

# Synthesis, Characterization and Antibacterial Studies of Some New 1, 4-bis (3-Phenyl-4,5-Dihydroisoxazol-5-yl)Benzene Derivatives

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## Introduction

Chalcones, analogs of 1,3-diarylprop-2-en-1-one, form a wide class of compounds containing two aromatic rings bound with vinyl ketone fragment. They are useful in synthesis of various heterocyclic compounds. Chalcones of plant origin are known [1]. Chalcones present great interest as compounds exhibiting antimalarial [2], antibacterial [3], antifibrogenic [4], anticancer [5], antitrichomonal [6], anti-inflammatory [7], antileishmanial [8], cytotoxic and antitrypanosoma cruzi [9] activities. While the flavonoid compounds are a group of natural products found in fruits, vegetables, nuts, seeds and flowers as well as in teas and are important constituent of human diet. They have been demonstrated to possess antioxidant [10], antihypertensive [11], antiallergic [12], antinociceptive [13], trypsin inhibitors [14], plant growth regulator [15], antibacterial and antifungal [16,17] activities.

In the last few years microwave induced organic reaction enhancement (MORE) chemistry has gained popularity as a nonconventional technique for rapid organic synthesis [18] and many researchers have described accelerated organic reactions, and a large number of papers has appeared. Proving the synthetic utility of SMORE chemistry in routine organic synthesis [19]. It has been termed as 'e-chemistry' because it is easy, effective, economical and ecofriendly and is believed to be a step toward green chemistry. In view of these observations and in continuation of our work on biologically active chalcones and their heterocycles [20], we have been planned to synthesize the new 1,4-bis(3-phenyl-4,5-dihydroisoxazol-5-yl)benzene derivatives from chalcones and also studied their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

## Experimental

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

### Synthesis of 4,5-dihydroisoxazol derivatives (2a-e)

**General procedure.** To a stirred solution of chalcone (**1a-e**) (which was prepared as mentioned in the literature) [21] (1.0 mmol) in 10 ml EtOH (96 %) was added hydroxylamine hydrochloride (2.0 mmol) and glacial acetic acid (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 recrystallized from EtOH to afford the pure products (**2a-e**).

#### 1,4-bis(3-phenyl-4,5-dihydroisoxazol-5-yl)benzene (2a)

was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-phenylprop-2-en-1-one) (**1a**) with hydrazine hydrate and gave a 70% yield with a m.p. (196-198) $^{\circ}\text{C}$ . The CHN analysis for  $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_2$ ; C 78.24; H 5.47; N 7.60 Found C 78.22; H 5.46; N 7.60, FT-IR spectra (KBr pellet)  $\nu(\text{cm}^{-1})$  1110  $\text{cm}^{-1}$  (N-O stretching of isoxazole ring), 3020 (C-H stretching of aromatic ring), 2880 (C-H stretching of aliphatic), 1614 (C=N stretching of isoxazoline ring), 1595 (C=C stretching of aromatic ring), 1219 (C-N stretching of pyrazoline ring),  $\delta_{\text{H}}(\text{CDCl}_3)$  (7.529-7.941) ppm (10H, m, 12, 12', 13, 13', 14, 14', 15, 15', 16, 16'); 7.293 ppm (4H, s, 6, 7, 9, 10); (5.041-5.600) ppm (2H, t, 4, 4'); (3.601-3.644) ppm (4H, d, 3, 3').

