A General Approach to the Synthesis of Polyamine Linked-Monoindolylmaleimides, a New Series of Trypanothione Reductase Inhibitors

Laurence SALMON,[a] Valérie LANDRY,[a] Oleg MELNYK,[a] Louis MAES,[b] Christian SERGHERAERT,[a] and Elisabeth DAVIOUD-CHARVET*,[a]

Institut de Biologie de Lille - Institut Pasteur de Lille - URA CNRS 1309,[a] 1 rue du Professeur Calmette, BP 447, 59021 Lille Cedex, France and Janssen Research Foundation,[b] Turnhoutseweg 30, B-2340 Beerse, Belgium.
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A simplified approach to the synthesis of 2-polyamine linked-monoindolylmaleimides has been achieved, leading to a new series of trypanothione reductase inhibitors. The conditions of access to *N*,2-bis(polyamine)-3-monoindolylmaleimides and *N*,*N*′-bis(monoindolylmaleimide) polyamines are described. Measured inhibitory activities towards trypanothione reductase from *Tryanosoma cruzi* show the importance of both aromatic moieties and polyamine chains for trypanothione reductase recognition.

Key words trypanosomatid; trypanothione reductase; polyamine; *N*-alkylmaleimide; 2-alkylamino-3-(indol-3-yl)maleimide ; protein kinase C

New drugs are required for the treatment of Chagas' disease, caused by the trypanosomatid parasite *Trypanosoma cruzi*, in South America and in this connection, we were interested in the trypanothione system, which interacts with a large number of known trypanocidal compounds. The preponderant thiol in the cytosol of the parasite is trypanothione or N^1,N^8-bis(glutathionyl)-spermidine, ($T(SH)_2$),[1-3] which has been shown to be involved in the maintenance of the thiol–disulfide redox balance in the parasite, but is absent in the host. Its regeneration from trypanothione disulfide ($T(S)_2$) is ensured by the NADPH-dependent trypanothione reductase (TR), a flavoprotein analogue of the human glutathione reductase (GR) enzyme. Despite 41% homology in their primary structures, human GR and *T. cruzi* TR show almost total mutual discrimination toward their respective substrates.[4] TR, therefore, appears to be an ideal target for drugs that disrupt the natural defences of the parasite without interfering with host enzymes, and several inhibitors based upon the structure of $T(S)_2$ have already been described.[5-7] Many ammonium functional side-chain-substituted compounds were also described as effective and selective inhibitors of TR *versus* GR.[8-16] The recognition of these ligands is based upon cation–π bonding and ionic interactions, which are responsible for the discrimination in substrate binding in the parasite TR and human GR, and include the interaction of Trp21 and Glu18, respectively, with the ammonium groups of ligands.[17] Given the limited structural data on ammonium-ligand–protein complexes, rational design strategies seemed inapplicable. For this reason, we focused upon two strategies. First, we developed a high-throughput assay to measure TR activity in microplates to screen new lead structures as potential TR inhibitors.[18] Secondly, an algorithm allowing the prediction of the binding affinity of ligands to TR was developed in our laboratory and used for the virtual screening of a data base of commercial and available molecules.[19] Based on this colorimetric TR assay, in addition to the phenothiazines and the 2-aminodiphenylsulfide series, we selected several 2-alkylamino-3-(indol-3-yl)maleimides from among a library of potential PKC inhibitors, synthesized in our laboratory.[20] Compound **4a** was chosen as a lead for TR inhibition ($IC_{50}=38$ μM), while it displays a weak PKC-inhibitory activity.

