Brief Articles

Structure−Activity Study on the in Vitro Antiprotozoal Activity of Glutathione Derivatives

Claudius D'Silva* and Sylvie Daunes

Department of Chemistry & Materials, Manchester Metropolitan University, Faculty of Science and Engineering, John Dalton Building, Chester Street, Manchester M1 5GD, U.K.

Peter Rock, Vanessa Yardley, and Simon L. Croft

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K.

Received July 14, 1999

A series of N-, S-, and COOH-blocked glutathione derivatives were evaluated against the pathogenic parasites *Trypanosoma brucei, Trypanosoma cruzi,* and *Leishmania donovani* in vitro, to identify the determinants necessary for activity and for further development into an active lead structure. The results show that N,S-blocked glutathione diesters are the most effective inhibitors of *T. brucei* with structures **14**−**16** being the most active, **14** having an $IC_{50} \sim 1.9\ \mu M$. The toxicity effects observed for glutathione derivatives **12**, **14,** and **16** have been correlated to the K562 antileukemic activity of these compounds and their inhibitory effects on the glyoxalase system of the host. Diester compounds based on *S*-2,4-dinitrophenylglutathione (**17**−**22**) were found to be significantly better inhibitors of *T. brucei* with ED_{50}'s in the range 16−0.19 μM. Compounds **19** and **20** were the two best inhibitors, with an ED_{50} of ∼1.07 and 0.19 μM, respectively; however **20** displayed toxicity in parasitic assays. Monoesters, monoamides, and diamides tested generally exhibited low in vitro activity. The compounds did not inhibit glutathionylspermidine synthetase and trypanothione reductase enzyme targets in the unique trypanothione pathway of these parasites. Diester compounds per se were considered to be ineffective inhibitors of trypanothione metabolism suggesting that these compounds might act as prodrugs, being hydrolyzed in situ into a variety of glutathione derivatives which include combinations of monoesters, free acids, and amines, some of which are inhibitors of trypanothione metabolism.

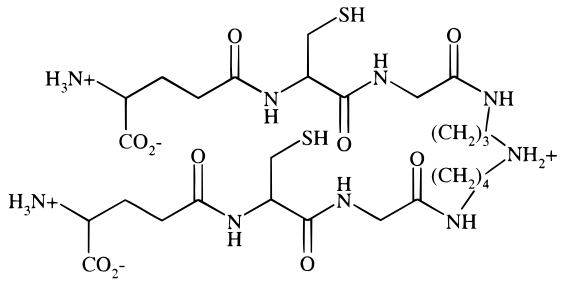
Introduction

The effects of trypanosomiasis and leishmaniasis are a serious problem to the health and economics of developing countries. Current chemotherapy is inadequate. One approach to the development of new drugs is to identify differences in metabolism between the host and pathogen and exploit these differences to design toxic agents. Trypanothione, like glutathione in the host, plays a central role in the metabolism of trypanosomatids acting both as a source of reducing equivalents and as a defense against oxidative stress.[1] Drugs effective against African trypanosomiasis[1,2] in humans and animals are few and include suramin (in human and camels), pentamidine (in humans), and berenil (in cattle), the arsenicals melarsoprol (in humans) and cymelarsan (in cattle), and eflornithine (D,L-α-difluoromethylornithine (DFMO); in humans only). The ionic nature of suramin and the diamidines however limits their usefulness as they are not therapeutically active in the late CNS stage of this disease as they cannot cross the blood−brain barrier. In late-stage sleeping sickness the only effective compounds are ones which create oxidative stress by altering trypanothione levels,[1,2] such as melarsoprol, which produces fatal side effects in ∼5% of treated cases,[3] or DFMO, an inhibitor of ornithine decarboxylase, an essential enzyme in spermidine synthesis and a precursor in trypanothione biosynthesis.

A key difference in the metabolism of the trypanosomatids[1,4] is the replacement of the tripeptide glutathione (GSH) and its dependent enzymes in the host by an analogous family utilizing trypanothione (N^1,N^8-bis(glutathionyl)spermidine, $T(SH)_2$). Alternative chemotherapeutic strategies to find less toxic drugs include the inhibition of trypanothione reductase with trypanothione mimics[5] and glutathionylspermidine synthetase (GSS) with glutathione-modified substrate analogues.[6] Despite the successful inhibition of these enzymes in situ these compounds are inactive against trypanosomes in vitro.[5,6] The inability of rational drug design studies to produce compounds active in vitro against parasitic cells led us to adopt a lead directed approach to identify potential antiparasitic compounds.[7] We present here structure−activity results aimed at identifying the

* Corresponding author. Tel: 00 44 161 2471416. Fax: 00 44 161 2476357. E-mail: C.DSilva@mmu.ac.uk.

10.1021/jm990259w CCC: $19.00
Published on Web 04/21/2000



Trypanothione ((TSH)$_2$)

necessary determinants required of glutathione analogues to act as toxic agents against trypanosomes in vitro.

Chemistry

Glutathione ester derivatives **1**−**10** and **12**−**13** were prepared using known methodology published in the literature.[9,11,12,21] Compound **11** was synthesized by the (dimethylamino)pyridine (DMAP) catalyzed acylation of **2** with benzyl chloroformate (CbzCl)[9] in a manner similar to **10**. Derivatives **14** and **16** were synthesized by the esterification of **9** and *N*,*S*-dibenzyloxycarbonylglutathione (mp lit.[8] 105−107 °C) with their respective alcohols according to literature procedures.[11] The *N*-acetyl derivative **15** was synthesized by DMAP acylation[9] of *S*-benzyloxycarbonylglutathione dimethyl ester with acetic anhydride in a manner similar to **8**.[9] *S*-Benzyloxycarbonylglutathione dimethyl ester was prepared by cleavage of the Cbz group of **16** with HBr/AcOH.[11]

S-2,4-Dinitrophenylglutathione derivatives **17**−**19**, **21** and **22** were synthesized by triethylamine (TEA) catalyzed N-acylation of a mixture of *S*-2,4-dinitrophenylglutathione mono- and diesters with CbzCl[9] and chromatographic separation. The mixture of mono- and diesters was prepared by $SOCl_2$ esterification of *S*-2,4-dinitrophenylglutathione in the appropriate alcohol as described for **3**;[11] however in this case monoesters[11] were not obtained as the exclusive product but a mixture of mono- and diesters was isolated. The monoesters were prepared for use in another study. Compound **20** was prepared in a manner similar to that described for **10**, **12**, **14** and **16** by esterification of *N*-benzyloxycarbonyl-*S*-2,4-dinitrophenylglutathione with cyclohexanol according to literature procedures.[11] *S*-Bromobenzylglutathione and *S*-2,4-dinitrophenylglutathione derivatives were primarily chosen as the basis of these investigations due to their previously reported efficacy against a range of glutathione-dependent enzymes.[12−15,22]

