Synthesis and study effects of 3,4-Dihydro-4-(p-anisyl)-6-phenyl pyrimidine-2(1H)-one on growth and morphology of *Leishmania tropica* promastigotes *in vitro*

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Abstract:
Most of the drugs used in the treatment of leishmaniasis have side effects, there is a need for a safe and effective drugs, therefore, in the present study heterocyclic compound containing pyrimidine ring (3,4-Dihydro-4-(p-anisyl)-6-phenyl pyramidine-2-(1H)-one) was synthesized, then tested for there antileishmanial activity against *Leishmania tropica* promastigotes *in vitro*. The effect of the compound on the growth, generation number and generation time of the parasite was determined using concentrations ranging from 1 to 10 µg/ml.

The effect of the compound concentration was found to be important to the parasite growth as the more the concentration the less the growth, generation number and increased the generation time. At the log phase the IC_{50} and IC_{90} were 2.5 and 10 µg/ml respectively and caused morphological distortions.

The toxicity of the synthesized compound was determined using Balb/c mice model. Dixon's up and down method (1980) was found to have an LD_{50} of 642.5 mg / Kg of body weight.

Key word: Phenyl pyrimidine , *Leishmania* , Growth.

Introduction:

Leishmaniasis is a vector – born disease that is transmitted by sand flies and caused by obligate intracellular protozoa of the genus *Leishmania*. Leishmaniasis is prevalent in 88 countries from Tropical to Mediterranean regions, where 12 million people are infected and approximately 350 million people are at risk, 1 – 2 million new cases registered annually [1]. At least there are four species of genus *Leishmania*, similar in morphology, but different in many aspects: cultural, characteristics, clinical manifestation, geographic distribution and sand fly vectors, can cause the disease in humans. *Leishmania donovani*, the etiologic agent of visceral leishmaniasis, or Kala-azar ; *Leishmania tropica,* of old world cutaneous leishmaniasis, or oriental sore ; the *Leishmania braziliensis,* complex of cutaneous and mucocutaneous disease in America and the *Leishmania maxicana,* complex are associated mainly with cutaneous lesions. Within each major group are strains and possibly subspecies with their own distinctive characteristics [2].

Cutaneous leishmaniasis is prevalent in some of the Middle - Eastern, Tropical African and Asian countries - including Iraq [3].

The drugs available for the treatment of leishmaniasis are in general, toxic, expensive and require long – term treatment. Large scale clinical resistance against the most commonly used antimonial agent, have been reported [4]. The usual form of treatment of leishmaniasis is still intravenously using highly poisonous antimony compounds, e.g., in pentostam 15% of the patients die from these injections, i.e. the therapy causes death in the advanced stage of the disease. In addition, patients are increasingly not reacting to the conventional poisonous antimonials. According to rough estimates, about 40% of the infected persons in India are already resistant to this therapy. Similar observations in Sudan supports this report [5]. In the present work, particular pyrimidinonic compounds were synthesized and tested for their antiparasitic activity against *Leishmania tropica* promastigotes *in vitro*.

Since pyrimidine bases are major constituents of nucleic acids, the chemistry of pyrimidines have been the subjects of many researches owing to their applications in molecular biology and medicine. They show many activities including antibacterial [6], antiviral activities [7], and antifungal effects [8]. Some pyrimidinone derivatives have anticancer activities [9], antidiabetic effects [10], immuonomodulator [11].

Thus, the present study drives at the synthesis of heterocyclic compound that contain pyrimidine ring and have a considerable inhibitory effect on the growth of *Leishmania tropica* promastigotes, and low toxicity *in vivo*.

Material and Methods:
1. Synthesis of 3,4-Dihydro-4-(p-anisyl) -6-phenyl pyrimidine-2(1H)-one
A. Preparation of 1-phenyl-3-(p-methoxy phenyl)-2-propen l-one:

1-phenyl-3-(p-methoxy phenyl)-2-propen l-one were prepared according to [12]. A mixture of 0.05 mol of sodium hydroxide pellets, 20 ml of water and 0.2 mol of ethanol was magnetically stirred in a 100 ml round bottomed flask which immersed in an ice – bath. A 0.043 mol of freshly distilled acetophenone was poured on stirred mixture followed by 0.043 mol freshly distilled anis aldehyde with vigorous stirring, the temperature was kept at 20 – 25 C^o for (2 – 3) hrs. until the mixture became thick. The thick mixture was kept in a refrigerator over night. The product filtered then, vacuum and washed with water until the neutralization of filtrates then washed with 20 ml ice – cold ethanol (See paragraph A of results).

B. Preparation of 3,4-Dihydro-4-(p-anisyl) -6-phenyl pyrimidine-2(1H)-one:
This was prepared applied according to [13]. In a 100 ml round bottomed flask, a mixture of 5 mmol of 1-phenyl-3-(p-methoxy phenyl) -2-propen l-one and 50 mmol of sodium hydroxide dissolved in 25 ml absolute ethanol

was stirred magnetically for 10 minutes. 5 mmol of urea which dissolved in 25 mmol absolute ethanol was added to the stirred mixture then cooled at room temperature and the solvent was separated to give the solid product of 3,4-Dihydro-4-(p-anisyl)
-6-phenyl pyrimidine-2(1H)-one (See paragraph B of results).

