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GLUTATHIONE SYNTHESIS IN FILARIAL WORMS: AN ATTRACTIVE TARGET FOR THE DESIGN AND SYNTHESIS OF NEW ANTIFILARIALS

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Abstract. A library of 363 glycoconjugates and C-nucleosides synthesized by our earlier reported methods were screened for their effect on isolated filarial glutamate cysteine ligase (GCL) and glutathione reductase (GR). Many of the compounds from the library including one glycosyl urea and one nucleoside showed inhibition of more than 50% on GCL, and seven glycosylated amino esters, two sugars, one glycosyl urea, five glycosyl hydroxamates, one glycosyl hydrantoin, three nucleoside and one N-protected glycopeptide compounds showed inhibition of more than 50% on GR from *S. cervi*. The effect of the most promising ones have been looked on the counterparts from mammalian sources and difference in the susceptibility towards enzyme activity inhibition were noted.

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Introduction

Lymphatic filariasis, a disease caused by filarial worms, is a major public health and socioeconomic problem in most of the south-eastern Asian developing countries. In India, an estimated population of 22 million is known to harbour circulating microfilariae and 16 million people suffer from filarial manifestations like elephantiasis of limbs, genitals and hydrocoele. Worldwide about 120 million people are infected with *Wuchereria bancrofti* and *Brugia malayi*. At present there is no effective and safe chemotherapy available against the adult filarial parasites, which raises an urgent need for the development of macrofilaricidal drugs. In the case of filarial worms, which survive for many years in the host, the question has been raised, how they manage this long-term persistence in spite of the host immune response, especially as filarial worms are reported to be sensitive towards oxidative stress.[1] Glutathione (GSH), a tripeptide (γ-glutamylcysteinyl-glycine) is known to guard the filarial worms from oxidative stress and attack by reactive oxygen species; a decrease in glutathione concentration is the most robust and significant alteration in the antioxidant defence.[2] It has been shown that interference with the filarial glutathione metabolism has curative effect on filarial infections in mice.[3] Work so far reveals that parasitic helminths are capable of GSH synthesis; they are known to have very active GSH-linked metabolism; GSH-reductase, GSH-peroxidases and GSH-transferases have been isolated and characterized from a variety of species, and several of these enzymes show chemo- and/or immunotherapeutic potential.[4,5,6] The intracellular concentration of GSH depends on numerous factors but is predominantly controlled by its *de novo* synthesis by glutamate cysteine ligase (GCL; EC 6.3.2.2) and glutathione synthetase, as well as by glutathione reductase (GR, EC 1.8.1.7) which recycles glutathione disulfide back into its reduced and active form.[7,8] Since most normal tissues have a large excess of GSH, and parasites have levels close to that required for survival, the interference with GSH metabolism (GSH synthesis and/ or GSSG reduction) may be more harmful to parasites than to normal tissues of the host. In view of the key role of GCL and GR in dictating GSH availability to filarial worms, inhibition of either one or both may serve as important targets in the design and synthesis of new antifilarial agents. In the present study, GR and GCL have been explored as possible targets for chemotherapeutic attack of this parasitic disease, in accordance with previous reports.[9, 10, 11]

Experimental

Chemistry

Synthesis of glycosyl amino esters (11-13, 15-18, acids (14, 19, 20), hydroxamic acids (21-28) and glycopeptides (29-31):

These were prepared following our earlier methods.[13, 14,15]

Fig 2

Table 2: Glycosyl amino ester acid, hydroxamic acid, N-protected glycopeptide and glycosyl ureids

Compound No.	R	R_1	R_2	R_3
11	CH_3	2-Propyl-imidazolyl		OCH_2CH_3
12 (*R*)	CH_3	H	C_7H_{15}	OCH_2CH_3
13 (*S*)	CH_3	H	C_7H_{15}	OCH_2CH_3
14	CH_3	H	C_7H_{15}	OH
15	CH_3	H	Furyl	OCH_2CH_3
16 (*R*)	CH_3	H	CH_2-2-OH-Ph	OCH_2CH_3
17 (*S*)	CH_3	H	CH_2-2-OH-Ph	OCH_2CH_3
18	CH_2Ph	H	Furyl	OCH_2CH_3
19	CH_3	H	3,5-di Me-hexyl	OH
20	CH_3	H	F-moc	OH
21	CH_3	H	C_7H_{15}	NHOH
22	CH_3	H	$C_{16}H_{33}$	NHOH
23	CH_2Ph	H	$C_{12}H_{25}$	NHOH
24	CH_2Ph	H	CH_2Ph	NHOH
25	CH_2Ph	H	C_7H_{15}	NHOH
26	CH_2Ph	H	$C_{16}H_{33}$	NHOH
27	CH_2Ph	H	CH_2Ph	NHOH
28	CH_2Ph	Morpholyl		NHOH
29	CH_3	Fmoc-Proline		OCH_2CH_3
30	CH_3	Proline		OCH_2CH_3
31	CH_3	Valine		OCH_2CH_3
32	CH_3	CH_2-2-OH-Ph	CONH-3-$COCH_3$-Ph	OCH_2CH_3
33	CH_3	CH_2-2-OH-Ph	$CONHCH_2Ph$	OCH_2CH_3

