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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, ^{1}H, and ^{13}C-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 IC_{50} values of 43.84 and 57.14 µg /mL, respectively. Furthermore, the pyridine compound **14** demonstrated cytotoxic action versus MCF-7 with an IC_{50} 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 pyridines [3-6]. 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 soluble[7]. Pyrimidines are essential biologically because they are connected to nucleic acids and are used to construct DNA and RNA[8]. 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 cells [9]. Chalcones and pyrimidines have been linked to a variety of biological and pharmacological activities, including antibacterial, anti-inflammatory, analgesic, anti-hypertensive, and CNS effects [10-12]. 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. ^{1}H spectra were recorded at Tehran University, Iran Bruker-400 MH_Z spectrometer operating at 400 MHz and ^{13}C-NMR spectra were recorded at Tehran University, Iran Bruker-125 MH_Z 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 synthesis[18] (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 un-reacted 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-fluoro acetophenone (**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 (*v*); 3363 (N-H str.), 1707 (C=O str.), 1676 (C=O str.), 1616 (C=C str.) cm^{-1}; ^{1}H 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-(1*H*-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 (*v*); 3379, 3275 (2NH str.), 1701 (C=O str.), 1612 (C=C str.); 1253 (C=S str.) cm^{-1}; ^{1}H 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 (*v*); 3300, 3203 (2NH str.), 1660 (C=O str.), 1627 (C=C str.); 1180 (C=S str.) cm^{-1}; ^{1}H 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 (*v*); 3452 (NH str.), 2210 (CN);1724, 1620 (2C=O str.), 1585 (C=C str.) cm^{-1}; ^{1}H 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 (*v*); 1660 (C=O str.), 1598 (CH=CH str.); 1510 (C=C str. arom); 748 (Ar-Br) cm^{-1}; ^{1}H 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 (*v*); 1695 (C=O str.), 1606 (CH=CH str.); 1566 (C=C str. arom); 1026 (Ar-F) cm^{-1}; ^{1}H 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 (*v*); 3356 (NH_2 str.), 1635 (C=N str.), 1587 (C=C str.), 777 (Ar-Br) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6 δ ppm) 6.91 (s, 2H, NH_2), 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 (H_2N-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 (*v*); 3450 (NH str.), 2222 (CN); 1720 (C=O str.), 1602 (C=C str.), 1018 (Ar-F) cm^{-1}; ^{1}H 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 (*v*); 3452 (NH str.),

1913 (CN); 1658 (C=O str.), 1602 (C=C str.), 1031 (Ar-F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6 δ ppm) 3.34 (s,3H, CH_3), 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 (CH_3), 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 technique [21]:

The bacterial inoculums were evenly distributed over a sterile Petri dish Mueller Hinton Agar (MHA) using a sterile cotton swab. Each well received 50 µl 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 37ºC 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 µg/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 RMPI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin in a 25 cm flask at 37ºC in a CO_2 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 µl/ well) in a complete RPMI 1640 culture medium at 37ºC in a CO_2 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 µl of 5m/ml MTT prepared in BPS was then added to each well, and protoscoleces were then incubated at 37ºC in a CO_2 incubator in the dark for two hours. The medium was withdrawn, and the protoscoleces' formazan crystals were then dissolved in 250 µl of 100% (v/v) dimethyl sulfoxide (DMSO). The absorbance was read at 570 nm using an ELISA reader. The half-maximal inhibitory concentration (IC_{50}) 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 µg/ml streptomycin). Cells were seeded at 37 °C in humidified air with 5% CO_2 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.

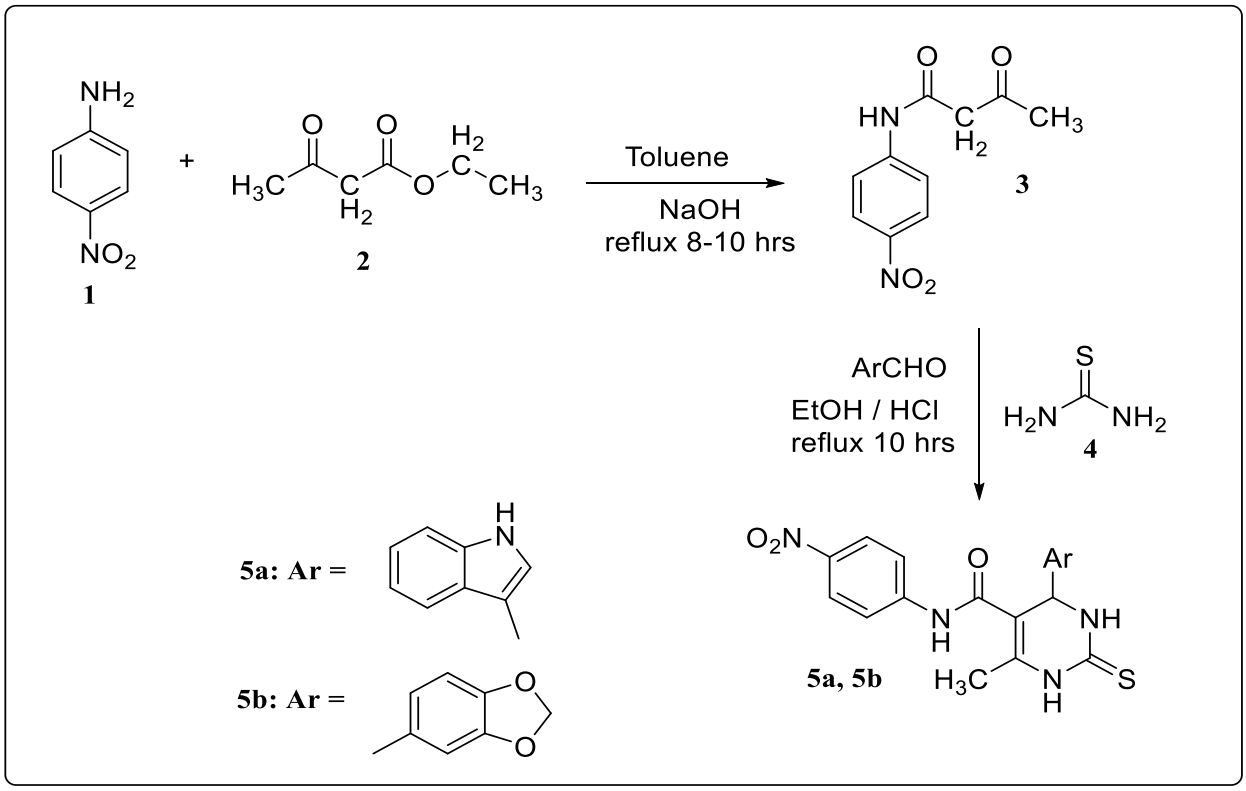
Methodology:

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 × 104 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 µg/ml of the compounds for 24 hours at 37 °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 µl/well of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was added and the plate was incubated at 37 °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 °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 procedure[28] 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, ^{1}H 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.^{1}H NMR of compounds 5a and 5b revealed singlet signals at δ 1.41and 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).

Table 1. The physical properties of prepared compounds.

No.	Molecular formula	M.Wt g/mol	Color	Time/h	m.p (ºC)	R_f	Yield (%)
3	$C_{10}H_{10}N_2O_4$	222.20	yellow	10	110- 112	0.52	**83**
5a	$C_{20}H_{17}N_5O_3S$	407.45	yellowish green	20	240-242	0.13	**54**
5b	$C_{19}H_{16}N_4O_5S$	412.42	yellow	10	195-197	0.1	**62**
9	$C_9H_5N_3O_2S$	219.22	brown	20	241dec.	0.6	**81**
11a	$C_{19}H_{13}BrO$	337.22	yellow	4	120-122	0.9	**67**
11b	$C_{18}H_{12}FNO$	277.30	yellow	4	131-133	0.7	**51**
13	$C_{20}H_{14}BrN_3$	376.26	yellowish white	10	146-148	0.15	**65**
14	$C_{21}H_{12}FN_3O$	341.35	yellow	9	236 dec.	0.7	**70**
16	$C_{19}H_{13}FN_2O$	304.32	white	8	173-175	0.2	**63**

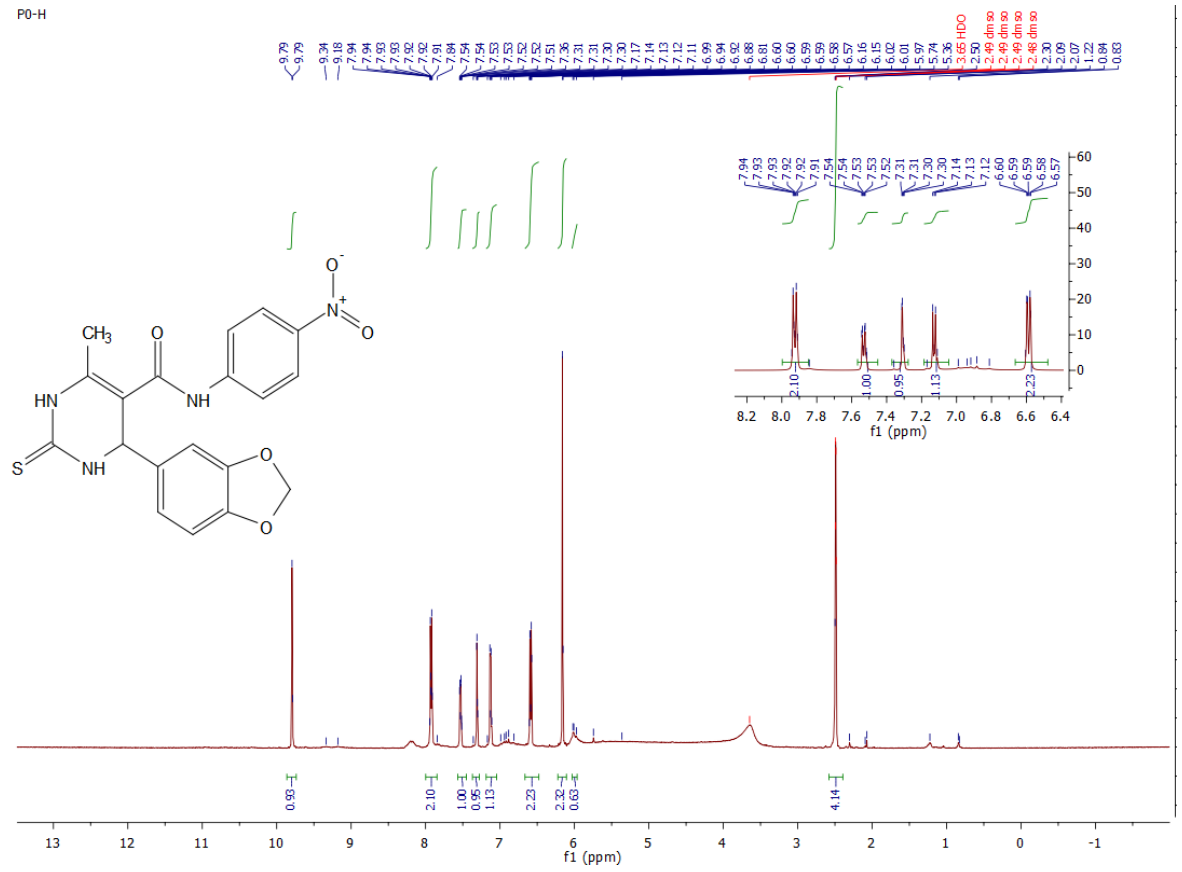
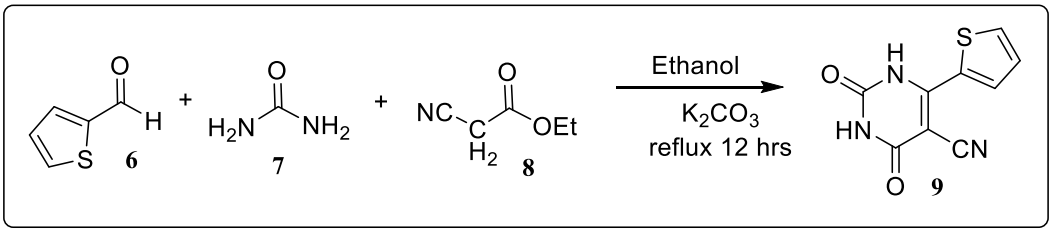


Figure 1. ^{1}H-NMR spectrum of compound (5b).

