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Solid-phase synthesis of a focused library of trypanothione reductase inhibitors

Stefania De Luca, Saraj Ulhaq, Mark J. Dixon, Jonathan Essex and Mark Bradley*

Department of Chemistry, University of Southampton, Southampton SO17 1BJ, UK

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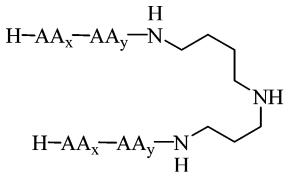
Abstract—A focused library of inhibitors of the enzyme trypanothione reductase was prepared using solid-phase synthesis. The inhibitors were based on a previously identified, non-competitive, lead compound comprising of two Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl) side-chain protected, *N*-capped arginine residues linked by a spermidine bridge. In total six protecting groups and four capping groups were used to generate a 24-membered library. All compounds bearing the 5-methoxyindole-3-acetic acid capping group were found to have good activity. The most potent inhibitor was observed to contain the Mtr (4-methoxy-2,3,6-trimethylbenzenesulphonyl) protecting group on the arginine residue, terminated with tryptophan as the capping group.

Trypanothione reductase (TR) is an enzyme unique to the trypanosomatid parasites,[1] the causative agents of diseases such as African sleeping sickness and Chagas' disease. TR and its product, N^1,N^8-bis(glutathionyl)-spermidine (trypanothione), help protect the parasite from excessive oxidative stress by maintaining intracellular redox balance in a manner analogous to glutathione reductase (GR) and glutathione in mammalian cells.[2] Despite the structural similarities of trypanothione and glutathione, trypanothione reductase and glutathione reductase demonstrate mutually exclusive substrate specificity, and consequently trypanothione reductase represents a promising target for the development of a range of anti-trypanosomal drugs.[3] This is a view enhanced by the vital role played by trypanothione in protecting the cellular environment from excessive oxidative stress.[4] Previous studies within our group had identified the spermidine conjugates **1**–**3** (Fig. 1) as inhibitors of TR, the most potent of which had the arginine side chain still bearing the Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl) protecting group.[5]

In order to evaluate and optimise this lead a 24-membered library consisting of six different Arg side-chain protecting groups (X) and four different capping groups (Y) was constructed using the T-bag methodology, introduced by Houghton as a convenient method of resin handling.[6] The readily available glass-coated transponders[7] and reader from Avid[8] provided the means of tracing the fate of the T-bags during synthesis.

The immobilised scaffold **4** (Fig. 2), consisting of bis-Fmoc protected spermidine attached to an aminomethyl polystyrene resin via an acid cleavable Wang type urethane linker, was prepared as previously reported.[9]

Following deprotection of the Fmoc-group the resin was split into 24 portions and each portion sealed into an individual polypropylene mesh bag with a heat sealer with the incorporation of a transponder in each bag. The bags were split into six (four bags in each



1: AA_x = Trp, AA_y = Arg, K_I = 16 μM
2: AA_x = Trp, AA_y = Arg/Arg(Pmc), K_I = 0.10 μM
3: AA_x = Trp, AA_y = Arg(Pmc), K_I = 0.19 μM

Figure 1. Inhibitors of trypanothione reductase with measured K_I's.

* Corresponding author.

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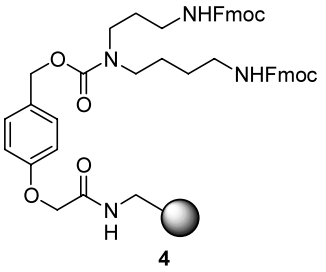

Figure 2. Polyamine scaffold used in library synthesis.

pool) and coupled to six different Fmoc-Arg(X)-OH derivatives[10] (the protecting groups chosen were the Pmc, Mtr (4-methoxy-2,3,6-trimethylbenzenesulfonyl), Pbf (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl), Mts (mesitylenesulfonyl), Tos (tosyl) and NO_2 (nitro) groups). Following coupling and the removal of the Fmoc protecting group the bags were sorted into the desired Vessel for incorporation of the capping group (Boc-tryptophan, indole-3-acetic acid, 5-bromoindole-3-acetic acid and 5-methoxyindole-3-acetic acid) (Fig. 3).

Identification of library members was accomplished by reading the tags, the 24 library members were cleaved individually and purified as the TFA salts by ether precipitation.[11] Analysis by RP-HPLC and LC ESMS was carried out on all products. Purities in all cases were found to be greater than 95% and all compounds gave the expected molecular ions.[12] The library was screened for TR inhibition at an inhibitor concentration of 10 μM.[13] Several potent inhibitors were identified (Fig. 4), the most active of which was **5** (X = Mtr, Y = tryptophan). Other active compounds included (**6**) X = NO_2, Y = 5-methoxyindole, (**7**) X = Pbf, Y = 5-methoxyindole and (**8**) X = Mtr, Y = 5-methoxyindole.

