



## Synthesis and Leishmanicidal Evaluation of 5-Arylidine-Immuno-Thiazolidine 4 one derivative: An *In-Vitro* study

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### ABSTRACT

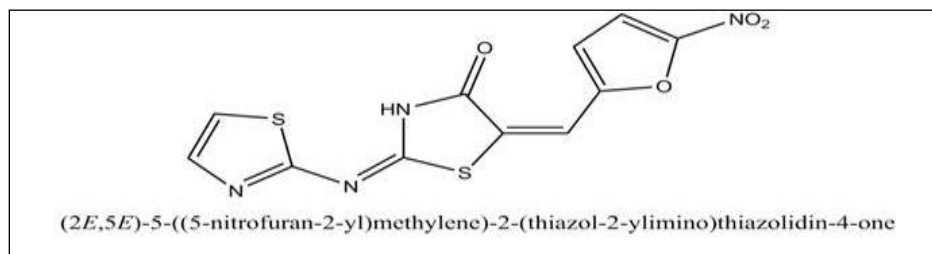
*Introduction:* Cutaneous Leishmaniasis (CL) is an endemic disease in developing countries. Glucantime has been recommended for CL treatment by W.H.O, there are some Restrictions in this case including high expense, side effects, frequent injections need, and incomplete efficacy. Cutaneous Leishmaniasis is an endemic disease in developing countries. Glucantime has been recommended for CL treatment by W.H.O, there are some Restrictions in this case including high expense, side effects, frequent injections need, and incomplete efficacy. The aim of the study was the efficacy of Synthesis and Leishmanicidal Evaluation of Aryl Immuno-5- Arylidine Thiazolidine 4- 1 derivative: An in vitro study. *Materials and Methods:* The design of the present probe was experimental (laboratory-trial); therefore, Iranian endemic species including Leishmania (L) tropica strain [MHOM/IR/NADIM3] was proliferated, and maintained in the standard culture. Then, the proper densities of Aryl Immuno-5- Arylidine Thiazolidine 4- 1 derivatives were provided and then sterilized and added to cultures containing parasite. Parasite numbers were counted in mentioned in cell proliferation ELISABrdu (Chemiluminescent). *Results:* Percentage of viability PMs Leishmania (L) tropica [MHOM/IR/NADIM3] in Concentrations of 1375, 2750, 5500 and 1100 mg/ml of 5-Arylidine-Immuno- Thiazolidine 4 one derivative and control showed statistically there was a significant difference, NOVA test, (P=0.000). And Percentage of viability PMs Leishmania (L) tropica [MHOM/IR/NADIM3] in Concentrations of 125, 250, 500 and 1000 mg/ml of Glucantime and control showed statistically there was a significant difference, ANOVA test, (P=0.000). *Conclusion:* According to the results, 5-Arylidine-Immuno-Thiazolidine 4 one derivative is effect and may make it possible to use them in the treatment of Cutaneous Leishmaniasis as a or alternative therapy; however, further studies are necessary and should be evaluated in cell culture and in vivo conditions to confirm it.

**Keywords:** Leishmania tropica; Thiazolidine; 5-Arylidine-Immuno- Thiazolidine 4 one; *In-vitro*

### INTRODUCTION

The term Leishmaniasis collectively refers to various clinical syndromes caused by obligate intracellular protozoa of the genus Leishmania. It is one of the major infectious diseases affecting the continuing countries populations throughout the world and there are two million annual new reports in 88 countries. Cutaneous Leishmaniasis (CL) is the most common form of Leishmaniasis. It causes ulcers on exposed parts of the body, leaving life-long scars and

serious disability. About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East and Central Asia. Over two-third of CL new cases occur in six countries: Afghanistan, Algeria, Brazil, Colombia, Iran and Syria. An estimated 0.7 million to 1.3 million new cases occur worldwide annually. For more than 60 years, pentavalent antimonials (Sbv) were the major therapeutic agents for the treatment of the disease. However, in the early 1980s, ineffectiveness of these agents was reported, but unfortunately, there is still no development in the production of newer drug. Although studies regarding the production of an effective vaccine to control Leishmaniasis have been extensively conducted over the past decades, there is still no vaccine against any form of Leishmaniasis for general human use. Since vaccines against this parasite are not yet in sight, its control relies mostly on chemotherapy, so, there is an urgent need to develop new and better drugs to combat this infectious disease [1-3]. Thiazole derivatives are known to possess anti-inflammatory, analgesic and antipyretic activities. Thiazolidine and 2-(p-chlorophenyl) thiazole-4-acetic acid are widely used as anti-inflammatory drugs. Scheme 1. Reagents and conditions: 5-Arylidine-Immuno- Thiazolidine 4 one derivative.



