Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Diaryl sulfide-based inhibitors of trypanothione reductase: inhibition potency, revised binding mode and antiprotozoal activities

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

Electronic Supplementary Information (ESI)

Table of contents

Figures referred to in text

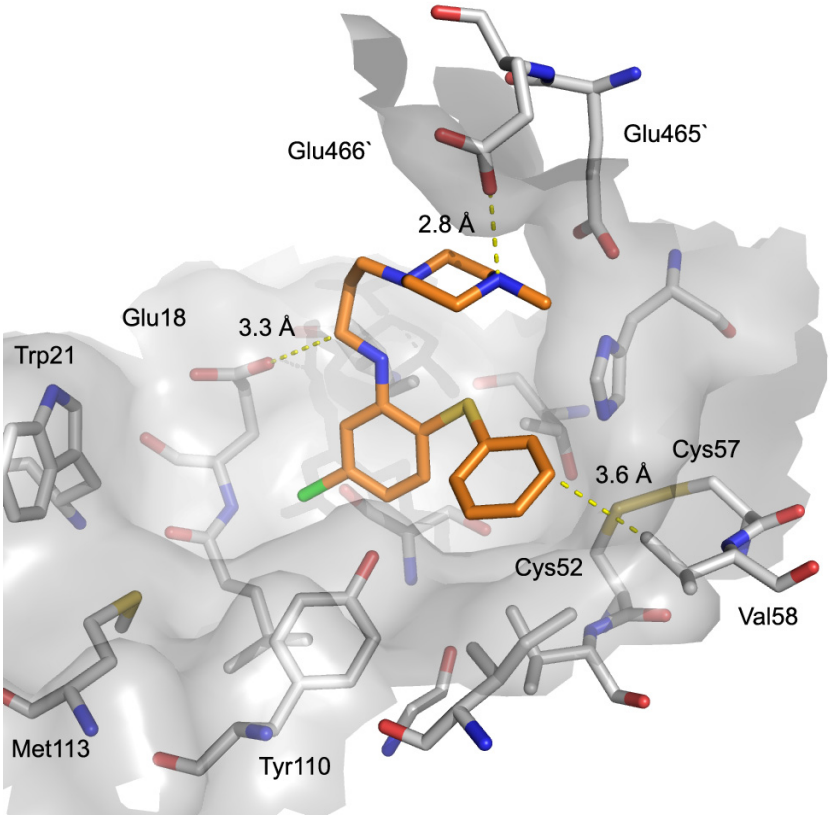

Figure 1 ESI. Model for the binding of diphenyl sulfide **3** to the TR active site as proposed by *Sergheraert et al.*[1]

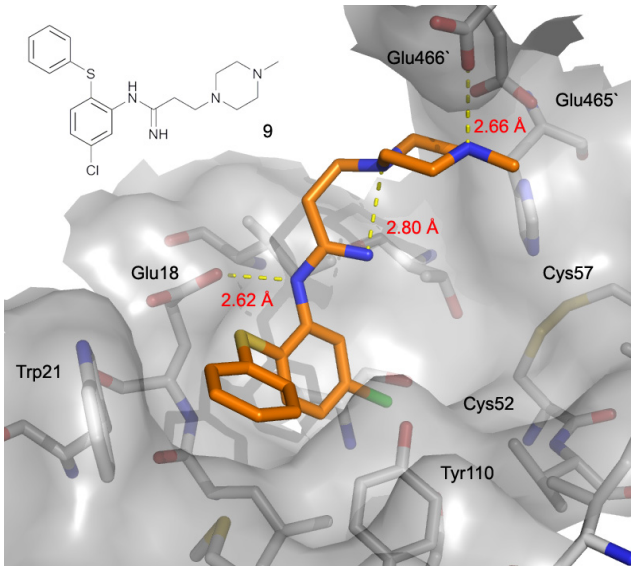

Figure 2a ESI: Model for the binding of amidine **9** in the TR active site. Two intermolecular interactions, namely with Glu 18 and Glu465'/Glu466', and one intramolecular H-bond stabilize the binding conformation.

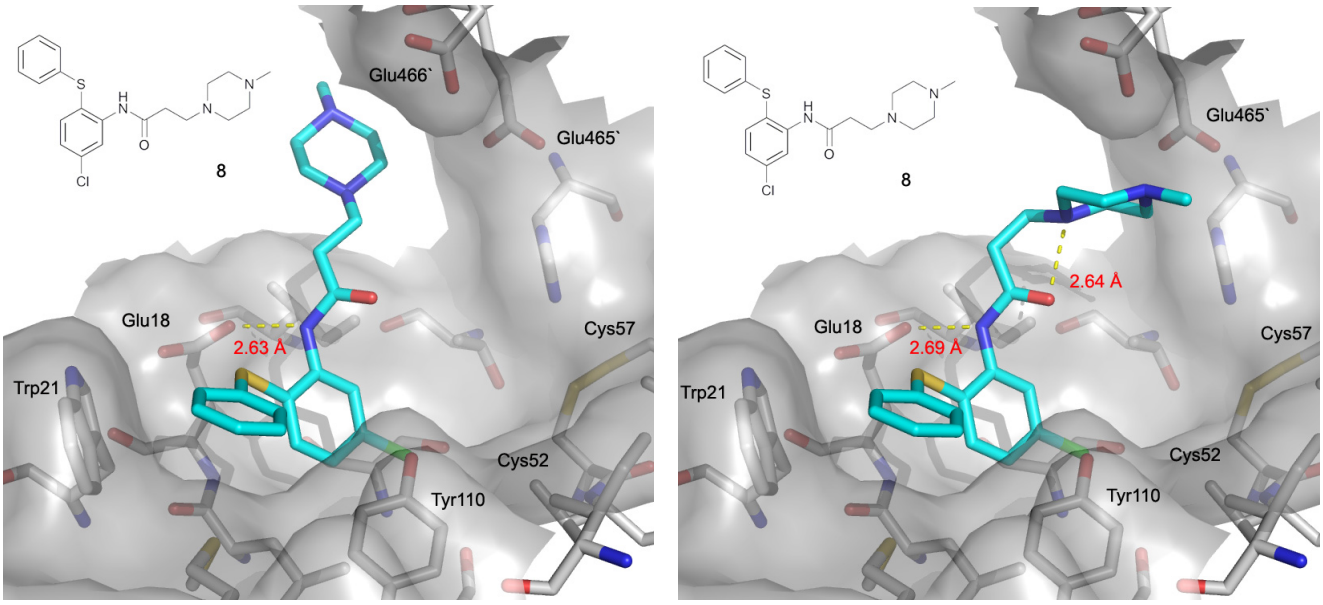

Figure 2b ESI: Two potential conformations of amide **8** docked into the TR active site. Left: **8** forms a H-bond to Glu18 but the piperazine moiety is not interacting with the enzyme. Right: **8** forms an intramolecular H-bond with the protonated piperazine nitrogen attached to the *N*-alkyl chain, leading to an unfavorable interaction be-

tween the unprotonated terminal piperazine nitrogen and Glu465'/Glu466'.

