QSAR Study on the Contribution of Log *P* and E_s to the in Vitro Antiprotozoal Activity of Glutathione Derivatives

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A series of N-S-blocked glutathione monoester and diester derivatives based on *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione were evaluated for activity against the pathogenic parasites *Trypanosoma brucei brucei*, *Trypanosoma cruzi*, and *Leishmania donovani* in vitro. Only monoesters **7**−**9** with a log *P* value of >2.7 were active inhibitors of *T.b. brucei* bloodstream form trypomastigotes. Diester compounds **10**−**15** and **17**−**27** in most cases were better inhibitors of *T.b. brucei* than monoester compounds, and some displayed high activity against *T. cruzi* **14** and *L. donovani* **17**, **19**, **29**. Compounds **14**, **24**, and **25** were the most active compounds identified against *T.b. brucei* having ED_{50} values of <0.4 μM. Analysis of the inhibition data (ED_{50}) vs calculated log *P* and E_s values provided evidence to support membrane penetration and steric factors as the key component in the activity of these compounds. The optimum values for log *P* and E_s determined were 5.8 and −0.70, respectively. A QSAR equation relating log-($1/ED_{50}$) vs log *P* and E_s was determined and interpreted within the proposed mechanism of activity for these compounds.

Introduction

Trypanosomiasis and leishmaniasis are important parasitic diseases in humans and domestic animals throughout the tropical and subtropical world. These infections pose serious health problems to the countries in these regions as well as damage to their economies.[1,2] The chemotherapy of these diseases is deficient as many drugs have either poor efficacy, toxic side effects, or are ineffective in the late chronic stages of the disease. Parasites display a large number of unique metabolic pathways not present in other mammalian cells. One general approach to the development of novel antiparasitic drugs is to identify key differences in metabolism between the host and pathogen and to exploit them in the design of selective toxic agents. Thiol metabolism of trypanosomatids[3] is characterized by a dependence on the hexapeptide trypanothione (N^1,N^8-bis(glutathionyl)spermidine) ($T(SH)_2$), an antioxidant replacing glutathione (GSH), the major antioxidant in other eukaryotic cells. The importance of trypanothione as an antiprotozoal drug target is highlighted by the fact that some trypanocidal drugs, notably the arsenicals (melarsoprol)[4] and difluoromethylornithine (eflornithine),[5] may work by interfering with the metabolism or synthesis of this hexapeptide. The central role of trypanothione in *Trypanosoma* and *Leishmania* parasites make thiol-dependent enzymes potential targets for the development of chemotherapeutic drugs. Despite the successful inhibition of enzymes such as trypanothione reductase[6] and glutathionylspermidine synthetase[7,8] in situ, some of these compounds have proved to be inactive against trypanosomes in vitro.[6−8] The poor success of rational drug design studies to produce compounds active in vitro against these types of protozoa led us to adopt a lead directed approach to identify potential antiparasitic compounds. In a recent paper[9] we reported the identification of several glutathione derivatives with activity against *Trypanosoma* and *Leishmania*. A structure−function study on *S*-bromobenzylglutathione derivatives identified the antiparasitic activity to be exclusively associated with N,S-blocked glutathione diester derivatives.[9] The nature of the N and S groups was found to contribute to the compounds' selectivity.[9] Glutathione diesters, due to their ease of membrane penetration and hydrolysis by nonspecific esterases to free acids, have been proposed as chemical delivery systems (CDS) for glutathione into cells.[10,11]

The nature of the ester group controls the hydrophobic character of derivatives and its ability to cross cell membranes, while the conformation and structure of the group may control its susceptibility to hydrolysis, due to its fit with the binding sites of esterases and as a consequence its receptor interactions. To evaluate the role of hydrophobicity and conformation on the *Trypanosoma brucei brucei* activity of glutathione derivatives, we synthesized a series of glutathione mono **3**−**9** and diester derivatives **10**−**32** of increasing hydrophobicity and steric variability as shown in Scheme 1. We present here a QSAR analysis of these results using the physicochemical parameters log *P* (an index of hydrophobicity) and E_s (Taft's steric parameter) to quantify the roles

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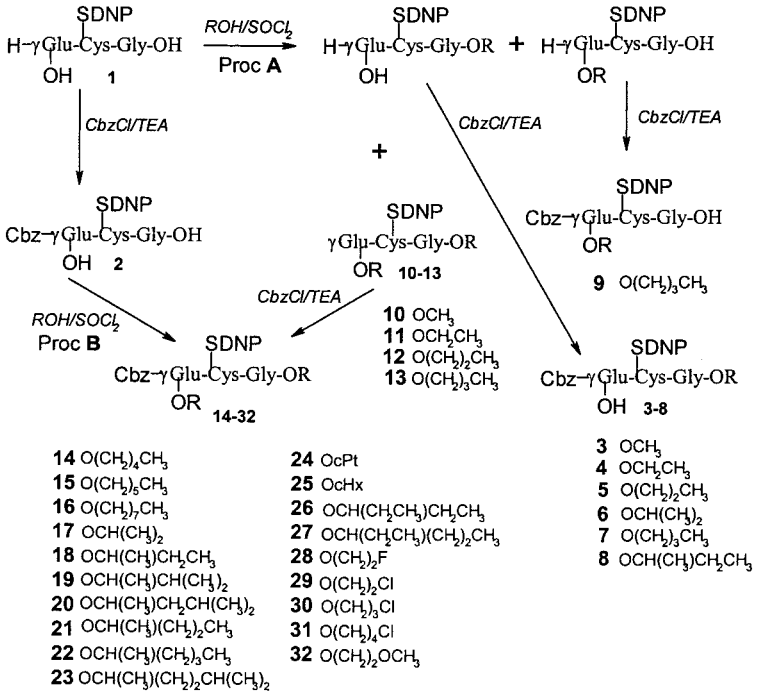
Scheme 1



played by each parameter to the in vitro activity of these compounds.

Chemistry

S-(2,4-Dinitrophenyl)glutathione **1** and *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl) glutathione **2** were prepared by standard procedures[12] and used as the starting materials for the synthesis of a series of mono- and diester derivatives **3**−**32**. Monoesters and diesters were synthesized using known methodology in the literature.[9,12,13] The synthesis of monoesters **3**−**9** was undertaken using *S*-(2,4-dinitrophenyl)glutathione **1** as the starting material with 1 mol equiv of thionyl chloride. An intimate mixture of mono- and diester glutathione derivatives was obtained which on reaction with benzylchloroformate was converted into the corresponding *N*-carbobenzyloxy derivatives and separated by preparative thin-layer chromatography (PTLC).[9] This procedure (A) allowed the synthesis of monoesters **3**−**9** and diesters **10**−**13** (see Scheme 1**)**. The remaining diesters **14**−**32** (see Scheme 1**)** were obtained by the reaction of *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione **2** with the appropriate alcohols and thionyl chloride (procedure B).

