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New potent inhibitors of trypanothione reductase from *Trypanosoma cruzi* in the 2-aminodiphenylsulfide series

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Summary — From a screening assay, 2-aminodiphenylsulfides were selected as leads for trypanothione reductase (TR) inhibition and studied by molecular modelling in the catalytic site of the enzyme. A series of analogues, monomers or bis-derivatives, were synthesized to improve binding energy and therefore inhibiting potency. These compounds appeared to be mixed competitive TR inhibitors and their inhibition profile could be explained when their aggregation in solution was taken into consideration. A bis-aminodiphenylsulfide with an IC_{50} of 0.55 μM was revealed to be the best TR inhibitor described so far.

trypanosomatid / trypanothione reductase / enzymatic inhibition / 2-aminodiphenylsulfide / molecular modelling

Introduction

Chagas' disease, which is caused by the trypanosomatid parasite *Trypanosoma cruzi*, constitutes an important health problem in South and Central America. Until now, there has been no effective chemotherapy. Nevertheless, a major breakthrough occurred with the discovery of a new redox defense system in the parasite and other trypanosomatids [1–3] based on a spermidine conjugate named trypanothione (N^1,N^8-bis-(glutathionyl) spermidine, $T(SH)_2$) (fig 1). $T(SH)_2$ is regenerated from $T(S)_2$ in its reduced dithiol form by an NADPH-dependent flavoprotein, trypanothione reductase (TR). Despite 41% homology in their primary structure, human glutathione reductase (GR) and *T cruzi* TR show almost total mutual discrimination towards their respective substrates [4]. Therefore, TR appears to be an ideal target for drugs that disrupt the natural defenses of the parasite without interfering with the host enzymes. Several inhibitors, analogues of the natural substrate $T(S)_2$, have already been described [5–7]. In order to screen TR inhibitors from libraries of compounds, we developed a microplate assay based on the measurement of the absorbance of a coloured thione [8]. Using this assay, three structurally related inhibitors of TR were discovered among other potential leads: the 2-aminodiphenylsulfide derivative **1** [9] and the phenothiazine derivatives **2** and **3** (fig 2). Several phenothiazines have previously been reported both as specific TR inhibitors ($K_i \approx 10$ μM) [10–14] and for their trypanocidal activity. However, the strong neuroleptic activity of these compounds at 40 μM precludes their use in humans as antiparasitic drugs [15]. Interestingly, compound **1** belongs to a series which was prepared almost 20 years ago, in an attempt to apply the theory of 'open rings' to phenothiazines but displayed disappointingly low neuroleptic activity [9, 16]. Compound **1** was competitive with trypanothione disulfide for TR while it did not inhibit human GR. Previously synthesized analogues of **1** (with different amino side chains, different substituents on the rings and different distances between the amine group and the aromatic

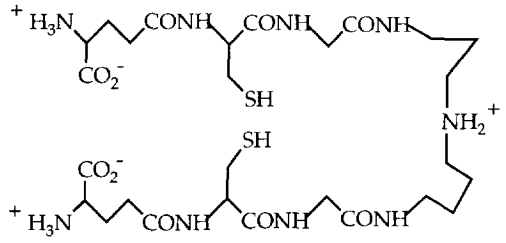

Fig 1. Trypanothione.

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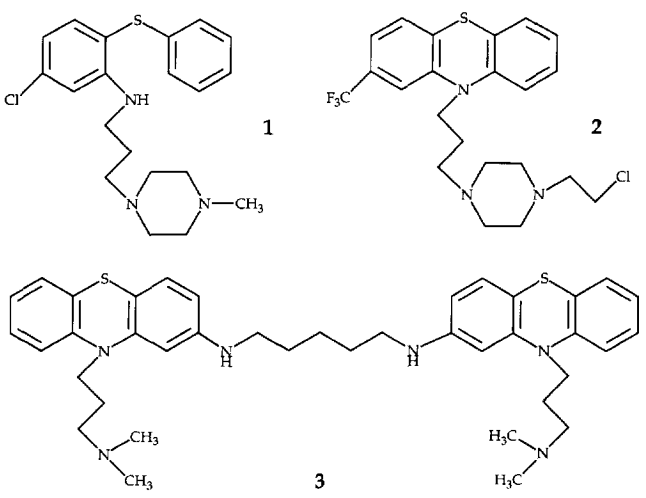


Fig 2. Structures of compounds **1–3**.

ring) were also tested to determine the pharmacophores of the structure and proved to be less active. Therefore **1** was selected as a lead to design TR inhibitors.

The position and the conformation of **1** in the catalytic site of TR from *Crithidia fasciculata* (2.4 Å resolution [17]) were studied by molecular dynamics simulation [18 and references therein]. Only two low potential energy conformations in a window of 3 kcal/ mol with respect to the minimal energy value were retained (fig 3). According to these results, in TR **1** is bound to two carboxylate groups of glutamic residues through its *N*-methylpiperazine extremity and with a hydrophobic pocket through the two aromatic rings. The presence of other acidic residues in the catalytic site of TR or in the close vicinity, while absent in GR, led us first to modify the side chain of **1** by replacing the piperazine ring with various linear and branched amine chains. As expected, this modification enabled us to increase the inhibitory potency towards TR while preserving the inactivity towards GR [18].

Molecular modelling clearly indicated that the hydrophobic pocket in the active site of TR could accommodate a bulky aromatic entity [18]. The additional binding energy, expected from the replacement of the piperazine side chain by several amino groups and responsible for better TR inhibition, could also be obtained by increasing the number of piperazine side chains.

In this report, we describe the synthesis and the inhibiting potency of analogues of **1**, in which two amino side chains were incorporated in various positions. These two chains were linked either simultaneously to the same aminodiphenylsulfide monomer

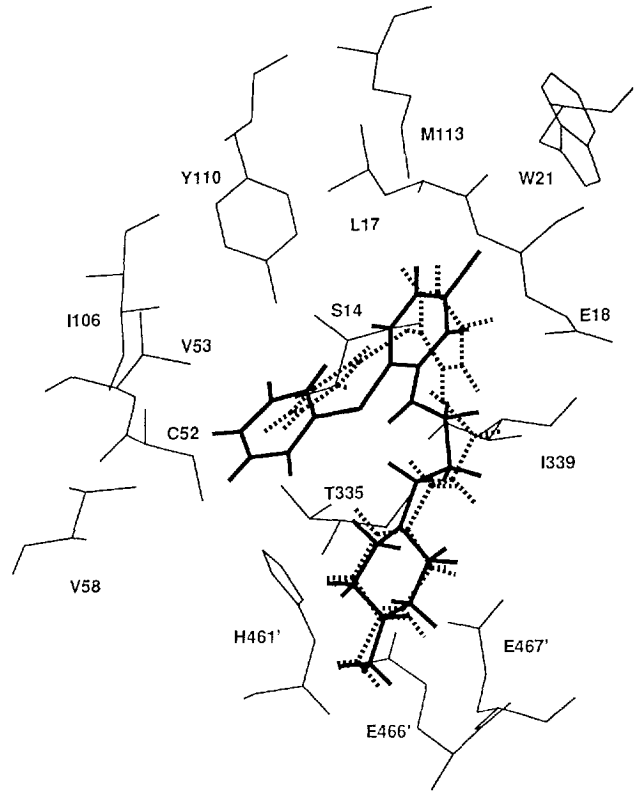


Fig 3. Orientation and binding of compound **1** in the catalytic site of TR as predicted by molecular dynamics calculations. The inhibitor molecule interacts with the glutamate residues Glu466' and Glu467' and with the hydrophobic region defined around amino acids Trp21 and Met113. In addition to Glu466' and Glu467', only the enzyme residues located at a distance lower than 4 Å from the aromatic diphenylsulfide unit are shown and labelled. The quotes (') designate amino acids belonging to the second monomer unit of TR.

(group C, fig 4) or separately to two aminodiphenylsulfide moieties bound at the ring level and thus mimicking **3** (group A). In the second approach, given the narrower catalytic site of GR, the bulkiness of the molecules should favour the TR/GR specificity. Furthermore, an intermediate series with two aminodiphenylsulfides bound by an amino side chain of varying length was also prepared and tested against TR (group B).

Chemistry

2-Nitrophenylthiophenylamine isomers **2a–c** were prepared by reaction of 1-chloro-2-nitrobenzene with the appropriate aminothiophenol as outlined in

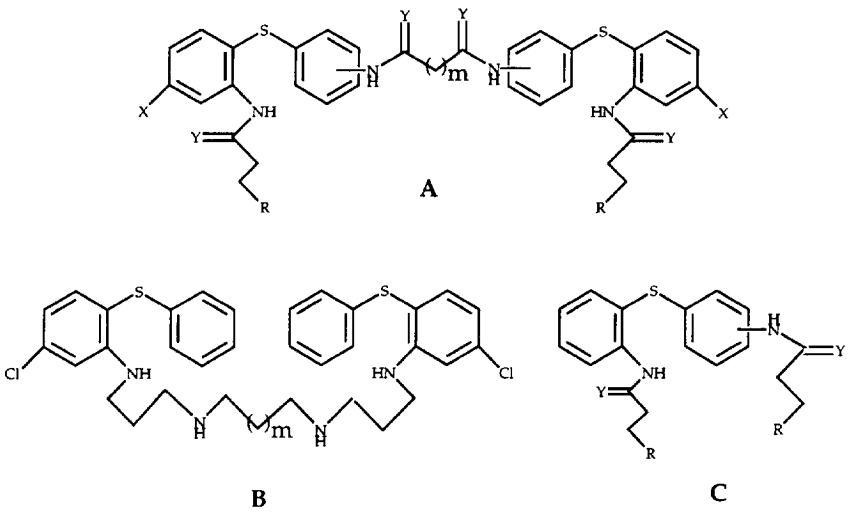


Fig 4. Orientation of the various amino side chains.

scheme 1. Reaction of **2a–c** with glutaryl chloride in pyridine led to the diamides **3a–c** whose nitro groups were reduced by catalytic hydrogenation (compounds **4a–c**). Conversion of the amino side chain was accomplished in two steps: first, reaction of **4a–c** with 3-chloropropionyl chloride (1-ethylpiperidine as base) and, second, substitution of the chloro group by *N*-methylpiperazine to form **5a–c** derivatives, or dimethylamine to form **5'a–c** derivatives. 1-Ethylpiperidine was preferred as a tertiary amine over pyridine which leads to a pyridinium salt not convertible into the methylpiperazine derivative. Complete reduction of the four amide groups to obtain **6a–c** or **6'a–c** was carried out by refluxing with a large excess of borane/tetrahydrofuran complex.

The preparation of compounds **9a–c** from **2a–c** via the corresponding amides **8a–c** (scheme 2) was carried out following the same protocol, except for the reduction of the nitro group carried out before the simultaneous assembly of both amino side chains.