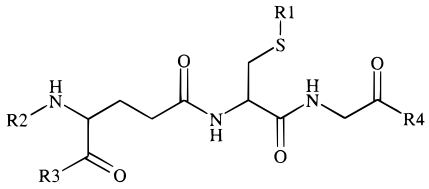
Results and Discussion

Glutathione derivatives **1**−**16** (see Table 1) were evaluated for antiparasitic activity against bloodstream form *Trypanosoma brucei* trypomastigotes and both *Leishmania donovani* and *Trypanosoma cruzi* amastigotes in mouse peritoneal macrophages. The inhibition of parasitic growth observed was expressed as a percentage of the untreated control at concentrations up to ∼30 μM (see Table 1). One of the 16 compounds evaluated, **2**, showed moderate activity against *T. brucei*, with another 3, the dibutyl esters of *N*-benzyloxycarbonyl-*S*-bromobenzylglutathione **14**, the dimethyl esters of *N*-acetyl-*S*-benzyloxycarbonylglutathione **15,** and *N*,*S*-dibenzyloxycarbonylglutathione **16** showing considerable activity with ED_{50}'s of 1.9, 3.23 and 17 μM, respectively. Compounds **14** and **16** had activity against *L. donovani* and *T. cruzi*, but in both cases toxicity to host macrophages was also observed. Examination of the data obtained for compounds **1**−**16** (Table 1) with *T. brucei* indicates that diester compounds such as **14**−**16** generally show higher activity relative to diamides **13**, monoamides **11,** and monoesters **10**. The nature of the ester group also appears important if we compare the dimethyl **12** versus the dibutyl esters **14**, with the latter showing greater activity against *T. brucei* and low-grade activity against *L. donovani*.

The presence and nature of the N-blocking group appear important with respect to toxicity against amastigotes especially if we compare **7**, **12,** and **15**. Replacement of the formyl group of **7** by an *N*-acetyl group **(15)** increases the activity of this compound against *T. brucei* while substitution with a benzyloxycarbonyl group **(16)** produces a lower degree of inhibition relative to **15** and toxicity effects. This result is not surprising in view of the inhibitory activity of the deesterified derivatives of **14**−**16** against enzymes of the mammalian glyoxalase system (GLI and GLII)[10,14,15] which are in the order **14** > **16** > **15**.[10,15] Inhibition of the glyoxalase system by *S*-bromobenzylglutathione diester derivatives has been investigated as a strategy to antileukemic agents.[18] The K562 antileukemic properties of diesters **1**−**16** (see Table 1**)** correlate well with the toxicity results observed for compounds **12**, **14** and **16** in parasitic assays. This result prevents us from improving the efficacy of the compounds by extrapolating data obtained in the study of glutathione diester antitumor agents,[18] due to their toxicity and general nonselectivity in their mode of action.

The nature of the S-blocking group appears important if we compare **12** with **16** as the latter compound with an *S*-benzyloxycarbonyl group has higher activity against *T. brucei*. However both the *S*-bromobenzyl and *S*-benzyloxycarbonyl groups are good inhibitors of the glyoxalase system[10,12−15,21] and so potentially have the ability to generate toxicity in parasitic assays depending on the nature of groups attached at the N-site. *S*-2,4-Dinitrophenylglutathione is a weak inhibitor of GLI (K_i ∼ 0.77 mM[21]) but a moderate inhibitor of glutathione reductase ($K_i^{50\%}$ ∼ 30 μM[22]), an enzyme structurally related to trypanothione reductase, and therefore possibly a better starting material for the synthesis of antiparasitic compounds. The antiparasitic activities of diesters **17**−**21** (see Table 2) are significantly better especially in the cases of **19** and **20**, with activities in the submicromolar range against *T. brucei*. The optimum length for the ester group appears to lie between a butyl group and a hexyl group. Precedence for the improvement in activity of butyl esters **(19)** has been reported for a series of alkyl ester prodrugs based on ibuprofren.[23] Compound **20**, the cyclohexyl diester**,** is one of the most active compounds as expected with an ED_{50} ∼ 0.21 μM and the most toxic based on the pharmacological responses of glutathione-based anticancer agents.[18] However the cytotoxicity in this case may be associated with the nature of the cyclohexyl group and its metabolism, as the K562 antileukemic

Table 1. Biological Properties of Glutathione Derivatives against *T. brucei, L. donovani, and T. cruzi*



compound					ED_{50} (μM)	% inhibition (μM)		ID_{50} (μM)
	R1	R2	R3	R4	*T. brucei*[a]	*L. donovani*[b]	*T. cruzi*[b]	K562
1	BrBz	H	OH	OH	>30 (40)[a]	na	na	>50
2	BrBz	H	OH	NH_2	<30 (60)[a]	n.a	na	>50
3	BrBz	H	OH	OMe	>30 (20)[a]	na	na	>50
4	BrBz	H	OMe	OMe	na	8.7	na	>50
5	BrBz	H	NH_2	NH_2	na	4.0	na	>50
6	BrBz	HCO	OH	OH	na	na	11	>50
7	BrBz	HCO	OMe	OMe	na	na	12	>50
8	BrBz	CH_3CO	OH	OH	>30 (40)[a]	na	na	>50
9	BrBz	Cbz	OH	OH	>30 (40)[a]	na	na	>50
10	BrBz	Cbz	OH	OMe	na	na	na	>50
11	BrBz	Cbz	OH	NH_2	na	na	na	>50
12	BrBz	Cbz	OMe	OMe	>30 (40)[a]	T/0	T/0	16.7
13	BrBz	Cbz	NH_2	NH_2	>30 (20)[a]	na	na	>50
14	BrBz	Cbz	OBu	OBu	1.9 ± 0.04	68	T/+	16.7
15	Cbz	CH_3CO	OMe	OMe	3.2 ± 2.4	na	na	>50
16	Cbz	Cbz	OMe	OMe	17 ± 0.66	T/100	T/100	1.26

[a] The percent (%) inhibition, in parentheses, at 30 μM is expressed as the percent (%) decrease in viable parasites relative to the control. [b] T/100, toxic to macrophages/100% inhibition of parasites; T/0, toxic to macrophages/0% inhibition of parasites; T/+, toxic to macrophages/inhibition of parasites; na, not active at ~30 μM.

