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Synthesis, Characterization and Antibacterial Studies of Some New 2-(1,5-Diphenyl-4,5-Dihydro-1H-Pyrazol-3-Yl)Pyridine Derivatives

KEYWORDS: Pyrazoline, Chalcone, antibacterial.

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ABSTRACT *An efficient and practical synthesis of five compounds of pyrazoline derivatives structures was achieved through cyclization of phenyl hydrazine with α,β--unsaturated ketones (chalcones) using glacial acetic acid as catalyst under thermal conditions. These compounds have been characterized by FT-IR, elemental analysis (C.H.N.) and 1H NMR spectroscopyand biological activity; the anti-bacterial activity indicated that compounds possessed a broad spectrum of activity.*

Introduction :

In the past, many decades since penicillin was discovered and introduced as apowerful antibacterial agent, antibiotics have become critical in the fight against infectious diseases caused by bacteria and other microbes. However, widespread antibiotic use has promoted the emergence of antibiotic-resistant pathogens, including multidrug resistant strains [1–3]. At present, the appearance of more and more pathogenetic bacterial species resistant to conventional antibiotics has resulted in either high expenses or failure in the treatment of infectious diseases. An alarming increase in resistance of bacteria that cause community acquired infections has also been documented, especially in Staphylococci and Pneumococci, which are prevalent causes of disease and mortality. In addition, the risk of opportunistic fungal infections increases rapidly accompanied with AIDS disease, and as an obviously consequence invasive infections represent a major cause of mortality for these patients [4]. With the emergence of new microbial strains resistant to many conventional available antibiotics there is growing interest in the discovery of new antibacterial agents [1,2]. According to the known structure and activity relationships, it is considered that certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules [5,6].

Chalcones (1,3-diphenyl-propene-1-one) belonging to the flavonoid family, are natural and synthetic products that have been reviewed for their wide range of biological activities as antibacterial [7,8], anti-tumor [9–11], anti-inflammatory [12–14] and antioxidant agents [15–18], etc. Although studies on the bioavailability of heterocyclic chalcones from natural sources are limited, they have been reported as having a wide range of biological activities, especially antibacterial [19–28], and antifungal activities [29]. In an effort to diversify the biological activities of conventional chalcones, Heterocyclic chalcone analogues in which an electron rich nitrogen replaces the benzene ring were synthesized. Herein, we report the synthesis of some novel heterocyclic chalcone analogues using a conventional base catalyzed Claisen Schmidt condensation reaction and their possible antibacterial activity alone and in combination with antibiotics.

Experimental

General. Melting points were uncorrected. FT.IR-8400,SHIMADZU. NMR spectra were acquired with a Bruker Ultra Shield (^{1}H : 300 MHz) (University of AL-al-Bayt,Jordan). The chemical shifts were referenced to tetra methyl silane (TMS) as an internal standard. The elemental analysis were performed by using Euro Vector EA3000A (University of AL-al-Bayt,Jordan).

Synthesis of pyrazoline derivatives (1-5)

General procedure. To a stirred solution of chalcone (a–e) (which was prepared as mentioned in the literature) [30] (1.0 mmol) in 10 ml EtOH (96 %) was added phenyl hydrazine (2.0 mmol) and glacialaceticacid (2.5 ml) at room temperature. The reaction mixture was heated to reflux overnight. The progress of the reaction was monitored by TLC (ethyl acetate/hexane, 8:2). The EtOH was removed under reduced pressure and the residue was recrystalized from EtOH to afford the pure products (1–5).

3-(1-phenyl-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-5-yl) phenol (1)

was prepared from the reaction of 3-(3-hydroxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-one (a) with phenyl hydrazine and gave a 73% yield with a m.p. (200-202)°c. The CHN analysis for $C_{20}H_{17}N_3O$; C 76.17; H 5.43; N 13.32; Found C 76.12; H 5.40; N 13.30, FT-IR spectra (KBr pellet) υ(cm^{-1}) 3450 (OH stretching of phenol), 3330 (NH stretching of pyrazoline ring), 3020 (C–H stretching of aromatic ring), 2880 (C–H stretching of aliphatic), 1614 (C=N stretching of pyrazoline ring), 1595 (C=C stretching of aromatic ring), 1219 (C–N stretching of pyrazoline ring), $\delta_H(CDCl_3)$ 9.5 ppm (1H,s,13), (7.960-7.990) ppm (1H,d,1); (7.790-7.820) ppm (1H,t,2); (7.640-7.690) ppm (1H,t,3) ; (8.650-8.690) ppm (1H,d,4); (3.600-3.620) ppm (2H,d,5,5′); (5.190-5.230) ppm (1H,t,6); (6.800-7.250) ppm (9H,m,7,8,9,10,11,12,14).