In our structure–activity studies on TR inhibitors among amino-2-diphenylsulfides,[10,13,15,16] like 1,4-naphthoquinones[8] and spermine- and spermidine-linked aromatic compounds,[12,15,16] we and others have found that two ammonium side chains are important for potent and selective TR recognition, since they provide additional cationic interactions in the active site of TR.[13,15,16] Moreover, we have shown by molecular modelling[13,19] that the hydrophobic pocket in the active site of TR can accomodate a bulky aromatic entity or a second hydrophobic moiety, which is consistent with the increased inhibitory potency of bis(aromatic)–bis(polyamine) derivatives towards TR.[11,12,15,16] These features prompted us to prepare from the lead structure **4a**, analogous derivatives and bis derivatives with alkyl side-chains containing varying numbers of nitrogens to increase both the hydrophobic and cationic character of the molecule, respectively. In this paper, we describe the synthesis of *N*,2-bis(polyamine)monoindolylmaleimides **5a—c**, and *N*,*N*′-bis(monoindolylmaleimide)polyamines **6a**, **b**, as potential TR inhibitors. In addition, an improved and simplified access to new 2-alkylamino-3-(indol-3-yl)maleimides **4a—c** is reported.

Results

Chemistry In the course of our studies of the synthesis of analogues of **4a**, we required the most direct strategy and highest yielding synthesis for a number of polycationic chain-substituted monoindolylmaleimides. We have already described a general method for the preparation of a series of monoindolylmaleimide derivatives.[20] This route involved the condensation of an indole Grignard reagent with an *N*-trityl-2,3-dibromomaleimide, protection of the indolic nitrogen by a *tert*-butyloxycarbonyl group and then addition of the polyamine with elimination of HBr. Since

* To whom correspondence should be addressed.

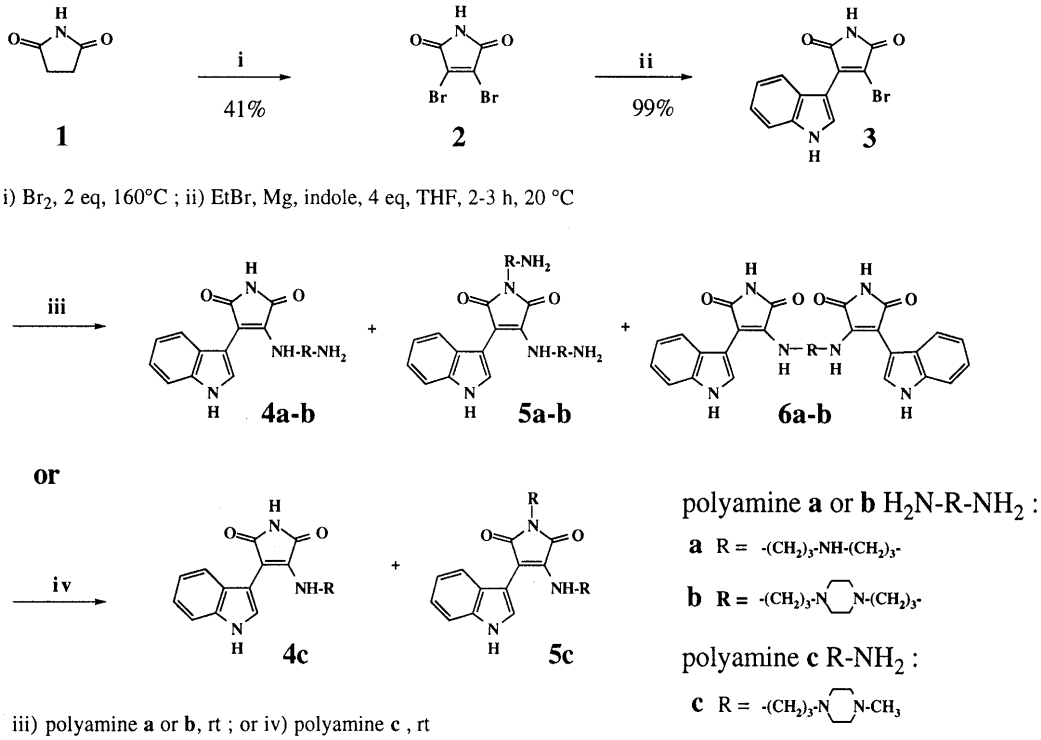

Chart 1. Synthesis of Polyamine Linked-Monoindolylmaleimide Derivatives **4**—**6**