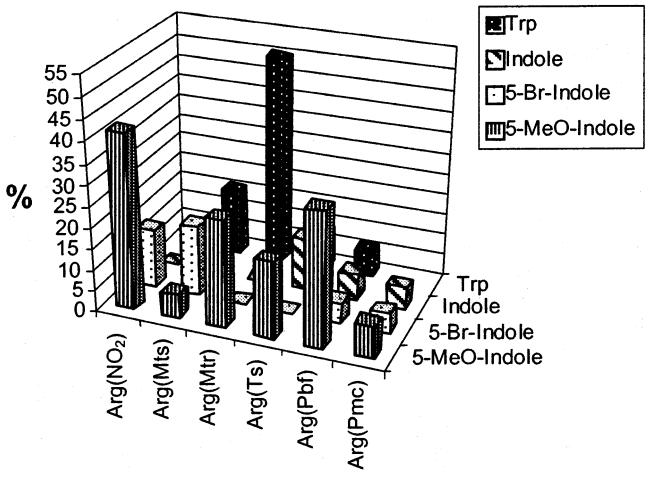

Figure 4. Inhibition of trypanothione reductase by library members.

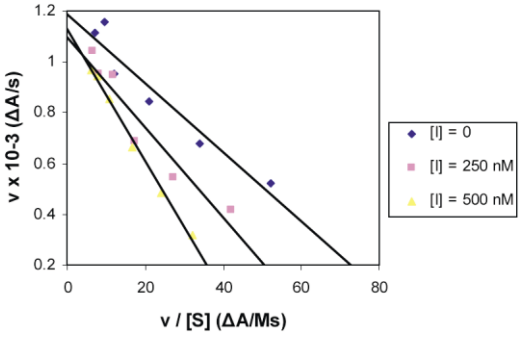

Figure 5. Eadie–Hofstee plot showing competitive behaviour of **5**.

Four of the most potent compounds in this series were fully characterised[14] and kinetically analysed. The most potent of these (**5**) was found to have a K_I of 530 nM but in this case displayed competitive inhibition under the assay conditions (Fig. 5).

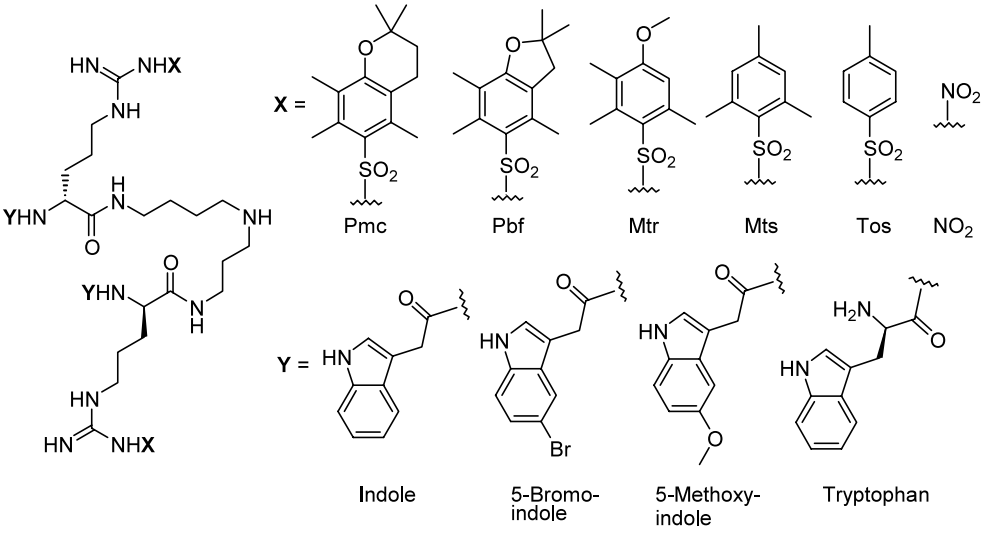

Figure 3. Monomers used in library construction.

This mode of action is clearly different from the mode of action of related compounds, including the lead compounds (**2** and **3**) which have been clearly shown to be non-competitive in nature. The data presented above shows how solid-phase synthesis can be used to prepare rapidly a small focused library of compounds for determination of SAR within a lead series.

