CHEMMEDCHEM
Chemistry & Drug Discovery

Supporting Information

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

by **Bernhard Stump, Marcel Kaiser, Reto Brun, R. Luise Krauth-Siegel,** and **François Diederich***

Supporting Information

Table of contents

Figures referred to in text

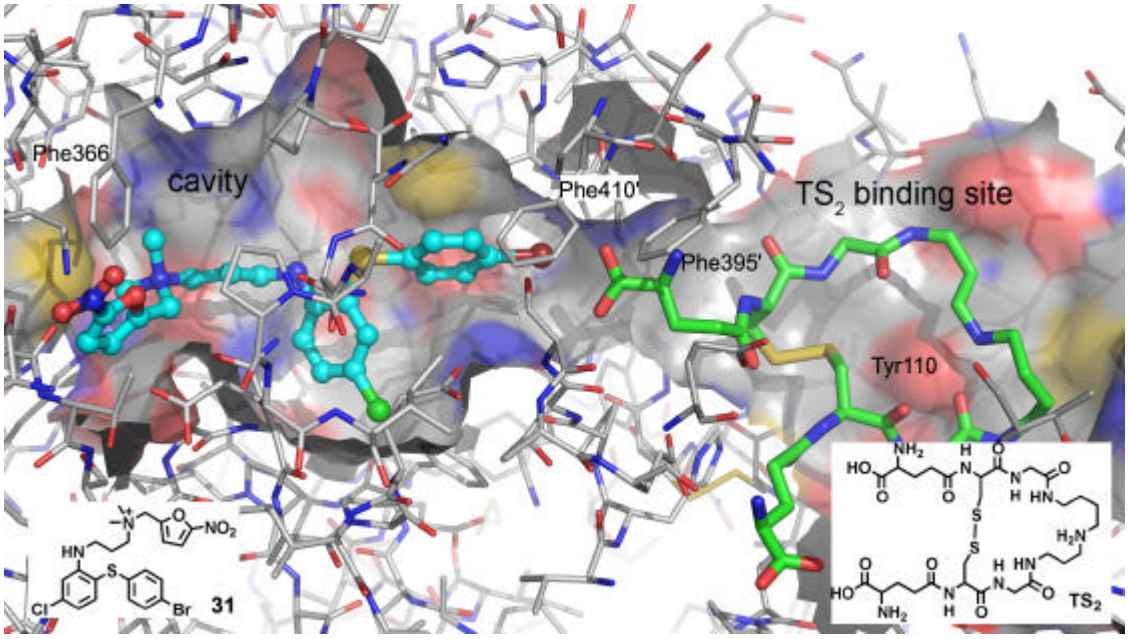
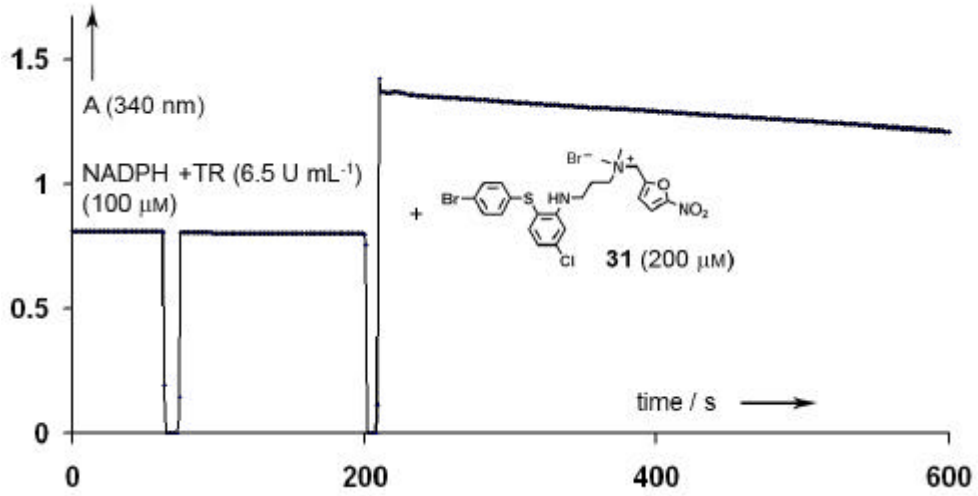


