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THERAPEUTIC POTENTIAL OF NOVEL HETEROCYCLIC THIAZOLIDINE COMPOUNDS AGAINST HUMAN LYMPHATIC FILARIAL PARASITE: AN *IN VITRO* STUDY

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Key words:

Lymphatic filariasis, thiazolidineones, *Brugia malayi*, anti-parasitic effect

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ABSTRACT: Objective: Following World Health Organization tropical disease research mandate, there is definitive need for search of new lead molecules for development of drug against human lymphatic filariasis. Thiazolidineone derivatives has shown versatile therapeutic potential. The present study has been undertaken to explore the possible antifilarial effect of such synthetic compounds against filarial parasite, *Brugia malayi*. **Methods:** A series of 12 thiazolidineone derivatives, following synthesis and characterization, were screened for antifilarial potential in vitro against microfilarial stage of *Brugia malayi*; those showed significant effect were tested against adult parasites also. The pharmacological response in terms of loss of parasitic motility was assessed under microscope. Further dose dependent response and potential lethality onto host cells for such effective drugs were carried out. **Result:** Out of the tested compounds, four such compounds showed significant activity and those were tested against the adult worms; one of these compounds showed marked effect. The corresponding IC_{50} and LD_{50} values were deduced for each of these four effective compounds. Most of them showed a good therapeutic index. **Conclusion:** Since the standard drug Diethyl Carbamazine Citrate is largely dependent on the host innate immune response and also not effective as microfilaricidal, hence the observed result with that thiazolidineone compounds have definitive potential for being considered as important therapeutic lead molecules against both microfilarial as well as the adult stage of the parasite.

INTRODUCTION: The cumulative burden of disability among the people affected with lymphatic filariasis (that is endemic in 73 tropical and subtropical countries with approximately 120 million already infected and an estimated 1.39 billion people at risk for infection), has compelled World Health Organization (WHO) to implement Global Programme to Eliminate Lymphatic Filariasis (GPELF) [1].

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Under this program, currently existing drug regimen used in 'Mass Drug Administration' strategy involving DEC (Diethyl Carbamazine Citrate) mostly as the sole drug that works almost empirically [2]. Moreover other limitations like side effects, lack of compliance and threat of emergence of possible drug resistance haunts to raise alarm for urgent need of research for drug development [3]. Therefore, WHO under its TDR program has recommended fresh research to find out new therapeutic modality [4].

It is well-known that DEC is able to exert its pharmacological effect *in vivo* but unable to display significant *in-vitro* activity [5], this is probably because of its proposed pre-requisite of harnessing

innate inflammatory response of host immune cells to achieve its effect. In recent years, genome sequencing of *Brugia malayi* parasite contributed towards unraveling of several pathways for exploring potential antifilarial therapeutics [6].

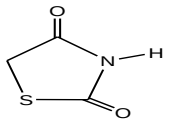
However, corresponding structural details of possible target proteins are yet to be deciphered. Hence screening of synthetic compounds to validate the candidate drug as well as to develop mechanistic insight might be rewarding. Our recent work with newly synthesized compounds i.e. bigaunides, dihydrotriazine, 2, 4-diaminopyrimidine, and 2, 4-diamino-s-triazine compounds showed potent activity against filarial parasite; moreover their proposed action as enzyme dihydro folate reductase (DHFR) inhibitors were also validated by suitable reversal experiments [7, 8]. Although initially several potential candidates show promise, but owing to the substantial technical intricacy most of them failed to reach actual stage to be developed into a viable product. Therefore it is quite imperative to continue exploring for novel lead molecules to have a sustainable back-up to develop and enrich the therapeutic repertoire for this particular parasitic disease, as of now which has a meager options.

During the recent decades, there has been intense investigation on thiazolidineones compounds, many of which are known to possess interesting therapeutic properties such as antibacterial [9], anticancer [10], antimalarial [11], and antiproliferative [12]. With the perspective mentioned above, the purpose of the present work was to explore the potential of this class of molecules and develop the novel candidate with improved efficacy for treating filariasis.

MATERIALS AND METHODS:

Materials:

All reagents and chemicals were obtained from commercial sources (Himedia Laboratories Pvt. Ltd, Mumbai and Sigma Aldrich Chemicals Pvt. Ltd, Mumbai). The thiazolidine derivative (**Fig. 1**) were synthesized and purified in our laboratory. The synthesis and characterization of these compounds has been provided as supplementary material.



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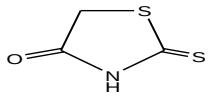

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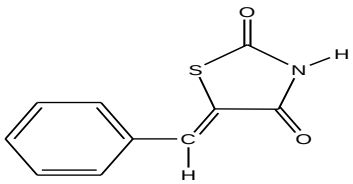

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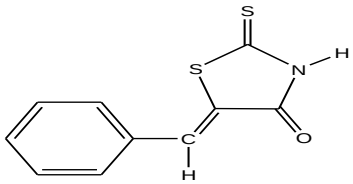

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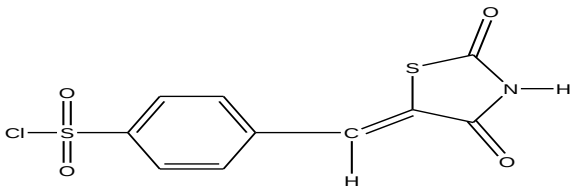

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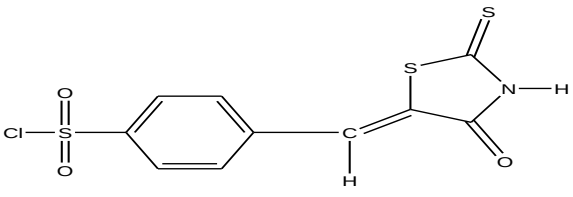

