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Synthesis and Characterization of Pyrrole, Pyridine and Pyrazoline Derivatives: Biological Activity against *Leishmania Tropica*, Human Lymphocytes, and Molecular Docking Study

Israa I. Salih [1*], ***Maha Salih Hussein*** [1]

[1] Department of Chemistry, College of Education, University of Samarra, Samarra, Iraq.

ABSTRACT

This study prepared seven compounds I1-7 containing pyrrole, pyridine, and pyrazoline rings. First, four chalcones I1-4 were prepared as precursors, and then three pyrazoline I5-7 cores were prepared from them. The chalcones compounds were synthesized by reacting with 4-ethoxy acetophenone or 3,4-(Methylenedioxy) acetophenone with Pyrrole-2-carboxaldehyde and 2-Pyridinecarboxaldehyde. The pyrazoline ring derivatives were obtained from reaction chalcones with hydrazine derivatives: (hydrazine,2-hydrazinobenzothiazole& phenyl hydrazine). All compounds were proven by FTIR, 1H& 13C NMR spectroscopies in addition to the physical properties. The biological activity of some compounds against *Leishmania tropica* and human lymphocytes was examined using the 3-[4,5-dimethylthiazole]-2,5-diphenyltetrazolium bromide (MTT) test. Considering the *Leishmania tropica* test, the prepared compound I3 showed the highest inhibition of 80-81%, While compound I2 showed the highest toxicity against lymphocytes of 54-70% at (1 to 0.0625) mg/ml, which was confirmed by their molecular docking study with three different enzymes (DNA polymerase iota, HSP 90, Lysine-specific demethylase) has been measured and compared with the binding energy of the drug Amphotericin B, and Azithromycin where the docking energy appeared close to that of the drugs that were used, which nominates them as good anti-leishmania and lymphocytes in the future.

Keywords: 5-Acetyl-1,3-benzodioxole, Claisen-Schmidt, pyrazoline, MTT assay.

1. INTRODUCTION:

The chemistry of chalcones has prompted a lot of scientific research around the world. The synthesis and biodynamic properties of chalcones have sparked particular interest. Kostanecki and Tambor came up with the term "Chalcones". These chemicals are also called benzalacetophenone and benzylidene acetophenone. Chalcones are composed of two aromatic rings connected by an aliphatic three carbon chain. A chalcone has a very excellent synthon, which allows the development of a wide range of new heterocycles [(1)] with promising medicinal properties [(2)].

Chalcones are a class of aromatic ketones which have two aromatic groups connected by an enone linkage (Ar-COCH=CH-Ar) [(3)]. Furthermore, chalcones (trans-1,3-diaryl-2-propen-1-ones) naturally occur in flavonoid compounds which are considered as intermediates in flavonoid biosynthesis and found in many plants. Many biologically active chemicals include the α, β-unsaturated ketone moiety in chalcones. Thus, natural or synthetic chalcone compounds demonstrated different pharmacological actions, such as antimicrobial [(4)].

Nitrogenous heterocycles such as pyridine, pyrrole and pyrazoline play an important role as a valuable source of clinically relevant compounds in medicinal chemistry

[*]***Corresponding author: Israa I. Salih***
esraa.ismaeel@uosamarra.edu.iq
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research. These distinct substrates have been continuously used in a wide range of FDA-approved drug candidates. These substrates have received increasing attention as antidotes for many pathological conditions due to their ease of preparation and the possibility of testing and evaluating their biological activity [5].

Leishmaniasis, a vector-borne infection is brought about by an intracellular protozoan parasite having a place with the class *Leishmania*. Additionally, it is spread by the nibble of a tainted female phlebotomine sandfly. Leishmaniasis is a vector-borne metazoonosis illness, caused by obligatory intra macrophage protozoa of the genus *Leishmania* [6].

Disease exists in three kinds: cutaneous (skin), the most common form; mucocutaneous (mucous membranes), a very uncommon form; and visceral (internal organs). Different species of *Leishmania* generally cause different forms of disease. Leishmaniasis is also named kalaazar, Oriental sore, Delhi boil, and espundia [7].

Chalcones derived from heterocyclic rings are unique and important compounds that have been studied in many research papers as anti-leishmaniasis [8] and human lymphocyte cell. The importance of these rings was highlighted by the preparation of a number of chalcones and the pyrazolines ring by ring-closure reaction, and a preliminary evaluation of their effectiveness against *L. tropica* and lymphocytes was conducted.

2. EXPERIMENTAL AND METHODS

2.1. Organic part

Melting Point Digital Advanced SMP30 device equipped by Stuart-United Kingdom, at the University of Samarra - Faculty of Applied Sciences - Laboratories of the Department of Applied Chemistry, and all measured melting points are not corrected.IR (infrared) spectra were recorded on Shimadzu FT-IR (Fourier transform infrared) 8400S instrument and were calibrated using a polystyrene film. Solid compounds were recorded in potassium bromide disks (KBr). ^{1}H-NMR, ^{13}C-NMR (nuclear magnetic resonance) spectra were recorded on 400 MHz (AV III-HD-800) bio spin spectrometer. Dimethyl sulfoxide (DMSO-d6) was used as a solvent and reference. Chemical shifts were quoted in ppm (parts per million).

2.1.1. General procedure for the synthesis of chalcones (1-4)

A solution of (0.0055 mol) NaOH dissolved in 13 ml distilled water/8 ml ethanol was mixed with (0.0244 mol) of aromatic ketone (4-ethoxyacetophenone or 1-(benzo[d][1,3] dioxol-5-yl)ethan-1-one), stirred for 20 minutes. Then (0.0244 mol) of aromatic aldehydes (pyrrole or pyridine carboxaldehyde) are added. After completing the addition, the reaction mixture was stirred at (15-30) °C for 3-7 hours. (Reference)

Furniss, B. S., Hannaford, A. J., Smith, P. W. G., & Tatchell, A. R. (1989). Vogel's TEXTBOOK OF PRACTICAL ORGANIC CHEMISTRY 5th ED Revised-Brian S. Furniss. *Journal of Polymer Science Part A: Polymer Chemistry*, *29*, 1034,1417.

***(E)-2-(3-(4-ethoxyphenyl)prop-1-en-1-yl)-1H-pyrrole* (1):**

Yield 33%, m.p: 142°C, FT-IR (νmax, cm^{-1}): 3236,3086, 2978, 1651, 1608, 1581, ^{1}H NMR (400 MHz, DMSO) δ 11.72 (s, 1H$_{17}$), 8.09 – 8.01 (m, 2H$_{6,8}$), 7.61 (s, 2H$_{11,12}$), 7.13 (dd, *J* = 2.5, 1.4 Hz, 1H$_{16}$), 7.10 – 7.02 (m, 2H$_{5,9}$), 6.71 (dd, *J* = 3.6, 1.4 Hz, 1H$_{14}$), 6.22 (dd, *J* = 3.6, 2.5 Hz, 1H$_{15}$), 4.10 (q, *J* = 7.0 Hz, 2H$_{2}$), 1.34 (t, *J* = 7.0 Hz, 3H$_{1}$).^{13}C NMR (101 MHz, DMSO) δ 187.21 (C-10), 162.57 (C-4), 133.89 (CH-12), 131.43 (C-7), 130.70 (CH-6,7), 129.73 (C-13), 124.32 (CH-16), 116.36 (CH-11), 115.09 (CH-14), 114.73 (CH-5,9), 110.92 (CH-15), 63.95 (CH$_2$-2), 14.96 (CH$_3$-1).

***(E)-2-(3-(4-ethoxyphenyl)prop-1-en-1-yl)pyridine* (2):**

Yield 82%, m.p: 103°C, FT-IR (νmax, cm^{-1}): 3047,2982, 1645, 1600, 1573, ^{1}H NMR (400 MHz, DMSO) δ 8.68 (d, *J* = 4.7, 1.4 Hz, 1H$_{18}$), 8.15 - 8.11 (m, 2H$_{6,8}$), 7.92 (t, 1H$_{16}$), 7.70 (d, *J* = 15.4 Hz, 1H$_{12}$), 7.41 (d, *J* = 6.1, 4.8, 2.7 Hz, 2H$_{11,15}$), 7.10 (d, 1H$_{17}$), 6.97 (d, 2H$_{5,9}$), 4.16 – 3.99 (q, 2H$_{2}$), 3.43 – 3.38 (dd, 1H), 1.34 (t, *J* = 6.9 Hz, 3H$_{1}$).^{13}C NMR (101 MHz, DMSO) δ 187.92 (C-10), 163.17 (C-4), 153.42 (C-13), 150.45 (CH-18), 142.68 (CH-12), 137.60 (CH-16), 131.40 (CH-6,8), 130.49 (C-7), 125.50 (CH-11), 125.24 (CH-15), 125.12 (CH-17), 114.95 (CH-5,9), 64.06 (CH$_2$-2), 14.93 (CH$_3$-1).

***(E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(pyridin-2-yl)prop-2-en-1-one* (3):**

Yield 60%, m.p: 124°C, FT-IR (ν max, cm^{-1}): 3078, 2924, 1685, 1612, 1579, ^{1}H NMR (400 MHz, DMSO) δ 8.68 (d, *J* = 4.6, 1.5 Hz, $1H_{14}$), 8.11 (d, *J* = 15.4 Hz, $1H_8$), 7.96 (d, $2H_{12,3}$), 7.80 (d, *J* = 8.2, 1.8 Hz, $1H_9$), 7.68 (d, *J* = 15.4 Hz, $1H_5$), 7.57 (d, *J* = 1.8 Hz, $1H_{11}$), 7.42 (ddd, *J* = 6.8, 4.8, 1.5 Hz, $1H_{13}$), 7.08 (d, *J* = 8.2 Hz, $1H_2$), 6.17 (s, $2H_{18}$).^{13}C NMR (101 MHz, DMSO) δ 187.65 (C-7), 153.41 (C-10), 152.26 (C-1), 150.44 (CH-4), 148.61 (C-6), 143.01 (CH-9), 137.59 (CH-12), 136.18 (CH-3), 132.47 (C-4), 125.76 (CH-8), 125.50 (CH-11), 125.13 (CH-13), 108.66 (CH-5), 108.16 (CH-2), 102.64 (CH_2-18).

***(E)-1-(benzo[d][1,3]dioxol-5-yl)-3-(1H-pyrrol-2-yl)prop-2-en-1-one* (4):**

Yield 57%, m.p: 211°C, FT-IR (ν max, cm^{-1}): 3244, 3093, 2978, 1647, 1570, 1546, ^{1}H NMR (400 MHz, DMSO) δ 11.67 (s, $1H_{17}$), 7.71 (dd, *J* = 8.1, 1.8 Hz, $1H_3$), 7.59 – 7.52 (m, $3H_{8,5,9}$), 7.17 – 7.06 (m, $2H_{16,2}$), 6.71 (dd, *J* = 3.6, 1.9 Hz, $1H_{14}$), 6.22 (t, *J* = 3.4 Hz, $1H_{15}$), 6.15 (s, $2H_{12}$).^{13}C NMR (101 MHz, DMSO) δ 186.76 (C-7), 151.52 (C-1), 148.39 (C-6), 134.14 (CH-9), 133.44 (C-4), 129.69 (C-10), 124.51 (CH-3), 124.48 (CH-16), 116.53 (CH-8), 114.95 (CH-14), 110.97 (CH-15), 108.57 (CH-5), 107.98 (CH-2), 102.45 (CH_2-12).

2.1.2. General procedure for the synthesis of compounds (5-7):

A solution of chalcones 1-3 (0.0019 mol) in absolute ethanol (10 mL) and hydrazine derivatives (hydrazine solution 80%, 2-hydrazinobenzothiazole, phenyl hydrazine) respectively with (0.0019 mol) were mixed. The reaction mixture was heated under reflux, then cooled to room temperature after completion. The separated crystalline substance was filtered, rinsed with cold ethanol and then dried. As a consequence, solid products were obtained and recrystallized using ethanol. (Reference)

Sun, Z. G., Zhou, X. J., Zhu, M. L., Ding, W. Z., Li, Z., & Zhu, H. L. (2015). Synthesis and biological evaluation of novel aryl-2H-pyrazole derivatives as potent non-purine xanthine oxidase inhibitors. *Chemical and Pharmaceutical Bulletin*, *63*(8), 603-607.

***2-(3-(benzo[d][1,3]dioxol-5-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine* (5):**

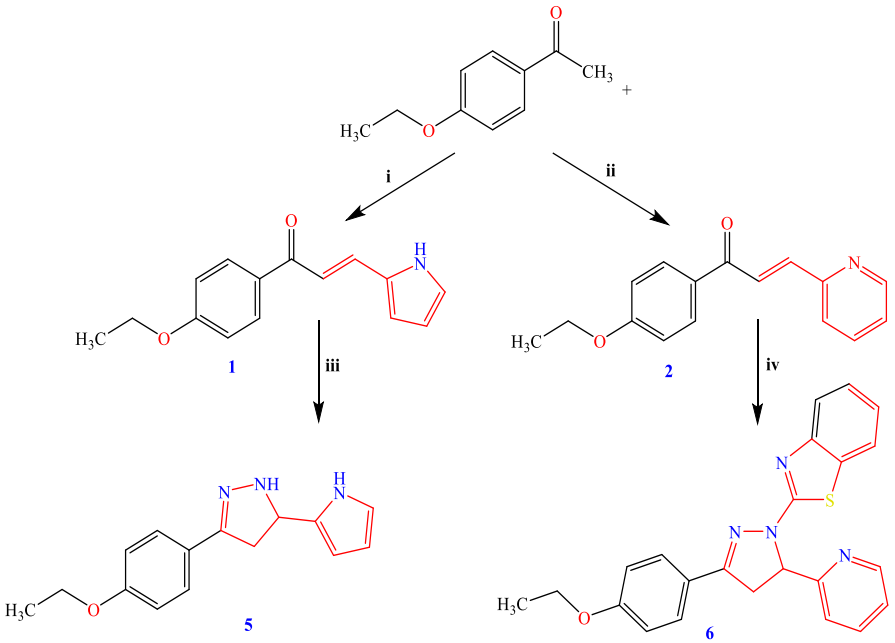
Yield 42%, m.p: 162°C, FT-IR (ATR) (ν max, cm-1): 3063, 2904, 1597, 1566, ^{1}H NMR (400 MHz, DMSO) δ 8.57 (d, *J* = 4.7, 1.5 Hz, $1H_{14}$), 7.73 (ddd, *J* = 7.7, 1.8 Hz, $1H_{12}$), 7.40 (d, *J* = 1.7 Hz, $1H_5$), 7.28 (m, *J* = 7.5, 5.1 Hz, $2H_{11,13}$), 7.21 – 7.10 (m, $3H_{3,23,25}$), 7.02 – 6.92 (m, $3H_{22,24,26}$), 6.74 – 6.66 (d, $1H_2$), 6.08 (s, $2H_{17}$), 5.45 (dd, *J* = 12.4, 6.5 Hz, $1H_9$), 3.89 (dd, *J* = 17.5, 12.4 Hz, $1H_8$), 3.20 (dd, *J* = 17.5, 6.5 Hz, $1H_8$).^{13}C NMR (101 MHz, DMSO) δ 161.65 (C-10), 150.13 (C-7), 148.45 (C-1), 148.19 (CH-14), 147.76 (C-6), 144.89 (C-21), 137.82 (CH-12), 129.38 (CH-23,25), 126.96 (C-4), 123.23 (CH-13), 121.10 (CH-3), 120.94 (CH-24), 118.87 (CH-11), 113.06 (CH-22,26), 108.79 (CH-5), 105.80 (CH-2), 101.81 (CH_2-17), 65.20 (CH-9), 42.22 (CH_2-8).