**Scheme 1: Reagents and conditions: 5-Arylidine-Immuno- Thiazolidine 4 one (5-Arylidine-Immuno- Thiazolidine 4 one derivative)**

Meloxicam, for example, is a new NSAID with a thiazolyl group in its structure. Furthermore, Niridazole<sup>13</sup> and some other thiazole derivatives<sup>14–20</sup> have been found to exhibit antimicrobial /antihelminthic activities [4-9]. Inflammation represents a fundamental host response to a wide range of stimuli such as trauma, tissue injury, microbial activity, burns, surgery, sepsis, toxic entities, ischemia–reperfusion, and post ischemic or autoimmune injury. The role of microorganisms in inflammation is well recognized, therapeutic strategies with effects on both the microorganism and associated inflammation have received considerably less attention. Identification of novel compounds which treat both infectious and inflammatory states more effectively, and which lack side effects associated with current therapies, remains a major challenge in biomedical research (10, 11). The present study was carried out to Synthesis and Evaluation of Aryl Immuno-5- Arylidine Thiazolidine 4- 1 Derivatives: An in vitro study.

## MATERIALS AND METHODS

### Preparing of 5-Arylidine-Immuno-Thiazolidine 4 one derivative

The synthesis of new 4-thiazolidinone derivatives incorporating two known bioactive heterocyclic nuclei such as thiazolyl in Aryl Immuno-5- Arylidine Thiazolidine 4- 1 and attempt to present also anti-inflammatory activities structural variations were selected by introducing, at the Aryl Immuno-5- Arylidine Thiazolidine 4- 1 position of thiazolidinone moiety, different Arylidine substituent's that we recently exploited as bioactive, on heterocyclic scaffolds, useful to encompass certain physico-chemical properties as and steric.

### Source of parasites

*Leishmania (L) tropica* strain [MHOM/IR/NADIM3] promastigotes (PM) were obtained from the medical Parasitology department/school of medicine/Shahid Sadoughi University of medical sciences. *Leishmania tropica* strain (MHOM/IR/NADIM3) was maintained in BALB/c mice. Amastigote were isolated from mice spleens, and then transformed to promastigotes in Novy-Nicolle-Mac Neal (NNN). The Third passage PM from NNN medium were progressively adapted to RPMI 1640 media (gibco) with antibiotics, glutamine and FCS supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) and 20% heat-inactivated fetal calf serum (FCS) at 25°C [12-14].

### Cell proliferation ELISA, Brdu (Chemiluminescent) method

The cell proliferation of enzyme-linked immunosorbent assay (ELISA), Brdu (Chemiluminescent) was performed as described by Roche Diagnostics GmbH Roche Applied Science 68298 Mannheim Germany (Version march 2016,

Cat. No. 1027640) the cell proliferation ELISABrd (Chemiluminescent) method is a Quantitative determination of DNA synthesis in cell cultures is now a routine procedure in many laboratories. Protocols are available for various applications, especially in cell culture systems. The effects of growth factors, inhibition of cell division by exogenous factors, stimulation/inhibition by cytokines, development of serum-free media, influence of hormones and receptor activity on proliferation, and selection advantages for aneuploid cells in vitro are just a few examples where the determination of DNA synthesis provides important information. That in brief is:

- A fixed initial density of the parasites was transferred to screw-capped vials containing 5 ml of liquid medium to which different concentrations of 1375, 2750, 5500 and 11,000  $\mu\text{g}$  of 5-Arylidine-Immuno-Thiazolidine 4 one (1 derivative) Derivative were added. Each concentration was done and each run included control
- It was stimulated with acetone in the period
- Dioxy bromoouridin was added and it was incubated at 37°C for 8 hours
- Supernatant was removed. Fixator was added to the permeable membrane
- Anti-oxibromoouridin conjugated with POD was Added and incubated for 3 hours
- Chromogen was added and incubated. And finally, it was terminated and read at 450 nm.

### Statistical analysis

The results were expressed as mean  $\pm$  SEM. Comparisons among the experimental groups were done by one-way ANOVA test using graph pad prism5 software program. The upper level of significance was chosen as  $P < 0.05$ .

## RESULTS

Lethal Concentration<sub>50</sub> (LC<sub>50</sub>) of 5-Arylidine-Immuno- Thiazolidine 4 one derivative and Glucantime against logarithmic and stationary phase's promastigotes calculated. Furthermore, the sensitivities of *Leishmania (L) tropica* strain [MHOM/IR/NADIM3] were tested using a simple slide method and compared to results of the standard method the in vitro sensitivities of promastigotes *Leishmania (L) tropica* strain [MHOM/IR/NADIM3] (Table1). There was statistically significant difference between LC<sub>50</sub> of 5-Arylidine-Immuno- Thiazolidine 4 one and LC<sub>50</sub> of Glucantime groups on PMs of Logarithmic and Stationary Phases ( $P=0.000$ ).