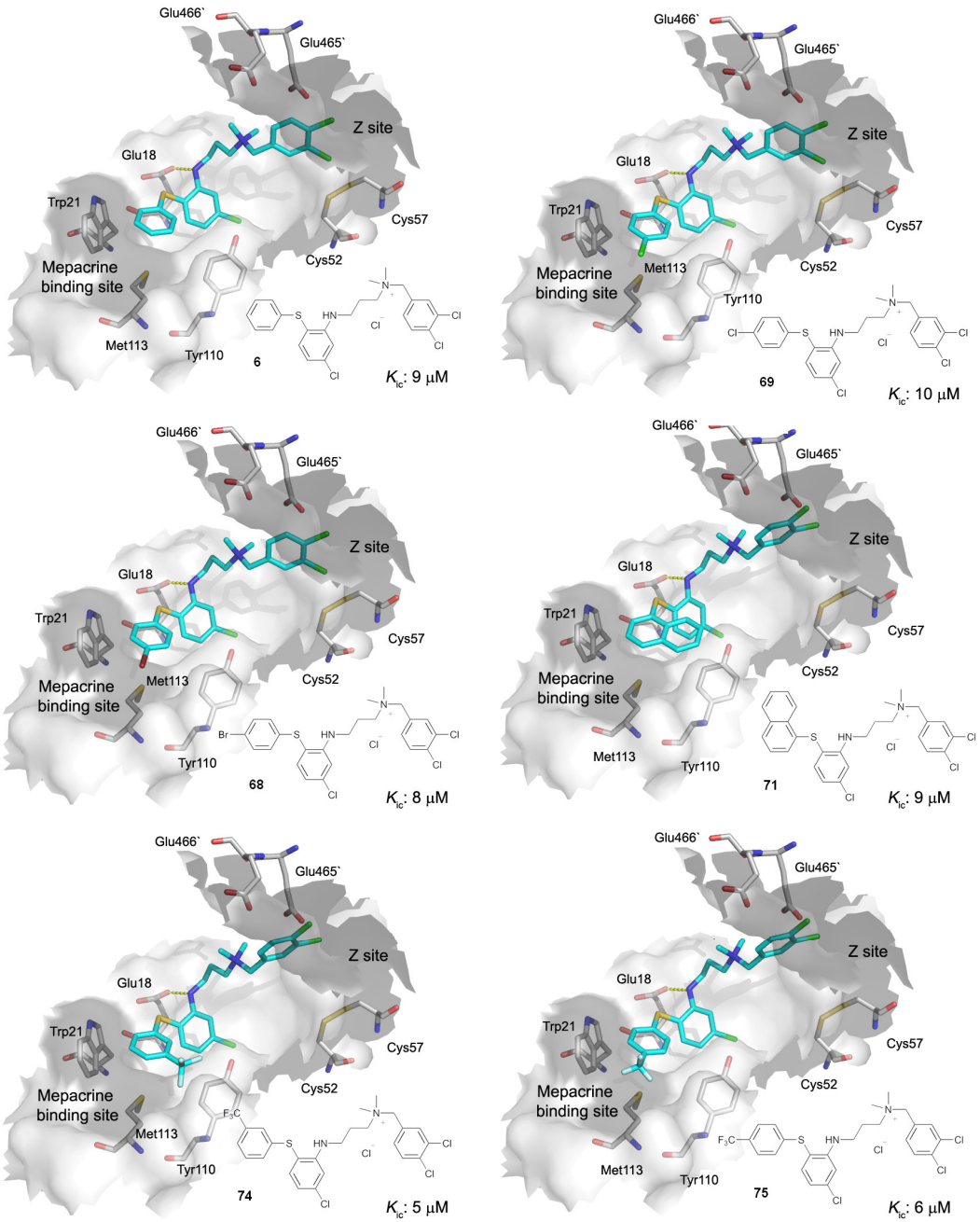

Figure 3 ESI. Models for the binding of the diphenyl sulfide inhibitors **6**, **69-71**, **74** and **75** in the TR active site. All compounds can be placed strain-free into the disulfide substrate binding site under occupation of the mepacrine binding site.

Experimental section

Biology: Trypanothione reductase assay

T. cruzi TR activity was measured at 25 °C in a total volume of 1 cm^3 in the presence of 100 µM NADPH and 5-10 mU enzyme in 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.[2]

Parasitology: *in vitro* bioassays, IC_{50} determination

Trypanosoma b. rhodesiense. Minimum Essential Medium (0.05 cm^3) supplemented according to *Baltz et al.*[3] 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 cm^{-3}. Then 10^4 bloodstream forms of *T. b. rhodesiense* STIB 900 in 0.05 cm^3 were added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. 0.01 cm^3 of Alamar Blue (12.5 mg resazurin dissolved in 100 cm^3 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.[4] 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.

Plasmodium falciparum. Antiplasmodial activity was determined using the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). A modification of the [^{3}H]-hypoxanthine incorporation assay was used.[5] Briefly, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in microtiter plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 μCi ^{3}H-hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a BetaplateTM liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. From the sigmoidal inhibition curves IC_{50} values were calculated. Assays were run in duplicate and repeated once.

Cytotoxicity using L-6 cells. L-6 cells (rat skeletal myoblasts) were used to assess cytotoxicity. The cells were grown in RPMI 1640 medium supplemented with 1% *L*-glutamine (200 mM) 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 0.1 cm^3 of culture medium with $4 \bullet 10^4$ 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 cm^{-3}. Each ligand 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, 0.01 cm^3 of Alamar blue (12.5 mg resazurin dissolved in 100 cm^3 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).

Modelling of inhibitors using MOLOC

Potential inhibitors were manually docked within the known structure of *T.* cruzi TR, cocrystallized in complex with trypanothione (pdb code: 1BZL[6]). The enzyme structure without trypanothione 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.[7] 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.

Chemistry: Synthetic procedures

General methods

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 characterisation. TLC: Aluminium sheets coated with SiO_2-60 UV_{254} from *Macherey-Nagel*, visualisation 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 cm^3) in H_2O (150 cm^3), or a solution of ninhydrin (0.3 g) in butanol (100 cm^3) and glacial acetic acid (3 cm^3). 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 cm^3 min^{-1} with UV detection at λ = 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 cm^3 min^{-1} with UV detection at λ = 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*, *Bruker ARX-300, AV-400 and DRX-400*; 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), *Varian's IonSpec FT-ICR* (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*)

The synthesis of compounds **3**, **7**, **26**, **45** and **61** has been described by *Sergheraert et al.*,[1] the preparation of cation **6** has been reported by *Douglas et al.*.[8] The derivatives **19**, **27**, **28**, **34**, **38**, **44**, **46**, **47**, **53**, **62**, **63**, **68**, **69**, **70**, **75** and **79-82** were synthesized as reported earlier.[9]

General procedure 1 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 2 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 3 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 4 for the microwave-assisted introduction of *N*-methylpiperazine

A suspension of the chloride (1 eq.), K_2CO_3 (2 eq.), NaI (0.5 eq.) and *N*-methylpiperazine (1.5 eq.) in DMF (2-3 cm^3) was stirred in a sealed microwave tube for 5 min at 80 °C, 60 min at 120 °C and 5 min at 110 °C.[10] After evaporation

of the solvent, the obtained residue was dissolved in a solvent mixture (CH_2Cl_2/*i*-PrOH 67:33) and washed (saturated aqueous $NaHCO_3$ solution). The aqueous layers were extracted (CH_2Cl_2/*i*-PrOH 67:33), the organic layers dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by column chromatography (CC) (SiO_2; CH_2Cl_2-MeOH 97:3) yielded the desired amines.

General procedure 5 for the alkylation of dimethylamine:

The appropriate chloride (1 eq.) was dissolved in DMF. $HNMe_2$ (40% solution in H_2O, ca. 50 eq.) was added and the mixture 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.

5-Chloro-2-(phenylthio)phenol (12)

A solution of $NaNO_2$ (100 mg, 1.45 mmol) in H_2O (1 cm^3) was added dropwise to a slurry of aniline **7** (240 mg, 1.02 mmol) in sulfuric acid (4 cm^3) and H_2O (4 cm^3) at 0 °C and the mixture was stirred for 2 h at 0 °C before $NaBF_4$ (291 mg, 2.65 mmol) was added. The reaction was stirred for 2 h at 0 °C, the precipitated crude diazonium fluoro-borate was

separated by filtration and suspended in a mixture of sulfuric acid (6 cm^3) and H_2O (5 cm^3). The reaction was left to stir for 12 h at 100 °C, cooled to 25 °C, diluted (Et_2O), washed (saturated aqueous $NaHCO_3$ and NaCl solution, H_2O), dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by CC (SiO_2; heptane-EtOAc 100:0→90:10) yielded the desired phenol **12** (136 mg, 56%) as an orange oil; ν_{max}(neat)/cm^{-1} 3413, 1588, 1562, 1471, 1305, 1189, 1081, 1023, 902, 802, 736, 688; δ_H(300 MHz, $CDCl_3$) 6.95 (br s, 1 H), 6.96 (dd, *J* = 7.8, 2.1, 1 H), 7.08-7.12 (m, 3 H), 7.18-7.28 (m, 3 H), 7.46 (d, *J* = 8.1, 1 H); δ_C(75 MHz, $CDCl_3$) 115.0, 115.8, 116.0, 121.5, 126.3, 126.8, 129.2, 135.0, 137.5, 157.5; EI-HR-MS: calcd for $C_{12}H_9ClOS^+$ (M^+): 236.0057; found: 236.0058.

(4-Chloro-2-(3-chloropropoxy)-1-(phenylthio)benzene (13)

1-bromo-3-chloropropane (0.042 cm^3, 0.42 mmol) was added to a solution of phenol **12** (50 mg, 0.21 mmol) and K_2CO_3 (64 mg, 0.46 mmol) in acetone (1 cm^3) and the reaction was left to stir for 5 h at 60 °C. Dilution (Et_2O), washing (saturated aqueous NaCl solution), extraction of the aqueous layers (Et_2O), drying of the combined organic layers ($MgSO_4$), filtration and concentration in vacuo, followed by purifica-

tion by CC (SiO_2; heptane-EtOAc 100:0→85:15) delivered ether **13** (37 mg, 54%) as a red oil; ν_{max}(neat)/cm^{-1} 2925, 1727, 1575, 1463, 1384, 1246, 1024, 959, 879, 740, 690; δ_H(300 MHz, $CDCl_3$) 2.14 (quint, J = 6.0, 2 H), 3.51 (t, J = 6.3, 2 H), 4.11 (t, J = 5.7, 2 H), 6.86-6.89 (m, 2 H), 7.10 (d, J = 8.4, 1 H), 7.27-7.31 (m, 5 H); δ_C(100 MHz, $CDCl_3$) 31.0, 40.2, 64.2, 111.7, 120.4, 121.8, 126.0, 128.2, 129.7, 131.9, 133.2, 133.6, 156.1; EI-HR-MS: calcd for $C_{15}H_{14}Cl_2OS^+$ (M^+): 312.0137; found: 312.0139.

4-Chloro-2-iodo-1-(phenylthio)benzene (15)

A solution of $NaNO_2$ (351 mg, 5.09 mmol) in H_2O (5 cm^3) was added dropwise to a slurry of aniline **7** (1.00 g, 4.24 mmol) in conc. HCl (12 cm^3) at -10 °C and the mixture was stirred 90 min at -10 °C. A solution of KI (1.41 mg, 8.48 mmol) in H_2O (5 cm^3) was added dropwise, and the reaction mixture was stirred for 30 min at -10 °C and for 2 h at 25 °C. The mixture was diluted with H_2O and extracted with Et_2O. The combined organic phases were washed (5% aqueous $NaHSO_3$ solution, H_2O and aqueous NaCl solution), dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by CC (SiO_2; heptane-EtOAc 100:0→98:2) yielded the desired aryl iodide **15** (977 mg, 66%) as a colourless oil; (Found:

C 41.9, H 2.5. calcd for $C_{12}H_8ClIS$: C 41.6, H 2.3%); ν_{max}(neat)/cm^{-1} 1560, 1540, 1474, 1439, 1358, 1106, 1097, 1014, 870, 748, 689; δ_H(300 MHz, $CDCl_3$) 6.85 (d, J = 8.4, 1 H), 7.16 (dd, J = 8.4, 2.1, 1 H), 7.37-7.43 (m, 5 H), 7.83 (d, J = 2.7, 1 H); δ_C(100 MHz, $CDCl_3$) 99.2, 128.6, 128.9, 129.8, 130.0, 132.3, 133.1, 133.7, 138.8, 141.2; EI-HR-MS: calcd for $C_{12}H_8ClIS^+$ (M^+): 345.9074; found: 345.9070.

