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Optimising Inhibitors of Trypanothione Reductase Using Solid-Phase Chemistry

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Abstract—A series of inhibitors of the enzyme trypanothione reductase has been identified using directed solid-phase chemistry. The compounds were based on a series of polyamine scaffolds and used the natural product kukoamine A as the lead structure. A compound with a K_i of 76 nM was identified, although somewhat surprisingly the compound appeared to be noncompetitive in nature.

The enzyme trypanothione reductase (TR) is unique to parasitic protozoa known as trypanosomes and leishmania.[1] This enzyme and its product, the key dithiol N^1,N^8-bis(glutathionyl) spermidine (trypanothione), play an essential role in the maintenance of a reducing cellular environment within these parasites. Mammalian cells utilise glutathione (GSH) and glutathione reductase (GR) for a similar protective function, thus trypanothione reductase is believed to offer an attractive medicinal target in the treatment of trypanosomal diseases because of its fundamental role in oxidative stress management and its compound specificity compared to the equivalent host protein.[2,3] The trypanothione reductase (TR) system is made even more attractive as a target for rational drug design since the active site of its human equivalent, GR, is profoundly different. GR has a much more closed, positively charged active site (to accommodate the glycine carboxylates), whereas TR's active site is more open and has greater hydrophobic character.[4] This hydrophobic character in turn allows the binding of the polyamine component of trypano thione disulfide probably via aromatic-π stacking interactions between the ammonium ion and a Trp residue, as well as more general hydrophobic interactions with the methylene bridge of the polyamine.[5] Indeed this difference probably explains the selectivity of many existing inhibitors, but also suggests that the polyamine component is an ideal place to gain selectivity.[6–8]

Using solid-phase combinatorial chemistry[6] and a defined polyamine template,[9] we can thus selectively target the unique parasitic enzyme trypanothione reductase (TR) over the human counterpart. Here we describe our recent solid-phase activities in the area, synthesising a range of immobilised polyamines, which allowed the synthesis of a number of polyamine derivatives followed by lead optimisation. The first series of compounds synthesised were based on the natural product kukoamine A (N^1,N^{12} bis(3,4-dihydroxyphenylpropanoyl)spermine) (**6a**) a known inhibitor of TR that shows no significant inhibition of human GR (K_i >10 mM) and thus provides a novel, selective lead.[6] The polyamine scaffolds were prepared as outlined in Scheme 1 and expand on our previously published procedures to give resin immobilised norspermine, spermidine, and spermine (**1**, **2** and **3**).

The three scaffolds (**1**, **2** and **3**) were then derivatised with a range of capping groups[10] to give, following cleavage from the solid phase with (TFA:H_2O (95:5)), the corresponding libraries (**4a**–**y**, **5a**–**y** and **6a**–**y**) which were purified by reverse-phase HPLC and fully characterised (NMR, MS, and HPLC) prior to screening against TR (*T. cruzi*).[11] All compounds were screened in triplicate, initially using a single point assay (100 μM inhibitor, 100 μM trypanothione disulfide, and 100 μM NADPH, and between 1–5 nM TR) and the four most potent compounds were fully analysed kinetically (Table 1).

All the inhibitors reported in this paper were analysed using the commercially available program Grafit. The kinetic data was fitted to the equation given in Eq. 1, for noncompetive inhibition, with the assays being carried out at six concentrations of substrate (oxidised

