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INHIBITORS OF FILARIAL GAMMA-GLUTAMYL CYCLE ENZYMES AS POSSIBLE MACROFILARICIDAL AGENTS

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Abstract. The sensitivity of two γ-glutamyl cycle enzymes i.e. glutamate cysteine ligase (GCL) and γ-glutamyl transpeptidase (γ-GT) from bovine filarial worms *Setaria cervi* and their counterparts from mammalian liver to known inhibitors i.e. buthionine sulfoximine vs. GCL and serine-borate vs. γ-GT were compared. Two chemical classes i.e. diglycosylated diaminoalkanes (DGDA) and coumarins were studied for their inhibition against filarial GCL, glutathione synthetase (GS) and γ-GT and the most effective inhibitors of filarial enzymes were also looked for their effect on GSH levels of bovine filariae *S. cervi in vitro*. Results revealed some definite correlation between the enzyme inhibition with GSH depletion in *S. cervi* females by certain DGDA and coumarin derivatives.

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Introduction

Glutathione metabolism is one of the main aspects of filarial biochemistry that can be attacked as a drug target. It seems useful to develop drugs that could selectively deplete glutathione stores in these parasites. Glutathione (GSH; L-γ-glutamyl-cysteinylglycine), a tripeptide has been identified as an important part of the antioxidant system of filarial worms to protect them from the oxidative stress created by the host.[1] The complex of six enzymatic reactions leading to the formation and breakdown of glutathione (GSH) constitutes the γ-glutamyl cycle.[2] The enzymes being involved are glutamate cysteine ligase (GCL; EC 6.3.2.2), glutathione synthetase (GS; EC 6.3.2.3), γ-glutamyl transpeptidase (γ-GT; EC 2.3.2.2), γ-glutamyl cyclotransferase (EC 2.3.2.4), 5-oxoprolinase (EC 3.5.2.9) and cysteinyl-glycine dipeptidase (EC 3.4.13.6). GCL and γ-GT are well studied in some species of filarrids[3, 4] but GS has not been studied in any of the filarial species at present.

GSH is synthesized intracellularly in two sequential steps of γ-glutamyl cycle catalysed by GCL and GS respectively.

L-Glutamate + L-Cysteine + ATP ⟷ L-γ-Glutamyl-L-cysteine + ADP + Pi

L-γ-Glutamyl-L-Cysteine + Glycine + ATP ⟷ GSH + ADP + Pi

De novo GSH synthesis is regulated by the level of GCL present in the cell[5], feedback inhibition by GSH on GCL[6] and the availability of its substrates, particularly L-cysteine.[7,8,9,10]

The degradation of extracellular GSH is initiated by γ-GT, one of the enzymes of γ-glutamyl cycle. This enzyme catalyzes transfer of the γ-glutamyl moiety of GSH, S-substituted glutathione derivatives and other γ-glutamyl compounds to a number of acceptors *viz.* aminoacids and dipeptides as well as GSH in case of

autotranspeptidation. γ-GT is largely localized at the outer surface of membranes[11] and is involved in the transport of amino acids.

γ-Glutamyl-cysteinyl-glycine + Acceptor → γ-Glutamyl-acceptor + Cysteinyl-glycine

By cleaving the γ-glutamyl bond in GSH, γ-GT provides the first step in catabolism of extracellular GSH. With the involvement of membrane dipeptidase activities, cysteinylglycine is cleaved and precursor amino acids glycine and cysteine are then released, which cross the plasma membrane and are re-utilized for intracellular GSH synthesis.[12] Since, it has been recognized that a continuous efflux of GSH occurs from a number of cell types through specific out-transporters[13], it is conceivable that the major function of γ-GT ectoactivity could be the salvage of extracellular GSH which would otherwise be lost from the cell.

In the present work special attention has been paid to the three most important enzymes, GCL, GS and γ-GT, of the γ-glutamyl cycle for the preliminary screening of compounds. Two classes of compounds, the glycoconjugates and coumarins have been evaluated for their GSH depletion potential in adult filarial worms.