1-(4-(5-Chloro-2-(phenylthio)phenyl)but-3-ynyl)-4-methylpiperazine (16)

Iodide **15** (0.11 g, 0.27 mmol) and Et_3N (200 mg, 0.56 mmol) were dissolved in THF (5 cm^3) and degassed in an ultrasonic bath under argon. 1-(But-3-ynyl)-4-methylpiperazine (106 mg, 0.70 mmol) and a mixture of $[PdCl_2(PPh_3)_2]$ (30 mg, 26 µmol) and CuI in degassed THF (5 cm^3) were added and the mixture was left to stir for 1 d at 60 °C. Dilution (EtOAc), washing (saturated aqueous $NaHCO_3$ solution), extraction of the aqueous layers (CH_2Cl_2), drying of the combined organic layers ($MgSO_4$), filtration and concentration in vacuo, followed by purification by CC (SiO_2; CH_2Cl_2-MeOH 99:1→90:10) delivered alkyne **16** (76 mg, 36%) as a colourless oil; ν_{max}(neat)/cm^{-1} 2794, 2227, 1575, 1449, 1385,

1165, 1141, 1098, 1010, 880, 751, 691; δ_H(300 MHz, $CDCl_3$) 2.29 (s, 3 H), 2.42-2.45 (m, 4 H), 2.45-2.62 (m, 4 H), 2.63-2.65 (m, 4 H), 6.86 (d, J = 8.4, 1 H), 7.07 (dd, J = 8.4, 2.4, 1 H), 7.32-7.42 (m, 6 H); δ_C(100 MHz, $CDCl_3$) 18.1, 46.1, 52.9, 55.1, 56.9, 78.3, 96.4, 124.8, 128.1, 128.4, 129.5, 129.8, 131.6, 132.2, 132.9, 133.3, 138.3; MALDI-HR-MS: calcd for $C_{21}H_{24}ClN_2S^+$ ($[M+H]^+$): 371.1343; found: 371.1340.

_N_-{5-Chlor-2-[(4-bromophenyl)thio]phenyl}-3-(4-methylpiperazin-1-yl)propanamide (17)

4-Bromothiophenol (**18**, 2.28 g, 11.46 mmol) was added portionwise to a suspension of Na (30-35% dispersion in paraffin wax, 0.97 g, 12.60 mmol) in MeOH (200 cm^3). The mixture was heated to 65 °C when 2,5-dichloronitrobenzene (2.20 g, 11.46 mmol) was added. The mixture was left to stirr at 60 °C for 6 h before it was cooled to 0 °C and NH_4Cl (6.13 g, 114.57 mmol), followed by Zn powder (7.49 g, 114.57 mmol), was carefully added and the mixture left to stir for at 65 °C for 4 h. Filtration over celite, concentration in vacuo, dissolving (AcOEt), washing (saturated aqueous $NaHCO_3$ solution), drying ($MgSO_4$), filtration and concentration under vacuo delivered a residue that was redissolved in THF (150 cm^3). 3-Chloropropionyl chloride (1.31 cm^3, 1.75 mmol)

and K_2CO_3 (4.75 g, 34.37 mmol) was added and the reaction was left to stir overnight before *N*-methylpiperazine (6.35 cm^3, 57.28 mmol) was added and the mixture was stirred for another 5 h at 50 °C. The solvent was removed under reduced pressure, the residue dissolved (AcOEt), washed (saturated aqueous $NaHCO_3$ and NaCl solutions) dried ($MgSO_4$) and concentrated under vacuo. The amide **17** (4.69 g, 87%) was obtained after flash chromatography (SiO_2, CH_2Cl_2/MeOH 95:5) as a white solid; (Found: C 51.49, H 5.06, N 9.08. calcd for $C_{20}H_{23}BrClN_3OS$: C 51.24, H 4.94, N 8.96%); mp: 85 °C. IR (neat): ν_{max}(neat)/cm^{-1} = 3114, 2934, 2817, 2795, 1681, 1566, 1507, 1417, 1444, 1397, 1282, 1252, 1236, 1215, 1161, 1131, 1083, 1052, 1006 cm^{-1}; δ_H(300 MHz, $CDCl_3$) 2.22 (s, 3 H), 2.31-2.57 (m, 12 H), 6.88-6.92 (m, 2 H), 7.07 (dd, *J* = 8.4, 2.4, 1 H), 7.34-7.38 (m, 3 H), 8.43 (d, *J* = 2.1, 1 H), 10.54 (br s, 1 H); δ_C(100 MHz, $CDCl_3$) 29.8, 33.3, 46.9, 52.8, 53.7, 54.5, 119.4, 119.8, 122.9, 124.9, 128.6, 132.2, 135.1, 136.3, 136.4, 141.2, 170.9; MALDI-HR-MS: calcd for $C_{20}H_{23}BrClN_3OS^+$ ($[M+H]^+$): 468.0507; found: 468.0499.

2-[(4-Bromophenyl)thio]-5-chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline (20)

General procedure 4, starting from chloride **47** (0.11 g, 0.28 mmol), K_2CO_3 (77 mg, 0.56 mmol), NaI (21 mg, 0.14 mmol) and *N*-methylpiperazine (0.047 cm^3, 0.42 mmol) in DMF (2 cm^3) delivered amine **20** (0.11 g, 82%) as pale yellow oil; ν_{max}(neat)/cm^{-1} 3388, 3246, 2936, 2794, 2683, 1586, 1501, 1471, 1420, 1282, 1082, 1040, 1006, 897, 809, 789, 726; δ_H(300 MHz, $CDCl_3$) 1.70 (quint, J = 6.6, 2 H), 2.27 (s, 3 H), 2.24-2.60 (m, 10 H), 3.15 (q, J = 6.0, 2 H), 5.34 (br s, 1 H), 6.61-6.66 (m, 2 H), 6.88 (d, J = 8.7, 2 H), 7.29-7.35 (m, 3 H); δ_C(75 MHz, $CDCl_3$) 25.8, 42.2 46.1, 53.4, 55.0, 56.3, 110.4, 111.4, 116.5, 119.0, 127.7, 131.9, 135.8, 137.7, 138.3, 150.3; MALDI-HR-MS: calcd for $C_{20}H_{26}BrClN_3S^+$ ($[M+H]^+$): 454.0714; found: 454.0707.

4-Chloro-2-nitro-1-(1-naphthylthio)benzene (29)

General procedure 1, starting from naphthalene-1-thiol (**23**, 0.67 g, 4.17 mmol), 2,5-dichloronitrobenzene (0.80 g, 4.17 mmol) and Na (30-35% dispersion in paraffin wax, 0.29 g, 4.17 mmol) in methanol (10 cm^3), left to stir for 5 h under reflux and purification by CC (SiO_2; hexane-EtOAc 100:0→0:100) delivered diaryl sulfide **29** (1.00 g, 80%) as yellow solid; (Found: C 60.6, H 3.1, N 4.4. calcd for $C_{16}H_{10}ClNO_2S$: C 60.9, H 3.2, N 4.4%); mp: 93 °C;

ν_{max}(neat)/cm^{-1} 3090, 3075, 1918, 1519, 1549, 1514, 1450, 1329, 1272, 1159, 1093, 1051, 885, 823, 792, 767, 667; δ_H($CDCl_3$, 300 MHz) 6.47 (d, *J* = 8.7, 1 H), 7.10 (dd, *J* = 8.7, 2.1, 1 H), 7.48-7.60 (m, 3 H), 7.92-7.97 (m, 2 H), 8.05 (d, *J* = 8.4, 1 H), 8.18 (d, *J* = 8.1, 1 H), 8.28 (d, *J* = 2.1, 1 H); δ_C($CDCl_3$, 75 MHz) 125.3, 125.6, 126.2, 127.0, 127.9, 128.9, 129.2, 130.5, 131.9, 133.5, 134.2, 134.4, 136.4, 137.7, 144.7 (1 signal not visible); EI-HR-MS: calcd for $C_{16}H_{10}ClNO_2S^+$ (M^+): 315.0115; found: 315.0114.

4-Chloro-2-nitro-1-(2-naphthylthio)benzene (30)

General procedure 1, starting from naphthalene-2-thiol (**24**, 3.0 g, 18.7 mmol), 2,5-dichloronitrobenzene (3.60 g, 18.7 mmol) and Na (30-35% dispersion in paraffin wax, 1.44 g, 18.72 mmol) in methanol (10 cm^3), left to stir for 3 h under reflux and purification by CC (SiO_2; hexane-EtOAc 100:0→0:100) delivered diaryl sulfide **30** (5.45 g, 92%) as yellow solid; mp: 85 °C; ν_{max}(neat)/cm^{-1} 3093, 3058, 1548, 1511, 1333, 1270, 1093, 884, 813, 744; δ_H(300 MHz, $CDCl_3$) 6.82 (d, *J* = 8.6, 1 H), 7.56-7.65 (m, 2 H), 7.51 (dd, *J* = 8.6, 3.0, 1 H), 7.56-7.65 (m, 2 H), 7.85-7.95 (m, 2 H), 8.17 (d, *J* = 1.2, 1 H), 8.24 (d, *J* = 2.1, 1 H); δ_C(75 MHz, $CDCl_3$) 125.4, 127.0, 127.5, 127.7, 127.8, 127.9, 129.6,

130.0, 130.7, 131.3, 133.4, 133.5, 133.8, 136.0, 138.0, 138.1; EI-HR-MS: calcd for $C_{16}H_{11}ClNO_2S^+$ (M^+): 315.0116; found: 315.0117.