Results and Discussion

The synthesis of monoesters **3**−**9** did not proceed as expected, and the major product formed was the diesters **10**−**13**, contaminated with a small quantity of monoester (<20%). The latter required a significant amount of purification to achieve homogeneity. This result was contrary to studies on the use of this technique in the preparation of *S*-bromobenzylglutathione monoesters[13] and probably a result of the poor solubility of **1** in the alcohols used resulting in the initially formed monoesters being immediately converted to diesters. The synthesis of *S*-(2,4-dinitrophenyl)glutathione 1-butyl glycinate **7** proved an anomaly, as *S*-(2,4-dinitrophenyl)-glutathione 1-butyl glutamate **9** was also isolated in small quantities in addition to the diester **13**.

Table 1. Monoester Derivatives

cpd	ED_{50} (μM) *T.b. brucei*	ED_{50} (μM) *L. donovani*	ED_{50} (μM) *T. cruzi*	log *P*
3	na[a]	>30	na	1.32
4	na	>30	na	1.82
5	na	>30	na	2.31
6	na	na	na	2.23
7	20.0	na	na	2.8
8	20.0	20.0	na	2.72
9	10.26	>30 (12)[f]	>30 (42)[f]	2.8

[a] na = not active at ∼30 μmol.

The inhibitory activities of *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl) glutathione monoesters **3**−**9** were investigated against *T.b. brucei*, *Leishmania donovani*, and *Trypanosoma cruzi* (see Table 1). Low grade activity was observed against *T.b. brucei* with compounds **7**−**9** and against *L. donovani* with **8**. These results indicate that monoesters based on *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione with a log *P* value of >2.7 are probably sufficiently hydrophobic to enter parasite cells.

Diester compounds **13**, **14**, **29**, **30** (Table 2) and **20**, **24**, **25** (Table 3) showed significant inhibitory activity against *T.b. brucei* (>μM). Of the linear glutathione diester derivatives (see Table 2), compound **14**, the pentyl diester (log *P* ∼ 6.04), proved the most active with an ED_{50} value of 0.38 μM against *T.b. brucei* and 6.7 μM against *T. cruzi*. For the heteroatom substituted diesters **28**−**30**, the most active linear diesters against *T.b. brucei* (ED_{50} ∼ 0.50 μM) were the chloroethyl and chloropropyl diesters **29** and **30**, respectively. However, the toxicity of these types of compounds to KB cells was high, as expressed by the small relative toxicity values (KB/*T. brucei*) of less than unity (see Table 2), except in the case of **29**.

In the branched series, compounds **20**, **24**, and **25** displayed the highest activity against *T.b. brucei* (0.25 μM, 0.18 μM, and 0.21 μM, respectively). The inhibitory

Table 2. Linear Diester Derivatives

cpd	ED_{50} (μM) *T.b. brucei*	ED_{50} (μM) *L. donovani*	ED_{50} (μM) *T. cruzi*	ED_{50} (μM) KB cells	rel. tox KB/T.b.	log *P*	E_s[c]
10	16.0 ± 0.64	>30	na	17.9	1.1	2.11	0.0
11	3.5 ± 0.1	>30	na	272	78	3.09	−0.07
12	6.0 ± 0.16	>30	na	205	34	4.07	−0.36
13	1.07 ± 2.1	>30 (36)[f]	na	95.2	89	5.06	−0.39
14	0.38	>30	6.7 ± 2.9	>300	789	6.04	−0.41[e]
15	5.8 ± 0.32	>30 (9.0)[f]	>30 (36)[f]	109.3	18.8	7.02	−0.43[e]
16	na	>30 (11)[f]	na	95.3		8.99	
28	21.8 ± 0.03	>30 (26)[f]	>30 (8.0)[f]	15.2	0.99	2.97	
29	0.49 ± 0.06	$9.92 \pm 1 \times 10^{-4}$	T/+[a]	49.5	101	3.6	
30	1.05	>30	>30 (35)[f]	0.39	0.8	4.58	
31	3.05	>30	28.0 ± 2.1	0.38	0.12	5.56	
32	10.04	20		0.70	0.07	1.56	

[a] T/+: toxic to macrophages (~20 μmol)/inhibition of parasites. [b] na = not active at ~30 μmol. [c] Taft steric factor. [d] Calculated value of E_s using equation shown in Appendix. [e] Graphically extrapolated values from a plot of E_s vs number of carbon atoms for the series of linear esters (Me, Et, Pr, Bu). [f] The percent (%) inhibition, in parentheses, at 30 μM is expressed as the percent (%) decrease in viable parasites relative to the control.

Table 3. Branched Diester Derivatives

cpd	ED_{50} (μM) *T.b. brucei*	ED_{50} (μM) *L. donovani*	ED_{50} (μM) *T. cruzi*	ED_{50} (μM) KB cells	rel. tox KB/T.b.	log *P*	E_s[c]
17	20 ± 0.33	7.8	na	269	13.5	3.93	−0.47
18	2.0	>30 (36)	na	>300	150	4.91	−1.13
19	6.0 ± 0.08	13.4 ± 0.15	<30 (64)[e]	245	24	5.75	−1.36
20	0.25 ± 0.02	>30 (8.0)[e]	>30 (31)[e]	43.2	173	6.73	−1.59[d]
21	3.34 ± 0.4	>30 (25)[e]	>30 (17)[e]	393	118	5.89	−1.15
22	5.2 ± 0.44	>30 (6.0)[e]	>30 (21)[e]	178	46	6.87	−1.37
23	34.8	>30 (70)[e]	T/+[a]	>300	>9	7.71	−1.8[d]
24	0.18 ± 0.004	29.6 ± 1.3	>30 (30)[e]	50.5	280	5.67	−0.51
25	0.21 ± 0.04	T/+[a]	T/+[a]	51.0	243	6.65	−0.67
26	3.74 ± 0.03	>30 (17)[e]	T/+[a]	213	57	5.89	−1.1
27	2.06 ± 0.05	>30 (12)[e]	T/+[a]	211	102	6.87	−1.3

[a] T/+: toxic to macrophages (~20 μmol)/inhibition of parasites. [b] na = not active at ~30 μmol. [c] Taft steric factor. [d] Calculated value of E_s using the equation shown in the Appendix. [e] The percent (%) inhibition, in parentheses, at 30 μM is expressed as the percent (%) decrease in viable parasites relative to the control.

activity of **24** (0.18 μM; log $P \sim 5.67$) and **25** (0.21 μM; log $P \sim 6.65$) against *T.b. brucei* (Table 3) was marginally better than **14** (0.38 μM; log $P \sim 6.04$), the best linear diester (see Table 2). However, **25** displayed toxicity against macrophages used in the assay of these parasites (see Table 3). The branched diester compounds **17** and **19** exhibited low activity against *T.b. brucei* but a high inhibitory activity against *L. donovani* (7.8 and 13.4 μM, respectively), indicating a major difference in the inhibitory behavior of these compounds. The assay of *L. donovani* and *T. cruzi* is undertaken in murine macrophages, so the inhibition pattern observed probably reflects differences both in the penetration of host macrophage and the intracellular parasite.

A large proportion of branched diesters tested (see Table 3) showed some degree of toxicity against macrophages not seen in KB cells which may reflect differences in the phase I metabolism of branched chain esters between different cell types[14] and the toxicity of the hydrolysis product generated.