Figure 1SI. Model for the binding of the non-competitive inhibitor **31** in the cavity of TR. The furfuryl inhibitor **31** (cyan, balls and sticks) was modeled into the cavity at the two-fold symmetry axis of *Trypanosoma cruzi* trypanothione reductase in complex with trypanothione (green). PDB-code: 1BZL.[1]

a)



b)

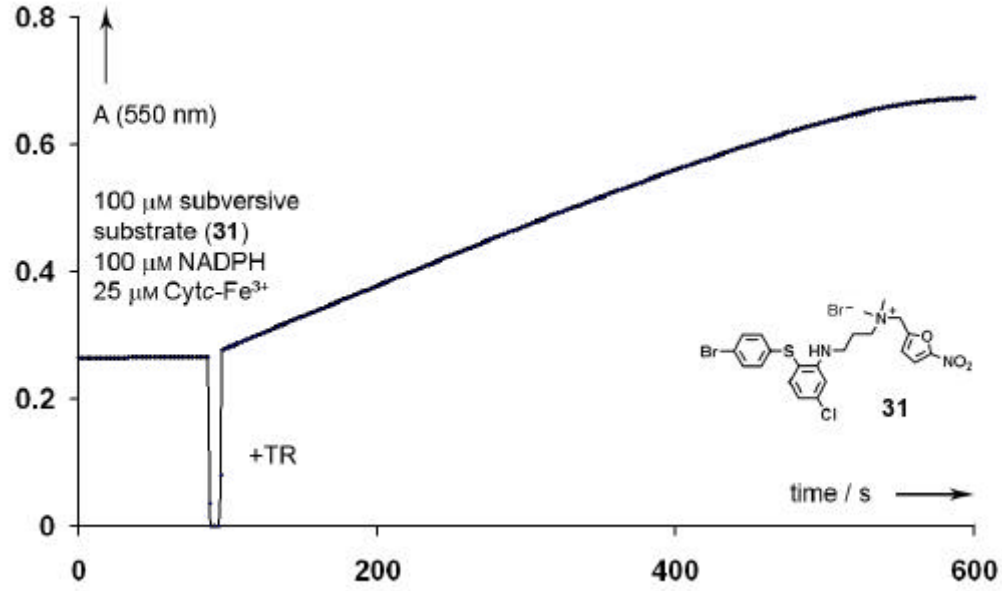


Figure 2SI. Oxidase activity assays. a) Induced NADPH consumption of TR by furfuryl derivative **31**, observed at 340 nm. b) Coupled assay with oxidized cytochrome c (Cyt*c*-Fe^{3+}). Cyt*c*-Fe^{3+} reduction was monitored at 550 nm. The assay was measured as described in the Supporting Information below.

Table 1SI. Reduction of nitrofuran derivative **31** by TR in presence and absence of superoxide dismutase (SOD). Only partial quenching of the Cyt*c*-Fe^{3+} reduction was observed in the coupled assay with SOD.

K_{ic}(= K_{iu}) [μM]	NADPH ox. [U mL^{-1}][a]	Cyt*c*-Fe^{3+} red. [U mL^{-1}][b]	+ SOD [U mL^{-1}][c]
7±1	0.60	0.60	0.36

[a] 100 μM NADPH, 200 μM inhibitor, 6.5 U mL^{-1} TR, 25 °C. [b] As [a], + 25 μM Cyt*c*-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.[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. CH_2Cl_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_2CO_3 (10 g), 5 % NaOH (2.5 mL) in H_2O (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_3CN in H_2O containing 0.1 % TFA over 50 min with a flow rate of 1 mL min^{-1} with UV detection at *l* = 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_3CN in H_2O containing 0.1 % TFA with a flow rate of 10 mL min^{-1} with UV detection at *l* = 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 (^1H, ^{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_NAr-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 $BH_3 \cdot THF$ (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 CH_2Cl_2. The combined organic phases were washed with saturated aqueous NaCl solution, dried over $MgSO_4$, 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 Et_2O, 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 Et_2O.