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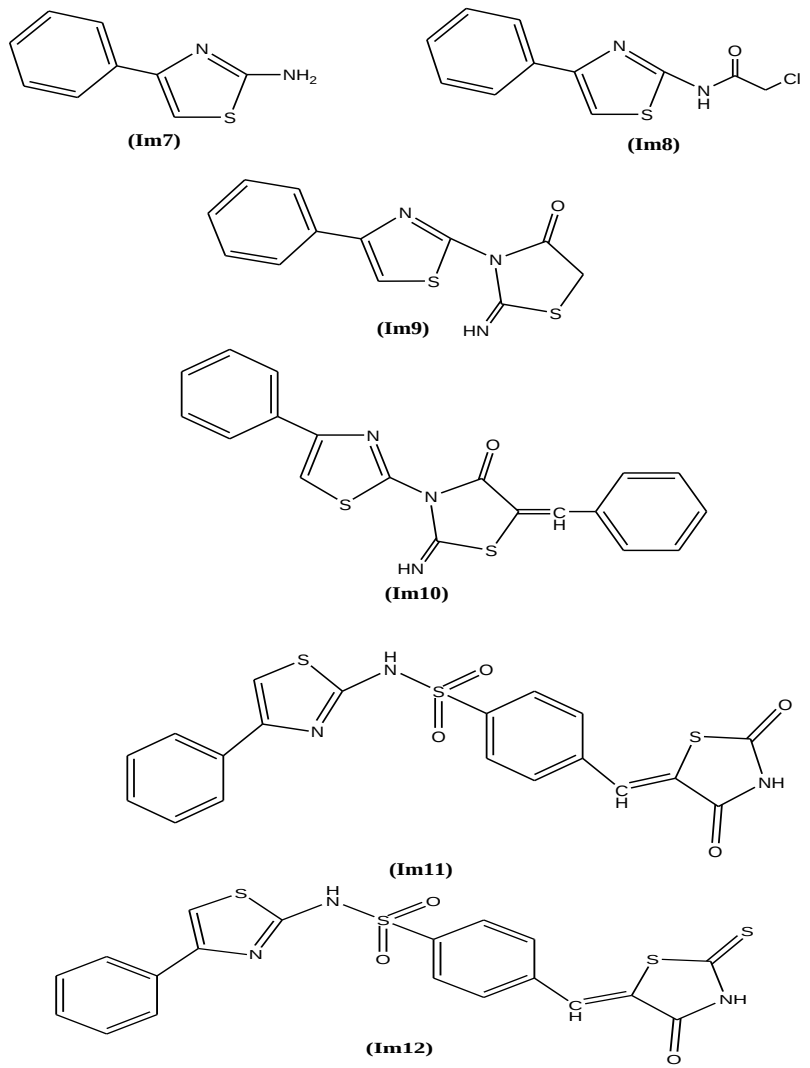

FIG.1: STRUCTURES OF SYNTHESIZED THIAZOLIDINE DERIVATIVES

Im1- thiazolidine-2,4-dione
Im2- 2-thioxothiazolidin-4-one
Im3- (Z)-5-benzylidenethiazolidine-2,4-dione
Im4- (Z)-5-benzylidene-2-thioxothiazolidin-4-one
Im5- 4-((Z)-(2,4-dioxothiazolidin-5-ylidene)methyl)benzene-1-sulfonyl chloride
Im6- 4-((Z)-(4-oxo-2-thioxothiazolidin-5-ylidene)methyl)benzene-1-sulfonyl chloride
Im7- 4-phenylthiazol-2-amine
Im 8- 2-chloro-*N*-(4-phenylthiazol-2-yl) acetamide
Im9- 2-imino-3-(4-phenylthiazol-2-yl) thiazolidin-4-one
Im10- 5-benzylidene-2-imino-3-(4-phenylthiazol-2-yl)thiazolidin-4-one
Im11- 4-(2,4-dioxo-thiazolidin-5-ylidenemethyl)-N-(4-phenylthiazol-2-yl)benzenesulfonamide
Im12- 4-(2-thioxo-4-oxo-thiazolidin-5-ylidenenemethyl)-N-(4-phenylthiazol-2-yl)benzenesulfonamide

Establishment and maintenance of *Brugia malayi* life cycle:

The human filarial parasite *B. malayi* life cycle was maintained in jirds (*Meriones unguiculatus*), mastomys (*Mastomys caucha*) using mosquitoes (*Aedes aegypti*) as vectors by standard methods as described earlier. The use of animals for this study was approved by the Institutional Animal Ethics Committee, which follows the norms of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) in India.

Microfilariae (Mf) and adult worms were freshly obtained from the peritoneal cavity of the jirds exposed to infective 3rd stage larvae (L_3) 4–5 months back. The microfilariae and adult worms were washed with RPMI 1640 medium (containing 20 μg mL^{-1} gentamycin, 100 μg mL^{-1} penicillin, 100 μg mL^{-1} streptomycin) plated on the sterile

plastic petri dishes and incubated at 37°C for 1 h to remove the peritoneal exudate cells of the jirds. The microfilariae were collected from the petri dishes, washed with RPMI 1640 medium and used for *in vitro* experiments [8].

***In vitro* screening of compounds for anti-filarial activity:**

Microfilariae:

The efficacy of compounds to affect the viability of Mf *in vitro* was assessed by the extent of parasite motility. A stock solution of 2 mM concentration was made for each thiazolidine compound in DMSO. Further dilutions were made to obtain the desired final concentration in the range of 0.5 µM to 500 µM. The highest concentration of DMSO used along with compound was <1% hence comparable vehicle control was also taken with 1% DMSO.

Approximately, 300 Mf in 1000 µl of sterile 0.9% saline were introduced into each vial for every test drug (over a dose range of 0.5 µM to 18 µM) along with above mentioned vehicle control and incubated on shaker incubator at 37°C for 30 minutes with 150 rpm (Scigenics Biotech, India). After incubation, Mf were washed with RPMI 1640 media and 100 Mf were plated in each well (each individual samples in triplicates) in sterile 24 well culture plates (Nunc, Denmark) containing 1000 µl of RPMI media. The plates were re-incubated at 37°C for 48h in 5% CO_2 incubator (pre-optimized conditions). Mf motility was assessed by microscopy (using Nikon Diaphot, TMD inverted microscope). Each experiment was repeated thrice to check the reproducibility.