***3-(4-ethoxyphenyl)-5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazole* (6):**

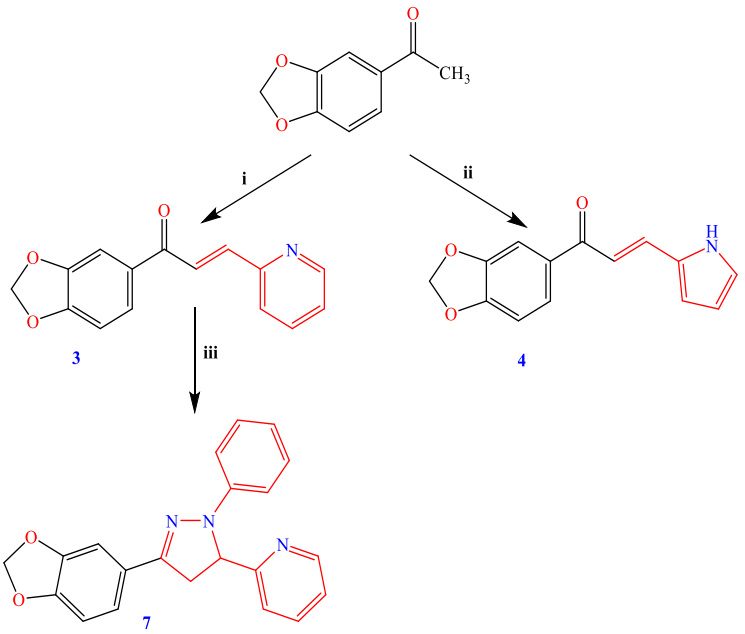
Yield 73%, m.p: 161°C, FT-IR (ATR) (ν max, cm^{-1}): 3298, 3070, 2928, 1608, 1593, ^{1}H NMR (400 MHz, DMSO) δ 10.85 – 10.79 (m, $2H_{15,19}$), 7.65 – 7.54 (m, $2H_{5,9}$), 6.98 – 6.90 (m, $2H_{6,8}$), 6.70 (td, *J* = 2.6, 1.5 Hz, $1H_{18}$), 5.97 (ddd, *J* = 7.8, 4.3, 2.2 Hz, $2H_{16,17}$), 4.78 (t, *J* = 10.8 Hz, $1H_{12}$), 4.03 (q, *J* = 7.0 Hz, $2H_2$), 3.25 (dd, *J* = 16.1, 10.2 Hz, $1H_{11}$), 2.96 (dd, *J* = 16.0, 11.4 Hz, $1H_{11'}$), 1.34 (t, *J* = 7.0 Hz, $3H_1$).^{13}C NMR (101 MHz, DMSO) δ 159.09 (C-4), 149.98 (C-10), 132.28 (C-13), 127.36 (CH-6,8), 126.38 (C-7), 117.88 (CH-18), 114.82 (CH-5,9), 107.49 (CH-14), 105.77 (CH-16), 63.54 (CH_2-2), 58.12 (CH-12), 39.35 (CH_2-11), 15.09 (CH_3-1).

***2-(3-(4-ethoxyphenyl)-5-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)benzo[d]thiazole* (7)**:

Yield 80%, m.p: 110-112°C, FT-IR (ATR) (ν max, cm^{-1}): 3059, 2982, 1600, 1566, ^{1}H NMR (400 MHz, DMSO) δ 8.54 (s, $1H_{29}$), 7.88 (dd, *J* = 17.9, 8.2 Hz, $1H_{15}$), 7.81 – 7.69 (m, $3H_{8,6,26}$), 7.66 (d, *J* = 15.0 Hz, $1H_{17}$), 7.31 – 7.22 (m, $3H_{4,16,28}$), 7.01 (dd, *J* = 17.4, 8.4 Hz, $2H_{27}$), 6.96 – 6.90 (m, $2H_{5,9}$), 4.07 (s, $1H_{12}$), 4.05 – 3.97 (m, $3H_{2,11,11'}$), 1.31 (d, *J* = 20.4 Hz, $3H_1$).^{13}C NMR (101 MHz, DMSO) δ 160.19 (C-21), 159.27 (C-4), 153.90 (C-13), 149.62 (C-10), 136.91 (C-24), 130.18 (CH-17), 128.22 (CH-15), 122.92 (C-23), 123..39 (CH-6,8), 122.07 (CH-7,28), 121.69 (CH-16,27), 119.65 (CH-14,26), 115.19 (CH-29), 114.66 (CH-5,9), 63.98 (CH-12), 42.28 (CH_2-2,11), 14.99 (CH_3-1).



Ragents and solvents: (i) pyrrole-2-carboxaldehyde , NaOH ,EtOH,r.t (ii) pyridine-2-carboxaldehyde, NaOH ,EtOH,r.t , (iii) Hyrazine 80%, EtOH,reflex (iv) 2-hydrazinobenzothiazole, EtOH,reflex



Ragents and solvents: (i) pyridine-2-carboxaldehyde , NaOH ,EtOH,r.t (ii) pyrrole-2-carboxaldehyde, NaOH ,EtOH,r.t , (iii) phenyl hydrazine, EtOH,reflex

Scheme (1): Synthesis of chalcones and Pyrazoline derivatives (1-7)

2.2. Biological part

2.2.1. Cytotoxic activity using the 3-[4,5-dimethylthiazoyl]-2, 5-diphenyltetrazolium bromide (MTT) test (in vitro)

2.2.1.1. Activity against *Leishmania tropica*

The activity was evaluated for compounds **(1-5)** against *L. tropica* using the methylthiazol tetrazolium (MTT) bioassay solution. Briefly, 100 µLwell^{-1} *Leishmania tropica* promastigotes (10^6 cell mL^{-1}) were cultured in 96-well tissue culture plate. Prepare different concentrations of compounds (1-5) solution (half concentrations), stock concentration was 1 mg.mL^{-1}, so the first concentration was 1 mg. mL^{-1} and so on until the sixth concentration.

100 µL was added of various concentrations to each well and incubated at 26∘C for 24h. After incubation, 10µL of (MTT) solution (5 mg mL^{-1}) was added to each well and incubation was continued for a further 4 hours. The experiment was performed in triplicate. (Reference)

Mahmoudvand, H., Ezzatkhah, F., Sharififar, F., Sharifi, I., & Dezaki, E. S. (2015). Antileishmanial and cytotoxic effects of essential oil and methanolic extract of Myrtus communis L. *The Korean journal of parasitology*, *53*(1), 21.

2.2.1.2. Collection and processing of human lymphocytes

A healthy 26-year-old male donor provided peripheral venous blood, and lymphocytes were collected in accordance with Rafael and Vaclav [(9)]. The suspension of cultured human lymphocytes was adjusted to approximately 1 × 104 cells/ml. To achieve a final cell count of 1000 cells per well, 100 µl of the cell suspension was added to each of the 96 wells of a microtiter plate. The plates were then incubated at 37 °C in an incubator with 5% CO_2 for 24 hours [(10)]. After incubation, prepared compounds (1-5) were added to each well at different concentrations and incubated for 24 h [(9)].

The ELISA reader was used to measure absorbance for each well at 620 nm. The mean absorbance for each group of replicates was calculated. The viable cells, percentage inhibition ratio was calculated according to the equation:

Growth Inhibition%=O.D. of control−O.D. of Sample O.D. of control×100

Where O.D. is the optical density

Viability=100-growth inhibition %

While activity against human lymphocyte

This test was performed by dissolving 3-[4,5-dimethylthiazoyl]-2, 5-diphenyltetrazolium bromide in phosphate buffered saline (PBS) at 2 mg/ml, filtrated by a 0.22 µm millipore filter. 50 µl of the MTT dye was added to each of the microtiter plate wells containing human lymphocytes treated with different concentrations of prepared compounds (1-5) solution (half concentrations), stock concentration was 1 mg.mL^{-1}, so the first concentration was 1 mg. mL^{-1} and so on until the sixth concentration for 24 h. The ELISA reader was used to measure absorbance for each well at 620 nm [(11)]. The mean absorbance for each group of replicates was calculated. The viable cells, percentage inhibition ratio was calculated according to the formula [(12)]:

Growth Inhibition%=O.D. of control−O.D. of Sample O.D. of control×100

Where O.D. is the optical density

Viability=100-growth inhibition %

Statistical analysis:

The significant differences are determined in rate of probability 5% as the statistical analysis includes one way analysis of variance (ANOVA).

▪ Chi-square to know the kind of relationship whether significant or not among type of concentration and compound used [(13)].

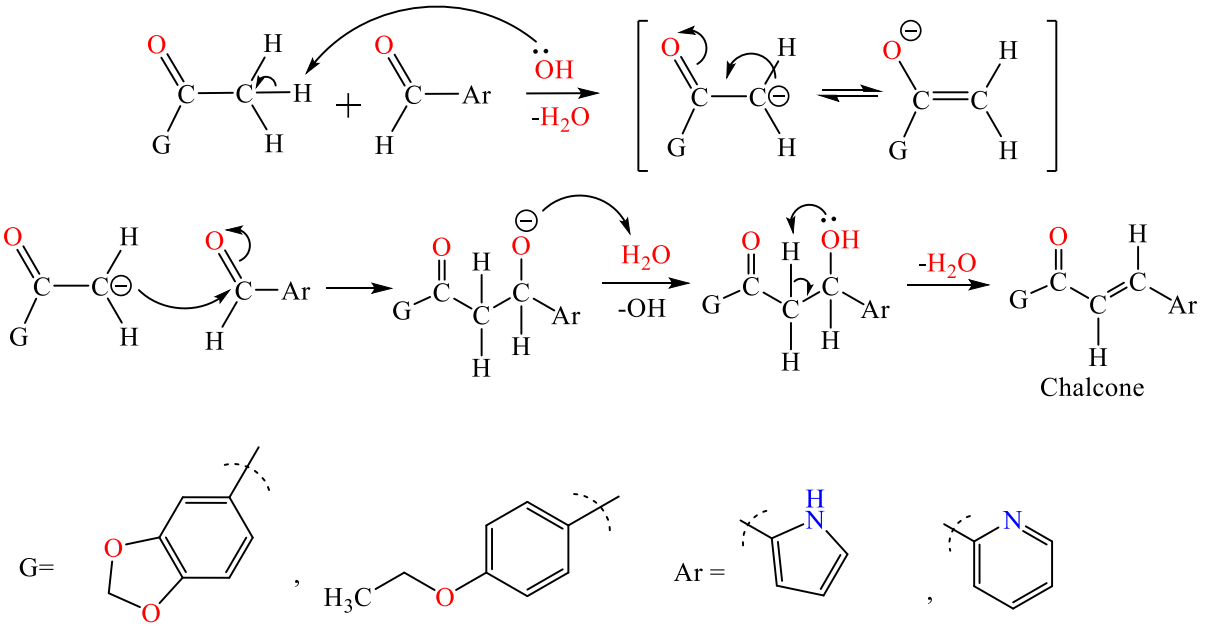
Results and discussion

Organic part:

In this work, four different chalcones and three of pyrazoline derivatives were synthesized (1-7). Four chalcones were synthesizes using the Claizen-Schmidt technique [(14)].

The reaction began by removing a proton from the alpha carbon atom in acetophenone derivative to form the

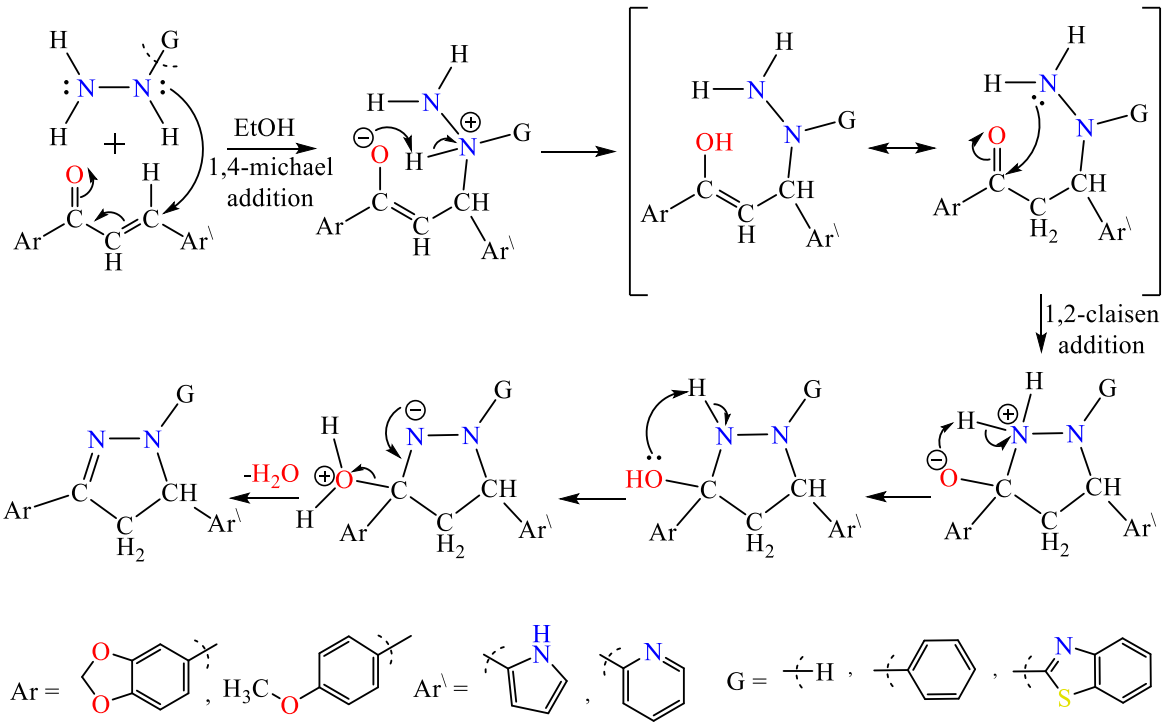
stable enolate ion due to resonance, followed by a nucleophilic attack by enolates on carbonyl carbon of benzaldehyde derivative leading to formation of a new carbon-carbon bond. This step followed by adding a proton and removing it using a hydroxide ion, which is the last step to form chalcones [15], as shown in diagram below:



The IR spectra is used for synthesized compounds and revealed a carbonyl (C=O) stretching absorption band for chalcones at about (1658 to 1554) cm-1. C=C bands appeared at approximately (1612 to 1570) cm-1, and a C=N absorption band at (1597 to 1546) cm-1. The ^{1}H-NMR spectra revealed significant signals, such as aromatic CH signals appeared at δ 6.22–8.1. The olefinic hydrogen atoms is shown at δ 7.2-8.2. Finally, signals of hydrogen NH group appeared at δ 11.67 and 11.72 ppm.