Chemistry

Synthesis of glycoconjugates-diglycosylated diaminoalkanes (1-14)

Synthesis of diglycosylated diaminoalkanes has been reported by us earlier.[14]

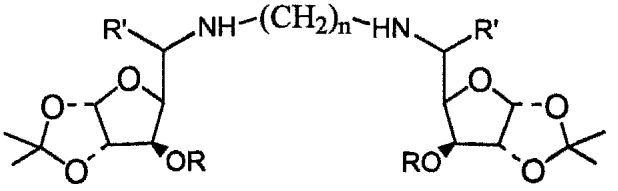

Table 1: N^1, N^n- bis-Xylofuranosylated Diaminoalkanes

Compound	R	R'	n
1	CH_3	H	7
2	CH_3	H	10
3	CH_3	H	12
4	$CH_2C_6H_5$	H	7
5	$CH_2C_6H_5$	H	10
6	$CH_2C_6H_5$	H	12
7	CH_3	CH_2COOEt	3
8	CH_3	CH_2COOEt	7
9	CH_3	CH_2COOEt	10
10	CH_3	CH_2COOEt	12
11	$CH_2C_6H_5$	CH_2COOEt	3
12	$CH_2C_6H_5$	CH_2COOEt	7
13	$CH_2C_6H_5$	CH_2COOEt	10
14	$CH_2C_6H_5$	CH_2COOEt	12

Synthesis of coumarin derivatives (15-20)

The compound 15 was synthesized as reported by Bhattacharya *et al*[15] and compounds 16, 17 and 18 were synthesized as reported earlier by Katiyar *et al.*[16] The compounds 19 and 20 were synthesized by Pechmann condensation of 3, 4 dimethylphenol and 3, 5 dimethylphenol with ethylacetoacetate respectively as reported previously.[17,18]

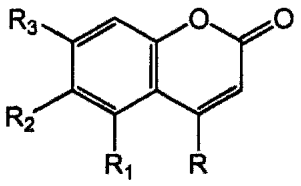

Table 2: Structural details of coumarin derivatives

Compound	R	R_1	R_2	R_3
15	CH_3	H	H	$-OCO(CH_2)_{12}CH_3$
16	CH_3	H	H	$-OCH_2CONH(CH)_{12}NH_2$
17	$-CH_2\ CH_2\ CH_3$	H	H	$-O\ CH_2COOC_2H_5$
18	CH_3	H	H	$-OCH_2CONH-(C_6H_{11})$
19	CH_3	H	CH_3	CH_3
20	CH_3	CH_3	H	CH_3

Biology

Experimental model

Adult bovine filariids *S. cervi* females were collected from the peritoneal folds of naturally infected water buffaloes (*Bubalus bubalis* Linn) at a local abbatoir and brought to laboratory in Ringer's saline. For enzymatic studies male/ female Wistar strain of albino rats (8-10 weeks of age: body weight 160 ± 20 gm) were procured from the animal colony of Central Drug Research Institute, Lucknow, India.

Chemicals

Glutathione (GSH), o-phthaldialdehyde, L-buthionine-[S,R]-sulfoximine (BSO), L-γ-glutamyl-p-nitroanilide, glycylglycine, Triton-X-100, L-serine, acivicin, glutamate, phenylmethylsulfonylfluoride (PMSF), L-α-aminobutyrate, EDTA, NADH, L-γ-glutamylcysteine, glycine, ATP, KCl, DTT, $MgCl_2$, pyruvate kinase, lactate dehydrogenase (LDH), phosphoenolpyruvate (PEP), ammonium sulfate and Tris were purchased from Sigma Chemical Co., USA. Hanks balanced salt solution (HBSS) and gentamycin were obtained from Hi Media Laboratories Pvt Ltd, Mumbai, India. Methanol (HPLC Grade) and sodium acetate used were from Merck India Ltd, Mumbai, India. All other chemicals used were of analytical grade. Spherisorb S5 ODS2 column (4.0 x 125 mm) was purchased from Waters Corporation, Milford, Massachusetts.

Experimental procedure for GSH estimation

Actively moving *S. cervi* females after being washed with sterile saline were allowed to revive for 1 h in sterile HBSS containing gentamycin (1 μg/ml). After revival three parasites were transferred to flask containing 25 ml of HBSS (pH 7.2-7.4) and gentamycin, with or without the synthetic chemical compounds/ standard GSH depletors for 6 h. All incubations were done at 37° C in Dubnoff metabolic shaker at a low speed. A 5% *S. cervi* homogenate (w/v) was prepared in 2.5% sulphosalicylic acid and was centrifuged at 10,000 x g for 10 min. The supernatant was saved and used for GSH analysis.