Percent inhibition in terms of loss of motility was determined as described earlier [13]. The IC_{50} value was also calculated for effective compounds [7].

Adult worms:

Considering the paucity of procuring adult worms, only compounds with high efficacy against microfilariae were further evaluated against adult worms. Procedure similar as above was followed with adult worms (1 male and 1 female) to evaluate efficacy of such compounds (using the concentration of the respective compounds that showed highest anti-microfilarial effect) along with DMSO as vehicle control. The plates were incubated at 37°C, with 5% CO_2, for 48 h and the motility of worms was assessed by microscopy, wherein the anti-parasitic effect was also assessed visually by direct microscopic observations (using a Nikon Diaphot, TMD inverted microscope as indicated previously) and the observations were scored as −, inactive; +, less active; ++,moderately active; and +++, highly active. Experiments were done in triplicate to check the reproducibility.

Evaluation of the *in vitro* cytotoxicity of various effective agents on the human Peripheral Blood Mononuclear Cells (PBMCs):

Whole blood samples were obtained from healthy subjects. Venous blood from each subject was overlaid carefully on histopaque - 1077 (Sigma Chem. Co., USA) in 1:1 ratio taken in screw cap tubes centrifuged at room temperature 400 X g for 30 minutes. Upper layer of plasma was discarded and opaque layer to within 0.5 cm was collected containing PBMCs. Opaque layer was re-suspended in RPMI 1640 medium supplemented with 2 mM L-glutamine. The cell suspension was then washed by adding RPMI 1640 medium followed by centrifugation at 250 X g for 10 min and the cell pellet was collected. The washing procedure was repeated twice and the cell pellet was re-suspended in RPMI medium supplemented with 10% fetal bovine serum. Viability of the cells was determined by trypan blue dye exclusion method before using these PBMCs for determination of cytotoxic effect of the proposed drugs.

Cytotoxicity was evaluated against human PBMCs (1X 10^5 PBMCs/mL) by MTT assay [14] following similar procedure as adapted for parasites with each of the agents. The dose at which 50% cytotoxicity was observed, has been denoted as LD_{50} concentration.

RESULT:

Out of the 12 compounds screened only four were found to be pharmacologically effective for others even with the use of highest dose (500 µM) no effective response was obtained as displayed by their impact on loss of motility of the parasites *in vitro*. The results obtained are shown in **Table 1**.

TABLE 1: *IN VITRO* EFFECT OF ACTIVE SYNTHETIC COMPOUNDS ON THE MOTILITY OF *B. MALAYI* MICROFILARIAE (MF)

Sr. No.	Mol ID	Mol Wt.	Activity*
1.	Im1	117.13	3.55 ±1.27
2.	Im2	133.19	8.04 ± 1.22
3.	Im3	205.23	6.36 ± 0.17
4.	Im4	221.3	5.575 ± 0.615
5.	Im5	303.74	10.125 ± 2.255
6.	Im6	319.81	100 ± 0
7.	Im7	176.24	2.415 ± 0.505
8.	Im8	252.72	100 ± 0
9.	Im9	275.35	4.62 ± 2.95
10.	Im10	363.46	100 ± 0
11.	Im11	443.52	1.43 ± 0.54
12.	Im12	459.58	100 ± 0
13.	Control 1	DMSO Control	2.21 ± 1.23
14.	Control 2	RPMI control	1.32 ± 1.32

* Activity in terms of % loss of Mf motility.

Consequently, these four pharmacologically effective compounds (namely Im6, Im8, Im10 and Im12) individually were tested for assessment of cytotoxicity against human PBMCs by MTT assay.

These four pharmacologically effective compounds were further screened against adult worms of which lm10 in particular showed quite significant effect (**Table 2**).

TABLE 2: *IN VITRO* EFFECT OF ACTIVE SYNTHETIC COMPOUNDS ON MOTILITY OF *B. MALAYI* ADULT WORMS.

Sr. No.	Compound	Motility of *B. malayi* adult worms after 48 h. incubation*
1.	Im6	+
2.	Im8	+
3.	Im10	-
4.	Im12	++
5.	Control (RPMI)	+++

* Two adult worms were incubated for each inhibitor concentration at 37^{o}C in an atmosphere of 5% CO_2. After the indicated time, worm motility was assessed and scored under an inverted microscope and designated as -, Inactive; +, Less Active; ++, moderately active; and +++, highly active. Results are from two independent experiments performed in duplicate.

The difference between IC_{50} (50% inhibitory concentration) and the LD_{50} (50% lethal dose) values for different effective agents as obtained from the *in vitro* filarial motility assay and the *in vitro* cytotoxicity assay (against PBMCs) respectively are shown in **Table 3**. The difference between observed IC_{50} and LD_{50} showed good therapeutic index for each of these agents, particularly the Im6 and Im8 compounds displayed widest intervals.

TABLE 3: ANTIFILARIAL ACTIVITY AND TOXICITY STUDIES

Sr. No.	Compound	IC_{50}	LD_{50}
1.	Im6	5.2 µM	349 µM
2.	Im8	2.4 µM	217 µM
3.	Im10	1.78 µM	17.59 µM
4.	Im12	14.8 µM	42 µM

The IC_{50} (50% inhibitory concentration) and the LD_{50} (50% lethal dose) values for different effective agents as obtained from the *in vitro* filarial motility assay and the *in vitro* cytotoxicity assay (against PBMCs) respectively.