The procedure mentioned above to obtain **6a–c** was further applied in the *meta* series. The only variation corresponded to the replacing of glutaryl dichloride with pimeloyl or malonyl dichloride to give the amides **12b** (m = 5) and **16b** (m = 1) and their reduced derivatives **13b** and **17b**, as well as the amides **28b** and **33b** (X = Cl and Br, respectively) and their reduced derivatives **29b** and **34b** (scheme 1).

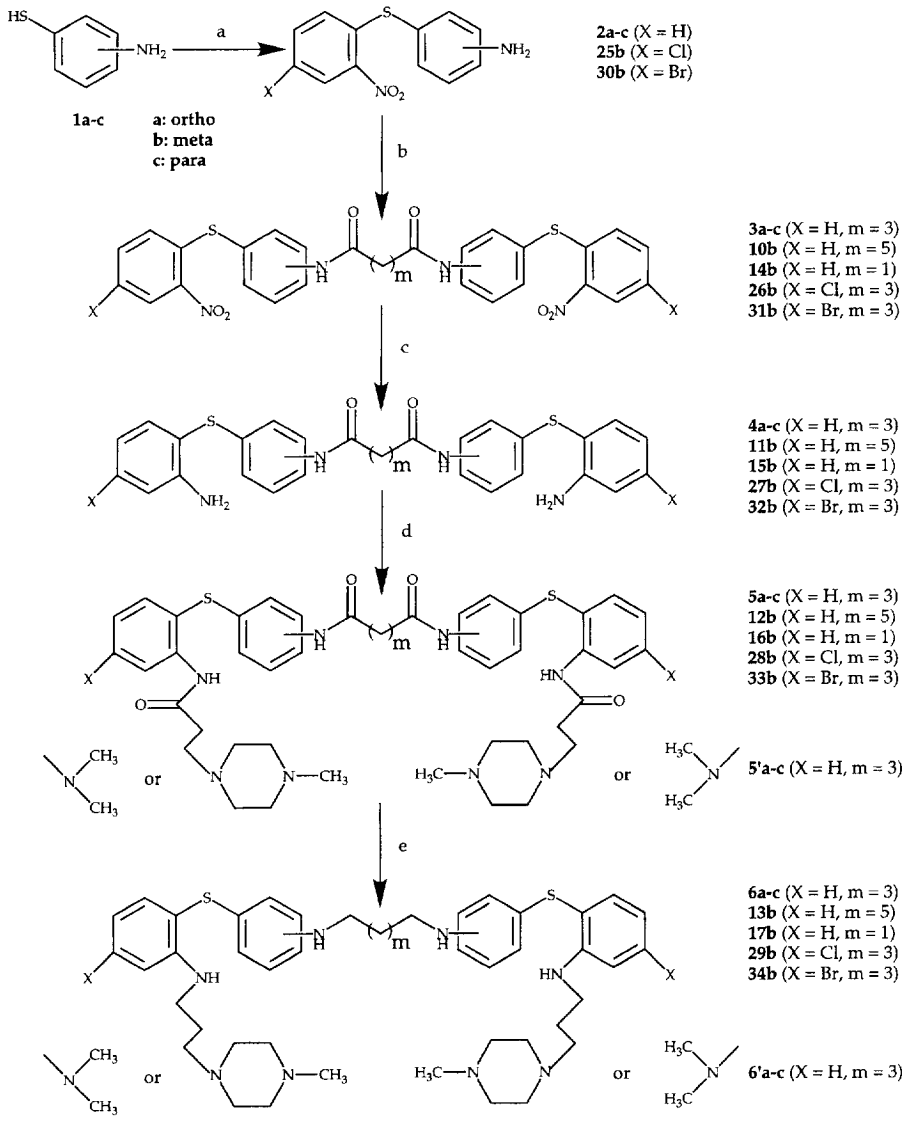
For the preparation of compounds **21–24** (scheme 3), the starting material was 2-amino-4-chlorodiphenylsulfide **18** obtained by reaction of 2,5-dichloronitrobenzene with thiophenol followed by reduction of the nitro group by catalytic hydrogenation. In this case, nucleophilic substitution of the halogenated derivative must be preceded by reduction of the amide group. The use of amide **19**, which is much more reactive than the corresponding non-reduced compound **20**, leads to polyalkylation of the polyamines.

Biochemistry

Inhibiting potency of the different compounds was evaluated by measuring the IC_{50} (table I) in the presence of 57 μM of $T(S)_2$ and increasing concentrations of inhibitor (0–114 μM).

To determine the nature of inhibition by the lead compounds in group A **5a–c**, the rate of $T(S)_2$ reduction was measured using several concentrations of $T(S)_2$ and inhibitor. The initial velocities of disappearance of NADPH at 340 nm in the presence of different concentrations of inhibitor $[I]_0$ (**5a**: 0–500 μM; **5b**: 0–40 μM, **5c**: 0–80 μM) were measured. With these data, the classical double-reciprocal plot and the corresponding slope report were obtained, establishing that **5a–c** were mixed competitive-type inhibitors (fig 5A). The K_i and K_{ii} values for mixed inhibitors can be deduced by considering the linearized dependence of the reciprocal reaction rate with respect to the reciprocal concentration in substrate at different inhibitor concentrations $[I]_0$ according to [1]:

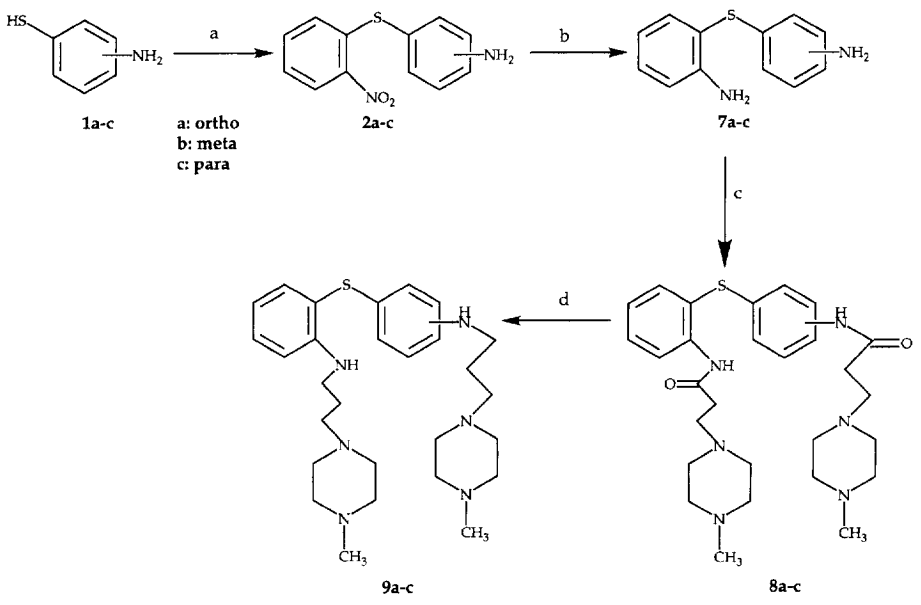
$$V_{max}/V = \{K_m \cdot [1 + ([I]/K_i)] \cdot 1/[S]\} + [1 + ([I]/K_{ii})] = \{A([I]) \cdot 1/[S]\} + B([I]) \quad [1]$$



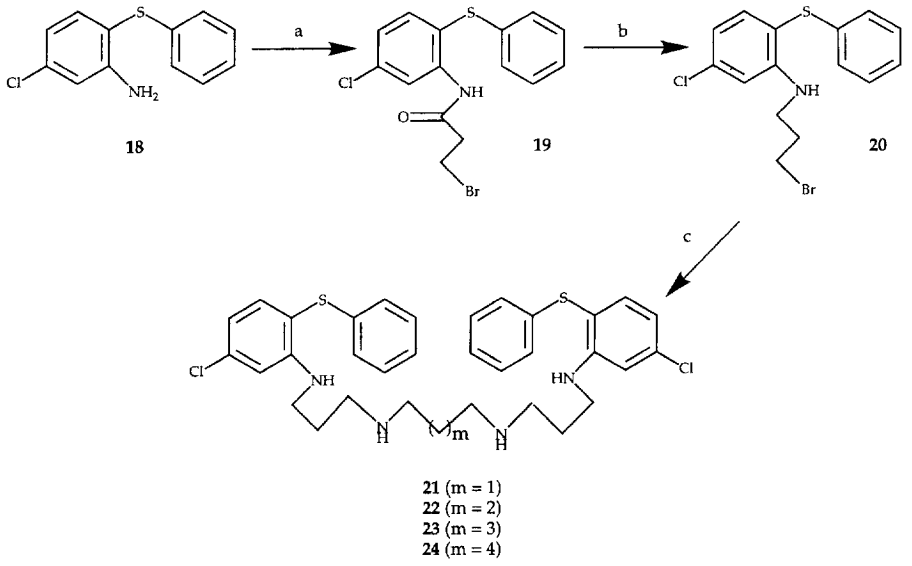
Scheme 1. Reagents: a) 1-chloro-2-nitrobenzene or 2,5-dichloronitrobenzene or 2,5-dibromonitrobenzene, anhydrous CH_3COONa, absolute EtOH, reflux, 36 h; b) diacyl halide, pyridine or ether/pyridine, rt, 7 h; c) H_2, activated Ni (according to Raney), absolute EtOH, 100 bar, 5 h or Fe, aqueous HCl 35%, EtOH 95 °C, reflux, 1 h; d) 3-chloropropionyl chloride, 1-ethylpiperidine, anhydrous THF, 0 °C, 2 h 30 min then 1-methylpiperazine or dimethylamine, anhydrous THF, reflux, 4 h; e) BH_3/THF 1 M, anhydrous THF, reflux, 4 h.

It can be seen that the slope $A([I])$ is a linear function of [I] which only depends on K_i, while the intercept $B([I])$ depends on the concentration of inhibitor only through the K_{ii} value. Therefore, the following approach has been tried at first in order to determine these inhibition constants.

1) The dependence of V_{max}/V was monitored in function of 1/[S] at different levels of [I]. A cross-validated linear regression approach was used to calculate the optimal values $A^0([I])$ and $B^0([I])$ of the slopes and respectively intercepts of those lines, as well as the standard errors $\sigma(A)$ and $\sigma(B)$ of these magnitudes.

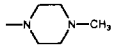
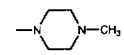


Scheme 2. Reagents: a) 1-chloro-2-nitrobenzene, anhydrous CH_3COONa, absolute EtOH, reflux, 36 h; b) H_2, activated Ni (according to Raney), absolute EtOH, 100 bar, 5 h; c) 3-chloropropionyl chloride, 1-ethylpiperidine, anhydrous THF, 0 °C, 2 h 30 min then 1-methylpiperazine, anhydrous THF, reflux, 4 h; d) BH_3/THF 1 M, anhydrous THF, reflux, 4 h.



Scheme 3. Reagents: a) 3-bromopropionyl chloride, pyridine, ether, 0 °C, 2 h; b) BH_3/THF 1 M, anhydrous THF, rt, 2 h; c) polyamine, THF, reflux, 15 h.

Table I. Inhibition of TR from *T cruzi* by 2-aminodiphenylsulfide derivatives.

