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Synthesis and Characterization of Some New Pyridine and Pyrimidine Derivatives and Studying Their Biological Activities

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Abstract:

Heterocyclic systems, which are essential in medicinal chemistry due to their promising cytotoxic activity, are one of the most important families of organic molecules found in nature or produced in the laboratory. As a result of coupling *N*-(4-nitrophenyl)-3-oxo-butanamide (**3**) using thiourea, indole-3-carboxaldehyde, or piperonal, the pyrimidine derivatives (**5a** and **5b**) were produced. Furthermore, pyrimidine **9** was synthesized by reacting thiophene-2-carboxaldehyde with ethyl cyanoacetate and urea with potassium carbonate as a catalyst. The chalcones **11a** and **11b** were synthesized by reacting equal molar quantities of 1-naphthaldehyde and 2-quinoline carboxaldehyde with 4-Bromo acetophenone and 4-fluoro acetophenone respectively. Pyrimidine **13** was synthesized by reacting chalcone **11a** with guanidine hydrochloride in the presence of potassium hydroxide. Pyridine derivative **14** was prepared from the reaction of chalcone **11b** with ethyl cyanoacetate and ammonium acetate in glacial acetic acid. In addition, the reaction of 4-methyl benzaldehyde and 4-fluoro acetophenone with ethyl cyanoacetate and ammonium acetate in *n*-butanol gave pyridine derivative **16**. Spectral investigations (FT-IR, ¹H, and ¹³C-NMR) and EI-MS spectra were used to determine the structure of the prepared compounds. The synthesized derivatives were tested *in vitro* for their potential cytotoxicity against two different human cancer cell types, MCF-7 (breast cancer cell) or HepG2 (liver cancer cell). Compounds **5a** and **14** displayed cytotoxic activity versus HepG2 cell line with IC₅₀ values of 43.84 and 57.14 μg /mL, respectively. Furthermore, the pyridine compound **14** demonstrated cytotoxic action versus MCF-7 with an IC₅₀ value of 50.84 g/mL. The antibacterial and anti-parasitic properties of the synthesized derivatives have also been described.

Keywords: Anti-bacterial, Anti-parasitic, Cyanopyridine, cells (HepG2, MCF-7), Pyrimidine.

Introduction:

Nitrogen-based heterocyclic compounds are regarded as an extremely important class of compounds that play an important role in health care and pharmaceutical drug design^{1,2}. Chalcones have been linked to a variety of biological activities. Furthermore, they are well-known intermediates in the synthesis of various heterocyclic compounds such as pyrimidines and pyridines³⁻⁶. Pyrimidines are the most significant six-membered heterocyclics, with two nitrogen atoms in positions 1 and 3. The pyrimidine was obtained from nucleic acid hydrolyses and is a substantially weaker base than pyridine and water soluble⁷. Pyrimidines are essential biologically because they are connected to nucleic acids and are used to construct DNA and RNA⁸. Pyrimidine

derivatives, such as cytarabine and 5-fluorouracil, are widely used as anticancer drugs because their toxicity is expressed in the S phase of the cell cycle, which kills only actively dividing cells⁹. Chalcones and pyrimidines have been linked to a variety of biological and pharmacological activities, including antibacterial, anti-inflammatory, analgesic, anti-hypertensive, and CNS effects¹⁰⁻¹². Additionally, Pyridines are an organic heterocyclic compound with a six-member ring with five carbons and one nitrogen atom. Pyridine and its derivatives are antimicrobial, antiviral, antioxidant, antidiabetic, anticancer, antimalarial, anti-inflammatory, analgesic, anti-convulsant, and anti-parkinsonian properties^{13, 14}. Cyanopyridines have piqued the interest of medicinal chemists due to their heterocyclic

chemistry and the pharmacological actions connected with them^{15, 16}. The goal of the current research is to synthesize pyridine and pyrimidine derivatives and investigate their anticancer, antibacterial, and antiparasitic effects. The evaluation of the synthesized compounds yielded encouraging results.

Materials and Methods:

All of the chemicals used are of the reagent grade. They were acquired from Sigma-Aldrich and utilized without further processing.

Melting points were measured with a thermo-scientific apparatus. The evolution of the reaction was checked by thin-layer chromatography. IR spectra were carried out at Basrah University, Department of Chemistry on a KBr disc in an FTIR-84005-SHIMADZU. ¹H spectra were recorded at Tehran University, Iran Bruker-400 MHz spectrometer operating at 400 MHz and ¹³C-NMR spectra were recorded at Tehran University, Iran Bruker-125 MHz spectrometer operating at 125 MHz in DMSO-*d*₆ as solvent using tetramethylsilane (TMS) as the internal standard with chemical shifts indicated in δ ppm. Mass spectra were run at 70 eV using Agilent technologies (Tehran University-Iran).

Synthesis of N-(4-nitrophenyl)-3-oxobutanamide (3)¹⁷

4-Nitroaniline (1) (0.01 mol) and ethyl acetoacetate (2) (0.01 mol) were mixed with a catalytic amount of 40% NaOH (0.05 mL) in toluene (25 mL) and heated for roughly 8 hours. The colorless liquid created was then boiled in a water bath to remove the alcohol produced by the process. Crude crystals were obtained after letting the reaction mixture cool. The crude crystals were refined by swirling them for about 15 minutes with cold diethyl ether using a mechanical stirrer. The target chemical was obtained after allowing the solution to remain for 15 minutes before filtering, resulting in the title compound.

The general method for the preparation of tetrahydro pyrimidines by Biginelli synthesis¹⁸ (5a, 5b, and 9):

First method: A mixture of *N*-(4-nitrophenyl)-3-oxobutanamide (3) (0.005 mol), thiourea (4) (0.0075 mol), indole-3-carboxaldehyde (5a), and piperonal (5b) (0.005 mol) respectively was mixed with a catalytic amount of hydrochloric acid (1 mL) in ethyl alcohol (15 mL) was heated under reflux for the required time. TLC was used to monitor the reaction, which was carried out with a 20% ethyl acetate: hexane solvent system. After

the reaction was finished, the precipitate was thoroughly washed with water to remove unreacted thiourea and left to dry. The target chemicals were obtained via recrystallization of the solid product with ethanol to produce derivatives (5a) and (5b).

Second method: A mixture of thiophene-2-carboxaldehyde (6) (0.56 g, 0.005 mol), urea (7) (0.30 g, 0.005 mol), ethyl cyanoacetate (8) (0.56 gm, 0.005 mol), and potassium carbonate (0.69 gm, 0.005 mol) in 25 ml of ethanol was refluxed for 8 hours. The completion of the reaction was monitored by thin-layer chromatography using 40% ethyl acetate: hexane as eluent and the precipitate was filtered, dissolved in hot water, and acidified by glacial acetic acid. The solid obtained was filtered, dried, and recrystallized from ethyl alcohol to get the desired compound (9).

The general method for the preparation of chalcones (11a) and (11b):

Aromatic aldehydes (0.01 mol) were combined with 4-bromoacetophenone (10a) and 4-fluoroacetophenone (10b) (0.01 mol) respectively, then dissolved in ethyl alcohol (10 mL). To this, 40% solution of sodium hydroxide (10 mL) was progressively added while swirling continuously. At room temperature, the reaction mixture was stirred for 3 hours. The completion of the reaction was monitored by thin-layer chromatography. The reaction mixture was refrigerated overnight after it was completed. The mixture was filtered and rinsed with cold water until the washings were litmus neutral, after which it was acidified with dilute HCl⁽¹⁹⁾. To obtain target chemicals, the product was dried and recrystallized from ethyl alcohol to give compounds (11a) and (11b).

Synthesis of pyrimidine (13) from chalcone:

A mixture of chalcone (11a) (0.34 g, 0.001 mol) with guanidine hydrochloride (12) (0.1 g, 0.001 mol) was stirred in ethyl alcohol (10 mL) and potassium hydroxide (0.002 mol) was then added to it. The reaction mixture was refluxed for 8 hours. The completion of the reaction was checked by TLC using a 20% ethyl acetate: hexane solvent system. After the reaction was completed, the resulting mixture was cooled and then put into ice-cold water before being neutralized with dilute HCl. The residue was then filtered, rinsed, and dried. The desired chemical was obtained by recrystallizing the product from ethyl alcohol (13).