Supplementary Information:

1. Synthesis of 2, 4- thiazolidinedione (Im1):

To a 500 mL three neck flask equipped with a thermometer, a stirrer and a condenser were added 2-chloroacetic acid (18.9 g, 0.2 mole), thiourea (15.2 g, 0.2 mole) and 40 mL of hydrochloric acid (35-36%). This mixture was stirred at room temperature for at least 1 h, and then was heated to reflux. The process of the reaction was monitored by TLC (silica gel, Rf = 0.36, elution: Benzene-methanol: 9.8:0.2) until the reaction was complete (10 h). Then the solution was allowed to cool down gradually to room temperature while still being stirred. The large amount of pale yellow crystalline solid was collected, washed with small amount of cold water to give the crude product as pale yellow crystals, which were recrystallized from water

(small amount of activated charcoal may be used) to give pure white crystals (Im1), mp 124–125°C. After about 4h of reflux, 2-imino-4-thiazolidone appeared as white solid, mp 254-256°C (dec.) which on further refluxing converted to (Im1). The synthesized compound was studied further by spectral analysis.

Spectral analysis:

The molecular formula was deduced to be $C_3H_3O_2NS$by mass spectral analysis, indicating the annulation of the reactants to form the desired 2, 4-thiazolidinedione. The ^{1}H NMR spectrum (CD_3OD, 300 MHz, ppm) exhibited two characteristic peaks δ 3.83 (2H, s) for the aliphatic methylene protons and a peak for NH proton at δ 9.98 (1H, s) and the reason for this deshielding is the presence of two electron-withdrawing carbonyl groups (C=O), on either side of NH group in the thiazolidine ring system. The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) showed 3 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established a peak at δ 36.78 for the methylene carbon, the rest two peaks of carbonyl carbon were assigned at δ 175.09 and 175.68. IR: 3132.18 cm^{-1} (*sec* N-H *str*), 2947.03 cm^{-1} (CH_2 *str*), 1735.81 cm^{-1} (C=O *str* at C_4), 1681.81 cm^{-1}(C=O *str* at C_2),1342.36 cm^{-1} (N–C_4*str*), 1161.07 cm^{-1} (C_2–N *str*), 889.12cm^{-1}(C_5–S*str*), 715.54cm^{-1} (C_2–S*str*); ESI-MS (positive mode): m/z 161.93 $[M+ 2 Na]^+$, 139.9 $[M+Na]^+$, 116.96$[M]^+$.

2. Synthesis of 2-thioxo-4-thiazolidineone (Im2):

2.1. Synthesis of Ammonium dithiocarbamate:

Gaseous ammonia was passed into 250 mL of 95% ethanol contained in a 1L Erlenmeyer flask immersed in an ice bath for three and one-half hours. To this solution, still cooled in the ice bath, was added a well-cooled mixture of 76 g (60 mL, 1 mole) of carbon disulfide and 200 mL of ether. The flask was stoppered loosely and allowed to remain in the ice bath for 2–3 hours. The contents of the flask then represent an almost solid cake of pale yellow crystals, which were then left at room temperature overnight. The mixture was again cooled in an ice bath or refrigerator, and the crystals collected by filtration, sucked dry and washed on the filter with 50 mL of cold alcohol, followed by 100 mL of ether. The product was used immediately without further treatment; on standing it deteriorates rapidly.

2.2. Synthesis of Sodium 2-(carbomodithioyl) acetate:

In the meantime a solution of sodium chloroacetate has been prepared by dissolving 71g. (45 mL, 0.75 mole) of chloroacetic acid in 150 mL of water contained in a 1-L wide-mouthed round-bottomed flask and neutralizing the acid with 40 g. (0.38 mole) of anhydrous sodium carbonate while stirring the solution mechanically. This solution was then cooled in an ice bath. Just before the filtration of the product in 2.1, a solution of sodium chloroacetate was prepared. To the solution of Sodium Chloroacetate, cooled in an ice bath, ammonium dithiocarbamate from the preceding Step 2.1 was added during 5 minutes with continual stirring. As soon as the first portion of ammonium dithiocarbamate was added the solution becomes very dark in color and after all the dithiocarbamate has been added, the ice bath was removed and stirring was discontinued. The solution was allowed to stand for 20–30 minutes longer, during which time the color changes from dark at first, gradually lightening and finally becoming a straw yellow if all had gone well.

2.3. Synthesis of 2-thioxo-4-thiazolidinone(Im2):

In a 1L beaker, 400 mL 6N hydrochloric acid was heated to boiling, and the above solution was poured slowly with stirring into the hot acid, and heating was continued until the solution has attained a temperature of 80–85°C, after which the solution was allowed to cool slowly to room temperature. The compound separates as nearly colorless long blades which are collected by filtration, washed well with water, and dried. The product yield 50% and melts at 167–168°C. Recrystallization was from boiling glacial acetic acid raises the melting point to 168–169°C. The compound was further studied by spectral analysis.

Spectral analysis:

The ^{1}H NMR spectrum (Py-d5, 300MHz) exhibited characteristic peaks δ 4.381(2H, s) for the aliphatic methylene protons. The ^{13}C NMR spectrum (Py-d5, 75 MHz) showed 3 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established a peak at δ 40.59 for the methylene

carbon, the rest two peaks were assigned at δ 177.92 and 206.39. IR: 3164.97 cm^{-1} (*sec* N-H *str*), 2960.53 cm^{-1} (CH_2 *str*), 1708.81 cm^{-1} (C=O *str* at C_4), 1442.66 cm^{-1}(C=S *str* at C_2), 1326.93 cm^{-1} (N–C_4*str*), 1188.07 cm^{-1} (C_2–N *str*), 819.69cm^{-1}(C_5–S*str*), 680.83cm^{-1} (C_2–S*str*).

3. Synthesis of 5-benzylidenethiazolidine-2,4 –dione (Im3) and 5-benzylidenethiazolidine- 2-Thioxo-4-one (Im4):

3.1. Synthesis of 5-benzylidenethiazolidine-2, 4 –dione (Im3):

2, 4-thiazolidinedione (Im1, 0.1 mole, 11.71 g), was dissolved in 50 mL of ethanol by warming, to the clear colorless solution was added benzaldehyde (0.1 mole, 10.16 mL) and piperidine (0.1 mole, 9.87 mL). The resultant clear solution was refluxed on water bath for 14 h. The complete reaction was monitored by TLC in Benzene: Methanol (9.5:0.5v/v).

