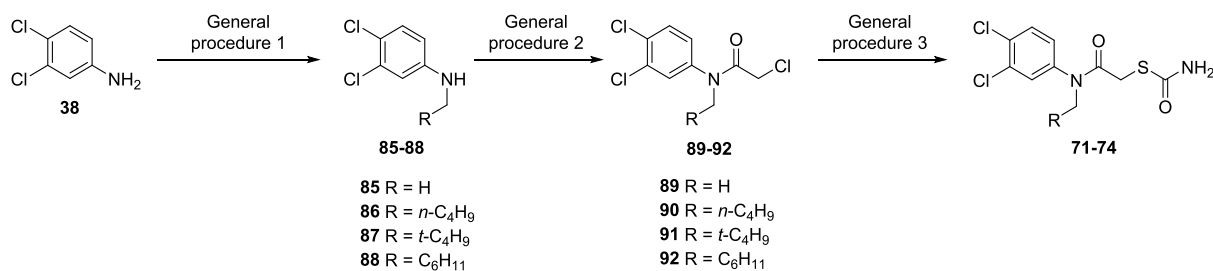


**54** was prepared according to general procedure 1 using 3,4-dichloroaniline (300 mg, 1.85 mmol), 4-fluoro-3-methoxybenzaldehyde (343 mg, 2.22 mmol), acetic acid (10.6 μL, 0.19 mmol) and sodium triacetoxylborohydride (785 mg, 3.70 mmol). The obtained oil (550 mg) was used without further purification. MS (ESI<sup>+</sup>) *m/z* 300 (M+H)<sup>+</sup>

**62** was prepared according to general procedure 2 using **54** (200 mg, 0.67 mmol) and chloroacetyl chloride (64 μL, 0.80 mmol). The obtained pale oil (162 mg) was used without further purification. MS (ESI<sup>+</sup>) *m/z* 376 (M+H)<sup>+</sup>

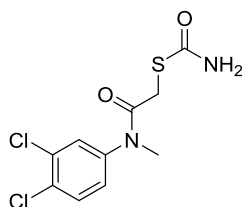
**70** was prepared according to general procedure 3 using **62** (162 mg, 0.43 mmol) and ammonium thiocyanate (36 mg, 0.47 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 30:70 to 100:0) to yield the title compound as a white powder after freeze-drying (38 mg, 14% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ ppm 3.54 (br. s, 2 H), 3.73 - 3.82 (s, 3 H), 4.85 (s, 2 H), 6.68 - 6.78 (m, 1 H), 6.98 (d, *J* = 8.0 Hz, 1 H), 7.09 (dd, *J* = 11.6, 8.3 Hz, 1 H), 7.25 (dd, *J* = 8.6, 2.4 Hz, 1 H), 7.36 - 7.98 (br. s, 2 H), 7.62 - 7.68 (m, 2 H); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) δ ppm 32.7, 51.8, 55.9, 113.3, 115.7 (d, *J*<sub>C-F</sub> = 17.9 Hz, 1 C), 120.2 (d, *J*<sub>C-F</sub> = 6.7 Hz, 1 C), 128.8, 130.4, 130.6, 131.2, 131.5, 133.6 (d, *J*<sub>C-F</sub> = 3.7 Hz, 1 C), 141.5, 147.0 (d, *J*<sub>C-F</sub> = 10.4 Hz, 1 C), 150.7 (d, *J*<sub>C-F</sub> = 242.9 Hz, 1 C), 166.0, 167.9; MS (ESI<sup>+</sup>) *m/z* 417 (M+H)<sup>+</sup>

## Synthesis of *S*-(2-((3,4-dichlorophenyl)(alkylamino)-2-oxoethyl) carbamothioates 71-74



Scheme S2. Synthesis of *N*-alkyl mercaptoacetamides.