Synthesis of pyridine derivatives: (14) and (16):

First method: A mixture of chalcone (11b) (2.8 g, 0.01 mol), ethyl cyanoacetate (8) (1.13 g, 0.01

mol) with ammonium acetate (5.9 g, 0.077 mol) were mixed in glacial acetic acid (20 mL) of, the mixture was heated for 6 hours. TLC was used to observe the reaction. After the reaction was completed, the acetic acid was evaporated to dryness, precipitated by crushed ice, filtered, and recrystallized from ethyl alcohol, providing the target chemical (14).

Second method: 4-Methyl benzaldehyde (15) (1.20 g, 0.01 mol) was mixed with 4-fluoro acetophenone (10b) (1.38 g, 0.01 mol), ethyl cyanoacetate (8) (1.13 g, 0.01 mol) and ammonium acetate (5.9 g, 0.077 mol) in n-butanol (25 mL) of, the reaction was heated for 8 hours. TLC was used to monitor the reaction. The solvent was evaporated to dryness after the reaction was completed, precipitated by crushed ice, and recrystallized from ethanol, yielding the target compound (16).

Physical and Spectral Data

N-(4-Nitrophenyl)-3-oxobutanamide (3): Yield: (83%), $R_f = 0.52$ (EtOAc: Hex., 4:6 v/v); yellow color; m.p 110-112°C; FTIR (ν); 3363 (N-H str.), 1707 (C=O str.), 1676 (C=O str.), 1616 (C=C str.) cm^{-1} ; ^1H NMR (DMSO- d_6) δ (ppm) 13.49 (s, H, NH amide), 7.81-8.25 (m, 4H, Ar-H), 3.66 (s, 2H, CH_2), 2.09 (s, 3H, CH_3) and MS: $m/z = 222$ (M+).

4-(1H-Indol-3-yl)-6-methyl-N-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (5a): Yield: (54%), $R_f = 0.13$ (EtOAc: Hex., 2:8 v/v); yellowish green color; m.p 240-242°C; FTIR (ν); 3379, 3275 (2NH str.), 1701 (C=O str.), 1612 (C=C str.); 1253 (C=S str.) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 1.41 (s, 3H, CH_3), 5.09 (s, 1H, CH-pyrimidine), 6.55-8.38 (m, 9H, Ar-H), 9.17 (s, 4H, NH); and ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm) 25.2 (CH_3), 62.0 (CH pyrimidine), 111.7-145.7 (Ar-C), 156.2 (C=C pyrimidine ring), 161.3 (C=O amide), 170.5 (C=S) and MS: $m/z = 408$ (M+).

4-(Benzo[d][1,3]dioxol-5-yl)-6-methyl-N-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (5b): Yield: (62%), $R_f = 0.1$ (EtOAc: Hex., 2:8 v/v); yellow color; m.p 195-197°C; FTIR (ν); 3300, 3203 (2NH str.), 1660 (C=O str.), 1627 (C=C str.); 1180 (C=S str.) cm^{-1} ; ^1H NMR (DMSO- d_6) δ (ppm) 2.50 (s, 3H, CH_3), 6.02 (s, 1H, CH-pyrimidine), 6.16 (s, 2H, CH_2 ring), 6.57-7.94 (m, 7H, Ar-H), 9.18 (s, 1H, NH), 9.34 (s, 1H, NH), 9.79 (s, 1H, NH) and MS: $m/z = 412$ (M+).

2,4-Dioxo-(6-thiophen-2-yl)-tetrahydropyrimidine-5-carbonitrile (9): Yield: (81%), $R_f = 0.6$ (EtOAc: Hex., 4:6 v/v); dark yellow color; m.p 241 dec. °C; FTIR (ν); 3452 (NH str.), 2210 (CN); 1724, 1620 (2C=O str.), 1585 (C=C str.) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 7.15-8.04 (m, 4H, Ar-H+ NH), 10.12 (s, 1H, NH); and ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm) 85.4 (C=C pyrimidine ring), 117.7 CN, 126.2-137.8 (Ar-C), 150.8 (C=O), 166.7 (C=O), 169.5 (C-NH ring) and MS: $m/z = 219$ (M+).

(E)-1-(4-Bromophenyl)-3-(naphthalen-1-yl) prop-2-en-1-one (11a): Yield: (67%), $R_f = 0.9$ (EtOAc: Hex., 2:8 v/v); yellow color; m.p 120-122 °C; FTIR (ν); 1660 (C=O str.), 1598 (CH=CH str.); 1510 (C=C str. arom); 748 (Ar-Br) cm^{-1} ; ^1H NMR (DMSO- d_6) δ (ppm) 7.61-8.32 (m, 12H, Ar-H), 8.60 (d, 1H, Ar-CH=); and ^{13}C NMR (DMSO- d_6) δ (ppm) 123.5 (Ar-CH=), 140.8 (=CH-Ar), 124.6-136.9 (Ar-C), 184.2 (C=O) and MS: $m/z = 337$ (M+).

(E)-1-(4-Fluorophenyl)-3-(quinolin-2-yl) prop-2-en-1-one (11b): Yield: (51%), $R_f = 0.7$ (EtOAc: Hex., 2:8 v/v); yellow color; m.p 131-133 °C; FTIR (ν); 1695 (C=O str.), 1606 (CH=CH str.); 1566 (C=C str. arom); 1026 (Ar-F) cm^{-1} ; ^1H NMR (DMSO- d_6) δ (ppm) 7.41-8.32 (m, 9H, Ar-H), 7.81 (d, 1H, =CH-Ar); and ^{13}C NMR (DMSO- d_6) δ (ppm) 125.1 (Ar-CH=), 140.7 (=CH-Ar), 116.3-137.5 (Ar-C), 142.9 (C-N ring), 164.7 (C=N ring), 166.7 (C-F), 188.1 (C=O) and MS: $m/z = 279$ (M+).

4-(4-Bromophenyl)-6-(naphthalen-1-yl) pyrimidin-2-amine (13): Yield: (65%), $R_f = 0.15$ (EtOAc: Hex., 2:8 v/v); yellowish white color; m.p 146-148 °C; FTIR (ν); 3356 (NH₂ str.), 1635 (C=N str.), 1587 (C=C str.), 777 (Ar-Br) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 6.91 (s, 2H, NH₂), 7.40 (s, 1H, CH-pyrimidine), 7.55-8.26 (m, 11H, Ar-H); and ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm) 106.5 (CH-pyrimidine), 124.2 (C-Br), 125.3-136.8 (Ar-C), 163.2 (C-N pyrimidine), 163.8 (H₂N-C=N pyrimidine), 168.1 (C=N pyrimidine) and MS: $m/z = 375$ (M+).

6-(4-Fluorophenyl)-2-oxo-4-(quinolin-2-yl)-1,2-dihydropyridine-3-carbonitrile (14): Yield: (70%), $R_f = 0.7$ (EtOAc: Hex., 1:9 v/v); yellow color; m.p 236 dec. °C; FTIR (ν); 3450 (NH str.), 2222 (CN); 1720 (C=O str.), 1602 (C=C str.), 1018 (Ar-F) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6) δ (ppm) 7.38-8.28 (m, 11H, Ar-H), 8.38 (s, 1H, NH); and ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm) 103.1

(C=C-NH pyridine), 115.9 (CN), 124.5 (NC-C=C), 115.5-142.2 (Ar-C), 153.9 (C=N quinoline), 161.7 (C=O pyridine), 164.2 (C-NH pyridine), 166.2 (C-F), 187.5 (N=C-C=C pyridine) and MS: $m/z = 341(M^+)$.

