Betraying the parasite's redox system: Diaryl sulfide-based inhibitors of trypanothione reductase: subversive substrates and antitrypanosomal properties

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Figure 1SI. Model for the binding of the non-competitive inhibitor 31 in the cavity of TR. The furfuryl inhibitor 31 (cyan, balls and sticks) was modeled into the cavity at the two-fold symmetry axis of Trypanosoma cruzi trypanothione reductase in complex with trypanothione (green). PDB-code: 1BZL. \[1\]
Figure 2SI. Oxidase activity assays. a) Induced NADPH consumption of TR by furfuryl derivative 31, observed at 340 nm. b) Coupled assay with oxidized cytochrome c (Cyt-c-Fe$^{3+}$). Cyt-c-Fe$^{3+}$ reduction was monitored at 550 nm. The assay was measured as described in the Supporting Information below.
Table 1SI. Reduction of nitrofuran derivative 31 by TR in presence and absence of superoxide dismutase (SOD). Only partial quenching of the Cytc-Fe\(^{3+}\) reduction was observed in the coupled assay with SOD.

<table>
<thead>
<tr>
<th>(K_{ic}=K_{iu})</th>
<th>NADPH ox. [U mL(^{-1})](^{[a]})</th>
<th>Cytc-Fe(^{3+}) red. [U mL(^{-1})](^{[b]})</th>
<th>+ SOD [U mL(^{-1})](^{[c]})</th>
</tr>
</thead>
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<tr>
<td>7±1</td>
<td>0.60</td>
<td>0.60</td>
<td>0.36</td>
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\(^{[a]}\) 100 µM NADPH, 200 µM inhibitor, 6.5 U mL\(^{-1}\) TR, 25 °C.  
\(^{[b]}\) As \([a]\), + 25 µM Cytc-Fe\(^{3+}\).  
\(^{[c]}\) As \([b]\), + 6 µg SOD.  
ox.: oxidation;  red.: reduction.
Experimental section of the Supporting Information

Modeling of inhibitors using MOLOC.
Potential inhibitors were manually docked within the known structures of TR. The enzyme structure was fixed (except for the side chain of Glu18), and the energy of the system was minimized using the MAB force field as implemented in the computer program MOLOC.\textsuperscript{[2]} Evaluation of different binding conformations of the inhibitors was based on i) avoidance of unfavorable steric contacts, ii) forming of H-bonding contacts, iii) complete filling of the space within binding pockets by use of maximal capacity of hydrophobic contacts between enzyme and ligand.

Synthesis.
General: Solvents and reagents were purchased reagent-grade and used without further purification. All reactions were carried out under a nitrogen atmosphere unless otherwise stated. \(\text{CH}_2\text{Cl}_2\) and toluene were freshly distilled over CaH\(_2\) and sodium, respectively. All products were dried under high vacuum (\(10^{-2}\) Torr) before analytical characterization. TLC: Aluminium sheets coated with SiO\(_2\)-60 UV\(_{254}\) from Macherey-Nagel, visualization by UV light at 245 nm and staining with a solution of KMnO\(_4\) (1.5 g), K\(_2\)CO\(_3\) (10 g), 5 % NaOH (2.5 mL) in H\(_2\)O (150 mL); or a solution of ninhydrin (0.3 g) in butanol (100 mL) and glacial acetic acid (3 mL). Column chromatography (CC): SiO\(_2\)-60 (230-400 mesh, 0.040-0.063 mm) from Fluka. Microwave-assisted reactions were carried out in a CEM discover series microwave reactor. Analytical HPLC was performed on a Knauer Prontosil 120 C\(_{18}\) column (259 x 4 mm, 5 µm, 100 Å); products were eluted with a linear gradient (50-100 %) of
CH$_3$CN in H$_2$O containing 0.1 % TFA over 50 min with a flow rate of 1 mL min$^{-1}$ with UV detection at $\lambda = 254$ nm. Preparative HPLC was performed on a Knauer Prontosil 120-5 C18 column (250 x 25 mm, 7 µm, 100 Å); products were eluted with a linear gradient (50-100 %) of CH$_3$CN in H$_2$O containing 0.1 % TFA with a flow rate of 10 mL min$^{-1}$ with UV detection at $\lambda = 254$ nm. Melting points (Mp): Büchi-510 apparatus; uncorrected. IR Spectra: Perkin Elmer Spectrum BX FTIR System spectrometer (ATR-unit, Attenuated Total Reflection, Golden Gate). NMR spectra ($^1$H, $^{13}$C, $^{19}$F): Varian Gemini-300 and Bruker ARX-300; spectra were recorded at 25 °C using the solvent peak as an internal reference. Coupling constants (J) are given in Hz. The resonance multiplicity is described as s (singlet), br. s (broad singlet), d (doublet), t (triplet), q (quartet), quint (quintet) and m (multiplet). High-resolution mass spectra (HRMS): IonSpec Ultima FT-ICR with 3-hydroxypicolinic acid (3-HPA) as matrix (MALDI); Micromass AutoSpec-Ultima (EI); Finnigan TSQ 7000 (ESI). Elemental analyses were performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH Zürich. The nomenclature was generated with the computer program ACD/Name (ACD/Labs).

**General procedure A for the quaternization of amines with 3,4-dichlorobenzyl chloride:** The mixture of the dimethylamine derivative (1 eq.) and 3,4-dichlorobenzyl chloride (10 eq.), dissolved in acetone (ca. 2 mL), was stirred in a closed vessel in a microwave reactor for 20 min at 120 °C before it was concentrated in vacuo. The residue was suspended in hexane, the solvent decanted, and the residue dried in vacuo.
General procedure B for the S_N Ar-reaction with 2,5-dichloronitrobenzene: The thiophenol derivative (1 eq.) was added portionwise to a suspension of Na (as a 30–35 % dispersion in paraffin wax, 1 eq.) in MeOH. The mixture was heated to 60 °C, before 2,5-dichloronitrobenzene (1 eq.) was added. The mixture was left to stir at 65 °C, cooled to 25 °C, diluted with EtOAc, washed with H_2O and saturated aqueous NaHCO_3 solution, dried over MgSO_4, filtered, and concentrated in vacuo.

General procedure C for the reduction of nitrobenzene derivatives: The nitrobenzene derivative (1 eq.), Zn powder (20 eq.), and NH_4Cl (20 eq.) were suspended in MeOH, and the mixture was stirred at 65 °C before it was filtered over Celite and concentrated in vacuo. The residue was dissolved in EtOAc, washed with H_2O and saturated aqueous NaHCO_3 solution, dried over MgSO_4, filtered, and concentrated in vacuo.