**Table1: LC<sub>50</sub> of 5-Arylidine-immuno- Thiazolidine 4 one and Glucantime, against logarithmic and stationary phases PMs of *Leishmania (L) tropica* [MHOM/IR/NADIM3]**

Organism	C Thiazolidine Derivative (mg/ml)	MA (mg/ml)
Logarithmic Phase PM	34.2	334.6
Stationary Phase PM	37.4	339

Growth average of Viability PM of *Leishmania (L) tropica* [MHOM/IR/NADIM3] in culture

According to 5-Arylidine-Immuno- Thiazolidine 4 one derivative gradients in comparing with control by the Cell proliferation ELISA, Brdu (Chemiluminescent) method:

Effect of 5-Arylidine-Immuno- Thiazolidine 4 one derivative against *Leishmania (L) tropica* [MHOM/IR/NADIM3] of stationary phase PMs shows the results of different concentrations of 5-Arylidine-Immuno- Thiazolidine 4 one derivative on stationary phase PMs of *Leishmania (L) tropica* [MHOM/IR/NADIM3], in geometrically increasing concentrations, dose dependently inhibited the growth of *Leishmania (L) tropica* [MHOM/IR/NADIM3] PMs. Growth average of *L. tropica* PMs in culture media contained different densities of 1375, 2750, 5500, 11000 $\mu\text{g}/\text{ml}$ , derived from 5-Arylidene 2- Aryl-Immuno-Thiazolidin-4-one (1) compared to the control group: Average growth of *L. tropica* PMs in culture media contained different densities of 1375, 2750, 5500, 11000  $\mu\text{g}/\text{ml}$ . Arylidine2. Aryl-Immuno thiazolidine-4-one compared to the control group showed that by increasing densities of 1375, 2750, 5500 and 11,000  $\mu\text{g}/\text{ml}$ , parasite growth will decrease; ANOVA test was run and statistically there was a significant difference ( $p=0.00$ ). The comparison of luminometer mean in the case of parasite in the culture among different densities was conducted by ANOVA tests, it showed  $p=0.000$  *Leishmania (L) tropica* strain [MHOM/IR/NADIM3] in which it was a statistically significance among different densities and controlling group (Figure 1).

Growth average of Viability PM of *Leishmania (L) tropica* [MHOM/IR/NADIM3] in culture

According to Glucantime gradients in comparing with control by the Cell proliferation ELISA, Nrd (Chemiluminescent) method:

Growth average of *L. tropica* PMs in culture media contained different densities of 125, 250, 500, 1000 µg/ml out of Glucantime compared to the control group: Average growth of *L. tropica* PMs in culture contained different densities of 125, 250, 500, 1000 µg/ml out of Glucantime compared to the control group showed that by increasing densities of 1375, 2750, 5500 and 11,000 µg/ml, parasite growth will decrease. The comparison of luminometer mean in the case of parasite in the culture among different densities was conducted by ANOVA tests, it showed  $p=0.000$  *Leishmania (L) tropica* strain [MHOM/IR/NADIM3] in which it was a statistically significance among different densities and controlling group (Figure 2).

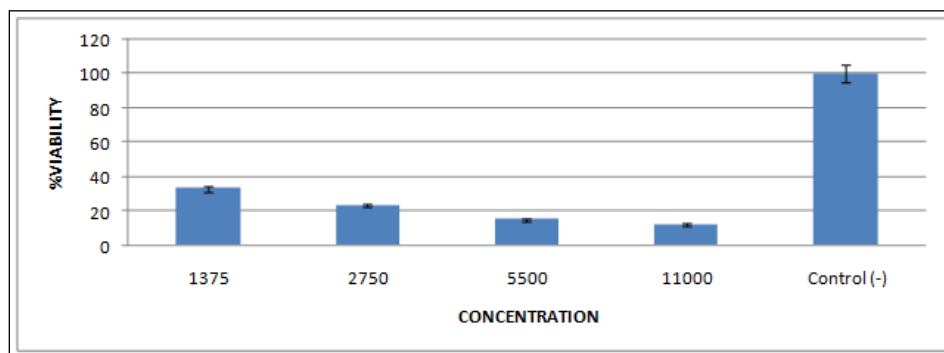


Figure1: Percent of viability PM *Leishmania (L) tropica* [MHOM/IR/NADIM3] in Concentrations of 1375, 2750, 5500 and 1100 mg/ml of 5-Arylidene-Immuno-Thiazolidine 4 one derivative and control, NOVA test, (P=0.000)