6-(4-Fluorophenyl)-2-oxo-4-(p-tolyl)-1,2-dihydropyridine-3-carbonitrile (16): Yield: (63%), $R_f = 0.2$ (EtOAc: Hex., 2:8 v/v); pale yellow color; m.p 173-175 °C; FTIR (ν); 3452 (NH str.), 1913 (CN); 1658 (C=O str.), 1602 (C=C str.), 1031 (Ar-F) cm^{-1} ; 1H NMR (400 MHz, DMSO- d_6 δ ppm) 3.34 (s, 3H, CH₃), 7.34-8.24 (m, 9H, Ar-H), 8.38 (s, 1H, NH); and ^{13}C NMR 100 MHz, DMSO- d_6 δ ppm) 26.3 (CH₃), 120.6 (C=C-NH pyridine), 120.8 (CN), 120.8 (NC-C=C), 121.1-149.5 (Ar-C), 160.6 (C=C-CN pyridine), 169.2 (C=O pyridine), 171.2 (C-NH pyridine), 192.8 (C-F) and MS: $m/z = 304(M^+)$.

Biological evaluation:

Antibacterial Activity

The antibacterial activity of target compounds was evaluated to be in vitro against two pathogenic microorganisms, namely *Staphylococcus aureus* (G+ve), and *Escherichia coli* (G-ve). Cultures were grown overnight. After 24 hours of incubation, the bacterial suspension (inoculum) was diluted with a neutral physiological solution to 10⁸ CFU/ml (turbidity = McFarland barium sulfate standard 0.5) for the diffusion test.²⁰

Agar well diffusion technique²¹:

The bacterial inoculums were evenly distributed over a sterile Petri dish Mueller Hinton Agar (MHA) using a sterile cotton swab. Each well received 50 μ l of chemical product at a concentration of 100 mg/ml (7 mm diameter holes cut in the agar gel, 20 mm apart from one alternative). The plates were then incubated at 37°C for 24 hours under aerobic conditions. Confluent bacterial growth was detected after incubation. Bacterial growth inhibition was measured in millimeters.

Effect of drugs/plant extracts on the viability of *Echinococcus granulosus* protoscoleces^{22, 23}.

E. granulosus protoscoleces were aseptically isolated from liver hydatid cysts obtained from infected sheep. Protoscoleces were washed five times with phosphate-buffered saline (PBS) containing 100 U/ml penicillin and 100 μ g/ml streptomycin. The viability of protoscoleces was determined by staining with 0.4% (w/v) trypan blue, and dead protoscoleces staining blue. Protoscoleces with > 95% viability were

subsequently cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μ g/ml streptomycin in a 25 cm flask at 37°C in a CO₂ incubator for 24 hours.

For evaluation of cytotoxicity of drugs/plant extracts against protoscoleces, the protoscoleces maintained as described above were seeded in 96-well plates (200 protoscoleces/250 μ l/ well) in a complete RPMI 1640 culture medium at 37°C in a CO₂ incubator for three days. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to determine viable protoscoleces. Viable protoscoleces with active metabolism reduce the MTT tetrazolium into a purple-colored formazan product. Therefore, 25 μ l of 5m/ml MTT prepared in BPS was then added to each well, and protoscoleces were then incubated at 37°C in a CO₂ incubator in the dark for two hours. The medium was withdrawn, and the protoscoleces' formazan crystals were then dissolved in 250 μ l of 100% (v/v) dimethyl sulfoxide (DMSO). The absorbance was read at 570 nm using an ELISA reader. The half-maximal inhibitory concentration (IC₅₀) value was calculated using GraphPad Prism version 8.

In vitro cytotoxic activity of synthesized compounds over HepG2 and MCF-7 cancer cell lines^{24, 25}.

Materials:

Cell lines and culture. MCF-7 (breast cancer cell) and HepG2 (liver cancer cell) cell lines were obtained from the National Cell Bank of Iran (Pasteur Institute, Iran). Cells were cultured in RPMI-1640 media (Gibco) containing 10% FBS (Gibco) and antibiotics (100 U/ml penicillin and 100 μ g/ml streptomycin). Cells were seeded at 37 °C in humidified air with 5% CO₂ and passaged with trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solution. The culturing fluids and conditions utilized to develop the cells as 3D colonies were identical to those employed for monolayer cell culture.

Methodology:

MTT cell viability assay in MCF-7 and HepG2 Cells^{26, 27}. Cell growth and cell viability were quantified using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium Bromide] (Sigma-Aldrich) assay. In brief, for monolayer culture, cells (MCF-7 & HepG2) were digested with trypsin, harvested, adjusted to a density of 1.4×10^4 cells/well, and seeded to 96-well plates filled

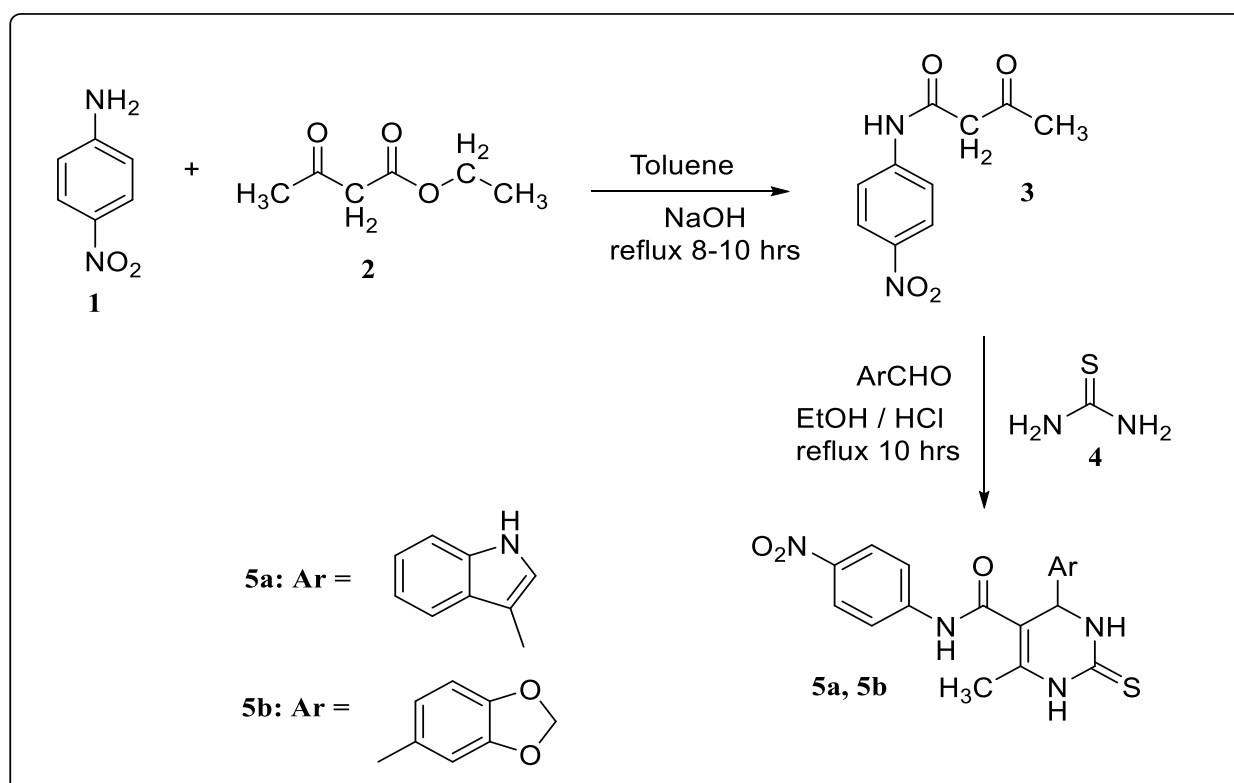
with 200 μl fresh medium per well for 24 hours. When cells formed a monolayer, they were treated with 400-25 $\mu\text{g/ml}$ of the compounds for 24 hours at 37 $^{\circ}\text{C}$ in 5% CO_2 . At the end of the treatment 24 hours, while the monolayer culture was left untouched in the original plate, the supernatant was removed and 200 $\mu\text{l/well}$ of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was added and the plate was incubated at 37 $^{\circ}\text{C}$ for an additional 4 hours. MTT solution (the supernatant of cells was removed and dimethyl sulfoxide was added (100 μl per well). Cells were incubated on a shaker at 37 $^{\circ}\text{C}$ until crystals were completely dissolved. Cell viability was quantified by measuring absorbance at 570 nm using an ELISA reader (Model wave xs2, BioTek, USA). The concentration of the compounds that resulted in 50% of cell death (IC_{50}) was determined from respective dose-response curves.