product isolation proved difficult in the case of polyamines (tri- or tetra-amines), a solid-phase process was also considered by linking maleimidyl nitrogen to a 2-chlorotrityl chloride resin or a trityl chloride resin,[21] in analogy with our previously described protection of 2,3-dibromomaleimide **2** with a trityl group.[20] However, protection of the 2-bromo-3-indolylmaleimide **3** with a trityl group did not occur either in a solid matrix, or in a homogenous phase. Therefore, we attempted to simplify the synthesis by subtracting the protection steps of maleimidyl and indolic nitrogens (Chart 1).

2,3-Dibromomaleimide **2** was prepared from succinimide by a previously described procedure.[22] 2-Bromo-3-indolylmaleimide **3** was obtained in 99% yield by condensation of 4 eq of indole-MgBr with 2,3-dibromomaleimide **2** in tetrahydrofuran (THF), with slight modifications applied to the reported general method.[23] The formation of bisindolylmaleimides, which is known to be solvent-dependent, was not observed in THF. The synthesis of *N*-substituted maleimides using amines as starting materials has already been reported *via* maleamic acid cyclization, but with moderate yield.[24] The conjugated addition of the polyamine to the bromo derivative **3** followed by the elimination of HBr was therefore examined. At this stage of the synthesis, the primary amine of the polyamines **a**—**c** could be expected to attack 2-bromo-3-indolylmaleimide **3** at two distinct positions, leading to 2-alkylamino-3-(indol-3-yl)maleimides **4** *via* elimination of HBr, and to imide derivatives **5** *via* an alkylamino-deamination reaction.

In THF as the solvent (Table 1, entry 1), alkylamino-deamination reaction occurred, leading to the formation of **5a**—**c** (yield 31—38%), which was disfavored when THF was replaced with dimethylformamide (DMF) (Table 1, entries 2—4). The synthesis of **4a**—**c** was optimized

Table 1. Conditions of Synthesis of **4**, **5**, and **6** in THF and in DMF

Entry	Polyamine (eq.)	Base (eq.)	[3] (mM)	Ratio[a] **3**/**4**/**5**/**6**	Isolated[b] yield (%)
1	**c** (5) in THF	—	14	nd	**4c**: 58% **5c**: 38%
2	**a** (2) in DMF	—	14	86/13/<1/1	nd
3	**a** (5) in DMF	—	14	4/94/<1/2	**4a**: 78%
4	**b** (5) in DMF	—	14	7/94/<1/2	**4b**: 79%
5	**a** (0,5) in DMF	NEt_3 (5)	275	44/32/<1/24	**6a**: 25%
6	**b** (0,5) in DMF	NEt_3 (5)	275	50/28/<1/22	**6b**: 30%

All reactions were stopped after 2 d, except for entries 3, 4 which were stopped after 24 h, and worked up by standard extractive methods. *a*) Ratios were estimated by measuring the peak areas obtained by HPLC, and corrected for the presence of 2 chromophores in **6**. HPLC conditions: Nucleosil cyanopropyl column (4.6 × 300 mm); eluent A, 0.05% TFA in water; eluent B, 0.05% TFA, 80% CH_3CN in water. Elution: eluent A for 5 min, then linear gradient 0 to 100% B over 30 min, 0.5 ml·min^{-1}, detection at 254 nm. *b*) After PTLC. nd: not determined.

(yield 78—79%) in DMF by using a large excess of the polyamine **a**—**c** to ensure the complete conversion of the starting bromo derivative **3** (entries 2, 3, 4). The solvent-dependent effect upon the formation of **5** was also examined in more detail. Changing the reaction conditions (addition of a base, or heating the reaction medium) did not increase the yield of **5** in THF or in DMF (data not shown). As **4a**, **b** and **5a**, **b** had exactly the same *Rf* values in the classical TLC solvent systems (acetone/NH_4OH, 90/10 to 98/2), it was necessary to introduce 0.26 M ammonium salt in the following migration solvent system (THF/AcOH/H_2O: 30/15/5) to check the reactions, otherwise it was not possible to separate the two compounds. The synthesis of **6** was achieved by decreasing the amount of polyamine and increasing the concentrations of compound **3** and NEt_3 (entries 5, 6).