2. Biological activity of 3,4-Dihydro-4-(p-anisyl)-6-phenyl pyrimidine-2(1H)-one

A. *Leishmania* used:

MHOM / IQ / 1992 / MREC3 *Leishmania tropica* stock culture was used. The culture was obtained from the College of Medicine AL-Nahreen University, which has been characterized using isoenzyme method according to [14]. The obtained culture was cultivated in Tobie`s medium [15].

B. Cultivation and estimation of numbers of parasites:

1.9 ml of liquid phase was added to McCantry vials that contained 5 ml solid phase slants, then 0.1 ml of *Leishmania* promastigotes inoculums was taken from stock culture during logarithmic phase, so that the initial density of the organism was 2×10^5 / ml, then the number of organisms of new culture incubated at 26 C^o for 4 days, was counted directly using a haemocytometer.

C. Effect of the synthesized pyrimidinone on growth, generation number and time of *Leishmania tropica* promastigotes:

Effect of the compound on growth was studied *in vitro* in comparison with untreated groups. The compound was dissolved in 2% dimethyl sulfoxide (DMSO). Five concentrations (1 , 2.5 , 5 , 7.5 , 10) μg / ml were used to determined the (IC_{50}) and the (IC_{90}) of the cultivated organisms. Numbers of promastigotes were determined at different time intervals (24 , 48 , 72 , 96) hours, then generation number and time of each culture at each time intervals were estimated using the following laws [16]:

$$n = \frac{\text{Log N} - \text{Log N}_0}{\text{Log 2}}$$

whereas, n = generation number , N = number of promastigotes at time (t) N0 = initial number at zero time

$$g = \frac{t}{n}$$

whereas, g = generation time (hours), t = exposure time (hours), n = generation number

D. Effect of the synthesized pyrimidinone on morphology and dimensions of the parasites:

Fixed film of *Leishmania tropica* promastigotes treated with IC_{50} and IC_{90} of the pyrimidinone prepared and stained with Giemsa stain, examined using Olympus compound light microscope, then the dimensions (length and width) of 15 organism for each treatment were measured using micrometer lens and oil immersion (100 x).

Photos of the treated promastigotes in addition to control group were taken using compound light microscope(Olympus) with UBS PC camera 301$^+$.

3. Determination of the 50% of lethal dose (LD50) of the synthesized pyrimidinone *in vivo*:

50% of the lethal dose of the pyrimidinone in Balb/c mice were determined using up-and-down method [17]. Male mice aged 4 – 6 weeks were injected intraperitonially with different doses of the pyrimidinone. Primary dose (0.5 mg / kg body weight) were chosen after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response. Testing continued until chosen "nominal" sample size was reached. LD_{50} were determined after reading final result (response-dead (X) or non response-alive(O)), then the following equation was applied

$$LD_{50} = XF + Kd$$

The estimate of LD_{50} is XF + Kd, where (XF) is the final test level and (K) is the interval between dose levels. (d) is the tabulated value. (Table 1):

Table(1): Dixon table for estimating medium lethal dose

2nd part of series	K for test series whose first part is:					Standard error of LD_{50}
	O	OO	OOO	OOOO		
XOOO	-0.157	-0.154	-0.154	-0.154	OXXX	0.61
XOOX	-0.878	-0.861	-0.860	-0.860	OXXO	
XOXO	0.701	0.373	0.741	0.741	OXOX	
XOXX	0.084	0.169	0.181	0.182	OXOO	
XXOO	0.305	0.372	0.380	0.381	OOXX	
XXOX	-0.305	-0.169	-0.169	-0.142	OOXO	
XXXO	1.288	1.500	1.544	1.549	OOOX	
XXXX	0.555	0.897	0.985	1.000	OOOO	
	X	XX	XXX	XXXX		2nd part of series
-K for test series whose first part is:						

$$LD_{50} = XF + Kd$$

Results and discussion:

Chalcone is an α , β- unsaturated ketonic carbonyl compound (1-phenyl-3-(p-methoxy phenyl)-2-propen l-one) that have wide applications in organic chemistry. In the present research chalcone was prepared then used as the chief starting material to synthesis the pyrimidinonic compound(3,4-Dihydro-4-(p-anisyl)-6-phenyl

pyrimidine-2(1H)-one), which was tested for it's antiparasitic activity against *Leishmania tropica* in albino mice *in vivo*.