The pyrimidine of compound (**9**) was synthesized by reacting thiophene-2-carboxaldehyde (**6**), urea (**7**), ethyl cyanoacetate (**8**), and potassium carbonate as a catalyst with a yield of 81% (Scheme 2). The FT-IR spectrum of compound (**9**) showed absorption bands at 3452 cm^{-1}, 1724, 1620 cm^{-1} or 1585 cm^{-1} attributed to stretching of the NH, 2C=O, or C=C respectively. ^{1}H NMR spectrum of compound (**9**) revealed multiplet signals at δ 7.15-8.04 ppm assigned to aromatic protons with NH, as well as a signal at δ 10.12 ppm assigned to NH (Fig. 2). The mass spectrum of the pyrimidine derivative (**9**), which showed a molecular ion peak at *m/z* 218.95 (M^+), agreed well with the predicted value of *m/z* 219.01.



Scheme 2. **Preparation of pyrimidine compound (9).**

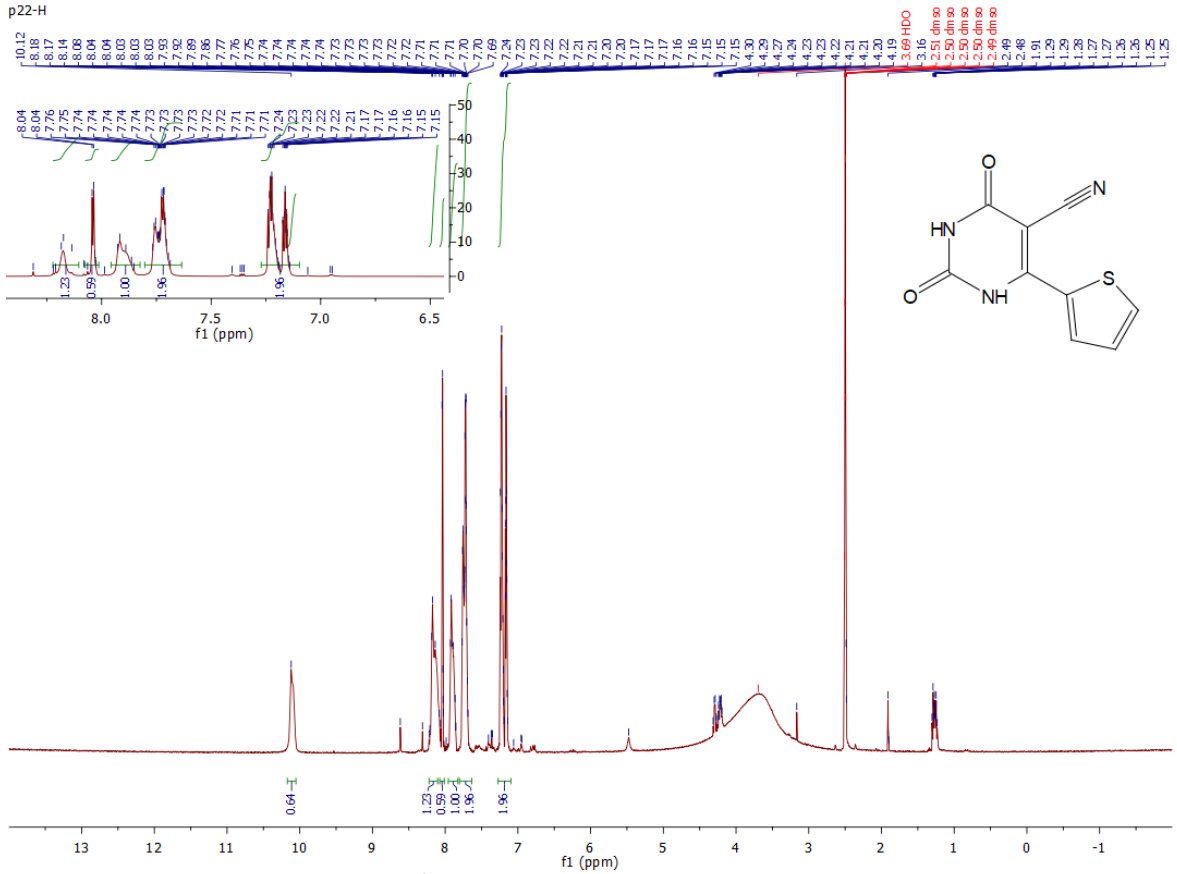
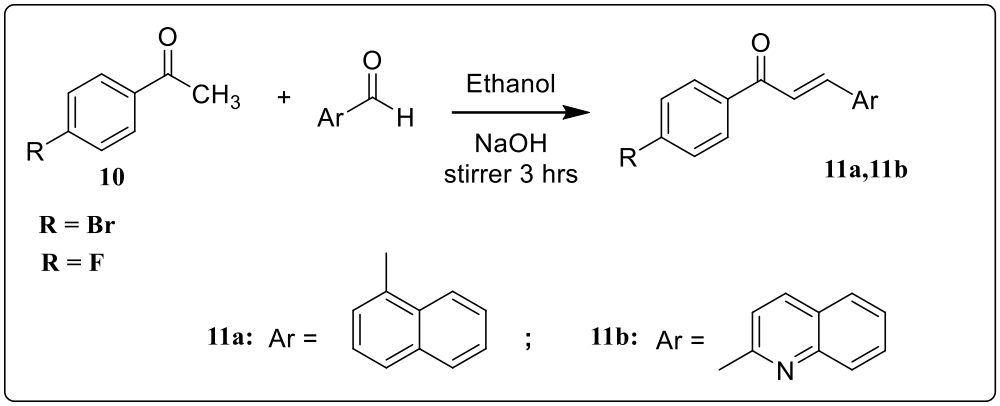


Figure 2. ^{1}H- NMR spectrum of compound (9).