### *S*-(2-((3,4-dichlorophenyl)(methyl)amino)-2-oxoethyl) carbamothioate (**71**)

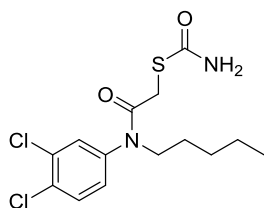


**85** was prepared according to general procedure 1 using 3,4-dichloroaniline (300 mg, 1.85 mmol), formaldehyde (81  $\mu$ L, 2.22 mmol), acetic acid (10.6  $\mu$ L, 0.19 mmol) and sodium triacetoxyborohydride (785 mg, 3.70 mmol). The obtained crude was purified using automated flash chromatography (petroleum ether:ethyl acetate 100:0 to 30:70) to yield the title compound as a pale powder (80 mg, 25%). MS (ESI<sup>+</sup>)  $m/z$  217 (M+H)<sup>+</sup>

**89** was prepared according to general procedure 2 using **85** (80 mg, 0.45 mmol) and chloroacetyl chloride (44  $\mu$ L, 0.55 mmol). The obtained pale oil (107 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  252 (M+H)<sup>+</sup>

**71** was prepared according to general procedure 3 using **89** (107 mg, 0.42 mmol) and ammonium thiocyanate (36 mg, 0.47 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 30:70 to 100:0) to yield the title compound as a white powder after freeze-drying (41 mg, 32% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.19 (br. s, 3 H), 3.55 (br. s, 2 H), 7.28 - 7.88 (br. s, 2H), 7.42 (dd,  $J$  = 8.6 Hz,  $J$  = 2.1 Hz, 1 H), 7.72 (d,  $J$  = 8.7 Hz, 1 H), 7.75 (br. s, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 32.6, 37.3, 127.7, 129.4, 131.2, 131.6, 143.4, 165.9, 167.5; MS (ESI<sup>+</sup>)  $m/z$  293 (M+H)<sup>+</sup>

*S*-2-((3,4-dichlorophenyl)(pentyl)amino)-2-oxoethyl carbamothioate (**72**)

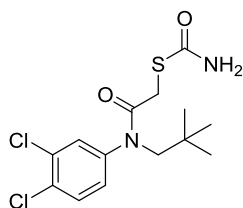


**86** was prepared according to general procedure 1 using 3,4-dichloroaniline (300 mg, 1.85 mmol), valeraldehyde (217  $\mu$ L, 2.04 mmol), acetic acid (10.6  $\mu$ L, 0.19 mmol) and sodium triacetoxyborohydride (785 mg, 3.70 mmol). The obtained oil (360 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  232 (M+H)<sup>+</sup>

**90** was prepared according to general procedure 2 using **86** (180 mg, 0.78 mmol) and chloroacetyl chloride (74  $\mu$ L, 0.93 mmol). The obtained pale oil (240 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  308 (M+H)<sup>+</sup>

**72** was prepared according to general procedure 3 using **90** (240 mg, 0.78 mmol) and ammonium thiocyanate (65 mg, 0.86 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 50:50 to 100:0) to yield the title compound as a white powder after freeze-drying (110 mg, 34% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 0.77 - 0.87 (m, 3 H), 1.10 - 1.30 (m, 4 H), 1.36 (m, 2 H), 3.45 (br. s, 2 H), 3.61 (t, *J* = 7.3 Hz, 2 H), 7.21 - 7.90 (br. s, 2 H), 7.39 (m, 1 H), 7.72 (s, 1 H), 7.75 (s, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 13.8, 21.7, 26.7, 28.1, 32.8, 38.7, 39.0, 39.2, 39.8, 40.1, 40.4, 48.7, 128.9, 130.4, 131.2, 131.7, 141.8, 165.9, 167.1; MS (ESI<sup>+</sup>)  $m/z$  349 (M+H)<sup>+</sup>

*S*-2-((3,4-dichlorophenyl)(neopentyl)amino)-2-oxoethyl carbamothioate (**73**)