General procedure D for the alkylation of aniline derivatives: 3-Chloropropionyl chloride (1.1 eq.) and pyridine (0.5 eq.) were added to a solution of the aniline derivative (1 eq.) in THF. The mixture was left to stir at 25 °C before BH_3·THF (1 M solution in THF, 7 eq.) was added. The reaction was stirred at 67 °C, cooled to 25 °C, quenched by addition of MeOH, and concentrated in vacuo.

General procedure E for the alkylation of dimethylamine: The appropriate chloride (1 eq.) was dissolved in DMF, HNMe_2 (40 % solution in H_2O, 50 eq.) was added, and the mixture was stirred overnight at 90 °C. The mixture was diluted
with a saturated aqueous NaCl solution and extracted with CH₂Cl₂. The combined organic phases were washed with saturated aqueous NaCl solution, dried over MgSO₄, filtered, and concentrated in vacuo.

**General procedure F for the quaternization of amines with (2-bromomethyl)-5-nitrofuran:** The appropriate dimethylamine (1 eq.) was suspended in Et₂O, and acetone was added until a clear solution was obtained. (2-Bromomethyl)-5-nitrofuran (1.5 eq.) was added, and the mixture was left to stir overnight, filtered, and the residue was washed with hexane and Et₂O.

3-({5-Chloro-2-[phenylthio]phenyl}amino)-N-(3,4-dichlorobenzyl)-N,N-dimethylpropan-1-ammonium chloride (5):⁴ General procedure A, starting from 22 (47 mg, 0.13 mmol) and 3,4-dichlorobenzyl chloride (0.18 mL, 1.29 mmol). Purification by reversed phase HPLC (CH₃CN/H₂O (0.1 % TFA) 50:0 → 100:0 in 50 min) to yield 5 (50 mg, 75 %) as a colorless solid. Mp.: 82 °C; ¹H NMR (300 MHz, CD₃OD): δ = 2.03-2.09 (m, 2 H), 2.93 (s, 6 H), 3.11-3.17 (m, 2 H), 3.29-3.45 (m, 2 H), 4.40 (s, 2 H), 5.11 (dd, J = 8.1, 2.2, 1 H), 5.71 (m, 5 H), 7.35-7.41 (m, 2 H), 7.59 (d, J = 8.4, 1 H), 7.69 ppm (d, J = 2.1, 1 H); ¹³C NMR (75 MHz, CD₃OD): δ = 23.5, 40.7, 50.8, 63.9, 67.4, 111.4, 114.5, 118.2, 127.0, 127.9, 129.2, 130.2, 132.5, 133.8, 134.5, 135.9, 136.5, 138.0, 138.9, 140.0, 151.3 ppm; IR (neat): ν = 3058, 2946, 1583, 1501, 1352, 1321, 1165, 1110, 1012, 826 cm⁻¹; HRMS (MALDI): calcd for C₂₄H₂₆Cl₃N₂S⁺ ([M-Cl]⁺): 479.0877, found: 479.0867.
4-Chloro-2-nitro-1-(phenylthio)benzene (9):[3, 4] General procedure B, starting from thiophenol (6, 1.6 mL, 15.6 mmol), Na (30-35 % dispersion in paraffin wax, 1.09 g, 15.6 mmol), and 2,5-dichloronitrobenzene (3.00 g, 15.6 mmol). The mixture was left to stir for 15 h. Purification by CC (hexane/EtOAc 100:0 → 0:100) to yield 9 (3.75 g, 90 %) as yellow crystals. Mp: 83 °C. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 6.78$ (d, $J = 8.7$, 1 H), 7.32 (dd, $J = 8.7$, 2.2, 1 H), 7.48-7.52 (m, 3 H), 7.56-7.59 (m, 2 H), 8.23 ppm (d, $J = 2.2$, 1 H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta = 125.5$, 129.5, 130.3, 130.3, 130.5, 130.8, 133.6, 135.9, 138.2, 145.1 ppm; IR (neat): $\tilde{\nu} = 3093, 3073, 1550, 1513, 1332, 1283, 1096, 876, 826, 747$ cm$^{-1}$; HRMS (EI): calcd for C$_{12}$H$_8$ClNO$_2$S$^+$ ($M^+$): 264.9959, found: 264.9956.

4-Chloro-2-nitro-1-[(4-chlorophenyl)thio]benzene (10):[5] General procedure B, starting from 4-chlorothiophenol (7, 2.26 g, 15.6 mmol), Na (30-35 % dispersion in paraffin wax, 1.09 g, 15.6 mmol), and 2,5-dichloronitrobenzene (3.00 g, 15.6 mmol). The mixture was left to stir for 28 h. Purification by CC (hexane/EtOAc 90:10) to yield 10 (4.20 g, 90 %) as yellow crystals. Mp: 153 °C; $^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 6.78$ (d, $J = 8.7$, 1 H), 7.32 (dd, $J = 8.7$, 2.1, 1 H), 7.45-7.52 (m, 4 H), 8.23 ppm (d, $J = 2.1$, 1 H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta = 125.4$, 125.5, 128.9, 129.2, 130.2, 130.5, 133.6, 135.8, 137.0, 145.0 ppm; IR (neat): $\tilde{\nu} = 3094, 3074, 1549, 1519, 1330, 1280, 1161, 1119, 1092, 1048, 1012, 878, 821, 747, 676$ cm$^{-1}$; HRMS (EI): calcd for C$_{12}$H$_8$Cl$_2$NO$_2$S$^+$ ($M^+$): 298.9569, found: 298.9570; anal.: calcd for C$_{12}$H$_8$Cl$_2$NO$_2$S: C 48.02, H 2.67, N 4.67, found: C 47.99, H 2.54, N 4.78.
4-Chloro-2-nitro-1-[(4-bromophenyl)thio]benzene \((11)\):

General procedure B, starting from 4-bromo thiophenol \((8, 1.97 \text{ g, } 10.4 \text{ mmol}), \) Na \((30-35 \% \text{ dispersion in paraffin wax, } 0.73 \text{ g, } 10.4 \text{ mmol}), \) and 2,5-dichloronitrobenzene \((2.00 \text{ g, } 10.4 \text{ mmol}). \) The mixture was left to stir for 24 h. Purification by CC (hexane/EtOAc 100:0 \(\rightarrow\) 0:100) to yield \(11\) \((3.52 \text{ g, } 95 \%)\) as yellow crystals. Mp: 155 °C; \(\textsuperscript{1}H\) NMR: \(\delta = 6.79 \text{ (d, } J = 8.7, 1 \text{ H}), 7.32 \text{ (dd, } J = 8.7, 2.1, 1 \text{ H}), 7.42-7.46 \text{ (m, } 2 \text{ H}), 7.60-7.64 \text{ (m, } 2 \text{ H), } 8.23 \text{ ppm (d, } J = 2.1, 1 \text{ H}); \) \(\textsuperscript{13}C\) NMR (CDCl\(_3\), 75MHz): \(\delta = 125.1, 125.5, 129.3, 129.6, 130.2, 133.5, 133.6, 135.8, 137.2, 145.1 \text{ ppm}; \) IR (neat): \(\tilde{\nu} = 3072, 2917, 2849, 1644, 1590, 1549, 1517, 1449, 1384, 1331, 1244, 1161, 1096, 1049, 1008, 878, 820, 754, 731 \text{ cm}^{-1}; \) HRMS (EI): calcd for C\(_{12}\)H\(_7\)BrClNO\(_2\)S\(^{+}\) \((M^{+})\): 342.9064, found: 342.9056.