The chalcone compounds of (**11a)** and (**11b)** were obtained in a yield of 67% and 51% respectively by reaction of aromatic aldehydes with 4-bromo acetophenone (**10a**) and 4- fluoro acetophenone (**10b**) respectively in ethanol and 40% aqueous solution of sodium hydroxide (Scheme 3). The FT-IR spectra of (**11a)** and (**11b)** revealed absorption bands at 1660 and 1695 cm^{-1} or 1598 and 1606 cm^{-1} respectively, attributed to stretching of the C=O and CH=CH. ^{1}H NMR of compounds (**11a)** and (**11b)** revealed a doublet signal at δ 7.81 ppm assigned to =CH-Ar and a doublet signal at δ 8.60 corresponding to Ar-CH= respectively (Fig. 3). The mass spectra of chalcone compounds (**11a)** and (**11b)**, which occurred at molecular ion peaks, were found to be in good alignment with the expected values of *m/z* 336.01 and 277.09.



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

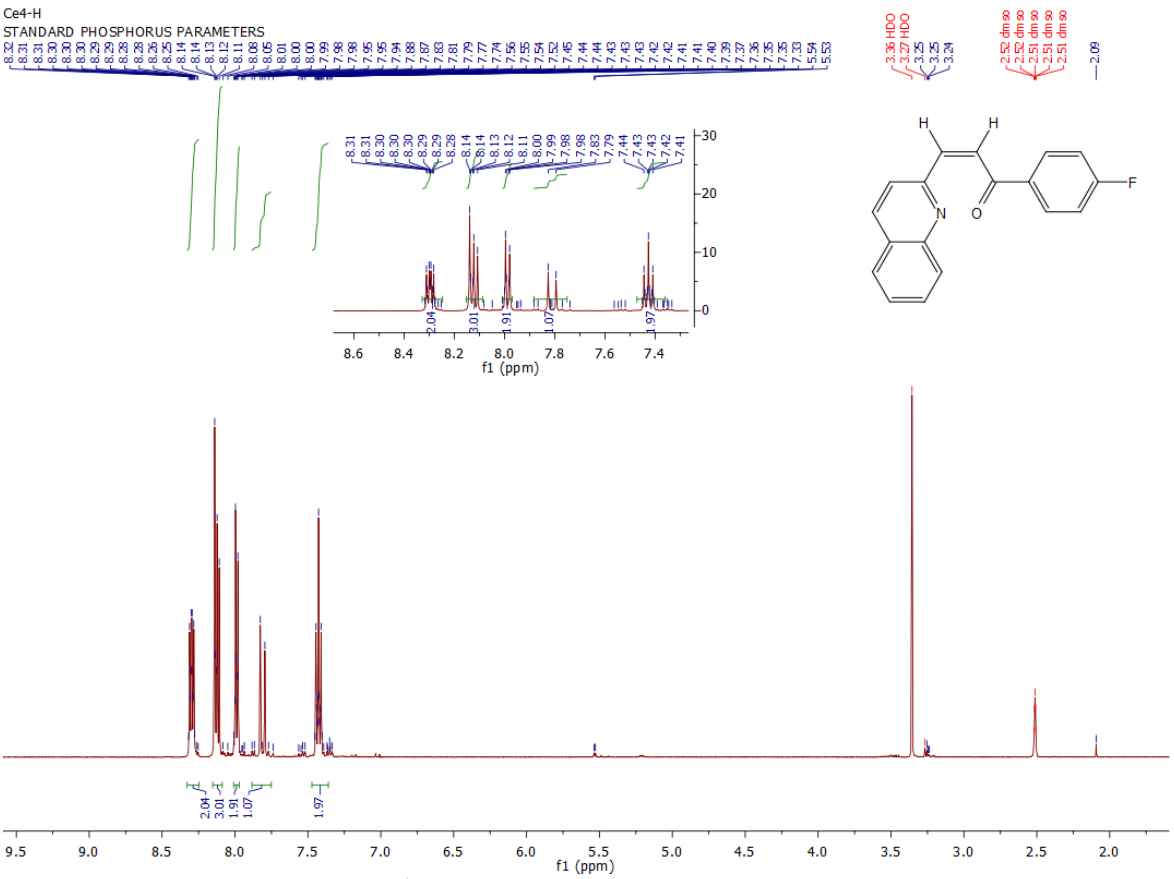


Figure 3. ^{1}H-NMR spectrum of compound (11b).

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}H NMR spectrum of (**13**), a signal at δ 6.91 ppm was ascribed to NH_2, a singlet signal at δ 7.40 ppm assigned to pyrimidine-CH, and multiplet signals at δ 7.55-8.26 ppm to aromatic protons (Fig. 4). ^{13}C NMR showed signals at δ 106.5 assigned to pyrimidine-CH, signals at δ 163.2, 163.8, and 168.1 assigned to C-N, H_2N-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.

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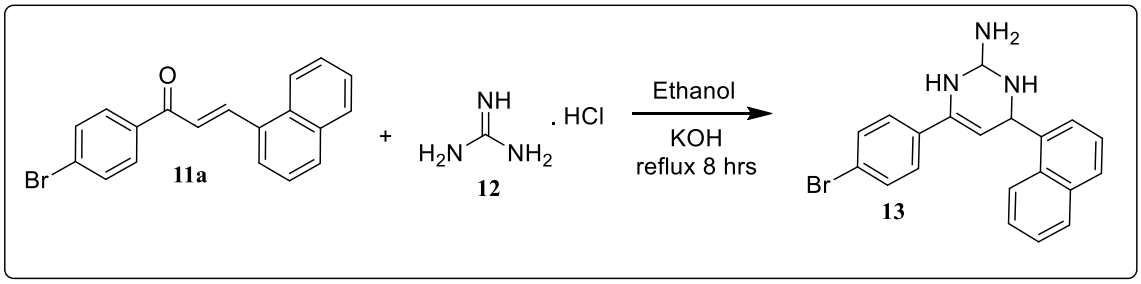

Scheme 4. Preparation of pyrimidine compound (13).