Molecule	*Group*	*m*	*Orientation*	*X*	*Y*	*-R*	*IC_{50} (μM)*
5a	A	3	*ortho*	H	O		60
5b	A	3	*meta*	H	O		1.8
5c	A	3	*para*	H	O		2.5
5'a	A	3	*ortho*	H	O		>60
5'b	A	3	*meta*	H	O		23
5'c	A	3	*para*	H	O		>60
6a	A	3	*ortho*	H	2H		a
6b	A	3	*meta*	H	2H		a
6c	A	3	*para*	H	2H		a
6'a	A	3	*ortho*	H	2H		a
6'b	A	3	*meta*	H	2H		a
6'c	A	3	*para*	H	2H		a
8a	C		*ortho*		O		>60
8b	C		*meta*		O		>60
8c	C		*para*		O		>60
9a	C		*ortho*		2H		>60
9b	C		*meta*		2H		>60
9c	C		*para*		2H		>60
12b	A	5	*meta*	H	O		7
13b	A	5	*meta*	H	2H		>60
16b	A	1	*meta*	H	O		>60
17b	A	1	*meta*	H	2H		>60
21	B	1					60[b]
22	B	2					>60[b]
23	B	3					13
24	B	4					8[b]
28b	A	3	*meta*	Cl	O		0.93
29b	A	3	*meta*	Cl	2H		6.9
33b	A	3	*meta*	Br	O		0.55
34b	A	3	*meta*	Br	2H		16.5

[a]Precipitate in the enzymatic buffer at 14.25 μM (2% DMSO); IC_{50} were less than 5 μM in the presence of precipitate; [b]difficult to solubilize; for A, B and C, see figure 4.

2) The dependence of $A([I])$ in function of [I] was monitored. For each level of inhibitor concentration [I], three data points: $A^0([I])$, $A^0([I]) + \sigma([I])$ and $A^0([I]) - \sigma([I])$ were considered in order to account for the error affecting the measured slopes.

However, the plot of the considered A values as a function of [I] was not linear as expected, but was better approximated by a parabolic function of [I]:

$$A([I]) = a + b \cdot [I] + c \cdot [I]^2 \qquad [2]$$

with a negative quadratic coefficient c. The same trend can be observed when plotting $B([I])$ against [I]. In other words, the inhibitory potency of the compounds appears to be relatively lower at higher concentrations than might have been extrapolated from the measurements at low [I]. This can be understood in terms of a decreased activity factor of the compounds in more concentrated solutions. We attempted to model this phenomenon by stipulating that an aggregation of the inhibitor molecules occurred according to $nI \rightleftarrows I_n$ characterized by the dissociation constant K_d which links the effective concentration of free inhibitor [I], the total concentration of added inhibitor $[I]_0$ and that of the aggregate I_n. Because **5c** showed the most pronounced effect, a more thorough study of its kinetics was conducted. Experimental evidence of the aggregation hypothesis

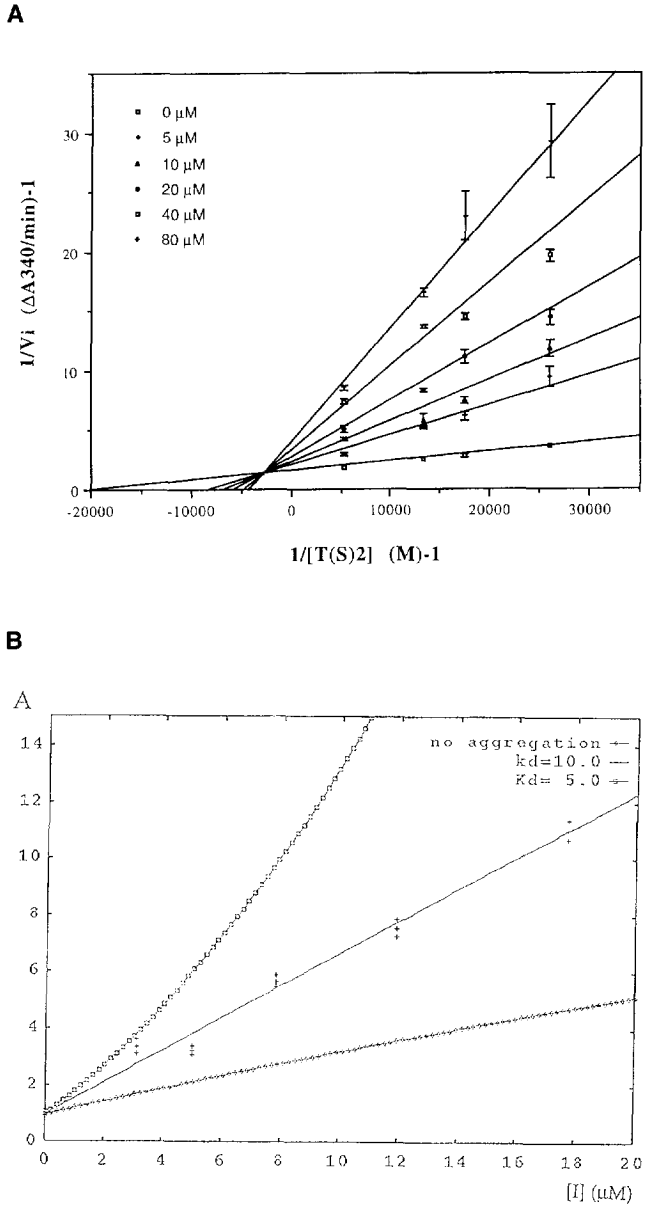


Fig 5. Mixed-type competitive inhibition of TR by compound **5c**. (**A**) Lineweaver–Burk plot of $1/V$ versus 1/[S] showing mixed-type competitive inhibition of TR observed with **5c**. (**B**) $A([I]) = f([I])$ see equation [1], when the effective inhibitor concentrations [I] were considered: i) equal to total concentration $[I]_0$ (no aggregation) or nearly equal to $[I]_0$ (understatement of aggregation) (bottom line); negative value of c; ii) in the presence of an aggregation at K_d = 10 (middle line) (c = 0), the three points represent coordinates of $A^0([I])$, $A^0([I]) + \sigma([I])$ and $A^0([I]) - \sigma([I])$ for each effective inhibitor concentration [I]; and iii) in the case of an aggregation at K_d = 5 (top line) (overstatement of aggregation); positive value of c.

was furnished by the observed shift of the absorption maxima (243–263 nm) in the UV spectra of **5c** at 5, 10 and 40 µM.

The aggregation process must not necessarily be understood in terms of the formation of a well-defined polymeric species I_n. Interactions between the protonated amino groups and the aromatic rings have already been reported to play an important role in the aggregation of such compounds [19]. If the values of n and K_d were known, the effective inhibitor concentrations [I] could be determined as a function of the total concentrations $[I]_0$ and would lead to linear plots of A and B as a function of [I]. Understating the aggregation of the inhibitor still leads to parabolic plots with negative c coefficients, while its overstatement leads to parabolic plots with a positive c value (fig 5B). Therefore, plotting the value of the quadratic coefficient c as a function of the log of K_d for different values of n allowed us to find the optimal K_d values corresponding to the degeneracy of $A([I])$ to a linear function (c = 0). The pair of values (n, K_d) for which the function becomes linear and displays a maximal correlation with the experimental points has been found using a systematic search technique, based on the variation of the linear correlation coefficient $r^2 = f(K_d)$. From kinetic data of **5c**, the linearization of the dependence of $A([I])$ as a function of [I] succeeds well for any n within the range 2 to 2.5. The quality of the regression lines $A([I]) = a' \cdot [I] + b'$, given by r^2 only slightly decreases when going from n = 2 (r^2 = 0.995) to n = 3 (r^2 = 0.990). Consistently, the same pairs of (n, K_d) values lead to the linearization of both dependences of $A([I])$ and $B([I])$ as a function of [I] (fig 5B). This confirmed the hypothesis that the observed non-linearities were due to a difference between total and effective inhibitor concentrations. In table II, we report the inhibition and aggregation constants obtained in the hypothesis of an aggregation with n = 2.

Discussion

Comparative studies of the catalytic sites of TR and GR show that, if some acidic residues are shared by both enzymes, others are present only in TR. From

Table II. Calculated inhibition constants of compounds **5b** and **5c**.

Compound	K_d	K_i (µM)	K_{ii} (µM)
5b	50	2.6	7.5
5c	10	2.0	7.0

compound **1**, selected in a screening assay on TR, it was conceivable to increase binding energy by generating additional ionic interactions with additional amine groups, while at the same time preserving the specificity. This possibility was tested by introducing a second amino side chain into the diphenylsulfide moiety (group C, compounds **8a–c** and **9a–c**). Given the size of the hydrophobic pocket in the catalytic site of TR, another possibility was to synthesize bis-diphenylsulfide derivatives in which each monomer carries one amino side chain (group A, compounds **5a–c** and **6a–c**). In addition, the dimethylamine derivatives **5'a–c** and **6'a–c** were synthesized to check whether, in this series, the previously observed range of effectiveness for **1** and some of its analogues [20] (methylpiperazine side chain > dimethyl side chain) was maintained. An intermediate series with two aminodiphenylsulfides bound by an amino side chain of varying length was also prepared (group B, compounds **8a–c** and **9a–c**).

Enzymatic studies show that the different inhibitors are specific towards TR from *T cruzi* over human GR. The results given in table I show that:

i) Group A represents the most potent inhibitors with efficiency depending on the orientation of the linker between the rings in the order *meta* > *para* ≫ *ortho* isomers and as already observed for analogues of **1** [20], with methylpiperazine moieties confering a greater activity than dimethylamine ones.

ii) In group B, the inhibiting potency can be correlated with an increase in the number of methylene groups.

iii) In group C, aminodiphenylsulfide monomers with two methylpiperazine side chains, either amides or the corresponding reduced derivatives, are totally devoid of activity at 60 μM, showing the importance of the second hydrophobic moiety.

For the most interesting group (A), our enzymatic studies give calculated K_i in the same range than IC_{50} but with a slightly better value for the *para* isomer **5c** (2 μM) than the *meta* isomer **5b** (2.6 μM) (table II). However, the highest aggregation in solution of **5c** (K_d = 50) led us to select *meta* position and methylpiperazine moiety as the most favourable chemical features to design further inhibitors in this series of bis-aminodiphenylsulfides.