To identify the nature of the relationship between log-($1/ED_{50}$) and log *P* for *T.b. brucei*, a plot was undertaken on the data in Tables 2 and 3. The plot was found to be predominantly parabolic in nature with an optimum value for log *P* in the range 5.0−6.0. However, diesters **19**, **21**, and **26**, and to a lesser extent **10** and **11**, exhibited a poor fit to the plot, indicating a more complex relationship between log($1/ED_{50}$) and log *P*, which involved other descriptors. The inhibition of *Trypanosoma brucei rhodesiense*[15] by compounds **10**−**32** showed a similar parabolic dependence between log-($1/ED_{50}$) and log *P*, but the fit in this case was better. The optimum value for log *P* (log P^o) was estimated from the graphical plot to be 5.8 in the case of *T.b. brucei*. This value was consistent with compounds possessing good membrane penetration, with log *P* values in the range ~4−7,[14,16] and also indicates that the *T.b. brucei* activity of these glutathione diester derivatives was controlled to some degree by their ability to enter cells, as in the case of the anaesthetic ethers.[17] To address the poor fit of the linear diesters **10** and **11** and their branched counterparts **19**, **21**, **22**, and **26** to the parabolic dependence, the QSAR analysis of these data was evaluated in addition to the hydrophobicity descriptor (log *P*), two steric descriptors, molar refractivity (MR), and Taft's steric factor (E_s).[18,19]. Inclusion of MR appeared to be nonbeneficial, but this was not the case for E_s. Due to the absence of E_s values for the ester group **20** and **23** in the literature,[18,19] a value for this parameter was derived using multiple regression analysis (see Appendix and Table 3).

In the case of the pentyl **14** and hexyl **15** esters, E_s values were obtained by graphical extrapolation of the nonlinear plot of ED_{50} vs E_s, for linear diesters. Due to the limitations of the SciQSAR program permitting the evaluation of only one user derived descriptor, analysis of log *P* and E_s was not possible with this package. The QSAR equation determined was therefore derived by multiple linear regression analysis using the LINEST function in Microsoft Excel with the descriptors log *P*, E_s, and SciQSAR calculated descriptors (see Appendix). The best linear QSAR equation derived by a rigorous

process of elimination, using a set of 17 compounds, excluding the outliers **17**, **19**, and **20**, was eq 1 with an $R^2 = 0.84$ and $F = 12.0$.

$$\log(1/ED_{50}) = -2.57 \log P + 1.87E_s + 0.20\ MW - 0.002W - 102 \quad (1)$$

The equation consisted of two types of descriptors, hydrophobicity (log *P*) and steric factors (E_s, MW, *W*). The WienI (*W*) descriptor is a topological parameter[20] and a measure of the branching of a molecule, the value being larger for extended molecules than compact ones. Equation 1 indicates that the therapeutic activity of these compounds decreases with increasing values of log *P* and E_s, the latter a result of the overall negative character of these parameters (the log *P* coefficient is negative (see eq 1), while E_s values are predominantly negative (see Appendix), thus these two parameters render the overall equation negative in character). This result is consistent with there being a maximum value for log *P* and E_s. A plot of $\log(1/ED_{50})$ vs E_s also showed a parabolic dependence between the parameters with an optimum value in this case of −0.70 for E_s. The significance of log *P* and E_s may be interpreted within the proposed mechanism[9] for the delivery of glutathione diesters into cells involving diester delivery (related to log *P* and E_s) and hydrolysis to free acid and monoester by nonspecific esterases (related to E_s). HPLC studies[15] have confirmed the fate of these diesters in *T.b. brucei* cells by the isolation of monoester and diacid, in the cell. Trypanothione reductase, the primary target for the design of antiparasitic drugs, was again tested and eliminated in this case by evaluation of compounds **1**, **2**, **7**, and **13 (**Scheme 1**)** against enzyme isolated from *Crithidia fasciculata* using 5,5′-dithiobis{*N*-[3-(dimethylamino)propyl]-2-nitrobenzamide}·HCl salt[21] as a substrate as previously reported.[21]

The pharmacological value of the compounds identified in this study is dependent on their specificity which is a consequence of three main factors: (a) differences in transport properties between parasite and host resulting in delivery of compound into the cell (dependent on log *P* and E_s in the former case (see text) and possibly E_s, in the case of KB cell (see Tables 2 and 3)); (b) differences in metabolism of the peptide into its several active forms[9] with or without the formation of toxic byproducts (see Table 3**)**; (*c*) differences in recognition by the target or targets responsible for cell death, present in the parasitic cell with respect to the host. The relative toxicity values given in Tables 2 and 3 are a suitable indicator of specificity for compounds **13** < **29** < **20** < **25** < **24** < **14**. The difference in relative toxicity values (KB/T.b.) for the above compounds fall in a range between 80 and 800, clearly indicating that by optimization of structures to exploit subtle differences in characteristics between parasite and host it is possible to develop therapeutically useful compounds based on glutathione. While branched glutathione diesters have been proposed as therapeutic agents for use in cancer chemotherapy,[11] this is not the case here and both linear diesters **13**, **14**, and **29** and branched diester derivatives **20**, **24**, and **25** show a good therapeutic value based on their specificity.

Experimental Section

Precursors and solvents were purchased commercially and purified appropriately. GSH, DMAP, and CbzCl were obtained from the Avocado Chemical Co. Ltd. (U.K). Glutathione derivatives **1**, **9**, **11**, **12**, **14**, **15** and **25** were prepared as previously described.[9] Compounds **3**−**9** and **11** were synthesized using literature procedure A[9] and **14**, **17**−**24**, and **26**−**32** using procedure B.[9] 5,5′-Dithiobis{*N*-[3-(dimethylamino)-propyl]-2-nitrobenzamide}·HCl salt[21] was synthesized and assayed against trypanothione reductase as described previously by standard assay on microtiter plates.[21]

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione (2)** was prepared by the modification of a previously reported method.[12] To a stirred solution of *S*-(2,4-dinitrophenyl)glutathione **(1)** (2 g; 4.2 mmol) in MeOH were added dropwise Et_3N (0.88 mL; 6.34 mmol) and benzylchloroformate (0.9 mL; 6.34 mmol). After 20 h the solution was evaporated, acidified (pH 2), extracted (Et_2O), dried ($MgSO_4$), and evaporated in vacuo. The residue was triturated with Et_2O and dried (P_2O_5) to give a yellow product (2.3 g; 90% yield): mp 120−125 °C; ^{1}H NMR (D_6MSO) δ 9.0 (s, ArH_3), 8.7 (m, glyN*H*), 8.5 (2H, m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.8 (d, GluN*H*), 7.3−7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.65 (m, GluC*H*), 4.1 (m, HN-C*H*-CH_2), 4.0 (d, glyCH_2), 3.6 (dd, CH_2-S), 2.2 (t, CH_2CO), 2.0 (td, GluCH_2CHCOO).

