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NOVEL POLYAMINE DERIVATIVES AS POTENT COMPETITIVE INHIBITORS OF *TRYPANOSOMA CRUZI* TRYPANOTHIONE REDUCTASE

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Abstract: The inhibiting effects of several spermidine and spermine derivatives on *T. cruzi* trypanothione reductase were assessed. Spermidine and spermine derivatives containing hydrophobic aromatic substituents were found to be competitive inhibitors of trypanothione reductase. The most effective compounds tested were N^1,N^8-bis(2-naphthylmethyl)spermidine, N^4,N^8-bis(2-naphthylmethyl)spermine and N^4,N^8-bis(3-phenylpropyl)spermine.

Trypanosoma cruzi is a protozoan parasite causing Chagas disease which currently infects 16-18 million people leading to more than 45,000 deaths each year.[1] The disease occurs predominantly in Central and South America, however about 100,000 people in the USA are also infected, probably due to transfusion of blood products originating from South America.[2] Current treatment of *T. cruzi* and other trypanosome infections is difficult and often ineffectual in controlling the chronic phase of these diseases.[3] Therefore effective antitrypanosomal drugs are needed. A promising strategy for the development of new drugs is to design compounds which interfere with the mechanisms by which trypanosomes maintain the levels of reduced glutathione necessary for defense against oxidative stress.[4,5,6] Levels of reduced glutathione in most organisms are maintained by the action of glutathione reductase. Trypanosomes do not contain glutathione reductase, but contain a unique enzyme, trypanothione reductase (TR).[7] TR is a NADPH-dependent, FAD-containing enzyme which reduces the disulfide group of N^1,N^8-bis(glutathionyl)spermidine (trypanothione).[7] It is presumed that in trypanosomes, glutathione (and possibly other thiols[8]) is reduced nonenzymatically by a thiol-disulfide exchange reaction with reduced trypanothione.[9] Given that the antioxidant defenses of trypanosomes are based on the action of TR, inhibitors of TR are potential antitrypanosomal agents.

In this paper we describe the synthesis and inhibiting effects of several novel spermidine and spermine derivatives on *T. cruzi* TR. The compounds N^1,N^8-bis(2-naphthylmethyl)spermidine (**3**), N^4,N^8-bis(2-naphthylmethyl)spermine (**7**) and N^4,N^8-bis(3-phenylpropyl)spermine (**6**) were found to be competitive inhibitors (K_i values of 9.5, 5.5 and 3.5 μM, respectively) with potencies of a similar magnitude to the most effective competitive inhibitor described previously.[10] These compounds are easily synthesized and could furthermore be modified to produce potentially more effective or irreversible inhibitors of TR.

Substrate specificity and inhibitor studies have indicated that several structurally diverse compounds bind reversibly to the active site of TR.[10,11] These studies have shown that these molecules do not require a cyclic structure, a disulfide or glutamyl residue(s) to bind to the active site. However, all compounds that bind to the active site contain amino group(s) and often have hydrophobic residue(s). Previously, the most potent inhibitor described was a tricyclic amine, clomipramine (with an approximate K_i of 6.5 μM against *T. cruzi* TR).[10]

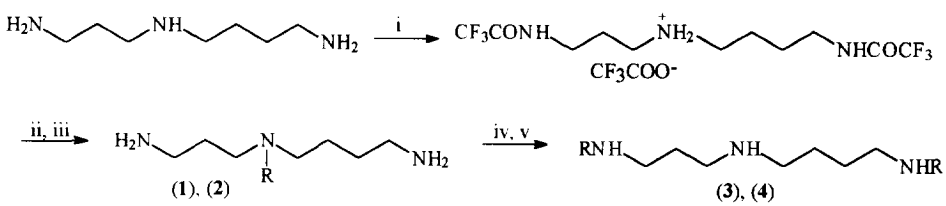
We were interested in developing competitive inhibitors of TR that are synthetically readily available. We envisioned that such compounds could be easily modified to contain chemically reactive groups leading to

compounds that may act as irreversible inhibitors of TR. Initially, we investigated the inhibiting effects of simple trypanothione analogs containing amino and hydrophobic groups, specifically N^1,N^8-bis-substituted spermidine derivatives. The results we obtained led us to synthesize and investigate the inhibiting effects of N^8-substituted spermidines and several substituted spermine derivatives.

The synthetic strategies leading to the spermidine derivatives are outlined in Scheme 1 and those used to prepare the spermine derivatives in Scheme 2. In both synthetic schemes, a critical reaction which has been described previously, is the one-step selective trifluoroacetylation of the primary amino groups of spermidine or spermine.[12] For each compound, the analytical data obtained (MS, ^{1}H and ^{13}C NMR) were in accordance with the structure proposed.

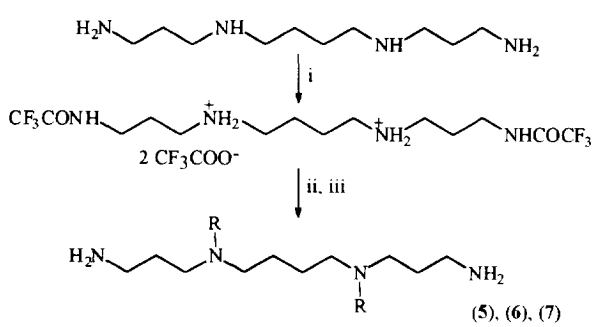
T. cruzi TR was purified following the procedure of Walsh et al.[13] from a SG5 *E. coli* strain (a glutathione reductase deletion mutant) containing the expression vector pIBITczTR described by Sullivan and Walsh.[14] The effects of prepared compounds on the rate of reduction of trypanothione by *T. cruzi* TR were assayed