Table 2. Biological Properties of *S*-2,4-Dinitrophenylglutathione Derivatives against *T. brucei, L. donovani, and T. cruzi*

compound					ED_{50} (μM)			ID_{50} (μM)
	R1	R2	R3	R4	*T. brucei*	*L. donovani*	*T. cruzi*	K562
17	2,4-DNP	Cbz	OMe	OMe	16.2 ± 0.64	>30	na	23.4
18	2,4-DNP	Cbz	OPr	OPr	6 ± 0.16	>30	na	17.6
19	2,4-DNP	Cbz	OBu	OBu	1.07 ± 2.1	>30	na	21.8
20	2,4-DNP	Cbz	OcHx	OcHx	0.19 ± 0.04	T/+[a]	3.0[a] ± 0.11	15.9
21	2,4-DNP	Cbz	OHx	OHx	5.8 ± 0.32	>50	<50	>50
22	2,4-DNP	Cbz	OOct	OOct	na	>50	na	24.7

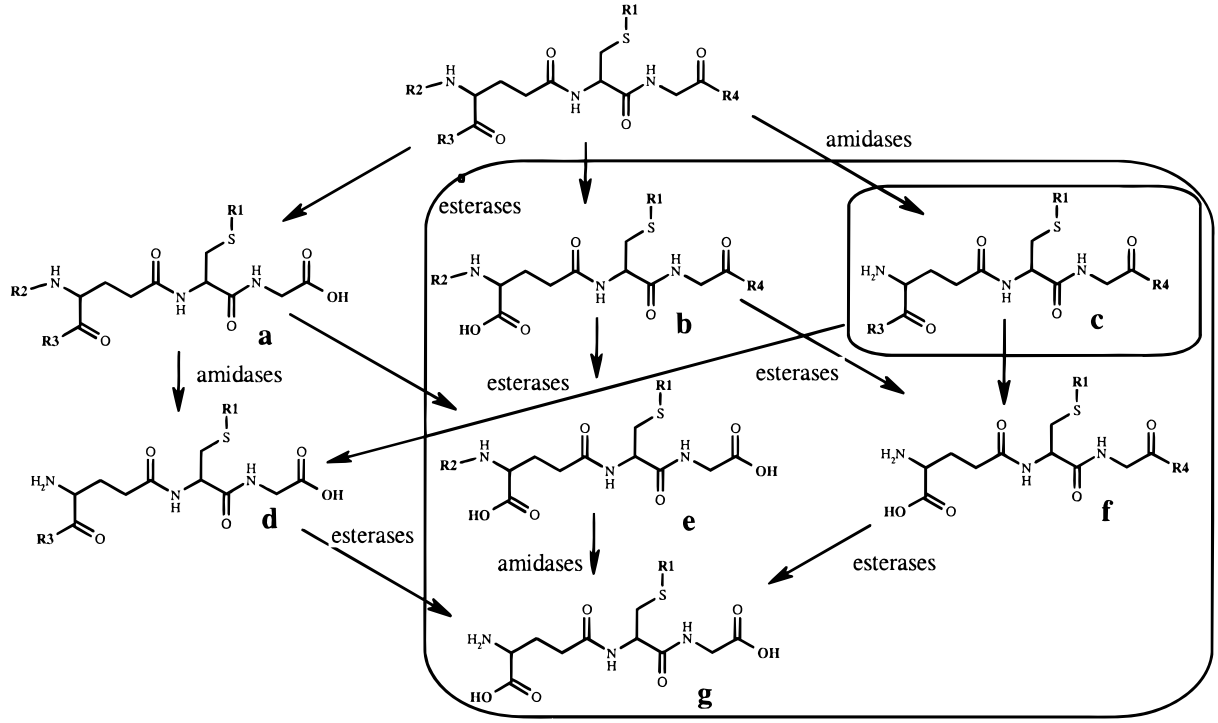
[a] T/+, toxic to macrophages (~20 μM)/inhibition of parasites; na, not active at ~30 μM.

activity of **20** is not significantly different from that of **17**−**19** and **22**. Comparison of compound **14** with **19** indicates that the presence of the *S*-2,4-dinitrophenyl group enhances the *T. brucei* activity of **19** by ~20-fold relative to the *S*-bromobenzyl group.

Based on the current understanding of glutathione chemistry and the determinants required for glutathione[11−15] and trypanothione[2,5,6] to bind to their respective enzymes, we speculate that compounds **14**−**21** are ineffective as inhibitors of trypanothione[2] or glutathione enzymes.[11−15] Diesters, per se, lack the minimum requirements for binding, the presence of a free α-Glu-COOH group.[12,13] To be effective as inhibitors these diester compounds would have to undergo partial or complete de-esterification by nonspecific esterases on entry into the parasite. The N-blocked monoesters or diacids are discounted as inhibitors of the two enzymes responsible for trypanothione biosynthesis, glutathionylspermidine synthetase[6,24] and trypanothione synthetase,[24] due to the requirement of the former for a glutathione derivative with a free α-amino group[6] and the latter for an intact glutamyl residue. This leaves trypanothione reductase as the final candidate, known to bind derivatives of glutathione lacking a γ-Glu group or a free COOH group as in the case of *N*-benzyloxycarbonyl-L-cysteinylglycyl-3-methylaminopropylamide disulfide.[16] Compounds **1**−**3, 8, 9, 12**−**16** and **19** were tested at a relatively high concentration of 100 μM as inhibitors of trypanothione reductase using 5,5′-dithiobis{*N*-[3-(dimethylamino)propyl]-2-nitrobenzamide} as a colorimetric substrate as described by Davioud-Charvet.[25] No loss in trypanothione reductase activity was observed suggesting that the target of these compounds was another trypanothione dependent enzyme.

The mechanism of action of these compounds is consistent with the proposed mechanism reported for cancer cells treated with GSH esters where diesters were found to be the most effective compounds for delivery of GSH to cells due to their partial de-esterification to free acid and monoester in situ by nonspecific esterases, within 1 h of addition.[17] These compounds (**c**; see Scheme 1) were considered prodrugs[17−20] functioning primarily by increasing the membrane penetration of glutathione derivatives prior to cleavage into three possible glutathione derivatives (**d**, **f**, **g;** Scheme 1), of which only two were observed[17] (**f**, **g**), as expected. However in this case seven derivatives (**a**−**g**; Scheme 1) are possible based on the current understanding of detoxification mechanisms, which may account for the diverse range of therapeutic activities found with these compounds.[26] Of these potential derivatives only four or five types (**b, e, f, g,** and possibly **c**) are expected to

Scheme 1



be observed in reality depending on the nature of the groups R2, R3, and R4, located on the glutathione inhibitor (see Scheme 1). A preliminary study on four of these glutathione derivatives (**b, e, f, g**) linked to sensors[27,28] tested against ammonium sulfate fractionated cell extracts of the insect trypanosome *Crithidia fasciculata* indicates that two of the four inhibitors **(f** and **e)** were strongly recognized by proteins present in the 40% and 60−100% $(NH_4)_2SO_4$ extracts, respectively. In both cases, up to 76% of the recognition could be reversed by the addition of inhibitor to the solution. The 40% extract contains proteins of high molecular weight associated with trypanothione biosynthesis (e.g. GSS and TSS[24]) and the latter low molecular weight proteins (<30 kDa) associated with the trypanosomal peroxidase cycle (e.g. tryparedoxin peroxidase[29] and the tryparedoxins[30,31]), while the 40−60% $(NH_4)_2SO_4$ extract contains the enzyme trypanothione reductase[32] which showed no binding to **e**, consistent with enzymic studies. The recognition by these different classes of proteins for particular inhibitors is consistent with the known binding preferences of enzymes associated with trypanothione biosynthesis and to some extent tryparedoxins in view of the latter's relationship to glutaredoxin[31] and ribonucleotide reductase which is known to be inhibited by glutathione derivatives[33] such as **e** or **g**. The recognition of **f** by a different type of proteins to **e** may explain the higher inhibitory activity of compound **15** containing an *N*-acetyl group than a benzyloxycarbonyl group (Cbz). The *N*-acetyl group is readily deacylated by amidases, and therefore this compound as a monoester could possibly inhibit a different parasitic target **(**e.g. GSS[6,24] or TRS[24]) to that inhibited by Cbz-protected glutathiones **(10**−**14**, **16)** where de-esterification occurs more readily than deacylation and the resulting compounds resemble **b** or **e** (see Scheme 1).