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione methyl glycinate (3)** was prepared using procedure A from *S*-(2,4-dinitrophenyl)glutathione methyl ester (1 g, 1.97 mmol), MeOH (30 mL), Et_3N (0.42 mL, 2.96 mmol), and benzylchloroformate (0.42 mL, 2.96 mmol) and isolated by PTLC (MeOH/$CHCl_3$; 1:3) as pale yellow crystals (48 mg; 4% yield): mp 128−130 °C; ^{1}H NMR (D_6MSO) δ 9.1 (t, glyN*H*), 9.0 (d, ArH_3), 8.7 (d, cysN*H*), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.3−7.4 (m, Ar-5H), 6.7 (d, GluN*H*), 5.1 (s, CH_2Ar), 4.65 (td, GluC*H*), 4.2 (td, HN-C*H*-CH_2), 3.95 (d, glyCH_2), 3.7(dd, CH_2-S), 3.6 (s, CH_3OCO), 2.25 (t, CH_2CO), 2.0 (t, GluCH_2CHCOO). Anal. ($C_{25}H_{27}N_5O_{12}S\cdot H_2O$) N; C: calcd, 46.86; found, 46.30; H: calcd, 5.48; found, 5.03.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione ethyl glycinate (4)** was prepared using procedure A from *S*-(2,4-dinitrophenyl)glutathione ethyl ester (1 g, 1.92 mmol), MeOH (30 mL), Et_3N (0.40 mL, 2.9 mmol), and benzylchloroformate (0.40 mL, 2.9 mmol) and isolated by PTLC (MeOH/$CHCl_3$; 1:3) as pale yellow crystals (72 mg; 6% yield): mp 105−110 °C; ^{1}H NMR (D_6MSO) δ 9.1 (t, glyN*H*), 9.0 (d, ArH_3), 8.7 (d, cysN*H*), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.3−7.4 (m, Ar-5H), 6.7 (d, GluN*H*), 5.1 (s, CH_2Ar), 4.65 (td, GluC*H*),4.2 (q, *CH*$_2$-CH_3) 4.0 (td, HN-C*H*-CH_2), 3.95 (d, glyCH_2), 3.7 (dd, CH_2-S), 2.25 (t, CH_2CO), 2.0 (t, GluCH_2CHCOO), 1.2 (t, *CH*$_3$-CH_2); FABMS *m*/*z* 636 ((M + H)$^+$, 18), 658 ((M + Na)$^+$, 37), 680 ((M + 2Na)$^+$, 7). HRFABMS Calcd for $C_{26}H_{30}N_5O_{12}S$ 636.1611, Found 636.1611. Anal. ($C_{26}H_{29}N_5O_{12}SNa\cdot 3H_2O$) C, H; N: calcd, 9.65. found, 8.64.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione diethyl ester (11)** was prepared using procedure A from *S*-(2,4-dinitrophenyl)glutathione diethyl ester (767 mg; 61% yield): mp 100−105 °C; ^{1}H NMR (D_6MSO) δ 9.0 (d, ArH_3), 8.7 (t, glyN*H*), 8.5 (d, cysN*H*), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.7 (d, GluN*H*), 7.3−7.4 (m, Ar-5H), 5.1 (d, CH_2Ar), 4.65 (td, GluC*H*), 4.2 (q, 2 × *CH*$_2$-CH_3), 4.1 (td, HN-C*H*-CH_2), 3.95 (d, glyCH_2), 3.7(dd, CH_2-S), 2.25 (t, CH_2CO), 2.0 (td, GluCH_2CHCOO), 1.2 (t, 2 × CH_3-CH_2); FABMS *m*/*z* 664 ((M + H)$^+$, 100), 686 ((M + Na)$^+$, 80). HRFABMS Calcd for $C_{28}H_{34}N_5O_{12}S$ 664.1924, Found 664.1931. Anal. ($C_{28}H_{33}N_5O_{12}S\cdot 3H_2O$) N; C: calcd, 46.86; found, 46.33; H: calcd 5.48; found, 5.03.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione 1-propyl glycinate (5)** was prepared using procedure A from *S*-(2,4-dinitrophenyl)glutathione *n*-propyl ester (i.e., **a**) (1 g, 1.9 mmol), MeOH (30 mL), Et_3N (0.40 mL, 2.82 mmol), benzylchloroformate (0.40 mL, 2.82 mmol) and isolated by PTLC (MeOH/$CHCl_3$; 3:17) as pale yellow crystals (24 mg; 2% yield): mp 130−132 °C; ^{1}H NMR (D_6MSO) δ 9.3 (t, glyN*H*), 9.0 (d, ArH_3), 8.7 (d, cysN*H*), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.3−7.4 (m, Ar-5H), 6.4 (d, GluN*H*), 5.1 (s, CH_2Ar), 4.65 (td,