2-[(4-Chloro-2-nitrophenyl)thio]pyridine (31)

General procedure 1, starting from 2-mercaptopyridine (**25**, 2.0 g, 18.0 mmol), 2,5-dichloronitrobenzene (3.45 g, 18.0 mmol) and Na (30-35% dispersion in paraffin wax, 1.09 g, 15.6 mmol) in methanol (10 cm^3), left to stir for 7 h under reflux and purification by CC (SiO_2; hexane-EtOAc 100:0→0:100) delivered diaryl sulfide **31** (3.42 g, 71%) as yellow solid; (Found: C 49.4, H 2.7, N 10.6. calcd for $C_{11}H_7ClN_2O_2S^+$: C 49.5, H 2.7, N 10.5%); mp: 82 °C; ν_{max}(neat)/cm^{-1} 3027, 1769, 1572, 1548, 1515, 1451, 1418, 1332, 1282, 1096, 1044, 880, 758, 703; δ_H($CDCl_3$, 300 MHz) 7.24-7.29 (m, 2 H), 7.41 (dd, *J* = 7.8, 2.3, 1 H), 7.50 (d, *J* = 7.8, 1 H), 7.71 (dd, *J* = 7.2, 2.1, 1 H), 8.13 (d, *J* = 2.3, 1 H), 8.57 (ddd, *J* = 5.0, 1.8, 0.6, 1 H); δ_C(75 MHz, $CDCl_3$) 122.9, 125.3, 127.3, 131.6, 132.7, 132.9, 133.1, 137.6, 150.7, 154.9 (one signal not visible); EI-HR-MS: calcd for $C_{11}H_7ClN_2O_2S^+$ (M^+): 265.9912; found: 265.9913.

4-Chloro-2-nitro-1-{[2-(trifluoromethyl)phenyl]thio}benzene (32)

To a solution of 2-(trifluoromethyl)bromobenzene (**35**, 1.50 cm^3, 11.0 mmol) and *tert*-BuLi (1.7 M in pentane, 16.9 cm^3, 22.0 mmol) in THF (20 cm^3), stirred for 10 min at -78 °C, sulfur (0.35 g, 11.0 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 (2.11 g, 11.0 mmol) was added and the reaction stirred for 3 h. Dilution (saturated aqueous $NaHCO_3$ solution), extraction (EtOAc), washing (saturated aqueous NaCl solution), extraction of the aqueous phases (CH_2Cl_2), drying of the combined organic phases ($MgSO_4$), filtration, concentration in vacuo, followed by purification by CC (SiO_2; hexane-CH_2Cl_2 90:10) delivered diphenyl sulfide **32** (2.60 g, 71%) as a red solid; (Found: C 46.7, H 2.0, N 4.1. calcd for $C_{13}H_7ClF_3NO_2S$: C 46.8, H 2.1, N 4.2%); mp: 89 °C; ν_{max}(neat)/cm^{-1} 3102, 3028, 1520, 1312, 1175, 1111, 1032, 825, 768, 644; δ_H(300 MHz, $CDCl_3$) 6.57 (d, *J* = 8.7, 1 H), 7.30 (dd, *J* = 8.4, 2.3, 1 H), 7.64-7.70 (m, 3 H), 7.90 (dd, *J* = 5.4, 3.6, 1 H), 8.24 (d, *J* = 2.3, 1 H); δ_C(75 MHz, $CDCl_3$) 123.2 (q, *J* = 272.4), 125.5, 127.9 (q, *J* = 5.4), 129.7, 129.8, 130.8, 131.2, 133.4, 133.7, 134.7 (q, *J* = 30.2), 137.2, 139.6, 145.2; δ_F(282

MHz, $CDCl_3$): –60.7 (s, 3 F); EI-HR-MS: calcd for $C_{13}H_7ClF_3NO_2S^+$ (M^+): 332.9833; found: 332.9831.

4-Chloro-2-nitro-1-{[3-(trifluoromethyl)phenyl]thio}benzene (33)

3-(Trifluoromethyl)bromobenzene (**36**, 1.00 cm^3, 7.16 mmol) was dissolved in THF (20 cm^3), cooled to at -78 °C, and *tert*-BuLi (1.3 M in pentane, 11.0 cm^3, 14.3 mmol) was added to this solution which then was stirred for 20 min at -78 °C. Sulfur (0.30 g, 7.16 mmol) was added and the mixture left to stir for 30 min while it was allowed to warm to 25 °C. After addition of 2,5-dichloronitrobenzene (1.37 g, 7.16 mmol), the mixture was stirred for 1.5 h. Dilution (saturated aqueous $NaHCO_3$ solution), extraction (EtOAc), washing (saturated aqueous NaCl solution), extraction of the aqueous phases (CH_2Cl_2), drying of the combined organic phases ($MgSO_4$), filtration and concentration in vacuo, followed by purification by CC (hexane-CH_2Cl_2 90:10), delivered diphenyl sulfide **33** (1.44 g, 60%) as a red solid; (Found: C 46.8, H 2.2, N 4.4. calcd for $C_{13}H_7ClF_3NO_2S$: C 46.8, H 2.1, N 4.2%); mp: 66 °C; ν_{max}(neat)/cm^{-1} 3101, 3026, 1552, 1516, 1451, 1321, 1305, 1169, 1121, 1106, 1072, 1049, 888, 800, 768, 704; δ_H(300 MHz, $CDCl_3$) 6.77 (d, J = 8.7, 1 H), 7.35 (dd, J = 8.7, 2.4, 1 H), 7.60-7.65 (m, 1 H),

7.75-7.78 (m, 2 H), 7.84 (s, 1 H), 8.24 (d, J = 2.4); δ_C(75 MHz, $CDCl_3$) 123.39 (q, J = 271.2), 125.7, 127.0 (q, J = 3.5), 129.6, 130.8, 131.6, 132.2 (q, J = 3.6), 132.4, 132.8 (q, J = 32.7), 133.8, 136.4, 138.9, 145.5; δ_F(282 MHz, $CDCl_3$) –62.6 (s, 3 F); MALDI-HR-MS: calcd for $C_{13}H_7ClF_3NO_2S^+$ ($[M+H]^+$): 332.9833; found: 332.9834.

5-Chloro-2-(1-naphthylthio)aniline (39)

General procedure 2, starting from nitrobenzene **29** (0.90 g, 2.85 mmol), NH_4Cl (3.05 g, 57.0 mmol), Zn (3.73 g, 57.0 mmol) in MeOH (50 cm^3). The mixture was left to stir under reflux for 5 h to yield aniline **39** (0.80 g, 98%) as orange solid; (Found: C 67.0, H 4.4, N 4.9. calcd for $C_{16}H_{12}ClNS$: C 67.2, H 4.2, N 4.9%); mp: 106 °C; ν_{max}(neat)/cm^{-1} 3464, 3367, 3051, 2922, 2851, 1603, 1553, 1475, 1416, 1380, 1258, 1131, 1078, 968, 909, 850, 789, 661; δ_H($CDCl_3$, 300 MHz) 4.35 (br s, 2 H), 6.75 (dd, J = 8.1, 2.1, 1 H), 6.82 (d, J = 2.1, 1 H), 6.97 (dd, J = 7.5, 1.2, 1 H), 7.30 (d, J = 7.5, 1 H), 7.37 (d, J = 8.1, 1 H), 7.50-7.60 (m, 2 H), 7.67 (d, J = 8.1, 1 H), 7.84-7.88 (m, 1 H), 8.30-8.33 (m, 1 H); δ_C($CDCl_3$, 75 MHz) 112.5, 114.9, 118.9, 123.8, 123.8, 123.8, 125.8, 126.2, 126.3, 128.6, 131.0, 132.9, 133.8, 136.6,

138.1, 149.3; EI-HR-MS: calcd for $C_{16}H_{12}ClNS^+$ (M^+): 285.0374; found: 285.0375.

5-Chloro-2-(2-naphthylthio)aniline (40)

General procedure 2, starting from nitrobenzene **30** (1.00 g, 3.17 mmol), NH_4Cl (3.39 g, 63.3 mmol), Zn (4.14 g, 63.3 mmol) in MeOH (100 cm^3). The mixture was left to stir under reflux for 2 h to yield aniline **40** (0.91 g, 99%); mp: 88 °C; ν_{max}(neat)/cm^{-1} 3466, 3369, 3048, 1600, 1474, 1416, 1250, 1089, 1059, 905, 843, 814, 790, 746; δ_H(300 MHz, $CDCl_3$) 4.38 (br s, 2 H), 6.76 (dd, *J* = 8.4, 2.2, 1 H), 6.81 (d, *J* = 2.2, 1 H), 7.22 (dd, *J* = 7.4, 2.0, 1 H), 7.38-7.47 (m, 4 H), 7.65-7.78 (m, 3 H); δ_C(75 MHz, $CDCl_3$) 112.7, 114.9, 118.7, 124.4, 124.9, 125.5, 126.5, 126.9, 127.6, 128.7, 131.5, 133.4, 133.6, 136.8, 138.2, 149.5; EI-HR-MS: calcd for $C_{16}H_{12}ClNS^+$ (M^+): 285.0374; found: 285.0375.