GSH was determined by reversed phase HPLC using Spherisorb S5 ODS2 column (4.0 x 125 mm). 50 μl aliquots of the 10,000 x g supernatant were mixed with 50 μl of o-phthalaldehyde (OPA) reagent and the reaction was stopped after 1 min with 100 μl of 0.1 M potassium phosphate-H_3PO_4 buffer (pH 7.0). The OPA reagent is composed of 40 mM OPA and 0.4 M sodium tetraborate (pH 9.0). In all cases 50 μl aliquot was subjected to the column for GSH analysis. Separation was performed at a flow rate of 1 ml/min with solvent A (0.15 M sodium acetate, pH adjusted to 7.0 with acetic acid/methanol (1/24, v/v)) and solvent B (100% methanol). Gradient (expressed as percentages of solvent B) used was: 8 min, 0; 12 min, 10%; 13 min, 25%; 27 min, 90%. For the next 8 min methanol was kept at 90% and then allowed to come to 0% in 5 min, and then solvent A was kept at 100% to reattain the initial conditions for 10 min. GSH in samples was estimated using the standard curve for GSH (0.5 to 5.0 μg/ml). Excitation and emission wavelengths used were 350 and 420 nm respectively. The mentioned procedure is as described by Hussain and Walter.[19] Protein was estimated as described by Lowry *et al* [20] using bovine serum albumin as standard.

Experimental procedure for GCL estimation

A 10% homogenate of actively motile bovine filarial worms was prepared in a Tris-HCl buffer (50 mM, pH 7.5) containing 1 mM glutamate, 5 mM $MgCl_2$ and 0.1 mM

PMSF using a Potter Elvehjem glass homogenizer fitted with a Teflon pestle. The homogenate was then subjected to subcellular fractionation; the cytosolic fraction obtained was salt fractionated. All the fractions obtained were analysed for GCL activity, which was found maximum in the 65-80% ammonium sulfate precipitate. The pellet obtained was dissolved in a solution of 50 mM Tris-HCl buffer (pH 7.4) containing glutamate (1 mM), $MgCl_2$ (5 mM) and PMSF (0.1 mM). This fraction was stored at 4°C and used as the source of GCL.

GCL activity was estimated according to the method of Seelig and Meister[21] with slight modifications. The method used is 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. Enzyme activity was determined at 37° C in reaction mixtures (1.0 ml) containing Tris HCl buffer; 100 mM (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, 5 mM acivicin, 1.6 μg/μl pyruvate kinase and 1.6 μg/μl LDH. Reaction was initiated by the addition of enzyme protein. The absorbance at 340 nm was monitored for 5 min at 30 sec intervals. Protein was estimated following the method of Lowry *et al.*[20] Effect of standard inhibitor BSO and chemical compounds on GCL activity of *S. cervi* females was studied by adding them directly in the assay systems 10 min prior to addition of the substrate.

Experimental procedure for γ-GT estimation

A 10% homogenate of actively moving worms was prepared in 50 mM Tris-HCl buffer (pH 7.5). The microsomal pellet obtained by differential centrifugation was suspended in 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl and 1% Triton-X-100 for 1 h and subsequently centrifuged at 100,000 x g for 1 h to obtain solubilized enzyme. Triton-X-100 was removed from the solubilized enzyme by overnight dialysis against two changes of 200 volumes of Tris-HCl buffer (pH 7.5) containing 0.1% Triton-X-100.

The activity of γ-GT was determined using the substrate, L-γ-glutamyl-p-nitroanilide as described by Meister *et al.*[22] The assay mixture in a final volume of 1.0 ml

contained 0.1 M Tris-HCl (pH 8.0), 20 mM glyclyglycine, 1 mM L-γ-glutamyl-p-nitroanilide and a suitable amount of the enzyme protein. The reaction mixtures were incubated at 37°C for desired period and the reaction was terminated with 10% acetic acid. p-Nitroaniline formed was estimated spectrophotometrically at 410 nm (Molar Extinction coefficient=8800 $M^{-1}cm^{-1}$). Protein was estimated by the method described by Lowry *et al.*[20] The effect of serine-borate and chemical compounds on γ-GT was studied by directly adding them in the assay systems 15 min prior to the addition of substrate L-γ -glutamyl-p-nitroanilide.