GluC*H*), 4.1 (q, 2 × CH_3-CH_2-*CH_2*), 4.0 (td, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.7 (dd, C*H_2*-S), 2.25 (t, C*H_2*CO), 2.0 (t, GluC*H_2*-CHCOO), 1.6 (q, CH_3-*CH_2*-CH_2), 1.0 (t, 2 × C*H_3*-CH_2). Anal. ($C_{27}H_{31}N_5O_{12}S·5H_2O$) C; H: calcd, 5.59; found, 5.16; N: calcd, 9.47; found, 8.92.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione 2-propyl glycinate (6)** was prepared using procedure A from *S*-(2,4-dinitrophenyl)glutathione 2-propyl ester (1 g, 1.9 mmol), MeOH (30 mL), Et_3N (0.40 mL, 2.82 mmol), and benzylchloroformate (0.40 mL, 2.82 mmol) and isolated by PTLC (MeOH/$CHCl_3$; 3:17) as pale yellow crystals (229 mg;18% yield): mp135−138 °C; ^{1}H NMR (D_6MSO) δ 9.1 (1H, t, glyN*H*), 9.0 (1H, d, ArH_3), 8.7 (1H, d, cysN*H*), 8.4 (1H, dd, ArH_5), 8.0 (1H, d, ArH_6), 7.3−7.4 (5H, m, Ar), 6.7 (1H, d, GluN*H*), 5.1 (2H, s, C*H_2*Ar), 5.0 (1H, m, *CH*(CH_3)$_2$), 4.65 (1H, td, GluC*H*), 4.0(1H, td, HN-C*H*-CH_2), 3.95 (2H, d, gly*CH_2*), 3.7(2H, dd, C*H_2*-S), 2.25 (2H, t, C*H_2*CO), 2.0 (2H, t, GluC*H_2*CHCOO), 1.2 (6H, d, 2 × C*H_3*); FABMS *m*/*z* 650 ((M + H)$^+$, 18), 672 ((M + Na)$^+$, 100), 694 ((M + 2Na)$^+$, 42). Anal. ($C_{27}H_{31}N_5O_{12}SNa·H_2O$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-2-propyl ester (17)** was prepared using procedure A from *S*-(2,4-dinitrophenyl)glutathione di-2-propyl ester (175.3 mg; 14% yield): mp 130−135 °C; ^{1}H NMR (D_6MSO) δ 9.0 (d, ArH_3), 8.7 (t, glyN*H*), 8.5 (d, cysN*H*), 8.4 (dd, ArH_5), 8.0 (d, ArH_6),7.7 (d, GluN*H*), 7.3−7.4 (m, Ar), 5.1 (d, C*H_2*Ar), 5.0(m, 2 × C*H*(CH_3)$_2$), 4.65 (td, GluC*H*), 4.0 (td, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.7(dd, C*H_2*-S), 2.25 (t, C*H_2*CO), 2.0 (td, GluC*H_2*-CHCOO), 1.2 (d, 4 × C*H_3*); CIMS *m*/*z* 709 ((MNH_4)$^+$, 20), 692 ((MH)$^+$, 30), 493 ((MH)$^+$ − $C_6H_3N_2O_4S$), 100), 558 ((MH)$^+$ − $C_8H_7O_2$), 30). HRFABMS Calcd for $C_{30}H_{38}N_5O_{12}S$ 692.2237, Found 692.2226. Anal. ($C_{30}H_{37}N_5O_{12}S·H_2O$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione 1-butyl glycinate (7)** was prepared using procedure A from a mixture of *S*-(2,4-dinitrophenyl)glutathione butyl esters (0.5 g, 0.91 mmol), MeOH (20 mL), Et_3N (0.20 mL, 1.3 mmol), and benzylchloroformate (0.20 mL, 1.3 mmol) and separated from **9** and **13** by PTLC (MeOH/$CHCl_3$: 3/7) as pale yellow crystals (100.3 mg; 17% yield): mp 173−175 °C; ^{1}H NMR (D_6-MSO) δ 9.1 (t, glyN*H*), 9.0 (d, ArH_3), 8.7 (d, cysN*H*), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.3−7.4 (m, Ar), 6.7 (d, GluN*H*), 5.1 (s, C*H_2*Ar), 4.65 (td, GluC*H*), 4.1 (t, C*H_2*-OCO), 4.0 (td, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.7(dd, C*H_2*-S), 2.25 (t, C*H_2*CO), 2.0 (t, GluC*H_2*CHCOO), 1.6 (m, CH_3-CH_2-*CH_2*), 1.15 (m, *CH_2*-CH_3), 0.95 (t, *CH_3*-CH_2). Anal. ($C_{28}H_{33}N_5O_{12}S$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione 1-butyl glutamate (9)** was prepared using procedure A from a mixture of *S*-(2,4-dinitrophenyl) butyl esters (0.5 g, 0.91 mmol), MeOH (20 mL), Et_3N (0.20 mL, 1.3 mmol), and benzylchloroformate (0.20 mL, 1.3 mmol) and separated from **7** and **13** by PTLC (MeOH/$CHCl_3$: 3/7) as pale yellow crystals (20.0 mg; 4% yield): mp 160−165 °C; ^{1}H NMR (D_6MSO) δ 9.1 (t, glyN*H*), 9.0 (d, ArH_3), 8.6 (m, cysN*H*, ArH_5), 8.0 (d, ArH_6), 7.6 (d, GluN*H*), 5.1 (s, C*H_2*Ar), 4.65 (m, GluC*H*), 4.1 (t, C*H_2*-OCO), 4.0 (td, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.7(m, C*H_2*-S), 2.25 (t, C*H_2*CO), 2.0 (td, GluC*H_2*CHCOO), 1.6 (m, CH_3-CH_2-*CH_2*), 1.15 (m, *CH_2*-CH_3), 0.95 (t, *CH_3*-CH_2); FABMS *m*/*z* 664 ((M + H)$^+$, 65), 686 ((M + Na)$^+$, 70). Anal. ($C_{28}H_{33}N_5O_{12}S·H_2O$): C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione 2-butyl glycinate (8)** was prepared using procedure A from *S*-(2,4-dinitrophenyl)glutathione 2-butyl ester (0.5 g, 0.91 mmol), MeOH (20 mL), Et_3N (0.20 mL, 1.3 mmol), and benzylchloroformate (0.20 mL, 1.3 mmol) and isolated by PTLC (MeOH/$CHCl_3$; 1:9) as pale yellow crystals. (42 mg; 7.0% yield): mp 130−135 °C; ^{1}H NMR (D_6MSO) δ 9.1 (t, glyN*H*), 9.0 (d, ArH_3), 8.7 (d, cysN*H*), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.3−7.4 (m, Ar), 6.6 (d, GluN*H*), 5.1 (s, C*H_2*Ar), 4.7 (m, C*H*), 4.65 (td, GluC*H*), 4.0 (td, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.7 (dd, C*H_2*-S), 2.25 (t, CH_2CO), 2.0 (td, GluC*H_2*CHCOO), 1.5 (td, *CH_2*-CH_3), 1.2 (d, *CH_3*-CH), 0.9 (t, *CH_3*-CH_2); FABMS *m*/*z* 686 ((M + Na)$^+$, 10), 708 ((M + 2Na)$^+$, 5). Anal. ($C_{28}H_{33}N_5O_{12}SNa$) C, N; H: calcd, 4.85; found, 5.67.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-2-butyl ester (18)** was prepared using procedure A from *S*-(2,4-dinitrophenyl)glutathione di-2-butyl ester (171.5 mg; 26.2% yield): mp 120−122 °C; ^{1}H NMR (D_6MSO) δ 9.0 (d, ArH_3), 8.7 (t, glyN*H*), 8.5 (d, cysN*H*), 8.4 (dd, ArH_5), 8.0 (d, ArH_6), 7.7 (d, GluN*H*), 7.3−7.4 (m, Ar), 5.1 (d, C*H_2*Ar), 4.7 (m, C*H*), 4.65 (td, GluC*H*), 4.0 (td, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.7 (dd, C*H_2*-S), 2.25 (t, C*H_2*CO), 2.0 (td, GluC*H_2*CHCOO), 1.5 (td, 2 × *CH_2*-CH_3), 1.2 (d, 2 × *CH_3*-CH), 0.9 (t, 2 × *CH_3*-CH_2); FABMS *m*/*z* 720 ((M + H)$^+$, 50), 742 ((M + Na)$^+$, 100). Anal. ($C_{32}H_{41}N_5O_{12}S$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione dipentyl ester (14)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl) glutathione **(2)** (0.3 g, 0.41 mmol), *n*-pentanol (25 mL), and thionyl chloride (0.085 mL, 0.97 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.24 g; 89% yield): mp 115−120 °C; ^{1}H NMR (D_6MSO) 9.0 (1H, s, ArH_3), 8.7 (1H, t, glyN*H*), 8.5 (1H, d, cysN*H*), 8.4 (1H, m, ArH_5), 8.0 (1H, d, ArH_6), 7.7 (1H, d, GluN*H*), 7.3−7.4 (5H, m, Ar), 5.1 (2H, d, C*H_2*Ar), 4.65 (1H, td, GluC*H*), 4.1(4H, t, 2 × C*H_2*OCO), 4.0 (1H, td, HN-C*H*-CH_2), 3.95 (2H, d, gly*CH_2*), 3.7(2H, dd, C*H_2*-S), 2.3 (2H, t, C*H_2*CO), 2.0 (2H, td, GluC*H_2*CHCOO), 1.6 (4H, t, 2 × *CH_2*-CH_2-OCO), 1.3 (8H, m, 2 × (*CH_2*)$_2$), 0.9 (6H, t, 2 × *CH_3*-CH_2); FABMS *m*/*z* 748.3 ((M + H)$^+$, 35), 770.3 ((M + Na)$^+$, 100). Anal. ($C_{34}H_{45}N_5O_{12}S·H_2O$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-3-methyl-2-butyl ester (19)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl) glutathione (**2**) (0.2 g, 0.33 mmol), 3-methyl-2-butanol (10 mL), and thionyl chloride (0.06 mL, 0.724 mmol) and isolated by trituration with Et_2O (2×) (0.17 g; 70% yield): mp 112−115 °C; ^{1}H NMR (D_6MSO) δ 9.0 (1H, s, ArH_3), 8.8 (1H, t, glyN*H*), 8.6 (1H, d, cysN*H*), 8.5 (1H, m, ArH_5), 8.1 (d, ArH_6), 7.8 (d, GluN*H*), 7.3−7.4 (m, Ar-5H), 5.1 (s, C*H_2*Ar), 4.7 (m, GluC*H* and 2 × CH_3-C*H*-OCO), 4.0 (td, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.7 (dd, C*H_2*-S), 2.3 (t, C*H_2*CO), 2.0 (td, GluC*H_2*CHCOO), 1.8 (m, 2 × *CH*(CH_3)$_2$), 1.2 (d, 2 × (*CH_3*-CH-OCO)), 0.9 (d, 2 × CH(C*H_3*)$_2$); FABMS *m*/*z* 748 ((M + H)$^+$, 70), 770 ((M + Na)$^+$, 85). HRFABMS Calcd for $C_{34}H_{46}N_5O_{12}S$ 748.2863, Found 748.2883. Anal. ($C_{34}H_{45}N_5O_{12}S·H_2O$) C; H: calcd, 6.19; found, 5.64; N: calcd, 9.15; found, 9.67.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-4-methyl-2-pentyl ester (20)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)-glutathione (**2**) (0.2 g, 0.33 mmol), 4-methyl-2-pentanol (10 mL), and thionyl chloride (0.06 mL, 0.72 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.16 g; 63% yield): mp 103−105 °C; ^{1}H NMR (D_6MSO) δ 9.0 (s, ArH_3), 8.8 (t, glyN*H*), 8.6 (d, cysN*H*), 8.5 (dd, ArH_5), 8.1 (d, ArH_6), 7.8 (d, GluN*H*), 7.3−7.4 (m, Ar-5H), 5.1 (s, C*H_2*Ar), 4.8 (m, GluC*H* and 2 × CH_3-C*H*-OCO), 4.1 (m, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.6 (dd, C*H_2*-S), 2.3 (2H, m, C*H_2*CO), 2.0 (m, GluC*H_2*-CHCOO), 1.8 (m, 2 × *CH*(CH_3)$_2$), 1.2 (m, 2 × (*CH_3*-CH-OCO) and 2 × C*H_2*-CH(CH_3)$_2$), 0.9 (d, 2 × CH(*CH_3*)$_2$); FABMS *m*/*z* 776 ((M + H)$^+$, 45), 798 ((M + Na)$^+$, 100). Anal. ($C_{36}H_{49}N_5O_{12}S·H_2O$) C, H; N: calcd, 8.83; found, 9.34.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-2-pentyl ester (21)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione (**2**) (0.3 g, 0.49 mmol), 2-pentanol (15 mL) and thionyl chloride (0.09 mL, 1.08 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.25 g; 69% yield): mp 123−125 °C; ^{1}H NMR (D_6MSO) δ 9.0 (s, ArH_3), 8.7 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.8 (d, GluN*H*), 7.3−7.4 (m, Ar-5H), 5.1 (s, C*H_2*Ar), 4.9 (m, 2 × CH_3-C*H*-OCO), 4.7 (m, GluC*H*), 4.1 (td, HN-C*H*-CH_2), 3.95 (d, gly*CH_2*), 3.6 (dd, C*H_2*-S), 2.3 (t, C*H_2*CO), 2.0 (m, GluC*H_2*CHCOO), 1.8 (m, 2 × *CH_2*-CH), 1.4 (m, 2 × *CH_2*-CH_3), 1.2 (m, 2 × *CH_3*-CH), 1.0 (m, 2 × *CH_3*-CH_2); ESIMS *m*/*z* 748 ((M + H)$^+$, 75), 770 (M + Na)$^+$, 746 (M − N)$^-$, 782 (M − Cl)$^-$. Anal. ($C_{34}H_{45}N_5O_{12}S$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-2-hexyl ester (22)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl glutathione **(2)**