The ^{13}C-NMR spectra of a similar compounds showed methyl group signals at δ 14.91-14.96 and CH_2 group signals at δ 42.22-102.64. Signals of CH groups close to carbonyl groups ranged from 116 to 125.7, whereas signals of CH or C near to NH groups or N atoms ranged from δ 124 to 152.26. Tertiary carbon atoms produced signals between δ 126 to 153.42. Aromatic carbon signals were detected between δ 105 to 152.26 ppm, whereas carbonyl group carbon produced significant signals at δ 186.76 to 187.92 ppm.

Pyrazoline derivatives were prepared according to nucleophilic addition by which the free electron pair located on one of the nitrogen atoms in the hydrazine molecule, attacking the beta carbon atom in the chalcone molecule, the product of Michael addition (1,4) is formed. Then the free electron pair of the other nitrogen atom is attacked on the carbon atom of the carbonyl group in the chalcone molecule, where this addition product is of the Claisen type (1,2), and by deleting the water molecule the compound was produced [16] as illustrated in the diagram below:



The IR spectra showed the evanescence of carbonyl (C=O) stretching absorption band for chalcones and revealed instead of that bands belong to C=N absorption band at (1593 to 1566) cm-1. The ^{1}H-NMR spectra revealed significant signals, such as CH_2 signals appeared at δ 2.96–4.05 ppm.

The ^{13}C-NMR spectra of a similar compound showed methylene group signals at δ 39.35-42.28, whereas signals of CH groups close to carbonyl groups were demised which ranged from δ 116.36 to 125.76. In addition to that carbonyl groups signals disappeared from their positions at δ 187.21 to 187.92 ppm.

Biological part:

Activity against *Leishmania tropica*

After the Leishmania parasite was treated in 96 wells on a plate with five compounds, each at five concentrations and in three duplicates, the inhibition rates varied. In this investigation, a negative control, the medication pentostam, was utilized alongside the five produced compounds at half concentrations. The control exhibited a reading of (0.330), whereas pentostam indicated an inhibition rate of (77%). While compound No. (2,3) had the maximum inhibition rate of (81%), compound No. (5) had the lowest inhibition rate of (6%). It is worth noting that the provided findings were read using ELISA equipment.

After treating the Leishmania parasite in 96 wells on a plate with five compounds, five concentrations and three duplicates, there was a variation in the rates of inhibition. In this investigation, the negative control, medication pentostam, were utilized alongside the five produced compounds at half concentrations. The control exhibited a reading of (0.330), whereas pentostam indicated an inhibition rate of (77%). While compounds No. (2 and 3) had the maximum inhibition rate of (81%), compound No. (5) had the lowest inhibition rate of (6%). It is worth noting that the provided findings appeared using the ELISA equipment.

Table (1) Cytotoxicity effect %, Mean ±SD of prepared compounds (1-5) against promastigote of *Leishmania tropica*.

Conc.mg/mL	Comp. No				
	Drug1(I1)	**Drug2(I2)**	**Drug3(I3)**	**Drug4(I4)**	**Drug5(I5)**
1	46.4± 0 A	81±0 A	80± 0 A	70±0.73 A	56± 0.34 A
0.5	45.8± 0 A	80±0 A	81± 0 A	48± 0 B	52±0.30 A
0.25	40.03±0.33 B	72±0 AB	81± 0 A	46± 0 B	49±0.29 A
0.125	35.3±0.33 B	51±0.65 C	81± 0 A	49± 0 B	6±0.12 B
0.0625	35.0±1.15 BC	39±±0.61 C	80± 0 A	50± 0 B	1±0.17 C
Pentostam	77±0.57 D	77±0.57 D	77±0.57 D	77±0.57 D	77±0.57 D
Control	100±0.28 E	100±0.28 E	100±0.28 E	100±0.28 E	100±0.28 E
IC_{50}	1.12	0.01	<0.001	0.26	0.68

*p. value =0.05

* IC50 is the antilogarithm of the concentration at which the curve passes through the 50% percent of inhibition.

• Different letters within a column imply a significant difference (P <0.05).

• Different letters within a Raw of IC_{50} presuppose a significant difference (P <0.05).

Clarifying how the statistical significance was determined would strengthen the credibility of the findings

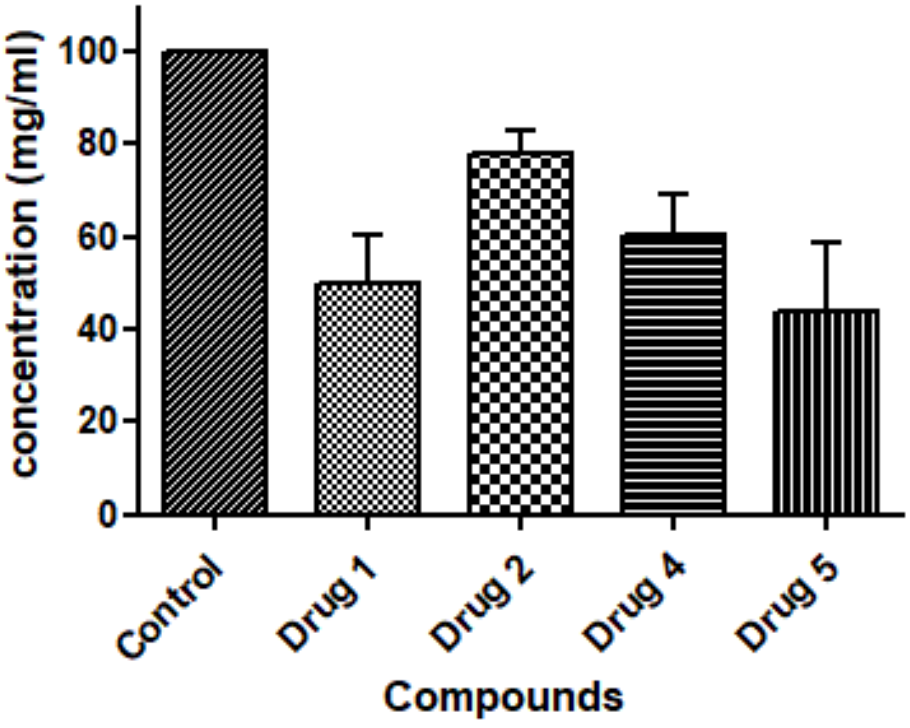


Figure (1) Cytotoxicity effect of prepared compounds (1-5) against promastigote of *Leishmania tropica*.

Activity against Human Lymphocyte

After the lymphocytes had been isolated from the blood, they were put in the plate, and the synthesized compounds were added at half concentrations, with five concentrations of substance. Three duplicates were taken for each concentration, and after one hour of incubation, the results were read in the ELISA equipment. The results were good when compared with the control, as the inhibition percentage of the compounds applied to the samples ranged from the highest inhibition percentage, which reached (70%), which returned to compound No. (2), to the lowest inhibition percentage, which was (% -78), which was for compound (5), the viability of compound (4,5) for the concentration 1 mg/mL reached to (120, 178) respectively.

After the lymphocytes had been isolated from the blood, they were put in the wells, then the synthesized compounds were added at half concentration, with five concentrations of substance for each. In addition, three duplicates were taken for each concentration, after one hour of incubation, the plate was inserted into the ELISA equipment, and the results appeared. The results were good compared with the control. As the inhibition percentage of the compounds ranged from the highest inhibition which reached (70%) - that attributed to compound No. (2) - to the lowest inhibition, which was (-78%), that attributed to compound (5). The viability of compound (4 and 5) for the concentration 1 mg/mL reached to (120 and 178) respectively.

Table (2) Cytotoxicity effect %, Mean ±SD of prepared compounds (1-5) against human lymphocyte.

Conc.mg/mL	Comp.No.				
	Drug1(I1)	**Drug2(I2)**	**Drug3(I3)**	**Drug4(I4)**	**Drug5(I5)**
1	37.87 ± 0.23 B	70.00 ± 0 A	73.02 ± 0.54 B	41.85 ± 0. 89 B	37.71 ± 0.96 B
0.5	36.70 ± 0.29 B	69.00 ± 0 A	72.08 ± 0.76 C	17.80 ± 0. 65 C	25.61 ± 0 .78 C
0.25	35.43 ± 0 A	49.00 ± 0 A	51.22 ± 0 A	14.08 ± 0. A	-11.53 ± 0.77 C
0.125	31.10 ± 0 A	41.20 ± 0.11 AB	32.25 ± 0 A	10.59 ± 0 A	-12.43 ± 0 .12 A
0.0625	28.20 ± 0 A	30.23 ± 0. 22 B	14.78 ± 0 A	4.61 ± 0 A	-61.53 ± 0 .14 D
Control	100±0 E	100±0 E	100± 0 E	100± 0 E	100± 0 E
IC_{50}	2.22	0.34	0.41	1.26	1.01

*p. value =0.05

• Different letters within a column imply a significant difference (P <0.05).

• Different letters within a Raw of IC_{50} imply a significant difference (P <0.05).

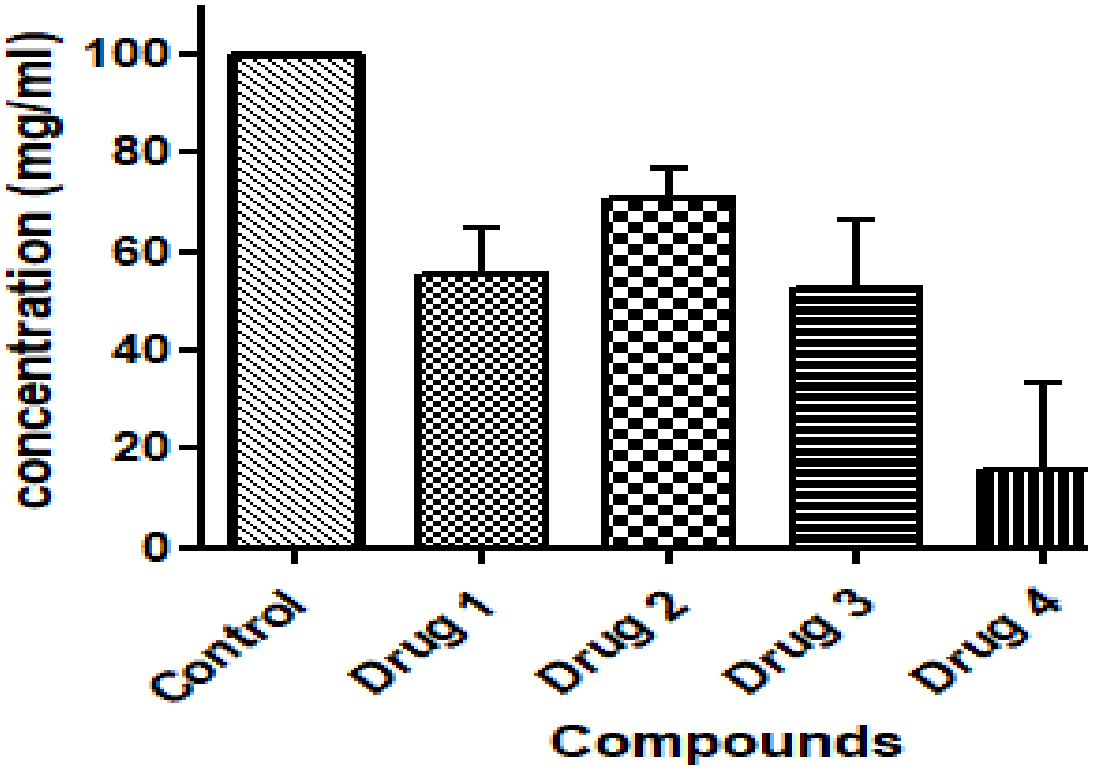


Figure (2) Cytotoxicity effect of prepared compounds (1-5) against human lymphocyte.

The growth inhibition observed on the growth of lymphocytes incubated with concentrations. The results clearly indicate that the rate of inhibition of growth was concentration dependent. The inhibitory effect was increased when compared with the negative control.

The colorimetric cytotoxic assay by MTT is widely used for the measurement of cell viability [19]. The assay is dependent on the fact that the MTT dye is reduced to a purple formazan product by live cells. The higher the number of live cells, therefore, the more MTT-formazan is produced. The quantity of MTT-formazan produced can be determined spectro-photometrically once it is solubilized in a suitable solvent (since it is insoluble in aqueous solution) [20].

The prepared compounds may be induce apoptosis through the elevation of the cAMP concentration [21]. Meanwhile, Shergalis et al. have described how is the compound (E)-3-(benzo[d][1,3]dioxol-5-yl)acrylic acid has anti-benign prostatic hyperplasia [22], so the prepared compounds may be cause morphological changes in normal cells due to a loss of adherence between the cells and their plastic container, which is inherently an apoptotic process. Nierman and Fedorova [23] have gone on to describe this phenomenon in lymphocytes, spleen cells, thymocytes and macrophages.

As they considered the prepared compounds, it is possible that these compounds passed through the cytoplasmic membrane and disrupted its structure at different layers, leading to membrane permeabilization. Consequently, the cytotoxic effect appeared to involve

such membrane damage. However, prepared compounds showed that in human lymphocyte and *L. tropica* cells, prepared compounds can act as prooxidants affecting inner cell membranes and organelles such as mitochondria [24].

As a result, there is an urgent need to develop a new, safe, effective, and economical antileishmanial medication. Several bioactive compounds with antileishmanial activity and various mechanisms of action have been synthesized [25,26]. A number of natural and synthetic chalcones have been found to have antileishmanial properties [27, 28, 29, 30]. Several studies [31, 32, 33,34] found chalcone derivatives effective against leishmanial targets. However, in this research, the synthesized chalcone analogues were shown to be more effective and safer for inhibiting Leishmania donovani than the reference compound Pentostam.

Antileishmanial activity in vitro and the connection between structural activity

Following the effective synthesis of the five chalcone analogues, the antileishmanial activity at concentrations ranging from 200 to 0.05 lg/ml was measured as a percentage of inhibition and 50% inhibitory concentration (IC50). The five prepared compounds had significant IC50 values ranging from 4.01 to 12.43. Conversely, the substitution with pyridine moiety in prepared compounds (1, 4, 5) showed decrease in IC50[35].

In addition to cancer cells, the tumor microenvironment (TME) contains immune cells (such as macrophages or T-cells) [36,37]. The interaction of all TME variables leads to the growth of the tumor. Macrophages constitute the bulk of immune cells in the TME, and the number and phenotype of tumor-associated macrophages (TAMs) influence tumor formation and progression [38,39]. Macrophages can have two distinct phenotypes: M1, which is conventionally active and produces pro-inflammatory TNF-α and IL-1β, and M2, which is activated and produces pro-tumoral IL-10 and TGF-β [38, 39, 40]. TAMs have an M2-like phenotype [38, 40].