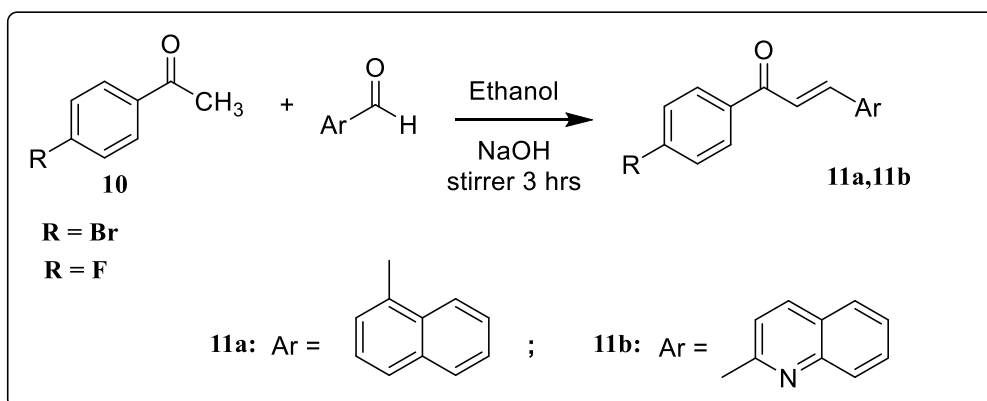
Results and Discussion

The physical properties of prepared derivatives data were listed in Table. 1. Biginelli synthetic procedure²⁸ involving a one-pot multicomponent reaction was performed to prepare pyrimidine

derivatives (**5a**) and (**5b**). In the first step, 4-nitroaniline (**1**) was reacted with ethyl acetoacetate (**2**) in toluene and 40% NaOH to produce *N*-(4-nitrophenyl)-3-oxo-butanamide (**3**) with an 83% yield. By reacting *N*-(4-nitrophenyl)-3-oxo-butanamide (**3**) with thiourea (**4**) and aryl or heteroaryl aldehyde in the presence of a catalytic quantity of HCl, the pyrimidine derivatives (**5a**) and (**5b**) were obtained with the yields of 54% and 62% respectively (Scheme 1). Establishing compounds (**5a**) and (**5b**) based on their MS spectra and spectral data (IR, ^1H NMR, ^{13}C NMR). The FT-IR spectra of compounds **5a** and **5b** showed characteristic absorption bands at 3379, 3275 and 3300, 3203 cm^{-1} , 1701, 1660 cm^{-1} , 1612, 1627 cm^{-1} or 1253, 1180 cm^{-1} related to stretching of the 2NH, C=O, C=C and C=S respectively. ^1H NMR of compounds **5a** and **5b** revealed singlet signals at δ 1.41 and 2.50 ppm assigned to CH_3 , signals at δ 5.09 and 6.02 ppm ascribed to pyrimidine-H, in addition to proton signals of aromatic structure, (Fig. 1). The mass spectra of pyrimidine derivatives (**5a**) and (**5b**), which appeared at molecular ion peaks at m/z 407.75 (M^+) and 412(M^+) respectively, were in good agreement with the expected values of m/z 407.11 and 412.08.

Scheme 1. Preparation of pyrimidine compounds (**5a**) and (**5b**).





Scheme 3. Preparation of chalcone compounds (11a) and (11b).

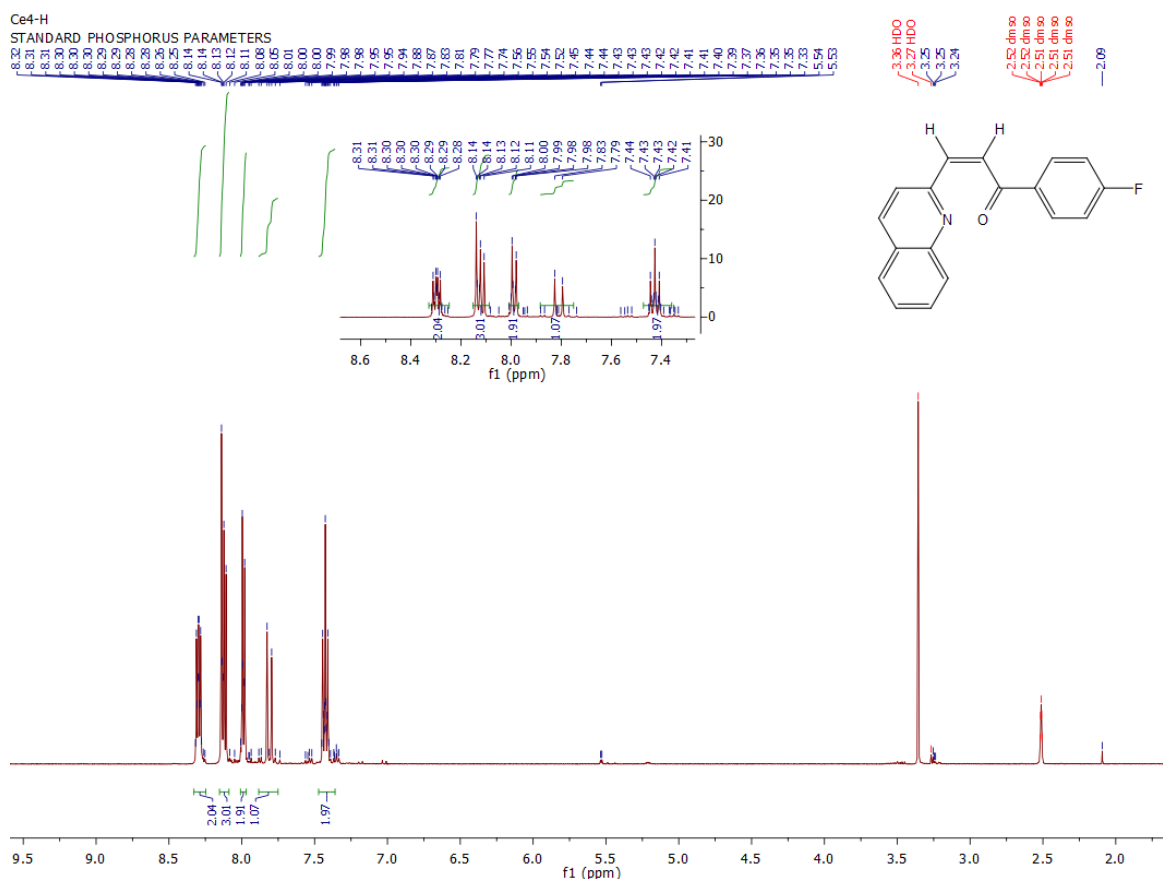
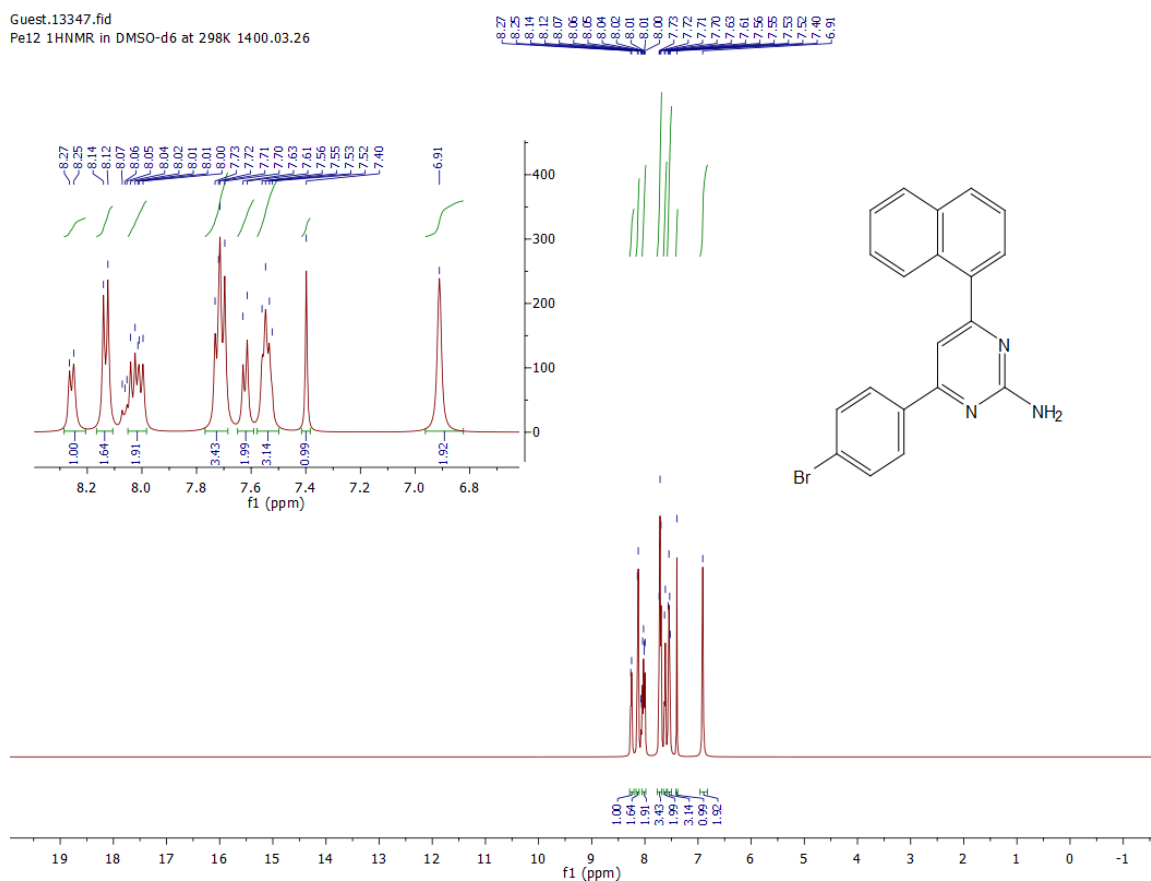
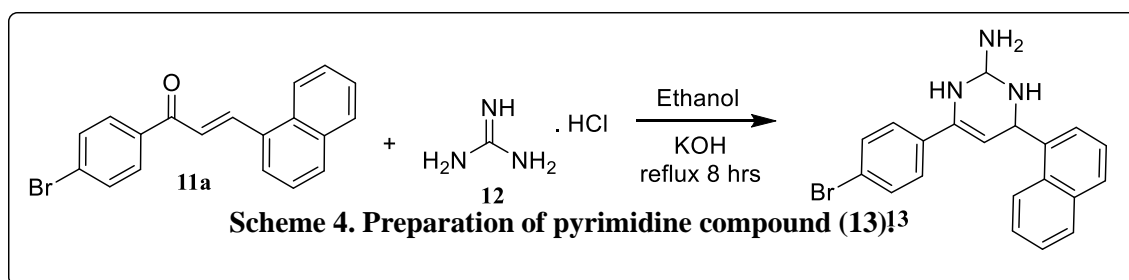