(0.2 g, 0.33 mmol), 2-hexanol (20 mL), and thionyl chloride (0.15 mL, 1.65 mmol). Removal of the solvent gave an oily residue which on trituration with petroleum ether (40:60) gave a yellow solid which was purified by PTLC ($CHCl_3$/AcOEt; 5/5) (0.157 g, 62% yield): mp 125−128 °C; ^{1}H NMR (D_6MSO) δ 9.0 (s, ArH_3), 8.75 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.8 (d, gluN*H*), 7.5 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.9 (m, 2 × CH_3-CHOCO), 4.8 (m, gluC*H*), 4.2 (m, HN-C*H*-CH_2), 4.0 (d, glyCH_2), 3.8−3.7 (dd, CH_2-S), 2.3 (m, CH_2CO), 2.0 (m, gluCH_2CHCOO), 1.6 (m, 2 × CH_2-CH), 1.4 (m, 4 × CH_2), 1.2 (d, 2 × CH_3-CH), 1.0 (m, 2 × CH_3-CH_2); FABMS *m*/*z* 776.4((M + H)$^+$, 85), 798.4 ((M + Na)$^+$, 100); Anal. ($C_{36}H_{49}N_5O_{14}S$) C, N; H: calcd, 6.37; found, 5.91.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-3-pentyl ester (23)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione (**2**) (0.3 g, 0.49 mmol), 3-pentanol (15 mL), and thionyl chloride (0.09 mL, 1.08 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.15 g; 43% yield): mp 153−155 °C; ^{1}H NMR (D_6MSO) δ 9.0 (s, ArH_3), 8.7 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.9 (d, GluN*H*), 7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.8 (m, 2 × CH_3-CH_2-C*H*-OCO and GluC*H*), 4.1 (m, HN-C*H*-CH_2), 4.0 (d, glyCH_2), 3.6 (dd, CH_2-S), 2.4 (m, CH_2CO), 2.0 (m, GluCH_2CHCOO), 1.6 (m, 4 × CH_2-CH), 0.9 (t, 4 × CH_3-CH_2); FABMS *m*/*z* 748 ((M + H)$^+$, 100), 770 ((M + Na)$^+$, 55). HRFABMS Calcd for $C_{34}H_{46}N_5O_{12}S$ 748.2863, Found 748.2840. Anal. ($C_{34}H_{45}N_5O_{12}S$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione dicyclopentyl ester (24)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione (**2**) (0.5 g, 0.82 mmol), cyclopentanol (25 mL), and thionyl chloride (0.18 mL, 2.05 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.18 g; 67% yield): mp 110−112 °C; ^{1}H NMR (D_6MSO) δ 8.9 (s, ArH_3), 8.6 (t, glyN*H*), 8.4 (m, cysN*H* and ArH_5), 8.0 (d, ArH_6), 7.7 (d, GluN*H*), 7.3−7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.6 (m, GluC*H* and 2 × CH_{cPt}), 4.0 (td, HN-C*H*-CH_2), 3.95 (d, glyCH_2), 3.7(dd, CH_2-S), 2.3 (t, CH_2-CO), 2.0 (m, GluCH_2CHCOO), 1.4 (m, 2 × (CH_2-CH-CH_2)), 1.2 (m, 2 × $(CH_2)_{3\ cPt}$); ESIMS *m*/*z* 744 (M + H)$^+$, 761 (M + NH_4)$^+$, 766 (M + Na)$^+$, 742 (M−H)$^-$, 778 (M−Cl)$^-$. Anal. ($C_{34}H_{41}N_5O_{12}S$·H_2O) H, N; C: calcd, 53.60; found, 54.13.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-3-hexyl ester (26)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl) glutathione (**2**) (0.3 g, 0.49 mmol), 3-hexanol (15 mL), and thionyl chloride (0.09 mL, 1.08 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.21 g; 57% yield): mp 135−138 °C; ^{1}H NMR (D_6MSO) δ 9.0 (s, ArH_3), 8.7 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.8 (d, GluN*H*), 7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.9 (m, 2 × CH_3-CH_2-C*H*-OCO and GluC*H*), 4.1 (m, HN-C*H*-CH_2), 3.95 (d, glyCH_2), 3.6 (dd, CH_2-S), 2.3 (m, CH_2CO), 2.0 (m, GluCH_2CHCOO), 1.7 (m, 4 × CH_2-CH), 1.3 (m, 2 × CH_2-CH_3), 0.9 (q, 4 × CH_3-CH_2). FABMS *m*/*z* 776 ((M + H)$^+$, 75), 798 ((M + Na)$^+$, 70). Anal. ($C_{36}H_{49}N_5O_{12}S$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-5-methyl-2-hexyl ester (27)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)-glutathione (**2**) (0.4 g, 0.65 mmol), 5-methyl-2-hexanol (20 mL), and thionyl chloride (0.12 mL, 1.44 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.35 g; 68% yield): mp 145−148 °C; ^{1}H NMR (D_6MSO) δ 8.95 (s, ArH_3), 8.7 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.7 (d, GluN*H*), 7.3−7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.8 (m, 2 × CH_3-C*H*-OCO), 4.7 (m, GluC*H*), 4.1 (m, HN-C*H*-CH_2), 3.95 (m, glyCH_2), 3.5 (dd, CH_2-S), 2.3 (m, CH_2CO), 2.0 (m, GluCH_2-CHCOO), 1.6 (m, 2 × CH-CH_2-CH_2 and 2 × CH(CH_3)$_2$), 1.2 (m, 2 × CH_2-CH-CH_3 and 2 × (CH_3-CH)), 0.9 (m, 4 × CH_3-CH); FABMS *m*/*z* 809 ((M + H)$^+$, 40), 826 ((M + Na)$^+$, 10). Anal. ($C_{38}H_{53}N_5O_{12}S$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-2-fluoroethyl ester (28)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl glutathione **(2)** (0.2 g, 0.33 mmol), 2-fluoroethanol (20 mL), and thionyl chloride (0.15 mL, 1.65 mmol). Removal of the solvent gave an oily residue which on trituration with Et_2O gave a yellow solid which was purified by PTLC ($CHCl_3$/MeOH; 9/1) (0.14 g, 61% yield): mp 125−128 °C; ^{1}H NMR (D_6MSO) δ 8.95 (s, ArH_3), 8.8 (t, glyN*H*), 8.6 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.9 (d, gluN*H*), 7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.6−4.8 (m, 2 × CH_2OCO and gluC*H*), 4.3−4.5 (m, 2 × CH_2F), 4.25 (m, gluCH_2OCO), 4.2 (m, HN-C*H*-CH_2), 4.0 (d, glyCH_2), 3.6 (dd, CH_2-S), 2.3 (m, CH_2CO), 2.0 (m, gluCH_2CHCOO); FABMS *m*/*z* 700.24 ((M + H)$^+$,100), 722.23 ((M + Na)$^+$, 45); Anal. ($C_{28}H_{31}N_5O_{12}SF_2$) N; C: calcd, 47.39; found, 46.94; H: calcd, 4.45; found, 3.97.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-2-chloroethyl ester (29)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione (**2**) (0.4 g, 0.66 mmol), chloroethanol (20 mL), and thionyl chloride (0.15 mL, 1.65 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.38 g; 75% yield): mp 123−125 °C; ^{1}H NMR (D_6MSO) δ 8.95 (s, ArH_3), 8.7 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.0 (d, ArH_6), 7.8 (d, GluN*H*), 7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.7 (m, GluC*H*), 4.3 (t, 2 × CH_2OCO), 4.1 (m, HN-C*H*-CH_2), 3.9 (d, glyCH_2), 3.8 (t, 2 × CH_2Cl), 3.6 (dd, CH_2-S), 2.3 (m, CH_2CO), 2.0 (m, GluCH_2CHCOO); FABMS *m*/*z* 732 ((M + H)$^+$, 60), 754 ((M + Na)$^+$, 100). HRFABMS Calcd for $C_{28}H_{32}N_5O_{12}SCl_2$ 732.1145, Found 732.1144. Anal. ($C_{28}H_{31}N_5O_{12}SCl_2$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-3-chloropropyl ester (30)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)-glutathione (**2**) (0.15 g, 0.25 mmol), 3-chloro-1-propanol (15 mL), and thionyl chloride (0.11 mL, 1.23 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.14 g; 72% yield): mp 110−115 °C; ^{1}H NMR (D_6MSO) δ 8.95 (s, ArH_3), 8.7 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.8 (d, GluN*H*), 7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.7 (m, GluC*H*), 4.3 (t, 2 × CH_2OCO), 4.1 (m, HN-C*H*-CH_2), 3.9 (d, glyCH_2), 3.8 (t, 2 × CH_2Cl), 3.6 (dd, CH_2-S), 2.3 (m, CH_2CO), 2.1 (t, 2 × CH_2-CH_2Cl), 2.0 (m, GluCH_2CHCOO); FABMS *m*/*z* 760 ((M + H)$^+$, 100), 782 ((M + Na)$^+$, 60). Anal. ($C_{30}H_{35}N_5O_{12}SCl_2$·$H_2O$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-4-chlorobutyl ester (31)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione (**2**) (0.3 g, 0.49 mmol), 4-chloro-1-butanol (20 mL), and thionyl chloride (0.22 mL, 2.4 mmol) and isolated by trituration with Et_2O (2×) as pale yellow crystals (0.19 g; 50% yield): mp 215−220 °C; ^{1}H NMR (D_6MSO) δ 9.0 (s, ArH_3), 8.8 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.8 (d, GluN*H*), 7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.7 (m, GluC*H*), 4.1 (t, 2 × CH_2-OCO), 4.0 (m, HN-C*H*-CH_2 and glyCH_2), 3.8 (t, 2 × CH_2Cl), 3.6 (dd, CH_2-S), 2.3 (m, CH_2CO), 2.0 (m, GluCH_2CHCOO), 1.8 (m, 2 × CH_2-CH_2-CH_2Cl); FABMS *m*/*z* 788 ((M + H)$^+$, 100), 810 ((M + Na)$^+$, 92). HRFABMS Calcd for $C_{32}H_{39}N_5O_{12}SCl_2$ 787.1692, Found 787.1673. Anal. ($C_{32}H_{39}N_5O_{12}SCl_2$) C, H, N.