The chalcones play an important role in macrophage polarization by influencing metabolic and inflammatory signaling pathways. The activity of chalcones on macrophages causes macrophage polarization to cell, enhanced pro-inflammatory cytokine production by Lipopolysaccharide -stimulated macrophages (LPS), and suppression of IL-4-induced polarization to M2 [41,42].

Molecular docking:

The molecular docking energy of some prepared compounds as (I2 and I3) has been studied and compared with the binding energy of some drugs which considered as a standard material for this comparison.

It is worth knowing that the binding energy of the compound (I3) -the compound that gave the highest inhibition rate against leishmaniasis - and the three different enzymes (DNA polymerase iota, HSP 90, Lysine-specific demethylase) have been measured and compared with the binding energy of the drug (Amphotericin B) - Amphotericin B is used to treat leishmaniasis. It is known that the mechanism of action of amphotericin B is mainly related to its binding to ergosterol, which damages cells by causing membrane permeability [17] - and the same three enzymes.

Then the molecular docking study of the compound (I3) has clarified the value of the binding energy which is (-8.6 Kcal/mole). When this energy is compared with the binding energy of the drug (AmphotericinB), (which is equal to 8.6 Kcal/mole), it is noted that the compound has a binding energy equal to the drug. The reason is that the compound (I3) has three hydrogen bonds (with the amino acids SER301, ARG357, SER407) in addition to the other bonds (Hydrophobic, Alkyl Hydrophobic Pi-Alkyl) and the drug has the same type of bonds but with different numbers.

This study also provided information about the molecular binding values of the compound (I3) and the same drug (AmphotericinB) but with two other enzymes.

One of which is (HSP 90). This one gave the drug a binding energy value of (-9.4 Kcal/mole), which is higher than the energy value of the compound (I3) which was (-

6.7 Kcal/mole). The cause behind this is the difference in the number of hydrogen bonds that the drug is bound to when compared to the hydrogen bonds that the compound is bound to with the same enzyme.

The other one (Lysine-specific demethylase) shows a molecular binding with the same drug along with the prepared compound(I3). It also gave a binding value with the drug (Amphotericin B) which is (-9.2 Kcal/mole) while the compound (I3) gave an energy of (-7.3 Kcal/mole) through its binding with the same enzyme.

To conclude, the first enzyme (DNA polymerase iota) gave the best binding value with the compound (I3). Its value was equal to the value of the drug used to eliminate leishmaniasis. it gave a promising vision about the activity and the effect of the prepared compound.

Table (1) shows all the information related to the binding energy and the types of bonds of the prepared compounds and the drugs.

The figures of compound (I3), the drug (AmphotericinB) and the enzymes (DNA polymerase iota, HSP 90, Lysine-specific demethylase) are presented in (3D, 2D) dimensions.

Concerning compound (I2) (which gave the highest inhibition rate against human lymphocytes), its binding energy with the enzyme (HSP 90-alpha) was compared with the binding energy of the drug (Azithromycin) with the same enzyme.

Azithromycin prevents the growth of bacteria by interfering in their protein synthesis as it binds to the 50S subunit of the bacterial ribosome and thus prevents the translation of mRNA.

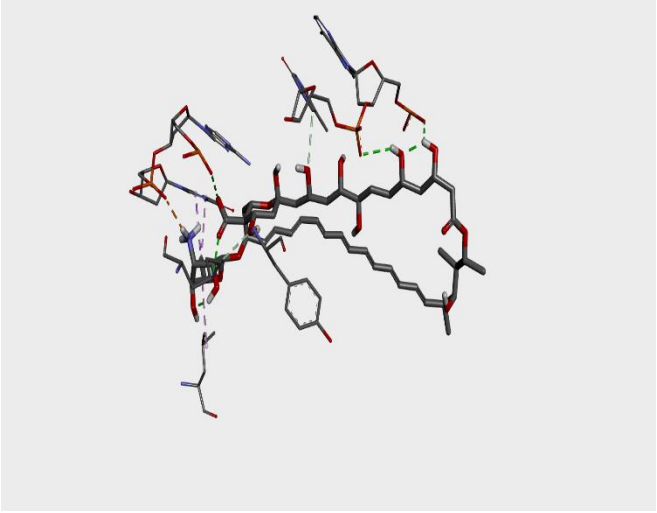
Azithromycin is used to treat or prevent some bacterial infections which cause middle ear infections, sore throat, pneumonia, typhoid, bronchitis, and sinusitis.

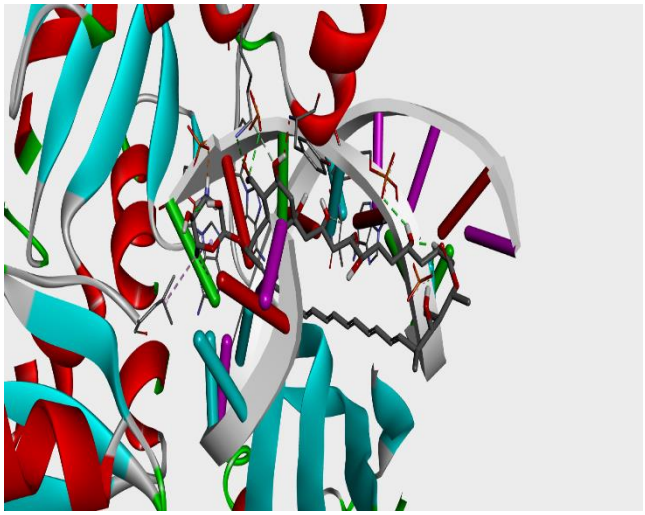
In recent years, it has been used primarily to prevent bacterial infections for infants and patients with weak immune systems [18]. The molecular docking study of compound (I2) provided information about the binding energy value. The binding energy given by the compound (I2) was equal to (-6.7 Kcal/mole). when it is compared with the binding energy of the drug (Azithromycin) (which is equal to -5.1 Kcal/mole), it is noted that the compound (I2) has a higher binding energy than the binding energy of the drug with the enzyme (HSP 90-alpha), as it is linked to the enzyme by a hydrogen bond and the alkyl bond with the amino acids (LYS58, MET98).

Table (1) information of molecular docking for (I2 and I3) and the drugs (Amphotericin B and Azithromycin).

Comp. Symb.	Type of Enzyme	Docking Score Kcal/mole	Interactions	Distance Å	Bonding	Bonding Types	Binding site of target	Binding site of ligand
Amphotericin B	dna polymerase iota	-8.6	D:LYS237:NZ - A:AmphotericinB:O	2.77422	Hydrogen Bond	Conventional Hydrogen Bond	D:LYS237:NZ	A:AmphotericinB:O
			A:AmphotericinB:H - A:AmphotericinB:O	1.89954	Hydrogen Bond	Conventional Hydrogen Bond	A:AmphotericinB:H	A:AmphotericinB:O
			A:AmphotericinB:O - E:DA872:OP2	3.0503	Hydrogen Bond	Conventional Hydrogen Bond	A:AmphotericinB:O	E:DA872:OP2
			A:AmphotericinB:H - A:AmphotericinB:O	1.88256	Hydrogen Bond	Conventional Hydrogen Bond	A:AmphotericinB:H	A:AmphotericinB:O
			A:AmphotericinB:C - D:LEU78	5.06512	Hydrophobic	Alkyl	A:AmphotericinB:C	D:LEU78
			E:DG873 - A:AmphotericinB:C	4.01549	Hydrophobic	Pi-Alkyl	E:DG873	A:AmphotericinB:C
I3	dna polymerase iota	-8.6	D:SER301:OG - A:UNL1:O2O2	3.11887	Hydrogen Bond	Conventional Hydrogen Bond	D:SER301:OG	A:UNL1:
			D:ARG357:NH1 - A:UNL1:O3	2.92132	Hydrogen Bond	Conventional Hydrogen Bond	D:ARG357:NH1	A:I3:O3
			D:SER407:OG - A:I3:O3	3.23997	Hydrogen Bond	Conventional Hydrogen Bond	D:SER407:OG	A:I3:O3
			A:I3:C15 - D:CYS409	4.32032	Hydrophobic	Alkyl	A:I3:C15	D:CYS409
			A:I3 - D:CYS409	4.34002	Hydrophobic	Pi-Alkyl	A:I3	D:CYS409
			A:I3 - D:ARG357	4.03185	Hydrophobic	Pi-Alkyl	A:I3	D:ARG357
AmphotericinB	HSP 90	-9.4	A:AmphotericinB:H - A:AmphotericinB:O	2.92584	Hydrogen Bond	Conventional Hydrogen Bond	A:AmphotericinB:H	A:AmphotericinB:O
			A:AmphotericinB:H - A:MET87:SD	2.68654	Hydrogen Bond	Conventional Hydrogen Bond	A:AmphotericinB:H	A:MET87:SD
			A:AmphotericinB:H - A:ASP91:OD1	2.95279	Hydrogen Bond	Conventional Hydrogen Bond	A:AmphotericinB:H	A:ASP91:OD1

Comp. Symb.	Type of Enzyme	Docking Score Kcal/mole	Interactions	Distance Å	Bonding	Bonding Types	Binding site of target	Binding site of ligand
I3	HSP 90	-6.7	A:ASN40:ND2 - A:I3:O1	3.34072	Hydrogen Bond	Conventional Hydrogen Bond	A:ASN40:ND2	A:I3:O1
			A:PHE128:N - A:I3:O1	3.19091	Hydrogen Bond Conventional	Hydrogen Bond	A:PHE128:N	A:I3:O1
			A:MET87:CE - A:I3	3.68211	Hydrophobic	Pi-Sigma	A:MET87:CE	A:I3
			A:MET87:SD - A:I3	3.97399	Other	Pi-Sulfur	A:MET87:SD	A:I3
			A:I3 - A:ALA44	4.46286	Hydrophobic	Pi-Alkyl	A:I3	A:ALA44
AmphotericinB	Lysine-specific demethylase	-9.2	A:GLN945:NE2 - A:AmphotericinB:O	2.61112	Hydrogen Bond	Conventional Hydrogen Bond	A:GLN945:NE2	A:AmphotericinB:O
			A:GLN945:NE2 - A:AmphotericinB:O	3.08449			A:GLN945:NE2	A:AmphotericinB:O
			A:AmphotericinB:H - B:GLN1007:OE1	2.42388			A:AmphotericinB:H	B:GLN1007:OE1
			A:AmphotericinB:H - B:THR1017:OG1	2.17382			A:AmphotericinB:H	B:THR1017:OG1
			A:AmphotericinB:H - A:ALA942:O	1.83753			A:AmphotericinB:H	A:ALA942:O
I3	Lysine-specific demethylase	-7.3	A:ARG938:NH1 - A:I3:O1	3.15161	Hydrogen Bond	Conventional Hydrogen Bond	A:ARG938:NH1	A:I3:O1
			B:THR946:N - A:I3:N1	3.24727	Hydrogen Bond	Conventional Hydrogen Bond	B:THR946:N	A:I3:N1
			A:I3 - B:PRO963	4.88149	Hydrophobic	Pi-Alkyl	A:I3	B:PRO963
			A:I3 - B:PRO963	5.22398	Hydrophobic	Pi-Alkyl	A:I3	B:PRO963
Azithromycin	HSP 90-alpha	-5.1	A:ASN79:ND2 - A:Azithromycin:O	3.05689	Hydrogen Bond	Conventional Hydrogen Bond	A:ASN79:ND2	A:Azithromycin:O
			A:Azithromycin:H - A:ASN79:OD1	2.12919	Hydrogen Bond	Conventional Hydrogen Bond	A:Azithromycin:H	A:ASN79:OD1
			A:Azithromycin:H - A:Azithromycin:O	2.59669	Hydrogen Bond	Conventional Hydrogen Bond	A:Azithromycin:H	A:Azithromycin:O
			A:Azithromycin:C - A:VAL92	4.96975	Hydrophobic	Alkyl	A:Azithromycin:C	A:VAL92
			A:HIS77 - A:Azithromycin:C	4.61305	Hydrophobic	Pi-Alkyl	A:HIS77	A:Azithromycin:C
I2	HSP 90-alpha	-6.7	A:LYS58:NZ - A:I2:O2	3.19598	Hydrogen Bond	Conventional Hydrogen Bond	A:LYS58:NZ	A:I2:O2
			A:I2 - A:MET98	5.2538	Hydrophobic	Pi-Alkyl	A:I2	A:MET98





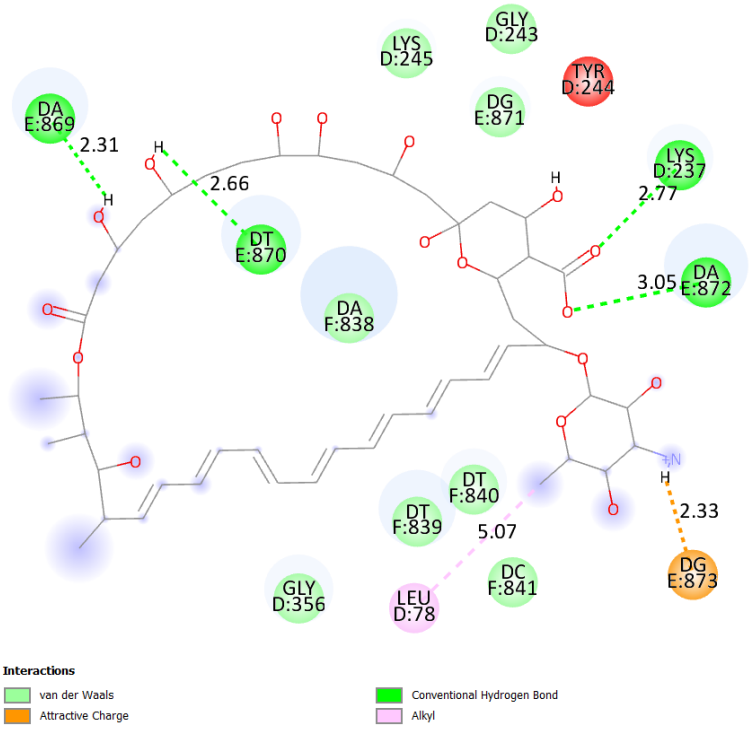
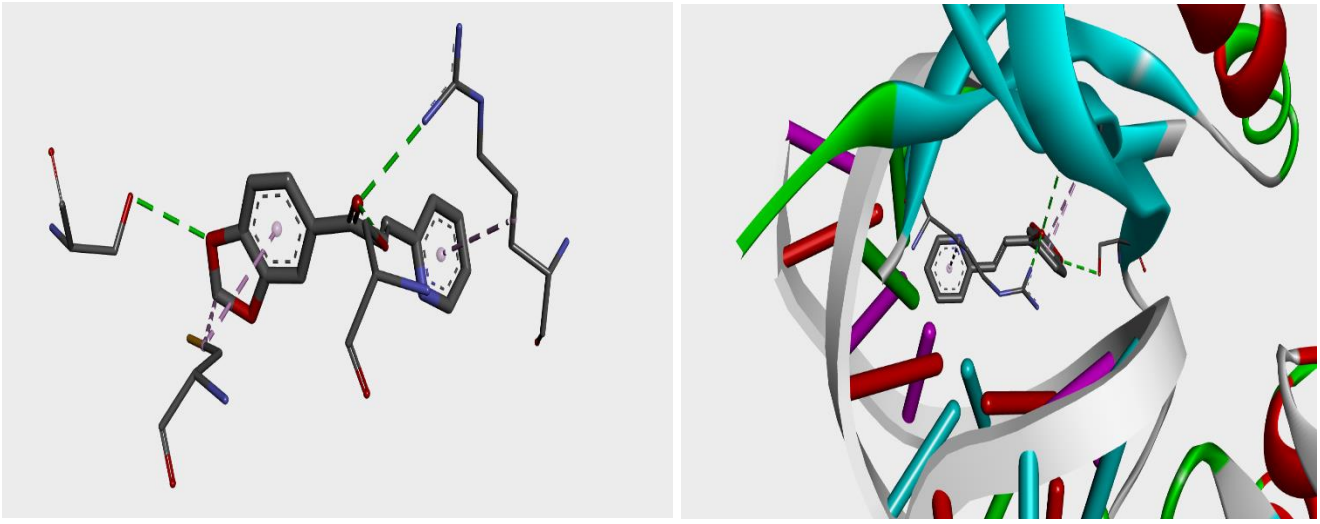


Figure (3): The interaction between the drug Amphotericin B and the enzyme DNA polymerase iota in 3D and 2D dimensions.