#### 1,4-bis(3-p-tolyl-4,5-dihydroisoxazol-5-yl)benzene (2b)

was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-p-tolylprop-2-en-1-one) (**1b**) with hydrazine hydrate and gave a 72% yield with a m.p. (202-204) $^{\circ}\text{C}$ . The CHN analysis for  $\text{C}_{26}\text{H}_{24}\text{N}_2\text{O}_2$ ; C 78.76; H 6.10; N 7.07 Found C 78.75; H 6.08; N 7.05, FT-IR spectra (KBr pellet)  $\nu(\text{cm}^{-1})$  1112  $\text{cm}^{-1}$  (N-O stretching of isoxazole ring), 3024 (C-H stretching of aromatic ring), 2885 (C-H stretching of aliphatic), 1610 (C=N stretching of isoxazoline ring), 1597 (C=C stretching of aromatic ring), 1218 (C-N stretching of pyrazoline ring),  $\delta_{\text{H}}(\text{CDCl}_3)$  (7.378-7.402) ppm (4H, d, 13, 13', 15, 15'); (7.710-7.925) ppm (4H, d, 12, 12', 16, 16'); 7.293 ppm (4H, s, 6, 7, 9, 10); (5.041-5.600) ppm (2H, t, 4, 4'); (3.601-3.644) ppm (4H, d, 3, 3'); 2.542 ppm (6H, s, 14, 14' (CH<sub>3</sub>)).

#### 1,4-bis(3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)benzene (2c)

was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-(4-methoxyphenyl)prop-2-en-1-one) (1c) with hydrazine hydrate and gave a 77% yield with a m.p. (205-207)<sup>o</sup>c. The CHN analysis for C<sub>26</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>; C 72.88; H 5.65; N 6.54 Found C 72.87; H 5.62; N 6.52, FT-IR spectra (KBr pellet)  $\nu(\text{cm}^{-1})$  1115  $\text{cm}^{-1}$  (N-O stretching of isoxazole ring), 3020 (C-H stretching of aromatic ring), 2884 (C-H stretching of aliphatic), 1611 (C=N stretching of isoxazoline ring), 1596 (C=C stretching of aromatic ring), 1210 (C-N stretching of pyrazoline ring),  $\delta_{\text{H}}(\text{CDCl}_3)$  (7.064-7.199) ppm (4H,d, 13,13',15,15'); (7.910-7.944) ppm (4H,d, 12,12',16,16'); 7.293 ppm (4H,s, 6,7,9,10); (5.041-5.600) ppm (2H,t,4,4'); (3.601-3.644) ppm (4H,d,3,3'); 3.833 ppm (6H,s,14,14' (OCH<sub>3</sub>)).

#### 1,4-bis(3-(4-bromophenyl)-4,5-dihydroisoxazol-5-yl)benzene (2d)

was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-(4-bromophenyl)prop-2-en-1-one) (1d) with hydrazine hydrate and gave a 82% yield with a m.p. (208-210)<sup>o</sup>c. The CHN analysis for C<sub>24</sub>H<sub>18</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub>; C 54.78; H 3.45; N 5.32 Found C 54.76; H 3.44; N 5.30, FT-IR spectra (KBr pellet)  $\nu(\text{cm}^{-1})$  1116  $\text{cm}^{-1}$  (N-O stretching of isoxazole ring), 3022 (C-H stretching of aromatic ring), 2880 (C-H stretching of aliphatic), 1611 (C=N stretching of isoxazoline ring), 1596 (C=C stretching of aromatic ring), 1211 (C-N stretching of pyrazoline ring),  $\delta_{\text{H}}(\text{CDCl}_3)$  (7.583-7.602) ppm (4H,d, 13,13',15,15'); (7.722-7.776) ppm (4H,d, 12,12',16,16'); 7.293 ppm (4H,s, 6,7,9,10); (5.041-5.600) ppm (2H,t,4,4'); (3.601-3.644) ppm (4H,d,3,3').