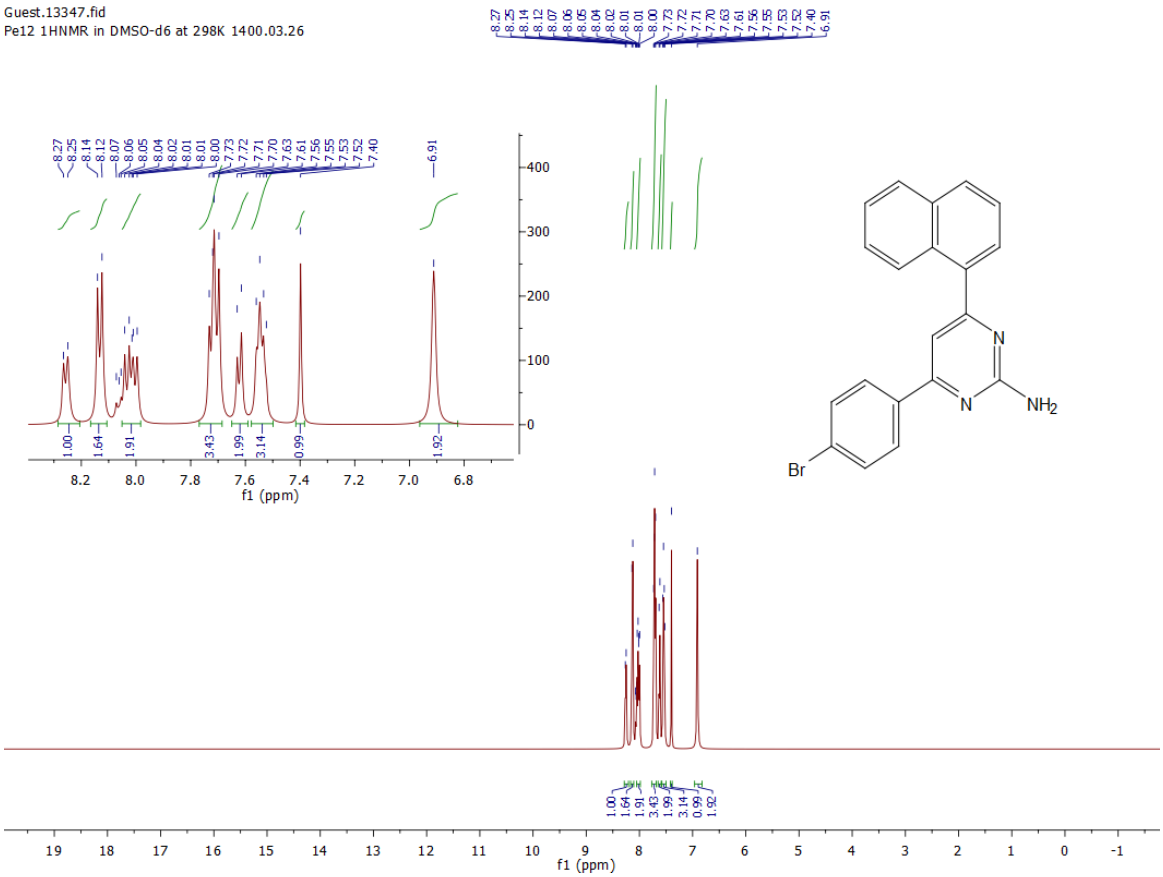

Figure 4. ^{1}H-NMR spectrum of compound (13).

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.^{1}H 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). ^{13}C 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=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.

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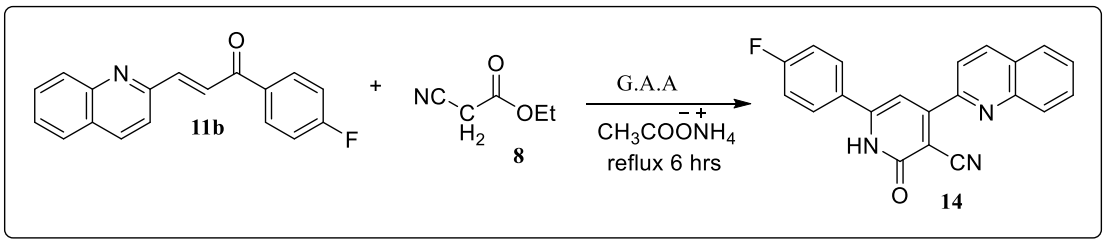


Scheme 5. Preparation of pyridine compound (14).