Based on this reasoning the overall mode for the in vitro action of these compounds can be related to the balance of three factors: a, delivery of the compound into the cell; b, cleavage of the peptide by nonspecific esterases and amidases in the cytosol into several inhibitory forms; c, recognition by the target or targets, inhibition, and cell death.

The activity of compounds **15, 17**−**19, 21** and **22** clearly suggests that N,S-blocked GSH diester derivatives fulfill many of the above requirements for in vitro activity, and optimization of their structure to utilize the prodrug mechanisms proposed to generate the therapeutically most important active structure of those considered should eventually lead to a useful chemotherapeutic agent.

Conclusion

Glutathione derivatives have been shown for the first time to possess significant antiparasitic activity in vitro with only a few compounds showing selectivity against *T. brucei*. A limited structure−activity study indicates that N,S-blocked glutathione diesters are active against *T. brucei* parasites and that *N*-acetyl-*S*-benzyloxycarbonylglutathione dimethyl ester **15** and the *N*,*S*-benzyloxycarbonyl-*S*-2,4-dinitrophenylglutathione diester derivatives **17**−**19** and **21** represent lead structures possessing minimal toxicity which potentially could be developed further to yield a therapeutically active agent for the treatment of trypanosomiasis or leishmaniasis.

Experimental Section

GSH, DMAP and CbzCl were obtained from the Avocado Chemical Co. Ltd. (U.K.). Silica gel 60 F_{234} and alumina TLC plates were purchased from BDH Chemicals (Poole, Dorset, U.K.). NMR spectra were obtained on a JEOL 270 MHz FT-NMR spectrometer using TMS as an internal standard and FAB mass spectra on a VG Autospec instrument using cesium bombardment at 25kV in a matrix of 3-nitrobenzyl alcohol (NOBA). *S*-(4-Bromobenzyl)glutathione **(1)** was synthesized by the method of Vince et al.[21] and *S*-2,4-dinitrophenylglutathione

by the method of Sokolovsky et al.[8] Glutathione derivatives **2**−**10**, **12**, and **13** were synthesized by previously reported methods.[11]

***N*-Benzyloxycarbonyl-*S*-(4-bromobenzyl)glutathione glycinamide** (**11**) was prepared by N-acylation of **2** (0.5 g; 1.0 mmol) with the CbzCl/DMAP salt (2 equiv) according to literature procedures[9] and recrystallized from CH_3CN to recover colorless crystals (0.24 g; 40%): mp 178−182 °C; ^{1}H NMR (D_6MSO) δ 8.34 (t, Gly-NH, J = 4.6), 8.2 (d, Cys-NH, J = 9.3), 7.5 (d, Ar-m-2H, J = 9.3), 7.35 (s, Ar-5H), 7.3 (d, Ar-o-2H, J = 9.3), 7.2 (s, NH), 5.0 (s, O−CH_2−Ar), 4.5 (m, CH), 4.0 (m, CH), 3.7 (s, Ar−CH_2−S), 3.6 (m, Gly-CH_2), 2.7 (m, CH), 2.5 (m, CH_2), 2.2 (t, 2H, γ-Glu-CH_2), 1.8−2.0 (m, 2H, γ-Glu-CH_2); FABMS m/z 609 ((M + H)$^+$, 40), 633 ((M + Na)$^+$, 32); HRFABMS calcd for $C_{25}H_{30}N_4O_7SBr$ 609.1018, found 609.1017. Anal. Calcd for $C_{25}H_{29}N_4O_7SBr\cdot{}^1/_2H_2O$: C, 48.6; H, 4.7; N, 9.0. Found: C, 49.14; H, 4.41; N, 8.49.

***N*-Benzyloxycarbonyl-*S*-(4-bromobenzyl)glutathione di-*n*-butyl ester** (**14**) was synthesized by $SOCl_2$ (0.093 g; 0.72 mmol) esterification of *N*-benzyloxycarbonyl-*S*-(4-bromobenzyl)glutathione (0.2 g; 0.33 mmol) (**9**) in *n*-butanol (15 mL) using literature procedures as described for **12**.[11] The solvent was removed by azeotropic vacuum distillation with water to give an oily residue which was triturated with Et_2O to afford colorless crystals (0.13 g; 56% yield): mp 110−115 °C; ^{1}H NMR (D_6MSO) δ 8.8 (m, glyNH), 8.2 (d, cysNH, J = 9.9), 7.8 (d, GluNH, J = 9.9), 7.6 (d, 2 × Ar-*m*-H, J = 9.1), 7.4 (m, Ar-5H), 7.3 (d, 2 × Ar-*o*-*H*, J = 9.1), 5.1 (s, CH_2Ar), 4.65 (m, GluCH), 4.1 (t, 2 × CH_2−OCO, J = 7.2), 4.0 (d, glyCH_2, J = 6.5), 3.7 (s, S−CH_2), 3.6 (m, HN-*CH*-CH_2), 2.8 (dd, CH_a-S, J = 6.6, J = 0.3), 2.7 (dd, CH_b-S, J = 6.6, J = 0.3), 2.3 (t, CH_2CO, J = 5.7), 2.0 (m, GluCH_2CHCOO), 1.6 (m, 2 × *C*H_2−CH_2−CH_3), 1.4 (m, 2 × *C*H_2−CH_3), 1.0 (t, 2 × -CH_2-*C*H_3, J = 9.6). Anal. Calcd for $C_{33}H_{44}N_3O_8SBr\cdot{}^3/_2H_2O$: C, 52.93; H, 6.28; N, 5.6. Found: C, 52.77; H, 6.47; N, 6.19.