Biochemistry Inhibitory potency of the different compounds toward parasitic TR was evaluated by measuring

Table 2. Inhibitory Activities of Compounds **4**—**6** towards TR from *T. cruzi*

Compound (57 μM)	IC_{50} (μM)
4a	38
4b	29
4c	>57
5a	26
5b	5.4
5c	>57
6a	>57
6b	16
Clomipramine	12

$[TS_2]=57\,\mu M$; $[E]=0.02\,U\cdot ml^{-1}$.

the initial velocities of the disappearance of NADPH at 340 nm in the presence of different concentrations of inhibitor, 57 μM $T(S)_2$ and TR. The IC_{50} values are given in Table 2 and compared with that of clomipramine, a known TR inhibitor. The results reveal that: i) monoindolylmaleimides **4** are weak inhibitors of TR, ii) addition of a second polyamine chain or addition of a second hydrophobic moiety increases the inhibitory potency with the same polyamine chain **b**. Compound **5b** is thus slightly more active than clomipramine. We checked that the maleimide derivatives **4**—**6** did not inhibit human GR by conducting an assay under similar conditions to those described for TR.

The compounds were also tested *in vitro* on *Trypanosoma cruzi* and *Trypanosoma brucei* trypomastigotes, and *Leishmania infantum* promastigotes.[13] Within the drug concentration range of 1.56—12.5 μM, all of them were inactive or cytotoxic, even the best TR inhibitors **5b** and **6b**. We have therefore terminated our investigation of this series. Nevertheless, the structure–activity relationships which are reported in this paper for this new series of TR inhibitors show the importance of both aromatic moieties and polyamine chains as a general feature for TR recognition.

Further studies for development of more potent and selective TR inhibitors are ongoing in order to find new potential trypanocidal drugs.

Experimental

All reactions were monitored by TLC (acetone/NH_4OH : 90/10 to 98/2; and 0.26 M CH_3COONH_4 in THF/AcOH/H_2O: 30/15/5) on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent. ^1H- and ^{13}C-spectra were obtained using a Brucker 300 MHz spectrometer; coupling constants are expressed in Hz and chemical shifts in ppm relative to TMS as an internal standard. Mass spectra were recorded on a time-of-flight plasma desorption mass spectrometer (TOF-PDMS) using a californium source.

General Procedure for the Synthesis of 2-Alkylamino-3-(indol-3-yl)maleimide 4 in DMF A solution of 2-bromo-3-indolylmaleimide **3** (0.5 g, 1.7 mmol) in dry DMF (122.5 ml) was added dropwise to a solution of the appropriate polyamine (8.6 mmol in 2.5 ml DMF) for 45 min at room temperature under argon. The mixture was stirred for 24 h at room temperature, then neutralized with aqueous AcOH (1 N) to pH 6.5. After evaporation of the solvent, the residue was dissolved in EtOH and subjected to preparative TLC for purification. Compounds **4** were obtained as yellow oils (yield 78—79%) and were tested for TR inhibition in the base form.

General Procedure for the Synthesis of *N*,2-Bis(alkylamino)-3-indolylmaleimide 5 in THF A solution of 2-bromo-3-indolylmaleimide **3** (0.3 g, 1.0 mmol) in dry THF (70 ml) was added dropwise to a solution of the appropriate polyamine (5.0 mmol in 5 ml THF) for 1 h at room temperature under argon. The mixture was stirred for 48 h at room temperature. After evaporation of the solvent, the residue was dissolved in EtOH and subjected to preparative TLC then preparative HPLC. Compounds **4** and **5** were obtained as yellow oils (yields 58% and 38% respectively) and were tested for TR inhibition in the base form.