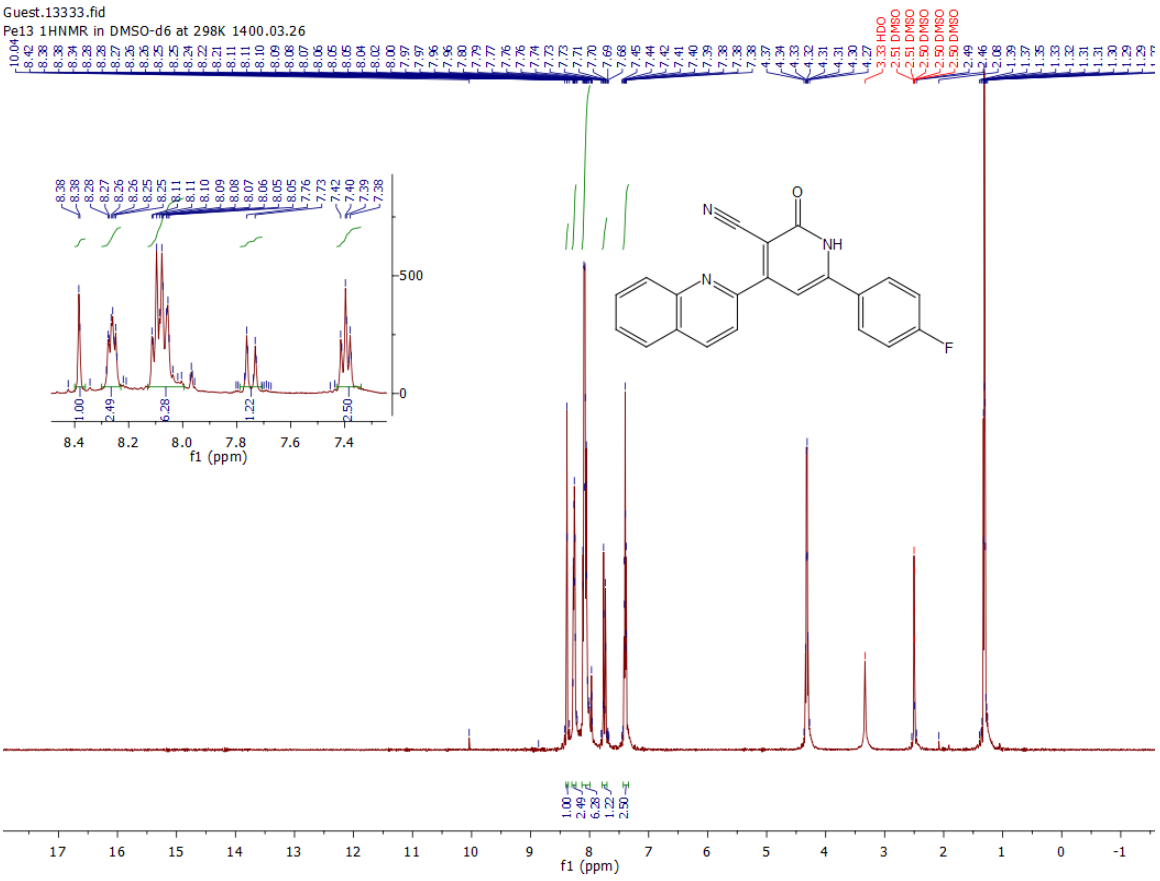
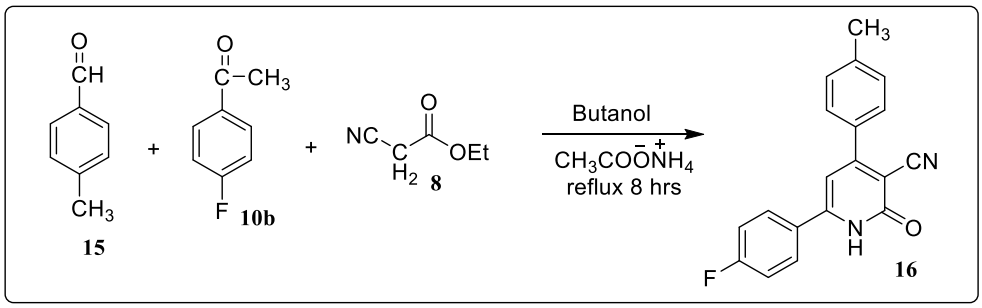


Figure 5. ^{1}H-NMR spectrum of compound (14).

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.^{1}H NMR spectrum of (**16)** revealed a signal at δ 3.34 ppm assigned to CH_3, multiplet signals at δ 7.34-8.24 ppm assigned to value of *m/z* 304.10. aromatic protons, and NH appeared at δ 8.38 (Fig. 6). ^{13}C NMR showed a signal at δ 26.3 assigned to CH_3, 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).

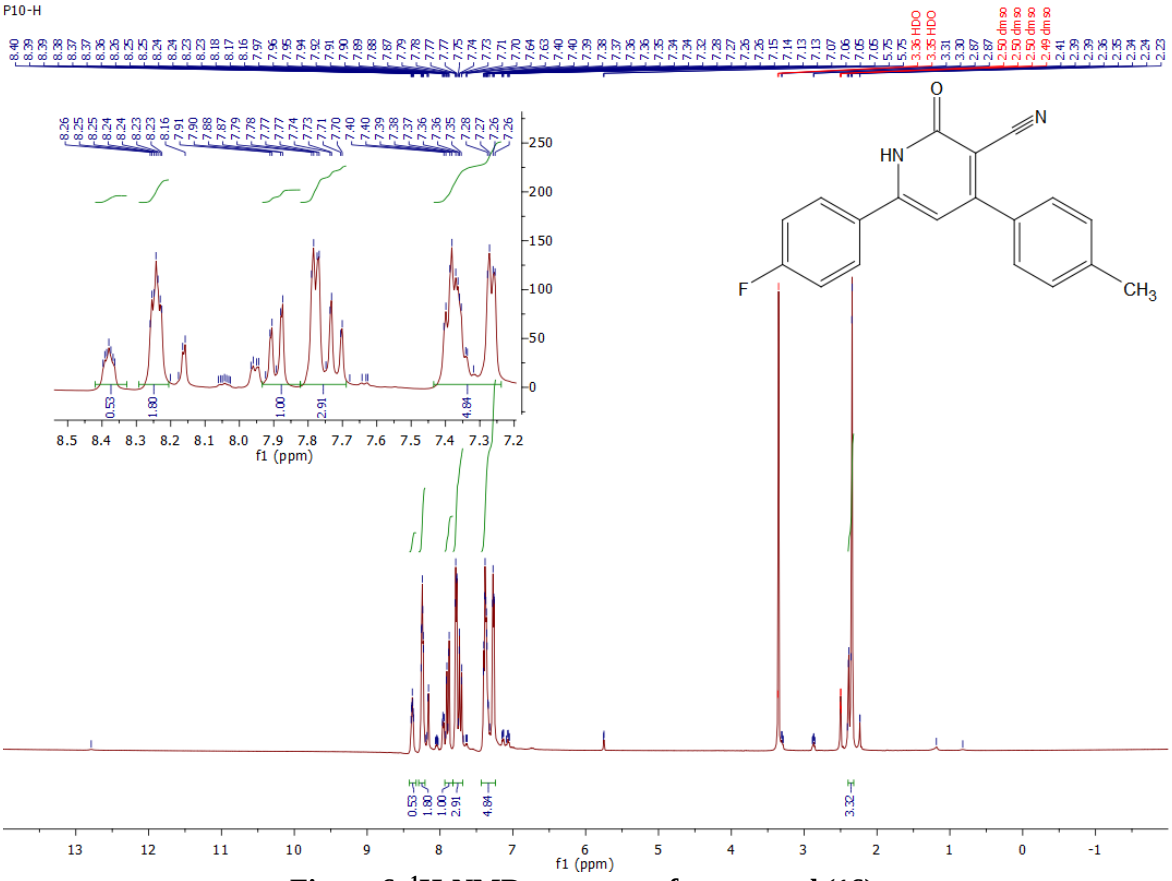


Figure 6. ^{1}H-NMR spectrum of compound (16).