***N*-Benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)glutathione di-2-methoxyethyl ester (32)** was prepared using procedure B from *N*-benzyloxycarbonyl-*S*-(2,4-dinitrophenyl)-glutathione (**2**) (0.3 g, 0.49 mmol), 2-methoxyethanol (20 mL), and thionyl chloride (0.22 mL, 2.4 mmol) and isolated by trituration with Et_2O/petroleum ether/$CHCl_3$ as a yellow solid which was purified by PTLC ($CHCl_3$/MeOH; 9/1) (0.21 g, 58% yield): mp 112−115 °C; ^{1}H NMR (D_6MSO) δ 8.95 (s, ArH_3), 8.8 (m, glyN*H*), 8.5 (m, cysN*H* and ArH_5), 8.1 (d, ArH_6), 7.8 (d, GluN*H*), 7.4 (m, Ar-5H), 5.1 (s, CH_2Ar), 4.7 (m, GluC*H*), 4.3 (t, 2 × CH_2OCO), 4.2 (m, HN-C*H*-CH_2), 4.0 (m, glyCH_2), 3.7 (m, CH_2-S), 3.6 (m, 2 × CH_2O-), 3.3 (s, 2 × OCH_3) 2.3 (m, CH_2CO), 2.0 (m, GluCH_2CHCOO); ESIMS *m*/*z* 746 (M + Na)$^+$, 758 (M + Cl)$^-$. Anal. ($C_{36}H_{49}N_5O_{12}S$) C, N; H: calcd, 5.16; found, 5.63.