***N*-Acetyl-*S*-(benzyloxycarbonyl)glutathione dimethyl ester** (**15**) was synthesized by cleavage of the Cbz group from compound **16** (1.5 g; 2.5 mmol) with HBr/AcOH as described for **7**,[11] followed by acetylation with acetic anhydride/DMAP (4 equiv) as described for **8**,[9] to afford colorless crystals (0.38 g), purified by PTLC (1:10; MeOH/$CHCl_3$) to afford an analytical sample (0.11 g; 8.7%): mp 193−195 °C; ^{1}H NMR (D_6MSO) δ 8.46 (t, Gly-NH, J = 5.1), 8.26 (d, 2 × NH, J = 7.1), 7.43 (s, Ar-5H), 5.28 (s, O−CH_2−Ar), 4.58 (m, CH), 4.26 (m, CH), 3.86 (m, Gly-CH_2), 3.66 (s, 2 × OCH_3), 2.96 (m, CH_2), 2.26 (t, 2H, γ-Glu-CH_2), 1.88 (m,s, 5H, γ-Glu-CH_2, −$COCH_3$); FABMS m/z 512 ((M + H)$^+$, 67), 534 ((M + Na)$^+$, 100), 1023 ((2M + H)$^+$, 3), 1045 ((2M + Na)$^+$, 5). Anal. Calcd for $C_{22}H_{29}N_3O_9S$: C, 51.65; H, 5.72; N, 8.22. Found: C, 51.51; H, 5.69; N, 8.27.

***N*,*S*-Dibenzyloxycarbonylglutathione dimethyl ester** (**16**) was synthesized by $SOCl_2$ (1.6 g; 12.5 mmol) esterification of *N*,*S*-dibenzyloxycarbonylglutathione[8] (1 g; 1.7 mmol) in MeOH (20 mL) as described for **12**[11] to afford colorless crystals on recrystallization from methanol (0.7 g; 66.7% yield): mp 167−168 °C; ^{1}H NMR ($CDCl_3$) δ 7.35 (s, 2 × ArH), 5.25 (s, SCO_2−CH_2-Ar), 5.1 (s, Ar-CH_2−S), 4.7 (m, CH), 4.0 (d, Ar−CH_2−S), 3.7 (s, 2 × OCH_3), 3.3 (m, CH), 2.1 (m, Gly-CH_2, γ-Glu-CH_2CH_2). Anal. Calcd for $C_{28}H_{33}N_3O_{10}S$: C, 55.71; H, 5.51; N, 6.96. Found: C, 55.7; H, 5.7; N, 6.9.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione Diesters. Procedure A.** A mixture of *S*-(2,4-dinitrophenyl)glutathione mono- and diesters was prepared by $SOCl_2$ esterification of *S*-(2,4-dinitrophenyl)glutathione with the appropriate alcohol using literature procedures as described for **3**.[11] The crude mixture of mono- and diesters on evaporation of solvent was triturated with diethyl ether and the residue N-acylated with CbzCl/TEA (1.1 equiv) in MeOH containing a catalytic amount of DMAP. After stirring overnight at 55 °C, the solution was evaporated in vacuo and the residue triturated with Et_2O to remove the monoester. The residue left was extracted into AcOEt, washed water, dried ($MgSO_4$) and evaporated in vacuo and then retriturated with Et_2O to give the diester. The Et_2O layer containing the crude monoester was acidified to pH 4 with dilute hydrochloric acid (10%) and extracted into EtOAc, washed H_2O, dried ($MgSO_4$) and evaporated in vacuo to give the monoester for use elsewhere. An analytical sample of monosubstituted and disubstituted products were prepared by purification using preparative thin-layer chromatography (PTLC).

Procedure B. This was prepared by $SOCl_2$ (2.2 equiv) esterification of *N*-benzyloxycarbonyl-*S*-2,4-dinitrophenylglutathione in the appropriate alcohol as described for **12**.[11]

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione dimethyl ester (17)** was prepared using procedure A from a mixture (1 g) of *S*-(2,4-dinitrophenyl)glutathione methyl mono- and diester and isolated by PTLC (MeOH/$CHCl_3$; 1:3) as pale yellow crystals (0.78 g; 63% yield): mp 142−145 °C; ^{1}H NMR (D_6MSO) δ 9.0 (d, ArH_3), 8.7 (t, glyNH), 8.5 (d, cysNH), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.7 (d, GluNH), 7.3−7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.65 (td, GluCH), 4.2 (td, HN−C*H*-CH_2), 3.95 (d, glyCH_2), 3.7 (dd, CH_2−S), 3.6 (s, 2 × CH_3OCO), 2.25 (t, CH_2CO), 2.0 (td, GluCH_2CHCOO); CIMS m/z 653 ((M + NH_4)$^+$, 20), 636 ((MH)$^+$, 35), 436 ((M − $C_6H_3N_2O_4S$), 100), 502 ((M − $C_8H_7O_2$), 50); HRFABMS calcd for $C_{26}H_{30}N_5O_{12}S$ 636.3802, found 636.3802. Anal. Calcd for $C_{26}H_{29}N_5O_{12}S$: C, 49.13; H, 4.60; N, 11.02. Found: C, 49.54; H, 4.57; N, 10.56.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione dipropyl ester (18)** was prepared using procedure A from a mixture (1 g) of *S*-(2,4-dinitrophenyl)glutathione *n*-propyl mono- and diester and isolated by PTLC (MeOH/$CHCl_3$; 3:17) as pale yellow crystals (0.83 g; 64% yield): mp 110 °C; ^{1}H NMR (D_6MSO) δ 9.0 (d, ArH_3), 8.7 (t, glyNH), 8.5 (d, cysNH), 8.4 (dd, ArH_5), 8.0 (d, ArH_6),7.7 (d, GluNH), 7.3−7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.65 (td, GluCH), 4.1 (q, 2 × CH_3−CH_2-*C*H_2), 4.0 (td, HN−C*H*-CH_2), 3.95 (d, glyCH_2), 3.7(dd, CH_2−S), 2.25 (t, CH_2CO), 1.6 (q, 2 × CH_3-*C*H_2−CH_2), 2.0 (td, GluCH_2CHCOO), 1.0 (t, 2 × *C*H_3−CH_2); CIMS m/z 709 ((M − NH_4)$^+$, 30), 692 ((MH)$^+$, 50), 493 ((MH)$^+$ − $C_6H_3N_2O_4S$), 80), 664 ((M − C_2H_4), 40), 464 (M − (C_2H_4 + $C_6H_3N_2O_4S$), 100); HRFABMS calcd for $C_{30}H_{38}N_5O_{12}S$ 692.2237, found 692.2234. Anal. Calcd for $C_{30}H_{37}N_5O_{12}S$ (M_r 691.2159): C, 52.1; H, 5.35; N, 10.13. Found: C, 52.04, H, 5.33; N, 10.25.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione dibutyl ester (19)** was prepared using procedure A from a mixture (0.5 g) of *S*-(2,4-dinitrophenyl)glutathione *n*-butyl mono- and diester and isolated by PTLC (EtOAc/$CHCl_3$; 1:1) as pale yellow crystals (0.179 g; 28% yield): mp 115 °C; ^{1}H NMR (D_6MSO) δ 9.0 (d, ArH_3), 8.7 (t, glyNH), 8.5 (d, cysNH), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.7 (d, GluNH), 7.3−7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.65 (td, GluCH), 4.1 (t, 2 × CH_2−OCO), 4.0 (td, HN−CH−CH_2), 3.95 (d, glyCH_2), 3.7 (dd, CH_2−S), 2.25 (t, CH_2CO), 2 (td, GluCH_2CHCOO), 1.6 (m, 2 × CH_3−CH_2−CH_2), 1.15 (m, 2 × CH_2−CH_3), 0.95 (t, 2 × CH_3−CH_2); FABMS m/z 720 ((M + H)$^+$, 61), 742 ((M + Na)$^+$, 80). Anal. Calcd for $C_{32}H_{41}N_5O_{12}S\cdot{}^1/_2H_2O$: C, 52.72; H, 5.76; N, 9.61. Found: C, 52.54; H, 5.95; N, 10.25.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione dicyclohexyl ester (20)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione (0.5 g, 0.82 mmol) in cyclohexanol (25 mL). Azeotropic removal of the solvent with water gave an oily residue which on trituration with Et_2O gave a hygroscopic yellow solid which was purified by PTLC (MeOH/$CHCl_3$; 1:9) (0.4 g; 63% yield): mp 135−137 °C; ^{1}H NMR (D_6MSO) δ 8.9 (s, ArH_3), 8.6 (t, glyNH), 8.4 (m, cysNH & ArH_5), 8.0 (d, ArH_6), 7.7 (d, GluNH), 7.3−7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.6 (m, GluCH & 2 × CH_{cHx}), 4.0 (td, HN−CH−CH_2), 3.95 (d, glyCH_2), 3.7 (dd, CH_2−S), 2.3 (t, CH_2CO), 2.0 (m, GluCH_2CHCOO), 1.4 (m, 2 × (CH_2−CH−CH_2)), 1.2 (m, 2 × $(CH_2)_{3\ cHx}$); FABMS m/z 772 (M + H)$^+$, 794 (M + Na)$^+$; HRFABMS calcd for $C_{36}H_{46}N_5O_{12}S$ 772.2863, found 772.2827. Anal. Calcd for $C_{36}H_{45}N_5O_{12}S\cdot 2H_2O$: C, 53.52; H, 6.11; N, 8.67. Found: C, 53.23; H, 5.52; N, 8.68.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione dihexyl ester (21)** was prepared using procedure A from a mixture (0.5 g, 0.89 mmol) of *S*-(2,4-dinitrophenyl)-glutathione *n*-hexyl mono- and diester and isolated by PTLC (MeOH/$CHCl_3$; 1:9) as pale yellow solid (0.56 g; 94% yield):