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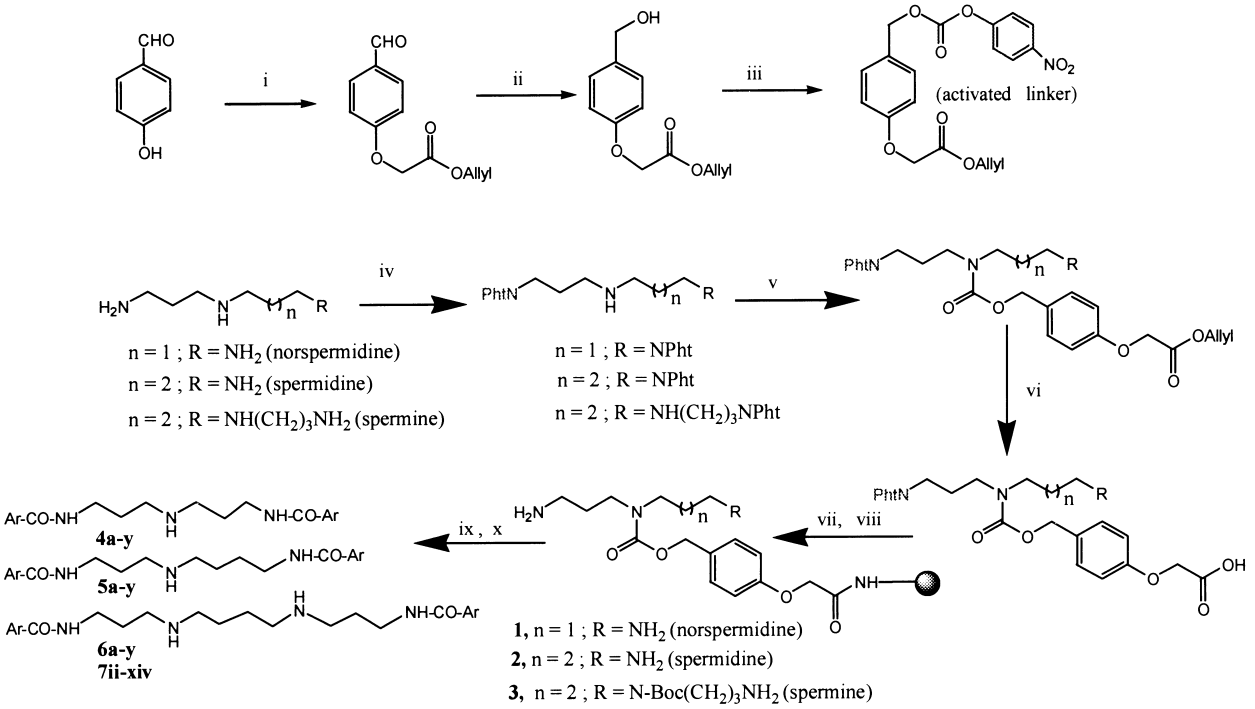

Scheme 1. The immobilisation of the polyamine scaffolds. (i) $ClCH_2CO_2Allyl$, K_2CO_3, KI, CH_3CN, 95%; (ii) $NaBH_3CN$, THF, H_2O (1:1), 90%; (iii) $pNO_2PhOCOCl$, pyridine, 0 °C, CH_2Cl_2, 90%; (iv) phthalimido-*N*-ethoxycarbonyl, $CHCl_3$, 76–84%; (v) activated linker, NEt_3, DMAP, DMF, 44–88% (1.1 equiv of Boc_2O added for spermine); (vi) $Pd(PPh_3)_4$, mercaptobenzoic acid, THF, CH_2Cl_2 (1:1), 72–95%; (vii) aminomethyl polystyrene resin, DIC, HOBt, CH_2Cl_2; (viii) N_2H_4, EtOH, reflux; (ix) RCO_2H, DIC, HOBt, CH_2Cl_2; (x) TFA, H_2O, (95:5), 4 h.

Table 1. The four most potent inhibitors in the initial library. (K_i values were determined in triplicate and analysed using the commercial program Grafit)

	Capping acid	Polyamine	K_i (μM) ±SD
(**6a**)	3,4-Dihydroxyhydocinnamic acid	Spermine	1.39±0.41
(**6m**)	4-Hydroxy-7-trifluoromethyl-3-quinoline-carboxylic acid	Spermine	0.75±0.22
(**5m**)	4-Hydroxy-7-trifluoromethyl-3-quinoline-carboxylic acid	Spermidine	0.85±0.22
(**6y**)	3-Indole acetic acid	Spermine	0.39±0.06

Table 2. The most potent inhibitors from the indole series. (K_i values were determined in triplicate and analysed using the commercial program Grafit)

	Capping acid	K_i (μM) ±SD
(**6y**)	3-Indole acetic acid	0.39±0.06
(**7iii**)	3-Indole propanoic acid	0.35±0.05
(**7vii**)	5-Methoxy-3-indole acetic acid	0.28±0.02
(**7xii**)	3-Indole butanoic acid	1.26±0.23
(**7xiii**)	5-Bromo-3-indole acetic acid	0.076±0.010

trypanothione, 0.25–5×K_m), and at four concentrations of inhibitor (determined experimentally) in triplicate. Without exception all inhibitors were shown to be non-competitive in nature.

$$v = \frac{V\max [S]/(1 + [I]/K_i)}{K_m + [S]} \quad (1)$$

This led to the identification of the indole spermine derivative (**6y**) as the most potent compound, which was some 3-fold more potent than the natural product kukoamine A (**6a**), which was prepared and screened at the same time. These results directed the synthesis of the second indole based library (**7ii**–**xiv**).[12] The five most active compounds of this series were fully characterised kinetically and the data is given in Table 2.