Some analogues of the amide **5b** and of its corresponding amine **6b** were therefore synthesized. The new compounds are different as regards the distance between the two diphenylsulfide moieties and the nature of the halogenated atom on the aniline ring. This second modification was introduced, given the strong activity of **1** compared to its non-halogenated derivative [20]. Compared to **5b** (m = 3), amides **12b** (m = 5) and especially **16b** (m = 1) are less active, indicating that a seven-membered sequence between the hydrophobic moieties is the most favourable linker. The same conclusion could be reached for amines **13b** and **17b** compared to **6b**. Halogenated analogues of **5b** and **6b** with a chlorine atom on the aniline ring (compounds **28b** and **29b**) were found better inhibitors than initial compounds; thus, IC_{50} for amide **28b** is twice as good as for the non-halogenated analogue. This improvement can be explained by a binding of one chloroaminodiphenylsulfide moiety similar to that of mepacrine in the TR active site, recently described from crystallographic studies by Krauth-Siegel et al [21]. The halogen atom was thought to interact with the positively polarized hydrogen of the indolyl nitrogen of Trp21. The brominated analogues of **5b** and **6b** (compounds **33b** and **34b**) were therefore synthesized to determine whether the favourable influence of the halogen atom was really due to this type of electronic effect or to a simple hydrophobic effect. The very potent activity of amide **33b** may justify the second hypothesis and underlines the influence of the ring substituents in the series. Work is now in progress to improve even further **33b**, which is the best specific TR inhibitor described so far.

Experimental protocols

Chemistry

All melting points were determined on a Büchi melting point apparatus and were uncorrected. All reactions were monitored by thin-layer chromatography (acetone/28% NH_4OH 8:2) carried out on 0.2 mm E Merck silica-gel plates (60F-254) using UV light as a visualizing agent and 10% ninhydrin in acetone or Reindel Hope (RH) [22] as developing agents; the purity of final compounds was checked by HPLC (cyanopropyl Nucleosil columm) before preparing oxalate salts. ^{1}H-NMR spectra were obtained using a Brucker 300 MHz spectrometer; mass spectra were recorded on a time of flight plasma desorption spectrometer using a californium source. Microanalyses were obtained from CNRS (France) and were calculated for oxalate salts. Elemental analyses indicated by the symbols of the elements were within ± 0.4% of theoretical values.

Procedure for oxalate salts

A saturated solution of oxalic acid in AcOEt was added dropwise to a saturated solution of amine in ethyl acetate (AcOEt). The mixture was kept at 4 °C for 3 h; the salt was isolated by filtration and successively washed with ice-cold water, AcOEt and ether.

General procedure for the synthesis of substituted diphenylsulfides

The appropriate aminothiophenol **1a–c** (5 g, 40 mmol, 1.5 equiv) and 1-chloro-2-nitrobenzene (4.2 g, 26.7 mmol, 1 equiv) were added to a solution of anhydrous sodium acetate (10.9 g, 133 mmol, 5 equiv) in 80 mL of absolute ethyl alcohol. After refluxing the mixture for 36 h, ethyl alcohol was evaporated. The solid residue was treated with a AcOEt/water mixture and the organic layer was separated, dried over $MgSO_4$ and evaporated to dryness. Absolute ethyl alcohol (10 mL) was

added to the oily residue. After storage over night at 4 °C, the mixture was filtered and washed with ethyl alcohol to yield **2a–c** which were used without further purification.

2-(2-Nitro)phenylthiophenylamine ***2a***. Yellow solid; yield: 81%; R_f (CH_2Cl_2/petroleum ether 5:5): 0.5; mp: 75 °C. ^{1}H-NMR (CD_2Cl_2) δ ppm: 8.30–8.26 (m, 1H, Ph); 7.50–7.25 (m, 4H, Ph); 6.93–6.82 (m, 3H, Ph); 4.35 (s, 2H exch D_2O, NH_2). *m/z*: 246.

3-(2-Nitro)phenylthiophenylamine ***2b***. Orange solid; yield: 70%; R_f (CH_2Cl_2/petroleum ether 5:5): 0.5; mp: 115 °C. ^{1}H-NMR (CD_2Cl_2) δ ppm: 8.24–8.20 (m, 1H, Ph); 7.44–7.38 (m, 1H, Ph); 7.31–7.22 (m, 2H, Ph); 7.06–7.02 (m, 1H, Ph); 6.99–6.91 (m, 2H, Ph); 6.85–6.80 (m, 1H, Ph); 3.90 (s, 2H exch D_2O, NH_2). *m/z*: 246.

4-(2-Nitro)phenylthiophenylamine ***2c***. Orange solid; yield: 74%; R_f (CH_2Cl_2/petroleum ether 5:5): 0.6; mp: 100 °C. ^{1}H-NMR (CD_2Cl_2) δ ppm: 8.24–8.20 (m, 1H, Ph); 7.42–7.34 (m, 3H, Ph); 7.26–7.19 (m, 1H, Ph); 6.97–6.92 (m, 1H, Ph); 6.83–6.77 (m, 2H, Ph); 4.07 (s, 2H exch D_2O, NH_2). *m/z*: 246.

General procedure for linking
Under N_2, glutaryl dichloride (260 mL, 2.03 mmol, 1 equiv) was added dropwise to a cooled solution of **2a–c** (1 g, 4.06 mmol, 2 equiv) in 7 mL of distilled pyridine. After stirring the mixture for 1 h at 0 °C then 7 h at room temperature, the pyridine was evaporated. The solid residue **3a–c** was washed with 1 M HCl, ether and used without further purification.

N,N'-(2-(2-Nitro)phenylthiophenyl)-1,5-pentylenediamide ***3a***. Yellow solid; yield: 47%; R_f (CH_2Cl_2/MeOH 9:1): 0.8, RH positive; mp: 135 °C. ^{1}H-NMR (DMSO-d_6) δ ppm: 9.35 (s, 2H exch D_2O, 2NH); 8.26–8.19 (m, 2H, Ph); 7.65–7.61 (m, 1H, Ph); 7.57–7.50 (m, 5H, Ph); 7.37–7.30 (m, 5H, Ph); 6.86–6.78 (m, 1H, Ph); 6.71–6.65 (m, 2H, Ph); 2.08–2.02 (t, J = 7.4 Hz, 4H, $2CH_2$); 1.51–1.43 (qt, J = 7.4 Hz, 2H, CH_2). *m/z*: 588.

N,N'-(3-(2-Nitro)phenylthiophenyl)-1,5-pentylenediamide ***3b***. Yellow solid; yield: 83%; R_f (CH_2Cl_2/MeOH 9:1): 0.6, RH positive; mp: 110 °C. ^{1}H-NMR (DMSO-d_6) δ ppm: 10.14 (s, 2H exch D_2O, 2NH); 8.27–8.23 (m, 2H, Ph), 7.92–7.91 (m, 1H, Ph); 7.61–7.57 (m, 1H, Ph); 7.50–7.46 (m, 2H, Ph); 7.42–7.38 (m, 4H, Ph); 7.31–7.27 (m, 2H, Ph); 6.96–6.92 (m, 2H, Ph); 6.83–6.79 (m, 2H, Ph); 2.42–2.37 (t, J = 7.1 Hz, 4H, $2CH_2$); 1.93–1.87 (qt, J = 7.2 Hz, 2H, CH_2). *m/z*: 588.

N,N'-(4-(2-Nitro)phenylthiophenyl)-1,5-pentylenediamide ***3c***. Yellow solid; yield: 69%; R_f (CH_2Cl_2/MeOH 9:1): 0.7, RH positive; mp: 160 °C. ^{1}H-NMR (DMSO-d_6) δ ppm: 10.25 (s, 2H exch D_2O, 2NH); 8.26–8.22 (m, 2H, Ph); 7.82–7.78 (m, 4H, Ph); 7.59–7.54 (m, 6H, Ph); 7.40–7.37 (m, 2H, Ph); 6.86–6.82 (m, 2H, Ph); 2.50–2.42 (t, J = 7.2 Hz, 4H, $2CH_2$); 1.98–1.92 (qt, J = 7.5 Hz, 2H, CH_2). *m/z*: 588.

General procedure for the reduction of nitro compounds
Activated nickel (1 g) was added to a solution of **3a–c** (9 g, 15.4 mmol) in 200 mL of absolute ethyl alcohol. After 5 h at 80 °C and under H_2 pressure (100 bar), the nickel was eliminated by filtration and the filtrate was evaporated to yield **4a–c**, which were used without further purification.

N,N'-(2-(2-Amino)phenylthiophenyl)-1,5-pentylenediamide ***4a***. Colourless oil; yield: 80%; R_f (CH_2Cl_2/MeOH 9:1): 0.5, RH positive. ^{1}H-NMR (DMSO-d_6) δ ppm: 9.83 (s, 2H exch D_2O, 2NH); 7.65–7.61 (m, 1H, Ph); 7.33–7.27 (m, 4H, Ph); 7.17–7.03 (m, 6H, Ph); 6.83–6.71 (m, 3H, Ph); 6.60–6.52 (m, 2H, Ph); 5.29 (s, 4H exch D_2O, $2NH_2$); 2.42–2.37 (t, J = 7.3 Hz, 4H, $2CH_2$); 1.99–1.94 (qt, J = 7.4 Hz, 2H, CH_2). *m/z*: 528.

N,N'-(3-(2-Amino)phenylthiophenyl)-1,5-pentylenediamide ***4b***. Colourless solid; yield: 80%; R_f (CH_2Cl_2/MeOH 9:1): 0.5, RH positive; mp: 90 °C. ^{1}H-NMR (DMSO-d_6) δ ppm: 9.89 (s, 2H exch D_2O, 2NH); 7.61–7.57 (m, 1H, Ph); 7.41–7.37 (m, 3H, Ph); 7.31–7.25 (m, 2H, Ph); 7.20–7.14 (m, 3H, Ph); 7.04–6.99 (m, 1H, Ph); 6.82–6.78 (m, 2H, Ph); 6.70–6.66 (m, 2H, Ph); 6.62–6.56 (m, 2H, Ph); 5.34 (s, 4H exch D_2O, $2NH_2$); 2.33–2.28 (t, J = 7.4 Hz, 4H, $2CH_2$); 1.89–1.80 (qt, J = 7.4 Hz, 2H, CH_2). *m/z*: 528.

N,N'-(4-(2-Amino)phenylthiophenyl)-1,5-pentylenediamide ***4c***. Colourless solid; yield: 80%; R_f (CH_2Cl_2/MeOH 9:1): 0.5, RH positive; mp: 175 °C. ^{1}H-NMR (DMSO-d_6) δ ppm: 9.92 (s, 2H exch D_2O, 2NH); 7.58 (m, 1H, Ph); 7.53–7.49 (m, 3H, Ph); 7.29–7.26 (m, 3H, Ph); 7.14–7.05 (m, 6H, Ph); 6.80–6.60 (m, 1H, Ph); 6.58–6.57 (m, 2H, Ph); 5.30 (s, 4H exch D_2O, $2NH_2$); 2.36–2.31 (t, J = 7.3 Hz, 4H, $2CH_2$); 1.92–1.81 (qt, J = 7.3 Hz, 2H, CH_2). *m/z*: 528.