2-(5-(2-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl) pyridine (2)

was prepared from the reaction of 3-(2-nitrophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (b) with phenyl hydrazine and gave a 87% yield with a m.p. (198-200)°c. The CHN analysis for $C_{20}H_{16}N_4O_2$; C 69.76; H 4.68; N 16.27; Found C 69.75; H 4.66; N 16.20, FT-IR spectra (KBr pellet) υ(cm^{-1}) 3333 (NH stretching of pyrazoline ring), 3024 (C–H stretching of aromatic ring), 2885 (C–H stretching of aliphatic), 1622 (C=N stretching of pyrazoline ring),

Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

1595 (C=C stretching of aromatic ring), 1214(C–N stretching of pyrazoline ring),, $\delta_H(CDCl_3)$ (7.960-7.990) ppm (1H,d,1); (7.790-7.820) ppm (1H,t,2); (7.640-7.690) ppm (1H,t,3) ; (8.650-8.690) ppm (1H,d,4); (3.600-3.620) ppm (2H,d,5,5′); (5.190-5.230) ppm (1H,t,6); (6.770-7.600) ppm (9H,m,7,8,9,10,11,12,13).

2-(1-phenyl-5-p-tolyl-4,5-dihydro-1H-pyrazol-3-yl)pyridine (3)

was prepared from the reaction of 1-(pyridin-2-yl)-3-p-tolylprop-2-en-1-one (c) with phenyl hydrazine and gave a 72 % yield with a m.p. (196-198)°c. The CHN analysis $C_{21}H_{19}N_3$; C 80.48; H 6.11; N 13.41; Found C 80.45; H 6.10; N 13.40, FT-IR spectra (KBr pellet) υ(cm^{-1}) 3332 (NH stretching of pyrazoline ring), 3028 (C–H stretching of aromatic ring), 2887 (C–H stretching of aliphatic), 1620 (C=N stretching of pyrazoline ring), 1590 (C=C stretching of aromatic ring), 1212(C–N stretching of pyrazoline ring), , $\delta_H(CDCl_3)$ (7.960-7.990) ppm (1H,d,1); (7.790-7.820) ppm (1H,t,2); (7.640-7.690) ppm (1H,t,3) ; (8.650-8.690) ppm (1H,d,4); (3.600-3.620) ppm (2H,d,5,5′); (5.190-5.230) ppm (1H,t,6); (6.800-7.250) ppm (9H,m,7,8,9,10,11,13,14); 2.34 ppm (3H,s,12).

2-(5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)pyridin (4)

was prepared from the reaction of 3-(4-chlorophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (d) with phenyl hydrazine and gave a 82 % yield with a m.p. (203-205)°c. The CHN analysis $C_{20}H_{16}Cl\ N_3$; C 71.96; H 4.83; N 12.59; Found C 71.95; H 4.80; N 12.57, FT-IR spectra (KBr pellet) υ(cm^{-1}) 3330 (NH stretching of pyrazoline ring), 3020 (C–H stretching of aromatic ring), 2885 (C–H stretching of aliphatic), 1618 (C=N stretching of pyrazoline ring), 1593 (C=C stretching of aromatic ring), 1210 (C–N stretching of pyrazoline ring), , $\delta_H(CDCl_3)$ (7.960-7.990) ppm (1H,d,1); (7.790-7.820) ppm (1H,t,2); (7.640-7.690) ppm (1H,t,3) ; (8.650-8.690) ppm (1H,d,4); (3.600-3.620) ppm (2H,d,5,5′); (5.190-5.230) ppm (1H,t,6); (6.800-7.250) ppm (9H,m,7,8,910,11,13,14),