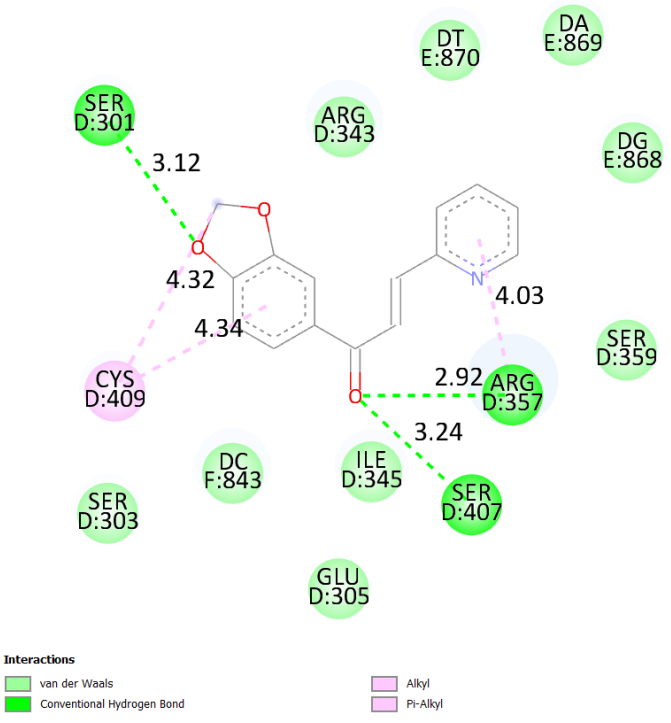
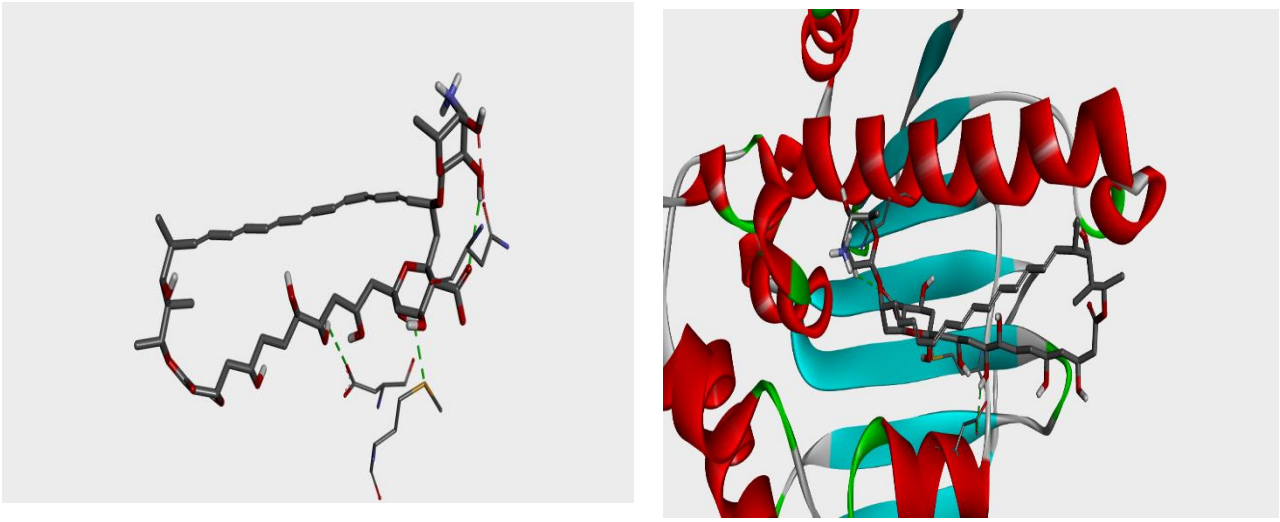


Figure (4): The interaction between I3 and the enzyme DNA polymerase iota in 3D and 2D dimensions.



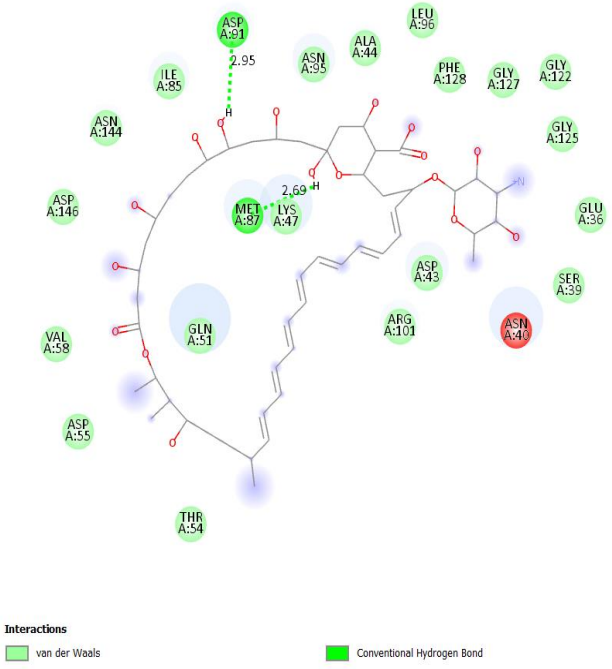
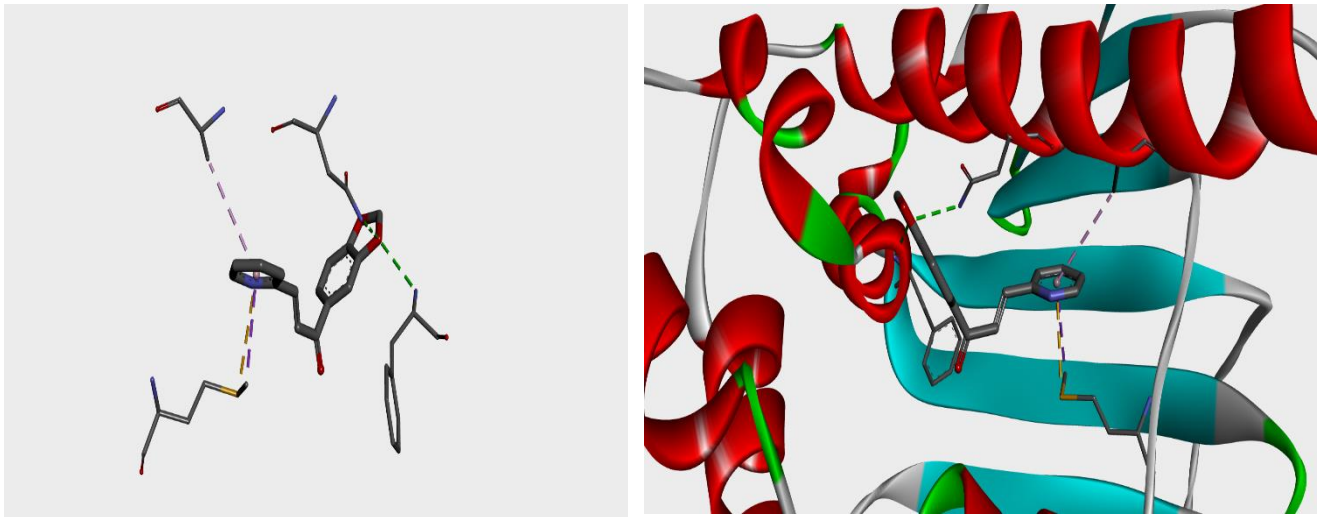


Figure (5): The interaction between the drug Amphotericin B and the enzyme HSP90 in 3D and 2D dimensions.



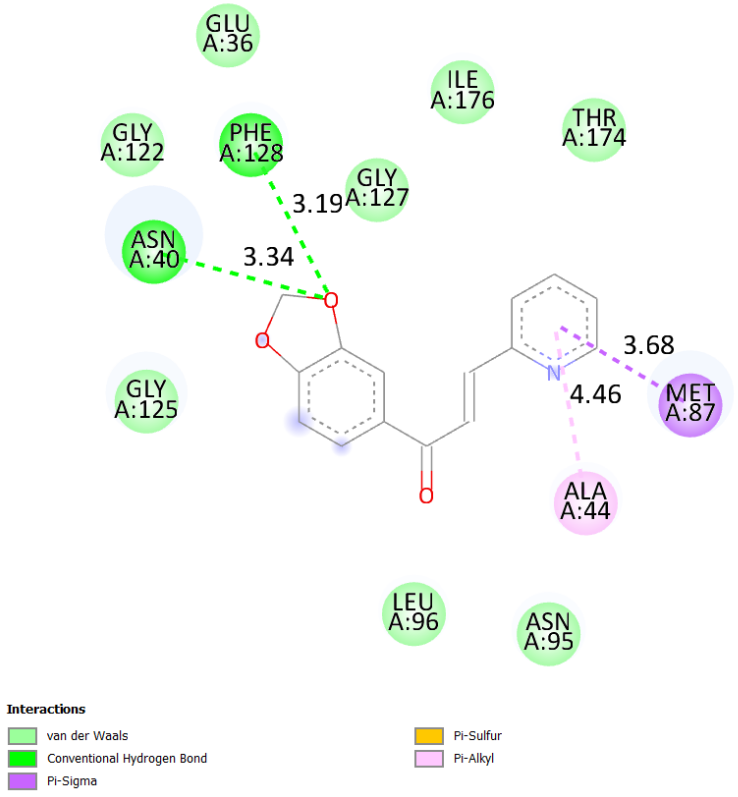
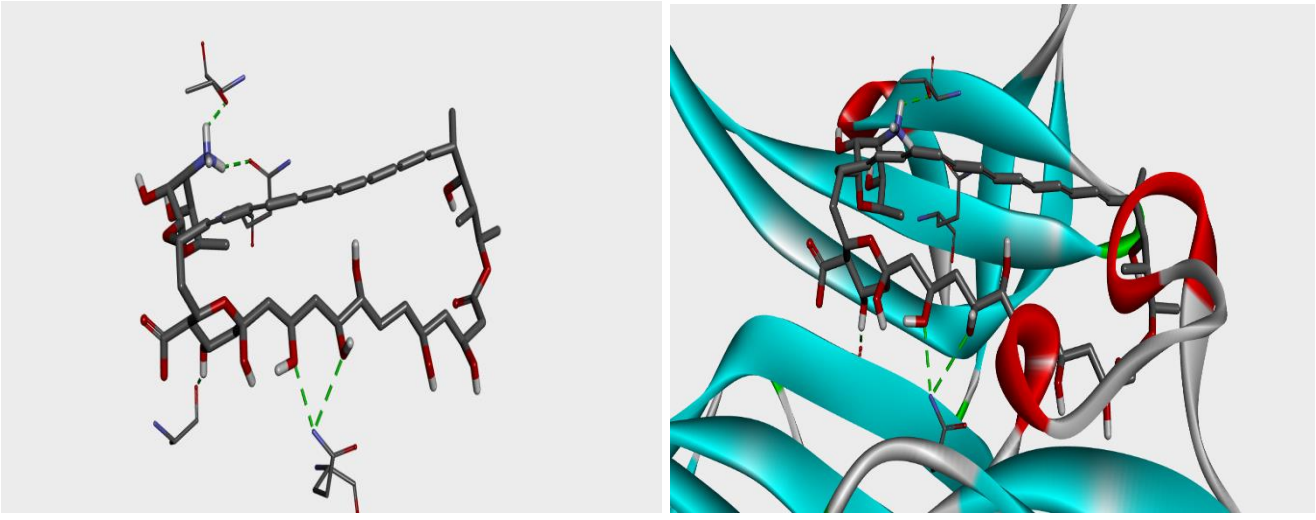


Figure (6): The interaction between I3 and the enzyme HSP 90 in 3D and 2D dimensions.



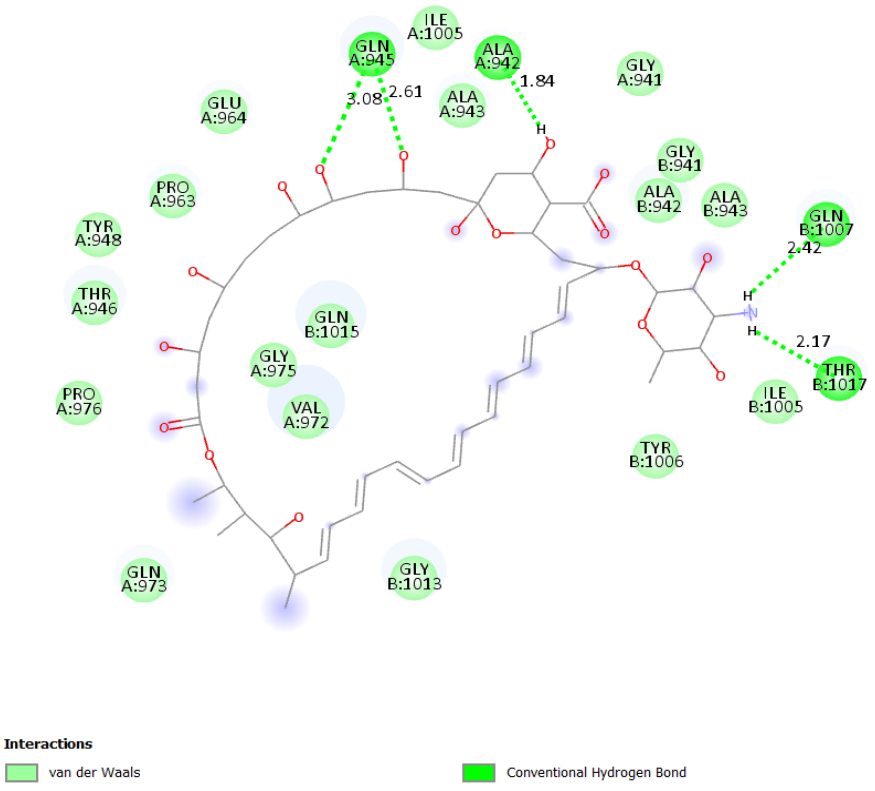


Figure (7): The interaction between the drug Amphotericin B and the enzyme Lysine-specific demethylase in 3D and 2D dimensions.