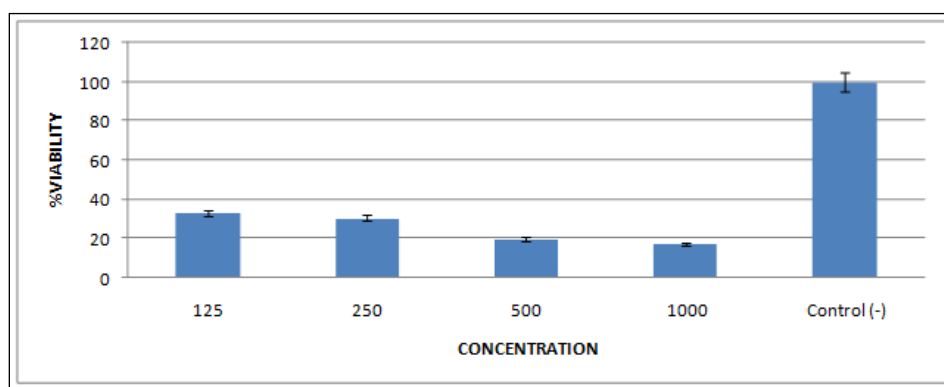


Figure2. Percent of viability PM *Leishmania (L) tropica* [MHOM/IR/NADIM3] in Concentrations of 125, 250, 500 and 1000 mg/ml of Glucantime and control, ANOVA test, (P=0.000)

Comparison between average of growth and life of *L. tropica* promastigotes in culture media containing derivatives of 5-Arylidene 2- Aryl-Immuno thiazolidine-4-one (1) with Control indicated that growth inhibition process is faster in 5. Arylidene 2. Aryl-Immuno Thiazolidine-4-ono compared to Control group.

## DISCUSSION

Standard treatment in the recent past of Leishmaniasis involved the use of pentavalent antimonials. It containing compounds that are the main drugs used to treat Leishmaniasis include: Sodium stibogluconate and Meglumine antimonite. Importance of antimony in the early medicine is well documented, due to the debate created around their utilization in this period. Despite the recent developments, the effective therapy for CL has been yet based on long parenteral courses of these drugs for six decades, even though these are fairly costly, toxic and inconvenient to use, along with inadequate knowledge on their Pharmacokinetics or mechanism of action [15-17].

During the past 5 decays significant efforts in the diagnosis and treatment of microbial diseases have been accomplished and identification of novel compounds which treat both inflammatory and infectious states more effectively, and which lack side effects associated with current therapies, remains a major challenge in biomedical research. New compounds of 5 Arylidene 2. Aryl-Immuno Thiazolidine- 4-one called (2E,5E)-5-((5-nitrofur-2-

yl)methylene)-2-(thiazol-2-ylimino)thiazolidin-4-one) which is derived from Thiazolidine group; previous studies showed that Thiazolidine an important group of compounds with significant pharmacological activity where nucleus has biological, anti-inflammatory, antibacterial, anti-tuberculosis, anti-fungal and anti-parasitic activities. As the previous studies proved antimicrobial effect of these compounds namely 5-Arylidine-2-Aryl-Immuno Thiazolidine-4-one, then we investigated Leishmania effects of these derivatives.

Thiazole derivatives are known to possess antipyretic activities, analgesic and anti-inflammatory [18,19]. Thiazolidine and 2-(pchlorophenyl) thiazole-4-acetic acid are widely used as anti-inflammatory drugs. Meloxicam, for example, is a new NSAID with a thiazolyl group in its structure [20,21]. Furthermore, Niridazole and some other thiazole derivatives [22,23] have been found to exhibit antimicrobial antifungal/antihelminthic activities [24,25]. Recent studies showed that 5-Arylidine-Immuno- Thiazolidine 4 one derivative has potent cytotoxic [26]. Until now, many studies have been carried out regarding the effect of herbal compounds on parasitic infections. Among the most important biological and pharmacological activities of Thiazolidine one derivative is: antibacterial [27,28], antifungal [29], anti-inflammatory [30], antioxidant and several other activities. Given that Cutaneous Leishmaniasis is limited to the skin typically, so the use of an effective topical medicine in treating this disease is very important.

### CONCLUSION

In the present study has suggested in geometrically increasing concentrations, dose dependently inhibited the growth of *Leishmania (L) tropica* strain [MHOM/IR/NADIM3] PMS. Studies showed that a number of herbs have moderate to strong anti-Leishmania activity. Considering the made compound safeness in comparison with Glucantime, its use possibility in the treatment of species, (*L) tropica* [MHOM/IR/NADIM3], is not far to reach. Although 5-Arylidine-Immuno- 4 one derivative in vitro was affected, but probes are mandatory in the cases of animal and sick persons, figure out its daily dosage, concentration, time and duration.

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**Conflicts of interest:** The authors have no conflict of interest.

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