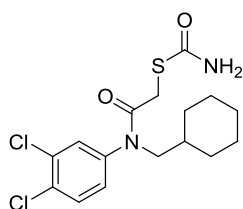


**87** was prepared according to general procedure 1 using 3,4-dichloroaniline (300 mg, 1.85 mmol), pivaldehyde (221  $\mu$ L, 2.04 mmol), acetic acid (10.6  $\mu$ L, 0.19 mmol) and sodium triacetoxyborohydride (785 mg, 3.70 mmol). The obtained oil (360 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  232 (M+H)<sup>+</sup>

**91** was prepared according to general procedure 2 using **87** (180 mg, 0.78 mmol) and chloroacetyl chloride (74  $\mu$ L, 0.93 mmol). The obtained pale oil (240 mg) was used without further purification. MS (ESI<sup>+</sup>)  $m/z$  308 (M+H)<sup>+</sup>

**73** was prepared according to general procedure 3 using **91** (240 mg, 0.78 mmol) and ammonium thiocyanate (65 mg, 0.86 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 30:70 to 100:0) to yield the title compound as a white powder after freeze-drying (20 mg, 6% over three steps). <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ ppm 0.77 (s, 9 H), 3.54 (br. s, 2 H), 3.61 (s, 2 H), 7.28 - 7.79 (br. s, 2 H), 7.45 - 7.55 (dd, *J* = 2.0, *J* = 8.5, 1 H), 7.70 (d, *J* = 8.7 Hz, 1 H), 7.85 (br. s, 1 H); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) δ ppm 28.1, 33.0, 33.8, 59.7, 128.8, 130.2, 131.0, 131.5, 143.9, 166.0, 168.1; MS (ESI<sup>+</sup>) *m/z* 349 (M+H)<sup>+</sup>

*S*-2-((cyclohexylmethyl)(3,4-dichlorophenyl)amino)-2-oxoethyl carbamothioate (**74**)



**88** was prepared according to general procedure 1 using 3,4-dichloroaniline (300 mg, 1.85 mmol), cyclohexanecarboxaldehyde (224 μL, 1.85 mmol), acetic acid (10.6 μL, 0.19 mmol) and sodium triacetoxyborohydride (654 mg, 3.09 mmol). The obtained oil (400 mg) was used without further purification. MS (ESI<sup>+</sup>) *m/z* 258 (M+H)<sup>+</sup>

**92** was prepared according to general procedure 2 using **88** (200 mg, 0.78 mmol) and chloroacetyl chloride (86 μL, 1.08 mmol). The obtained pale oil (215 mg) was used without further purification. MS (ESI<sup>+</sup>) *m/z* 334 (M+H)<sup>+</sup>

**74** was prepared according to general procedure 3 using **92** (215 mg, 0.64 mmol) and ammonium thiocyanate (49 mg, 0.64 mmol). The crude was purified using automated flash chromatography (reversed-phase: acetonitrile + 0.1% formic acid:water + 0.1% formic acid 40:60 to 100:0) to yield the title compound as a white powder after freeze-drying (71 mg, 25% over three steps). <sup>1</sup>H NMR (300 MHz, Chloroform-d) δ ppm 0.84 - 1.07 (m, 2 H), 1.07 - 1.29 (m, 3 H), 1.30 - 1.62 (m, 6 H), 3.47 (s, 2 H), 3.55 (d, *J* = 7.3 Hz, 2 H), 6.03 (br. s, 2 H), 7.15 (d, *J* = 7.8 Hz, 1 H), 7.38 (br. s, 1 H), 7.52 (d, *J* = 8.4 Hz, 1 H); <sup>13</sup>C NMR (75 MHz, Chloroform-d) δ ppm 32.7, 51.0, 127.9, 128.5, 128.9, 129.2, 130.2, 130.9, 131.3, 131.7, 132.3, 132.9, 138.3, 141.3, 165.9, 168.3; MS (ESI<sup>+</sup>) *m/z* 377 (M+H)<sup>+</sup>