General procedure for the addition of amino side chains
Under N_2, 5 equiv of 3-chloropropionyl chloride (904 mL, 9.47 mmol), were added, dropwise, to a cooled solution of **4a–c** (1 g, 1.9 mmol, 1 equiv) in 35 mL of anhydrous THF. After stirring the mixture for 30 min at 0 °C, 3 equiv of 1-ethylpiperidine (781 mL, 5.69 mmol) were added. Amine (3 equiv) was then added twice at an interval of 30 min. After stirring the mixture for 1 h at 0 °C then 30 min at room temperature, a large excess of 1-methylpiperazine (4.2 mL, 37.88 mmol, 20 equiv) was added and the mixture was refluxed for 4 h. The solvent was removed and the oily residue was treated with a CH_2Cl_2/water mixture. The organic layer was separated, dried over $MgSO_4$, evaporated to dryness and the oily residue purified by thick-layer chromatography (SiO_2, CH_2Cl_2/MeOH 8.5:1.5) to yield **5a–c**. For the synthesis of **5'a–c**, dimethylamine (2.0 M solution in MeOH, 19 mL, 37.88 mmol, 20 equiv) was used instead of 1-methylpiperazine.

N,N'-(2-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)-amino)phenylthio)phenyl)-1,5-pentylenediamide ***5a***. Colourless oil; yield: 40%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.4, RH positive; anal $C_{53}H_{64}N_8O_{20}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.40 (s, 2H exch D_2O, 2NH); 8.51 (s, 2H exch D_2O, 2NH); 8.20–8.17 (m, 2H, Ph); 7.72–7.69 (m, 2H, Ph); 7.44–7.37 (m, 4H, Ph); 7.25–6.95 (m, 8H, Ph); 2.76–2.72 (t, J = 6 Hz, 4H, $2CH_2$); 2.64–2.47 (m, 24H, $12CH_2$); 2.26 (s, 6H, $2CH_3$); 2.18–2.15 (qt, J = 5.2 Hz, 2H, CH_2). *m/z*: 836.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)-amino)phenylthio)phenyl)-1,5-pentylenediamide ***5b***. Colourless solid; yield: 40%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.4, RH positive; mp: 82 °C; anal $C_{53}H_{64}N_8O_{20}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.54 (s, 2H exch D_2O, 2NH); 8.48 (s, 2H exch D_2O, 2NH); 8.19–8.15 (m, 2H, Ph); 7.59–7.55 (m, 2H, Ph); 7.45–7.39 (m, 4H, Ph); 7.26–7.12 (m, 6H, Ph); 6.91–6.87 (m, 2H, Ph); 2.55–2.39 (m, 24H, $12CH_2$); 2.38–2.33 (t, J = 6.9 Hz, 4H, $2CH_2$); 2.23 (s, 6H, $2CH_3$); 2.07–1.99 (qt, J = 7 Hz, 2H, CH_2). *m/z*: 836.

N,N'-(4-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)-amino)phenylthio)phenyl)-1,5-pentylenediamide ***5c***. Colourless solid; yield: 59%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.4, RH

positive; mp: 68 °C; anal $C_{53}H_{64}N_8O_{20}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.38 (s, 2H exch D_2O, 2NH); 8.23 (s, 2H exch D_2O, 2NH); 8.20–8.16 (m, 2H, Ph); 7.51–7.48 (m, 4H, Ph); 7.43–7.36 (m, 4H, Ph); 7.13–7.07 (m, 6H, Ph); 2.64–2.42 (m, 28H, $14CH_2$); 2.22 (s, 6H, $2CH_3$); 2.08–2.03 (qt, J = 6.9 Hz, 2H, CH_2). *m/z*: 836.

N,N'-(2-(2-(N-(3-Dimethylaminopropyloxy)amino)phenylthio)-phenyl)-1,5-pentylenediamide ***5'a***. Colourless oil; yield: 42%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.5, RH positive; anal $C_{43}H_{50}N_6O_{12}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.77 (s, 2H exch D_2O, 2NH); 8.57 (s, 2H exch D_2O, 2NH); 8.12–8.09 (m, 2H, Ph); 7.90–7.87 (m, 2H, Ph); 7.39–7.22 (m, 6H, Ph); 7.14–7.03 (m, 6H, Ph); 2.79–2.71 (t, J = 6.6 Hz, 6H, $3CH_2$); 2.64–2.60 (t, J = 6 Hz, 4H, $2CH_2$); 2.56–2.51 (t, J = 6.3 Hz, 2H, CH_2); 2.37 (s, 12H, $4CH_3$); 1.88–1.80 (qt, J = 7.2 Hz, 2H, CH_2). *m/z*: 726.

N,N'-(3-(2-(N-(3-Dimethylaminopropyloxy)amino)phenylthio)-phenyl)-1,5-pentylenediamide ***5'b***. Colourless solid; yield: 54%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.5, RH positive; mp: 56 °C; anal $C_{43}H_{50}N_6O_{12}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 11.21 (s, 2H exch D_2O, 2NH); 8.40–8.36 (m, 2H, Ph); 8.27 (s, 2H exch D_2O, 2NH); 7.61–7.57 (m, 2H, Ph); 7.47–7.39 (m, 4H, Ph); 7.24–7.10 (m, 6H, Ph); 6.88–6.84 (m, 2H, Ph); 2.55–2.33 (m, 12H, $6CH_2$); 2.15 (s, 12H, $4CH_3$); 2.04–1.99 (qt, J = 6.9 Hz, 2H, CH_2). *m/z*: 726.

N,N'-(4-(2-(N-(3-dimethylaminopropyloxy)amino)phenylthio)-phenyl)-1,5-pentylenediamide ***5'c***. Colourles solid; yield: 68%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.5, RH positive; mp: 48 °C; anal $C_{43}H_{50}N_6O_{12}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 11.17 (s, 2H exch D_2O, 2NH); 8.68 (s, 2H exch D_2O, 2NH); 8.35–8.31 (m, 2H, Ph); 7.51–7.34 (m, 8H, Ph); 7.11–7.02 (m, 6H, Ph); 2.61–2.55 (m, 4H, $2CH_2$); 2.49–2.40 (m, 8H, $4CH_2$); 2.37 (s, 12H, 4CH3); 2.05–2.00 (qt, J = 7.2 Hz, 2H, CH_2). *m/z*: 726.

General procedure for the reduction of amides
Commercial 1 M BH_3/THF solution (7.5 mL, 7.5 mmol, 15 equiv) was added, dropwise for 1 h under N_2 to a cooled solution of **5a–c** (448 mg, 0.5 mmol, 1 equiv) in 1.5 mL of anhydrous THF. The mixture was stirred at 0 °C for 30 min, allowed to react 1 h at room temperature and refluxed for 4 h. Excess BH_3 was neutralized, at 0 °C, by an aqueous 1 M Na_2CO_3 solution and the mixture was evaporated to dryness. The solid residue was treated with a CH_2Cl_2/aqueous 1 M NaOH mixture. The organic layer was separated, dried over $MgSO_4$, evaporated and the oily residue was purified on thick-layer chromatography (SiO_2, CH_2Cl_2/AcOEt 10:0.15) to yield **6a–c**. Compounds **6'a**, **6'b** and **6'c** were synthesized from **5'a**, **5'b** and **5'c** according to this method.

N,N'-(2-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino)-phenylthio)phenyl)-1,5-pentylenediamine ***6a***. Colourless oil; yield: 50%; R_f (CH_2Cl_2/AcOEt 10:0.15): 0.3; anal $C_{53}H_{72}N_8O_{16}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.30–7.15 (m, 8H, Ph); 6.67–6.60 (m, 8H, Ph); 3.27–3.22 (t, J = 6.4 Hz, 4H, $2CH_2$); 3.12–3.09 (m, 8H, $4CH_2$); 2.82–2.76 (m, 10H, $5CH_2$); 2.56 (s, 6H, $2CH_3$); 2.53–2.49 (m, 4H exch D_2O and 4H, 4NH and $2CH_2$); 1.83–1.79 (qt, J = 6.4 Hz, 4H, $2CH_2$); 1.60–1.57 (qt, J = 7.3 Hz, 4H, $2CH_2$); 1.37–1.36 (m, 4H, $2CH_2$). *m/z*: 780.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino)-phenylthio)phenyl)-1,5-pentylenediamine ***6b***. Colourless oil; yield: 56%; R_f (CH_2Cl_2/AcOEt 10:0.15): 0.3; anal $C_{53}H_{72}N_8O_{16}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.84–7.44 (m, 2H, Ph); 7.35–7.28 (m, 2H, Ph); 7.04–6.99 (m, 2H, Ph); 6.73–6.64 (m, 4H, Ph); 6.41–6.31 (m, 6H, Ph); 3.24–3.19 (t, J = 6.4 Hz, 4H, $2CH_2$); 3.06–3.01 (t, J = 6.8 Hz, 4H, $2CH_2$); 2.80–2.60 (m, 4H, $2CH_2$); 2.44–2.30 (m, 4H exch D_2O and 16H, 4NH and $8CH_2$); 2.29 (s, 6H, $2CH_3$); 1.76–1.70 (m, 4H, $2CH_2$); 1.61–1.56 (qt, J = 7.4 Hz, 4H, $2CH_2$); 1.50–1.40 (m, 2H, CH_2). *m/z*: 780.

N,N'-(4-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino)-phenylthio)phenyl)-1,5-pentylenediamine ***6c***. Colourless oil; yield: 59%; R_f (CH_2Cl_2/AcOEt 10:0.15): 0.3; anal $C_{53}H_{72}N_8O_{16}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.37–7.31 (m, 2H, Ph); 7.25–7.18 (m, 2H, Ph); 7.13–7.08 (m, 4H, Ph); 6.66–6.59 (m, 4H, Ph); 6.56–6.50 (m, 4H, Ph); 3.25–3.19 (m, 4H, $2CH_2$); 3.13–3.03 (m, 8H, $4CH_2$); 2.83–2.71 (m, 8H, $4CH_2$); 2.58 (s, 6H, $2CH_3$); 2.51–2.43 (m, 4H exch D_2O and 4H, 4NH and $2CH_2$); 2.39–2.34 (t, J = 6.8 Hz, 4H, $2CH_2$); 1.82–1.75 (m, 4H, $2CH_2$); 1.69–1.61 (qt, J = 7.1 Hz, 4H, $2CH_2$); 1.53–1.51 (m, 2H, CH_2). *m/z*: 780.

N,N'-(2-(2-(N-(3-Dimethylaminopropyl)amino)phenylthio)-phenyl)-1,5-pentylenediamine ***6'a***. Colourless oil; yield: 60%; R_f (CH_2Cl_2/AcOEt 10:0.15): 0.3; anal $C_{43}H_{58}N_6O_8S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.32–7.16 (m, 8H, Ph); 6.72–6.61 (m, 8H, Ph); 4.60 (s, 4H exch D_2O, 4NH); 3.25–3.14 (m, 8H, $4CH_2$); 2.75–2.69 (m, 4H, $2CH_2$); 2.52 (s, 12H, $4CH_3$); 2.07–1.98 (m, 6H, $3CH_2$); 1.68–1.62 (qt, J = 7.3 Hz, 4H, $2CH_2$). *m/z*: 670.