After 14 h, the resultant pale yellow color clear solution was poured in crushed ice and triturated vigorously, white turbidity appears, and pH was alkaline, the turbid solution was acidified with acetic acid to give white solid, which was filtered, and washed with water and dried below 50°C in oven. The white product was recrystallized from methanol. White crystals, Yield 65%, mp 240-242°C was obtained, which was subjected to spectral analysis.

Spectral analysis:

The molecular formula was deduced to be $C_{10}H_7NO_2S$ by mass spectral analysis, indicating the annulation of the reactants to form the desired 5-benzylidenethiazolidine-2, 4 –dione. The ^1H NMR spectrum (Py-d5, 300MHz) exhibited characteristic peaks at δ 7.31-7.49 (5H, m) for aromatic protons and δ7.60 (1H, d, *J*=7.5 Hz) for =CH proton. The ^{13}C NMR spectrum (Py-d5, 75 MHz) showed 10 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established 6 peaks at δ 129.76x2C, δ130.02, δ130.64, δ130.71, δ132.38 for the methylene carbon, the two peaks at δ169. 42 and δ169.02 are of C=O at C_2 and C_4, respectively and peak at δ125.40 and δ134.20 is for quaternary carbon; ESI-MS (positive mode): m/z 227.99 $[M+Na]^+$, 206.01$[M+1]^+$, 177.11$[M-CO]^+$, 144.99$[C_9H_7NO]^+$, 115.00$[C_3HNO_2S]^+$.

3.2. Synthesis of 5-benzylidenethiazolidine- 2-Thioxo-4-one (Im4):

2-thioxo 4-thiazolidineone (Im2, 0.01 mole, 1.3319 g), was dissolved in 50mL of glacial acetic acid by warming, to the clear yellow color solution was added benzaldehyde (0.02 mole, 2.03 mL) and sodium acetate (0.02 mole, 1.64 g). The resultant yellow color solution was refluxed on water bath for 8 h. The complete reaction was monitored by TLC in Benzene: Methanol (9.5:0.5v/v).

After 8 h, the resultant yellow color clear solution was poured in crushed ice and triturated vigorously, allowed to stand, to give yellow solid, which was filtered, and washed with water and dried below 50°C in oven. The yellow product was recrystallized from methanol. Yellow shining crystals, Yield 55%, mp 220-224°C was obtained, which was subjected to spectral analysis.

Spectral analysis:

The molecular formula was deduced to be $C_{10}H_7NOS_2$ by mass spectral analysis, indicating the annulation of the reactants to form the desired 5-benzylidene-2-thixothiazolidine- 4 –one. The ^1H NMR spectrum ($CDCl_3$, 300 MHz) exhibited characteristic peaks at δ 7.49 (5H, s) for aromatic protons and δ7. 68 (1H, s) for =CH proton and broad peak at δ9.47 (1H, s) for N-H proton. The ^1H NMR spectrum (DMSO-d6, 300MHz) exhibited characteristic peaks at δ 7.47- δ 7.62 (5H, m) for aromatic protons and δ7. 65 (1H, s) for =CH proton and broad peak at δ13.86 (1H, s) for N-H proton. The ^{13}C NMR spectrum (Py-d5, 75 MHz) showed 10 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established 6 peaks at δ 129.94x2C, δ131.03x3C, δ131.09, δ131.89 for the methylene carbon, and the two peaks at δ197.54 and δ171.38 are of C=S at C_2 and C=O at C_4, respectively and peak at δ127.88 and δ134.27 is for quaternary carbon; ESI-MS (positive mode): m/z 244.10 $[M+Na]^+$, 221.10$[M]^+$, 203.14 $[M-H_20]^+$, 135.00$[C_8H_6S]^+$, 113.94 $[C_4H_4NOS]^+$.

4. Synthesis of4-((Z)-(2, 4-dioxothiazolidin-5-ylidene) methyl) benzene-1-sulfonyl chloride (Im5) and 4-((Z)-(4-oxo-2-thioxothiazolidin-5-

ylidene) methyl) benzene-1-sulfonyl chloride (Im6):

4.1. Synthesis of 4-((Z)-(2, 4-dioxothiazolidin-5-ylidene) methyl) benzene-1-sulfonyl chloride (Im5): 5-benzylidenethiazolidine-2, 4 - dione (Im3, 0.0388 mole, 7.954 g) was placed in 250 mL round bottom flask equipped with a condenser and a dropping funnel. Chlorosulfonic acid (0.155 mole, 10.37 mL) was added at room temperature using the dropping funnel. The reaction was exothermic. After addition of chlorosulfonic acid was over the reaction mass was refluxed for one and one half hour on a water bath. The blackish viscous solution was cooled and poured in crushed ice with vigorous trituration. A white product was obtained, which was filtered and washed with water and dried in oven below 50°C. The product was recrystallized from ethanol. White crystals, yield 50%, mp 180-181°C was obtained. The compound was studied by spectral analysis.

Spectral analysis:

The molecular formula was deduced to be $C_{10}H_6ClNO_4S_2$ by mass spectral analysis, indicating the annulation of the reactants to form the desired 4-((Z)-(2, 4-dioxothiazolidin-5-ylidene) methyl) benzene-1-sulfonyl chloride. The ^1H NMR spectrum ($CDCl_3$, 300MHz) exhibited characteristic peaks at δ 8.61 (1H, s) broad peak of N-H, δ 8.13(1H, d, *J*=8.4Hz), δ 7.64-7.73 (2H, m), δ8.00 (1H, d, *J*=8.4Hz) four aromatic protons and δ7.86 (1H, s) for =CH proton. The ^{13}C NMR spectrum (Py-d5, 75 MHz) showed 10 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established 5 peaks at δ 127.97x2C, δ130.48x2C, δ131.82 for the methylene carbon, and the two peaks at δ169. 32 and δ168.89 are of C=O at C_2 and C_4, respectively and peak at δ125.94 and δ134.72 is for quaternary carbon; ESI-MS (positive mode): m/z: 628.86 $[2M+Na]^+$, 325.92 $[M+Na]^+$ Base Peak, 302.94 $[M]^+$.