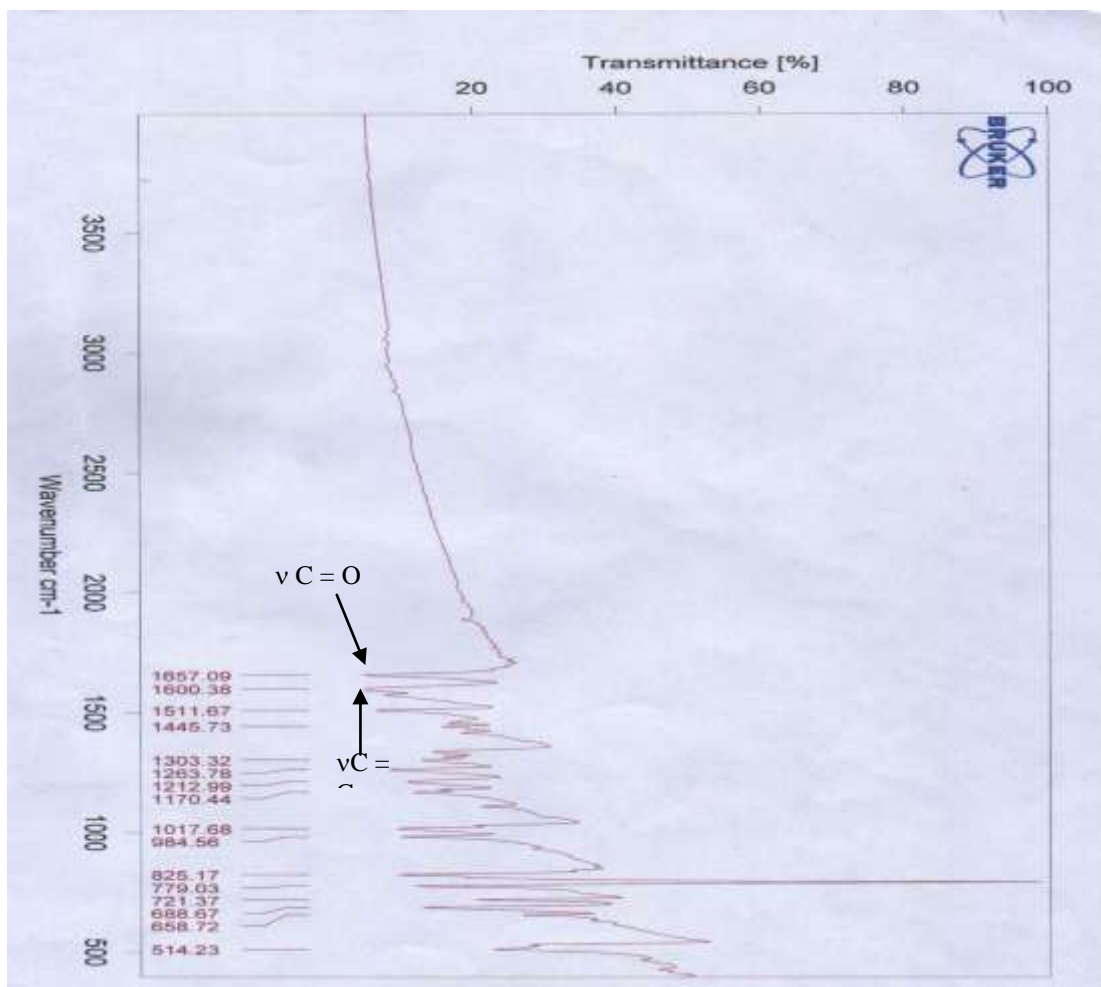**A. 1-phenyl-3-(p-methoxy phenyl)-2-propen l-one:**
Chalcone was prepared by the condensation of the aromatic aldehyde Anis aldehyde ; with the aromatic keton ; Acetophenon ; in the presence of sodium hydroxide using Claisen – Schmidt condensation depending on method (12) (Equation. 1).

The resultant chalcone was using spectroscopic method as well as melting point. It gave yellow organic powder

with melting point $(75 – 77)C^o$. The infrared spectrum (Fig. 1) shows a sharp band at (1657.09) cm^{-1} which is related to the carbonyl (C = O) stretching vibration, whereas the carbon – carbon double bond (C = C) was at (1600.38) cm^{-1}, both variations were within the range of similar groups [18]. Ultraviolet spectrum according to method [19] gave many wave lengths at different absorbance, but the wave length at maximum absorption (λ max) was seen at 348 nm. (it may be worthy when compared with cyclic product later).
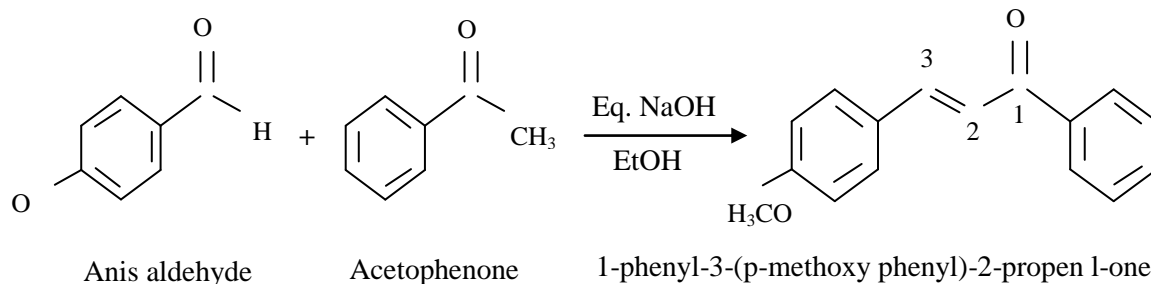
Equation(1): Synthesis of the chalcone via Claisen – Schmidt condensation