mp 130−132 °C; ^{1}H NMR (D_6MSO) δ 9.0 (d, ArH_3), 8.7 (t, glyNH), 8.5 (d, cysNH), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.7 (d, GluNH), 7.3−7.4 (m, Ar), 5.1 (s, CH_2Ar), 4.65 (td, GluCH), 4.1 (t, 2 × CH_2OCO), 4.0 (td, HN−CH−CH_2), 3.95 (d, glyCH_2), 3.7 (dd, CH_2−S), 2.3 (t, CH_2CO), 2.0 (td, GluCH_2CHCOO), 1.6 (t, 2 × CH_2−CH_2−OCO), 1.3 (m, 2 × $(CH_2)_3$), 0.9 (t, 2 × CH_3−CH_2); FABMS *m*/*z* 776 ((M + H)$^+$, 75), 798 ((M + Na)$^+$, 100); HRFABMS calcd for $C_{36}H_{50}N_5O_{12}S$ 776.3176, found 776.3664. Anal. Calcd for $C_{36}H_{49}N_5O_{12}S$: C, 55.72; H, 6.37; N, 9.03. Found: C, 55.75; H, 6.58; N, 9.00.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione dioctyl ester (22)** was prepared using procedure A from a mixture (0.5 g, 0.81 mmol) of *S*-(2,4-dinitrophenyl)-glutathione *n*-octyl mono- and diester and isolated by PTLC (MeOH/$CHCl_3$; 1:9) as a pale yellow solid (0.57 g; 82% yield): mp 144 °C; ^{1}H NMR (D_6MSO) δ 9.0 (d, ArH_3), 8.7 (t, glyNH), 8.5 (d, cysNH), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.7 (d, GluNH), 7.3−7.4 (m, ArH), 5.1 (s, CH_2Ar), 4.65 (td, GluCH), 4.1 (t, 2 × CH_2OCO), 4.0 (td, HN−CH−CH_2), 3.95 (d, glyCH_2), 3.7 (dd, CH_2−S), 2.3 (t, CH_2CO), 2.0 (td, GluCH_2CHCOO), 1.6 (t, 2 × CH_2−CH_2−OCO), 1.3 (m, 2 × $(CH_2)_5$), 0.9 (t, 2 × CH_3−CH_2); FABMS *m*/*z* 832 ((M + H)$^+$, 60), 854 ((M + Na)$^+$, 100). Anal. Calcd for $C_{40}H_{57}N_5O_{12}S$: C, 57.75; H, 6.91; N, 8.42. Found: C, 57.29; H, 7.10; N, 8.27.

Evaluation of the Parasitic Activity of Glutathione Derivatives in Vitro. Parasites: *T. brucei* (strain S427) bloodstream form trypomastigotes were maintained in HMI-18 medium[34,35] with 20% heat inactivated foetal calf serum (HIFCS) (Harlan Sera-Lab, Crawley, U.K.) at 37 °C in 5% CO_2−air mixture. *L. donovani* (strain MHOM/ET/67/L82) was maintained routinely in special pathogen free (SPF) female golden hamsters by serial passage every 6−8 weeks. *T. cruzi* (strain MHOM/BR/OO/Y) trypomastigotes were derived from MDCK fibroblasts in Dulbecco's modified Eagle medium (Life Technologies Ltd., Paisley, Scotland) with 10% HICFS at 37 °C in a 5% CO_2−air mixture.

In vitro assays: *T. brucei:* All compounds were tested in triplicate in a 3-fold dilution series from a top concentration of 30 μM. Parasites were diluted to 2 × 10^5/mL and added in equal volumes to the test compounds in 96-well, flat bottom Microtest III tissue culture plates (Becton Dickinson and Co., NJ). Appropriate controls with pentamidine isethionate (Rhone-Poulenc-Rorer) as the positive were set up in parallel. Plates were maintained for 3 days at 37 °C in a 5% CO_2−air mixture. Compound activity was determined by the use of a tetrazolium salt colorimetric assay[37] on day 3.