5-Chloro-2-(pyridin-2-ylthio)aniline (41)

General procedure 2, starting from nitrobenzene **31** (1.00 g, 3.75 mmol), NH_4Cl (4.01 g, 75.0 mmol), Zn (4.90 g, 75.0 mmol) in MeOH (100 cm^3). The mixture was left to stir under reflux for 2 h to yield aniline **41** (0.87 g, 98%); mp: 77 °C; ν_{max}(neat)/cm^{-1} 3441, 3334, 3026, 2915, 1624, 1572,

1555, 1478, 1447, 1417, 1260, 1129, 909, 788, 753; δ_H(300 MHz, $CDCl_3$) 4.35 (br s, 2 H), 6.72-6.81 (m, 3 H), 7.00 (ddd, *J* = 7.5, 4.8, 1.0, 1 H), 7.38-7.48 (m, 2 H), 8.41 (ddd, *J* = 4.8, 1.8, 0.8, 1 H); δ_C(75 MHz, $CDCl_3$) 110.9, 114.9, 118.7, 120.9, 119.9, 136.8, 137.4, 138.6, 149.5, 149.9, 159.6; EI-HR-MS: calcd for $C_{11}H_8ClN_2S^+$ (M^+): 235.0092; found: 235.0092.

5-Chloro-2-{[2-(trifluoromethyl)phenyl]thio}aniline (42)

General procedure 2, starting from nitrobenzene **32** (2.08 g, 6.23 mmol), NH_4Cl (6.66 g, 0.12 mol), Zn (8.14 g, 0.12 mol) in MeOH (50 cm^3). The mixture was left to stir under reflux for 4 h to yield aniline **42** (1.76 g, 93%); (Found: C 51.3, H 3.0, N 4.5. calcd for $C_{13}H_9ClF_3NS$: 51.4, H 3.0, N 4.6%); mp: 61 °C; ν_{max}(neat)/cm^{-1} 3480, 3379, 3027, 1606, 1476, 1310, 1258, 1174, 1112, 1030, 759, 703; δ_H(300 MHz, $CDCl_3$) 4.38 (br s, 2 H), 6.76 (dd, *J* = 8.1, 2.1, 1 H), 6.80 (d, *J* = 2.1, 1 H), 6.90 (d, *J* = 7.5, 1 H), 7.21 (t, *J* = 7.5, 1 H), 7.31 (t, *J* = 7.5, 1 H), 7.40 (d, *J* = 8.1, 1 H), 7.64 (d, *J* = 7.5, 1 H); δ_C(75 MHz, $CDCl_3$) 111.1, 114.9, 118.7, 123.9 (q, *J* = 271.1), 125.2, 126.6 (q, *J* = 5.6), 127.3 (q, *J* = 30.5), 127.6, 132.0, 136.0, 137.5, 138.9, 149.7; δ_F(282

MHz, $CDCl_3$) –61.2 (s, 3 F); EI-HR-MS: calcd for $C_{13}H_9ClF_3NS^+$ (M^+): 303.0091; found: 303.0091.

5-Chloro-2-{[3-(trifluoromethyl)phenyl]thio}aniline (43)

General procedure 2, starting from nitrobenzene **33** (0.97 g, 2.91), NH_4Cl (3.11 g, 58.1 mmol), Zn (2.85 g, 43.6 mmol) in MeOH (30 cm^3). The mixture was left to stir for 3 h at 50 °C to yield aniline **43** (0.40 g, 45%) as red solid after purification by CC (SiO_2; hexane-CH_2Cl_2 80:20→60:40); (Found: C 51.6, H 2.9, N 4.8. calcd for $C_{13}H_9ClF_3NS$: C 51.4, H 3.0, N 4.6%); mp: 45 °C; ν_{max}(neat)/cm^{-1} 3482, 3379, 3027, 2917, 1604, 1582, 1476, 1418, 1320, 1165, 1120, 1071, 907, 791, 699; δ_H(300 MHz, MeOD) 4.38 (br s, 2 H), 6.75 (dd, *J* = 8.1, 2.1, 1 H), 6.82 (d, *J* = 2.1, 1 H), 7.13-7.17 (m, 1 H), 7.30-7.39 (m, 4 H); δ_C(75 MHz, $CDCl_3$) 111.2, 115.1, 122.2 (q, *J* = 3.7), 123.0 (q, *J* = 3.8), 123.8 (q, *J* = 272.3), 129.2, 129.5, 131.5 (q, *J* = 32.2), 137.6, 138.1, 138.7, 149.8; δ_F(282 MHz, $CDCl_3$) –62.7 (s, 3 F, CF_3); EI-HR-MS: calcd for $C_{13}H_9ClF_3NS^+$ (M^+): 303.0091; found: 303.0090.

2-(1-Naphthylthio)-5-chloro-*N*-(3-chloropropyl)aniline (48)

General procedure 3, starting from aniline **39** (0.70 g, 2.45 mmol), pyridine (0.99 cm^3, 1.23 mmol), 3-

chloropropionyl chloride (0.28 cm^3, 2.94 mmol) and $BH_3 \cdot THF$ (1 M in THF, 15.0 cm^3, 15.0 mmol) in THF (10 cm^3). The mixture was left to stir for 4 h for the first step, then for 4 h. Purification by CC (SiO_2; hexane-EtOAc 100:0→90:10) delivered chloride **48** (0.85 g, 95%) as a pale red oil; ν_{max}(neat)/cm^{-1} 3389, 2964, 1559, 1475, 1419, 1273, 1094, 968, 906, 789, 731, 661, 633; δ_H($CDCl_3$, 300 MHz) 1.90 (quint, J = 6.3, 2 H), 3.25-3.36 (m, 4 H), 4.93 (br s, 1 H), 6.70-6.73 (m, 2 H), 6.91 (dd, J = 7.5, 1.2, 1 H), 7.28 (d, J = 7.8, 1 H), 7.43 (d, J = 8.4, 1 H), 7.51-7.62 (m, 2 H), 7.66 (d, J = 8.4, 1 H), 7.87 (d, J = 7.5, 1 H), 8.32 (d, J = 8.7, 1 H); δ_C($CDCl_3$, 75 MHz) 31.4, 40.2, 42.1, 110.4, 112.3, 117.2, 123.8, 125.8, 126.4, 127.7, 128.7, 129.2, 131.2, 132.9, 133.9, 137.5, 138.4, 138.6, 149.7; EI-HR-MS: calcd for $C_{19}H_{17}Cl_2NS^+$ (M^+): 361.0453; found: 361.0453.

2-(2-Naphthylthio)-5-chloro-*N*-(3-chloropropyl)aniline (49)

General procedure 3, starting from aniline **40** (0.31 g, 1.08 mmol), pyridine (0.10 cm^3, 1.30 mmol), 3-chloropropionyl chloride (0.12 cm^3, 1.30 mmol) and $BH_3 \cdot THF$ (1 M in THF, 8.63 cm^3, 8.63 mmol) in THF (10 cm^3). The mixture was left to stir for 2 h for the first step, then for

3 h. Purification by CC (SiO_2; hexane-CH_2Cl_2 80:20) delivered chloride **49** (0.33 g, 83%) as a colourless oil; ν_{max}(neat)/cm^{-1} 3379, 3052, 2957, 2865, 1580, 1500, 1418, 1271, 1095, 812, 743; δ_H(300 MHz, $CDCl_3$) 1.89-1.98 (m, 2 H), 3.27-3.38 (m, 4 H), 5.05 (br s, 1 H), 6.71-6.73 (m, 2 H), 7.17-7.21 (m, 1 H), 7.41-7.48 (m, 4 H), 7.64-7.78 (m, 3 H); δ_C(75 MHz, $CDCl_3$) 31.4, 40.2, 42.0, 110.4, 112.5, 117.0, 124.4, 124.9, 125.6, 126.7, 127.0, 127.7, 128.8, 131.7, 133.4, 133.7, 137.6, 138.5, 149.7; MALDI-HR-MS: calcd for $C_{19}H_{18}Cl_2NS^+$ ($[M+H]^+$): 362.0532; found: 362.0526.

5-Chloro-*N*-(3-chloropropyl)-2-(pyridin-2-ylthio)aniline (50)

General procedure 3, starting from aniline **41** (0.79 g, 3.34 mmol), pyridine (0.14 cm^3, 1.70 mmol), 3-chloropropionyl chloride (0.35 cm^3, 3.67 mmol) and BH_3·THF (1 M in THF, 26.70 cm^3, 26.70 mmol) in THF (10 cm^3). The mixture was left to stir for 2 h for the first step, then for 3 h. Purification by CC (SiO_2; hexane-CH_2Cl_2-EtOAc 90:10:0→50:0:50) delivered chloride **50** (0.60 g, 57%) as a colourless oil; ν_{max}(neat)/cm^{-1} 3379, 3026, 2958, 2912, 1579, 1502, 1415, 1274, 1122, 1095, 1043, 758; δ_H(300 MHz, $CDCl_3$) 1.95-2.03 (m, 2 H), 3.27-3.33 (m, 2 H), 3.47 (t, J =

6.0, 2 H), 4.95 (t, *J* = 5.8, 1 H), 6.68-6.79 (m, 3 H), 7.15 (dd, *J* = 6.3, 6.3, 1 H), 7.39 (dd, *J* = 8.4, 0.9, 1 H), 7.59 (t, *J* = 7.8, 1 H), 8.63 (d, *J* = 5.7, 1 H); δ_C($CDCl_3$, 75 MHz) 31.3, 40.3, 42.0, 109.3, 111.0, 117.8, 120.3, 133.1, 138.4, 138.7, 139.2, 149.1, 150.0, 160.1; EI-HR-MS: calcd for $C_{14}H_{14}Cl_2N_2S^+$ (M^+): 312.0250; found: 312.0251.