4-Chloro-2-nitro-1-\{(4-\text{(trifluoromethyl)phenyl})thio\}benzene \((12)\): To a solution of 4-(trifluoromethyl)bromobenzene \((13, 0.60 \text{ mL, } 4.27 \text{ mmol})\) and tert-BuLi \((1.7 \text{ M in pentane, } 5.02 \text{ mL, } 8.53 \text{ mmol})\) in THF \((20 \text{ mL}), \) stirred for 10 min at \(-78 \text{ °C}, \) sulfur \((0.14 \text{ g, } 4.27 \text{ mmol})\) was added and the mixture was left to stir for 30 min while it was allowed to warm to \(25 \text{ °C}. \) 2,5-Dichloronitrobenzene \((0.66 \text{ g, } 3.41 \text{ mmol})\) was added, and the reaction was stirred for 5 h, quenched by addition of saturated aqueous NaHCO\(_3\) solution, and extracted with EtOAc. The organic phases were washed with saturated aqueous NaCl solution, the aqueous phases were extracted with CH\(_2\)Cl\(_2\), the combined organic phases were dried over MgSO\(_4\), filtered, and concentrated in vacuo. Purification by CC (hexane/CH\(_2\)Cl\(_2\) 90:10) to yield \(12\) \((0.88 \text{ g, } 77 \%)\) as an
orange solid. Mp: 100 °C; $^1$H NMR (300 MHz, CDCl$_3$): $\delta = 6.84$ (d, $J = 8.7$, 1 H), 7.35 (dd, $J = 8.7$, 2.1, 1 H), 7.67-7.76 (m, 4 H), 8.22 ppm (d, $J = 2.1$, 1 H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 125.6$, 126.9 (q, $J = 3.7$), 129.7, 129.8, 131.7, 133.6, 135.3, 135.7, 145.7 ppm (CCF$_3$ signals not visible); $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta = -62.8$ ppm (s, 3 F); IR (neat): $\tilde{\nu} = 3104$, 1549, 1512, 1318, 1175, 1121, 1061, 1014, 840, 820, 768 cm$^{-1}$; HRMS (EI): calcd for C$_{13}$H$_7$ClF$_3$NO$_2$S$^+$ ($M^+$): 332.9833, found: 332.9830.

5-Chloro-2-(phenylthio)aniline (14):$^{[3, 4]}$ General procedure C, starting from nitrobenzene 9 (2.00 g, 7.55 mmol), NH$_2$Cl (8.07 g, 0.15 mol), Zn (9.87 g, 0.15 mol) in MeOH (55 mL). The mixture was left to stir for 2 h to yield 14 (1.67 g, 94 %) as an orange solid. Mp: 63 °C; $^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 4.36$ (br. s, 2 H, NH$_2$), 6.72 (dd, $J = 8.1$, 2.2, 1 H), 6.78 (d, $J = 2.2$, 1 H), 7.06-7.16 (m, 3 H), 7.21-7.24 (m, 2 H), 7.38 ppm (d, $J = 8.1$, 1 H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta = 112.7$, 114.8, 118.7, 125.6, 126.4, 129.0, 136.1, 136.8, 138.3, 149.5 ppm; IR (neat): $\tilde{\nu} = 3478$, 3375, 1600, 1551, 1472, 1415, 1257, 1072, 905, 785, 738, 687 cm$^{-1}$; HRMS (EI): calcd for C$_{13}$H$_7$ClF$_3$NO$_2$S$^+$ ($M^+$): 332.9833, found: 332.9830.

5-Chloro-2-[(4-chlorophenyl)thio]aniline (15):$^{[5]}$ General procedure C, starting from nitrobenzene 10 (3.00 g, 10.00 mmol), NH$_2$Cl (10.7 g, 0.20 mol), Zn (13.1 g, 0.20 mol) in MeOH (75 mL). The mixture was left to stir for 5 h to yield 15 (2.63 g, 98 %) as an orange solid. Mp: 66 °C; $^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 4.35$ (br. s, 2 H), 6.73 (dd, $J = 8.1$, 2.1, 1 H), 6.79 (d, $J = 2.1$, 1 H), 6.98-7.02 (m, 2 H), 7.17-7.21 (m, 2 H), 7.36 ppm (d, $J = 8.1$, 1 H); $^{13}$C NMR
(CDCl$_3$, 75 MHz): $\delta = 112.2, 114.9, 118.8, 127.6, 129.1, 131.5, 134.7, 137.1, 138.3, 149.5$ ppm; IR (neat): $\tilde{\nu} = 3493, 3389, 1891, 1596, 1555, 1473, 1418, 1389, 1253, 1090, 1076, 1008, 908, 843, 798$ cm$^{-1}$; HRMS (EI): calcd for C$_{12}$H$_9$Cl$_2$NS$^+$ ($M^+$): 268.9828, found: 268.9850.

5-Chloro-2-[(4-bromophenyl)thio]aniline (16): General procedure C, starting from nitrobenzene 11 (3.00 g, 8.70 mmol), NH$_4$Cl (9.31 g, 0.17 mol), Zn (11.4 g, 0.17 mol) in MeOH (75 mL). The mixture was left to stir for 4 h to yield 16 (2.00 g, 73 %) as an orange solid. Mp: 73 °C; $^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 4.35$ (br. s, 2 H), 6.73 (dd, $J = 8.4, 2.0, 1$ H), 6.79 (d, $J = 2.0, 1$ H), 6.90-6.95 (m, 2 H), 7.31-7.37 ppm (m, 3 H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta = 112.0, 115.0, 118.8, 119.3, 127.8, 132.0, 135.4, 137.1, 138.3, 149.5$ ppm; IR (neat): $\tilde{\nu} = 3487, 3383, 1597, 1555, 1471, 1417, 1386, 1309, 1274, 1253, 1087, 1072, 1003, 907, 841, 798, 739$ cm$^{-1}$; HRMS (EI): calcd for C$_{12}$H$_9$BrClNS$^+$ ($M^+$): 312.9223, found: 312.9224.