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34	CH_2Ph	H	CONHPh	OCH_2CH_3
35	CH_2Ph	CH_2Ph	$CONHCH_2Ph$	OCH_2CH_3
36	CH_3	3,5-di Me-hexyl	$CONHCH_2Ph$	OCH_2CH_3
37	CH_2Ph	Furyl	CONH-4-Cl-Ph	OCH_2CH_3
38	CH_3	n-butyl	C=SNH-3-Cl-Ph	OCH_2CH_3
39	CH_3	n-butyl	C=SNH-2-Cl-Ph	OCH_2CH_3
40L	CH_3	3-O-Me Sugar	CONH-3-Cl-4-Me-Ph	NH_2
41L	CH_3	3-O-Me Sugar	CONH-3-$COCH_3$-Ph	NH_2
42L	CH_3	3-O-Me sugar	$CONHCH_2Ph$	NH_2
43L	CH_3	3-O-Benzyl sugar	CONH-2-Me-Ph	NH_2
44L	CH_3	3-O-Benzyl sugar	CONH-4-Me-Ph	NH_2
45L	CH_3	3-O-Benzyl sugar	CONH-3-Cl-4-Me-Ph	NH_2
46L	CH_3	3-O-Benzyl sugar	CONH-3-$COCH_3$-Ph	NH_2
47L	CH_3	CH_2Ph	CONH-4-Me-Ph	NH_2
48L	CH_3	CH_2Ph	CONH-3-Cl-4-Me-Ph	NH_2
49L	CH_3	CH_2Ph	CONH-3-$COCH_3$-Ph	NH_2
50L	CH_3	CH_2Ph	$CONHCH_2Ph$	NH_2
51L	CH_3	Furyl	CONH-4-Me-Ph	NH_2
52L	CH_3	Furyl	$CONHCH_2Ph$	NH_2
53L	CH_3	Furyl	CONH-2-Me-Ph	NH_2
54L	CH_3	CH_2-4-OCH_3-Ph	CONH-3-Cl-4-Me-Ph	NH_2
55L	CH_3	CH_2-4-OCH_3-Ph	$CONHCH_2Ph$	NH_2
56L	CH_3	CH_2-2-OH-Ph	CONH-4-Me-Ph	NH_2
57L	CH_3	CH_2-2-OH-Ph	CONH-3-Cl-4-Me-Ph	NH_2
58L	CH_3	CH_2-2-OH-Ph	CONH-3-$COCH_3$-Ph	NH_2
59L	CH_3	H	$CONHCH_2Ph$	NH_2
60L	CH_3	H	CONH-2-Me-Ph	NH_2
61L	CH_3	H	CONH-4-Me-Ph	NH_2
62L	CH_3	H	CONH-3-Cl-4-Me-Ph	NH_2
63L	CH_3	H	CONH-4-Me-Ph	NH_2
64L	CH_3	CH_2-4-OCH_3-Ph	H	NH_2

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Synthesis of glycosyl ureas (32-39) and nucleosides (89-99)

Sugar derived N-substituted and N-unsubstituted amino esters were reacted with substituted phenyl isocyanate/isothiocyanate in anhydrous DCM to give N-glycosyl N-substituted urea/thiourea respectively, and the method has been patented both on solid phase as well as by conventional method of synthesis and the chemistry of nucleosides has been published by us.[16,17,18,19,20]

Solid phase synthesis of glycosyl ureas (40L-64L):

These compounds were synthesized as a library on sieber amide resin in an advanced Chemtech robotic synthesizer in vacuo. Fluorenylmethoxycarbonyl-3-amino- [(1*R*,