Against yeast glutathione reductase there was less than 20% inhibition by these compounds at concentrations of 50 μM.

Having optimised the side chain a series of compounds was then prepared and screened in which the most potent side chains were coupled onto all three polyamine backbones. All compounds were screened at a single concentration of 10 μM and gave the data shown in Table 3. Although there is undoubtedly some levelling of activity for the most potent compounds of the series, the data shows quite clearly the important influence of the polyamine moiety. Thus although the natural polyamine spermidine is found in oxidized trypanothione (the presumed natural substrate for trypanothione reductase) this is not observed to be the basis of the most potent inhibitors, with the tetra-amine spermine being by far the most active. The subtle differences between spermidine and norspermidine are also of interest, with a large variation being observed between the two compounds although not in a consistent manner. With the indole series the natural polyamine spermidine is generally the more active of the two, although this is reversed with the capping groups (**a** and **m**). Interestingly, it was one

Table 3. Screening of the three polyamine conjugates with the most potent capping groups[10,12]

	Capping acid	Norspermidine	Spermidine	Spermine
(**a**)	3,4-Dihydroxyhydocinnamic acid	38%	20%	82%
(**m**)	4-Hydroxy-7-trifluoromethyl-3-quinoline-carboxylic acid	85%	62%	69%
(**y**)	3-Indole acetic acid	97%	95%	97%
(**ii**)	5-Hydroxy-3-indole acetic acid	24%	65%	95%
(**iii**)	3-Indole propanoic acid	60%	72%	96%
(**vii**)	5-Methoxy-3-indole acetic acid	96%	85%	96%
(**xii**)	3-Indole-butanoic acid	63%	93%	97%
(**xiii**)	5-Bromo-3-indole acetic acid	54%	72%	98%

of these compounds that was more active than the spermine analogue, the only example where this is the case across the whole series.

In conclusion, a number of immobilised polyamines were prepared which were derivatised with a range of aromatic carboxylates. Compound **7xiii** was the most potent of the series having a K_i of 76 nM. The effectiveness of the indole functionality fits with other inhibitors reported elsewhere[9] and suggests that despite the natural substrate of trypanothione reductase lacking aromatic residues the active site is able to accommodate electron rich aromatics. The kinetic behaviour of all these inhibitors was not simple competitive inhibition but non-competitive and thus the possibility exists that binding is taking place at an alternative active site, perhaps that of the co-factor NADPH. It is even possible that it is competing with the reduced product. These possibilities, and X-ray crystallographic studies are now under active investigation.

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10. Capping acids used in initial library (**4**, **5** and **6**): (a) 3,4-dihydro xyhydocinnamic acid, (b) 2,3-dimethoxyhydocinnamic acid, (c) 4-hydroxy-3,5-dimethoxycinnamic acid, (d) 4-methoxycinnamic acid, (e) 3-methoxycinnamic acid, (f) 4-hydroxyhydrocinnamic acid, (g) 2,4-dihydroxycinnamic acid, (h) 3,4-(methylenedioxy)cinnamic acid, (i) 2,5-dihydroxyphenylacetic acid, (j) 3-hydroxyphenylacetic acid, (k) 2-quinoxalinecarboxylic acid, (l) 3-hydroxy-2-quinoxalinecarboxylic acid, (m) 4-hydroxy-7-trifluoromethyl-3-quinoline-carboxylate, (n) 2-hydroxyphenylacetic acid, (o) 2-pyrrolecarboxylic acid, (p) 2-thiophenecarboxylic acid, (q) 2-thiopheneacetic acid, (r) 5-bromo-2-furancarboxylic acid, (s) 2-methoxy-4-hydroxyphenylacetic acid, (t) benzoic acid, (u) 3,4-(methylenedioxy)phenylacetic acid, (v) 4-chlorobenzoic acid, (w) 6-chloro-3-pyridinecarboxylic acid, (y) 3-indole acetic acid.
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12. Capping acids used in optimisation library (**7ii**–**xiv**): (ii) 5-hydroxy-3-indole acetic acid, (iii) 3-indole propanoic acid, (iv) 2-methyl-3-indole acetic acid, (v) 5-hydroxy-2-indole carboxylic acid, (vi) E-3-indole-acrylic acid, (vii) 5-methoxy-3-indole acetic acid, (viii) 2-indole carboxylic acid, (ix) tryptophan, (x) 2-methyl-5-methoxy-3-indole acetic acid, (xi) 3-indole carboxylic acid, (xii) 3-indole-butanoic acid, (xiii) 5-bromo-3-indole acetic acid, (xiv) 2,3-dihydro-2-indole carboxylic acid.