Experimental procedure for GS estimation

A 10% homogenate was prepared in 50 mM imidazole/HCl buffer containing 10 mM $MgCl_2$. The cytosolic fraction obtained by differential centrifugation was used for the activity determination.

The reaction mixture for GS activity estimation was prepared according to slight modifications in procedures by Parmentier *et al*[23] and Nardi *et al.*[24] Assay system (1ml) consisted of 0.1 M Tris HCl buffer (pH 8.2), 6 mM ATP, 50 mM KCl, 6 mM DTT, 20 mM $MgCl_2$, 0.3 mM γ-glutamylcysteine, 3 mM glycine and 5 mM acivicin. The reaction was initiated by the addition of enzyme aliquot and after incubation for desired period at 37°C, the reaction was stopped by the addition of 10% sulfosalicylic acid. After centrifugation at 10,000 x g for 10 min, the 50 µl of supernatant was derivatized with o-phthalaldehyde and analysed for GSH as mentioned above. One unit of enzyme activity is defined as the amount that catalysed the formation of 1 µmol of GSH per minute at 37°C. The procedure for product (GSH) estimation is as described by Hussain and Walter.[19] The effect of chemical compounds on GS was studied by directly adding them in the assay systems 15 min prior to the addition of substrate L-γ -glutamyl-L-cysteine.

Results and Discussion

Under the specified assay conditions the specific activities of GCL and GS responsible for GSH biosynthesis were estimated to be around 0.12-0.15 µmol/min

and 0.021-0.023 μmol/min in crude homogenates of adult female filarial worms *S. cervi*. The specific activity of γ-GT was estimated to be around 0.015-0.02 μmol/min in filarial worms *S. cervi*. Figures 1-4 represent the comparative effects of standard mammalian enzyme inhibitors on filarial counterparts in terms of Ki.

Comparative susceptibility studies on the effect of the standard γ-GT inhibitor serine-borate indicated that Ki value towards filarial enzyme (0.4 mM) was around 10 times higher as compared to the rodent enzyme (0.05 mM). Similar comparative inhibition studies were done with standard GCL inhibitor BSO, and it was found that Ki value towards rodent enzyme (13 mM) was twice as high as the filarial enzyme (7.7 mM). Serine-borate[25] and BSO[26] are the known transition state inhibitors of γ-GT and GCL respectively. Data from the present study reveals that serine-borate is a weak inhibitor of γ-GT from filarial source as compared to the same from the mammalian source. The lower Ki value obtained for the *S. cervi* GCL as compared to that for the rodent enzyme suggests that BSO is relatively good inhibitor of filarial GCL as compared to mammalian enzyme, and can be exploited as chemotherapeutic agent against filariasis.

In an effort towards the search for the specific inhibitors of filarial GCL, GS and γ-GT, many of the synthetic compounds belonging to various chemical series i.e. diglycosylated diaminoalkanes (DGDA) and coumarin derivatives were tested for their *in vitro* effect on these enzymes.

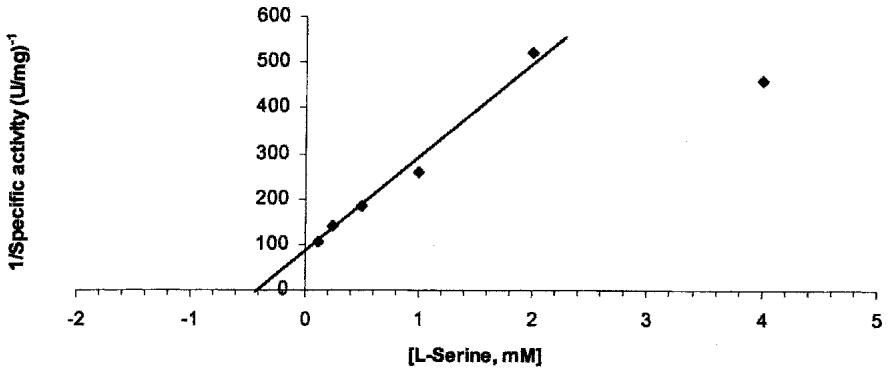

Fig. 1 Inhibition of γ-GT from *Setaria cervi* by L-serine in the presence of borate