3-({5-Chloro-2-[phenylthio]phenyl}amino)-*N*-(3,4-dichlorobenzyl)-*N,N*-dimethylpropan-1-ammonium chloride (5):[3] 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 (CH_3CN/H_2O (0.1 % TFA) 50:0 → 100:0 in 50 min) to yield **5** (50 mg, 75 %) as a colorless solid. Mp.: 82 °C; ^{1}H NMR (300 MHz, CD_3OD): 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), 6.71 (dd, *J* = 8.1, 2.2, 1 H), 6.81 (d, *J* = 2.2, 1 H), 7.01-7.21 (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); ^{13}C NMR (75 MHz, CD_3OD): 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): $\tilde{n}$ = 3058, 2946, 1583, 1501, 1352, 1321, 1165, 1110, 1012, 826 cm^{-1}; HRMS (MALDI): calcd for $C_{24}H_{26}Cl_3N_2S^+$ ([*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): δ = 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): δ = 125.5, 129.5, 130.3, 130.3, 130.5, 130.8, 133.6, 135.9, 138.2, 145.1 ppm; IR (neat): $\tilde{n}$ = 3093, 3073, 1550, 1513, 1332, 1283, 1096, 876, 826, 747 cm^{-1}; HRMS (EI): calcd for $C_{12}H_8ClNO_2S^+$ (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): δ = 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): δ = 125.4, 125.5, 128.9, 129.2, 130.2, 130.5, 133.6, 135.8, 137.0, 145.0 ppm; IR (neat): $\tilde{n}$ = 3094, 3074, 1549, 1519, 1330, 1280, 1161, 1119, 1092, 1048, 1012, 878, 821, 747, 676 cm^{-1}; HRMS (EI): calcd for $C_{12}H_7Cl_2NO_2S^+$ (M^+): 298.9569, found: 298.9570; anal.: calcd for $C_{12}H_7Cl_2NO_2S$: 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-brompphenyl)thio]benzene (11): General procedure B, starting from 4-bromothiophenol (**8**, 1.97 g, 10.4 mmol), Na (30-35 % dispersion in paraffin wax, 0.73 g, 10.4 mmol), and 2,5-dichloronitrobenzene (2.00 g, 10.4 mmol). The mixture was left to stir for 24 h. Purification by CC (hexane/EtOAc 100:0 → 0:100) to yield **11** (3.52 g, 95 %) as yellow crystals. Mp: 155 °C; ^{1}H NMR: δ = 6.79 (d, *J* = 8.7, 1 H), 7.32 (dd, *J* = 8.7, 2.1, 1 H), 7.42-7.46 (m, 2 H), 7.60-7.64 (m, 2 H), 8.23 ppm (d, *J* = 2.1, 1 H); ^{13}C NMR ($CDCl_3$, 75MHz): δ = 125.1, 125.5, 129.3, 129.6, 130.2, 133.5, 133.6, 135.8, 137.2, 145.1 ppm; IR (neat): $\tilde{n}$ = 3072, 2917, 2849, 1644, 1590, 1549, 1517, 1449, 1384, 1331, 1244, 1161, 1096, 1049, 1008, 878, 820, 754, 731 cm^{-1}; HRMS (EI): calcd for $C_{12}H_7BrClNO_2S^+$ (M^+): 342.9064, found: 342.9056.

4-Chloro-2-nitro-1-{[4-(trifluoromethyl)phenyl]thio}benzene (12): To a solution of 4-(trifluoromethyl)bromobenzene (**13**, 0.60 mL, 4.27 mmol) and *tert*-BuLi (1.7 M in pentane, 5.02 mL, 8.53 mmol) in THF (20 mL), stirred for 10 min at -78 °C, sulfur (0.14 g, 4.27 mmol) was added and the mixture was left to stir for 30 min while it was allowed to warm to 25 °C. 2,5-Dichloronitrobenzene (0.66 g, 3.41 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_2Cl_2, the combined organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by CC (hexane/CH_2Cl_2 90:10) to yield **12** (0.88 g, 77 %) as an

orange solid. Mp: 100 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = –62.8 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 3104, 1549, 1512, 1318, 1175, 1121, 1061, 1014, 840, 820, 768 cm^{-1}; HRMS (EI): calcd for $C_{13}H_7ClF_3NO_2S^+$ (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_4Cl (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): δ = 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): δ = 112.7, 114.8, 118.7, 125.6, 126.4, 129.0, 136.1, 136.8, 138.3, 149.5 ppm; IR (neat): $\tilde{n}$ = 3478, 3375, 1600, 1551, 1472, 1415, 1257, 1072, 905, 785, 738, 687 cm^{-1}; HRMS (EI): calcd for $C_{12}H_{10}ClNS^+$ (M^+): 235.0217, found: 235.0215.