2*R*, 3*S*, 4*R*) -3-*O*-methyl-1, 2-*O*-isopropylidene-1, 4-tetrahydrofuranos-4-yl)-propanoic acid [19] was loaded onto the resin following the standard peptide bond coupling methods (DIC, HOBT and DMAP combination) procedure. The Fmoc protection was removed using 20 % piperidine: DMF. This was followed by reductive alkylation using aromatic and sugar aldehydes in anhydrous trimethyl orthoformate followed by addition of a suspension of $NaCNBH_3$. These resin bound compounds were again reacted with different isocyanates in anhydrous DCM to give resin bound glycosyl ureas. Finally, the compounds were cleaved using 2% TFA/DCM and collected in cleavage vials. Compounds of the library, thus obtained, were lyophilized by dissolving in *t*-Butanol/ water (4:1) and drying under freeze gave the title compounds.

(1*R*, 2*R*, 3*S*, 4*R*, 5*S*)- 5,6-dideoxy-1, 2-*O*-isopropylidene- 3-*O*-methyl-5-N^1-2'-hydroxy benzyl {N^3-4'-chloro phenyl-1-ureidyl}-1,4-heptofuranos-5-yl - uronamide [56L]:

MS (FAB): 500 $(M+H)^+$; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.30, 7.16 and 7.05 (m, 10H, Ar-H and NH), 7.02 and 6.34 (bs, 2H, D_2O exchangeable NH), 5.82 (d, J = 3.3 Hz, 1H, H-1), 4.55 (d, J = 3.3 Hz, 1H, H-2), 4.30-4.19 (m, 4H, H-4, H-5 and NCH_2), 3.67 (d, J = 2.8 Hz, 1H, H-3), 3.39 (s, 3H, $-OCH_3$), 3.28 (bs, 1H, NH), 2.60 (m, 2H, H-6), 1.45 and 1.28 [each s, each 3H, $C(CH_3)_2$].

Solid phase synthesis of glycopeptidyl ureas (65L-88L):

The synthetic strategy starts with, *N*-Fmoc-3-amino-[(1R,2R,3S,4R)-3-O-methyl (benzyl)-1,2-O-isopropylidene-1,4-tetrahydrofuranos-4-yl]-propanoic acids were anchored on to the activated sieber amide resin using standard protocol. The deprotection of F-moc group of resin bound sugar amino acid was carried out with 30 % piperidine in DMF to give free amine on amino acyl resin. Further coupling of *N*-F-moc α–amino acids was done using the previous method to get resin bound *N*-F-moc glycopeptides compound. Similar deprotection of the F-moc group resulted in free amine functionality. This was followed by sequential reaction with different aldehydes and sodium cyanoborohydride (reductive alkylation) separately resulted in the resin bound glycopeptids. The separate reaction of this resin bound compound

with different substituted aromatic isocyanates resulted in formation of resin bound glycopeptidyl ureids.

Finally the compounds were cleaved off the resin using 2% TFA/DCM cleavage reagent to get glycopeptidyl ureids (15L-16L). Compounds, thus obtained were lyophilized by dissolving them in a mixture of *t*-BuOH and water (4:1) followed by drying under freeze.

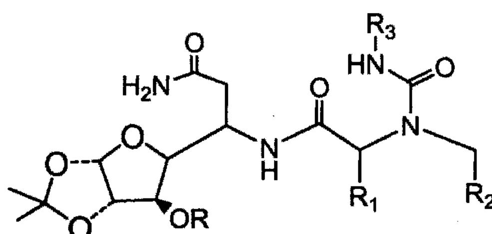

Fig 3: Core structure of glycoeptidyl urea

Table 3: Glycopeptidyl ureids synthesized on solid phase

Compound No.	R	R_1 Residue	R_2	R_3
65L	CH_3	Aspargine	CH_2-4-Br-Ph	4-Cl-Ph
66L	CH_3	Glutamine	CH_2Ph	*o*-Tolyl
67L	CH_3	Glutamine	CH_2-4-Br-Ph	4-Cl-Ph
68L	CH_3	Isoleucine	CH_2-4-OCH_3-Ph	Ch_2Ph
69L	CH_3	Isoleucine	CH_2Ph	*o*-Tolyl
70L	CH_3	Methionine	CH_2-4-OCH_3-Ph	Ch_2Ph
71L	CH_3	Phenyl alanine	CH_2-4-OCH_3-Ph	Ch_2Ph
72L	CH_3	Phenyl alanine	CH_2Ph	*o*-Tolyl
73L	CH_3	Proline	CH_2-4-Br-Ph	4-Cl-Ph
74L	CH_3	Valine	CH_2-4-Br-Ph	4-Cl-Ph
75L	CH_2Ph	Isoleucine	CH_2-4-OCH_3-Ph	Ch_2Ph
76L	CH_2Ph	Isoleucine	CH_2Ph	*o*-Tolyl
77L	CH_2Ph	Isoleucine	Furyl	*p*-Tolyl
78L	CH_2Ph	Isoleucine	CH_2-4-Br-Ph	4-Cl-Ph

(Continued next page.)