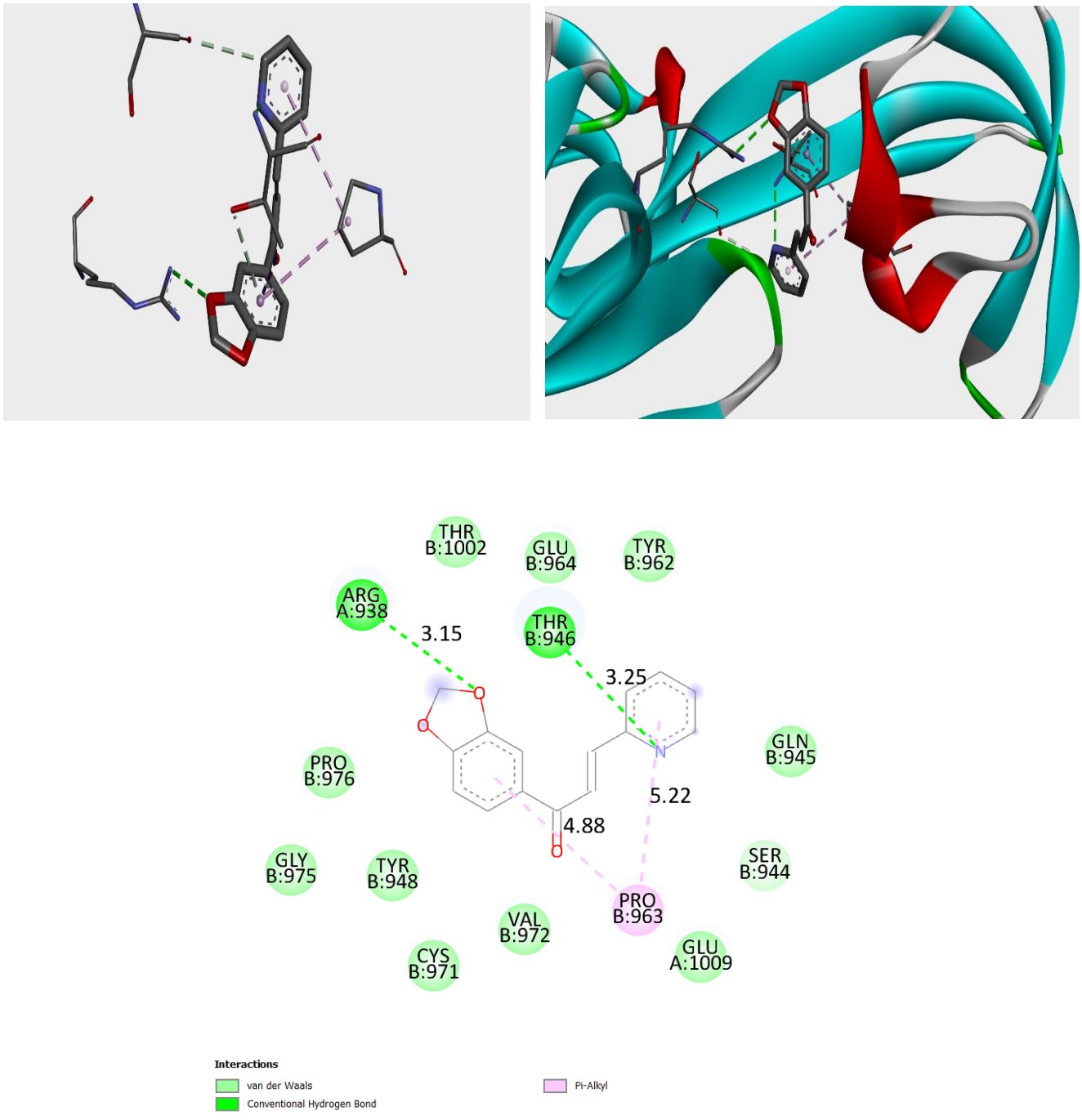


Figure (8): The interaction between I3 and the enzyme Lysine-specific demethylase in 3D and 2D dimensions.

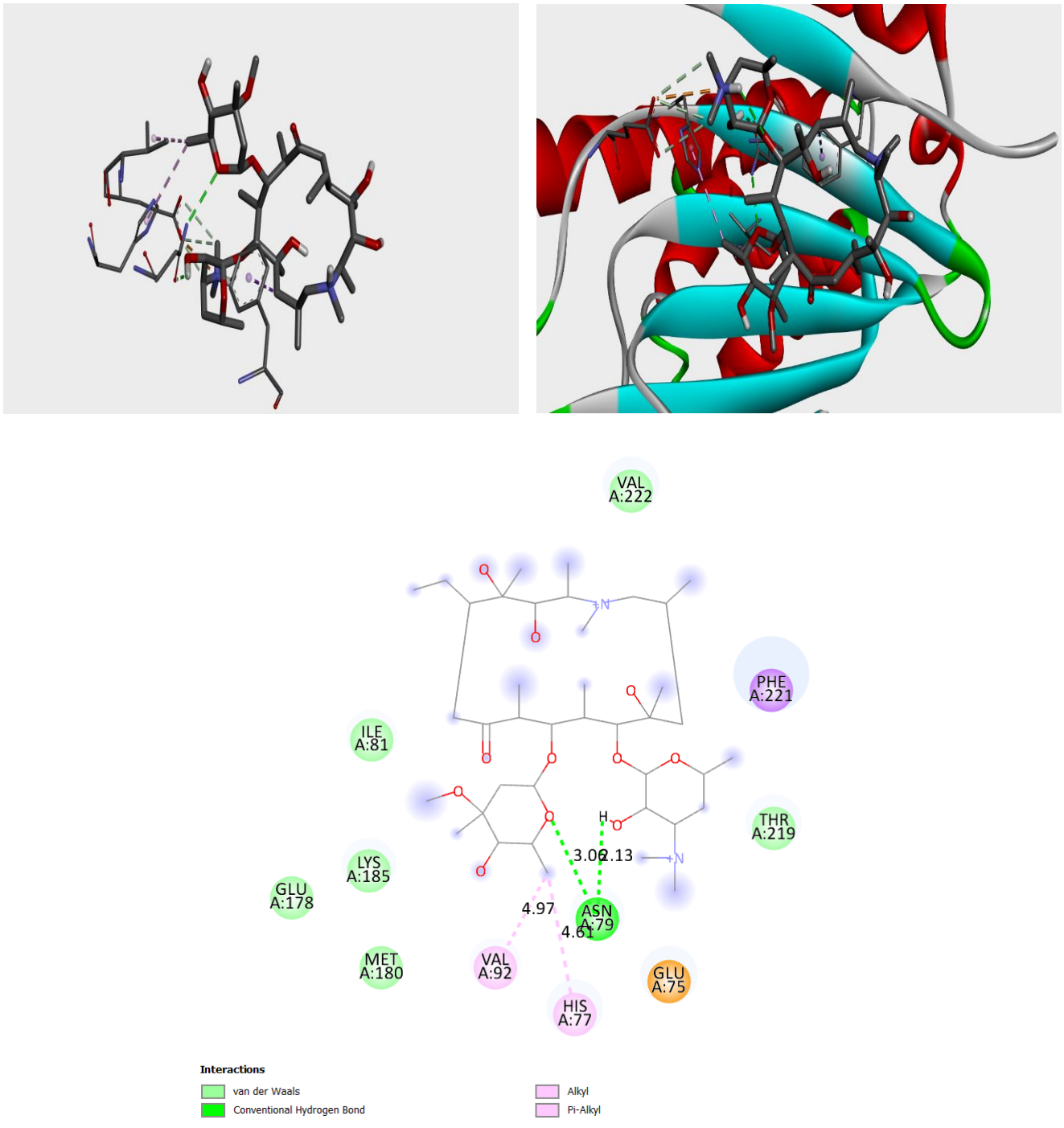


Figure (9): The interaction between the drug Azithromycin and the enzyme HSP 90-alpha in 3D and 2D dimensions.

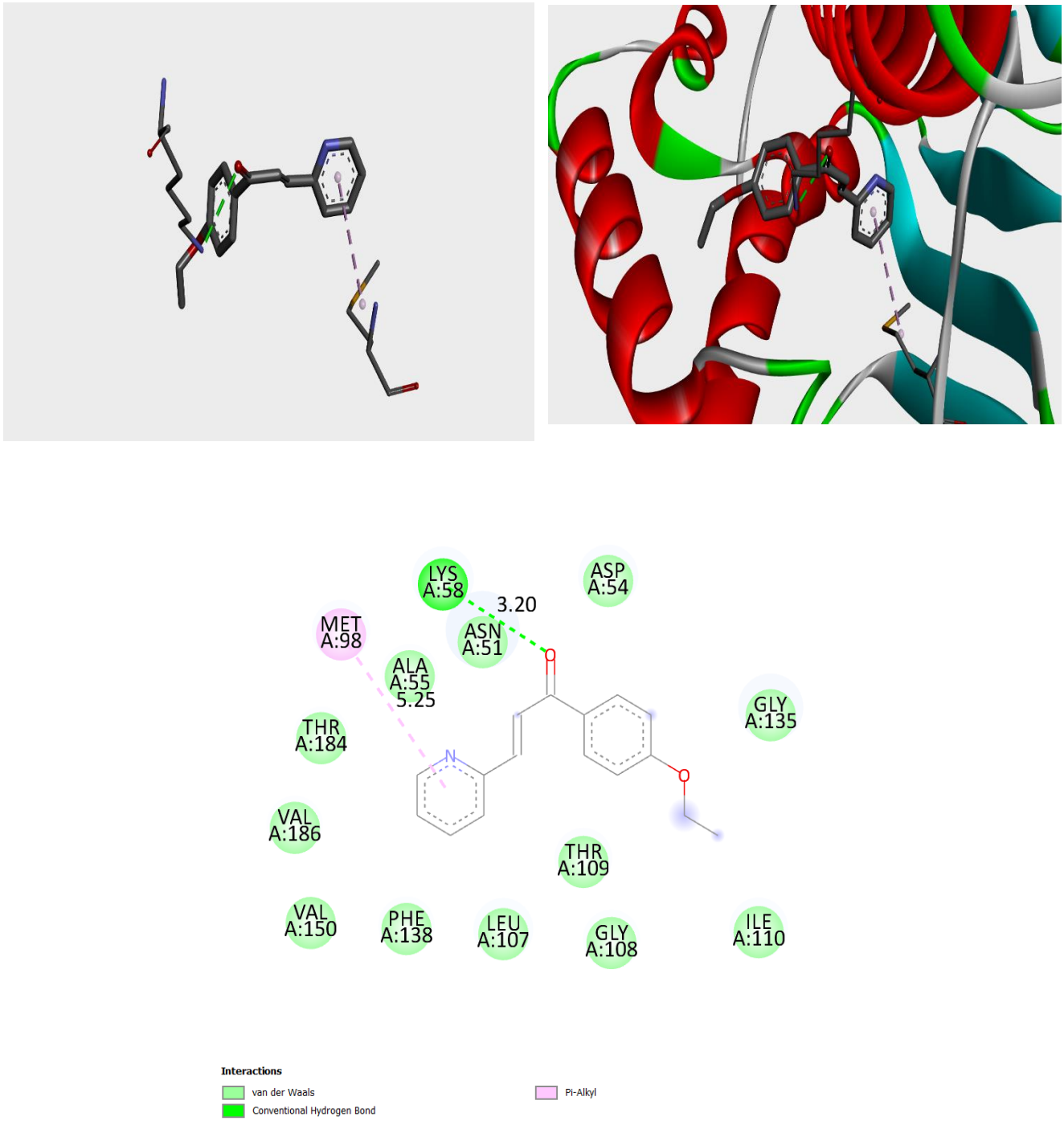


Figure (10): The interaction between I2 and the enzyme HSP 90-alpha in 3D and 2D dimensions.

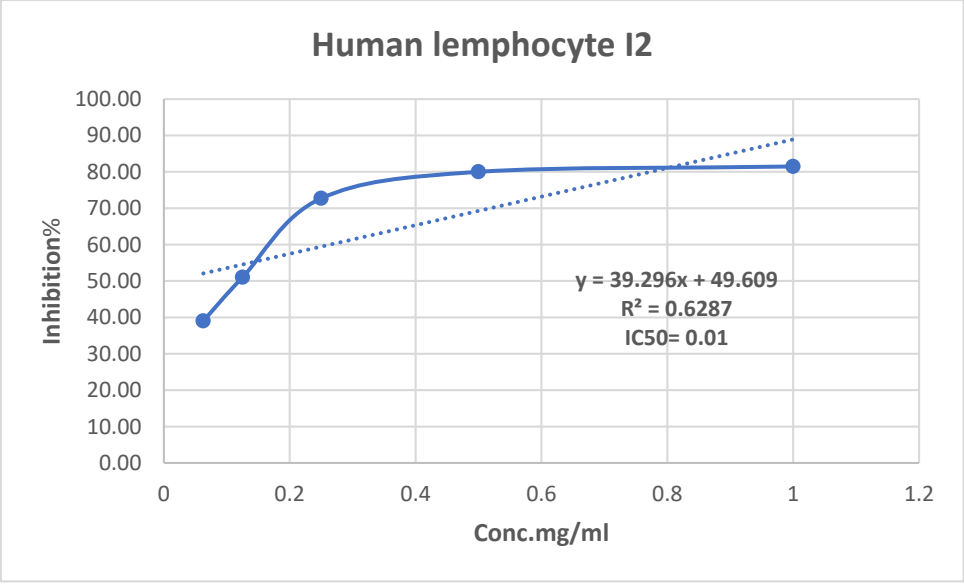
CONCLUSIONS

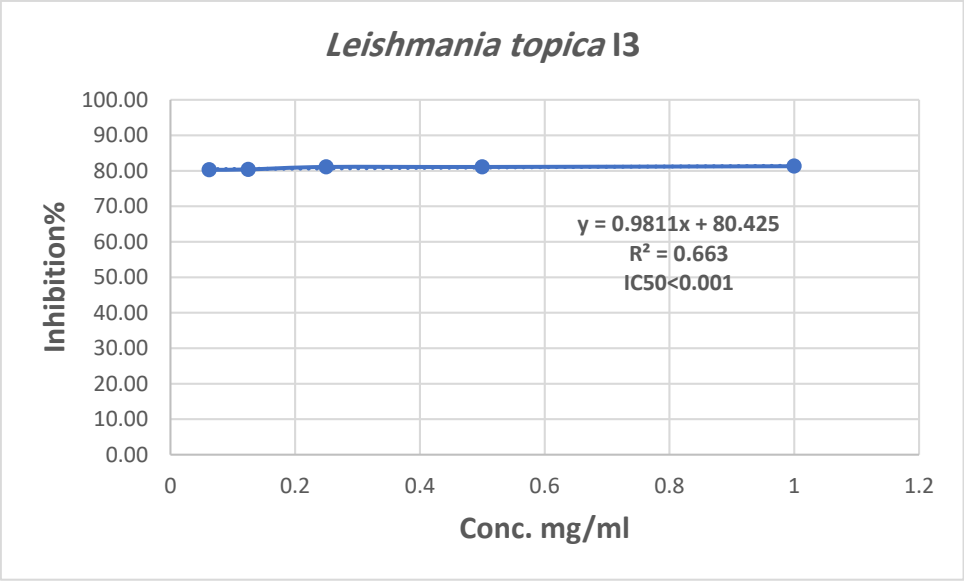
In the current study, the prepared chalcones and its derivatives (1-7) were synthesized using distinct procedures, and they have activity against *L. tropica* and human lymphocyte. The biological activity increased in a concentration-dependent manner. Compound (3) revealed the high significant activity against *L. tropica* and human lymphocyte. They revealed that they have an increased biological significance, in addition to their value as novel and effective organic chemicals.