Spectral Data for TR Inhibitors in the Series 4a: Yield, 78% (0.45 g). TOF-PDMS (m/z) = 341 (M^+), 297. ^1H-NMR (DMSO-d_6): 11.30 (s, 1H, exchanged in D_2O, NH_{indole}), 7.38 (d, 1H, J=8.0 Hz, H_7), 7.35 (d, 1H, J=8.0 Hz, H_4), 7.32—7.25 (m, 2H, H_2, CO–C–NH, exchanged in D_2O), 7.09 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_6), 6.99 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_5), 3.06 (q, 2H, J=6.0 Hz, CH_2), 2.73—2.64 (m, 2H, CH_2), 2.38—2.28 (m, 2H, CH_2), 2.15—2.05 (m, 2H, CH_2), 1.55—1.47 (m, 2H, CH_2), 1.38—1.28 (m, 2H, CH_2). ^{13}C-NMR (DMSO-d_6): 174.3, 169.9, 144.5, 136.4, 129.3, 126.7, 121.9, 120.4, 119.6, 112.3, 105.1, 92.9, 48.2, 47.7, 42.5, 38.5, 28.5, 27.8.

4b: Yield, 79% (0.56 g). TOF-PDMS (m/z) = 410 (M^+), 368, 282. ^1H-NMR (DMSO-d_6, T = 340 °K): 11.05 (s, 1H, exchanged in D_2O, NH_{indole}), 7.39 (d, 1H, J=8.0 Hz, H_7), 7.37 (d, 1H, J=8.0 Hz, H_4), 7.25 (s, 1H, H_2), 7.09 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_6), 6.99 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_5), 6.95 (s large, 1H, exchanged in D_2O, CO–C–NH–), 3.25 (qt, 2H, J=7.0 Hz, CH_2), 3.15—3.03 (m, 4H, CH_2, NH_2, exchanged in D_2O), 2.39—2.15 (m, 10H, $4CH_{2\text{-piperazine}}$, CH_2), 2.01 (t, 2H, J=6.0 Hz, CH_2), 1.64 (t, 2H, J=7.0 Hz, CH_2), 1.39 (qt, 2H, J=7.0 Hz, CH_2). ^{13}C-NMR (DMSO-d_6): 174.0, 169.9, 144.5, 136.6, 129.3, 126.6, 121.9, 120.4, 119.6, 112.3, 105.2, 92.9, 56.6, 56.5, 53.6, 53.5, 42.8, 42.7, 27.1, 27.0.

4c: Yield, 58% (0.22 g). TOF-PDMS (m/z) = 367 (M^+), 268. ^1H-NMR (DMSO-d_6, T = 340 °K): 11.03 (s, 1H, exchanged in D_2O, NH_{indole}), 7.39 (d, 1H, J=8.0 Hz, H_7), 7.37 (d, 1H, J=8.0 Hz, H_4), 7.25 (d, 1H, J=2.5 Hz, H_2), 7.10 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_6), 6.99 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_5), 6.94 (t, 1H, J=6.0 Hz, exchanged in D_2O, CO–C–NH–), 3.05 (q, 2H, J=7.0 Hz, NH–C$\underline{H}_2$), 2.18—2.10 (m, 11H, CH_3, $4CH_{2\text{-piperazine}}$), 1.99 (t, 2H, J=4.0 Hz, NH–CH_2–CH_2–C$\underline{H}_2$), 1.38 (qt, 2H, J=8.0 Hz, NH–CH_2–C$\underline{H}_2$). ^{13}C-NMR (DMSO-d_6): 174.3, 169.9, 144.5, 136.5, 129.3, 126.7, 121.9, 120.4, 119.7, 112.3, 105.1, 92.7, 56.4, 55.3, 53.2, 46.6, 42.6, 26.9.