Acknowledgements

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10. Couplings were carried out using standard DIC/HOBT coupling conditions (see: König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798). Reactions were monitored using a qualitative ninhydrin test on a small quantity of resin (see: Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147–157).
11. Cleavage conditions were as follows: compounds bearing the Arg(NO_2) moiety were cleaved with 90% TFA in DCM for 1.5 h; compounds bearing the Arg(Tos) moiety were cleaved with 90% TFA in DCM for 1 h; compounds bearing the Arg(Mtr) and Arg(Mts) moieties were cleaved with 50% TFA in DCM for 15 min; compounds bearing the Arg(Pbf) and Arg(Pmc) moieties were cleaved with 20% TFA in DCM for 2×15 min.
12. RP-HPLC analysis was carried out on a Hewlett Packard HP1100 Chemstation, using a Phenomenex Luna C_{18} column (150 mm×3 mm), gradient from 95% 0.1% TFA/H_2O to 95% 0.04% TFA/MeCN over 20 min at a flow rate of 0.5 mL min^{-1}, detected by ELS and UV at 220 nm. ES-MS data was obtained using a VG platform quadrupole electrospray ionisation mass spectrometer.
13. The kinetic assays were performed using 20% DMSO in phosphate buffer (50 mM K_2HPO_4, 1 mM EDTA, pH 7.5), 100 μM NADPH and various known concentrations of trypanothione (16 μM to 150 μM). Initial rates were recorded at 340 nm, following addition of the substrate. Data was collected in triplicate and analysed using GraFit.
14. ***N*¹,*N*⁸ - Bis(Tryptophanylarginyl(4 - methoxy - 2,3,6 - trimethylbenzenesulfonyl))spermidine**: δ_H (d_6-DMSO, 400 MHz, J/Hz): 7.62 (2H, d, J=8.0, 2×ArH), 7.32 (2H, d, J=8.0, 2×ArH), 7.16 (2H, d, J=2.0, 2×ArH), 7.05 (2H, t, J=7.5, 2×ArH), 6.95 (2H, t, J=7.5, 2×ArH), 6.64 (2H, s, 2×ArH), 4.26 (2H, m, Arg αH), 4.03 (2H, m, Trp αH), 3.74 (6H, s, 2×OCH_3), 3.16–2.96 (12H, m, 6×CH_2), 2.89–2.80 (4H, m, 2×CH_2), 2.55 (6H, s, 2×CH_3), 2.48 (6H, s, 2×CH_3), 2.01 (6H, s, 2×CH_3), 1.76–1.66 (2H, m, CH_2), 1.66–1.57 (4H, m, 2×CH_2), 1.57–1.46 (4H, m, 2×CH_2), 1.46–1.32 (4H, m, 2×CH_2). MS (ES+) m/z: 1256.3 (100%) $[M+H]^+$.
***N*¹,*N*⁸-Bis((2-(5-methoxy-1*H*-indol-2-yl)-acetyl)arginyl-(4-methoxy-2,3,6-trimethylbenzenesulfonyl))spermidine**: δ_H (d_6-DMSO, 400 MHz, J/Hz): 7.21 (2H, d, J=8.5, 2×ArH), 7.13 (2H, s, 2×ArH), 7.04 (2H, s, 2×ArH), 6.69 (2H, dd, J=8.5, 2.5, 2×ArH), 6.67 (2H, s, 2×ArH), 4.17 (2H, m, Arg αH), 3.78 (6H, s, 2×OCH_3), 3.73 (6H, s, 2×OCH_3), 3.52 (4H, s, 2×CH_2), 3.17–2.97 (8H, m, 4×CH_2), 2.83–2.65 (4H, m, 2×CH_2), 2.59 (6H, s, 2×CH_3), 2.51 (6H, s, 2×CH_3), 2.04 (6H, s, 2×CH_3), 1.68–1.53 (6H, m, 3×CH_2), 1.53–1.30 (8H, m, 4×CH_2). MS (ES+) m/z: 1256.4 (100%) $[M+H]^+$.
***N*¹,*N*⁸-Bis([2-(5-bromo-1*H*-indol-2-yl)-acetyl]arginyl(2,2,4,6,7 - pentamethyldihydrobenzofuran - 5 - sulfonyl))spermidine**: δ_H (d_6-DMSO, 400 MHz, J/Hz): 7.83 (2H, s, 2×ArH), 7.39 (2H, d, J=8.5, 2×ArH), 7.32 (2H, s, 2×ArH), 7.24 (2H, dd, J=8.5, 2.0, 2×ArH), 4.24 (2H, m, Arg αH), 4.10 (4H, s, 2×CH_2), 3.63 (4H, s, 2×CH_2), 3.28–3.06 (8H, m, 4×CH_2), 2.95–2.80 (4H, m, 2×CH_2), 2.56 (6H, s, 2×CH_3), 2.50 (6H, s, 2×CH_3), 2.08 (6H, s, 2×CH_3), 1.80–1.55 (14H, m, 7×CH_2), 1.48 (12H, s, 4×CH_3). MS (ES+) m/z: 718.0 (100%) $[M+2H]^{2+}$.
***N*¹,*N*⁸-Bis([2-(5-methoxy-1*H*-indol-2-yl)-acetyl]arginyl-(nitro))spermidine**: δ_H (d_6-DMSO, 400 MHz, J/Hz): 7.17 (2H, d, J=9.0, 2×ArH), 7.09 (2H, s, 2×ArH), 7.01 (2H, s, 2×ArH), 6.65 (2H, dd, J=9.0, 2.0, 2×ArH), 4.14 (2H, m, Arg αH), 3.69 (6H, s, 2×OCH_3), 3.49 (4H, s, 2×CH_2), 3.12–3.01 (6H, m, 3×CH_2), 3.01–2.94 (2H, m, CH_2), 2.82–2.65 (4H, m, 2×CH_2), 1.65–1.57 (4H, m, 2×CH_2), 1.57–1.28 (10H, m, 5×CH_2). MS (ES+) m/z: 923.7 (100%) $[M+H]^+$.