Figure(1): IR. spectrum of the synthesized chalcone 1-phenyl-3-(p-methoxy phenyl)-2-propen l-one
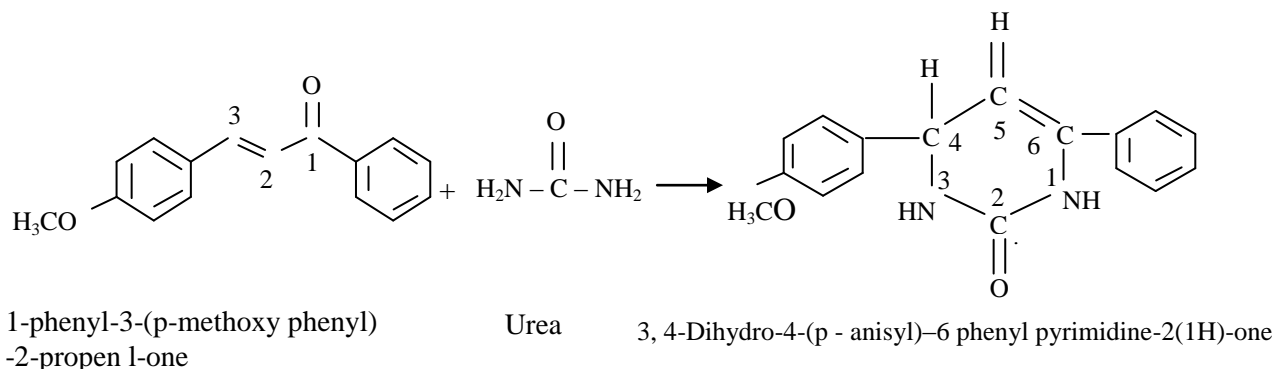
B. 3,4-Dihydro-4-(p-anisyl)-6-phenyl pyrimidine-2(1H)-one:
Michael - Claisen condensation or Claisen - Michael route of chalcone with urea under strong basic conditions

yielded the corresponding heterocyclic 3,4-Dihydro-4-(p-anisyl)-6-phenyl pyrimidine-2(1H)-one as yellow crystals, with melting point (60 – 64) C^o (Equation. 2).

The structure of the final product was established by the analysis of it's spectral data according to method [20], and to [21], and the UV spectrum of the end product showed a maximum absorption at wavelength of (210) nm. Upon comparison with the value of λ max of the product, it is concluded that the disappearance of conjugation of the carbonyl group with C = C bond and the aromatic ring caused in decrement λ max value of the product. This result is in agreement with [22].

The infrared spectrum (Fig. 2) manifested broad band at (3441.75) cm^{-1}, which belongs to the stretching vibration of nitrogen - hydrogen bond (ν N – H), whereas the vibration at (1682.52) cm^{-1} related to the carbonyl stretching (ν C = O). Finally carbon - carbon double bond (ν C = C) reflects a stretching vibration at (1598.53) cm^{-1} which is in a good agreement with reported values by [20].

1-phenyl-3-(p-methoxy phenyl) -2-propen l-one   Urea   3, 4-Dihydro-4-(p - anisyl)–6 phenyl pyrimidine-2(1H)-one

**Equation(2):** Synthesis of heterocyclic via Michael - Claisen condensation.

C. Effect of the synthesized pyrimidinone on the growth, generation number and generation time of *Leishmania tropica* promastigotes *in vitro*:

Table. 2 show the inhibitory effect of different concentrations of the synthesized pyrimidinone on *Leishmania tropica* growth in comparison with control group during different time intervals. 2.5 µg / ml seemed to be the inhibitory concentration of 50% of the promastigotes (IC_{50}) at the log phase (96) hrs., 10 µg / ml is the inhibitory concentration of 90% of the promastigotes (IC_{90}). Relatively all concentrations used demonstrated high inhibitory effects against *Leishmania* promastigotes.

On the light of the growth indices (Table 2), effect of the pyrimidinone on generation number and generation

time of *Leishmania tropica* promastigotes were estimated (see paragraph C of materials and methods). As for the effect of different concentrations of the synthesized pyrimidinone on generation number (Table 3), inverse correlation between generation number and concentration were observed, generation number at log phase ranged from 6.07 generations at 1.0 µg / ml to 1.76 generations at 10 µg / ml, when compared with control group (6.62 generations).