#### 1,4-bis(3-(4-nitrophenyl)-4,5-dihydroisoxazol-5-yl)benzene (2e)

Was prepared from the reaction of 3,3'-(1,4-phenylene)bis(1-(4-nitrophenyl)prop-2-en-1-one) (1f) with hydrazine hydrate and gave a 85% yield with a m.p. (210-212)<sup>o</sup>c. The CHN analysis for C<sub>24</sub>H<sub>18</sub>N<sub>4</sub>O<sub>6</sub>; C 62.88; H 3.96; N 12.22 Found C 62.85; H 3.94; N 12.20, FT-IR spectra (KBr pellet)  $\nu(\text{cm}^{-1})$  1114  $\text{cm}^{-1}$  (N-O stretching of isoxazole ring), 3023 (C-H stretching of aromatic ring), 2881 (C-H stretching of aliphatic), 1613 (C=N stretching of isoxazoline ring), 1597 (C=C stretching of aromatic ring), 1213 (C-N stretching of pyrazoline ring),  $\delta_{\text{H}}(\text{CDCl}_3)$  (8.332-8.426) ppm (4H,d, 13,13',15,15'); (8.009-8.039) ppm (4H,d, 12,12',16,16'); 7.293 ppm (4H,s, 6,7,9,10); (5.041-5.600) ppm (2H,t,4,4'); (3.601-3.644) ppm (4H,d,3,3').

#### 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 streaking 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<sup>6</sup> 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 nitron and isoxazolidine compounds (concentration 1000 g/ml) were added to (M.H.A.) medium by clean forceps, then incubated at 37 °C for 24 hours.

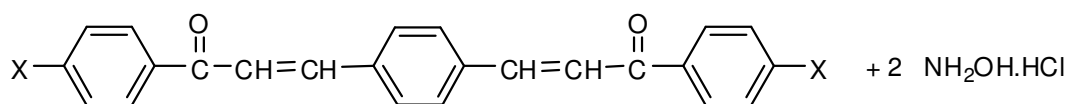
The dishes were examined for the presence or absence of bacterial growth and the averages of inhibition zone diameters around each disc in millimeters were measured (the zone where there is no bacterial growth) [22], as shown in table (1).

#### Results and discussion

Treatment of chalcones derivatives (1a-e) with hydroxylamine hydrochloride in boiling ethanol gave 4,5-dihydroisoxazole derivatives compounds, after purification by recrystallization from ethanol, pure 4,5-dihydroisoxazole derivatives compounds as shown in (scheme 1) in (70-85)% yield were obtained. The structures of these products were established from their elemental analysis, FT-IR, C.H.N and <sup>1</sup>H NMR spectra. The FT-IR spectra of 4,5-dihydroisoxazole compounds were characterized by the disappearance of the absorption band that was attributed to the (C=O) stretching which appeared at (1672-1710)  $\text{cm}^{-1}$ . These fact confirmed the correct expected chemical structure of these compounds. All the IR spectra of 4,5-dihydroisoxazole derivatives showed a peak at (1610-1614)  $\text{cm}^{-1}$  which related to (C=N) stretching of 4,5-

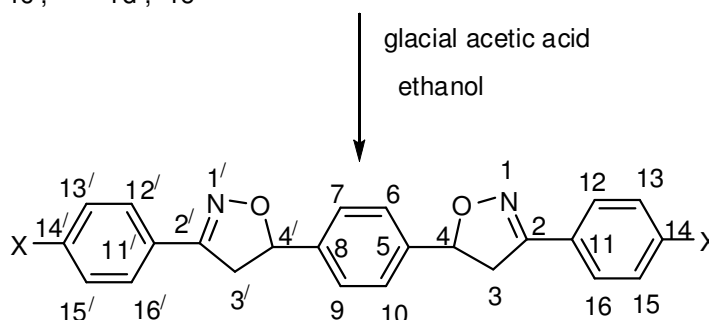
dihydroisoxazole ring, a peak at (1210-1219)  $\text{cm}^{-1}$  which appeared due to (C-N) stretching of pyrazoline ring and a peak at (1595-1597)  $\text{cm}^{-1}$  which appeared due to (C=C) stretching of aromatic ring). While, the C-H stretching aromatic rings showed a peak within the range (3020-3024)  $\text{cm}^{-1}$  and the C-H stretching aliphatic showed a peak within the range (2880-2885)  $\text{cm}^{-1}$ . The N-O stretching showed a peak within the range (1110-1116)  $\text{cm}^{-1}$ .