5-Chloro-2-[(4-chlorophenyl)thio]aniline (15):[5] General procedure C, starting from nitrobenzene **10** (3.00 g, 10.00 mmol), NH_4Cl (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): δ = 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): δ = 112.2, 114.9, 118.8, 127.6, 129.1, 131.5, 134.7, 137.1, 138.3, 149.5 ppm; IR (neat): $\tilde{n}$ = 3493, 3389, 1891, 1596, 1555, 1473, 1418, 1389, 1253, 1090, 1076, 1008, 908, 843, 798 cm^{-1}; HRMS (EI): calcd for $C_{12}H_9Cl_2NS^+$ (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_4Cl (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; ^1H NMR ($CDCl_3$, 300 MHz): δ = 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): δ = 112.0, 115.0, 118.8, 119.3, 127.8, 132.0, 135.4, 137.1, 138.3, 149.5 ppm; IR (neat): $\tilde{n}$ = 3487, 3383, 1597, 1555, 1471, 1417, 1386, 1309, 1274, 1253, 1087, 1072, 1003, 907, 841, 798, 739 cm^{-1}; HRMS (EI): calcd for $C_{12}H_9BrClNS^+$ (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_4Cl (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. ^1H NMR (300 MHz, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$):

δ = –62.2 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 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 $C_{13}H_9ClF_3NS$: 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·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_2Cl_2 90:10) to yield **18** (1.31 g, 87 %) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 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): δ = 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): $\tilde{n}$ = 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-chlor-*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·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): δ = 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): δ = 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): $\tilde{n}$ = 3405, 2964, 1583, 1559, 1506, 1419, 1390, 1363, 1273, 1204, 1176, 1132, 1088, 1041, 1010, 813, 792, 740 cm^{-1}; HRMS (EI): calcd for $C_{15}H_{14}Cl_3NS^+$ (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): δ = 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), 7.30-7.39 ppm (m, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 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): $\tilde{n}$ = 3383, 2958, 2868, 2361, 1582, 1560, 1503, 1471, 1419, 1280, 1082, 1041, 1006, 810, 631 cm^{-1}; HRMS (EI): calcd for $C_{15}H_{14}BrCl_2NS^+$ (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 $BH_3 \cdot THF$ (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. ^{1}H NMR (300 MHz, $CDCl_3$): 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); ^{13}C NMR (75 MHz, $CDCl_3$): 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 (CF_3-signal not visible); ^{19}F NMR (282 MHz, $CDCl_3$): δ = –62.2 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 3388, 3027, 2961, 1605, 1583, 1504, 1326, 1164, 1123, 1087, 1013, 828, 699, 631 cm^{-1}; HRMS (EI): calcd for $C_{16}H_{14}Cl_2F_3NS^+$ (M^+): 379.0171, found: 379.0169.

***N*`-[5-Chloro-2-(phenylthio)phenyl]-*N,N*-dimethylpropane-1,3-diamine (22):**[7] General procedure E, starting from **18** (0.16 g, 0.51 mmol) and $HNMe_2$ (40 % solution in H_2O, 2.85 mL, 25.30 mmol) in DMF (10 mL) to yield **22** (0.15 g, 91 %) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): 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); ^{13}C NMR (75 MHz, $CDCl_3$): 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): $\tilde{n}$ = 3058, 2943,

2857, 2816, 2767, 1583, 1502, 1478, 1419, 1281, 1096, 1042, 829, 737, 689, 633 cm^{-1}; HRMS (MALDI): calcd for $C_{17}H_{22}ClN_2S^+$ (MH^+): 321.1187, found: 321.1181.

***N`*-{5-Chloro-2-[(4-chlorophenyl)thio]phenyl}-*N,N*-dimethylpropan-1,3-diamine (23):**[5] General procedure E, starting from **19** (0.57 g, 1.64 mmol) and $HNMe_2$ (40 % solution in H_2O, 9.26 mL, 82.20 mmol) in DMF (20 mL) to yield **23** (0.47 g, 81 %) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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): $\tilde{n}$ = 2944, 2858, 2817, 2767, 1586, 1559, 1502, 1474, 1420, 1281, 1089, 1010, 812, 668, 621 cm^{-1}; HRMS (MALDI): calcd for $C_{17}H_{21}Cl_2N_2S^+$ (M^+): 355.0797, found: 355.0792; anal.: calcd for $C_{17}H_{20}Cl_2N_2S$: C 57.46, H 5.67, N 7.88, found: C 57.69, H 5.51, N 8.08.