Scheme 1

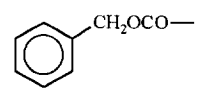
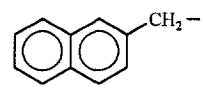
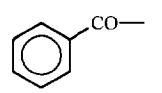
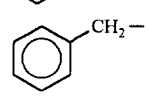
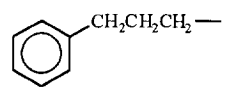


i) CF_3COOEt, H_2O, CH_3CN, reflux.[12] **ii)** for preparation of (**1**) benzyl chloroformate, K_2CO_3, H_2O, THF; for preparation of (**2**) 2-(bromomethyl)naphthalene, triethylamine, CH_3CN, reflux; for preparation of (**3**) and (**4**) di-*t*-butyl dicarbonate, triethylamine, THF. **iii)** either K_2CO_3, MeOH, H_2O; or ammonium hydroxide, MeOH. **iv)** for preparation of (**3**) 2-naphthaldehyde, CH_3CN, 3 h followed by $NaBH_4$, EtOH; for preparation of (**4**) benzoyl chloride, triethylamine, CH_3CN. **v)** trifluoroacetic acid.

Scheme 2



i) CF_3COOEt, H_2O, CH_3CN, reflux.[12] **ii)** for preparation of (**5**) benzyl bromide, triethylamine, CH_3CN; for preparation of (**6**) 1-bromo-3-phenylpropane, triethylamine, CH_3CN; for preparation of (**7**) 2-(bromomethyl)naphthalene, triethylamine, CH_3CN. **iii)** ammonium hydroxide, MeOH.

Compound	R
(1)	
(2), (3), (7)	
(4)	
(5)	
(6)	

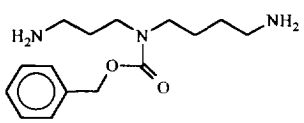
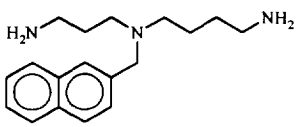
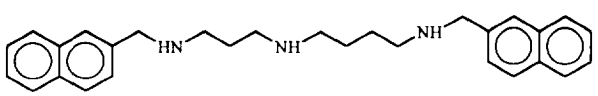
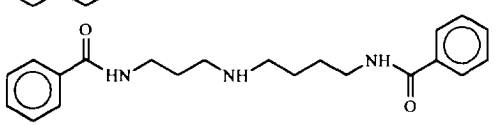
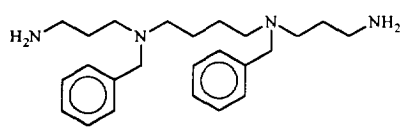
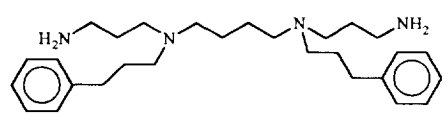
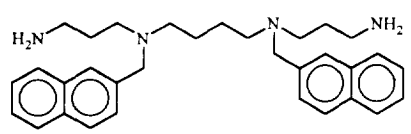
spectrophotometrically by monitoring the oxidation of NADPH at 340 nm.[15] TR activity was measured at 23 °C in HEPES buffer (100 mM, pH 7.25) containing EDTA (1 mM), NADPH (0.18 mM) and oxidized trypanothione with an enzyme concentration of 1.22 μg/mL.

Inhibition type was assessed by the patterns of three classes of plots: $1/v$ against $1/[S_o]$ for various [I]; $1/v$ against [I] for various $[S_o]$; and $[S_o]/v$ against [I] at various $[S_o]$. All compounds tested showed linear competitive inhibition against TR reduction of trypanothione. For each inhibitor concentration $K_{m(obs)}$ and V_{max} were determined from a least-squares linear regression analysis of the plot of $1/v$ against $1/[S_o]$. (The correlation confidence value, R, of all lines was greater than 0.93). K_i values were determined for each inhibitor concentration using the equation:

$$K_i = \frac{[I]}{\{(V_{max} K_{m(obs)})/(V_{max(obs)} K_m)\} - 1}$$

The mean K_i value for each compound was calculated from the K_i values obtained at five different inhibitor concentrations. The mean K_i values for each compound and their standard deviations are given in the Table.

Table. Mean K_i values for the competitive inhibition by spermidine and spermine derivatives of trypanothione reduction by recombinant TR from *T. cruzi*

	Compound	K_i (μM) ± SD
(1)		280 ± 17
(2)		108 ± 7
(3)		9.5 ± 2.1
(4)		greater than 2,000
(5)		19 ± 5
(6)		3.5 ± 0.4
(7)		5.5 ± 0.2

None of the compounds tested caused the TR mediated oxidation of NADPH in the absence of trypanothione. Thus, as expected, compounds tested were not TR substrates. Compounds (**3**), (**6**) and (**7**) did not inhibit the reduction of glutathione by yeast glutathione reductase (EC 1.6.4.2), assayed in similar conditions as described with TR.

In conclusion, spermidine and spermine derivatives with hydrophobic aromatic substituents are potent competitive inhibitors of *T. cruzi* TR. The most effective compounds tested were N^1,N^8-bis(2-naphthylmethyl)-spermidine (**3**), N^4,N^9-bis(2-naphthylmethyl)spermine (**7**) and N^4,N^9-bis(3-phenylpropyl)spermine (**6**). Trypanosome infections occur predominantly in lower socio-economic populations, therefore new chemotherapeutics to combat trypanosomal diseases in these groups should be inexpensive.[1] The inhibitors described in this paper are easy to prepare and inexpensive and thus may provide a new direction for the development of affordable, effective antitrypanosomal agents. A full account of this work containing the results of all polyamine derivatives tested is in preparation.

Acknowledgements

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