N,N'-(3-(2-(N-(3-Dimethylaminopropyl)amino)phenylthio)-phenyl)-1,5-pentylenediamine ***6'b***. Colourless oil; yield: 60%; R_f (CH_2Cl_2/AcOEt 10:0.15): 0.3; anal $C_{43}H_{58}N_6O_8S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.54–7.46 (m, 2H, Ph); 7.39–7.29 (m, 2H, Ph); 7.07–7.00 (m, 2H, Ph); 6.77–6.65 (m, 4H, Ph); 6.45–6.34 (m, 6H, Ph); 4.90 (s, 2H exch D_2O, 2NH); 3.75 (s, 2H exch D_2O, 2NH); 3.24–3.17 (m, 4H, $2CH_2$); 3.07–3.01 (t, J = 6.9 Hz, 4H, $2CH_2$); 2.67–2.61 (m, 4H, $2CH_2$); 2.48 (s, 12H, $4CH_3$); 2.32–2.27 (t, J = 6.9 Hz, 2H, CH_2); 2.03–1.94 (m, 4H, $2CH_2$); 1.76–1.71 (qt, J = 6.7 Hz, 4H, $2CH_2$). *m/z*: 670.

N,N'-(4-(2-(N-(3-Dimethylaminopropyl)amino)phenylthio)-phenyl)-1,5-pentylenediamine ***6'c***. Colourless oil; yield: 60%; R_f (CH_2Cl_2/AcOEt 10:0.15): 0.3; anal $C_{43}H_{58}N_6O_8S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.45–7.41 (m, 2H, Ph); 7.29–7.22 (m, 2H, Ph); 7.13–7.08 (m, 4H, Ph); 6.70–6.62 (m, 4H, Ph); 6.56–6.50 (m, 4H, Ph); 4.90 (s, 2H exch D_2O, 2NH); 3.80 (s, 2H exch D_2O, 2NH); 3.23–3.16 (t, J = 6.1 Hz, 4H, $2CH_2$); 3.12–3.07 (t, J = 6.9 Hz, 4H, $2CH_2$); 2.72–2.66 (m, 4H, $2CH_2$); 2.50 (s, 12H, $4CH_3$); 2.16–2.14 (m, 2H, CH_2); 2.04–1.92 (m, 4H, $2CH_2$); 1.68–1.60 (qt, J = 7.1 Hz, 4H, $2CH_2$). *m/z*: 670.

Compounds 7a–c
Compounds **7a–c** were synthesized from the corresponding **2a–c** according to the method described for **4a–c**.

2-(2-Amino)phenylthiophenylamine ***7a***. Colourless solid; yield: 75%; R_f (CH_2Cl_2/MeOH 9:1): 0.9; mp: 60 °C. ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.26–7.13 (m, 4H, Ph); 6.78–6.68 (m, 4H, Ph); 4.30 (s, 2H exch D_2O, NH_2); 3.73 (s, 2H exch D_2O, NH_2). *m/z*: 216.

3-(2-Amino)phenylthiophenylamine ***7b***. Colourless oil; yield: 80%; R_f (CH_2Cl_2/MeOH 9:1): 0.8. ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.23–7.15 (m, 2H, Ph); 6.85–6.69 (m, 4H, Ph); 6.56–6.41 (m, 2H, Ph); 4.39 (s, 2H exch D_2O, NH_2); 3.73 (s, 2H exch D_2O, NH_2). *m/z*: 216.

4-(2-Amino)phenylthiophenylamine ***7c***. Colourless oil; yield: 92%; R_f (CH_2Cl_2/MeOH 9:1): 0.75. ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.38–7.33 (m, 1H, Ph); 7.19–7.07 (m, 3H, Ph); 6.84–6.59 (m, 4H, Ph); 4.32 (s, 2H exch D_2O, NH_2); 3.74 (s, 2H exch D_2O, NH_2). *m/z*: 216.

Compounds ***8a–c***
Compounds **8a–c** were synthesized from the corresponding **7a–c** according to the method described for **5a–c**.

N-(2-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)amino)-phenylthio)phenyl)-3-(N-methylpiperazinyl)propylamide ***8a***. Yellow solid; yield: 72%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.2, RH positive; mp: 84 °C; anal $C_{36}H_{48}N_6O_{18}S$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.43 (s, 2H exch D_2O, 2NH); 8.11–8.07 (m, 2H, Ph); 7.35–7.29 (m, 2H, Ph); 7.17–7.04 (m, 4H, Ph); 2.67–2.44 (m, 24H, $12CH_2$); 2.29 (s, 3H, CH_3); 2.25 (s, 3H, CH_3). *m/z*: 524.

N-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)amino)-phenylthio)phenyl)-3-(N-methylpiperazinyl)propylamide ***8b***. Light-orange oil; yield: 64%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.2, RH positive; anal $C_{36}H_{48}N_6O_{18}S$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 11.00 (s, 1H exch D_2O, NH); 10.47 (s, 1H exch D_2O, NH); 8.31–8.28 (m, 1H, Ph); 7.59–7.53 (m, 1H, Ph); 7.45–7.39 (m, 2H, Ph); 7.33–7.31 (m, 1H, Ph); 7.24–7.10 (m, 2H, Ph); 6.83–6.79 (m, 1H, Ph); 2.69–2.42 (m, 24H, $12CH_2$); 2.29 (s, 3H, CH_3); 2.19 (s, 3H, CH_3). *m/z*: 524.

N-(4-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)amino)-phenylthio)phenyl)-3-(N-methylpiperazinyl)propylamide ***8c***. Light-orange oil; yield: 50%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.15, RH positive; anal $C_{36}H_{48}N_6O_{18}S$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 11.12 (s, 1H exch D_2O, NH); 10.35 (s, 1H exch D_2O, NH); 8.24–8.20 (m, 1H, Ph); 7.56–7.51 (m, 2H, Ph); 7.40–7.32 (m, 2H, Ph); 7.19–7.06 (m, 3H, Ph); 2.74–2.47 (m, 24H, $12CH_2$); 2.31 (s, 3H, CH_3); 2.23 (s, 3H, CH_3). *m/z*: 524.

Compounds ***9a–c***
Compounds **9a–c** were synthesized from the corresponding **8a–c** according to the method described for **6a–c**.

N-(2-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino)-phenylthio)phenyl)-N'-methylpiperazinylpropylenediamine ***9a***. Light-yellow oil; yield: 50%; R_f (CH_2Cl_2/AcOEt 10:0.2): 0.7; anal $C_{36}H_{52}N_6O_{16}S$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.22–7.17 (m, 4H, Ph); 6.69–6.59 (m, 4H, Ph); 3.26–3.22 (t, J = 6.4 Hz, 4H, $2CH_2$); 3.10–3.02 (m, 4H, $2CH_2$); 2.84–2.74 (m, 8H, $4CH_2$); 2.58 (s, 6H, $2CH_3$); 2.56–2.41 (m, 2H exch D_2O and 8H, 2NH and $4CH_2$); 1.86–1.75 (qt, J = 6.5 Hz, 4H, $2CH_2$). *m/z*: 496.

N-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino)-phenylthio)phenyl)-N'-methylpiperazinylpropylenediamine ***9b***. Light-yellow oil; yield: 50%; R_f (CH_2Cl_2/AcOEt 10:0.2): 0.7; anal $C_{36}H_{52}N_6O_{16}S$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.48–7.44 (m, 1H Ph); 7.35–7.29 (m, 1H, Ph); 7.04–6.98 (m, 1H, Ph); 6.74–6.62 (m, 2H, Ph); 6.41–6.32 (m, 3H, Ph); 3.25–3.01 (m, 8H, $4CH_2$); 2.81–2.68 (m, 8H, $4CH_2$); 2.62 (s, 3H, CH_3); 2.58 (s, 3H, CH_3); 2.55–2.39 (m, 2H exch D_2O and 8H, 2NH and $4CH_2$); 1.81–1.69 (qt, J = 6.4 Hz, 4H, $2CH_2$). *m/z*: 496.

N-(4-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino)-phenylthio)phenyl)-N-methylpiperazinylpropylenediamine ***9c***. Light-yellow oil; yield: 60%; R_f (CH_2Cl_2/AcOEt 10:0.2): 0.65; anal $C_{36}H_{52}N_6O_{16}S$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.37–7.30 (m, 1H, Ph); 7.25–7.17 (m, 1H, Ph); 7.14–7.08 (m, 2H, Ph); 6.67–6.50 (m, 4H, Ph); 3.24–3.06 (m, 8H, $4CH_2$); 2.86–2.72 (m, 8H, $4CH_2$); 2.62 (s, 3H, CH_3); 2.58 (s, 3H, CH_3); 2.56–2.38 (m, 2H exch D_2O and 8H, 2NH and $4CH_2$); 1.83–1.74 (qt, J = 6.5 Hz, 4H, $2CH_2$). *m/z*: 496.

N,N'-(3-(2-Nitro)phenylthiophenyl)-1,5-heptylenediamide ***10b***
Compound **10b** was synthesized from **2b** and pimeloyl dichloride according to the method described for **3b**; orange solid; yield: 93%; R_f (CH_2Cl_2/MeOH 9:1): 0.8, RH positive; mp: 58 °C. ^{1}H-NMR (CD_3COCD_3) δ ppm: 9.12 (s, 2H exch D_2O, 2NH); 8.26–8.20 (m, 2H, Ph); 8.01–7.99 (m, 2H, Ph); 7.84–7.80 (m, 2H, Ph); 7.58–7.27 (m, 8H, Ph); 7.06–7.01 (m, 2H, Ph); 2.43–2.37 (t, J = 7.3 Hz, 4H, $2CH_2$); 1.76–1.60 (qt, J = 7.6 Hz, 4H, $2CH_2$); 1.48–1.38 (m, 2H, CH_2). *m/z*: 616.