4.2. Synthesis of 4-((Z)-(4-oxo-2-thioxothia zolidin-5-ylidene) methyl) benzene-1-sulfonyl chloride (Im6):

5-benzylidenethiazolidine-2-thioxo-4-one (Im4, 0.0388 mole, 8.5924 g) was placed in 250 mL round bottom flask equipped with a condenser and a dropping funnel. Chlorosulfonic acid (0.155 mole, 10.37 mL) was added at room temperature using the dropping funnel. The reaction was exothermic. After addition of chlorosulfonic acid was over the reaction mass was refluxed for one and one half hour on a water bath. The blackish viscous solution was cooled and poured in crushed ice with vigorous trituration. A pale orange product was obtained, which was filtered and washed with water and dried in oven below 50°C. The product was recrystallized from ethanol. White crystals, yield 50%, mp 198-200°C was obtained. The compound was further studied by spectral analysis.

Spectral analysis:

The molecular formula was deduced to be $C_{10}H_6ClNO_4S_2$ by mass spectral analysis, indicating the annulation of the reactants to form the desired 4-((Z)-(4-oxo-2-thioxothiazolidin - 5 - ylidene) methyl) benzene-1-sulfonyl chloride. The ^1H NMR spectrum ($CDCl_3$, 300MHz) exhibited characteristic peaks at δ 8.61 (1H, s) broad peak of N-H, δ 8.13(1H, d, *J*=8.4Hz), δ 7.64-7.73 (2H, m), δ8.00 (1H, d, *J*=8.4Hz) four aromatic protons and δ7.86 (1H, s) for =CH proton. The ^{13}C NMR spectrum (Py-d5, 75 MHz) showed 10 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established 5 peaks at δ 127.97x2C, δ130.48x2C, δ131.82 for the methylene carbon, and the two peaks at δ169. 32 and δ168.89 are of C=O at C_2 and C_4, respectively and peak at δ125.94 and δ134.72 is for quaternary carbon; ESI-MS (positive mode): m/z: 628.86 $[2M+Na]^+$, 325.92[M+Na] $^+$ Base Peak, 302.94 $[M]^+$.

5. Synthesis of 5-benzylidene-2-imino-3-[4-phenylthiazol-2-yl] thiazolidin-4-one (Im9):

5.1. Synthesis of 2-amino-4-phenyl thiazole (Im7):

A finely powdered thiourea (0.2 mole, 15.2 g) and iodine (0.1 mole, 25.4 g) were triturated and mixed with acetophenone (0.2 mole, 24.0 g) in 250 mL round bottom flask and refluxed for 6h on water bath with occasional stirring. The obtained black solid was triturated with diethyl ether to remove un-reacted acetophenone, yellow color compound was obtained, which was filtered at vacuum pump and washed with aqueous sodium thiosulfate to remove excess of iodine and then washed with water, a white compound was obtained. The crude product was then dissolved in hot water, filtered to remove sulfones; to the clear filtrate ammonia was

added drop wise with stirring, white gelatinous precipitate was obtained. The separated solid was filtered, washed with water and dried in oven below 50°C and recrystallized from ethanol to give white shining crystals, Yield 55%, mp 149°C. The synthesized compound was studied further by spectral analysis.

Spectral Analysis:

The molecular formula was deduced to be $C_9H_8N_2S$ by mass spectral analysis, indicating the annulation of the reactants to form the desired 2-amino-4-phenyl thiazole. The ^{1}H NMR spectrum (CDCl3, 300MHz) exhibited characteristic peaks at δ 5.22 (1H, s) for NH_2, δ 6.71(1H, s) for =CH, δ 7.28-7.30 (1H, m), δ7.37 (2H, t, *J*=6.8Hz) and δ7.77 (2H, t, *J*=1.5Hz) for aromatic protons. The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) showed 9 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established 5 peaks at δ 102.6, δ125.90x2C, δ127.6, δ128.50x2C for the methylene carbon, and the three peaks at δ134.6, δ151.2 and δ167.5 for quaternary carbon; ESI-MS (positive mode): m/z: 176.98$[M]^+$Base Peak.

5.2. Synthesis of 2-chloroacetamido-4-phenylthiazole (Im8):

Solution of 2-amino-4-phenylthiazole (Im**7**, 0.02 mole, 3.7 g) in dry benzene (60 mL) was cooled to 0-5°C. Chloroacetyl chloride (0.04 mole, 5 mL) dissolved in dry benzene (20 mL) was slowly added to the solution with vigorous stirring. When the addition was complete, the reaction mixture was refluxed for 3 h, till pale yellow clear solution was obtained. Benzene was removed on rotary, and the residue was washed with 5% solution of sodium-bi-carbonate and then with water. The crude white product was filtered, and dried in oven below 50°C and recrystallized from ethanol to give white crystals, Yield 50%, mp 160°C. The compound was further studied by spectral analysis.

Spectral Analysis:

The molecular formula was deduced to be $C_{11}H_9ClN_2OS$ by mass spectral analysis, indicating the annulations of the reactants to form the desired 2-chloroacetamido-4-phenylthiazole. The ^{1}H NMR spectrum ($CDCl_3$, 300MHz) exhibited characteristic broad peak at δ 10.08 (1H, s) for N-H, δ 4. 20 (2H, s) for CH_2, δ 7.20 (1H, s) for =CH, δ 7.34 (3H, t, *J*= 7.2Hz), δ7.82 (2H, d, *J*=7.2Hz) for aromatic protons. The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) showed 11 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established inverted –CH_2 peak at δ 41.85, 6 peaks at δ 108.42, δ126.13x2C, δ128.32, δ128.86x2C for the methylene carbon, and four peaks at δ134.04, δ150.19, δ157.27 and δ164.24 for quaternary carbon; ESI-MS (positive mode): m/z: 274.95$[M+Na]^+$Base Peak, 252.97$[M]^+$.