L. donovani and *T. cruzi:* Peritoneal macrophages were harvested from female CD1 mice (Charles Rivers Ltd., Margate, U.K.) by peritoneal lavage 24 h after starch (Merck Ltd., Leics, U.K.) induced recruitment. After washing cells were dispensed into 16-well Lab-tek tissue culture slides (Nunc Inc., IL) at 4 × 10^4/well in a volume of 100 μL of RPMI-1640 medium (Sigma-Aldrich Co. Ltd., Dorset, U.K.) and 10% HIFCS. After 24 h, macrophages were infected at a ratio of 10:1 (4 × 10^5/well) with *L. donovani* amastigotes or 5:1 (2 × 10^5/well) with *T. cruzi* trypomastigotes. Infected macrophages were then maintained in the presence of drug in a 3-fold dilution series in quadruplicate for 5 days in the case of *L. donovani* and 3 days with *T. cruzi*. Drug activity was evaluated from the percentages of macrophages cleared of amastigotes in treated cultures. Sodium stibogluconate ($NaSb^v$) (Glaxo-Wellcome, Dartford, U.K.) and nifurtimox (Bayer, U.K.) were used as the respective controls.[36]

Evaluation of the Cytotoxicity of Glutathione Derivatives in Vitro. Cytotoxicity testing on K562 (human, chronic myelogenous leukemia) was undertaken independently on our behalf by the Christie Patterson Institute for Cancer Research, Manchester. The cytotoxicity of drugs were evaluated using the semi automated MMT assay developed by the National Cancer Institute (NCI), Maryland, based on the original work of Mosmann.[37] Cell cultures were grown in RPMI medium with 10% calf fetal serum (CFS) at 37 °C in a 5% CO_2−air mixture in a humidified incubator. Plates were incubated with compound for 5 days at 37 °C in a 5% CO_2-humidified air mixture prior to determination of activity by the use of a tetrazolium salt colorimetric assay[35,37] on day 5.

Trypanothione Reductase: Isolation and Assay. Trypanothione reductase was isolated as previously described[32] from *Crithidia fasciculata* grown on Schneider's insect medium (Sigma-Aldrich Co. Ltd., Dorset, U.K.), containing 0.0002% streptomycin sulfate, FMN (2.4 mg/L) and 10% heat inactivated CFS (Sigma-Aldrich Co. Ltd., Dorset, U.K.). All enzymatic and nonenzymatic reactions were conducted in a flat-bottomed 96-well microtiter plates (Sterilin) in a total volume of 100 μL. The plates on incubation at room temperature were then read using a 410 nm filter in a Dynatech MR5000 multiscan microplate reader connected to a Star Multipart LC-10 printer. Inhibition studies were undertaken on compounds **1**−**3**, **8**, **9** and **12**−**16** using a one-spot enzyme inhibition assay as previously described[25] with 5,5′-dithiobis{*N*-[3-(dimethylamino)propyl]-2-nitrobenzamide}·HCl salt[25] as the colorimetric substrate. The reaction was initialized by the addition of 80 μL of enzyme solution containing 42 × 10^{-4} U of trypanothione reductase and the reaction stopped after 10 min by the addition of 20 μL of acetonitrile. Measurements were undertaken against suitable controls, with and without enzyme, NADPH, or substrate.

5,5′-Dithiobis{*N*-[3-(dimethylamino)propyl]-2-nitrobenzamide}·HCl salt was prepared in a manner similar to the literature[25] and obtained as a pale yellow amorphous powder: ESIMS *m*/*z* 565 ([MH]$^+$, 15), 283 (100); HRESIMS calcd for $C_{24}H_{33}N_6O_6S_2$ [MH]$^+$ 565.1903, found 565.1905.

Acknowledgment. This investigation received financial support from the UDNP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). The authors also thank EPSRC for support under Grant GR/F 20937 and Dr. Ballantine and B. Stein of the EPSRC Mass Spectrometry Service Center, Swansea, for FABMS and HRFABMS measurements. The Christie Paterson Institute for Cancer Research, Manchester, is thanked for toxicity testing of compounds and H. Kendrick (LSHTMS) for statistical analysis of the data. The authors also thank the Biological Sciences Department (MMU) and Microbiology Section for access to their facilities and 21C Technology Ltd. (Cheshire) for use of their sensor.

References

(1) Barrett, M. P.; Fairlamb, A. H. The Biochemical Basis of Arsenical-Diamidine Crossresistance in African Trypanosomes. *Parasitol. Today* **1999**, *15* (4), 136−140.
(2) Krauth-Siegel, R. L.; Coombs, G. H. Enzymes of Parasite Thiol Metabolism as Drug Targets. *Parasitol. Today* **1999**, *15* (10), 404−409.
(3) Hajduk, S. L.; Englund, P. T.; Smith, D. H. In *Tropical and Geographic Medicine;* Warren, K. S., Mahmoud, A. A. F., Eds.; McGraw-Hill: New York, NY, 1989; pp 268−281; *Tropical Disease Science at Work* (World Health Organisation, Geneva, Switzerland, 1986).
(4) Fairlamb, A. H.; Cerami, A. Metabolism and functions of trypanothione in the kinetoplastid. *Annu. Rev. Microb.* **1992**, *46*, 695−729.
(5) Kosower, E. M.; Radkowsky, A. E.; Fairlamb, A. H.; Croft, S. L.; Neal, R. A. Bimane cyclic esters, possible stereologues of trypanothione as antitrypanosomal agents. Bimanes 29. *Eur. J. Med. Chem.* **1995**, *30*, 659−671.
(6) DeCraecker, S.; Verbruggen, C.; Rajan, P. K.; Smith, K.; Haemers, A.; Fairlamb, A. H. Characterization of the peptide substrate specificity of glutathionylspermidine synthetase from *Crithidia fasciculata*. *Mol. Biochem. Parasitol.* **1997**, *84*, 25−32.
(7) D'Silva, C.; Daunes, S. Glutathione peptides as anti-parasitic drugs. Symposium abstracts, 1st COST-B9 Congress on Antiprotozoal Chemotherapy, Sierra Nevada, Granada, Spain, May 31−June 3, 1998.
(8) Sokolovsky, M.; Wilchek, M.; Patchornik, A. On the synthesis of cysteine peptides. *J. Am. Chem. Soc.* **1964**, *86*, 1202−1206.
(9) D'Silva C.; Timari A. A.; Douglas K. T. Synthesis of N-acyl glutathione derivatives by (dimethylamino)pyridine catalysis. *Biochem. J.* **1982**, *207*, 329−332.