The reaction mixtures (1.0 ml) contained 0.1 M Tris-HCl (pH 8.0), 20 mM glycylglycine and 1 mM L-γ-glutamyl-p-nitroanilide. The concentration of L-serine was varied from 0.125 to 4 mM whereas borate was maintained at 10 mM. The Ki was determined to be 0.4 mM as the mean of three independent experiments. One unit (U) of enzyme activity is defined as the amount that catalyses the formation of 1 μmol of p-nitroaniline per minute at 37°C. Specific activity is expressed as μmol p-nitroaniline released/min/mg protein.

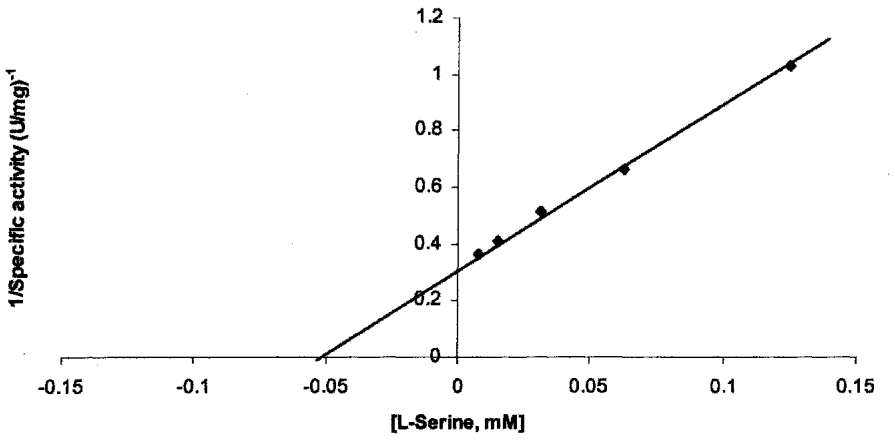

Fig. 2 Inhibition of γ-GT from rat liver by L-serine in the presence of borate

The reaction mixtures (1.0 ml) contained 0.1 M Tris-HCl (pH 8.0), 20 mM glycylglycine and 1 mM L-γ-glutamyl-p-nitroanilide. The concentration of L-serine was varied from 0.0078-0.125 mM whereas borate was maintained at 10 mM. The Ki was determined to be 0.05 mM as the mean of three independent experiments. One unit (U) of enzyme activity is defined as the amount that catalyses the formation of 1 μmol of p-nitroaniline per minute at 37°C. Specific activity is expressed as μmol p-nitroaniline released/min/mg protein.

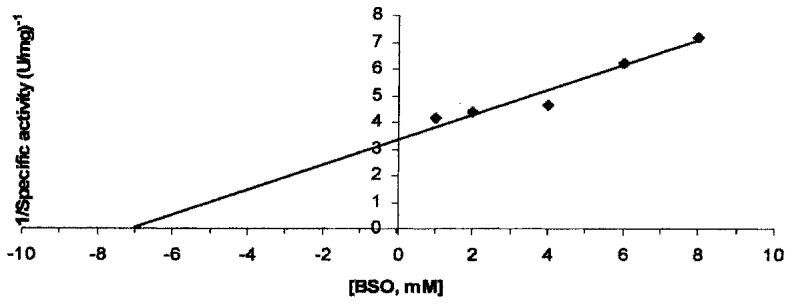

Fig. 3 Inhibition of GCL from *Setaria cervi* by BSO

Reaction mixtures (final volume 1.0 ml) contained 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, 5 mM acivicin, 1.6 μg/μl pyruvate kinase and 1.6 μg/μl lactate dehydrogenase. The concentration of BSO was varied from 1-8 mM to study the effect on enzyme activity and Ki was determined to be 7.7 mM as the mean of three independent experiments. One unit (U) of enzyme activity is defined as the amount that catalyses the formation of 1 μmol of NAD per minute at 37°C. Specific activity is expressed as μmol NAD released/min/mg protein.