***N`*-{5-Chlor-2-[(4-bromphenyl)thio]phenyl}-*N,N*-dimethylpropan-1,3-diamin (24):** General procedure E, starting from **20** (0.25 g, 0.64 mmol) and $HNMe_2$ (40 % solution in H_2O, 3.62 mL, 32.14 mmol) in DMF (10 mL) to yield **24** (0.21 g, 83 %) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 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), 5.69 (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, $CDCl_3$): δ = 26.2, 42.4, 45.3, 57.7, 110.2, 111.1, 116.3, 118.8, 127.5, 131.8, 135.9, 137.7,

138.4, 150.3 ppm; IR (neat): $\tilde{n}$ = 2945, 2858, 2817, 2767, 1587, 1503, 1471, 1419, 1281, 1082, 1006, 809, 608 cm^{-1}; HRMS (MALDI): calcd for $C_{17}H_{21}BrClN_2S^+$ (*M*H$^+$): 399.0292, found: 399.0281; anal.: calcd for $C_{17}H_{20}BrClN_2S$: C 51.07, H 5.04, N 7.01, found: C 51.08, H 5.08, N 7.04.

***N*`-(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_2O, 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = –62.2 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 2949, 2862, 2820, 2781, 1583, 1505, 1420, 1323, 1280, 1160, 1121, 1086, 1062, 1012, 825, 789 cm^{-1}; HRMS (MALDI): calcd for $C_{18}H_{21}ClF_3N_2S^+$ (*M*H$^+$): 389.1061, found: 389.1055; anal.: calcd for $C_{18}H_{20}ClF_3N_2S \cdot HCl$: 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_3CN/H_2O (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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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): $\tilde{n}$ = 3349, 2952, 2922, 2871, 1578, 1506, 1474, 1418, 1291, 1136, 1088, 1010, 825, 785 cm^{-1}; HRMS (MALDI): calcd for $C_{24}H_{25}Cl_4N_2S^+$ ([*M*-Cl]$^+$): 513.0487, found: 513.0485; anal.: calcd for $C_{24}H_{25}Cl_4N_2S^+Cl^-$: 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 µmol) and 3,4-dichlorobenzyl chloride (87 µL, 0.63 mmol). Purification by reversed-phase HPLC (CH_3CN/H_2O (0.1 % TFA) 50:0 → 100:0 in 50 min) to yield **27** (20 mg, 54 %) as a white solid. Mp: 105 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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): $\tilde{n}$ = 3008, 2956, 2920,

1565, 1506, 1473, 1137, 1035, 1006, 822, 785 cm^{-1}; HRMS (MALDI): calcd for $C_{24}H_{25}Cl_3N_2S^+$ ([*M*-Cl]$^+$): 556.9982, found: 556.9980; anal.: calcd for $C_{24}H_{25}Cl_3N_2S^+Cl^-$: C 48.43, H 4.23, N 4.71, found: C 48.24, H 4.49, N 4.40.

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_3CN/H_2O (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 $C_{25}H_{25}Cl_3F_3N_2S^+$ ([*M*-Cl]$^+$): 547.0751, found: 547.0753;