Figure 3. $^1\text{H-NMR}$ spectrum of compound (11b).

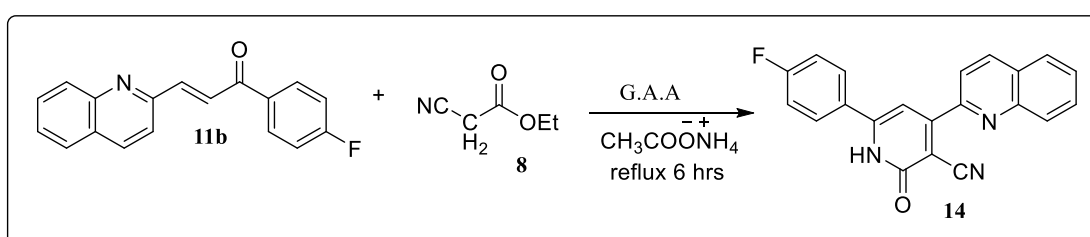
The pyrimidine of compound (13) was synthesized by reaction of chalcone derivative (11a) and guanidine hydrochloride (12) in ethanol and potassium hydroxide with a yield of 65% (Scheme 4). The FT-IR spectrum of (13) presented absorption bands at 3356 cm^{-1} , 1635 or 1587 cm^{-1} respectively, attributed to stretching of the NH_2 , C=N , or C=C . From the $^1\text{H NMR}$ spectrum of (13), a signal at $\delta 6.91\text{ ppm}$ was ascribed to NH_2 , a singlet signal at $\delta 7.40\text{ ppm}$ assigned to

pyrimidine-CH, and multiplet signals at $\delta 7.55$ - 8.26 ppm to aromatic protons (Fig. 4). $^{13}\text{C NMR}$ showed signals at $\delta 106.5$ assigned to pyrimidine-CH, signals at $\delta 163.2$, 163.8 , and 168.1 assigned to C-N , $\text{H}_2\text{N-C=N}$, C=N pyrimidine groups respectively, in addition to carbon signals of aromatic structure. The mass spectrum of the pyrimidine derivative (13), which revealed a molecular ion peak at $m/z 375.2 (M^+)$, matched the expected value of $m/z 375.04$.



Also, the reaction of ethyl cyanoacetate (**8**) with chalcone derivative (**11b**) and ammonium acetate in glacial acetic acid yield the pyridine derivative (**14**) (Scheme 5). The FT-IR spectrum of compound (**14**) revealed absorption bands at 3450 cm⁻¹, 1720 or 1602 cm⁻¹ respectively, related to stretching of the NH, C=O, or C=C. ¹H NMR of compound (**14**) revealed multiplet signals at δ 7.38-8.28 ppm assigned to aromatic protons and

signals at δ 8.38 ppm assigned to NH (Fig. 5). ¹³C NMR showed signals at δ 115.9 assigned to the CN group and signals at δ 153.9, 161.7, 164.2, 166.2, and 187.5 assigned to C=N quinoline, C=O, C-NH, C-F, and N=C-C pyridine groups respectively, in addition to carbon signals of aromatic structure. The mass spectrum of the pyridine derivative (**14**), which indicated a molecular ion peak at m/z 341.1 (M⁺), matched the expected value of m/z 341.10.



Scheme 5. Preparation of pyridine compound (14).