All the  $^1\text{H}$  NMR spectra of 4,5-dihydroisoxazole ring were characterized [23-25] by the presence protons (3,3') of 4,5-dihydroisoxazole ring showed doublet signals within the range (3.601-3.644) ppm because interaction with protons in (4,4') position and showed triplet signals within the range (5.041-5.600) ppm which appeared to protons in (4,4') position because interaction with two protons in (3 and 3') position, while The proton in position (1) of furan ring showed doublet signals at (7.900-7.921) ppm. The protons of aromatic rings in compound (2a) showed singlet signal within the range 7.293 ppm which appeared to four protons in (6,7,9,10), while the other protons in (12,12',13,13',14,14',15,15',16,16') showed multiplet signals within the range (7.529-7.941) ppm. The aromatic protons in compounds (2b,2c,2d and 2e) showed doublet signals within the range (7.064-8.426) ppm which appeared to the two protons in (13 and 15) positions. The other two protons in positions (12 and 16) showed doublet signals within the range (7.710-8.039) ppm. The  $\text{CH}_3$  protons showed singlet signal for six protons in the region 2.542 ppm. The  $\text{OCH}_3$  protons showed singlet signal for six protons at 3.833 ppm.



X = H,  $\text{CH}_3$ ,  $\text{OCH}_3$ , Br,  $\text{NO}_2$

1a, 1b, 1c, 1d, 1e



X = H,  $\text{CH}_3$ ,  $\text{OCH}_3$ , Br,  $\text{NO}_2$

2a, 2b, 2c, 2d, 2e

Scheme (1)

### The biological activity

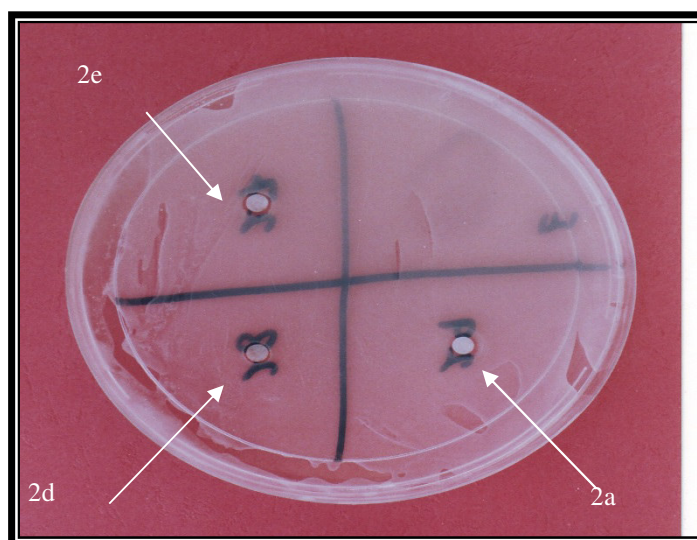
The antibacterial activities of the synthesized 4,5-dihydroisoxazole compounds against the tested organisms; *Staphylococcus aureus* and *Escherichia coli* using Hahn method[26], were summarized in table (1) and figures (1-4). This method was based on the disc diffusion for testing chemical agents and 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 [27].

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 [28].

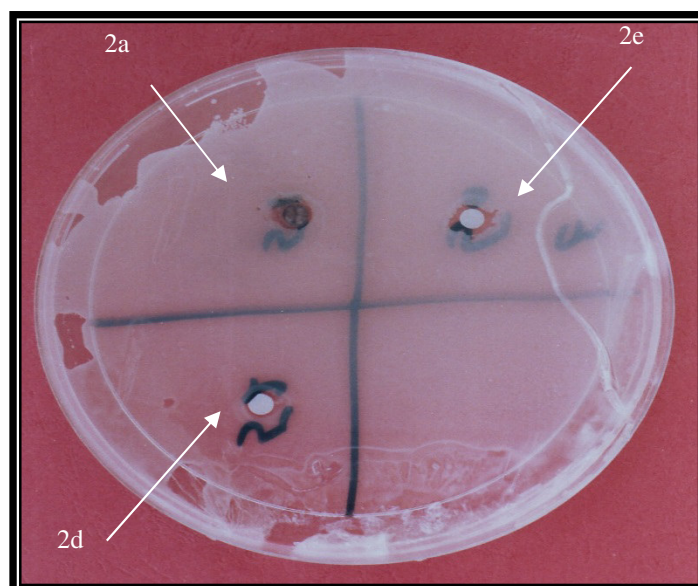
Table (1) shows the values of inhibition zones in (mm) of the 4,5-dihydroisoxazole compounds against the two tested microorganisms.