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_2O (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); ^{13}C NMR (75 MHz, $CDCl_3$): 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): $\tilde{n}$ = 3363, 3064, 2958, 1581, 1500, 1350, 1246, 1092, 1022, 900, 810, 734, 691 cm^{-1}; HRMS (MALDI): calcd for $C_{22}H_{25}ClN_3O_3S^+$ ([*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 Et_2O (5 mL) and acetone (1 mL) to yield **30** (0.19 g, 79 %) as a pale yellow solid. Mp: 146 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 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); ^{13}C NMR (75 MHz, $CDCl_3$): 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): $\tilde{n}$ = 3376, 3009, 2936, 1581, 1500, 1473, 1351, 1246, 1090, 1011, 889, 814, 746 cm^{-1}; HRMS (MALDI): calcd for $C_{22}H_{24}Cl_2N_3O_3S^+$ ([*M*-Br]$^+$): 480.0910, found: 480.0914; anal.: calcd for $C_{22}H_{24}Cl_2N_3O_3S^+Br^-$: 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 Et_2O (5 mL) and acetone (1 mL) to yield **31** (0.25 g, 84 %) as a pale yellow solid. Mp: 154 °C; ^{1}H NMR (300 MHz, CD_3OD): 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); ^{13}C NMR (75 MHz, $CDCl_3$): 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): $\tilde{n}$ = 3364, 3059, 3003, 2974, 2938, 2910, 2856, 1582, 1499, 1471, 1422, 1354, 1289, 1252, 1238, 1081, 1007, 903, 812 cm^{-1}; HRMS (MALDI): calcd for $C_{22}H_{24}BrClN_3O_3S^+$ ([*M*-Br]$^+$): 524.0405, found: 524.0405; anal.: calcd for $C_{22}H_{24}BrClN_3O_3S^+Br^-$: C 43.62, H 3.99, N 6.94, found: C 43.89, H 4.38, N 6.97.

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 Et_2O (10 mL) and acetone (3 mL) to yield **32** (0.66 g, 67 %) as a pale yellow solid. Mp: 136 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 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_3OD): δ = 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, $CDCl_3$): δ = –62.3 ppm (s, 3 F); IR (neat): $\tilde{n}$ = 3372, 3060, 2944, 2909, 2858, 2654, 1584, 1501, 1407, 1352, 1321, 1167, 1111, 1012, 964, 901, 828 cm^{-1}; HRMS (MALDI): calcd for $C_{23}H_{24}ClF_3N_3O_3S^+$ ([*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 μM 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_{max} was calculated using a K_m value of 18 μM 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 Cyt*c*-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 Cyt*c*-Fe^{2+}] was measured. An absorption coefficient of 18.9 $mM^{-1}cm^{-1}$ was used to calculate the Cyt*c*-Fe^{3+} reduction activity, which represents the difference in absorption between reduced and oxidized Cyt*c* at 550 nm.[10] In order to distinguish between $O_2^{-?}$ mediated and direct reduction of Cyt*c*-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[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_{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_2 in air. Assays were performed in 96-well microtiter plates, each well receiving 100 µL of culture medium with $4 \cdot 10^4$ 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^{-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_{50} values were determined using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA).

Literature

[1] C. S. Bond, Y. Zhang, M. Berriman, M. L. Cunningham, A. H. Fairlamb, W. N. Hunter, *Struct. Fold. Des.* **1999**, *7*, 81-89.

[2] P. R. Gerber, K. Müller, *J. Comput. Aid. Mol. Des.* **1995**, *9*, 251-268.

[3] S. Parveen, M. O. F. Khan, S. E. Austin, S. L. Croft, V. Yardley, P. Rock, K. T. Douglas, *J. Med. Chem.* **2005**, *48*, 8087-8097.

[4] J. D. Loudon, *J. Chem. Soc.* **1939**, 902-906.

[5] A. Burger, J. L. Stanmyer, *J. Org. Chem.* **1956**, *21*, 1382-1385.

[6] S. Baillet, E. Buisine, D. Horvath, L. Maes, B. Bonnet, C. Sergheraert, *Bioorg. Med. Chem.* **1996**, *4*, 891-899.

[7] R. Fernandez-Gomez, M. Moutiez, M. Aumercier, G. Bethegnies, M. Luyckx, A. Ouaissi, A. Tartar, C. Sergheraert, *Int. J. Antimicrob. Ag.* **1995**, *6*, 111-118.

[8] F. X. Sullivan, C. T. Walsh, *Mol. Biochem. Parasitol.* **1991**, *44*, 145-147.

[9] M. C. Jockers-Scherübl, R. H. Schirmer, R. L. Krauth-Siegel, *Eur. J. Biochem.* **1989**, *180*, 267-272.

[10] K.-G. Paul, *Method. Enzymol.* **1955**, *Volume 2*, 749-755.

[11] T. Baltz, D. Baltz, C. Giroud, J. Crockett, *EMBO J.* **1985**, *4*, 1273-1277.

[12] B. Räz, M. Iten, Y. Grether-Bühler, R. Kaminsky, R. Brun, *Acta Trop*. **1997**, *68*, 139-147.