5-Chloro-*N*-(3-chloropropyl)-2-{[2-(trifluoromethyl)phenyl]thio}aniline (51)

General procedure 3, starting from aniline **42** (0.79 g, 3.34 mmol), pyridine (0.13 cm^3, 1.65 mmol), 3-chloropropionyl chloride (0.38 cm^3, 3.96 mmol) and BH_3·THF (1 M in THF, 26.4 cm^3, 26.4 mmol) in THF (20 cm^3). The mixture was left to stir for 1.5 h for the first step, then for 2 h. Purification by CC (SiO_2; hexane-CH_2Cl_2 90:10) delivered chloride **51** (0.33 g, 83%) as a white solid; (Found: C 50.6, H 3.9, N 3.7. calcd for $C_{16}H_{14}Cl_2F_3NS$: C 50.5, H 3.7, N 3.7%); mp: 69-70 °C; ν_{max}(neat)/cm^{-1} 3394, 3143, 2873, 1584, 1506, 1474, 1420, 1311, 1257, 1184, 1126, 1089, 1029, 790, 766; δ_H(300 MHz, $CDCl_3$) 1.90-1.99 (m, 2 H), 3.21-3.31 (m, 2 H), 3.35 (t, *J* = 6.2, 2 H), 4.95 (br s, 1 H), 6.67 (d, *J* = 2.0, 1 H), 6.71 (dd, *J* = 8.1, 2.0, 1 H), 6.91 (d, *J* = 7.5, 1 H), 7.22 (dd, *J* = 7.5, 7.5, 1 H),

7.30 (ddd, J = 7.5, 7.5, 1.2, 1 H), 7.44 (d, J = 8.1, 1 H), 7.65 (dd, J = 7.5, 1.2, 1 H); δ_C(75 MHz, $CDCl_3$) 31.4, 40.2, 41.8, 110.7, 111.6, 117.2, 124.1 (q, J = 272.2), 125.6, 126.7 (q, J = 5.6), 127.7 (q, J = 30.5), 128.1, 132.2, 136.2, 138.3, 139.1, 149.8; δ_F(282 MHz, $CDCl_3$) –61.1 (s, 3 F); MALDI-HR-MS: calcd for $C_{16}H_{15}Cl_2F_3NS^+$ ($[M+H]^+$): 379.0171; found: 379.0168.

5-Chloro-*N*-(3-chloropropyl)-2-{[3-(trifluoromethyl)phenyl]thio}aniline (52)

General procedure 3, starting from aniline **43** (0.20 g, 0.66 mmol), pyridine (0.027 cm^3, 0.33 mmol), 3-chloropropionyl chloride (0.075 cm^3, 0.79 mmol), and BH_3·THF (1 M in THF, 5.27 cm^3, 5.27 mmol) in THF (15 cm^3). The mixture was left to stir for 3 h for the first step, then for 2 h. Purification by CC (SiO_2; hexane-CH_2Cl_2 90:10) delivered chloride **52** (0.21 g, 82%) as a colourless oil; (Found: C 50.7, H 3.8, N 3.9. calcd for $C_{16}H_{14}Cl_2F_3NS$: C 50.5, H 3.7, N 3.7%); ν_{max}(neat)/cm^{-1} 3392, 3026, 2962, 1582, 1503, 1320, 1272, 1166, 1124, 1072, 792, 703, 631; δ_H(300 MHz, $CDCl_3$) 1.94-2.02 (m, 2 H), 3.30-3.41 (m, 2 H), 3.43 (t, J = 6.3, 2 H), 4.99 (t, J = 5.6, 1 H), 6.69-6.72 (m, 2 H), 7.15 (d, J = 7.2, 1 H), 7.31-7.43 (m, 4 H); δ_C(75

MHz, $CDCl_3$) 31.6, 40.2, 42.0, 110.5, 110.9, 117.2, 122.2 (q, *J* = 3.7), 122.6 (q, *J* = 3.7), 123.6 (q, *J* = 70.8), 128.9, 129.3, 131.3 (q, *J* = 32.2), 137.8, 138.1, 138.6, 149.6; δ_F(282 MHz, $CDCl_3$) –63.2 (s, 3 F); EI-HR-MS: calcd for $C_{16}H_{14}Cl_2F_3NS^+$ (M^+): 379.0171; found: 379.0173.

2-[(4-Chlorophenyl)thio]-5-chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]aniline (54)

General procedure 4, starting from chloride **46** (0.20 g, 0.58 mmol), K_2CO_3 (0.16 g, 1.16 mmol), NaI (43 mg, 0.29 mmol) and *N*-methylpiperazine (0.096 cm^3, 0.87 mmol) in DMF (2 cm^3) delivered amine **54** (0.18 g, 76%) as pale yellow oil; ν_{max}(neat)/cm^{-1} 3386, 3248, 3053, 2936, 2876, 2794, 2682, 1585, 1501, 1474, 1282, 1163, 1089, 1011, 895, 814, 791, 740; δ_H(300 MHz, $CDCl_3$) 1.69 (quint, *J* = 6.6, 2 H), 2.15 (s, 3 H), 2.20-2.50 (m, 10 H), 3.14 (q, *J* = 6.3, 2 H), 5.35 (br s, 1 H), 6.59-6.65 (m, 2 H), 7.93 (d, *J* = 8.3, 2 H), 7.15 (d, *J* = 8.3, 2 H), 7.32 (d, *J* = 8.1, 1 H); δ_C(75 MHz, $CDCl_3$) 25.8, 42.2, 46.1, 53.3, 55.0, 56.3, 110.4, 111.6, 116.5, 127.4, 129.0, 131.2, 135.1, 137.6, 138.3, 150.2; MALDI-HR-MS: calcd for $C_{20}H_{26}Cl_2N_3S^+$ ($[M+H]^+$): 410.1219; found: 410.1212.

5-Chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]-2-(1-naphthylthio)aniline (55)

General procedure 4, starting from chloride **48** (0.20 g, 0.55 mmol), K_2CO_3 (0.15 g, 1.10 mmol), NaI (41 mg, 0.28 mmol) and *N*-methylpiperazine (0.092 cm^3, 0.83 mmol) in DMF (3 cm^3) delivered amine **55** (0.13 g, 51%) as pale yellow oil; ν_{max}(neat)/cm^{-1} 3388, 3305, 3260, 3051, 2935, 2878, 2799, 2681, 1586, 1562, 1498, 1281, 1095, 1010, 968, 895, 834, 787, 769, 662; δ_H(300 MHz, $CDCl_3$) 1.66 (quint, *J* = 6.3, 2 H), 2.15 (s, 3 H), 2.20-2.50 (m, 10 H), 3.16 (q, *J* = 6.3, 2 H), 5.39 (s, 1 H), 6.65 (dd, *J* = 8.3, 2.1, 1 H), 6.69 (d, *J* = 2.1, 1 H), 6.91 (dd, *J* = 7.5, 0.9, 1 H), 7.26-7.29 (m, 1 H), 7.36 (d, *J* = 8.3, 1 H), 7.52-7.65 (m, 3 H), 7.85 (dd, *J* = 7.5, 1.8, 1 H), 8.31 (dd, *J* = 7.8, 1.8, 1 H); δ_C(75 MHz, $CDCl_3$) 25.6, 42.3, 45.6, 52.9, 54.7, 56.2, 110.3, 111.8, 116.7, 123.5, 123.8, 125.8, 126.0, 126.2, 127.4, 128.6, 129.0, 133.3, 133.8, 137.2, 138.2, 150.3; MALDI-HR-MS: calcd for $C_{24}H_{29}ClN_3S^+$ ($[M+H]^+$): 426.1765; found: 426.1766.

5-Chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]-2-(2-naphthylthio)aniline (56)

General procedure 4, starting from chloride **49** (48 mg, 0.13 mmol), K_2CO_3 (36 mg, 0.26 mmol), NaI (10 mg, 65 µmol) and *N*-methylpiperazine (0.022 cm^3, 0.20 mmol) in DMF (3 cm^3) delivered amine **56** (42 mg, 75%) as pale yellow oil; ν_{max}(neat)/cm^{-1} 3383, 3252, 2935, 2875, 2793, 1584, 1500, 1458, 1420, 1282, 1163, 1096, 812, 631; δ_H(300 MHz, $CDCl_3$) 1.62-1.70 (m, 2 H), 2.17-2.44 (m, 13 H), 3.15 (dt, *J* = 6.3, 5.9, 2 H), 5.37 (t, *J* = 5.9, 1 H), 6.65-6.69 (m, 2 H), 7.18 (dd, *J* = 8.9, 2.1, 1 H), 7.36-7.45 (m, 4 H), 7.62-7.76 (m, 3 H); δ_C(75 MHz, $CDCl_3$) 25.6, 42.0, 45.7, 52.7, 54.7, 55.9, 110.2, 112.0, 116.4, 124.0, 124.7, 125.4, 126.5, 126.8, 127.6, 128.5, 131.4, 133.6, 133.7, 137.3, 138.2, 150.2; MALDI-HR-MS: calcd for $C_{24}H_{29}ClN_3S^+$ ($[M+H]^+$): 426.1765; found: 426.1758.