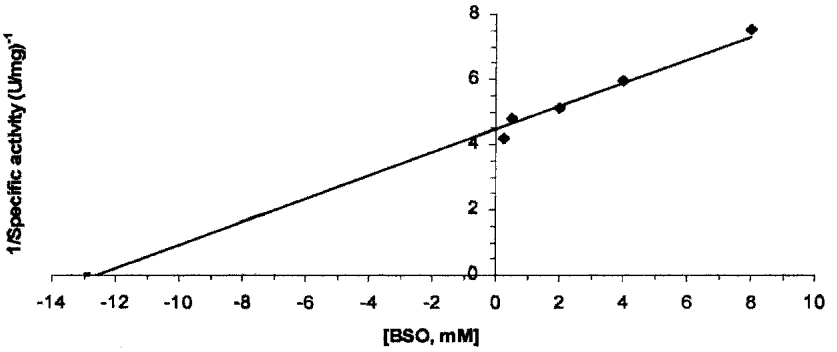

Fig. 4 Inhibition of GCL from rat liver by BSO

Reaction mixtures (final volume 1.0 ml) contained 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, 5 mM acivicin, 1.6 μg/μl pyruvate kinase and 1.6 μg/μl lactate dehydrogenase. The concentration of BSO was varied from 0.25-8 mM to study the effect on enzyme activity and Ki was determined to be 13 mM as the mean of three independent experiments. One unit (U) of enzyme activity is defined as the amount that catalyses the formation of 1 μmol of NAD per minute at 37°C. Specific activity is expressed as μmol NAD released/min/mg protein.

Prospective compounds which inhibited the activity of either of the three gamma glutamyl cycle enzymes *viz* GCL, GS and γ-GT were of relevance and were further screened for their effect on overall GSH homeostasis in filarial parasites by incubation studies. In such studies, filarial worms *S. cervi* were incubated in the presence of such test compounds for 6 h at 250 μM concentration. Table 3 is a selective list of those compounds for which all the four parameters were done. The compounds 2, 5-9, 11-13 and 18-20 caused overall decrease in GSH content of filarial worms and have promising future in design and synthesis of antifilarial drugs. In this regard compounds 5, 6, 9, 11, 13, 18, 19 and 20 are of special importance. They caused around 52%, 60%, 48%, 59%, 58%, 46%, 42% and 65% GSH depletion, respectively. Table 3 also depicts GSH depletion in *S. cervi* females by standard mammalian GSH depletors at 250 μM concentration in 6h.

Compounds 5, 6, 9, 11 and 13 are basically the amino acid analogs grafted on to the sugar backbone and it is presumed that they may serve as substrate analogs for GSH biosynthesis and hamper its *de novo* synthesis. Sugar component offers better stability, less toxicity, better pharmacokinetic parameters and facilitates the transport of drugs.[27,28]

Table 3. Effect of synthetic compounds on Glutathione (GSH), Glutamate cysteine ligase, Glutathione synthetase and γ-Glutamyl transpeptidase from *S.cervi.*

Compound	Class	% GSH[a] Modulation	% GCL[b] Modulation	% γ-GS[c] Modulation	% γ-GT[d] Modulation
1	DGDA	(+) 52.54*	21.00*	40.75	6.49
2	DGDA	33.14*	56.80*	7.35	(+) 15.26
3	DGDA	(+) 39.00*	27.40*	27.83	6.70
4	DGDA	(+) 69.29*	35.70*	51.16	8.24
5	DGDA	52.50*	20.40*	12.84	7.22
6	DGDA	60.00*	31.00*	7.97	Nil
7	DGDA	14.55*	Nil*	(+) 36.42	26.40
8	DGDA	39.04*	19.70*	(+) 13.95	2.30
9	DGDA	48.00*	29.60*	Nil	8.30
10	DGDA	(+) 37.43*	25.00*	33.28	(+) 28.35
11	DGDA	59.12*	11.50*	(+) 19.23	(+) 22.49
12	DGDA	15.9*	75.90*	24.45	3.61
13	DGDA	58.17*	29.60*	2.63	19.72
14	DGDA	(+) 67.00*	15.80*	35.80	Nil
15	Coumarin	Nil	Nil	45.42	(+) 54.61
16	Coumarin amide	3.22	Nil	Nil	10.84
17	Coumarin	5.75	Nil	Nil	(+) 18.93
18	Coumarin amide	46.00	Nil	(+) 8.12	28.64
19	Coumarin	42.07	Nil	(+) 9.26	(+) 18.83
20	Coumarin	65.00	Nil	(+) 6.44	Nil
Dimethylmaleate	Standard GSH depletor	95.82	-	-	-
Phorone	Standard GSH depletor	93.70	-	-	-
Menadione	Standard GSH depletor	96.04	-	-	-
Methylene Blue	Standard GSH depletor	34.68	-	-	-
BSO	Standard GSH depletor	35.78	-	-	-
Carmustine	Standard GSH depletor	68.97	-	-	-