However, generation time appeared to depend upon the concentration of the pyrimidinone (Table 4). Generation time increased when concentration increased (direct correlation). At log phase, generation time values ranged from 15.8 hrs. at 1.0 µg / ml to 54.7 hrs. at 10 µg / ml when compared with control group (14.5) hrs.
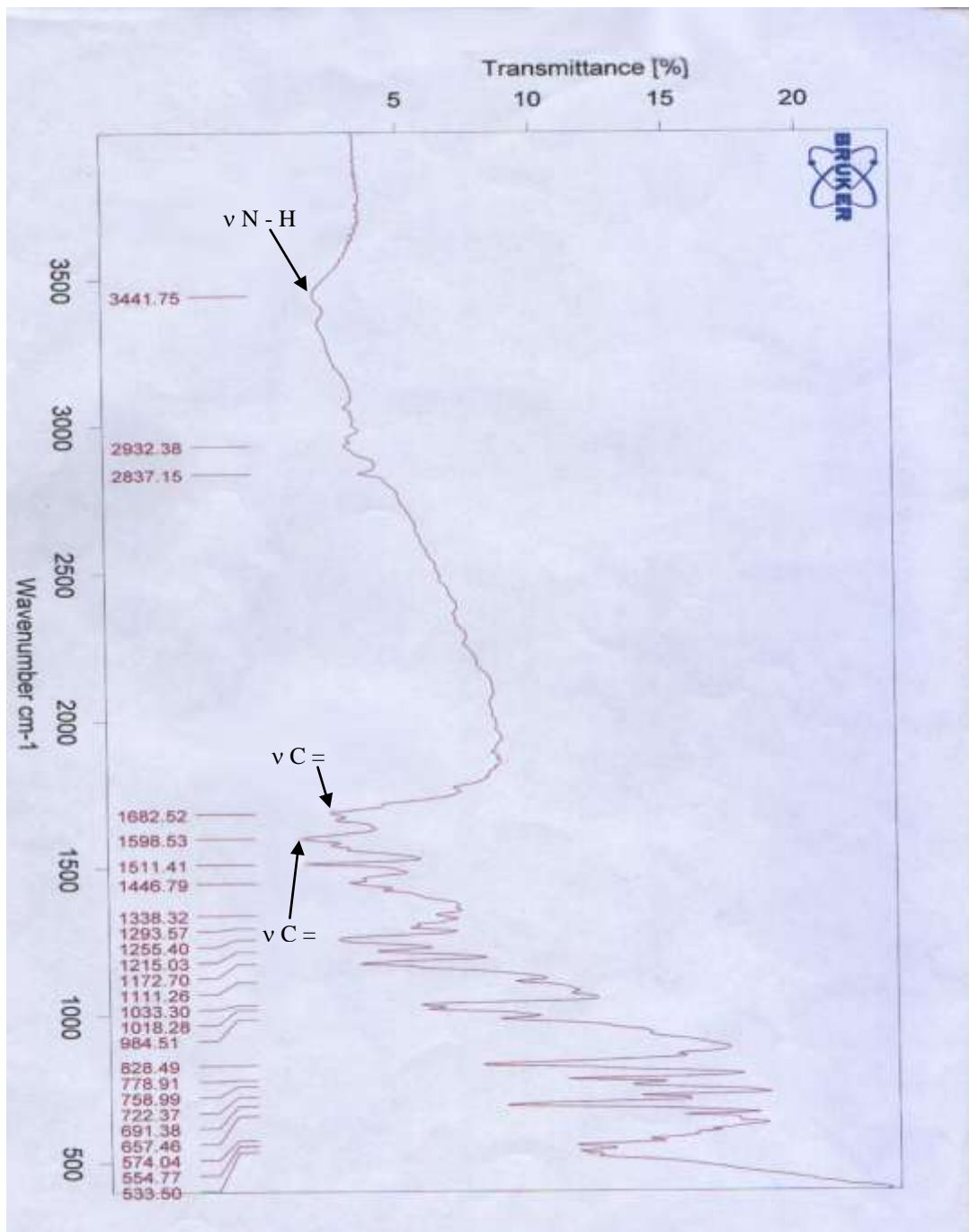
Figure(2): IR. spectrum of the synthesized 3,4-Dihydro-4-(p-anisyl)-6-phenyl pyrimidine-2(1H)-one

Table(2): Effect of different concentrations of the synthesized pyrimidinone on numbers of *Leishmania tropica* promastigotes at different time intervals

Exposure time (hrs.) Treatments (μ g / ml)	24 Mean* ± SE	% inhibition	48 Mean* ± SE	% inhibition	72 Mean* ± SE	% inhibition	96 Mean* ± SE	% inhibition
Control	0.45 ± 0.01 [bc]	--	1.78 ± 0.04 [e]	--	6.82 ± 1.50 [d]	--	19.8 ± 1.55 [f]	--
1.0	0.40 ± 0.02 [b]	11	1.23 ± 0.001 [b]	31	3.25 ± 0.12 [c]	52	13.06 ± 1.46 [e]	34
2.5	0.37 ± 0.001 [b]	18	1.18 ± 0.04 [bc]	34	3.01 ± 0.23 [c]	56	10.07 ± 0.04 [d]	49
5.0	0.30 ± 0.07 [ab]	33	1.12 ± 0.21 [b]	37	1.95 ± 0.57 [b]	71	7.51 ± 2.10 [c]	62
7.5	0.28 ± 0.01 [a]	38	0.84 ± 0.11 [a]	53	1.54 ± 0.05 [ab]	77	3.78 ± 1.28 [b]	81
10	0.25 ± 0.01 [a]	44	0.68 ± 0.28 [a]	62	1.17 ± 0.01 [a]	83	0.74 ± 0.15 [a]	96

* Three replicates were used for each treatment. Mean and Standard Error were multiplied x 10^6.

** Initial number of promastigotes used in each culture = 2 x 10^5.

*** Different letter refers to presence of significant differences between treatment at P ≤ 0.05, according to Duncan - test.