**Table (1): Inhibition zones (mm) of the synthesized 4,5-dihydroisoxazole derivatives against – standard microorganisms**

| Compounds | IZ ( <i>E. coli</i> ) mm | IZ ( <i>S. aureus</i> ) mm |
|-----------|--------------------------|----------------------------|
| 2a        | 14                       | 11                         |
| 2b        | 9                        | 10                         |
| 2c        | 8                        | 16                         |
| 2d        | 10                       | 10                         |
| 2e        | 7                        | 10                         |



**Figure 1 : inhibition zone for compounds 2a,2d,2e against E.Coli**



**Figure 2 : inhibition zone for compounds 2a,2d,2e against S.aureus**



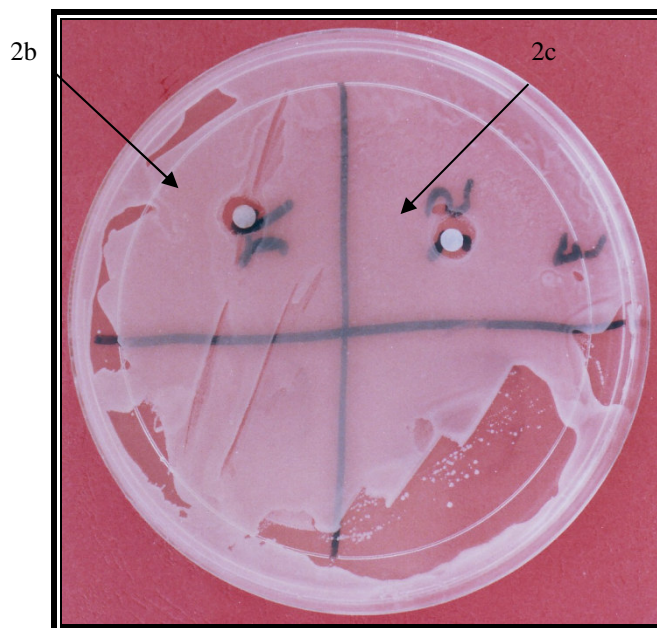


Figure 3 : inhibition zone for compounds 2b,2c against E.Coli

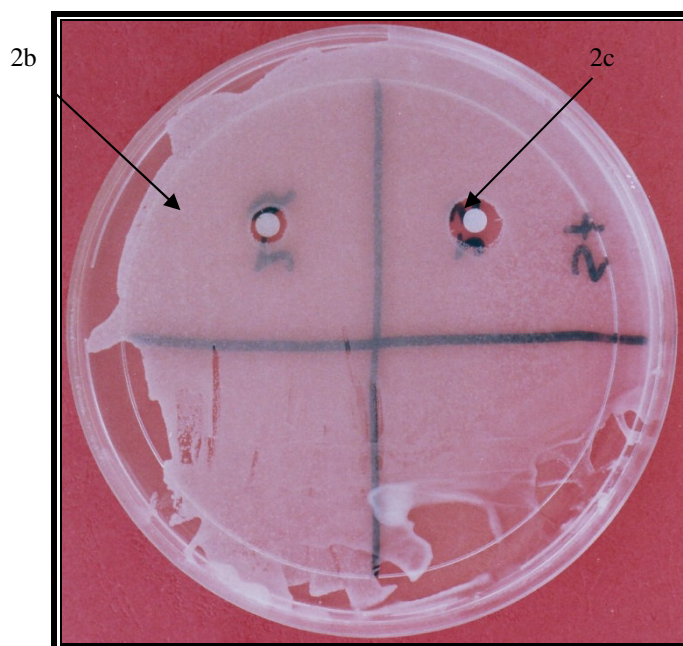


Figure 4 : inhibition zone for compounds 2b,2c against S. aureus

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