Antibacterial activity

The in vitro antibacterial activity of the synthesized compounds was evaluated using the agar disc diffusion method [18] against the growth of two pathogenic bacterial isolates, Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*, and the inhibition zone of the prepared compounds was calculated within three concentrations (1, 0.1 and 0.01 mg/mL) and compared with a standard antibiotic as a control (ampicillin) (Table. 2). Compounds **5a**, **5b**, **9**, **13**, **14,** and **16** demonstrated antibacterial activity against Staphylococcus aureus tested bacterium. On the other hand, compounds **5a**, **5b**, **9**, **13**, **14,** and **16** did not present any activity against *Escherichia coli* (Fig. 16).

Table 2. Antibacterial activities of the investigated compounds against pathogenic bacterial isolates by disc diffusion assay.

Compound No	Conc. mg/mL	*Inhibition zone diameter, (mm)	
		Gram +ve bacteria	Gram -ve bacteria
		S. aureus	*E. coli*
5a	1	1.8	0
	0.1	1.7	0
	0.01	1.7	0
5b	1	1.2	0
	0.1	1.2	0
	0.01	1.2	0
9	1	1.6	0
	0.1	1.6	0
	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

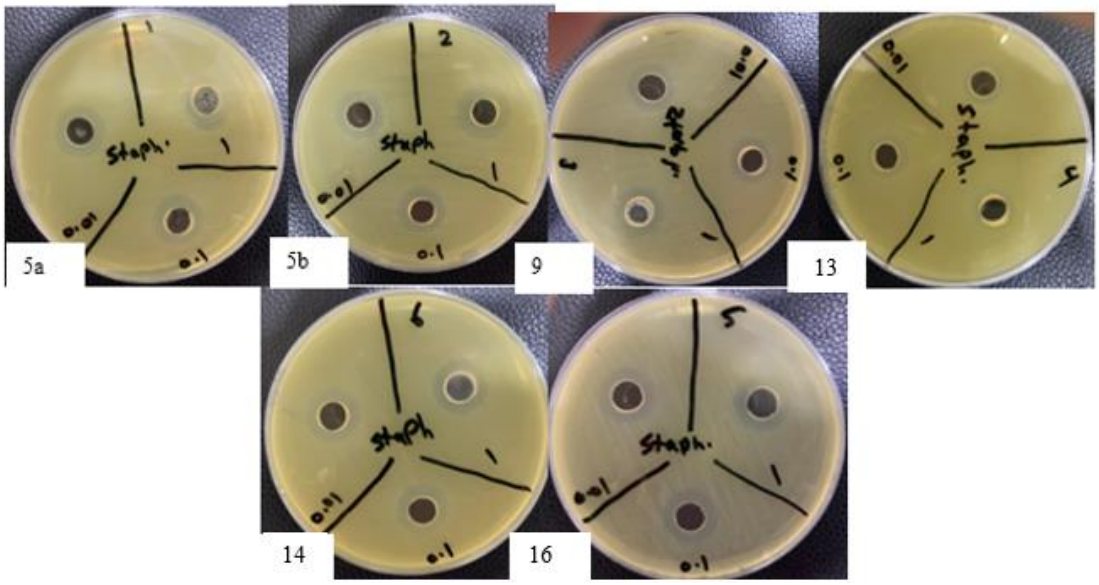


Figure 16. Inhibition zones of *S. aureus* G+ by the effects of the synthesized compounds.

Effect of drugs/plant extracts on the viability of *E. granulosus* protoscoleces[19, 20]

The antiparasitic activity of synthesized compounds **5a**, **5b,** and **16** were tested against *E. granulosus* protoscoleces, within three concentrations (1, 0.1, and 0.01 µg/mL). The cytotoxicity of drugs/plant extracts against protoscoleces 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 protoscoleces with an active metabolism. As a result, 25 µL of 5 m/mL MTT produced in BPS was injected into each well, and protoscoleces were maintained for two hours at 37°C in a CO2 incubator in the dark. The medium was replaced, and the protoscoleces' 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 µg/ml against *E. granulosus* (Fig. 17).

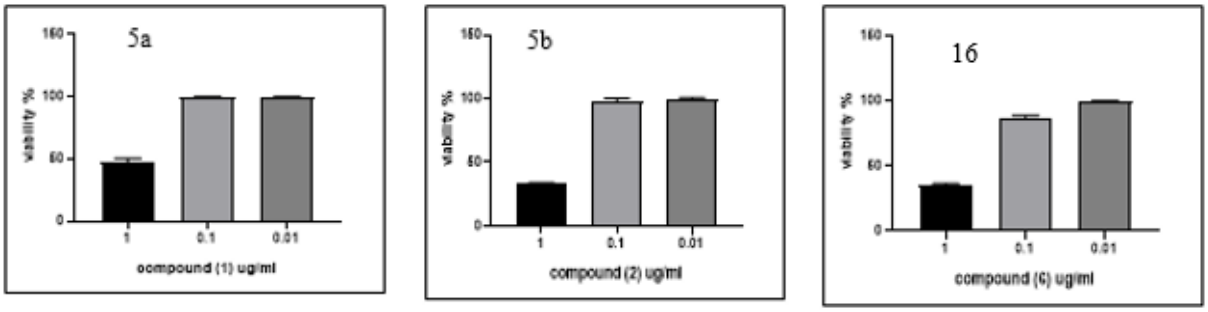


Figure 17. The viability of E. granulosus protoscoleces effects of the synthesized compounds.

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 µg /mL). Cytotoxicity results over HepG2 cells, compounds **5a** and **14** had a moderate cytotoxic activity with IC_{50} values of 43.84 µg /mL and 57.14 µg /mL respectively, when compared to the other synthesized compounds (Fig. 18).