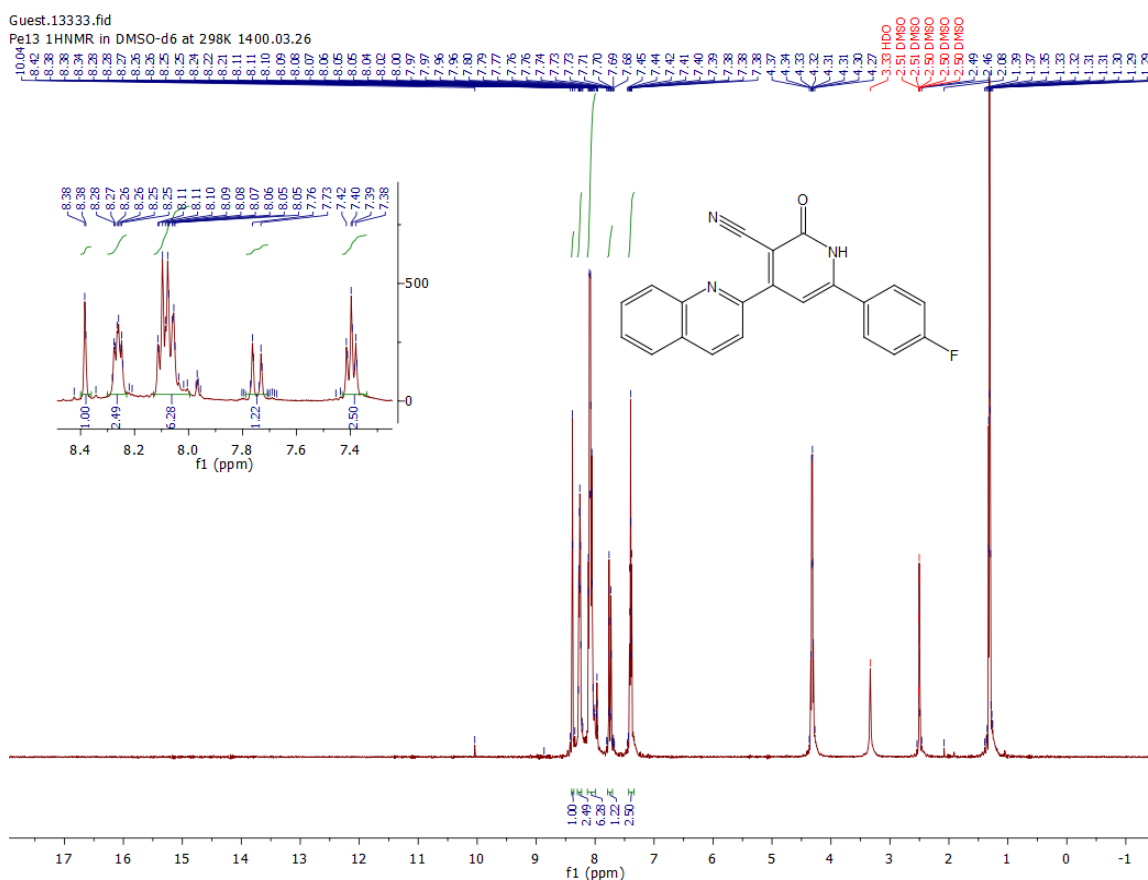
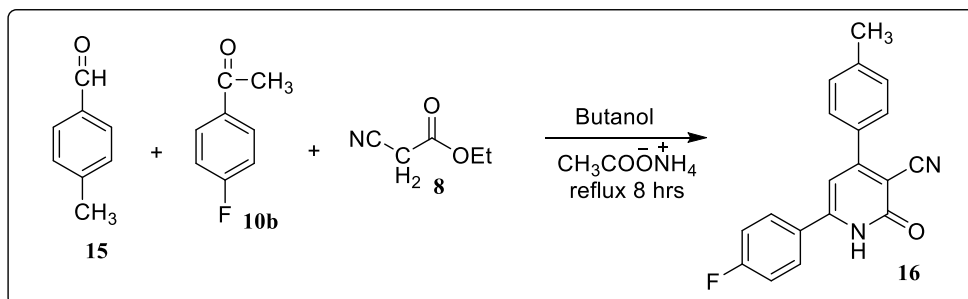


Figure 5. ¹H-NMR spectrum of compound (14).

On the other hand, the reaction of 4-methyl benzaldehyde (**15**), 4-fluoro acetophenone (**10b**), ethyl cyanoacetate (**8**), and ammonium acetate in *n*-butanol gave the pyridine derivative (**16**) with a yield of 63% (Scheme 6). FT-IR spectrum of compound (**16**) displayed absorption bands at 3452 cm⁻¹, 1658 or 1602 cm⁻¹ respectively, attributed to stretching of the NH, C=O, or C=C. ¹H NMR spectrum of (**16**) revealed a signal at δ 3.34 ppm assigned to CH₃, multiplet signals at δ 7.34-8.24 value of *m/z* 304.10.

ppm assigned to aromatic protons, and NH appeared at δ 8.38 (Fig. 6). ¹³C NMR showed a signal at δ 26.3 assigned to CH₃, cyano group was detected at δ 120.8 ppm and signals at δ 160.6, 169.2, 171.2, and 192.8 assigned to C=C-CN, C=O, C-NH, and C-F pyridine groups respectively, in addition to carbon signals of aromatic structure. The mass spectrum of the pyridine derivative (**16**), which demonstrated a molecular ion peak at *m/z* 304.1 (M⁺), equaled the expected



Scheme 6. Preparation of pyridine compound (16).

	0.01	1.5	0
13	1	1.7	1.3
	0.1	1.6	1.3
	0.01	1.3	0
14	1	1.8	0
	0.1	1.6	0
	0.01	1.6	0
16	1	1.3	0
	0.1	1.3	0
	0.01	1.3	0
Ampicillin	1	3.5	6.5
	0.1	2.7	5
	0.01	1.8	3.5

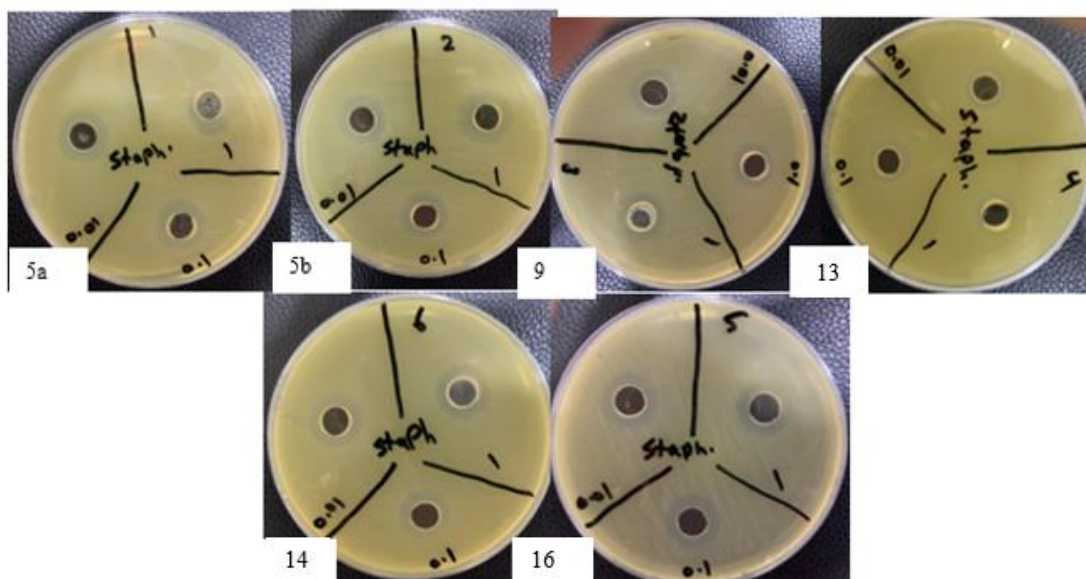


Figure 16. Inhibition zones of *S. aureus* G+ by the effects of the synthesized compounds.

Effect of drugs/plant extracts on the viability of *E. granulosus* protoscolexes^{19, 20}

The antiparasitic activity of synthesized compounds **5a**, **5b**, and **16** were tested against *E. granulosus* protoscolexes, within three concentrations (1, 0.1, and 0.01 $\mu\text{g/mL}$). The cytotoxicity of drugs/plant extracts against protoscolexes was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. MTT tetrazolium is converted into a purple formazan product by viable protoscolexes

with an active metabolism. As a result, 25 μL of 5 m/mL MTT produced in BPS was injected into each well, and protoscolexes were maintained for two hours at 37°C in a CO₂ incubator in the dark. The medium was replaced, and the protoscolexes' formazan crystals were dissolved in 250 μL of 100% (v/v) dimethyl sulfoxide (DMSO). From the evaluated compound series, derivatives **5a**, **5b** and **16** were effective with the concentration of 1 $\mu\text{g/ml}$ against *E. granulosus* (Fig. 17).

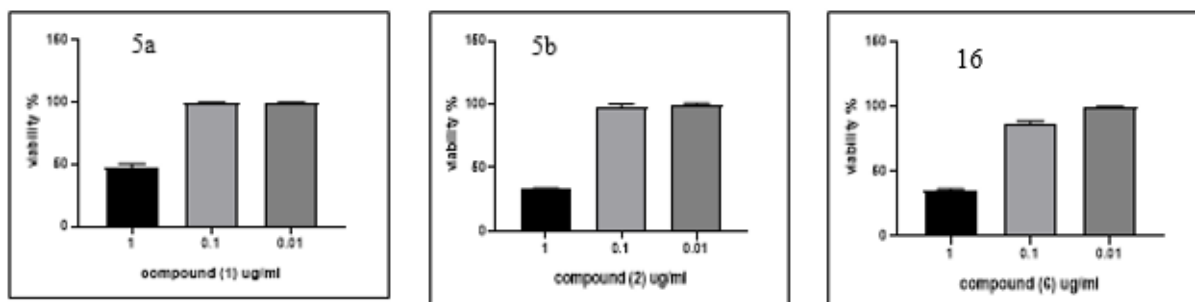


Figure 17. The viability of *E. granulosus* protoscolexes effects of the synthesized compounds.