5a: Yield, 31% (0.29 g). TOF-PDMS (m/z) = 455 (M^+), 411. ^1H-NMR (DMSO-d_6): 11.20 (s, 1H, exchanged in D_2O, NH_{indole}), 7.38 (dd, 1H, J=8.0, 1.0 Hz, H_7), 7.35 (dd, 1H, J=8.0, 1.0 Hz, H_4), 7.30—7.25 (m, 2H, H_2, CO–C–NH, exchanged in D_2O), 7.09 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_6), 6.99 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_5), 3.44—3.21 (m, 6H, exchanged in D_2O, $2NH_2$, 2NH), 2.79 (q, 2H, J=7.0 Hz, CH_2), 2.28 (t, 2H, J=7.0 Hz, N–CH_2), 2.22—2.05 (m, 12H, $6CH_2$), 1.95 (qt, 2H, J=6.0 Hz, CH_2), 1.64 (qt, 2H, J=6.5 Hz, CH_2), 1.33—1.23 (m, 4H, $2CH_2$). ^{13}C-NMR (DMSO-d_6): 174.3, 169.9, 144.5, 136.5, 129.5, 126.7, 121.9, 120.4, 119.6, 112.3, 105.0, 93.5, 56.8, 55.9, 53.4, 53.3, 53.2, 42.7, 42.0, 38.7, 26.2, 24.8.

5b: Yield, 31% (0.38 g). TOF-PDMS (m/z) = 593 (M^+), 496, 456. ^1H-NMR (DMSO-d_6, T = 340 °K): 11.29 (s, 1H, exchanged in D_2O, NH_{indole}), 7.41 (d, 1H, J=8.0 Hz, H_7), 7.37 (d, 1H, J=8.5 Hz, H_4), 7.29—7.23 (m, 2H, H_2, CO–C–NH, exchanged in D_2O), 7.11 (dd, 1H, J=8.0, 7.0 Hz, H_6), 7.00 (dd, 1H, J=8.0, 7.0 Hz, H_5), 3.51 (q, 2H, J=6.5 Hz, NH–C$\underline{H}_2$), 3.29—2.97 (m, 6H, CH_2, $2NH_2$, exchanged in D_2O), 2.67—2.51 (m, 16H, $8CH_{2\text{-piperazine}}$), 2.52 (t, 2H, J=6.5 Hz, N–CH_2), 2.36 (t, 2H, J=7.0 Hz, CH_2), 2.25 (qt, 2H, J=6.5 Hz, CH_2), 1.81—1.73 (m, 2H, CH_2), 1.67 (qt, 2H, J=7.0 Hz, CH_2), 1.60—1.45 (m, 6H, $3CH_2$), 1.44—1.33 (m, 4H, $2CH_2$). ^{13}C-NMR (DMSO-d_6): 173.2, 168.5, 144.3, 136.5, 129.3, 126.8, 121.9, 120.4, 119.7, 112.3, 104.9, 91.5, 48.6, 47.3, 42.6, 40.3, 40.1, 36.3, 32.6, 32.4, 32.2, 32.1, 29.9, 29.6, 29.4.

5c: Yield, 38% (0.20 g). TOF-PDMS (m/z) = 507 (M^+), 393. ^1H-NMR (DMSO-d_6, T = 340 °K): 11.25 (s, 1H, exchanged in D_2O, NH_{indole}), 7.44 (t, 1H, exchanged in D_2O, J=6.0 Hz, CO–C–NH–), 7.39 (d, 1H, J=8.0 Hz, H_7), 7.34 (d, 1H, J=8.0 Hz, H_4), 7.28 (d, 1H, J=2.5 Hz, H_2), 7.09 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_6), 6.98 (ddd, 1H, J=8.0, 7.0, 1.0 Hz, H_5), 3.44 (t, 2H, J=6.5 Hz, N–CH_2), 3.07 (q, 2H, J=6.5 Hz, CH_2), 2.81 (t, 2H, J=6.5 Hz, CH_2), 2.41—2.20 (m, 16H, $8CH_{2\text{-piperazine}}$), 2.14 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 1.93 (t, 2H, J=6.5 Hz, CH_2), 1.68 (qt, 2H, J=6.5 Hz, CH_2), 1.34 (qt, 2H, J=6.5 Hz, CH_2). ^{13}C-NMR (DMSO-d_6): 173.2, 166.5, 144.3, 136.5, 129.3, 126.7, 121.9, 120.4, 119.7, 112.3, 105.2, 90.8, 56.6, 55.5, 53.3, 53.2, 46.2, 42.8, 36.7, 35.5, 26.8, 26.1.