5-Chloro-2-[(4-(trifluoromethyl)phenyl)thio]aniline (17): General procedure C, starting from nitrobenzene 12 (0.88 g, 2.64 mmol), NH$_4$Cl (2.82 g, 52.80 mmol), Zn (3.45 g, 52.80 mmol) in MeOH (20 mL). The mixture was left to stir for 5 h to yield 17 (0.71 g, 88 %) as a yellow oil. $^1$H NMR (CDCl$_3$, 300 MHz, CDC$_{13}$): $\delta = 4.37$ (br. s, 2 H), 6.76 (dd, $J = 8.1, 2.1, 1$ H), 6.82 (d, $J = 2.1, 1$ H), 7.11 (dd, $J = 8.7, 0.6, 2$ H), 7.37 (d, $J = 8.1, 1$ H), 7.46 ppm (dd, $J = 8.7, 0.6, 2$ H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 110.7, 115.0, 119.0, 124.0$ (q, $J = 269.5$), 125.6, 125.7 (q, $J = 3.6$), 127.4 (q, $J = 33.0$), 137.6, 138.6, 141.5, 149.7 ppm; $^{19}$F NMR (282 MHz, CDCl$_3$):
$\delta = -62.2$ ppm (s, 3 F); IR (neat): $\tilde{\nu} = 3488, 3384, 3026, 2933, 1606, 1477, 1327, 1165, 1123, 1063, 1013, 908, 827, 702, 631$ cm$^{-1}$; HRMS (EI): calcd for $C_{13}H_9ClF_3NS^+$ ($M^+$): 303.0091, found: 303.0091; anal.: calcd for $C_{13}H_9ClF_3NS$: C 51.41, H 2.99, N 4.61, found: C 51.51, H 2.99, N 4.61.

2-(Phenylthio)-5-chloro-N-(3-chloropropyl)aniline (18): [6]
General procedure D, starting from aniline 14 (1.13 g, 4.82 mmol), pyridine (0.16 mL, 2.41 mmol), 3-chloropropionyl chloride (0.55 mL, 5.79 mmol), and $BH_3\cdot THF$ (1 M in THF, 38.57 mL, 38.57 mmol) in THF (50 mL). The mixture was left to stir for 1 h for the first step, then for 2 h. Purification by CC (hexane/CH$_2$Cl$_2$ 90:10) to yield 18 (1.31 g, 87 %) as a colorless oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 1.96$ (quint, $J = 6.3$, 2 H), 3.31 (q, $J = 6.3$, 2 H), 3.39 (t, $J = 6.3$, 2 H), 4.99 (br. s, 1 H), 6.66-6.69 (m, 2 H), 7.03-7.07 (m, 2 H), 7.10-7.16 (m, 1 H), 7.20-7.25 (m, 2 H), 7.39-7.42 ppm (d, $J = 8.4$, 1 H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta = 31.5$, 40.2, 42.1, 110.3, 112.5, 116.9, 125.7, 126.4, 129.0, 136.0, 137.4, 138.4, 149.5 ppm; IR (neat): $\tilde{\nu} = 3383, 3278, 3059, 2953, 2922, 2858, 1580, 1502, 1477, 1419, 1272, 1095, 1024, 834, 793, 738, 689$ cm$^{-1}$; HRMS (EI): calcd for $C_{15}H_{15}Cl_2NS^+$ ($M^+$): 311.0297, found: 311.0296.

2-[(4-Chlorophenyl)thio]-5-chloro-N-(3-chloropropyl)aniline (19): General procedure D, starting from aniline 15 (1.50 g, 5.55 mmol), pyridine (0.22 mL, 2.78 mmol), 3-chloropropionyl chloride (0.64 mL, 6.66 mmol), and $BH_3\cdot THF$ (1 M in THF, 44.40 mL, 44.40 mmol) in THF (20 mL). The mixture was left to stir for 4 h for the first step, then for 4 h. Purification by CC (hexane/EtOAc 100:0 → 90:10)
to yield 19 (1.83 g, 95 %) as a colorless oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 1.95-2.02 (m, 2 H), 3.28-3.34 (m, 2 H), 3.42-3.45 (m, 2 H), 4.99 (t, $J = 5.4$, 1 H), 6.66-6.70 (m, 2 H), 6.95-7.00 (d, $J = 6.9$, 2 H), 7.19 (d, $J = 6.9$, 2 H), 7.38 ppm (dd, $J = 8.4$, 2.1, 1 H); $^{13}$C NMR (CDCl$_3$, 75MHz): $\delta$ = 31.5, 40.4, 42.2, 110.4, 112.0, 117.1, 127.6, 129.1, 131.5, 134.7, 137.8, 138.5, 149.5 ppm; IR (neat): $\tilde{\nu}$ = 3405, 2964, 1583, 1559, 1506, 1419, 1390, 1363, 1273, 1204, 1176, 1132, 1088, 1041, 1010, 813, 792, 740 cm$^{-1}$; HRMS (EI): calcd for C$_{15}$H$_{14}$Cl$_2$ NS$^+$ ($M^+$): 344.9908, found: 344.9911.