Log *P*, MR. Theoretical values of log K_{ow} (log *P*) were calculated using an interactive demo program established at the Environmental Service Center, Syracuse Research Corporation (SRC), in which structures were entered in SMILES notation (limited to a 100 characters). The program estimated

the octanol/water partition coefficient (log *P*) using an atom/fragment contribution method developed at SRC.[22] Calculated values of MR were undertaken using an online demo program (CMR) established at Daylight Chemical Information Systems, Inc.,[23] in which structures were entered in SMILES notation.

Quantitative Structure−Activity Regression (QSAR). Data analysis was undertaken using SciQSAR a module of Alchemy 2000 (Tripos). This program searched Alchemy 2000 for low energy molecular conformations and used the information to calculate several molecular descriptors. These descriptors were used in the programs regression approach for predicting QSAR parameters. The program allowed the creation of regression equations derived from a specific set of molecules or to alter existing equations by the addition of new agents (e.g., log *P*) to a group of molecules. Statistical regression analysis of data was undertaken using the LINEST curve fitting routine provided with Excel (Microsoft Corporation).

Evaluation of the Parasitic Activity of Glutathione Derivatives in Vitro: Parasites. *Trypanosoma brucei brucei* (strain S427) bloodstream form trypomastigotes were maintained in HMI-18 medium[24,25] with 20% heat inactivated foetal calf serum (HIFCS) (Harlan Sera-Lab., Crawley, U.K.) at 37 °C in 5% CO_2−air mixture. *Leishmania donovani* (strain MHOM/ET/67/L82) was maintained routinely in special pathogen free (SPF) female Golden hamsters by serial passage every 6 to 8 weeks. *Trypanosoma 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.b. 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 Company, NJ). Appropriate controls with pentamidine isothionate (Aventis, U.K.) 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 the Alamar Blue assay[26] on day three.

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 being washed, cells were dispensed into 16-well Lab-tek tissue culture slides (Nunc Inc., IL) at 4×10^4/well in a volume of 100 mL of RPMI-1640 medium (Sigma-Aldrich Company 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.[27]

Evaluation of the Cytotoxicity of Glutathione Derivatives in Vitro. Cytotoxicity testing on KB cells (human oral pharyngeal carcinoma) was evaluated using the Almar Blue assay.[28] Cell cultures were grown in RPMI medium with 10% calf foetal serum (CFS) at 37 °C in a 5% CO_2−air mixture in a humidified incubator. Plates were incubated with compound for 3 days at 37 °C in a 5% CO_2−humidified air mixture prior to determination of activity.

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R	Es	R	Es	R	Es	R	Es
Cl_3C	-2.06	H	+1.24	$PhCH_2$	-0.38	cyclo-C_5H_9	-0.51
Cl_2CH	-1.54	Ph	-2.55	PhMeCH	-1.19	Bu^s	-1.13
FCH_2	-0.24	$MeOCH_2$	-0.19	$Ph[CH_2]_2$	-0.38	Bu^t	-1.54
$ClCH_2$	-0.24	PhCH:CH	-1.89	Pr^i	-0.47	Bu^n	-0.39
$BrCH_2$	-0.27	Ph_2C	-1.76	Bu^i	-0.93		
ICH_2	-0.37	MeCH:CH	-1.63	neopentyl	-1.74		
Me	0.0	Et	-0.07	Pr^n	-0.36		

Figure 1.

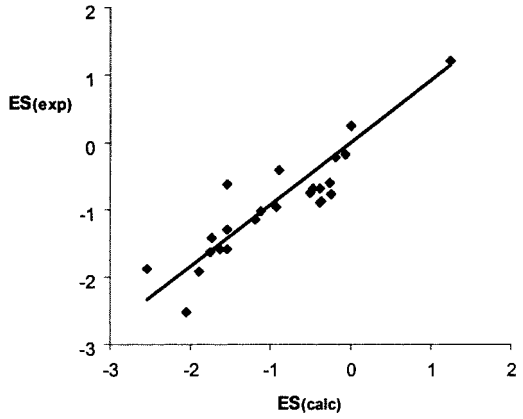

Figure 2. Experimental and calculated E_s values.

Appendix

Extrapolation of Taft's Steric Factor. QSAR analysis was made on the 25 methyl esters listed in Figure 1 with known experimental values,[18] and the regression equation was used to calculate values for the unknown branched esters. The E_s values calculated for the unknown linear structures using this equation were much larger than expected, and their values were obtained by graphical extrapolation of the experimental observed trend between E_s vs number of carbon atoms for the series of linear esters (Me, Et, Pr, and Bu); see Figure 2.

$$E_s = 120 + 1.59(X1) - 0.72(Polar) + 0.0218(MW) + 29.31(ABSQon) - 219(MaxQp) + 192(MaxNeg)$$

$$RMSD = 0.19;$$

$$\text{multiple correlation coefficient } R^2 = 0.95$$

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