Table(3): Effect of different concentrations of the synthesized pyrimidinone on generation number of *Leishmania tropica* promastigotes at different time intervals

Exposure time (hrs.) Treatments (µ g / ml)	24 Mean* ± SE	48 Mean* ± SE	72 Mean* ± SE	96 Mean* ± SE
Control	1.14 ± 0.04 [e]	3.1 ± 0.26 [c]	5.09 ± 1.50 [c]	6.62 ± 0.11 [d]
1.0	0.99 ± 0.09 [e]	2.29 ± 0.00 [b]	4.02 ± 0.22 [b]	6.07 ± 0.12 [d]
2.5	0.86 ± 0.00 [d]	2.01 ± 0.06 [b]	3.89 ± 0.11 [b]	5.65 ± 0.003 [c]
5.0	0.55 ± 0.02 [ab]	1.88 ± 0.05 [a]	3.21 ± 0.23 [b]	5.03 ± 0.46 [c]
7.5	0.38 ± 0.01 [b]	1.85 ± 0.16 [a]	2.94 ± 0.04 [a]	3.86 ± 0.60 [b]
10	0.25 ± 0.01 [a]	1.15 ± 0.05 [a]	2.54± 0.01 [a]	1.76 ± 0.03 [a]

* Three replicates were used for each treatment. Mean and Standard Error were multiplied x 10^6.
** Different letter refers to presence of significant differences between treatment at P $\leq$ 0.05, according to Duncan - test.

Table(4): Effect of different concentrations of the synthesized pyrimidinone on generation time (hours) of *Leishmania tropica* promastigotes at different time intervals

Exposure time (hrs.) Treatments (µ g / ml)	24 Mean* ± SE	48 Mean* ± SE	72 Mean* ± SE	96 Mean* ± SE
Control	21.0 ± 0.82 [a]	15.9 ± 1.31 [a]	14.3 ± 0.94 [a]	14.5 ± 0.24 [a]
1.0	24.2 ± 2.11 [b]	18.5 ± 0.00 [b]	17.9 ± 0.98 [b]	15.8 ± 0.29 [ab]
2.5	27.9 ± 0.00 [c]	23.9 ± 0.71 [c]	18.5 ± 0.53 [b]	17.0 ± 0.01 [b]
5.0	44.0 ± 1.84 [d]	25.6 ± 0.62 [d]	22.4 ± 1.63 [c]	19.1 ± 1.73 [c]
7.5	63.5 ± 2.35 [e]	26.0 ± 2.28 [d]	24.5 ± 0.32 [cd]	24.9 ± 3.88 [d]
10	97.5 ± 4.36 [f]	41.8 ± 1.73 [e]	28.3± 0.12 [d]	54.7 ± 0.89 [e]

* Three replicates were used for each treatment. Mean and Standard Error were multiplied x 10^6.
** Different letter refers to presence of significant differences between treatment at P $\leq$ 0.05, according to Duncan - test.

The pyrimidinonic compound showed a strong inhibitory effect on growth, generation number and generation time of *Leishmania tropica* promastigotes depending upon the concentration, which is agreement with [23] his demonstrated that *Leishmania tropica* promastigotes when treated with 5 mg / ml of *melia azedarach* aqueous extract inhibited 97% of the growth occurred. Other researchers used low molecular weight compound as antileishmanial agents. For example, [24] used anhydrous copper sulfate, they concluded that copper sulfate resulted in gradual inhibition in the growth of *Leishmania major* promastigotes depending upon concentration and time. In addition, other researchers tried drugs that were used for different disease, [25] used chlorpromazine which is used for psychosis cases, as an antileishmanial agent, which reduced 90% of *Leishmania major* and *Leishmania donovani* growth *in vitro* at log phase.

It is worthy to mention that bleomycin (2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone), the antineoplastic drug was used as anticutaneous leishmaniasis by [26], the lesions of the patients dried out within 4 – 6 weeks and healed both clinically and parasitologically after 5 – 8 weeks of therapy.

Since pyrimidine bases are minor constituents of nucleic acids, the chemistry of pyrimidines has been the subject of much research owing to their application in molecular biology and medicine [27]. In the last few years, pyrimidinone and it's derivatives which are substituted at both C-5 and C-6 positions have emerged in the field of chemotherapy. In this context, the C-6 substituted pyrimidinone and it's derivatives showed selective antitumor, antiviral, antitubercular and antifungal activities which suggested the importance of testing this family of compounds as broad – spectrum drugs [28].

The synthesized pyrimidinone exhibited relatively high antileishmanial activity. However the mechanism by which the pyrimidinone killed the parasite is not known. The ability of the tested compound to inhibit Leishmanial growth *in vitro* may be related to the resemblance of this compound to nucleotide analogs. [29] demonstrated that pyrazolpyrimidines reduced the activity of enzymes that take part in the metabolism of *Leishmania major* nucleotides. He conclude that the antileishmanial activity of these compounds may related to ability to imitate nucleotide analogs. On the other hand the antileishmanial activity of the synthesized pyrimidinone may be due to the presence of anisyl group (-OCH$_3$) at Para location of the phenyl ring at C-6 position or / and carbonyl group (C = O) at C-2 of the pyrimidinone ring, these active group are rich with electron pairs, that have the ability to attack and distort specific locations in the parasitic cells.

This may occur within cellular membranes or within cellular organelles. These effects were enhanced by morphological changes of the treated promastigotes (see picture 2 and 3). [30] synthesized 1,4-dihydro-3-ethyl-4-(2-presitylenyl)-6
-(O-chlorophenyl) pyrimidine -(1H)-one, then tested it's antileishmanial activity, and found that this compound had a high inhibitory effect against *Leishmania tropica* promastigotes with IC_{50} of 0.6 µg / ml, and the ability of such compound to affect (reduce) energy, proteins and nucleic acids metabolism.

Thus there is a need for subsequent biochemical studies to determinate the molecular location that may targeted by this antileishmanial agent.