(10) Douglas K. T.; Timari A. A.; D'Silva, C.; Gohel D. I. Role of the N-terminus of glutathione in the action of yeast glyoxalase I. *Biochem. J.* **1982**, *207*, 323−329.
(11) D'Silva, C. Synthesis of carboxy-residue-modified coenzyme derivatives as probes to the mechanism of glutathione enzymes. *Biochem. J.* **1990**, *271*, 167−169. D'Silva, C. Correction. *Biochem. J.* **1990**, *3*, 483.
(12) D'Silva C. Reinvestigation of the roles of the carboxyl groups of glutathione with yeast glyoxalase I: Implications to the mechanism and coenzymic role of glutathione. *FEBS Lett.* **1986**, *202*, 240−244.
(13) D'Silva C. Inhibition and Recognition Studies on the glutathione binding site of Equine liver glutathione-S-transferase. *Biochem. J.* **1990**, *271*, 161−165.
(14) Norton, S. J.; Elia, A. C.; Chyan, M. K.; Gillis, G.; Frenzel, C.; Princpato, G. B. Inhibitors and inhibition studies of mammalian glyoxalase II. *Biochem. Trans.* **1993**, *21*, 545−549.
(15) Al-Timari, A.; Douglas, K. T. Inhibition by glutathione derivatives of bovine liver glyoxalase II (hydroxyacylglutathione hydrolase) as a probe of the N- and S-sites for substrate binding. *Biochim. Biophys. Acta* **1986**, *870*, 219−225.
(16) El-Waer, A. F.; Smith, K.; McKie, J. H.; Benson, T.; Fairlamb, A. H.; Douglas, K. T. The glutamyl binding site of trypanothione reductase from *Crithidia fasciculata*: enzyme kinetic properties of γ-glutamyl modified substrate analogues. *Biochim. Biophys. Acta* **1993**, *1203*, 93−98.
(17) Thornalley, P. J.; Edwards, L. G.; Kang, Y.; Wyatt, C.; Davies, N.; Ladan, M. J.; Double, J. Antitumour activity of S-*p*-bromobenzylglutathione cyclopentyl diester in vitro and in vivo. *Biochem. Pharmacol.* **1996**, *51*, 1365−1372.
(18) Thornalley, P. J.; Ladan, M. J.; Ridgway, J. S.; Kang, Y. Antitumour Activity of S-(*p*-Bromobenzyl)glutathione diesters in *Vitro:* A Structure−Activity Study. *J. Med. Chem.* **1996**, *39*, 3409−3411.
(19) Levy, E. J.; Anderson, M. E.; Meister, A. Transport of glutathione diethyl esters in human cells. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9171−9175.
(20) Minhas, H. M.; Thornalley, P. J. Comparison of the delivery of reduced glutathione into $P388D_1$ cells by reduced glutathione and its mono and di-ethyl ester derivatives. *Biochem. Pharmacol.* **1995**, *49*, 1475−1482.
(21) Vince, R.; Daluge; S.; Wadd. W. B. Studies on the inhibition of glyoxalase I by S-substituted glutathiones. *J. Med. Chem.* **1971**, *14*, 402−405.
(22) Blizer, M.; Krauth-Siegel, R. L.; Schirmer, R. H.; Akerboom, T. P. M.; Sies, H and Schulz, G. E. *Eur. J. Biochem.* **1984**, *138*, 373−378.
(23) Bansal, A. K.; Dubey, R.; Khar, R. K. Quantitation of Activity of Alkyl ester Prodrugs of Ibuprofen. *Drug Dev. Ind. Pharm.* **1994**, *20* (12), 2025−2034.
(24) Smith, K.; Nadeau, K.; Bradley, M.; Walsh, C.; Fairlamb, A. H. Purification of glutathionylspermidine and trypanothione synthetases from *Crithidia fasciculata*. *Protein Sci.* **1992**, *1*, 874−883.
(25) Davioud-Charvet, E.; Becker. K.; Landry, V.; Gromer, S.; Loge′, C.; Sergheraert, C.; Synthesis of 5,5′-Dithiobis(2-nitrobenzamides) as Alternative Substrates for Trypanothione Reductase and Thioredoxin Reductase: A Microtitre Colorimetric Assay for Inhibitor Screening. *Anal. Biochem.* **1999**, *268*, 1−8.
(26) S-Blocked Glutathiones. U.K. Patent filed Sept 1,1997, Pub. no. GB2332676A.
(27) D'Silva, C. Receptor based Biosensors. In *Biosensors* (Current Topics in Biophysics); Frangopol, P. T., Sanduloviciu, M., Eds.; Iasy University Press: Iasy, Romania, 1995; Vol. 5, pp 1−19.
(28) Barnes, C.; D'Silva. C.; Jones, J. P.; Lewis, T. J. The Theory of operation of Piezoelectric Crystal Sensors for Biochemical Applications. *Sensors Actuators A.* **1992**, *31*, 159−163.
(29) Montemartin, M.; Nogoceke, E.; Singh, M.; Steinert, P.; Flohe, L.; Kalisz, H. M. Sequence analysis of the Tryparedoxin Peroxidase Gene from *Crithidia fasciculata* and its Functional Expression in *Escherichia coli. J. Biol. Chem.* **1998**, *273*, 4864−4871.
(30) Gommel, D. U.; Nogoceke, E.; Morr, M.; Kiess, M.; Kalisz, H. M.; Flohe, L. Catalytic characteristics of tryparedoxin. *Eur J. Biochem.* **1997**, *248*, 913−918.
(31) Ludemann, H.; Dormeyer, M.: Sticherling, C.; Stallmann, D.; Follmann, H.; Krauth-Siegel, R. L. *Trypanosoma brucei* tryparedoxin, a thioredoxin-like protein in African trypanosomes. *FEBS Lett.* **1998**, *431*, 381−385.
(32) Shames, S. L.; Fairlamb, A. H.; Cerami, A.; Walsh, C. T. Purification and Characterisation of Trypanothione Reductase from *Crithidia fasciculata* a Newly Discovered Member of the family of Disulphide-Containing Flavoprotein Reductases. *Biochemistry* **1986**, *25*, 3519−3526.
(33) Hoog, J.-O.; Holmgren, A.; D'Silva, C.; Douglas, K. T.; Seddon, A. P. Glutathione Derivatives as inhibitors of Glutaredoxin and ribonucleotide Reductase from *Escherichia coli. FEBS Lett.* **1982**, *138*, 59−61.
(34) Hirumi-H.; Hirumi-K. Continuous cultivation of *Trypanosoma brucei* blood stream forms in a medium containing a low concentration of serum proteins without feeder cell layers. *J. Parasitol.* **1989**, *75*, 985−989.
(35) Ellis, J. A.; Fish, W. R.; Sileghem, M.; McOdimba, F. A colorimetric assay for trypanosome viability and metabolic function. *Vet. Parasitol.* **1993**, *50*, 143−149.
(36) Neal, R. A.; Croft, S. L. An in vitro system for determining the activity of compounds against the intracellular amastigote form of *Leishmania donovani. J. Antimicrob. Chemother.* **1984**, *14*, 463−475.
(37) Mossmann, T. Rapid Colorimetric assay for cellular growth and Survival. Application to Proliferation and Cytotoxicity Assay. *J. Immunol. Methods* **1983**, *63*, 55−66.

JM990259W