79L	CH_2Ph	Leucine	CH_2Ph	*o*-Tolyl
80L	CH_2Ph	Leucine	CH_2-4-Br-Ph	4-Cl-Ph
81L	CH_2Ph	Methionine	CH_2-4-OCH_3-Ph	Ch_2Ph
82L	CH_2Ph	Methionine	Furyl	*p*-Tolyl
83L	CH_2Ph	Methionine	CH_2-4-Br-Ph	4-Cl-Ph
84L	CH_2Ph	Phenyl alanine	CH_2-4-OCH_3-Ph	Ch_2Ph
85L	CH_2Ph	Phenyl alanine	CH_2Ph	*o*-Tolyl
86L	CH_2Ph	Proline	CH_2-4-Br-Ph	4-Cl-Ph
87L	CH_2Ph	Valine	CH_2Ph	*o*-Tolyl
88L	CH_2Ph	Valine	Furyl	*p*-Tolyl

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Solid phase synthesis of C-nucleosides (100L-119L): C-nucleosides were synthesized as reported in our previous paper.[21]

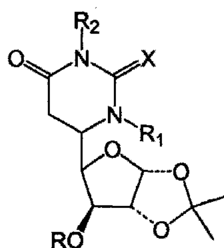

Fig 4: Core structure of Nucleosides

Table 4: C-Nucleosides synthesized by conventional method and on solid phase

Compound No.	R	R_1	R_2	X
89	CH_3	Furyl	4-n-Bu-Ph	O
90	CH_3	CH_2-2-OH-Ph	3-$COCH_3$-Ph	O
91	CH_2Ph	H	CH_2Ph	O
92	CH_3	$C_{12}H_{25}$	CH_2Ph	O
93	CH_3	3,5-di Me-hexyl	CH_2Ph	O
94 (*S*)	CH_2Ph	CH_2Ph	CH_2Ph	O
95	CH_2Ph	3,5-di Me-hexyl	4-F-Ph	O

(Continued next page.)

96 (*R*)	CH_2Ph	CH_2Ph	CH_2Ph	O
97	CH_2Ph	Furyl	4-Cl-Ph	O
98	CH_3	n-butyl	3-Cl-Ph	S
99	CH_3	n-butyl	2-Cl-Ph	S
100L	CH_3	CH_2Ph	2,4-di-Cl-Ph	O
101L	CH_3	CH_2Ph	4-n-Bu-Ph	O
102L	CH_3	3-OH,4-OMe-Ph	3,4-di-Cl-Ph	O
103L	CH_3	3-OH,4-OMe-Ph	3,5-di-Cl-Ph	O
104L	CH_3	Furyl	4-n-Bu-Ph	O
105L	CH_2Ph	CH_2-4-OCH_3-Ph	4-Acetyl Ph	O
106L	CH_3	CH_2-4-OCH_3-Ph	3-Acetyl Ph	O
107L	CH_3	CH_2Ph	3,5-di-Me-Ph	O
108L	CH_3	CH_2Ph	CH_2Ph	O
109L	CH_3	CH_2Ph	3-Cl-4-Me-Ph	O
110L	CH_3	CH_2Ph	3,5-di-Me-Ph	O
111L	CH_3	Furyl	3-Acetyl Ph	O
112L	CH_3	Furyl	4-CN-Ph	O
113L	CH_3	CH_2-2-OH-Ph	3-Acetyl Ph	O
114L	CH_3	CH_2-2-OH-Ph	CH_2Ph	O
115L	CH_3	CH_2-2-OH-Ph	3-Cl-4-Me-Ph	O
116L	CH_3	CH_2-2-OH-Ph	4-CN-Ph	O
117L	CH_2Ph	H	4-Cl-Ph	O
118L	CH_3	H	4-Cl-Ph	O
119L	CH_2Ph	H	4-Acetyl Ph	O

(Continued from previous page.)