* Already tested previously by us[18]
(-) denotes depletion/ inhibition; (+) denotes increase/ activation
[a] Concentration of test compound/ standard GSH depletor in incubation medium = 250μM
[b] Concentration of test compound in assay system = 200μM
[c & d] Concentration of test compound in assay system = 250μM
DGDA = Diglycosylated diaminoalkanes; GSH = Reduced glutathione; GCL = Glutamate cysteine ligase; γ-GT = γ-Glutamyl transpeptidase; GS = Glutathione synthetase; BSO = Buthionine sulfoximine

GSH depleting compounds 18, 19 and 20, with 2H-1-benzopyrone nucleus, are generally called coumarins. Such a coumarin derivative has been reported to reduce lymphatic oedema when administered along with diethylcarbamazine.[29] In previous studies coumarin derivatives were evaluated for filaricidal activity against *L. carinii*

and *A. viteae* infections in *Sigmodon hispidus* and *Mastomys coucha*, and significant macrofilaricidal effects were detected.[30] Studies have also been made regarding the effect of these compounds on glutathione metabolism of filarial worms. *In vitro* studies by Srivastava *et al*[18] using adult *S. cervi* revealed the inhibitory effect of benzopyrones on glutathione-S-transferases.

In the present study, these compounds have affected the GSH metabolism of filarial worms in a drastic way and can be categorised into various groups depending upon their *in vitro* effect on key γ-glutamyl cycle enzymes and their potential to alter GSH content of filarial worms *S. cervi*:

(i) Compound numbers 2, 5-6, 8-9 and 11-13 have the potential to decrease the GSH content of *S. cervi* worms by inhibiting GSH synthesizing enzymes. Compounds 2, 5-6 and 12-13 inhibited both the enzymes involved in the *de novo* biosynthesis of GSH *i.e.* GCL and GS whereas compounds 8, 9 and 11 inhibited only the regulatory enzyme, GCL of the γ-glutamyl cycle.

(ii) Compound numbers 7 and 18 to 20 have the potential to decrease the GSH content of *S. cervi* without affecting any of the synthesizing enzymes. Compound numbers 7 and 18 inhibited extracellular GSH salvaging enzyme, γ-GT. Whereas in the case of compounds 19 and 20, inhibition of intracellular salvage reaction catalysed by GR might be the reason for drastic GSH depletion and need to be studied further.

(iii) Compounds numbers 1, 3-4, 10 and 14 have the potential to increase the GSH content of filarial worms, although these compounds inhibited both the GSH synthesizing enzymes, GCL and GS and have very mild effect on γ-GT (except compound number 10, which activated γ-GT). The result implies that after the inhibition of both the synthesizing enzymes, filarial parasites in order to cope with the GSH demand might have shifted their metabolic scenario in such a way so as to get all the GSH from the intracellular salvage reaction by activating GR, which catalyses the reduction of GSSG back to GSH using NADPH as reducing cofactor. Boost in the GSH level might be the defensive stratagem adopted by the filarial parasite to surmount the drastic effects of test compounds before its complete subjugation. In the

case of compound number 10, increase in γ-GT is the evident reason for increase in GSH content.

(iv) Compound numbers 15-17 have negligible or no effect on GSH content of *S. cervi* filarial worms. These compounds also have insignificant or no effect on the key γ-glutamyl cycle enzymes. In the case of compound number 15, inhibition of GS and activation of γ-GT to an approximately same tune might have negated the overall effect on GSH homeostasis.

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