The effect of synthesized pyrimidinone on the morphology and dimensions of treated with IC_{50} and IC_{90} and untreated promastigotes were studied after 96 hrs. of incubate (Table. 5 and Picture. 1).

The synthesized pyrimidinone made remarkable changes in the morphology of the parasite, as it decreased the length and increased the width of the parasite treated with IC_{50} i.e. they swelled (Picture. 2). On the other hand, parasites treated with IC_{90} of the synthesized pyrimidinone underwent sizable blow up (Picture. 3).
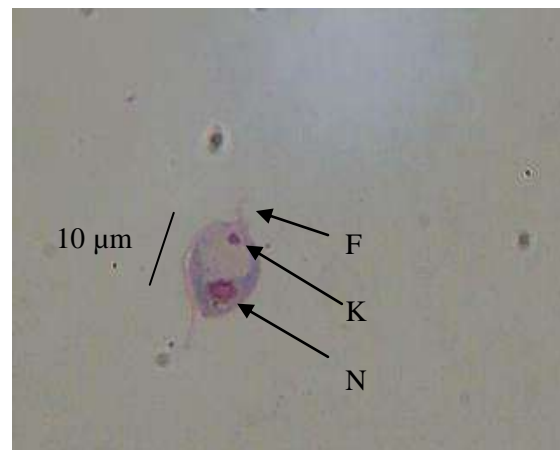
Table(5): Effect of IC_{50} and IC_{90} of the synthesized pyrimidinone on dimensions of *Leishmania tropica* promastigotes at log phase

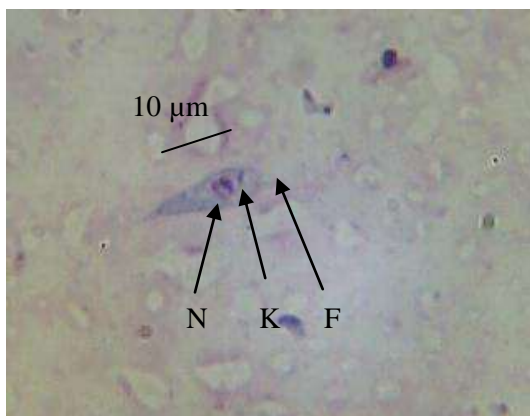| Treatment	concentrations (µg / ml)	Length (µm)	% Change		Width (µm)	% Change	
		Mean* ± SE	decrease	increase	Mean* ± SE	decrease	increase
Control	---	17.2 ± 0.98	---	---	3.26 ± 0.51	---	---
IC50	2.5	14.0 ± 0.86	19	---	4.93 ± 0.44	---	51
IC90	10	10.8 ± 1.36	37	---	5.93 ± 0.67	---	82

15 Replicates were used for each treatment.
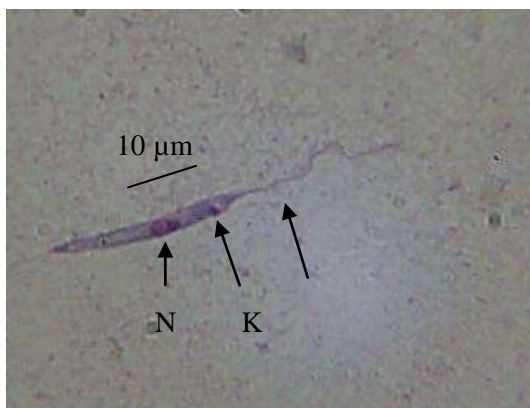
Picture(1): Untreated Giemsa stained promastigotes, body 17-19.2µm in length and 2.1-3.7µm in width, with pale blue cytoplasm and relatively large oval deep purple nucleus (N) near the middle of the cell. kinetoplast (K) rod shaped, lie at the base of the flagellum (F).

Picture(2): *Leishmania tropica* promastigotes, treated IC_{50} of the synthesized pyrimidinone. Note, swelling of the organism.

Picture(3): *Leishmania tropica* promastigotes, treated IC_{90} of the synthesized pyrimidinone. Note, the blow up of the organism.

Previous results appear to be consistent with those manifested by [31] who demonstrated that *Leishmania donovani* promastigotes treated with pentostam *in vitro*, produced few deformities, i.e. appeared smaller in size, shrunken in body and the peculiar phenomenon of biflagellation, while promastigotes treated with metronidazole underwent biflagellation, or loss of flagellum, multi nucleation, vaculation and degeneration. Similarly, [32] revealed that *Leishmania* promastigotes treated with ketokenazole and metronidazole, underwent slow motion, rounded promastigote appeared instead of the spindle shaped from the second till the sixth day of treatment. [33] demonstrated that *Leishmania major* and *Leishmania donovani* promastigotes showed morphological changes after 96 hrs. of treatment with chloropromazine, promastigotes became rounded or oval, in addition to the breakdown and degradation of the plasma membrane.

Researchers differ in their opinion about explanation of morphological changes, that result from treating promastigotes with antileishmanial agents. [34] illustrated the ability of allopuranol to alter the spindle shape of promastigotes to the rounded shape and observed weak motion of the parasite, and suggested that these effects may be due to the deficiency in the formation of parasitic RNA. Also it was found that amphotercin B changed sterol membrane composition of *Leishmania*, changing membrane permeability and finally killing the parasite [35]. [36] indicated that the inhibitory effect of antileishmanial agents on the membrane permeability might be related to the effect of such compound on the activity of some enzymes that take part in membrane transport regulation, and demonstrated that the aqueous extract of *Capparis spinosa* lowered osmotic pressure of *Leishmania major* promastigotes *in vitro*, then the cell swelled ; these events were accompanied with increase of acid phosphates activity.