However, during our synthetic strategy for the synthesis of glycosyl urea, when the glucose derived aldehyde was reacted with leucine methyl ester hydrochloride in presence of triethyl amine and sodium borohydride followed by the reaction of the intermediate with 3-acetylphenyl isocyanate resulted in the corresponding cyclised urea which on refluxing in presence of DBU and TBAB gave a five-membered glycosyl hydrantoin (compound 120) in good yield.

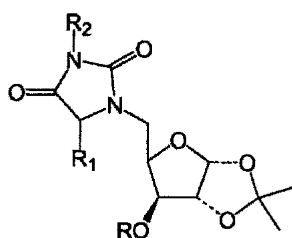

Fig 5: Structure of compound 120

R=CH_2Ph, R1= Leucine, R2 = 3-Acetyl Ph

The synthesis of compounds 121 and 122 has already been reported in our earlier paper.[22]

Biological

Chemicals: Tris, phosphoenol pyruvate, sodium L–glutamate, L-α-aminobutyric acid, sodium EDTA, β-nicotinamide adenine dinucleotide (reduced), adenosine-triphosphate, L-buthionine-[S,R]-sulfoximine (BSO), cystamine, carmustine and methylene blue were purchased from Sigma Chemical Co., USA. Potassium chloride, magnesium chloride, pyruvate kinase, lactate dehydrogenase, oxidised glutathione and β-nicotinamide adenine dinucleotide phosphate (reduced) were purchased from Sisco Research Laboratories Pvt. Ltd Mumbai, India. All other chemicals used were of analytical grade.

Biological Material: Adult *Setaria cervi* females of average body weight 35±5 mg and length 6.0±1.0 cm were collected from the peritoneal cavity of freshly slaughtered naturally infected water buffaloes *Bubalus bubalis* (Linn.) at a local abattoir. They were thoroughly washed with saline and kept in Hanks balanced salt solution with sodium bicarbonate and glucose (5.55 mM) for 60 min for complete revival before being used in enzymic studies.

Methods

Preparation of GCL and GR from bovine filarial worms *S. cervi*

A 10% homogenate of actively motile bovine filarial worms was prepared in a Tris-HCl buffer, 50 mM, pH 7.5 containing 1mM glutamate, 5 mM $MgCl_2$ and 0.1 mM PMSF using a Potter Elvehjem glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 1,000 x g for 15 min, 10,000 x g for 30 min, and subsequently at 100,000 x g for 60 min to obtain mitochondrial, post mitochondrial and cytosolic fractions, respectively. The cytosolic fraction was then subjected to ammonium sulfate precipitation. For the goal, the fraction was brought to a final concentration of 0-45% saline saturation with solid ammonium sulfate. The sample was stirred in cold for 30 min and then centrifuged at 13,000 x g for 15 min to collect the precipitate and the supernatant separately. The supernatant solution was then saturated with solid ammonium sulfate to a concentration of 65%. The sample was again stirred for 30 min and centrifuged at 13,000 x g for 15 min to collect the precipitate and the supernatant separately. The supernatant solution was then saturated with solid ammonium sulfate to a final concentration of 80%. After stirring in cold for 30 min, the suspension was centrifuged at 13,000 x g for 15 min. All the pellets were dissolved separately in the homogenisation buffer. All the samples were analysed for GCL and GR activities, which were found enriched in the 65-80% ammonium sulfate precipitate. This fraction was stored at 4°C and used as the source of GCL and GR.

GCL Activity determination

GCL activity was estimated in the filarial extracts following a coupled enzyme procedure in which the rate of ADP formation in the presence of pyruvate kinase, LDH, PEP and NADH is obtained from the decrease in absorbance of NADH at 340 nm.[23] Enzyme activity was monitored at 37° C using a Shimadzu double beam UV spectrophotometer in reaction mixtures (final volume 1.0 ml) containing 100 mM Tris HCl buffer; pH 8.4, 150 mM KCl, 5 mM ATP, 2 mM PEP, 10 mM L-glutamate, 10 mM L-α-aminobutyrate, 20 mM $MgCl_2$, 2 mM Na_2EDTA, 0.2 mM NADH, 1.6 μg/μl pyruvate kinase and 1.6 μg/μl lactate dehydrogenase. Reaction was initiated by the addition of enzyme protein. The absorbance at 340 nm was monitored for 5 min at 30 sec intervals.