Cytotoxic activity

The synthesized compounds **5a**, **5b**, **9**, **13**, **14**, and **16** were tested in vitro against two human tumor cancer cell kinds, MCF-7 (breast cancer cell) and HepG2 (liver cancer cell), using the MTT assay, as previously described^{21, 22}. The following experimental findings are based on an analysis of

cytotoxicity outcomes against two cancer cell lines at five concentrations (25, 50, 100, 200, and 400 $\mu\text{g}/\text{mL}$). Cytotoxicity results over HepG2 cells, compounds **5a** and **14** had a moderate cytotoxic activity with IC_{50} values of 43.84 $\mu\text{g}/\text{mL}$ and 57.14 $\mu\text{g}/\text{mL}$ respectively, when compared to the other synthesized compounds (Fig. 18).

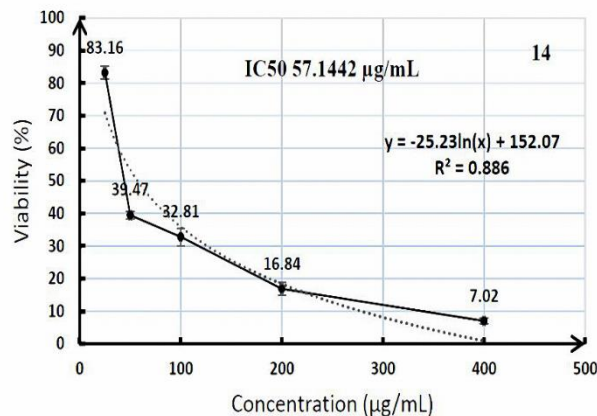
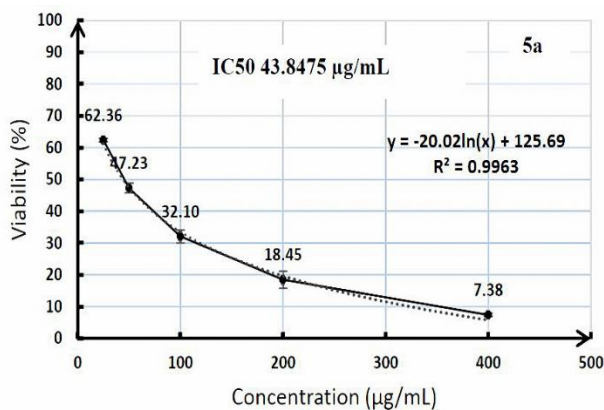


Figure 18. IC_{50} values of compounds **5a** and **14** against HepG2 cell.

Regarding the MCF7 cell line, pyridine compound **14** had a moderate cytotoxic activity with an IC_{50} value of 50.84 $\mu\text{g}/\text{mL}$, when

compared to the other synthesized compounds (Fig. 19).

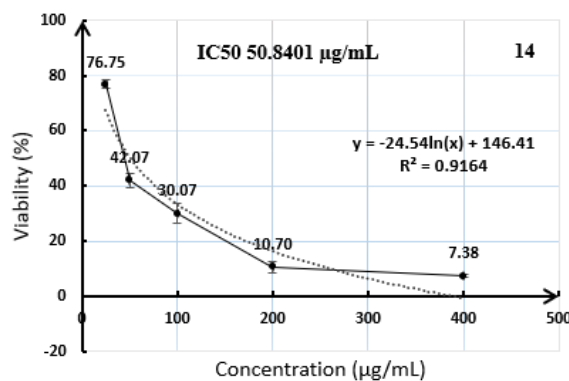
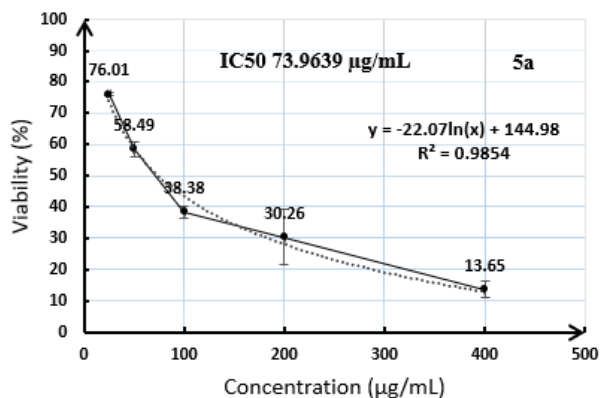


Figure 19. IC_{50} values of compounds **5a** and **14** against MCF7 cell.

Conclusions:

Based on the findings, it can be concluded that the prepared compounds of various substituted pyrimidines, pyridine, have high antibacterial action against the bacteria *staphylococcus aureus* but no activity against *Escherichia coli*. The anti-parasitic effectiveness of prepared compounds against *E. granulosus* protozoa was also investigated; of the evaluated chemical series, derivatives **5a**, **5b**, and **16** were effective. *In vitro* tests were performed on the prepared derivatives versus two human tumor cancer cell lines, MCF-7 and HepG2. Across all the compounds examined for cytotoxicity in HepG2 cells, compounds **5a** and

14 showed actions. Furthermore, compound **13** demonstrated efficacy against the MCF-7 cell line.

Authors' declaration:

- Conflicts of Interest: None.
- We affirm that all of the figures and tables in the manuscript are ours. Furthermore, the Figures and images, that are not ours have been granted permission for re-publication and are connected to the article.
- Ethical Clearance: The Local Ethical Committee at the University of Basrah accepted the project.

Authors' contributions statement:

The current research topic was proposed and planned by ZAM. S. The experiments were conceived and carried out by I RA. AH. All authors participated in the analysis, discussion, and drafting of the manuscript.

References:

1. Ansar R.S, Mazahar F, Satpute R.H, Syed A. Overview on Nitrogen containing compounds and their assessment based on 'International Regulatory Standards'. *J. Drug Deliv Ther.* 2018; 8(6-s):424-428.
2. Nagaraju K, Lalitha G, Suresh M, Kranthi KG, Sreekantha BJ. A Review on Recent Advances in Nitrogen Containing Molecules and Their Biological Applications. *Molecules.* 2020; 25: 1909.
3. Mangoud MM, Mohamed ZH, Eman AE. Design and Synthesis of Novel Pyrazoles, Pyrazolines, and Pyridines from Chalcone Derivatives with Evaluation of Their In Vitro Anticancer Activity Against T-47D and UACC-257 Cell Lines. *Egypt J. Chem.* 2020; 63(12): 5203-5218.
4. Mohammed KA, Noha R, Al-Shorbagy MY, Manal RM, Mohammed MI, Salwa E. Design, Synthesis and Screening of 4, 6-Diaryl Pyridine and Pyrimidine Derivatives As Potential Cytotoxic Molecules. *Chem. Pharm. Bull.* 2018; 66(10): 939-952; DOI:10.1248/cpb.c18-00269.
5. Ali K A A. Synthesis and Characterization of Some New Pyrazoline and Isoxazoline Derivatives as Antibacterial Agents. *Baghdad Sci J.* 2016;13(3): 568-577.
6. Abdel-Amir M F, Hala M S. Synthesis and Characterization of Some Novel Oxazine, Thiazine and Pyrazol Derivatives. *Baghdad Sci J.* 2016; 13: 244-252.
7. Sanjiv K, Balasubramanian N. Therapeutic potential of heterocyclic pyrimidine scaffolds. *Chem. Cent J.* 2018; 12: 38.
8. Ajmal R B, Rajendra S D, Aabid H S, Gowhar A N, Israr Ul H. Computational analysis for antimicrobial active pyrano[2,3-d]pyrimidine derivatives on the basis of theoretical and experimental ground. *J. Assoc. Arab Univ. Basic Appl. Sci.* 2016; 20: 19-25.
9. Ellen JBD, Jos HB. Intracellular Pharmacokinetics of Pyrimidine Analogues used in Oncology and the Correlation with Drug Action. *Clin. Pharm.* 2020; 59: 1521-1550.
10. Eswara R G, Srinivasa B P, Sai K O, Sharmila R, Maruthi Kumar S S S. Synthesis, Characterization and Biological Evaluation of Pyrimidines from Chalcones. *Int. J. Rec. Sci. Res.* 2016; 7(4): 10238-10241.
11. Ruaa WA, Hutham MYA, Israa NK, Ahmed AJA. Synthesis, characterization, and antibacterial activity of some new pyrimidine derivatives from chalcone derivatives. *Drug Invent Today.* 2019; 11 (7): 1732-1739.
12. Biswa MS, Mullangi R., Panda J, Sahoo B. Green Expedient Synthesis of Pyrimidine Derivatives via Chalcones and Evaluation of their Anthelmintic Activity. *Indian J. Pharm. Educ. Res.* 2017; 51: 5700-5706.
13. Mangoud MM, Mohamed ZH, Eman AE. Design and Synthesis of Novel Pyrazoles, Pyrazolines, and Pyridines from Chalcone Derivatives with Evaluation of Their In Vitro Anticancer Activity Against T-47D and UACC-257 Cell Lines. *Egypt J. Chem.* 2020; 63: 5203 – 5218.
14. Tehreem T, Muhammad A, Muhammad S, Muhammad R, Mirza IS, Katarzyna KM, Mariusz M. Pyridine Scaffolds, Phenols and Derivatives of Azo Moiety: Current Therapeutic Perspectives. *Molecules.* 2021; 26(16): 4872.
15. Rehab S, Marwa FH, Omkulthom M A, Najla A. Discovery of Novel 3-Cyanopyridines as Survivin Modulators and Apoptosis Inducers. *Molecules.* 2020; 25(21): 4892.
16. Pareshkumar UP, Vipul PG, Purohit DM, Patoliaa VN. Synthesis and biological evaluation of some new cyano pyridine derivatives. *J. Chem. Pharm. Res.* 2015; 7(1): 182-186.
17. Vladimir LG, Tatiana MZ, Maksim VD. Sodium hydrogen sulfate as a catalyst for the synthesis of N,4-diaryl-6-methyl-1-methyl(phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamides via the Biginelli reaction. *Chem. Heterocycl Compd.* 2018; 54(2): 177-182.
18. Giovanna B, Fiona C, Riccardo DN, Nicole M. Efficient One-Pot Synthesis of 3,4-Dihydropyrimidin-2(1H)-ones via a Three-Component Biginelli Reaction. *Molecules.* 2021; 26: 3753.
19. Eswara RG, Srinivasa BP, Sai KO, Sharmila R, Maruthi KSSS. Synthesis, Characterization and Biological Evaluation of Pyrimidines from Chalcones. *Int. J. Rec. Sci. Res.* 2016; 7(4): 10238-10241.
20. Cynthia BS. Updating Antimicrobial Susceptibility Testing Methods, *Clin Lab Sci.* 2012; 25(4): 233-239.
21. Mounyr B, Moulay S, Saad KI. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* 2016; 6: 71-79.
22. Ali T, Fatemeh G, John H, Abdolhossein D, Zohreh S. Silybum marianum ethanolic extract: in vitro effects on protoscolices of *Echinococcus granulosus* G1 strain with emphasis on other Iranian medicinal plants. *Trop Med Health.* 2021; 49: 71.
23. Sumbal H, Sadaf N, Naveeda AQ, Riaz U, Hafiz MM, Abdelaaty AS. Phytochemical analysis, Antioxidant and Anti-protoscolices potential of ethanol extracts of selected plants species against *Echinococcus granulosus*: In-vitro study. *Open Chem.* 2019; 17: 874-883.
24. Naglaa MA, Mahmoud MY, Moustafa KS, Ahmed MS. Design, Synthesis, Molecular Modeling and Antitumor Evaluation of Novel Indolyl-Pyrimidine

- Derivatives with EGFR Inhibitory Activity Molecules. 2021; 26: 1838.
25. Beata T, Benita W, Žaneta C, Aneta CN, Elżbieta G, Anna JK. Novel Pyrimidine Derivatives as Potential Anticancer Agents: Synthesis, Biological Evaluation and Molecular Docking Study. Int. J. Mol. Sci. 2021; 22(8): 3825.
26. Senthilraja P, Kathiresan K. In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. J. Appl. Pharm. Sci. 2015; 5 (03): 080-084.
27. Ebtesam SA, Nouf AA, Mai MA, Shaza MA, Ali AE, Nida NF. Evaluation of cytotoxicity, cell cycle arrest and apoptosis induced by Anethum graveolens L. essential oil in human hepatocellular carcinoma cell line. Saudi Pharm. J. 2019; 27(7): 1053-1060.
28. Abdel HMH, Abu-Bakr AME, Ahmed AME, Ahmed AK. Synthesis and Antibacterial Evaluation of Some New Pyrimidine, Pyridine and Thiophene Derivatives. Drug Des Int Prop Int J. 2021; 4(1): 446-454.

تحضير وتشخيص بعض مشتقات البيريدين والبيريدين الجديدة ودراسة فعاليتها البيولوجية

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الخلاصة:

تعتبر الأنظمة الحلقية غير المتجانسة ضرورية في الكيمياء الطبية بسبب نشاطها الواعد للسام للخلايا، واحدة من أهم عائلات الجزيئات العضوية الموجودة في الطبيعة أو المحضرة في المختبر. نتيجة لذلك، تم مفاعلة المركب 3 (*N*-(4-nitrophenyl)-3-oxobutanamide) مع الثيوبوريا و اندول-3-كاربوكسالديهييد و piperonal على التوالي لتحضير بعض مشتقات البيريدين 5a و 5b. كذلك حضر المركب 9 من تفاعل ثايوفين-2-كربوكسالديهييد مع اثيل سيانواستينيت واليوريا بوجود كاربونات البوتاسيوم كعامل مساعد. وحضرت مركبات الجالكونات 11a و 11b من تفاعل كميات مولية متساوية من كل من 1-نفثالديهييد و كوينولين-2-كربوكسالديهييد على التوالي من كل من 4-برومواسيتوفينون و 4-فلورواسيتوفينون على التوالي. وحضر مركب البيريدين 13 من تفاعل مركب الجالكون 11a مع كواندين هيدروكلوريد 12 بوجود هيدروكسيد البوتاسيوم. كما حضرت مشتقات البيريدين 14 و 16 من تفاعل مركب الجالكون 11b مع اثيل سيانواستينيت و خلات الامونيوم في حامض الخليك الثلجي، حضر بهذه الطريقة المركب 14. كذلك تم مفاعلة 4-مثيل بنزالديهييد مع 4-فلورواسيتوفينون و اثيل سيانواستينيت و خلات الامونيوم في ن-بيوتانول لتحضير المركب 16. شخضت جميع المشتقات المحضرة باستعمال التقنيات الطيفية (FT-IR و ¹H-NMR و ¹³C-NMR)، فضلا عن أطيايف الماس (EI-MS) للتحقق من الصيغ التركيبية للمشتقات المحضرة. كما فحصت فعالية المشتقات المحضرة المضادة للسرطان ضد نوعين من السلالات المختلفة من الخلايا السرطانية البشرية، MCF-7 (خلايا سرطان الثدي) و HepG2 (خلايا سرطان الكبد). فقد أظهر المركبين 5a و 14 أنشطة سامة للخلايا ضد خلايا HepG2 بقيم IC₅₀ تبلغ 43.84 و 57.14 ميكروغرام / مل على التوالي. بالإضافة إلى ذلك، فقد أظهر مركب البيريدين 14 نشاطاً ساماً للخلايا ضد خلايا MCF-7 بقيمة IC₅₀ تبلغ 50.84 ميكروغرام/ مل. كما درست الخصائص المضادة للبكتيريا الموجبة والسالبة لصبغة كرام والطفيليات (*E. granulosus* protoscolec) للمشتقات المحضرة.

الكلمات المفتاحية: بيريدين، جالكونات، خلايا HepG2 و MCF-7، سيانوبيريدين، مضاد للبكتيريا، مضاد للطفيليات.