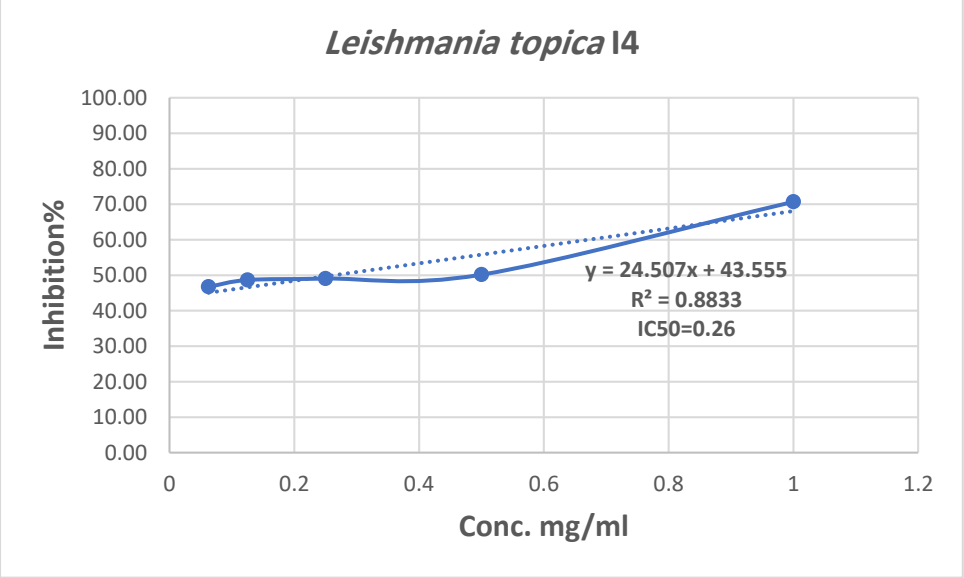
This study reflects that the chalcones were prepared by a distinct procedure and it shows that they have activity against *L. tropica* and human lymphocyte. The biological activity increased in a concentration-dependent manner. Compound (3) revealed the high significant activity against *L. tropica* and human lymphocyte. In addition, they exhibit that they have an increased biological indication, besides to their novel and effective organic chemicals.

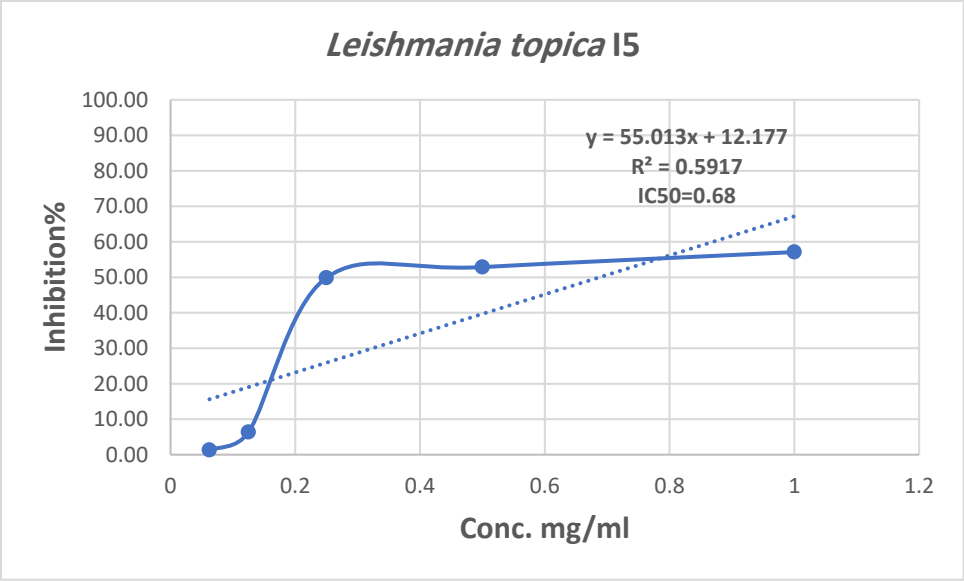
Acknowledgments

The authors would like to thank the Department of Applied Chemistry, College of Applied Sciences, University of Samarra, Iraq, for providing the necessary facilities.

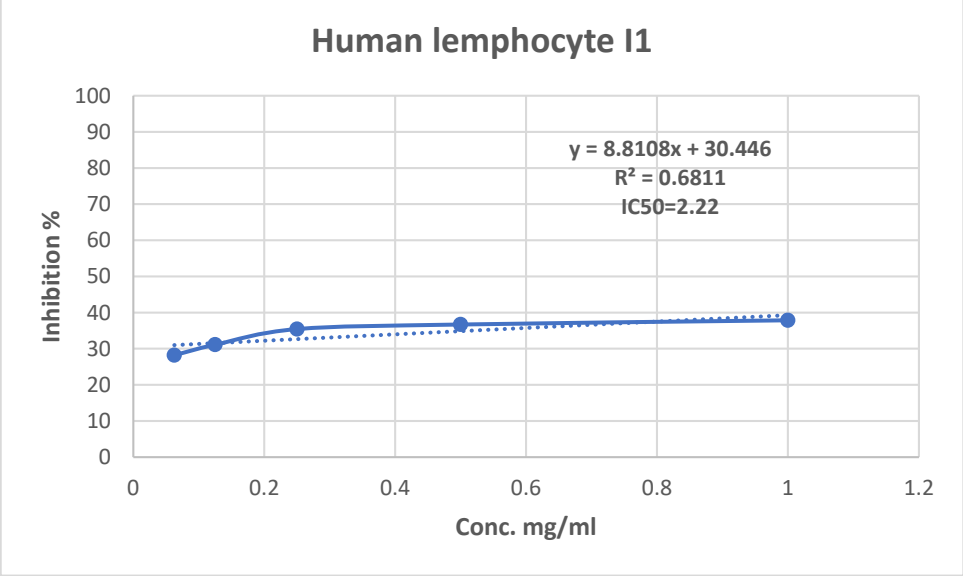


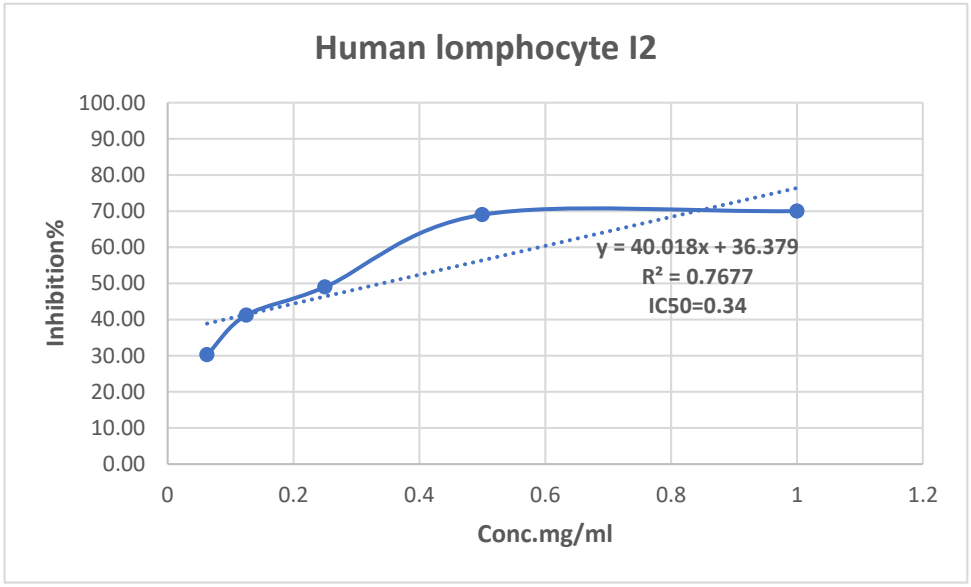

Leishmania topica I3
Inhibition%
Conc. mg/ml
y = 0.9811x + 80.425
R² = 0.663
IC50<0.001
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
0.2
0.4
0.6
0.8
1
1.2

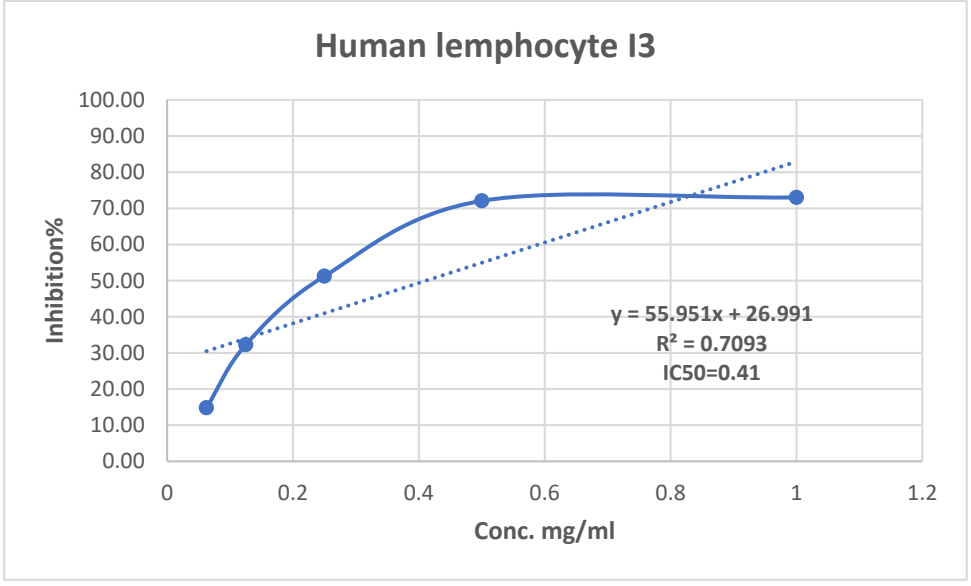

Leishmania topica I4
Inhibition%
Conc. mg/ml
y = 24.507x + 43.555
R² = 0.8833
IC50=0.26
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
0.2
0.4
0.6
0.8
1
1.2

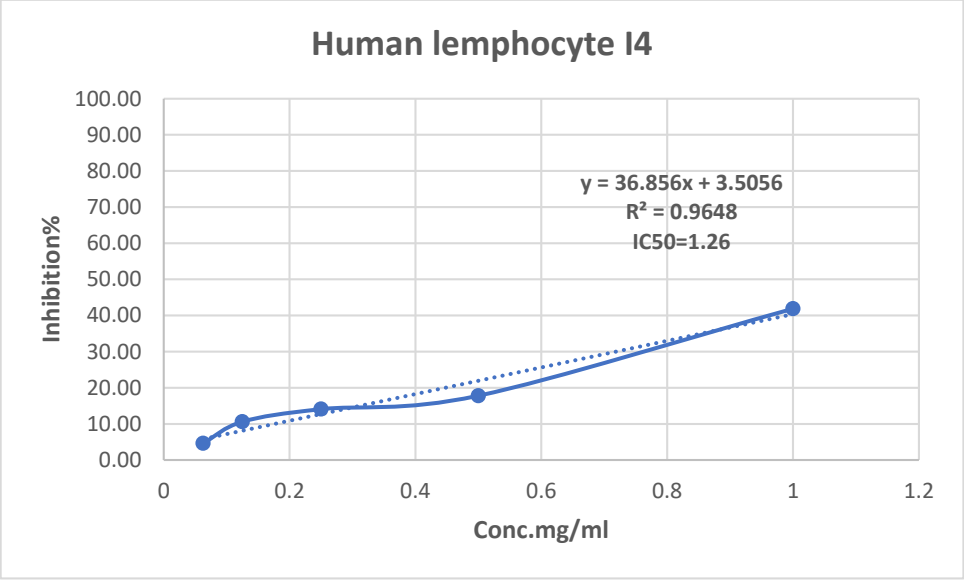


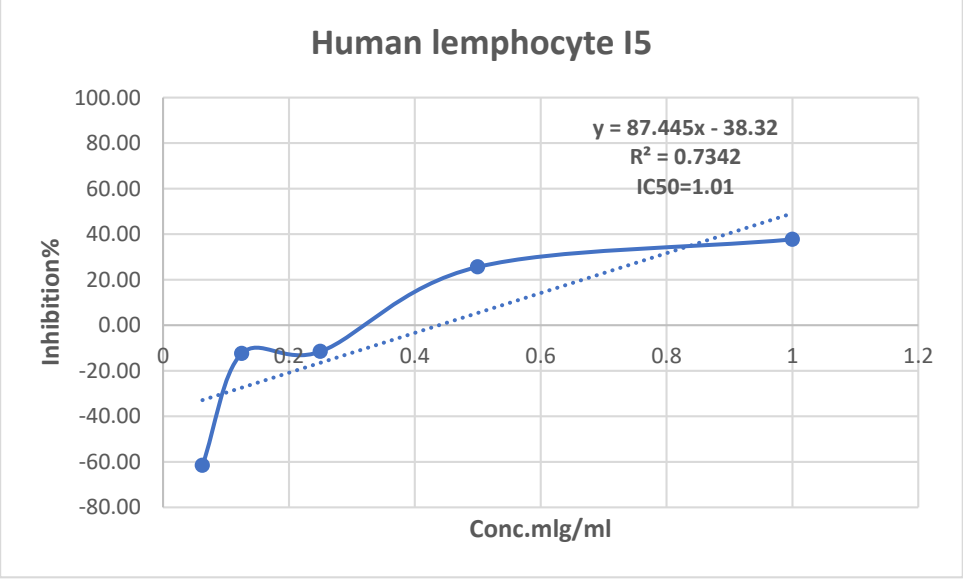
Human lymphocytes




Human lomphocyte I2
Inhibition%
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
0.2
0.4
0.6
0.8
1
1.2
Conc.mg/ml
y = 40.018x + 36.379
R² = 0.7677
IC50=0.34


Human lemphocyte I3
Inhibition%
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
0.2
0.4
0.6
0.8
1
1.2
Conc. mg/ml
y = 55.951x + 26.991
R² = 0.7093
IC50=0.41


Human lemphocyte I4
Inhibition%
Conc.mg/ml
y = 36.856x + 3.5056
R² = 0.9648
IC50=1.26
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
0.2
0.4
0.6
0.8
1
1.2