N,N'-(3-(2-Amino)phenylthiophenyl)-1,5-heptylenediamide ***11b***
This compound was synthesized from **10b** according to the method described for **4a**; colourless oil; yield: 88%; R_f (CH_2Cl_2/MeOH 9:1): 0.5. ^{1}H-NMR (CD_3COCD_3) δ ppm: 9.16 (s, 2H exch D_2O, 2NH); 7.69–7.66 (m, 2H, Ph); 7.55–7.50 (m, 1H, Ph); 7.43 (m, 1H, Ph); 7.39–7.35 (m, 1H, Ph); 7.31–7.13 (m, 4H, Ph); 7.07–7.02 (m, 2H, Ph); 6.90–6.86 (m, 1H, Ph); 6.77–6.74 (m, 1H, Ph); 6.69–6.59 (m, 3H, Ph); 5.04 (s, 4H exch D_2O, $2NH_2$); 2.37–2.32 (m, 4H, $2CH_2$); 1.80–1.77 (m, 4H, $2CH_2$); 1.50–1.40 (m, 2H, CH_2). *m/z*: 556.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)-amino)phenylthio)phenyl)-1,5-heptylenediamide ***12b***
This compound was synthesized from **11b** according to the method described for **5a**; light-yellow solid; yield: 44%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.3, RH positive; mp: 55 °C; anal $C_{55}H_{68}N_8O_{20}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.30 (s, 2H exch D_2O, 2NH); 8.22–8.18 (m, 1H, Ph); 8.05 (s, 2H exch D_2O, 2NH); 7.60–7.53 (m, 4H, Ph); 7.48–7.42 (m, 2H, Ph); 7.34–7.31 (m, 1H, Ph); 7.24–7.06 (m, 6H, Ph); 6.86–6.80 (m, 2H, Ph); 2.58–2.37 (m, 28H, $14CH_2$); 2.21 (s, 6H, $2CH_3$); 1.75–1.65 (m, 4H, $2CH_2$); 1.41–1.38 (m, 2H, CH_2). *m/z*: 864.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino)-phenylthio)phenyl)-1,5-heptylenediamine ***13b***
This compound was synthesized from **12b** according to the method described for **6a**; colourless oil; yield: 54%; R_f (CH_2Cl_2/MeOH 10:0.5): 0.6; anal $C_{55}H_{76}N_8O_{16}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.47–7.46 (m, 2H, Ph); 7.34–7.31 (m, 2H, Ph); 7.03–7.00 (m, 2H, Ph); 6.73–6.67 (m, 4H, Ph); 6.40–6.32 (m, 6H, Ph); 3.25–3.21 (m, 4H, $2CH_2$); 3.05–3.02 (m, 2H exch D_2O and 8H, 2NH and $4CH_2$); 2.76–2.68 (m, 2H exch D_2O and 8H, 2NH and $4CH_2$); 2.58 (s, 6H, $2CH_3$); 2.42–2.39 (m, 4H, $2CH_2$); 1.77–1.72 (m, 6H, 3 CH_2); 1.59–1.55 (m, 4H, $2CH_2$); 1.40–1.37 (m, 8H, $4CH_2$). *m/z*: 808.

N,N'-(3-(2-Nitro)phenylthiophenyl)-1,5-heptylenediamide ***14b***
Malonyl dichloride (198 µL, 2.03 mmol, 1 equiv) was added dropwise, under N_2, to a cooled solution of **2b** (1 g, 4.06 mmol, 2 equiv) in 80 mL of ether and 326 µL of distilled pyridine (4.06 mmol, 2 equiv). After stirring the mixture for 1 h at 0 °C then 7 h at room temperature, ether was evaporated. The solid residue was treated with a CH_2Cl_2/1 M HCl mixture. The organic layer was separated, dried over $MgSO_4$, evaporated to dryness to yield **14b** (73%) as a yellow solid which was used without further purification; R_f (CH_2Cl_2/MeOH 9:1): 0.75, RH positive; mp: 68 °C. ^{1}H-NMR (CD_3COCD_3) δ ppm: 9.30 (s, 2H exch D_2O, 2NH); 8.26–8.20 (m, 2H, Ph); 8.05–8.03 (m, 2H, Ph); 7.85–7.81 (m, 2H, Ph); 7.59–7.34 (m, 8H, Ph); 7.10–7.02 (m, 2H, Ph); 3.60 (s, 2H, CH_2). *m/z*: 560.

N,N'-(3-(2-Amino)phenylthiophenyl)-1,5-propylenediamide ***15b***
This compound was synthesized from **14b** according to the method described for **4a**; colourless oil; yield: 80%; R_f (CH_2Cl_2/MeOH 9:1): 0.55, RH positive. ^{1}H-NMR (CD_3COCD_3) δ ppm: 9.72 (s, 2H exch D_2O, 2NH); 7.71–7.66 (m, 3H, Ph); 7.51–7.29 (m, 6H, Ph); 7.12–7.02 (m, 3H, Ph); 6.78–6.77 (m, 1H, Ph); 6.68–6.54 (m, 3H, Ph); 5.10 (s, 4H exch D_2O, $2NH_2$); 3.57 (s, 2H, CH_2). *m/z*: 500.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)-amino)phenylthio)phenyl)-1,5-propylenediamide ***16b***
This compound was synthesized from **15b** according to the method described for **5a**; light-orange oil; yield: 64%; R_f (CH_2Cl_2/MeOH 8.5:1.5): 0.45, RH positive; anal $C_{51}H_{60}N_8O_{20}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.43 (s, 2H exch D_2O, 2NH); 9.47 (s, 2H exch D_2O, 2NH); 8.26–8.22 (m, 1H, Ph); 7.61–7.52 (m, 3H, Ph); 7.44–7.32 (m, 7H, Ph); 7.23–7.12 (m, 4H, Ph); 6.88–6.85 (m, 1H, Ph); 3.51 (s, 2H, CH_2); 2.64–2.47 (m, 24H, $12CH_2$); 2.27 (s, 6H, $2CH_3$). *m/z*: 808.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino)-phenylthio)phenyl)-1,5-propylenediamine ***17b***
This compound was synthesized from **16b** according to the method described for **6a**; colourless oil; yield: 66%; R_f (CH_2Cl_2/MeOH 10:0.5): 0.3; anal $C_{51}H_{68}N_8O_{16}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.48–7.44 (m, 1H, Ph); 7.36–7.32 (m, 1H, Ph); 7.24–7.15 (m, 4H, Ph); 7.02–6.99 (m, 1H, Ph); 6.81–6.61 (m, 7H, Ph); 6.41–6.35 (m, 2H, Ph); 3.24–3.18 (m, 2H exch D_2O and 4H, 2NH and $2CH_2$); 3.11–3.07 (m, 6H, $3CH_2$); 2.84–2.76 (m, 4H, $2CH_2$); 2.71–2.69 (m, 2H, CH_2); 2.62 (s, 6H, $2CH_3$); 2.51–2.45 (m, 6H, $3CH_2$); 2.41–2.37 (m, 2H exch D_2O and 4H, 2NH and $2CH_2$); 1.76–1.69 (m, 8H, $4CH_2$). *m/z*: 752.

N-(3-Chloropropyl)-5-chloro-2-phenylthiophenylamine ***19***
3-Bromopropanoyl chloride (0.7 mL, 7 mmol) was added, dropwise, to a cooled solution of 5-chloro-2-phenylthiophenylamine **18** (1 g, 4.2 mmol) in 18 mL of ether and 0.7 mL of pyridine. The mixture was stirred for 2 h, then allowed to reach room temperature and was extracted successively with saturated NaCl solution, 1 M HCl and 2.5 M NaOH. The organic layer was separated, dried over $MgSO_4$ and evaporated to dryness to yield **19** (100%) as a colourless solid which was used without purification; R_f (petroleum ether/ether 10:3): 0.4, RH positive; mp: 91 °C; anal $C_{15}H_{13}BrClNOS$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 8.58–8.57 (m, 1H, Ph); 8.30 (s, 1H exch D_2O, NH); 7.59–7.56 (m, 1H, Ph); 7.31–7.12 (m, 6H, Ph); 3.63–3.58 (t, J = 6.5 Hz, 2H, CH_2); 2.88–2.83 (t, J = 6.5 Hz, 2H, CH_2). *m/z*: 370.1.

3-Bromo(N-(2-phenylthio-5-chloro)phenyl)propylamine ***20***
Commercial 1 M BH_3/THF solution (25 mL, 25 mmol) was added dropwise for 1 h under N_2 to a cooled solution of **19** (1.2 g, 3.2 mmol) in 3.3 mL of anhydrous THF. The mixture was stirred for 30 min and allowed to react for 2 h at room temperature. Excess BH_3 was neutralized by water at 0 °C and the solution was evaporated to dryness. The oily residue was treated with a CH_2Cl_2/brine mixture. The organic layer was evaporated and the oily residue was purified by thick-layer chromatography (SiO_2, petroleum ether/ether 10:2) to yield **20** (74%) as a colourless solid; R_f (petroleum ether/ether 10:2): 0.8; mp: 48 °C; anal $C_{15}H_{15}BrClNS$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.47–7.46 (m, 1H, Ph); 7.44–7.08 (m, 5H, Ph); 6.73–6.69 (m, 2H, Ph); 5.06 (s, 1H exch D_2O, NH); 3.44–3.26 (m, 4H, $2CH_2$); 2.11–2.02 (qt, J = 6.5 Hz, 2H, CH_2). *m/z*: 355.6.

General procedure for the substitution of ***20*** *by polyamines*
Polyamine (5 equiv) was added to a solution of **20** (450 mg, 1.26 mmol) in 25 mL of anhydrous THF. After refluxing the mixture for 15 h, the solvent was removed and the oily residue was treated with a CH_2Cl_2/brine mixture. Products of mono- and disubstitution were obtained as colourless oils by thick-layer chromatography (SiO_2, acetone/28% NH_4OH 9.5:0.5). Overall yields were around 55% with a typical ratio of 80:20, for mono/disubstituted products; 40% of the starting material **20** was recovered.

The characteristics of disubstituted products only are given here; TLC purifications were eluted with petroleum ether/ether (9.5:0.5).

1,3-(N,N'-(3-(N-(5-Chloro-2-phenylthio)phenyl)aminopropyl))-diaminopropane ***21***. R_f: 0.3; anal $C_{37}H_{42}Cl_2N_4O_8S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.43–7.09 (m, 12H, Ph); 6.69–6.66 (m, 4H, Ph); 3.50 (s, 4H exch D_2O, 4NH); 3.24–3.20 (m, 4H, $2CH_2$); 2.71–2.56 (m, 8H, $4CH_2$); 1.84–1.75 (m, 4H, $2CH_2$); 1.33–1.28 (m, 2H, CH_2). *m/z*: 625.9.

1,4-(N,N'-(3-(N-(5-Chloro-2-phenylthio)phenyl)aminopropyl))-diaminobutane ***22***. R_f: 0.3; anal $C_{38}H_{44}Cl_2N_4O_8S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.45–7.41 (m, 2H, Ph); 7.27–7.21 (m, 4H, Ph); 7.17–7.09 (m, 6H, Ph); 6.71–6.67 (m, 4H, Ph); 5.45 (s, 4H exch D_2O, 4NH); 3.30–3.25 (m, 4H, $2CH_2$); 2.63–2.57 (m, 8H, $4CH_2$); 1.94–1.84 (m, 4H, $2CH_2$); 1.78 (m, 4H, $2CH_2$). *m/z*: 639.9.

1,5-(N,N'-(3-(N-(5-Chloro-2-phenylthio)phenyl)aminopropyl))-diaminopentane ***23***. R_f: 0.3; anal $C_{39}H_{46}Cl_2N_4O_8S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.42–7.09 (m, 12H, Ph); 6.69–6.66 (m, 4H, Ph); 4.70 (s, 4H exch D_2O, 4NH); 3.26–3.22 (m, 4H, $2CH_2$); 2.68–2.63 (m, 8H, $4CH_2$); 1.97–1.93 (m, 4H, $2CH_2$); 1.72–1.68 (m, 4H, $2CH_2$); 1.45–1.41 (m, 2H, CH_2). *m/z*: 653.9.