GR Activity determination

GR activity in filarial extracts was determined by the method described by Carlberg and Mannervik.[24] All enzyme kinetics experiments were carried out at 37°C using a Shimadzu double beam UV spectrophotometer. Enzyme activity was spectrophotometrically monitored from the rate of conversion of NADPH to NADP (e_{340} = 6.2 mM^{-1} cm^{-1}). For each assay the absorbance of NADPH in the reaction mixture was measured over a period of 5 min. Assays were carried out in 1 ml of the following solution: 100 mM potassium phosphate buffer, pH 7.0; 1mM EDTA; 1 mM GSSG; 0.2 mM NADPH and enzyme protein. Enzyme aliquots were preincubated in the presence of NADPH for 5 min in order to trace the non-enzymatic oxidation, after which GSSG was added to start the reaction. To correct for non-specific oxidation of NADPH, the blank cuvette contained all the assay components except GSSG.

Protein was estimated following the method of Lowry *et al.*[25] A unit of enzyme activity was expressed as the amount that catalyzes the consumption of 1µmol of substrate per minute, using a molar extinction coefficient of 6.2 x 10^{-6} for NADH/ NADPH.

Inhibition studies

Effect of standard inhibitors (BSO, cystamine, methylene blue and carmustine) and compounds belonging to various chemical series on GCL and GR activity of *S. cervi* females was studied by adding them directly in the assay systems at 200 µM concentration 10 min prior to addition of the substrate. The percentage inhibition/ stimulation of the enzyme activity by compounds were calculated by comparing with control tube.

Results and Discussion

Bovine filarial worms, *S. cervi*, were found equipped with both the GSH synthetic as well as GSSG reductive pathways and the specific activities of the GCL and GR in crude filarial extracts were calculated to be around 0.12 - 0.15 and 0.8 - 1.0 µmol/min/mg protein, respectively.

Figs 6 and 7 show the pattern of inhibition of standard mammalian inhibitors i.e., buthionine sulfoximine and cystamine on filarial GCL and menadione and carmustine

on GR at 200 μM concentration. Under the specified assay conditions the maximum inhibition of BSO was found to be 16.1% at 200 μM concentration which indicates that BSO is a weak inhibitor of filarial GCL. It inhibited GCL in confirmation of most[26] but not of all living cells.[27] Many of the published reports also indicate that BSO is a relatively poor inhibitor of bacterial GCL, particularly in the presence of physiological levels of L-glutamate. In contrast, cystamine shows strong inhibition on GCL from *S. cervi*, even at low concentrations. *S. cervi* GCL was inhibited by cystamine to the tune of 19-67% when studied in the concentration range of 0.25-200 μM. It has previously been found that the disulfide cystamine (2, 2'-dithiobis (ethylamine)) is a potent inactivator of GCL, and the susceptibility of this enzyme to inactivation is markedly influenced by the presence of magnesium ions, substrates and products.[28] Carmustine (1,3-bis[2-chloroethyl]-1-nitrosourea) (BCNU), a potent inhibitor of GR,[29] inhibited *S. cervi* GR to the tune of 20-58.5% when tested in 10 - 200 μM range. Methylene blue,[30] known mammalian GR inhibitor also showed appreciable inhibition to the tune of 24-94% when tested in the 2 - 200 μM range on *S. cervi* GR.

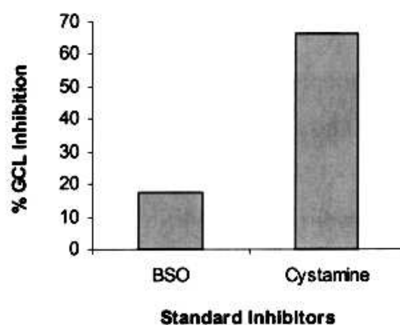

Fig 6: Effect of standard inhibitors on *S. cervi* GCL

* Concentration of inhibitors in the assay mixture- 200μM

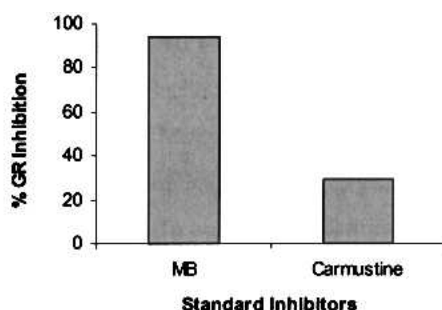

Fig 7: Effect of standard inhibitors on *S. cervi* GR

* Concentration of inhibitors in the assay mixture- 200μM

In an effort to search for novel inhibitors of filarial GCL and GR, many of the synthetic compounds that may serve as substrate analogs for GSH biosynthesis and hamper its *de novo* synthesis, belonging to various chemical series i.e. glycosylated amino acids (GAA), glycosylated amino esters (GAE), sugars, diglycosylated amino esters, glycosylated sulfone derivatives, thioglycosylated esters, FMOC derivatives of GAE, glycosyl ureas and thioureas, oxime, glycosylated hydroxamic acid, glycosylated hydrantoin, glycopeptides, nucleosides, C-nucleosides and S-containing nucleoside, thiazidine thiones, N-protected glycopeptide were looked for their *in vitro* effect at 200 μM concentration in the assay systems. Table 5 represents the comparative effects of selective synthetic compounds and standard mammalian inhibitors on filarial and mammalian counterparts.