5.3. Synthesis of 2-imino-3-[4-phenylthiazol-2-yl] thiazolidin-4-one (Im9):

A mixture of 2-chloroacetamido-4-phenylthiazole (Im8, 0.03mole, 10g), potassium thiocynate (0.06mole, 6g) and dry acetone (100mL) was refluxed for 3h. Excess of acetone was removed in vacuo; yellowish residue was stirred with 50mL water. Yellowish product was filtered, washed with water and dried in oven below 50°C. The yellowish product was recrystallized from ethanol. Yellow solid, Yield 45%, mp 172°C was obtained.

Spectral Analysis:

The molecular formula was deduced to be $C_{12}H_9N_3OS_2$ by mass spectral analysis, indicating the annulations of the reactants to form the desired 2-imino-3-[4-phenylthiazol-2-yl] thiazolidin-4-one. The ^{1}H NMR spectrum ($CDCl_3$, 300MHz) exhibited characteristic peak at δ 4.20 (2H, s) for –CH_2, δ 8.25 (2H, d, *J*=7.5Hz), δ 7.54 (2H, t, *J*=7.2Hz), δ 7.40 (1H, t, *J*= 7.2Hz), for aromatic protons, δ7.67 (1H, s, thiazole). The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) showed 12 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established inverted –CH_2 peak at δ 30.88, 6 peaks at δ 110.63, δ126.98x2C, δ128.78, δ129.58x2C for the methylene carbon, and five peaks at δ135.20, δ152.66, δ165.00, δ171.00 and δ175.48 for quaternary carbon; ESI-MS (positive mode): m/z: 275.975$[M+1]^+$Base Peak, 202.00$[C_{10}H_6N_2OS]^+$.

6. Synthesis of 5-benzylidene-2-imino-3-[4-phenylthiazol-2-yl] thiazolidin-4-one (Im10):

2-imino-3-[4-phenylthiazol-2-yl] thiazolidin-4-one (Im9, 0.01 mole, 2.7535 g), was dissolved in 50mL of glacial acetic acid by warming, to the clear yellow color solution was added benzaldehyde (0.02 mole, 2.03 mL) and sodium acetate (0.02

mole, 1.64 g). The resultant yellow color solution was refluxed on water bath, after every hour few mL was added to crushed ice with vigorous trituration, the process was continued till the compound appears; total time required was 6h.

After 6hours of refluxing, the resultant dark yellowish color clear solution was poured in crushed ice and triturated vigorously, to give dark yellow solid, which was filtered, and washed with water and dried below 50°C in oven. The dark yellow product was recrystallized from methanol. Dark Yellow compound, Yield 40%, mp 210-212°C was obtained, which was subjected to spectral analysis.

Spectral Analysis:

The molecular formula was deduced to be $C_{19}H_{13}N_3OS_2$ by mass spectral analysis, indicating the annulation of the reactants to form the desired 5-benzylidene-2-imino-3-[4-phenylthiazol – 2 - yl] thiazolidin-4-one. The ^{1}H NMR spectrum ($CDCl_3$, 300MHz) exhibited characteristic peak at δ 6.75 (1H, d, *J*=7.8Hz) for ethylene proton, ten aromatic protons appear as multiplet from δ 7.04-7.29 (10H, m), δ 7.79 (1H, s, thiazole), broad peak at δ12.01 (1H, s, NH). The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) showed 19 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established 12 peaks at δ 110.63, δ126.44x2C, δ127.43x2C δ128.00, δ128.78x3C, δ130.07x2C and δ142.00 for the methylene carbon, and seven peaks at δ115.90, δ133.10, δ135.20, δ148.20, δ151.66, δ166.90, and δ174.48 for quaternary carbon; ESI-MS (positive mode): m/z: 364.11 $[M+1]^+$, 338.09 $[M-C_2H_2]^+$, 273.05$[M-90]^+$, 233.00 $[C_{10}H_7N_3S_2]^+$, 203.05 $[C_{10}H_7N_2OS]^+$, 202.07 $[C_{10}H_7N_2OS]^+$, 177.06$[C_9H_6N_2S]^+$, 130.04$[C_9H_60]^+$ Base Peak.

7. Synthesis of 4- (2-thioxo-4-oxo-thiazolidin-5-ylidenemethyl)-N - (4-phenyl-thiazol – 2 - yl) benzene sulfonamide (Im11) & 4-(2, 4-dioxo - thiazolidin-5-ylidenemethyl) - N - (4 - phenyl-thiazol-2-yl) benzene sulfonamide (Im12):

2-amino-4-phenyl thiazole (Im7, 0.01 mole, 1.76 g) and 4-chlorosulfonyl-5-benzylidene - 2, 4-thiazolidinedione (Im5, 0.01 mole, 3.03 g) were added to a mixture of 4mL of dry pyridine and 20mL of acetic anhydride. The mixture was refluxed for 6h, till completely clear yellowish solution was obtained, which was then poured into crushed ice with vigorous trituration. Solid obtained was filtered and purified from ethanol to give white crystalline compound, Yield 35-37%, mp 190-192°C. Similarly, 2-amino-4-phenyl thiazole (Im7, 0.01 mole, 1.76 g) and 4-chlorosulfonyl-5-benzylidene – 2 – thioxo – 4 - thiazolidineone (Im6, 0.01 mole, 3.19 g)were also reacted to give pale orange colored compound which on recrystallization from ethanol gives white Solid, Yield 30-32%, mp 200-205°C.