[5-Chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]-2-(pyridin-2-ylthio)aniline (57)

General procedure 4, starting from chloride **50** (32 mg, 0.10 mmol), K_2CO_3 (28 mg, 0.20 mmol), NaI (8 mg, 51 µmol) and *N*-methylpiperazine (0.017 cm^3, 0.15 mmol) in DMF (2 cm^3) delivered amine **57** (25 mg, 57%) as pale yellow oil; ν_{max}(neat)/cm^{-1} 2991, 2972 2796, 1523, 1473, 1323, 1172, 1145, 1102, 1023, 1009, 987, 882; δ_H(300 MHz, $CDCl_3$) 1.68-

1.76 (m, 2 H), 2.38 (t, *J* = 6.5, 2 H), 2.41-2.68 (m, 11 H), 3.15-3.21 (m, 2 H), 5.78 (br s, 1 H), 6.64-6.70 (m, 3 H), 7.00 (ddd, *J* = 7.5, 5.0, 0.9, 1 H), 7.39-7.46 (m, 2 H), 8.40 (ddd, *J* = 5.0, 1.8, 0.9, 1 H); δ_C(75 MHz, $CDCl_3$) 25.2, 42.9, 45.1, 52.2, 54.3, 56.5, 110.3, 110.5, 116.6, 119.5, 119.9, 136.8, 138.1, 138.8, 149.3, 150.7, 160.2; MALDI-HR-MS: calcd for $C_{19}H_{26}ClN_4S^+$ ($[M+H]^+$): 377.1561; found: 377.1558.

5-Chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]-2-{[2-(trifluoromethyl)phenyl]thio}aniline (58)

General procedure 4, starting from chloride **51** (0.22 g, 0.58 mmol), K_2CO_3 (0.16 g, 1.16 mmol), NaI (44 mg, 0.24 mol) and *N*-methylpiperazine (0.097 cm^3, 0.87 mmol) in DMF (3 cm^3) delivered amine **58** (0.20 g, 77%) as pale yellow oil; ν_{max}(neat)/cm^{-1} 3397, 2938, 2797, 1586, 1503, 1311, 1166, 1126, 1031, 761; δ_H(300 MHz, $CDCl_3$) 1.64-1.73 (m, 2 H), 2.24-2.52 (m, 13 H), 3.16-3.18 (m, 2 H), 5.23 (s, 1 H), 6.67 (d, *J* = 2.0, 1 H), 6.65-6.68 (m, 1 H), 6.89 (d, *J* = 7.5, 1 H), 7.20 (t, *J* = 7.5, 1 H), 7.28 (t, *J* = 7.5, 1 H), 7.39 (d, *J* = 6.9, 1 H), 7.63 (d, *J* = 7.5, 1 H); δ_C(75 MHz, $CDCl_3$) 25.8, 41.8, 46.0, 53.3, 55.0, 55.9, 110.6, 111.1, 116.7, 124.0 (*q*, *J* = 272.2, CF_3); 125.3, 126.7 (q, *J* = 5.6),

127.7 (q, J = 30.7), 128.0, 132.1, 136.7, 138.2, 139.1, 150.5. δ_F(282 MHz, $CDCl_3$) −60.9 (s, 3 F); MALDI-HR-MS: calcd for $C_{21}H_{26}ClF_3N_3S^+$ ($[M+H]^+$): 444.1483; found: 444.1483.

5-Chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]-2-{[3-(trifluoromethyl)phenyl]thio}aniline (59)

General procedure 4, starting from chloride **52** (52 mg, 0.14 mmol), K_2CO_3 (39 mg, 0.28 mmol), NaI (11 mg, 70 µmol) and *N*-methylpiperazine (0.023 cm^3, 0.21 mmol) in DMF (2 cm^3) delivered amine **59** (0.50 g, 82%) as pale yellow oil; ν_{max}(neat)/cm^{-1} 2941, 2801, 1584, 1501, 1420, 1320, 1164, 1124, 791, 731, 703; δ_H(300 MHz, $CDCl_3$) 1.67-1.75 (m, 2 H), 2.29-2.60 (m, 13 H), 3.15-3.21 (m, 2 H), 5.43 (t, J = 5.4, 1 H), 6.65 (dd, J = 8.1, 2.1, 1 H), 6.69 (d, J = 2.1, 1 H), 7.11 (d, J = 7.5, 1 H), 7.28-7.37 (m, 4 H); δ_C(75 MHz, $CDCl_3$) 25.6, 42.2, 45.6, 52.6, 54.7, 56.1, 110.6, 116.9, 122.2 (q, J = 3.8), 122.7 (q, J = 3.9), 124.8 (q, J = 270.9), 129.0, 129.4, 131.4 (q, J = 32.0), 138.2, 138.4, 138.7, 150.6 (one signal not visible); δ_F(282 MHz, $CDCl_3$) −62.61 (s, 3 F); MALDI-HR-MS: calcd for $C_{21}H_{26}ClF_3N_3S^+$ ($[M+H]^+$): 444.1483; found: 444.1475.

5-Chloro-*N*-[3-(4-methylpiperazin-1-yl)propyl]-2-{[4-(trifluoromethyl)phenyl]thio}aniline (60)

General procedure 4, starting from chloride **53** (82 mg, 0.22 mmol), K_2CO_3 (61 mg, 0.44 mmol), NaI (16 mg, 0.11 mmol) and *N*-methylpiperazine (0.037 cm^3, 0.33 mmol) in DMF (2 cm^3) delivered amine **60** (70 mg, 76%) as colourless oil; ν_{max}(neat)/cm^{-1} 2937, 2796, 1585, 1499, 1323, 1161, 1129, 1085, 1062, 825; δ_H(300 MHz, $CDCl_3$) 1.67-1.75 (m, 2 H), 2.28-2.53 (m, 13 H), 3.17 (dt, *J* = 6.5, 5.7, 2 H), 5.38 (t, *J* = 5.3, 1 H), 6.65 (dd, *J* = 8.1, 2.1, 1 H), 6.68 (d, *J* = 2.1, 1 H), 7.07 (d, *J* = 8.2, 2 H), 7.35 (d, *J* = 8.1, 1 H), 7.43 (d, *J* = 8.3); δ_C(75 MHz, $CDCl_3$) 25.6, 42.2, 45.8, 52.9, 54.7, 56.1, 110.1, 110.4, 116.7, 123.9 (q, *J* = 269.5), 125.5, 125.6 (q, *J* = 3.8), 127.2 (q, *J* = 32.5), 138.0, 138.6, 141.9, 150.4; δ_F(282 MHz, $CDCl_3$) –62.2 (s, 3 F); MALDI-HR-MS: calcd for $C_{21}H_{26}ClF_3N_3S^+$ ($[M+H]^+$): 444.1483; found: 444.1476.

***N*`-[5-Chloro-2-(1-naphthylthio)phenyl]-*N,N*-dimethylpropan-1,3-diamine (64)**

General procedure 5, starting from **48** (59 mg, 0.16 mmol) and $HNMe_2$ (40% solution in H_2O, 0.18 cm^3, 1.63 mmol) in DMF (2 cm^3) to yield **64** (57 mg, 94%) as pale red solid; mp:

85 °C; ν_{max}(neat)/cm^{-1} 3174, 2938, 2816, 2771, 1586, 1505, 1383, 1281, 1160, 1133, 1091, 1042, 994, 839, 787, 768; δ_H(300 MHz, $CDCl_3$) 1.57-1.66 (m, 2 H), 1.90 (s, 6 H), 2.11 (t, J = 6.6, 2 H), 3.14 (q, J = 6.3, 2 H), 5.80 (t, J = 6.3, 1 H), 6.64-6.67 (m, 2 H), 6.88 (d, J = 7.2, 1 H), 7.26 (t, J = 7.8, 1 H), 7.37 (d, J = 8.1, 1 H), 7.50-7.64 (m, 3 H), 7.84 (dd, J = 7.8, 1.5, 1 H), 8.31 (dd, J = 8.1, 0.9, 1 H); δ_C(75 MHz, $CDCl_3$) 26.2, 42.6, 45.2, 57.8, 110.1, 111.3, 116.4, 122.9, 123.7, 125.7, 126.0, 126.1, 128.5, 130.8, 133.3, 133.7, 137.3, 138.2, 150.4 (one signal not visible); MALDI-HR-MS: calcd for $C_{21}H_{24}ClN_2S^+$ ([M+H]$^+$): 371.1343; found: 371.1336.

_N_`-[5-Chloro-2-(2-naphthylthio)phenyl]-_N,N_-dimethylpropan-1,3-diamine (65)

General procedure 5, starting from **49** (73 mg, 0.20 mmol) and $HNMe_2$ (40% solution in H_2O, 0.23 cm^3, 2.02 mmol) in DMF (2 cm^3) to yield **65** (69 mg, 92%) as a colourless oil after purification by CC (SiO_2; CH_2Cl_2-MeOH/NEt_3 99:0:1→98:1:1); ν_{max}(neat)/cm^{-1} 3389, 3054, 2944, 2816, 2770, 1587, 1502, 1460, 1420, 1282, 1132, 1096, 1042, 850, 812; δ_H(300 MHz, $CDCl_3$) 1.60-1.68 (m, 2 H), 1.99 (s, 6 H), 2.13 (t, J = 6.9, 2 H), 3.12-3.18 (m, 2 H), 5.71 (t, J = 5.1, 1 H), 6.65-6.68

(m, 2 H), 7.20 (dd, *J* = 8.1, 2.0, 1 H), 7.35-7.45 (m, 4 H), 7.63-7.76 (m, 3 H); δ_C(75 MHz, $CDCl_3$) 26.4, 42.3, 45.3, 57.5, 110.1, 111.8, 116.2, 123.8, 125.7, 125.3, 126.4, 126.8, 127.6, 128.4, 131.4, 133.6, 133.9, 137.3, 138.3, 150.4; MALDI-HR-MS: calcd for $C_{21}H_{24}ClN_2S^+$ ($[M+H]^+$): 371.1343; found: 371.1337.