2-[(4-Bromophenyl)thio]-5-chloro-N-(3-chloropropyl)aniline (20): General procedure D, starting from aniline 16 (1.50 g, 4.77 mmol), pyridine (0.19 mL, 2.38 mmol), 3-chloropropionyl chloride (0.55 mL, 5.72 mmol), and BH$_3$·THF (1 M in THF, 38.14 mL, 38.14 mmol) in THF (10 mL). The mixture was left to stir for 4 h for the first step, then for 2 h. Purification by CC (hexane/EtOAc 100:0 → 90:10) to yield 20 (1.54 g, 83 %) as a colorless oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 1.97 (quint, $J = 6.3$, 2 H), 3.28-3.33 (m, 2 H), 3.44 (t, $J = 6.3$, 2 H), 4.96 (br. s, 1 H), 6.66-6.70 (m, 2 H), 6.65-6.90 (m, 2 H), 7.30 ppm (dd, $J = 8.4$, 2.1, 1 H); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ = 31.6, 40.4, 42.3, 110.5, 111.9, 117.1, 119.4, 127.9, 132.0, 135.5, 137.8, 138.5, 149.6 ppm; IR (neat): $\tilde{\nu}$ = 3383, 2958, 2868, 2361, 1582, 1560, 1503, 1471, 1419, 1280, 1082, 1041, 1006, 810, 631 cm$^{-1}$; HRMS (EI): calcd for C$_{15}$H$_{14}$BrCl$_2$NS$^+$ ($M^+$): 388.9402, found: 388.9402.
5-Chloro-N-[(3-chloropropyl)-2-[(4-(trifluoromethyl)phenyl)thio]aniline (21): General procedure D, starting from aniline 17 (0.60 g, 1.98 mmol), pyridine (80 µL, 0.99 mmol), 3-chloropropionyl chloride (0.23 mL, 2.37 mmol), and BH₃·THF (1 M in THF, 15.80 mL, 15.80 mmol) in THF (20 mL). The mixture was left to stir for 1 h for the first step, then for 2 h. Purification by CC (hexane/EtOAc 100:0 → 90:10) to yield 21 (0.69 g, 92%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃): δ = 1.94-2.03 (m, 2 H), 3.29-3.36 (m, 2 H), 3.42-3.46 (m, 2 H), 4.97 (t, J = 5.7, 1 H), 6.69-6.73 (m, 2 H), 7.09 (d, J = 8.1, 2 H), 7.40 (d, J = 8.7, 1 H), 7.46 ppm (d, J = 8.1, 2 H); ¹³C NMR (75 MHz, CDCl₃): δ = 31.4, 40.3, 42.0, 110.7, 117.4, 122.3, 125.8, 125.9 (q, J = 3.7), 127.7 (q, J = 32.5), 138.4, 138.9, 141.6, 149.9 ppm (CF₃-signal not visible); ¹⁹F NMR (282 MHz, CDCl₃): δ = -62.2 ppm (s, 3 F); IR (neat): ν = 3388, 3027, 2961, 1605, 1583, 1504, 1326, 1164, 1123, 1087, 1013, 828, 699, 631 cm⁻¹; HRMS (EI): calcd for C₁₆H₁₄Cl₂F₃NS⁺ (M⁺): 379.0171, found: 379.0169.

N’-[5-Chloro-2-(phenylthio)phenyl]-N,N-dimethylpropane-1,3-diamine (22): General procedure E, starting from 18 (0.16 g, 0.51 mmol) and HNMe₂ (40 % solution in H₂O, 2.85 mL, 25.30 mmol) in DMF (10 mL) to yield 22 (0.15 g, 91%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃): δ = 1.73 (quint, J = 6.5, 2 H), 2.16 (s, 6 H), 2.27 (t, J = 6.9, 2 H), 3.18 (q, J = 6.3, 2 H), 5.57 (br. s, 1 H), 6.62-6.65 (m, 2 H), 7.02-7.13 (m, 3 H), 7.18-7.24 (m, 2 H), 7.38 ppm (d, J = 8.7, 1 H); ¹³C NMR (75 MHz, CDCl₃): δ = 26.0, 41.9, 45.0, 57.3, 110.1, 112.0, 116.4, 125.3, 126.1, 128.9, 136.5, 137.3, 138.4, 150.1 ppm; IR (neat): ν = 3058, 2943,
2857, 2816, 2767, 1583, 1419, 1281, 1096, 1042, 829, 737, 689, 633 cm\(^{-1}\); HRMS (MALDI): calcd for C\(_{17}\)H\(_{22}\)Cl\(_2\)N\(_2\)S\(^+\) (M\(^+\)): 321.1187, found: 321.1181.

\(N`-\{5\text{-}\text{Chloro}2-\{\text{4\text{-}chlorophenyl}\text{thio}\}\text{phenyl}\}-N, N\text{-dimethylpropan-1,3-diamine} \ (23)\)\footnote{[5]}: General procedure E, starting from \(19\) (0.57 g, 1.64 mmol) and HNMe\(_2\) (40 \% solution in H\(_2\)O, 9.26 mL, 82.20 mmol) in DMF (20 mL) to yield \(23\) (0.47 g, 81 \%) as a colorless oil. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 1.68\) (quint, \(J = 6.6, 2\) H), 2.10 (s, 6 H), 2.22 (t, \(J = 6.6, 2\) H), 3.12–3.18 (m, 2 H), 5.73 (t, \(J = 4.5, 1\) H), 6.61–6.64 (m, 2 H), 6.92–6.97 (m, 2 H), 7.15–7.18 (m, 2 H), 7.35 ppm (dd, \(J = 7.5, 0.9, 1\) H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta = 26.3, 42.5, 45.4, 57.7, 110.2, 111.3, 116.3, 127.2, 128.9, 131.0, 135.2, 137.6, 138.3, 150.4\) ppm; IR (neat): \(\tilde{\nu} = 2944, 2858, 2817, 2767, 1586, 1559, 1502, 1474, 1420, 1281, 1089, 1010, 812, 668, 621\) cm\(^{-1}\); HRMS (MALDI): calcd for C\(_{17}\)H\(_{21}\)Cl\(_2\)N\(_2\)S\(^+\) (M\(^+\)): 355.0797, found: 355.0792; anal.: calcd for C\(_{17}\)H\(_{20}\)Cl\(_2\)N\(_2\)S: C 57.46, H 5.67, N 7.88, found: C 57.69, H 5.51, N 8.08.

\(N`-\{5\text{-}\text{Chloro}2-\{\text{4\text{-}bromophenyl}\text{thio}\}\text{phenyl}\}-N, N\text{-dimethylpropan-1,3-diamine} \ (24)\): General procedure E, starting from \(20\) (0.25 g, 0.64 mmol) and HNMe\(_2\) (40 \% solution in H\(_2\)O, 3.62 mL, 32.14 mmol) in DMF (10 mL) to yield \(24\) (0.21 g, 83 \%) as a colorless oil. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 1.67-1.71\) (m, 2 H), 2.11 (s, 6 H), 2.24 (t, \(J = 6.6, 2\) H), 3.13–3.16 (m, 2 H), 6.89 (br. s, 1 H), 6.61–6.64 (m, 2 H), 6.88 (d, \(J = 8.7, 2\) H), 7.30–7.36 ppm (m, 3 H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta = 26.2, 42.4, 45.3, 57.7, 110.2, 111.1, 116.3, 118.8, 127.5, 131.8, 135.9, 137.7, 150.4\) ppm; IR (neat): \(\tilde{\nu} = 2944, 2858, 2817, 2767, 1586, 1559, 1502, 1474, 1420, 1281, 1089, 1010, 812, 668, 621\) cm\(^{-1}\); HRMS (MALDI): calcd for C\(_{17}\)H\(_{21}\)Cl\(_2\)N\(_2\)S\(^+\) (M\(^+\)): 355.0797, found: 355.0792; anal.: calcd for C\(_{17}\)H\(_{20}\)Cl\(_2\)N\(_2\)S: C 57.46, H 5.67, N 7.88, found: C 57.69, H 5.51, N 8.08.
18.4, 150.3 ppm; IR (neat): $\tilde{\nu}$ = 2945, 2858, 2817, 2767, 1587, 1503, 1471, 1419, 1281, 1082, 809, 608 cm$^{-1}$; HRMS (MALDI): calcd for C$_{17}$H$_{23}$BrClN$_2$S$^+$ (M$^+$): 399.0292, found: 399.0281; anal.: calcd for C$_{17}$H$_{20}$BrClN$_2$: C 51.07, H 5.04, N 7.01, found: C 51.08, H 5.08, N 7.04.