1,6-(N,N'-(3-(N-(5-Chloro-2-phenylthio)phenyl)aminopropyl))-diaminohexane ***24***. R_f: 0.4; anal $C_{40}H_{48}Cl_2N_4O_8S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.42–7.08 (m, 12H, Ph); 6.69–6.64 (m, 4H, Ph); 5.62 (s, 2H exch D_2O, 2NH); 3.25–3.20 (m, 2H exch D_2O and 4H, 2NH and $2CH_2$); 2.64–2.53 (m, 8H, $4CH_2$); 1.83–1.76 (m, 4H, $2CH_2$); 1.53–1.30 (m, 8H, $4CH_2$). *m/z*: 667.4.

3-(4-Chloro-2-nitro)phenylthiophenylamine ***25b***
This compound was synthesized from **1b** and 2,5-dichloronitrobenzene according to the method described for **2b**; orange solid; yield: 38%; R_f (CH_2Cl_2): 0.6; mp: 94 °C. ^{1}H-NMR (CD_2Cl_2) δ ppm: 8.24–8.23 (m, 1H, Ph); 7.39–7.34 (m, 1H, Ph); 7.31–7.26 (m, 1H, Ph); 6.99–6.90 (m, 3H, Ph); 6.86–6.81 (m, 1H, Ph); 3.93 (s, 2H exch D_2O, NH_2). *m/z*: 280.5.

N,N'-(3-(4-Chloro-2-nitro)phenylthiophenyl)-1,5-pentylene-diamide ***26b***
This compound was synthesized from **25b** according to the method described for **3b**; yellow solid; yield: 68%; R_f (CH_2Cl_2/MeOH 9:1): 0.6, RH positive; mp: 145 °C. ^{1}H-NMR (DMSO-d_6) δ ppm: 10.25 (s, 2H exch D_2O, 2NH); 8.88–8.85 (m, 2H, Ph); 7.95–7.93 (m, 2H, Ph); 7.77–7.67 (m, 4H, Ph); 7.52–7.46 (m, 2H, Ph); 7.30–7.26 (m, 2H, Ph); 6.94–6.90 (m, 2H, Ph); 2.43–2.38 (m, 4H, $2CH_2$); 1.92–1.87 (qt, J = 7.2 Hz, 2H, CH_2). *m/z*: 657.

N,N'-(3-(2-Amino-4-chloro)phenylthiophenyl)-1,5-pentylene-diamide ***27b***
Aqueous HCl 35% (423 μL, 5.09 mmol, 2.5 equiv) was added dropwise to a solution of **26b** (1.4 g, 2.16 mmol, 1 equiv) and

iron powder (754 mg, 13.5 mmol, 6 equiv) in 15 mL of ethyl alcohol 95 °C. After refluxing the mixture for 1 h, iron was eliminated by filtration and the filtrate was evaporated to yield **27b** as a colourless solid (96%) which was used without further purification; R_f (CH_2Cl_2/MeOH 9:1): 0.55, RH positive; mp: 75 °C. ^{1}H-NMR (CD_3COCD_3) δ ppm: 9.23 (s, 2H exch D_2O, 2NH); 7.56–7.53 (m, 2H, Ph); 7.41–7.35 (m, 4H, Ph); 7.23–7.17 (m, 2H, Ph); 6.93 (m, 2H, Ph); 6.80–6.77 (m, 2H, Ph); 6.73–6.66 (m, 2H, Ph); 5.32 (s, 4H exch D_2O, $2NH_2$); 2.42–2.37 (t, J = 7 Hz, 4H, $2CH_2$); 1.99–1.94 (m, 2H, CH_2). *m/z*: 597.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)-amino-4-chloro)phenylthio) phenyl)-1,5-pentylenediamide ***28b***
This compound was synthesized from **27b** according to the method described for **5b**; colourless solid; yield: 59%; R_f (CH_2Cl_2/MeOH 7:3): 0.25, RH positive; mp: 75 °C; anal $C_{53}H_{62}Cl_2N_8O_{20}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.69 (s, 2H exch D_2O, 2NH); 8.44 (s, 2H exch D_2O, 2NH); 8.34–8.33 (m, 2H, Ph); 7.52–7.48 (m, 2H, Ph); 7.41–7.38 (m, 2H, Ph); 7.31 (m, 2H, Ph); 7.27–7.21 (m, 2H, Ph); 7.13–7.09 (m, 2H, Ph); 6.90–6.86 (m, 2H, Ph); 2.54–2.41 (m, 24H, $12CH_2$); 2.40–2.35 (t, J = 7 Hz, 4H, $2CH_2$); 2.24 (s, 6H, $2CH_3$); 2.05–2.00 (qt, J = 6.8 Hz, 2H, CH_2). *m/z*: 905.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino-4-chloro)phenylthio)phenyl)-1,5-pentylenediamide ***29b***
This compound was synthesized from **28b** according to the method described for **6b**; colourless oil; yield: 49%; R_f (CH_2Cl_2/MeOH 10:0.5): 0.35; anal $C_{53}H_{70}Cl_2N_8O_{16}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.40–7.37 (m, 2H, Ph); 7.05–6.99 (m, 2H, Ph); 6.70–6.62 (m, 4H, Ph); 6.41–6.30 (m, 6H, Ph); 5.60 (s, 2H exch D_2O, 2NH); 3.24–3.17 (m, 4H, $2CH_2$); 3.07–2.98 (m, 2H exch D_2O and 6H, 2NH and $3CH_2$); 2.78–2.63 (m, 8H, $4CH_2$); 2.57 (s, 6H, $2CH_3$); 2.42–2.36 (t, J = 6.5 Hz, 8H, $4CH_2$); 1.77–1.70 (qt, J = 6.4 Hz, 8H, $4CH_2$); 1.62–1.57 (qt, J = 7.1 Hz, 4H, $2CH_2$). *m/z*: 849.

3-(4-Bromo-2-nitro)phenylthiophenylamine ***30b***
This compound was synthesized from **1b** and 2,5-dibromonitrobenzene according to the method described for **2b**; orange solid; yield: 84%; R_f (CH_2Cl_2): 0.55; mp: 105 °C; ^{1}H-NMR (CD_2Cl_2) δ ppm: 8.38–8.37 (m, 1H, Ph); 7.52–7.48 (m, 1H, Ph); 7.32–7.26 (m, 1H, Ph); 6.96–6.82 (m, 4H, Ph); 3.93 (s, 2H exch D_2O, NH_2); *m/z*: 325.

N,N'-(3-(4-Bromo-2-nitro)phenylthiophenyl)-1,5-pentylenediamide ***31b***
This compound was synthesized from **30b** according to the method described for **3b**; orange solid; yield: 95%; R_f (CH_2Cl_2/MeOH 9:1): 0.5, RH positive; mp: 110 °C. ^{1}H-NMR (DMSO-d_6) δ ppm: 10.23 (s, 2H exch D_2O, 2NH); 8.41–8.40 (m, 2H, Ph); 7.94–7.92 (m, 2H, Ph); 7.83–7.73 (m, 4H, Ph); 7.52–7.46 (m, 2H, Ph); 7.30–7.26 (m, 2H, Ph); 6.87–6.83 (m, 2H, Ph); 2.43–2.37 (t, J = 7.4 Hz, 4H, $2CH_2$); 1.93–1.87 (qt, J = 7.8 Hz, 2H, CH_2). *m/z*: 746.

N,N'-(3-(2-Amino-4-bromo)phenylthiophenyl)-1,5-pentylenediamide ***32b***
This compound was synthesized from **31b** according to the method described for **27b**; colourless solid; yield: 100%; R_f (CH_2Cl_2/MeOH 9:1): 0.5, RH positive; mp: 55 °C. ^{1}H-NMR (DMSO-d_6) δ ppm: 9.91 (s, 2H exch D_2O, 2NH), 8.00–7.98 (m, 2H, Ph); 7.41–6.69 (m, 8H, Ph); 6.71–6.68 (m, 4H, Ph); 5.63 (s, 4H exch D_2O, $2NH_2$); 2.30–2.26 (m, 4H, $2CH_2$); 1.83– 1.79 (m, 2H, CH_2). *m/z*: 686.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyloxy)-amino-4-bromo)phenylthio)phenyl)-1,5-pentylenediamide ***33b***
This compound was synthesized from **32b** according to the method described for **5b**; colourless solid; yield: 62%; R_f (CH_2Cl_2/MeOH 7:3): 0.3, RH positive; mp: 70 °C; anal $C_{53}H_{62}Br_2N_8O_{20}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 10.65 (s, 2H exch D_2O, 2NH); 8.47 (s, 2H exch D_2O, 2NH); 7.52–7.48 (m, 13H, Ph); 6.89–6.86 (m, 3H, Ph); 2.64–2.34 (m, 20H, $7CH_2$ and $2CH_3$); 2.33–2.20 (m, 10H, $5CH_2$); 2.15–2.05 (m, 4H, $2CH_2$); 1.45–1.35 (m, 2H, CH_2). *m/z*: 994.

N,N'-(3-(2-(N-(3-(N-Methylpiperazinyl)aminopropyl)amino-4-bromo)phenylthio)phenyl)-1,5-pentylenediamide ***34b***
This compound was synthesized from **33b** according to the method described for **6b**; colourless solid; yield: 60%; R_f (CH_2Cl_2/MeOH 10:0.5): 0.35; mp: 50 °C; anal $C_{53}H_{70}Br_2N_8O_{16}S_2$ (C, H, N). ^{1}H-NMR (CD_2Cl_2) δ ppm: 7.33–7.30 (m, 2H, Ph); 7.05–6.99 (m, 2H, Ph); 6.85–6.76 (m, 4H, Ph); 6.42–6.29 (m, 6H, Ph); 3.83 (s, 4H exch D_2O, 4NH); 3.24–3.17 (m, 4H, $2CH_2$); 3.07–2.98 (m, 6H, $3CH_2$); 2.78–2.63 (m, 8H, $4CH_2$); 2.57 (s, 6H, $2CH_3$); 2.44–2.28 (m, 10H, $5CH_2$); 1.75–1.70 (qt, J = 6.4 Hz, 8H, $4CH_2$); 1.62–1.55 (qt, J = 7.2 Hz, 4H, $2CH_2$); 1.47–1.44 (qt, J = 6.4 Hz, 2H, CH_2). *m/z*: 938.

Assays for TR activity

Recombinant TR was produced from the SG5 *Escherichia coli* strain with the overproducing expression vector pIBITczTR. TR activity was measured at 21 °C in a 0.02 M Hepes buffer, pH 7.25 containing 0.15 M KCl, 1 mM EDTA and 0.2 mM NADPH with an enzyme concentration of 0.02 U mL^{-1}. The reaction was started by adding the enzyme and the absorbance decrease was followed at 340 nm. Inhibiting potency of the different compounds was evaluated by measuring IC_{50} in the presence of 57 μM of $T(S)_2$ and increasing concentrations of inhibitor (0–114 μM).

Inhibitory potencies of the compounds at four concentrations (from 0.3 to 10 μM) were also determined with regard to human glutathione reductase, in presence of 44 μM of GSSG, in 40 mM Hepes, 50 mM KCl and 1 mM EDTA, pH 7.4, and 180 μM of NADPH.

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