4-bromo-2-(1-phenyl-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol (5)

was prepared from the reaction of 3-(5-bromo-2-hydroxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-one (e) with phenyl hydrazine and gave a 80 % yield with a m.p. (205-207)°c. The CHN analysis $C_{20}H_{16}BrN_3O$; C 60.93; H 4.09; N 10.66; Found C 60.90; H 4.07; N 10.65, FT-IR spectra (KBr pellet) υ(cm^{-1}) 3500 (OH stretching of phenol), 3330 (NH stretching of pyrazoline ring), 3020 (C–H stretching of aromatic ring), 2885 (C–H stretching of aliphatic), 1618 (C=N stretching of pyrazoline ring), 1593 (C=C stretching of aromatic ring), 1210 (C–N stretching of pyrazoline ring), , $\delta_H(CDCl_3)$ 9.620 ppm (1H,s,10); (7.960-7.990) ppm (1H,d,1); (7.790-7.820) ppm (1H,t,2); (7.640-7.690) ppm (1H,t,3) ; (8.650-8.690) ppm (1H,d,4); (3.600-3.620) ppm (2H,d,5,5′); (5.190-5.230) ppm (1H,t,6); (6.800-7.250) ppm (8H,m,7,8,9,11,12,14).

Biological activity

Kirby Bauer method was carried out by using discs (6 mm diameter) of paper chromatography which were sterilized by the autoclave apparatus at 121 °C for 15 minutes under 1 atm and kept in clean sterilized glass screw plugs.

The primary screening of the antibacterial activity of the synthesized pyrazoline compounds was investigated by the method of plate agar diffusion with slight modification by using Muller-Hinton agar medium which was sterilized by autoclave apparatus at 121 °C for 15 minutes under 1 atm and then cooled and poured into sterilized Petri dishes.

Two bacterial species were used, Gram positive Staphylococcus aureus and Gram negative Escherichia coli. The isolated bacteria were cultured by strecking on nutrient agar (N. A.) in order to obtain youth colonies of 24 hours age.

One colony from the fresh agar culture of each organism was inoculated into 5 ml sterile nutrient broth (N. B) and was incubated at 37 °C for 6 hrs. In order to obtain germ growth of optical density = 0.1 (equivalent to 10^6 cell/ml), 0.1 ml of each growing organism was spread on the Muller-Hinton Agar (M. H. A.) surface by sterilized swabs in three directions to get homogenous growth. The discs saturated with the prepared nitrone and isoxazolidine compounds (concentration 1000 µg/ml) were added to (M.H.A.) medium by clean forceps, then incubated at 37 °C for 24 hours.

The dishes were examined for the presence or absence of bacterial growth and the averages of inhibition zone diameters around each disc in millimeters were measured (the zone where there is no bacterial growth) [31] ,as shown in table (1).

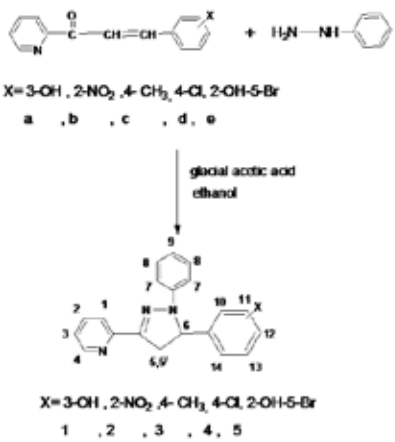
Results and discussion

Treatment of chalcones derivatives (a-e) with phenyl hydrazine in boiling ethanol gave pyrazoline derivatives compounds, after purification by recrystallization from ethanol, pure pyrazoline derivatives compounds as shown in (scheme 1) in (72-87)% yield were obtained. The structures of these products were established from their elemental analysis, FT-IR,C.H.N and ^{1}H NMR spectra. The FT-IR spectra of pyrazoline compounds were characterized by the disappearance of the absorption band that was attributed to the (C=O) stretching which appeared at (1672-1710) cm^{-1}. These fact confirmed the correct expected chemical structure of these compounds. All the IR spectra of pyrazoline derivatives showed a peak at (1614-1622) cm^{-1} which related to (C=N) stretching of pyrazoline ring , a peak at (1210-1219) cm^{-1} which appeared due to (C-N) stretching of pyrazoline ring and a peak at (1590-1595) cm^{-1} which appeared due to (C=C stretching of aromatic ring). While, the C-H stretching aromatic rings showed a peak within the range (3020-3028) cm^{-1} and the C-H stretching aliphatic showed a peak within the range (2880-2885) cm^{-1}. The N-H stretching showed a peak within the range (3330-3333) cm^{-1}.while the O-H stretching showed a peak within the range (3450-3500) cm^{-1}.