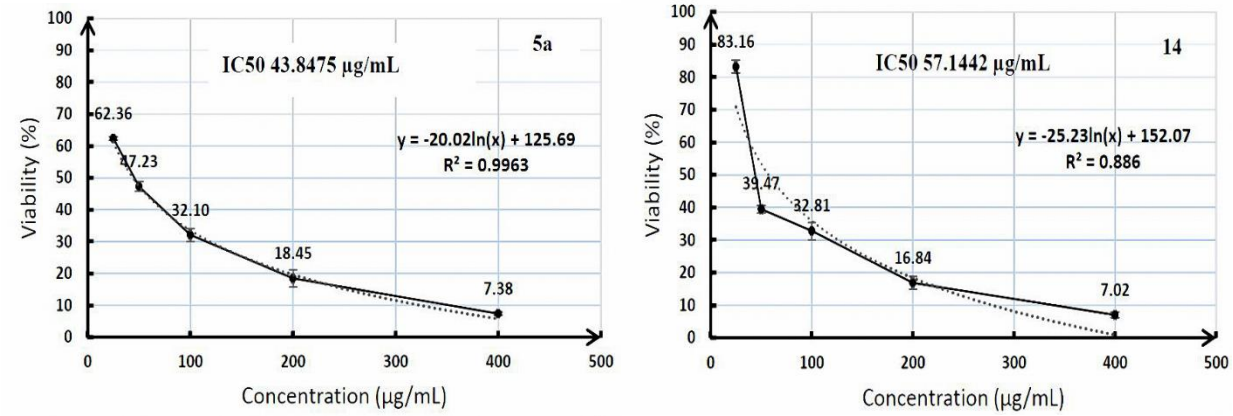


Figure 18. IC_{50} values of compounds 5a and 14 against HepG2 cell.

Regarding the MCF7 cell line, pyridine compound **14** had a moderate cytotoxic activity with an IC_{50} value of 50.84 µg /mL), when compared to the other synthesized compounds (Fig. 19).

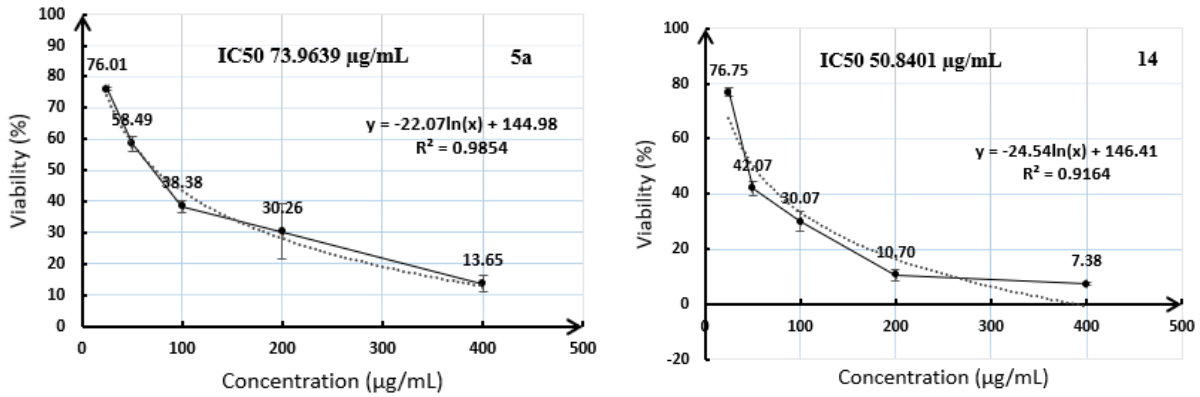


Figure 19. IC_{50} values of compounds 5a and 14 against MCF7 cell.

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 aurous* but no activity against *Escherichia coli*. The anti-parasitic effectiveness of prepared compounds against *E. granulosus* protoscoleces 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.

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تحضير وتشخيص بعض مشتقات البيريدين والبيريميدين الجديدة ودراسة فعاليتها البيولوجية

اسراء راضي عبدالحسين[1] **زينب عبدالامير محمد صالح[2]**

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الخلاصة:

تعتبر الأنظمة الحلقية غير المتجانسة ضرورية في الكيمياء الطبية بسبب نشاطها الواعد السام 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 الثيويوريا و اندول-3-كاربوكسالديهيد و piperonal على التوالي لتحضير بعض مشتقات البيريميدين 5a و 5b. كذلك حضر المركب 9 من تفاعل ثايوفين-2-كاربوكسالديهيد مع اثيل سيانواسيتيت واليوريا بوجود كاربونات البوتاسيوم كعامل مساعد. وحضرت مركبات الجالكونات 11a و 11b من تفاعل كميات مولية متساوية من كل من 1-نفثالديهيد و كوينولين-2-كاربوكسالديهيد على التوالي من كل من 4-بروموا سيتوفينون و 4-فلورواسيتوفينون على التوالي . وحضر مركب البيرميدين 13من تفاعل مركب الجالكون 11a مع كواندين هيدروكلوريد 12 بوجود هيدروكسيد البوتاسيوم. كما حضرت مشتقات البريدين 14 و16 من تفاعل مركب الجالكون 11b مع اثيل سيانواسيتيت وخلات الامونيوم في حامض الخليك الثلجي، حضر بهذه الطريقة المركب .14 كذلك تم مفاعلة 4-مثيل بنزالديهيد مع 4-فلورواسيتوفينون واثيل سيانواسيتيت وخلات الامونيوم في ن-بيوتانول لتحضير المركب 16. شخصت جميع المشتقات المحضرة باستعمال التقنيات الطيفية (FT-IR و ^{1}H-NMR و ^{13}C-NMR)، فضلا عن أطياف 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) وHepG2 (خلايا سرطان الكبد). فقد أظهر المركبين 5a و14 أنشطة سامة للخلايا ضد خلايا HepG2 بقيم IC_{50} تبلغ 43.84 و57.14 ميكروغرام / مل على التوالي. بالإضافة إلى ذلك، فقد أظهر مركب البريدين 14 نشاطًا سامًا للخلايا ضد خلايا MCF-7 بقيمة IC_{50} تبلغ 50.84 ميكروغرام/ مل. كما درست الخصائص المضادة للبكتيريا الموجبة والسالبة لصبغة كرام والطفيليات (*E. granulosus* protoscoleces) للمشتقات المحضرة.

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