Of the various compounds tested for inhibitory effect on filarial GCL and GR, eighty one compounds inhibited GCL to the tune of 10 - 82% of which one glycosyl urea (compound 49L) and one nucleoside (compound 108L) showed more than 50% inhibition on intact GCL, while ninety five compounds inhibited GR to the tune of 10-92% of which, seven glycosylated amino esters (compound 11, 13(s), 14, 15, 16(R), 17(S) and 18), two sugars (compound 121, 122), one glycosyl urea (compound 36), five glycosyl hydroxamates (compound 22, 23, 24, 25, 28), one glycosyl hydrantoin (compound 120), three nucleosides (compound 93, 94, 105L) and one N-protected glycopeptide (compound 29) compounds showed more than 50% inhibition on GR from *S. cervi.* Noteworthy in this connection are fifteen compounds that showed

simultaneous inhibition on GCL and GR. The compounds which showed more than 50% inhibition on GCL/ GR were screened on the counterparts from mammalian sources for the optimization of the leads. Majority of the compounds showed no inhibitory activity on the mammalian enzymes except glycosyl urea (compound 49L) which showed 18.0% inhibition on mammalian GCL, and glycosyl hydroxamate (compound 22), which showed 71.4% inhibition on mammalian GR.

Table 5: Comparative susceptibility of filarial and mammalian GCL and GR towards synthetic compounds and standard inhibitors

Compound No.	% GCL Inhibition		% GR Inhibition	
	S. cervi	Mice liver	*S. cervi*	Mice liver
11	32	ND	69	NIL
12 (*R*)	NIL	ND	42	ND
13 (*S*)	NIL	ND	77	NIL
14	NIL	ND	67	NIL
15	27	ND	67	NIL
16 (*R*)	27	ND	55	NIL
17 (*S*)	49	ND	67	NIL
18	20	ND	67	NIL
19	34	ND	55	NIL
20	8	ND	42	ND
21	4	ND	44	ND
22	NIL	ND	75	71
23	22	ND	50	NIL
24	NIL	ND	56	NIL
25	NIL	ND	54	NIL
26	17	ND	NIL	ND
27	13	ND	NIL	ND
28	23	ND	69	NIL
29	13	ND	92	NIL
30	22	ND	NIL	ND
31	21	ND	NIL	ND
32	NIL	ND	+29	ND
33	NIL	ND	+3	ND
34	NIL	ND	11	ND
35	25	ND	NA	ND
36	NIL	ND	68	NIL
37	21	ND	NIL	ND
38	28	ND	NIL	ND
39	26	ND	NIL	ND
40L	NIL	ND	21	ND
41L	NIL	ND	18	ND
42L	NIL	ND	29	ND
43L	NIL	ND	32	ND
44L	NIL	ND	29	ND
45L	NIL	ND	16	ND
46L	NIL	ND	19	ND

(Continued next page.)

47L	NIL	ND	16	ND
48L	NIL	ND	18	ND
49L	82	18	14	ND
50L	NIL	ND	33	ND
51L	NIL	ND	16	ND
52L	NIL	ND	38	ND
53L	NIL	ND	25	ND
54L	NIL	ND	35	ND
55L	NIL	ND	19	ND
56L	NIL	ND	43	ND
57L	NIL	ND	21	ND
58L	NIL	ND	27	ND
59L	NIL	ND	27	ND
60L	NIL	ND	16	ND
61L	16	ND	29	ND
62L	14	ND	NA	ND
63L	17	ND	NA	ND
64L	NIL	ND	30	ND
65L	34	ND	NA	ND
66L	NIL	ND	25	ND
67L	21	ND	NA	ND
68L	NIL	ND	18	ND
69L	NIL	ND	17	ND
70L	+34	ND	NA	ND
71L	NIL	ND	40	ND
72L	NIL	ND	25	ND
73L	NIL	ND	29	ND
74L	NIL	ND	27	ND
75L	29	ND	NA	ND
76L	27	ND	NA	ND
77L	15	ND	NA	ND
78L	32	ND	NA	ND
79L	19	ND	NA	ND
80L	40	ND	NA	ND
81L	25	ND	NA	ND
82L	28	ND	NA	ND
83L	24	ND	NA	ND
84L	18	ND	NA	ND
85L	30	ND	NA	ND
86L	NIL	ND	35	ND
87L	NIL	ND	38	ND
88L	27	ND	33	ND
89	22	ND	NIL	ND
90	NIL	ND	46	ND
91	NIL	ND	+86	ND
92	NIL	ND	36	ND
93	NIL	ND	75	NIL
94	NIL	ND	72	NIL
95	37	ND	NIL	ND
96	21	ND	NA	ND
97	17	ND	NIL	ND
98	10	ND	NIL	ND
99	19	ND	40	ND