Spectral Analysis:

The molecular formula was deduced to be $C_{19}H_{13}N_3OS_2$ by mass spectral analysis, indicating the annulation of the reactants to form the desired 5-benzylidene-2-imino-3-[4-phenylthiazol- 2 - yl] thiazolidin-4-one. The ^{1}H NMR spectrum ($CDCl_3$, 300MHz) exhibited characteristic peak at δ 6.75 (1H, d, *J*=7.8Hz) for ethylene proton, ten aromatic protons appear as multiplet from δ 7.04-7.29 (10H, m), δ 7.79 (1H, s, thiazole), broad peak at δ12.01 (1H, s, NH). The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) showed 19 distinct peaks in agreement with the proposed structure. ^{13}C DEPT data established 12 peaks at δ 110.63, δ126.44x2C, δ127.43x2C δ128.00, δ128.78x3C, δ130.07x2C and δ142.00 for the methylene carbon, and seven peaks at δ115.90, δ133.10, δ135.20, δ148.20, δ151.66, δ166.90, and δ174.48 for quaternary carbon; ESI-MS (positive mode): m/z: 364.11 $[M+1]^+$, 338.09 $[M-C_2H_2]^+$, 273.05$[M-90]^+$, 233.00$[C_{10}H_7N_3S_2]^+$, 203.05 $[C_{10}H_7N_2OS]^+$, 202.07 $[C_{10}H_7N_2OS]^+$, 177.06 $[C_9H_6N_2S]^+$, 130.04$[C_9H_60]^+$ Base Peak.

DISCUSSION: The present study recorded certain synthetic compounds of thiazolidine group to be effective against the human lymphatic parasite in *in vitro* condition of cell free system. The popular drug, DEC although widely used as the standard antifilarial drug could not be used as a positive control for *in vitro* studies as opposed to the test compounds. The results suggest direct action of these agents on the parasites unlike DEC. Moreover the observed effect was not only limited to the microfilarial stage, rather a convincing effect on adult worms was also found with at least one of the compounds, namely Im10. Such compounds containing heterocyclic ring systems possessing sulfur, oxygen and nitrogen are of great importance

and receiving special attention as they belong to a class of compounds with proven utility in medicinal chemistry [15]. In the past decade, the number of patents describing plethora of the different biological activities of thiazolidinone-based compounds has been on the rise.

Taking hint from the literature survey, a research program on synthesis, spectral characterization and biological evaluation of novel heterocycles, was undertaken in the laboratory. Out of 12 such compounds screened, only four compounds (Im6, Im8, Im10 and Im12) showed promising pharmacological efficacy as displayed by their impact on loss of motility of the parasites *in vitro*. Whereas the first compound in the series are the parent thiazolidine, the majority of others are having a rhodanine moiety. Usually such biologically active derivatives contain the exocyclic double bond, which is conjugated to the carbonyl group at position four of the rhodanine moiety, such compounds are electrophilic and potentially reactive due to possible Michael addition of the nucleophilic residues to the exocyclic double bond [16]. Overall, such Michael adducts are known to be chemically as well as physiologically significant as evidenced from activated xenobiotic conjugation of GSH [17].

In this study remarkably most of the compounds showing higher activity are derivative of thiazolidine with such Michael derivatization. That might explain their higher therapeutic potential as opposed to the others in this series. The deduced IC_{50} values displayed an impressive dose range of 2.5-15 µM for the four effective compounds. The corresponding LD_{50} values were quite higher as compared to the respective IC_{50} values indicating their level of safety. However, Im6 and Im8 showed great potential in terms of the wide therapeutic window (the relative difference between the corresponding IC_{50} and LD_{50} value), whereas that of Im12 did not show such promising result. It might be tempting to speculate that the later compound having a bulkiest shape with various additions may have some nonspecific action leading to the observed low therapeutic index.

The thiazolidine-dione and its derivatives are popular in anti-diabetic therapeutics [18], however relatively recent evidences reported its antimicrobial potential, mainly explored against bacteria and fungus [19].

To the best of our knowledge, this study provides experimental result displaying pharmacological effect against metazoan parasite for the first time. Possible role of oxidative stress has been implicated as the major proposed mechanism of the antimicrobial effect by these agents [20]. Interestingly, as mentioned before the most popular postulate about the rationale of DEC involves the promotion of host inflammatory response [21]. Such impact is mainly associated with oxidative mechanism [22].

However, DEC is unable to generate oxidative onslaught in cell free system unlike thiazolidine derivatives. Although not carried out in this study, it could be worthwhile to assess the level of reactive oxygen species and its possible impact. One major issue which is of concern in this rationale based on oxidative effect in context to this particular parasitic model is that reportedly, the parasite is endowed with several antioxidant armament [23]. Therefore, even if level of oxidants gets increased, presumably the oxidative damage to macromolecules seems not to play very significant role. However, more subtle nexus between oxidative mechanism and the apoptotic effect might not be ruled out. Particularly, DEC has been shown to induce weak apoptotic effect in filarial parasite under *in vitro* condition which was insufficient to kill the parasite [24].

Our experimental work established that such effect can be augmented by using H_2O_2, known oxidant and apoptotic agent to produce significant synergistic response but failed to show reversal of oxidative onslaught by conventional anti-oxidants [25]. This suggests that in this model beyond oxidative effect, more plausible associated apoptotic mechanism might have significance in developing anti-parasitic therapeutic modality. We have shown apoptosis as a significant anti-parasitic rationale with synthetic DHFR inhibitors earlier [8].

Thiazolidineone and its derivatives are shown to not only be potent as antiviral agents [26] but also broadly evaluated for anticancer activity [10]. Strikingly, cytotoxic effect in the form of apoptosis has been evidenced for thiazolidine derivatives both by ROS dependent as well as independent manner; the latter apoptotic effect without involvement of oxidative mechanism has been thought to be due to PPAR-γ receptor agonist effect [20]. Although argued against in prokaryotic system due to reported absence of such receptors in bacteria, but in eukaryotic system including this parasite the presence of this receptor has been confirmed [27].

CONCLUSION: Therefore such effect seems plausible in this observed anti-parasitic action. Pending mechanistic study towards this end which is underway for these agents in our lab, our results validates this compounds as safe and potential macrofilaricidal as well as macrofilaricidal lead.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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