$N^\prime$-(5-Chloro-2-[(4-(trifluoromethyl)thio)phenyl]N,N-dimethylpropan-1,3-diamin (25): General procedure E, starting from 21 (0.63 g, 1.66 mmol) and HNMe$_2$ (40 % solution in H$_2$O, 9.34 mL, 82.85 mmol) in DMF (25 mL) to yield 25 (0.59 g, 91 %) as a colorless oil. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 1.68 (quint, $J$ = 6.4, 2 H), 2.06 (s, 6 H), 2.23 (t, $J$ = 6.3, 2 H), 3.16 (q, $J$ = 6.3, 2 H), 5.88 (t, $J$ = 5.1, 1 H), 6.63-6.66 (m, 2 H), 7.07 (d, $J$ = 8.3, 2 H), 7.36 (dd, $J$ = 7.5, 0.9, 1 H), 7.43 (d, $J$ = 8.3, 2 H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 26.1, 42.6, 45.3, 57.8, 110.1, 110.4, 116.6, 124.2 (q, $J$ = 269.8), 125.5, 125.7 (q, $J$ = 3.8), 127.3 (q, $J$ = 32.5), 138.3, 138.8, 142.3, 150.8 ppm; $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta$ = -62.2 ppm (s, 3 F); IR (neat): $\tilde{\nu}$ = 2949, 2862, 2820, 2781, 1583, 1505, 1420, 1323, 1280, 1160, 1121, 1086, 1062, 1012, 825, 789 cm$^{-1}$; HRMS (MALDI): calcd for C$_{18}$H$_{21}$ClF$_3$N$_2$S$^+$ (M$^+$): 389.1061, found: 389.1055; anal.: calcd for C$_{18}$H$_{20}$ClF$_3$N$_2$S·HCl: C 50.83, H 4.98, N 6.59, found: C 50.56, H 5.01, N 6.50.

3-{(5-Chloro-2-[(4-chlorophenyl)thio]phenyl)amino}-N-(3,4-dichlorobenzyl)-N,N-dimethylpropan-1-ammonium chloride (26): General procedure A, starting from 23 (27 mg, 76 µmol) and 3,4-dichlorobenzyl chloride (0.15 mL, 0.76 mmol). Purification by reversed phase HPLC (CH$_3$CN/H$_2$O (0.1 % TFA) 50:0 → 100:0 in 50 min) to yield 26 (31 mg, 74 %) as
a white solid. Mp: 109 °C; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 2.00-2.18\) (m, 2 H), 3.13 (s, 6 H), 3.29-3.31 (m, 2 H), 3.46-3.52 (m, 2 H), 5.03-5.06 (m, 3 H), 6.63 (d, \(J = 2.1\), 1 H), 6.71 (dd, \(J = 8.3, 2.1, 1\) H), 6.97 (td, \(J = 8.9, 2.4, 2\) H), 7.14 (td, \(J = 8.9, 2.4, 2\) H), 7.38 (d, \(J = 8.3, 1\) H), 7.44 (d, \(J = 8.3, 1\) H), 7.54 (dd, \(J = 8.3, 1.9, 1\) H), 7.69 ppm (d, \(J = 1.9, 1\) H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta = 22.4, 39.8, 49.7, 62.7, 66.5, 110.3, 112.7, 117.8, 126.5, 128.0, 128.9, 131.3, 131.4, 132.0, 133.7, 134.2, 134.8, 135.9, 137.9, 138.3, 148.7 ppm; IR (neat): \(\tilde{\nu} = 3349, 2952, 2922, 2871, 1578, 1506, 1474, 1418, 1291, 1136, 1088, 1010, 825, 785\) cm\(^{-1}\); HRMS (MALDI): calcd for C\(_{24}\)H\(_{25}\)Cl\(_4\)N\(_2\)S\(#^+\) ([M-Cl]?): 513.0487, found: 513.0485; anal.: calcd for C\(_{24}\)H\(_{25}\)Cl\(_4\)N\(_2\)S\(#^+\)Cl\(-\): C 52.34, H 4.57, N 5.09, found: C 52.07, H 4.61, N 4.90.

3-({5-Chloro-2-[(4-bromophenyl)thio]phenyl}amino)-\(N\)-(3,4-dichlorobenzyl)-\(N, N\)-dimethylpropan-1-ammonium chloride (27): General procedure A, starting from 24 (25 mg, 63 \(\mu\)mol) and 3,4-dichlorobenzyl chloride (87 \(\mu\)L, 0.63 mmol). Purification by reversed-phase HPLC (CH\(_3\)CN/H\(_2\)O (0.1 \% TFA) 50:0 \(\rightarrow\) 100:0 in 50 min) to yield 27 (20 mg, 54 \%) as a white solid. Mp: 105 °C; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 2.07-2.12\) (m, 2 H), 3.13 (s, 6 H), 3.26-3.32 (m, 2 H), 3.44-3.50 (m, 2 H), 5.01-5.04 (m, 3 H), 6.62 (d, \(J = 2.0, 1\) H), 6.71 (dd, \(J = 8.1, 2.1, 1\) H), 6.97 (td, \(J = 8.6, 2.1, 2\) H), 7.27 (td, \(J = 8.6, 2.1, 2\) H), 7.37 (d, \(J = 8.1, 1\) H), 7.44 (d, \(J = 8.4, 1\) H), 7.53 (dd, \(J = 8.4, 1.8, 1\) H), 7.70 ppm (d, \(J = 1.8, 1\) H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta = 22.7, 40.0, 49.7, 62.4, 65.4, 110.4, 112.4, 117.8, 119.2, 127.1, 128.2, 131.2, 131.9, 132.4, 133.5, 134.4, 135.5, 135.6, 137.9, 138.5, 148.7 ppm; IR (neat): \(\tilde{\nu} = 3008, 2956, 2920,\)
3-[(5-Chloro-2-[(4-
(trifluoromethyl)phenyl)thio]phenyl)amino]-N-(3,4-
dichlorobenzyl)-N,N-dimethylpropan-1-ammonium chloride (28): General procedure A, starting from 25 (13 mg, 33 µmol) and 3,4-dichlorobenzyl chloride (46 µL, 0.33 mmol). Purification by reversed phase HPLC (CH$_3$CN/H$_2$O (0.1 % TFA) 50:0 → 100:0 in 50 min) to yield 28 (14 mg, 72 %) as a white solid. Mp: 153 °C; $^1$H NMR (300 MHz, CDCl$_3$): δ = 2.09-2.17 (m, 2 H), 3.15 (s, 6 H), 3.27-3.30 (m, 2 H), 3.54-3.61 (m, 2 H), 5.05-5.09 (m, 3 H), 6.66 (d, $J$ = 2.1, 1 H), 6.73 (dd, $J$ = 8.1, 2.1, 1 H), 7.06 (d, $J$ = 8.1, 2 H), 7.37-7.44 (m, 4 H), 7.53 (dd, $J$ = 8.4, 1.8, 1 H), 7.70 ppm (d, $J$ = 1.8, 1 H); $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 22.8, 40.2, 49.7, 62.3, 65.9, 110.7, 111.3, 118.1, 125.8, 126.1, 127.3, 127.7 (q, $J$ = 32.6), 131.3, 132.6, 133.7, 134.6, 135.9, 138.5, 139.0, 141.7, 149.4 ppm (CF$_3$-signal not visible); $^{19}$F NMR (282 MHz, CDCl$_3$): δ = -62.2 ppm (s, 3 F); HRMS (MALDI): calcd for C$_{25}$H$_{25}$Cl$_3$N$_2$S$^+$ ([M-Cl]$^+$): 556.9982, found: 556.9980; anal.: calcd for C$_{25}$H$_{25}$Cl$_3$N$_2$S$^+$Cl$^-$: C 48.43, H 4.23, N 4.71, found: C 48.24, H 4.49, N 4.40.