[37] showed that the aqueous extract of *Nerium oleander* and *Melia azedarach* affected the function of plasma membrane of *Leishmania tropica* promastigotes, in such manner that the output of K^+ and acid phosphates were increased. They concluded that these changes resulted in osmoregulation changes.

In the present research, there is no defined reason that may explain the changes in dimensions and morphology of *Leishmania* promastigotes after treatment with different concentration of synthesized pyrimidinone, They may be due to the changes in the osmolarity which happened when the materials added. On the other hand, the synthesized compounds may affect the activity of certain enzymes or physiological activities of the promastigotes in such manner that the organism became stumpy.

In conclusion there is a need for extensive radiolabilling study to trace routes of action of the synthesized pyrimidinone inside the parasite which may show the accurate role of this compound in the morphological changes and growth inhibition of the parasite *in vitro*.

As for the effect of (LD_{50}) of the synthesized pyrimidinone which was 642.9 mg / kg body weight. The most characteristic symptoms of the treated mice were quietness, trembling in addition walking difficulties (Table. 6):

Table(6): Estimating LD_{50} synthesized pyrimidinone in Balb/c mice

Measurements	Values
Medium lethal dose (mg / kg)body weight	642.9
Dose range (mg / kg) body weight	600 - 800
Number of mice used	8
Result after 24 hrs.	XXXX OOXO
Final test level (mg / kg)body weight	650

X: For died animal after 24 hrs.   **O:** for alive animal after 24 hrs.

In toxicology, the quintal dose – response is used extensively, The median lethal dose (LD_{50}) is usually the first experiment performed with a new chemical. According to toxicity rating chart [38] the synthesized pyrimidinone considered to have moderate toxicity so that, the present synthesized compound can be classified according to their poisoning potential as moderately toxic material.

LD_{50} of the existing compound is relatively similar to that of [39] who studied the effectiveness of 1-morpholinomethyl- tetrahydro-2(1H)-pyrimidinone (DD-13) as a selective inhibitor of the alpha viral reproduction *in vitro*. They demonstrated that LD_{50} of such compound in intraperitoneally injected white mice was 720 mg / kg body weight with a selectivity ratio (LD_{50} / ED_{50}) of 385. On the other hand broprimine, a pyrimidinone derivatives, is an immunomodulator agent with antiviral and antitumer activities. [40] demonstrated that this pyrimidinone has been found to be lethal to embryos at doses (200 and 400 mg / kg body weight).

The toxicity of the compound to cutaneous *Leishmania* was studied as the toxicity indicates the efficiency of the compound as an antileishmanial agent. As the compound was a good inhibitor for *Leishmania in vitro* and gave moderate toxicity in experimental mice, therefore, there is a need for expanded biochemical study to determined metabolically weak point/s in the parasite that may be attacked by the synthesized compound. Beside, there is a need for *in vivo* study to determined therapeutic indices of such compound in mice.

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بناء ودراسة تأثيرات المركب ٣ ، ٤ – داي هايدرو– ٤ –(بارا– أنيسايل)–٦ –فينيل بايريميدين–٢
(١ H)– ١ على نمو وشكل أمامي السوط لطفيلي اللشمانيا الاستوائية
Leishmania tropica في الزجاج

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الملخص:

أغلب الأدوية المستخدمة في معالجة داء اللشمانيا لها تأثيرات جانبية لذلك بات من الضروري وجود علاج آمن وفعال ضد هذا المرض ، في البحث الحالي تم تصنيع مركب يحوي على حلقة البريميدين (٣ ، ٤ – داي هايدرو–٤–(بارا–انيسايل)–٦– فينايل بايريميدين–٢–(١ H)– واحد) ومن ثم اختباره وملاحظة فعاليته على نمو اللشمانيا الجلدية *Leishmania tropica*.

تضمنت الدراسة تأثير المركب على النمو وعدد الجيل وزمن الجيل فضلاً عن المظهر الخارجي وأبعاد هذه الطفيليات إذ استخدمت تراكيز تراوحت ما بين ١ – ١٠ مايكروغرام / مل. أظهرت النتائج بأن هناك تأثير واضح على نمو الطفيليات إذ كلما زاد التركيز قل النمو وكذلك عدد الجيل فضلاً عن زيادة زمن الجيل ، وقد أظهرت النتائج بأن التركيز الذي يقتل ٥٠% من الطفيليات IC₅₀ هو ٢,٥ مايكروغرام / مل وأن التركيز القاتل لـ ٩٠% من الطفيليات IC₉₀ هو ١٠ مايكروغرام / مل عند الطور اللوغاريتمي من النمو والذي أدى إلى حدوث تغييرات واضحة في المظهر الخارجي.

كذلك اختبرت سمية المركب المصنع باستخدام الفئران من نوع Balb/c بطريقة دكسون (١٩٨٠) وقد ظهر بأن التركيز الذي يقتل ٥٠% من الفئران هو ٦٤٢,٥ ملي غرام / كغم من وزن الجسم.