All the ^{1}H NMR spectra of pyrazoline ring were characterized [32-34] showed triplet signals within the range (5.190-5.230) ppm which appeared to proton in (6) position because interaction with two protons in (5 and 5\) position , while the two protons in (5 and 5\) position showed doublet signals within the range (3.600-3.620) ppm because interaction with protons in (6) position. These peaks confirmed the correct expected chemical structure of pyrazoline compounds. The proton in position (1) of pyridine ring showed doublet signals at (7.960-7.990) ppm , while the proton in position (2) of pyridine ring showed triplet signals within the range (7.790-7.820) ppm.The proton in position (3) in pyridine ring showed triplet signals within the range (7.640-7.690) ppm. The other proton in position (4) in pyridine ring showed doublet signals within the range (8.650-8.690) ppm. The protons of aromatic rings showed multiplet signals within the range (6.800-7.250) ppm which appeared to 8 or 9 protons in positions (7,8,9,10,11,12,13 and 14). While the compounds

Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

(7) showed multiplet signals within the range (6.770-7.600) ppm which appeared to the 9 protons in positions (7,8,9,10,11,12 and 13) positions. The CH_3 protons showed singlet signal for three protons at 2.340 ppm. The OH protons showed singlet signal in the region δ= 9.500 ppm in compound 1 and δ= 9.620 ppm in compound 5.



Scheme (1)

The biological activity

The antibacterial activities of the synthesized pyrazoline compounds against the tested organisms; Staphylococcus aureus and Escherichia coli using Hahn method[35], were summarized in table (1) and figures (1-5). This method was based on the disc diffusion for testing chemical agents and antimicrobial effectiveness by measuring and determining the agents zones of inhibitions which sizes are proportional to how sensitive the organism is to the particular antibiotic in the disc [36].

Generally, it is clear that Gram negative bacteria (E. coli) are more affected than Gram positive bacteria (S. aureus). It has been postulated that the cell membrane of E. coli contains many condensed fat layers as compared to S. aureus. Accordingly, chemicals, antibiotics or antiseptics face difficulty in penetrating these membranes and therefore their effectiveness is diminished [37].

Table (1) shows the values of inhibition zones in (mm) of the pyrazoline compounds against the two tested microorganisms.

Table (1): Inhibition zones (mm) of the synthesized pyrazoline compounds against – standard microorganisms

Compounds	IZ (E. coli) mm	IZ (S. aureus) mm
1	12	10
2	-	-
3	12	9
4	14	11
5	-	-

Figure (1) Inhibition Zones of 1and 2 against E.coli

Figure (2) Inhibition Zones of 3,4 and 5 against E.coli

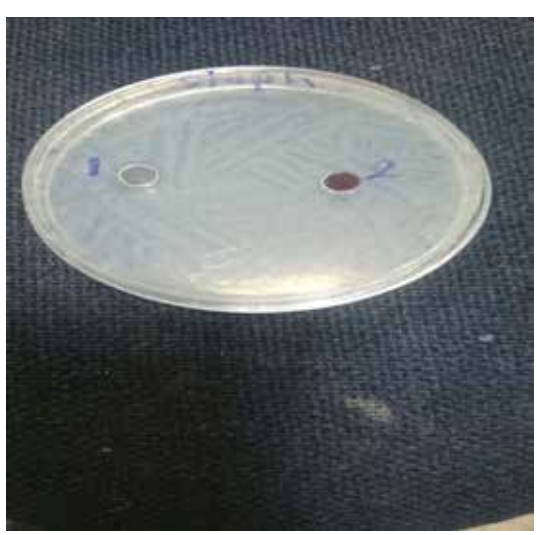


Figure (3) Inhibition Zones of 1and 2 against S. aureus

Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

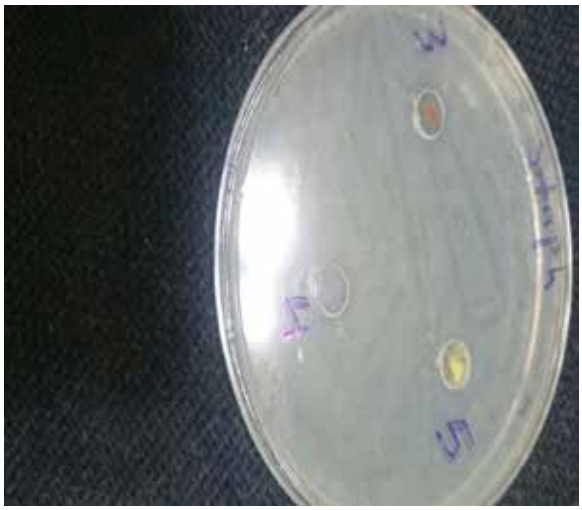


Figure (4) Inhibition Zones of 3,4 and 2 against S. aureus

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