3-[(5-Chloro-2-(phenylthio)phenyl)amino]-N,N-dimethyl-N-
[(5-nitro-2-furyl)methyl]-propan-1-ammonium bromide (29): General procedure F, starting from 22 (62 mg, 0.17 mol) and (2-bromomethyl)-5-nitrofuran (54 mg, 0.26 mmol) in Et$_2$O (10 mL) and acetone (8 mL) to yield 29 (58 mg, 63 %) as a pale yellow solid. Mp: 78 °C; $^1$H NMR (300 MHz, CDCl$_3$):
δ = 2.04-2.16 (m, 2 H), 3.21 (s, 6 H), 3.34-3.41 (m, 4 H),
5.03 (t, J = 5.7, 1 H), 5.21 (s, 2 H), 6.66 (d, J = 2.1,
1 H), 6.71 (dd, J = 8.1, 2.1, 1 H), 7.03 (dd, J = 8.4, 1.2,
2 H), 7.10-7.15 (m, 1 H), 7.19-7.24 (m, 2 H), 7.29 (d,
J = 3.8, 1 H), 7.40 (d, J = 8.1, 1 H), 7.44 ppm (d, J =
3.8, 1 H); ¹³C NMR (75 MHz, CDCl₃): δ = 22.8, 39.9, 51.2,
58.9, 63.2, 110.6, 112.1, 113.3, 117.8, 120.8, 126.0,
126.9, 129.2, 136.3, 137.6, 138.6, 145.0, 149.1, 153.2 ppm;
IR (neat): ν = 3363, 3064, 2958, 1581, 1500, 1350, 1246,
1092, 1022, 900, 810, 734, 691 cm⁻¹; HRMS (MALDI): calcd
for C₂₂H₂₅ClN₃O₃S⁺ ([M-Br]⁺): 446.1300, found: 446.1293.

3-{[5-Chloro-2-[(4-chlorophenyl)thio]phenyl}amino]-N,N-
dimethyl-N-[5-nitro-2-furyl]-methyl]propan-1-ammonium
bromide (30): General procedure F, starting from 23
(0.15 g, 0.43 mmol) and (2-bromomethyl)-5-nitrofuran (0.13
g, 0.65 mmol) in Et₂O (5 mL) and acetone (1 mL) to yield 30
(0.19 g, 79 %) as a pale yellow solid. Mp: 146 °C; ¹H NMR
(300 MHz, CDCl₃): δ = 2.13-2.16 (m, 2 H), 3.31-3.35 (m,
8 H), 3.49-3.54 (m, 2 H), 5.01 (t, J = 6.0, 1 H), 5.33 (s,
2 H), 6.66 (d, J = 1.9, 1 H), 6.70 (dd, J = 8.1, 2.0, 1 H),
6.98 (td, J = 8.6, 2.4, 2 H), 7.10 (td, J = 8.6, 2.4, 2 H),
7.30 (d, J = 3.8, 1 H), 7.37 (d, J = 8.1, 1 H), 7.42 ppm
(d, J = 3.8, 1 H); ¹³C NMR (75 MHz, CDCl₃): δ = 22.8, 40.0,
51.1, 58.8, 63.2, 110.5, 111.9, 112.7, 117.9, 120.7, 128.0,
129.0, 131.4, 134.7, 137.8, 138.4, 144.8, 148.7, 153.0 ppm;
IR (neat): ν = 3376, 3009, 2936, 1581, 1500, 1473, 1351,
1246, 1090, 1011, 889, 814, 746 cm⁻¹; HRMS (MALDI): calcd
for C₂₂H₂₅ClN₃O₃S⁻ ([M-Br]⁻): 480.0910, found: 480.0914;
anal.: calcd for C₂₂H₂₄Cl₂N₃O₃SBr⁻: C 47.07, H 4.31, N 7.49,
Br 14.23, Cl 12.63, found: C 47.09, H 4.41, N 7.43, Br 14.05, Cl 12.60.

3-({5-Chloro-2-[{4-bromophenyl}thio]phenyl}amino)-N,N-

dimethyl-N-[(5-nitro-2-furyl)-methyl]propan-1-ammonium

bromide (31):  General procedure F, starting from 24

(0.17 g, 0.50 mmol) and (2-bromomethyl)-5-nitrofuran (0.15

g, 0.75 mmol) in Et₂O (5 mL) and acetone (1 mL) to yield 31

(0.25 g, 84 %) as a pale yellow solid.  Mp: 154 °C; ¹H NMR

(300 MHz, CD₃OD): δ = 2.08-2.15 (m, 2 H), 3.11 (s, 6 H),

3.20-3.34 (m, 4 H), 4.72 (s, 2 H), 6.70 (dd, J = 8.1, 2.1,

1 H), 6.84 (d, J = 2.1, 1 H), 6.96 (td, J = 7.2, 2.0, 2 H),

7.10 (d, J = 3.8, 1 H), 7.36-7.42 (m, 3 H), 7.50 ppm (d, J

= 3.8, 1 H) (NH signal not visible); ¹³C NMR (75 MHz,

CDCl₃): δ = 22.6, 40.9, 51.4, 59.0, 63.1, 111.9, 112.2,

114.1, 119.0, 119.7, 120.9, 129.0, 132.2, 135.2, 137.7,

138.4, 145.0, 148.0, 153.2 ppm;  IR (neat): ν = 3364, 3059,

3003, 2974, 2938, 2910, 2856, 1582, 1499, 1471, 1422, 1354,

1289, 1252, 1238, 1081, 1007, 903, 812 cm⁻¹;  HRMS (MALDI):

calcld for C₂₂H₂₄BrClN₃O₃S⁺ ([M-Br]⁺): 524.0405, found:

524.0405;  anal.: calcld for C₂₂H₂₄BrClN₃O₃S⁺Br⁻: C 43.62, H


3-{{5-Chloro-2-{{4-

(trifluoromethyl)phenyl}thio}phenyl}amino}-N,N-

-dimethyl-N-[(5-nitro-2-furyl)-methyl]propan-1-ammonium

bromide (32):  General procedure F, starting from 25

(0.64 g, 1.65 mmol) and (2-bromomethyl)-5-nitrofuran (0.51 g, 2.47 mmol) in Et₂O

(10 mL) and acetone (3 mL) to yield 32 (0.66 g, 67 %) as a

pale yellow solid.  Mp: 136 °C; ¹H NMR (300 MHz, CDCl₃):

δ = 2.04-2.13 (m, 2 H), 3.13 (s, 6 H), 3.28-3.34 (m, 2 H),
3.38-3.44 (m, 2 H), 4.97 (s, 2 H), 6.68-6.72 (m, 2 H), 7.05 (d, \( J = 8.9 \), 2 H), 7.28-7.35 (m, 3 H), 7.40 ppm (d, \( J = 8.9 \), 2 H) (NH signal not visible); \(^{13}\)C NMR (75 MHz, CD\(_3\)OD): \( \delta = 25.1, 43.6, 51.7, 56.9, 64.1, 111.8, 111.9, 112.3, 112.8, 118.3, 118.4, 121.0, 125.7 (q, \( J = 270.5 \)), 126.9 (q, \( J = 3.7 \)), 127.2, 128.5 (q, \( J = 32.8 \)), 139.6, 140.4, 144.0, 146.3 ppm; \(^{19}\)F NMR (282 MHz, CDCl\(_3\)): \( \delta = -62.3 \) ppm (s, 3 F); IR (neat): \( \tilde{\nu} = 3372, 3060, 2944, 2909, 2858, 2654, 1584, 1501, 1407, 1352, 1321, 1167, 1111, 1012, 964, 901, 828 \text{ cm}^{-1} \); HRMS (MALDI): calcd for C\(_{23}\)H\(_{24}\)ClF\(_3\)N\(_3\)O\(_3\)S\(^+\) ([M-Br]\(^+\)): 514.1174, found: 514.1174.

**Enzymatic assays.**

**Materials.** Trypanothione disulfide (TS\(_2\)) was purchased from Bachem. Trypanothione reductase (TR) was prepared as described earlier\(^8\). NADPH was purchased from Biomol. Cytochrome c (Fe\(^{3+}\)) and superoxide dismutase (SOD) were from Sigma.

**Trypanothione reductase assay.** TR activity was measured at 25 °C in a total volume of 1 mL in the presence of 100 \( \mu \)M NADPH and 5-10 mU enzyme in TR assay buffer (40 mM HEPES, 1 mM EDTA, pH 7.5) containing 5 % DMSO. The reaction was started by adding TS\(_2\); NADPH consumption was followed spectrophotometrically at 340 nm. \( V_{\text{max}} \) was calculated using a \( K_m \) value of 18 \( \mu \)M for TS\(_2\)\(^9\).

**Oxidase assay.** The oxidase activity of TR was measured spectrophotometrically at 25 °C by following the
consumption of NADPH at 340 nm. The assay mixture (1 mL total volume) contained 100 µM NADPH in TR assay buffer containing 5 % DMSO. 6.5 U (ca. 1.8 mg) enzyme was added and after monitoring the baseline for 1 min, the reaction was started by adding 200 µM of the subversive substrate.

Cytochrome c-coupled oxidase assay. The assay contained in a total volume of 1 mL TR assay buffer 25 µM Cytc-Fe$^{3+}$, 100 µM NADPH, 100 µM subversive substrate and 5 % DMSO. When a stable baseline was reached, the reaction was started by adding 6.5 U TR and the absorption increase at 550 nm [formation of Cytc-Fe$^{2+}$] was measured. An absorption coefficient of 18.9 mm$^{-1}$cm$^{-1}$ was used to calculate the Cytc-Fe$^{3+}$ reduction activity, which represents the difference in absorption between reduced and oxidized Cytc at 550 nm.$^{[10]}$

In order to distinguish between O$_2$$^{-}$ mediated and direct reduction of Cytc-Fe$^{3+}$, the reaction was followed in the presence or absence of 6 µg SOD.

Parasitology: in vitro bioassays, IC$_{50}$ determination.

Trypanosoma b. rhodesiense. Minimum Essential Medium (50 µL) supplemented according to Baltz et al.$^{[11]}$ with 2-mercaptoethanol and 15 % heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were prepared covering a range from 90 to 0.123 µg mL$^{-1}$. Then 104 bloodstream forms of T. b. rhodesiense STIB 900 in 50 µL were added to each well and the plate incubated at 37 °C under a 5 % CO$_2$ atmosphere for 72 hours. 10 µL of Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and incubation continued for a further 2-4 hours.
The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm\textsuperscript{[12]}. Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic programme Softmax Pro (Molecular Devices) which calculated IC\textsubscript{50} values.

**Cytotoxicity L-6 cells.** The rat skeletal myoblast cell line (L-6 cells) was used to assess cytotoxicity. The cells were grown in RPMI 1640 medium supplemented with 1 % L-glutamine (200 nM) and 10 % fetal bovine serum at 37 °C in 5 % CO\textsubscript{2} in air. Assays were performed in 96-well microtiter plates, each well receiving 100 µL of culture medium with 4•10\textsuperscript{4} cells. After 24 h, the medium was removed from all wells and serial drug dilutions were prepared covering a range from 90 to 0.123 µg mL\textsuperscript{-1}. Each drug was tested in duplicate. After 72 h of incubation, the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. Then, 10 µL of Alamar blue (12.5 mg resazurin dissolved in 100 mL distilled water) was added to each well and the plates were incubated for another 2 h. The plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. EC\textsubscript{50} values were determined using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA).
Literature