Human lemphocyte I5
Inhibition%
Conc.mlg/ml
y = 87.445x - 38.32
R² = 0.7342
IC50=1.01
100.00
80.00
60.00
40.00
20.00
0.00
-20.00
-40.00
-60.00
-80.00
0
0.2
0.4
0.6
0.8
1
1.2

REFERENCES

1. Hadi AA. *Synthesis and microbial studies of pyrazoline and its derivatives*. Department of Chemistry, Fergusson College, Pune University, India; 2012.
2. Kumar V., Dhawan S., Girase P.S., Awolade P., Shinde S.R., Karpoormath R., Singh P. Recent advances in chalcone-based anticancer heterocycles: A structural and molecular target perspective. *Current Medicinal Chemistry* 2021; 28(33):6805–6845. https://doi.org/10.2174/0929867328666210322102836
3. Mohammad H.J., Salih I.I., Mahmood R.T. Synthesis of heterocyclic nitrogen compounds using cyclohexene derivative with various primary amines. *Systematic Reviews in Pharmacy* 2020; 11(9):124–129.
4. Prasad Y.R., Kumar P.R., Deepti C.A., Ramana M.V. Synthesis and antimicrobial activity of some novel chalcones of 2-hydroxy-1-acetonaphthone and 3-acetyl coumarin. *Journal of Chemistry* 2006; 3(4):236–241. https://doi.org/10.1155/2006/395386
5. De S., SK A.K., Shah S.K., Kazi S., Sarkar N., Banerjee S., Dey S. Pyridine: The scaffolds with significant clinical diversity. *RSC Advances* 2022; 12(24):15385–15406. https://doi.org/10.1039/D2RA01571D
6. Casalle N., de Barros Pinto Grifoni L., Bosco Mendes A.C., Delort S., Massucato E.M.S. Mucocutaneous leishmaniasis with rare manifestation in the nasal mucosa and cartilage bone septal. *Case Reports in Infectious Diseases* 2020; Article ID 8876020. https://doi.org/10.1155/2020/8876020
7. Meena A.K. *International Journal of Contemporary Research and Review.* 2010; 1(02).
8. Ortalli M., Ilari A., Colotti G., De Ionna I., Battista T., Bisi A., et al. Identification of chalcone-based antileishmanial agents targeting trypanothione reductase. *European Journal of Medicinal Chemistry* 2018; 152:527–541. https://doi.org/10.1016/j.ejmech.2018.04.057
9. Fernandez-Botran R., Vetvicka V. *Advanced Methods in Cellular Immunology.* CRC Press; 2000. https://doi.org/10.1201/9781420039238
10. Wichmann G., Herbarth O., Lehmann I. The mycotoxins citrinin, gliotoxin, and patulin affect interferon-γ rather than interleukin-4 production in human blood cells. *Environmental Toxicology* 2002; 17(3):211–218. https://doi.org/10.1002/tox.10050
11. Freshney R.I. *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications.* Wiley; 2015. https://doi.org/10.1002/9780470649367
12. Wang B., Relling M.V., Storm M.C., Woo M.H., Ribeiro R., Pui C.H., Hak L.J. Evaluation of immunologic cross-reaction of antiasparaginase antibodies in acute lymphoblastic leukemia and lymphoma patients. *Leukemia* 2003; 17(8):1583–1588.
13. Al-Rawi K.M., Khalafallah A.M. *Design & Analysis of Agricultural Experiments.* The Book House for Printing and Publishing, University of Mosul; 2000. 488 pp.
14. Al-Jaber N.A., Bougasim A.S., Karah M.M. Study of Michael addition on chalcones and chalcone analogues. *Journal of Saudi Chemical Society* 2012; 16(1):45–53. https://doi.org/10.1016/j.jscs.2010.10.017
15. Hadi AA. Synthesis and microbial studies of pyrazoline and its derivatives. Department of Chemistry, Fergusson College, Pune University, India; 2012.
16. Hadi AA. Synthesis and microbial studies of pyrazoline and its derivatives. Department of Chemistry, Fergusson College, Pune University, India; 2012.
17. Vahedpour T., Hamzeh-Mivehroud M., Hemmati S., Dastmalchi S. Synthesis of 2-pyrazolines from hydrazines: Mechanisms explained. *ChemistrySelect* 2021; 6(25):6483–6506. https://doi.org/10.1002/slct.202101467

18. Larabi M., Yardley V., Loiseau P.M., Appel M., Legrand P., Gulik A., et al. Toxicity and antileishmanial activity of a new stable lipid suspension of amphotericin B. *Antimicrobial Agents and Chemotherapy* 2003; 47(12):3774–3779. https://doi.org/10.1128/aac.47.12.3774-3779.2003
19. Bakheit A.H., Al-Hadiya B.M., Abd-Elgalil A.A. Azithromycin. *Profiles of Drug Substances, Excipients and Related Methodology* 2014; 39:1–40. https://doi.org/10.1016/B978-0-12-800173-8.00001-5
20. Cetin Y., Bullerman L.B. Cytotoxicity of *Fusarium* mycotoxins to mammalian cell cultures as determined by the MTT bioassay. *Food and Chemical Toxicology* 2005; 43(5):755–764. https://doi.org/10.1016/j.fct.2005.01.016
21. Abraham V.C., Towne D.L., Waring J.F., Warrior U., Burns D.J. Application of a high-content multiparameter cytotoxicity assay to prioritize compounds based on toxicity potential in humans. *SLAS Discovery* 2008; 13(6):527–537. https://doi.org/10.1177/1087057108318428
22. Waring P., Khan T., Sjaarda A. Apoptosis induced by gliotoxin is preceded by phosphorylation of histone H3 and enhanced sensitivity of chromatin to nuclease digestion. *Journal of Biological Chemistry* 1997; 272(29):17929–17936.
23. Shergalis A., Xue D., Gharbia F.Z., Driks H., Shrestha B., Tanweer A., et al. Characterization of aminobenzylphenols as protein disulfide isomerase inhibitors in glioblastoma cell lines. *Journal of Medicinal Chemistry* 2020; 63(18):10263–10286. https://doi.org/10.1021/acs.jmedchem.0c00728
24. Nierman W.C., Fedorova N. Subtelomeric diversity as a major force in evolution of *Aspergillus* secondary metabolism and virulence pathways. *3rd Advances Against Aspergillosis*, Miami; 16–19 January 2008.
25. Wu X.J., Stahl T., Hu Y., Kassie F., Mersch-Sundermann V. The production of reactive oxygen species and the mitochondrial membrane potential are modulated during onion oil–induced cell cycle arrest and apoptosis in A549 cells. *The Journal of Nutrition* 2006; 136(3):608–613. https://doi.org/10.1093/jn/136.3.608
26. Boeck P., Bandeira Falcão C.A., Leal P.C., Yunes R.A., Filho V.C., Torres-Santos E.C., Rossi-Bergmann B. *Bioorganic & Medicinal Chemistry* 2006; 14(15):1–2. *(Note: Original reference incomplete; retained as provided)*
27. Torres-Santos E.C., Sampaio-Santos M.I., Buckner F.S., Yokoyama K., Gelb M., Urbina J.A., Rossi-Bergmann B. Altered sterol profile induced in *Leishmania amazonensis* by a natural dihydroxymethoxylated chalcone. *Journal of Antimicrobial Chemotherapy* 2009; 63(3):469–472.
28. Chen M., Christensen S.B., Theander T.G., Kharazmi A. Antileishmanial activity of licochalcone A in mice infected with *Leishmania major* and in hamsters infected with *Leishmania donovani*. *Antimicrobial Agents and Chemotherapy* 1994; 38(6):1339–1344.
29. Ortalli M., Ilari A., Colotti G., De Ionna I., Battista T., Bisi A., et al. Identification of chalcone-based antileishmanial agents targeting trypanothione reductase. *European Journal of Medicinal Chemistry* 2018; 152:527–541.
30. Passalacqua T.G., Torres F.A., Nogueira C.T., de Almeida L., Del Cistia M.L., dos Santos M.B., et al. 2′,4′-Dihydroxychalcone as a potential inhibitor of glycerol-3-phosphate dehydrogenase from *Leishmania* species. *Bioorganic & Medicinal Chemistry Letters* 2015; 25(17):3564–3568.
31. Ogungbe I.V., Erwin W.R., Setzer W.N. Antileishmanial phytochemical phenolics: Molecular docking to potential protein targets. *Journal of Molecular Graphics and Modelling* 2014; 48:105–117.

32. Chen M., Zhai L., Christensen S.B., Theander T.G., Kharazmi A. Inhibition of fumarate reductase in *Leishmania major* and *L. donovani* by chalcones. *Antimicrobial Agents and Chemotherapy* 2001; 45(7):2023–2029.
33. Garcia A.R., Oliveira D.M., Jesus J.B., Souza A.M., Sodero A.C.R., Vermelho A.B., et al. Identification of chalcone derivatives as inhibitors of *Leishmania infantum* arginase and promising antileishmanial agents. *Frontiers in Chemistry* 2021; 8:624678.
34. Souza J.M., de Carvalho É.A., Candido A.C.B., de Mendonça R.P., da Silva M.F., Parreira R.L., et al. Licochalcone A exhibits leishmanicidal activity in vitro and in experimental model of *Leishmania (Leishmania) infantum*. *Frontiers in Veterinary Science* 2020; 7:527.
35. Tirona R.G., Kim R.B. Introduction to clinical pharmacology. In: *Clinical and Translational Science*. Academic Press; 2017:365–388.
36. Osman M.S., Awad T.A., Shantier S.W., Garelnabi E.A., Osman W., Mothana R.A., et al. Identification of some chalcone analogues as potential antileishmanial agents: An integrated in vitro and in silico evaluation. *Arabian Journal of Chemistry* 2022; 15(4):103717.
37. Yan S., Wan G. Tumor-associated macrophages in immunotherapy. *The FEBS Journal* 2021; 288(21):6174–6186.
38. Petrova V., Annicchiarico-Petruzzelli M., Melino G., Amelio I. The hypoxic tumour microenvironment. *Oncogenesis* 2018; 7(1):10.
39. Zhou J., Tang Z., Gao S., Li C., Feng Y., Zhou X. Tumor-associated macrophages: recent insights and therapies. *Frontiers in Oncology* 2020; 10:188.
40. Fu L.Q., Du W.L., Cai M.H., Yao J.Y., Zhao Y.Y., Mou X.Z. The roles of tumor-associated macrophages in tumor angiogenesis and metastasis. *Cellular Immunology* 2020; 353:104119.
41. Liu Y., Li L., Li Y., Zhao X. Research progress on tumor-associated macrophages and inflammation in cervical cancer. *BioMed Research International* 2020; 2020:6842963.
42. Vergadi E., Ieronymaki E., Lyroni K., Vaporidi K., Tsatsanis C. Akt signaling pathway in macrophage activation and M1/M2 polarization. *The Journal of Immunology* 2017; 198(3):1006–1014.
43. Byles V., Covarrubias A.J., Ben-Sahra I., Lamming D.W., Sabatini D.M., Manning B.D., Horng T. The TSC–mTOR pathway regulates macrophage polarization. *Nature Communications* 2013; 4:2834.
44. Horta B., Freitas-Silva J., Silva J., Dias F., Teixeira A.L., Medeiros R., et al. Antitumor effect of chalcone derivatives against human prostate (LNCaP and PC-3), cervix HPV-positive (HeLa) and lymphocyte (Jurkat) cell lines and their effect on macrophage functions. *Molecules* 2023; 28(5):2159.
45. Lee J.S., Bukhari S.N.A., Fauzi N.M. Effects of chalcone derivatives on players of the immune system. *Drug Design, Development and Therapy* 2015; 4761–4778.
46. Horta B., Freitas-Silva J., Silva J., Dias F., Teixeira A.L., Medeiros R., et al. Antitumor effect of chalcone derivatives against human prostate (LNCaP and PC-3), cervix HPV-positive (HeLa) and lymphocyte (Jurkat) cell lines and their effect on macrophage functions. *Molecules* 2023; 28(5):2159.
47. Rao Y.K., Fang S.H., Tzeng Y.M. Differential effects of synthesized 2′-oxygenated chalcone derivatives: modulation of human cell cycle phase distribution. *Bioorganic & Medicinal Chemistry* 2004; 12(10):2679–2686.

"تخليق وتوصيف مشتقات البيرول، البيريدين، والبيرازولين: النشاط البيولوجي ضد طفيل الليشمانيا المدارية، والخلايا الليمفاوية البشرية، ودراسة الرسو الجزيئي"

إسراء آي. صالح[1*] ، مها صالح حسي[1]

[1] قسم الكيمياء، كلية التربية، جامعة سامراء، سامراء، العراق.

ملخص

قامت هذه الدراسة بتحضير سبعة مركّبات I1–I7تحتوي على حلقات البيرول والبيريدين والبيرازولين. في البداية، تم تحضير أربعة مركّبات شالكون I1–I4كمركّبات أولية، ثم تم تحضير ثلاث نوى بيرازولين I5–I7منها. تم تصنيع مركّبات الشالكون من خلال تفاعل -4إيثوكسي أسيتوفينون أو -3,4ميثيلين ديوكسي أسيتوفينون مع بيرول–2–كاربوكسي ألدهيد و -2بيريدين كاربوكسي ألدهيد. أما مشتقات البيرازولين فقد تم الحصول عليها من خلال تفاعل مركّبات الشالكون مع مشتقات الهيدرازين :الهيدرازين، 2–هيدرازينو بنزوثيازول، وفينيل هيدرازين. تم إثبات بنية جميع المركّبات باستخدام أطياف FTIR و ^{1}H NMR و ^{13}C NMRإضافةً إلى دراسة الخواص الفيزيائية لها. تم تقييم النشاط البيولوجي لبعض المركّبات ضد طفيلي Leishmania tropicaوخلايا اللمف البشرية باستخدام اختبار MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). وأظهر المركّب I3أعلى نسبة تثبيط للطفيلي بلغت 81%–80، بينما أظهر المركّب I2أعلى سمّية تجاه الخلايا اللمفاوية بنسبة 70%–54عند تراكيز تتراوح بين (1 إلى 0.0625) ملغم/مل. تم دعم هذه النتائج بدراسة الارتباط الجزيئي (Molecular Docking) مع ثلاثة إنزيمات مختلفة هي DNA polymerase iota، HSP90، و lysine-specific demethylase.وقد تمت مقارنة طاقات الارتباط مع دواءي Amphotericin Bو Azithromycin، وكانت نتائج الارتباط قريبة من تلك التي حققها الدواءان، مما يشير إلى إمكانية ترشيح هذه المركّبات كمضادات واعدة لليشمانيا وربما كعوامل مؤثرة في الخلايا اللمفاوية مستقبلاً.

الكلمات الدالة: 5–أسيتيل–1,3–بنزودايأوكسول، تفاعل كلايزن–شميت، بيرازولين، اختبار MTT.

[*] ***المؤلف المراسل: إسراء آي. صالح***

esraa.ismaeel@uosamarra.edu.iq

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