_N`_-(5-Chloro-2-{[2-(trifluoromethyl)phenyl]thio}phenyl)-_N,N_-dimethylpropan-1,3-diamine (66)

General procedure 5, starting from **51** (100 mg, 0.26 mmol) and $HNMe_2$ (40% solution in H_2O, 0.30 cm^3, 2.63 mmol) in DMF (2 cm^3) to yield **66** (98 mg, 96%) as a colourless solid after purification by CC (SiO_2; CH_2Cl_2-NEt_3-MeOH 99:1:0→97:1:2); (Found: 55.3, H 5.2, N 7.0. calcd for $C_{18}H_{20}ClN_2F_3S$: C 55.6, H 5.2, N 7.2%); mp: 66 °C; ν_{max}(neat)/cm^{-1} 2946, 2860, 2819, 2771, 1586, 1503, 1311, 1257, 1125, 1114, 1031, 760; δ_H(300 MHz, $CDCl_3$) 1.26-1.70 (m, 2 H), 2.05 (s, 6 H), 2.19 (t, *J* = 6.6, 2 H), 3.11-3.19 (m, 2 H), 5.73 (br s, 1 H), 6.64-6.68 (m, 2 H), 6.85 (d, *J* = 7.8, 1 H, 7.15-7.20 (m, 1 H), 7.25-7.31 (m, 1 H), 7.37-7.40 (m, 1 H), 7.62 (dd, *J* = 7.8, 1.5, 1 H); δ_C(75 MHz, $CDCl_3$) 26.2, 42.4, 45.3, 57.7, 110.3, 116.4, 123.9 (q, *J* = 273.5), 124.9, 126.4 (q, *J* = 39.2), 126.5 (q, *J* = 5.5), 127.2, 131.9, 136.6, 138.1,

138.9, 150.7 (one signal not visible); δ_F(282 MHz, $CDCl_3$) –61.2 (s, 3 F); MALDI-HR-MS: calcd for $C_{18}H_{21}F_3ClN_2S^+$ ($[M+H]^+$): 389.1061; found: 389.1055.

_N_`-(5-Chloro-2-{[3-(trifluoromethyl)phenyl]thio}phenyl)-_N,N_-dimethylpropan-1,3-diamine (67)

General procedure 5, starting from **52** (25 mg, 66 µmol) and $HNMe_2$ (40% solution in H_2O, 0.074 cm^3, 0.66 mmol) in DMF (1 cm^3) to yield **67** (22 mg, 82%) as a pale yellow oil; ν_{max}(neat)/cm^{-1} 2946, 2862, 2820, 2772, 1585, 1503, 1422, 1320, 1273, 1165, 1123, 1072, 789, 695; δ_H(300 MHz, $CDCl_3$) 1.68 (quint, J = 6.3, 2 H), 2.05 (s, 6 H), 2.23 (t, J = 6.6, 2 H), 3.17 (q, J = 6.3, 2 H), 5.90 (t, J = 4.8, 1 H), 6.62-6.67 (m, 2 H), 7.01-7.12 (m, 1 H), 7.27-7.38 (m, 4 H); δ_C(75 MHz, $CDCl_3$) 26.1, 42.6, 45.3, 57.9, 110.3, 110.4, 116.5, 122.0 (q, J = 3.7), 122.5 (q, J = 3.9), 123.9 (q, J = 263.9), 127.1, 128.8, 129.3, 131.3 (q, J = 32.2), 138.2, 138.6, 150.8; δ_F(282 MHz, $CDCl_3$) –62.6 (s, 3 F); MALDI-HR-MS: calcd for $C_{18}H_{21}ClF_3N_2S^+$ ($[M+H]^+$): 389.1061; found: 389.1055.

_N_`-{5-Chloro-2-[4`-methylbiphenyl-4-yl)thio]phenyl}-_N,N_-dimethylpropan-1,3-diamine (76)

Bromide **63** (0.11 g, 0.27 mmol), Cs_2CO_3 (0.43 g, 1.33 mmol) and 4-tolylboronic acid (45 mg, 0.32 mmol) were suspended in a mixture of DME (10 cm^3) and H_2O (1 cm^3), degassed in an ultrasonic bath under Ar. $[Pd(PPh_3)_4]$ (30 mg, 26 µmol) was added and the mixture left to stir for 2.5 d at 80 °C; Dilution (EtOAc), washing (saturated aqueous $NaHCO_3$ solution), extraction of the aqueous layers (CH_2Cl_2), drying of the combined organic layers ($MgSO_4$), filtration and concentration in vacuo, followed by purification by CC (SiO_2; CH_2Cl_2-NEt_3 99:1) delivered biphenyl **76** (87 mg, 80%) as a colourless oil; ν_{max}(neat)/cm^{-1} 2952, 1675, 1592, 1479, 1443, 1193, 1130, 1021, 842, 802, 723; δ_H(300 MHz, MeOD) 1.87-1.92 (m, 2 H), 2.33 (s, 3 H), 2.66 (s, 6 H), 2.84-2.90 (m, 2 H), (t, J = 6.3, 2 H), 6.69 (dd, J = 8.1, 2.1, 1 H), 6.76 (d, J = 2.1, 1 H), 7.12 (td, J = 8.4, 2.0, 2 H), 7.20 (d, J = 8.4, 2 H), 7.41-7.51 (m, 5 H); δ_C(75 MHz, $CDCl_3$) 20.8, 23.6, 39.6, 42.5, 55.4, 110.2, 112.9, 117.3, 126.3, 127.0, 127.2, 129.6, 135.0, 136.8, 137.3, 137.5, 138.5, 149.1 (one signal not visisble); MALDI-HR-MS: calcd for $C_{24}H_{28}ClN_2S^+$ ($[M+H]^+$): 411.1656; found: 411.1655.

4-(3-{[5-Chloro-2-naphthylthio)phenyl]amino}propyl)-1-(3,4-dichlorobenzyl)-1-methylpiperazin-1-ium chloride (78)

3,4-Dichlorobenzyl chloride (0.013 cm^3, 94 µmol) was added to piperazine **56** (40 mg, 94 µmol) in acetone (2 cm^3). The mixture was stirred for 1 d at 45 °C. Concentration in vacuo followed by purification by reversed phase HPLC (C_{18}, CH_3CN-0.1% TFA in H_2O 0:100→100:0 in 50 min) delivered piperazinium salt **78** (35 mg, 60%) as a white solid; mp: 68 °C; ν_{max}(neat)/cm^{-1} 3361, 2958, 2924, 2852, 1684, 1584, 1502, 1472, 1420, 1283, 1199, 1123, 1035, 908, 819, 717; δ_H(300 MHz, $CDCl_3$) 1.60 (quint, *J* = 6.3, 2 H), 2.17-2.26 (m, 4 H), 2.48-2.52 (m, 2 H), 3.08 (s, 3 H), 3.14 (q, *J* = 6.0, 2 H), 3.29-3.42 (m, 4 H), 4.95 (s, 2 H), 5.21 (t, *J* = 5.4, 1 H), 6.64-6.70 (m, 2 H), 7.17 (dd, *J* = 8.7, 2.0, 1 H), 7.38-7.44 (m, 6 H), 7.51 (s, 1 H), 7.61-7.75 (m, 3 H); δ_C(75 MHz, $CDCl_3$) 25.4, 41.2, 46.1, 46.2, 54.7, 59.0, 65.1, 110.2, 112.0, 116.8, 124.1, 124.8, 125.7, 126.6, 126.8, 127.7, 128.7, 131.1, 131.5, 132.7, 133.3, 133.6, 133.8, 134.5, 135.6, 137.5, 138.3, 149.8 (one signal not visible); MALDI-HR-MS: calcd for $C_{31}H_{33}Cl_3N_3S^+$ ($[M+H]^+$): 584.1455; found: 584.1464.

Literature

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

2 M. C. Jockers-Scherübl, R. H. Schirmer and R. L. Krauth-Siegel, *Eur. J. Biochem.*, 1989, **180**, 267.

3 T. Baltz, D. Baltz, C. Giroud and J. Crockett, *EMBO J.*, 1985, **4**, 1273.

4 B. Räz, M. Iten, Y. Grether-Bühler, R. Kaminsky and R. Brun, *Acta Trop.*, 1997, **68**, 139.

5 H. Matile and J. R. L. Pink, *Plasmodium falciparum malaria parasite cultures and their use in immunology*. In *Immunological Methods* (Eds.: I. Lefkovits, B. Pernis), Academic Press, San Diego, 1990.

6 P. R. Gerber and K. Müller, *J. Comput. Aided Mol. Des.*, 1995, **9**, 251.

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

8 S. Parveen, M. O. F. Khan, S. E. Austin, S. L. Croft, V. Yardley, P. Rock and K. T. Douglas, *J. Med. Chem.*, 2005, **48**, 8087.

9 B. Stump, M. Kaiser, R. Brun, R. L. Krauth-Siegel and F. Diederich, *ChemMedChem*, 2007, **2**, 1708.

10 The microwave protocol for the alkylation was adopted from G. Caliendo, F. Fiorino, E. Perissutti, B. Severino, S. Gessi, E. Cattabriga, P. A. Borea, V. Santagada, *Eur. J. Med. Chem.*, 2001, **36**, 873.