6a: Yield, 25% (38 mg). TOF-PDMS (m/z) = 551 (M^+). ^1H-NMR (DMSO-d_6): 11.29 (s, 2H, exchanged in D_2O, $2NH_{indole}$), 10.40 (s, 2H,

exchanged in D_2O, $2NH_{maleimide}$), 7.38 (dd, 2H, $J=8.0$, 1.5 Hz, $2H_7$), 7.32 (dd, 2H, $J=8.0$, 1.5 Hz, $2H_4$), 7.31 (d, 2H, $J=2.0$ Hz, $2H_2$), 7.09—7.03 (m, 4H, $2H_6$, 2CO–C–NH, exchanged in D_2O), 6.95 (ddd, 2H, $J=8.0$, 7.0, 1.5 Hz, $2H_5$), 3.09 (q, 4H, $J=6.5$ Hz, 2NH–C$\underline{H}_2$), 2.85 (s large, 1H, exchanged in D_2O, NH), 2.22 (q, 4H, $J=7.5$ Hz, C$\underline{H}_2$–NH–C$\underline{H}_2$), 1.37—1.27 (m, 4H, $2CH_2$–C$\underline{H}_2$–CH_2). ^{13}C-NMR (DMSO-d_6): 174.3, 169.9, 144.5, 136.4, 129.3, 127.7, 122.0, 121.4, 119.6, 112.4, 105.0, 92.9, 45.0, 40.4, 26.9.

6b: Yield, 30% (52 mg). TOF-PDMS (m/z)=620 (M^+). ^{1}H-NMR (DMSO-d_6): 11.19 (s, 2H, exchanged in D_2O, $2NH_{indole}$), 10.31 (s, 2H, exchanged in D_2O, $2NH_{maleimide}$), 7.37 (dd, 2H, $J=8.0$, 1.0 Hz, $2H_7$), 7.34 (dd, 2H, $J=8.0$, 1.0 Hz, $2H_4$), 7.24 (t, 2H, exchanged in D_2O, $J=6.0$ Hz, 2CO–C–N$\underline{H}$–), 7.17 (d, 2H, $J=2.5$ Hz, $2H_2$), 7.08 (ddd, $J=8.0$, 7.0, 1.0 Hz, $2H_6$), 6.98 (ddd, 2H, $J=8.0$, 7.0, 1.0 Hz, $2H_5$), 3.01 (q, 4H, $J=6.5$ Hz, 2NH–C$\underline{H}_2$), 2.01—1.77 (m, 12H, 2NH–$(CH_2)_2$–C$\underline{H}_2$, $4CH_{2\text{-piperazine}}$), 1.28 (qt, 4H, $J=6.5$ Hz, 2NH–CH_2–C$\underline{H}_2$). ^{13}C-NMR (DMSO-d_6): 174.3, 169.9, 144.5, 136.4, 129.3, 126.7, 121.9, 120.4, 119.7, 112.3, 105.0, 92.9, 56.4, 53.1, 42.7, 26.8.

Assays for TR Activity Recombinant trypanothione reductase was produced from the SG5 *Escherichia coli* strain with the overproducing expression vector pIBITczTR. TR activity was measured at 22 °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. Inhibitory potency of the different compounds was evaluated by measuring the IC_{50} in the presence of 57 μM of $T(S)_2$ and increasing concentrations of inhibitor (0 to 57 μM).

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