(Continued next page.)

100L	+21	ND	NA	ND
101L	+25	ND	NIL	ND
102L	NIL	ND	6	ND
103L	NIL	ND	49	ND
104L	+20	ND	NIL	ND
105L	NIL	ND	60	NIL
106L	+36	ND	8	ND
107L	NIL	ND	19	ND
108L	57	NIL	28	ND
109L	NIL	ND	28	ND
110L	NIL	ND	26	ND
111L	25	ND	19	ND
112L	NIL	ND	19	ND
113L	NIL	ND	43	ND
114L	+22	ND	NIL	ND
115L	NIL	ND	26	ND
116L	NIL	ND	34	ND
117L	+23	ND	26	ND
118L	NIL	ND	17	ND
119L	NIL	ND	15	ND
120	25	ND	88	NIL
121	18	ND	52	NIL
122	13	ND	71	NIL

(Continued from previous page.)

*Concentration of test sample in the assay mixture-200μM

NIL= No inhibition; NA= Not available; ND= Not done

The antiparasitic agents known till date, act either on the membrane receptors or on enzyme/metabolic pathways crucial for the survival of the parasites.[31] Both available filaricides DEC and ivermectin are able to eliminate microfilaraemia in human lymphatic filariasis, however, the adult parasites mostly remain unaffected resulting in relapse of microfilaraemia.[32] Therefore, there is an urgent need to develop an antifilarial drug which can kill the adult parasites slowly, has microfilaricidal activity and at the same times sterilize the female worms. GSH dependent enzymes and GSH itself have a key role in protecting tissues against oxidative stress caused by free radicals and their reaction products. In helminths, this is of particular interest with respect to protection against respiratory burst of activated cells of the host's immune system and susceptibility to drugs whose mode of action involves the generation of free radicals.[33] In view of the high probability that the maintenance of an optimal GSH/GSSG ratio is vital to filariae, it seems worthwhile to develop drugs that could selectively deplete or distort the glutathione stores in these parasites.

In an approach towards this end, the present study was designed to try a preliminary rating of target attractiveness within the area of GSH-dependent parasitic antioxidant defence. The proposed compounds in the present study are expected to inhibit two crucial enzymes, GCL and GR, which are essential for the survival and propagation of these parasites.

It has been shown that certain sugar derivatives posses very good activity against filarial glutathione metabolizing enzymes *in vitro*.[12,34,35,36] These compounds possess immunomodulatory activity[37] and *in vitro* antifilarial activity too. As sugars are known to offer better pharmacokinetics, better transport and above all less toxicity[38,39] to the drugs, it is presumed that amino acid analogues if grafted on sugar backbone may serve as substrate for GSH biosynthesis and may hamper its *de novo* synthesis. C-nucleosides having C-C linkage instead of carbon to nitrogen linkage between the aglycon and the sugar moiety are known mainly for their anticancer,[39] antiviral[40] and antileukemic[41] activities. A C-C bond between the sugar and aglycon makes them more stable towards enzymatic degradation, and therefore the half-lives of these compounds in the body are far greater than the corresponding N-nucleosides. In addition, sugar derivatives are known to offer better stability, better pharmacokinetic parameters, facilitate the transport of drugs, and at the same time are less toxic.

If the present strategy i.e. inhibition of parasite GCL/GR activity, affects the survival of the parasites or helps in the enhancement of activity of presently available antifilarial/antimalarial drugs, it will help in the design of new target based chemotherapeutic agents. The compounds which, in the present study, inhibit GCL/ GR more than 50% and those which show simultaneous inhibitory effect on GCL and GR can thus emerge as lead molecules for further exploration to develop new class of antifilarial drugs.

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