Arabian Journal of Chemistry (2015) 8, 174–180

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Synthesis, studies and in-vitro antibacterial activity of N-substituted 5-(furan-2-yl)-phenyl pyrazolines

Mamta Rani [a,*], Mohamad Yusuf [a], Salman Ahmad Khan [b], P.P. Sahota [c], G. Pandove [c]

[a] *Department of Chemistry, Punjabi University Patiala, P.O. Box 147002, Punjab, India*
[b] *Chemistry Department, Faculty of Science, King Abdul Aziz University, P.O. Box 80203, Jeddah 21413, Saudi Arabia*
[c] *College of Basic Sciences and Humanities, Department of Microbiology, PAU Ludhiana, P.O. Box 141004, Punjab, India*

Received 6 July 2010; accepted 6 October 2010
Available online 15 February 2011

KEYWORDS

Pyrazoline;
Phenyl hydrazine;
Chalcones;
Antibacterial activity

Abstract Novel compounds with antibacterial properties: pyrazoline derivatives were synthesized by the cyclization of various -1-[2-(alkoxy)phenyl]-3-(furan-2-yl) prop-2-en-1-one **1a**–**1d** with N-substituted phenyl hydrazine in the presence of CH_3COOH in ethanol. The structures of these compounds were elucidated by, IR, ^1H NMR, ^{13}C NMR, ESI-MS spectral data and their purities were confirmed by elemental analyses. The in vitro antibacterial activity of these compounds was evaluated against two Gram-positive and two Gram-negative bacteria *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Staphylococcus aureus* by microdilution method and then the minimum inhibitory concentration (MIC) of compounds were determined. The results showed that compounds (5*R*)-5-(furan-2-yl)-1-phenyl-3-[2-(benzyloxy)phenyl]-4, 5-dihydro-1*H*-pyrazole (**2b**) and (5*R*)-5-(furan-2-yl)-1-phenyl-3-[2-(naphthalen-2-ylmethoxy prop-2-en-1-yloxy)phenyl]-4,5-dihydro-1*H*-pyrazole (**2d**) showing most promising antibacterial activities as compared to Gentamicin and Tetracycline are given.

1. Introduction

Bacterial infections such as food poisoning, salmonellosis and diarrhea are caused by multidrug-resistant Gram-positive and Gram-negative *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Listeria monocytogenes* and *Staphylococcus aureus* pathogens (Vivekanandhan et al., 2002). One million people in the subtropical regions of the world are infected and 20,000 deaths every year due to these parasitic bacterial infections. The spread of drug resistance *Aeromonas* is of concern because recent surveys indicate the emergence of these organisms as primary human pathogens and pose a public health risk and an etiological agent of gastroenteritis in epidemics. It is speculated that resistance to multiple antibiotics in *Aeromonas* isolate may be mediated by several conducible enzymes under the selection pressure of certain widely

* Corresponding author. Tel.: +91 1675264235 (R)/+91 9417636159 (M); fax: +91 175 2283073.
E-mail address: Drmamtaphd@gmail.com (M. Rani).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2010.10.036
1878-5352 © 2011 Production and hosting by Elsevier B.V. on behalf of King Saud University.

prescribed antibiotics. Resistance to β-lactam antibiotics is due to the production of multiple inducible, chromosomally encoded β-lactames (Goñi-Urriza et al., 2000a,b) Multiple antibiotic resistances among *Aeromonas* sp. have been reported from many parts of the world (WHO, 2006; Goñi-Urriza et al., 2000b). Amoxicillin, norfloxacin, ciprofloxacin are the principal drugs of choice in the treatment of bacterial infection since they are effective against extraintestinal and intestinal wall infections (Datta et al., 1974), but these are associated with several side effects such as nausea, metallic taste, dizziness, hypertension, etc. as well as resistance that have been reported (Alauddin and Smith, 1962). The development of nitrogen-containing five-membered heterocycles, pyrazolines is well known, representing a class of compounds of great importance of pyrazoline derivatives that possess biological activities like, antidepressant (Prasad et al., 2005), anticonvulsant (Amnerkar and Bhusari, 2010) antimicrobial (Ozdemir et al., 2007), analgesic (Aysel Gursoy et al., 2000), and antitumor (Brzozwskim et al., 2000), activities and also serve as human acyl-CoA: cholesterol acyltransferase inhibitors (Jeong et al., 2004). After the pioneering works of Fischer and Knövenagel in the late 19th century, 8 the reaction of α,β-unsaturated aldehydes and ketones with hydrazines became one of the most popular methods for the preparation of 2-pyrazolines (Sammour, 1964; Bhatnagar and George, 1968; Aubagnac et al., 1969; Andotra et al., 1993; Bilgin et al., 1994 and Mishriky et al., 1996). As a result, numerous substituted 2-pyrazolines have been synthesized, which has made possible structure–activity relationship investigations of these substances. In view of the therapeutic importance of these pyrazole heterocycles, the goal is to investigate the synthesis of pyrazoline. The present work is an extension of our ongoing efforts toward the development and identification of new pyrazole molecules to act as antibacterial agents. Here in we describe the synthesis of new pyrazoline derivative from various chalcones and screened for antibacterial Activity.

2. Experimental

2.1. Instrumentation

The entire chemicals were purchased from Aldrich Chemical Company (USA) and were used without further purification. The reactions were monitored by percolated aluminium Silica Gel 60F 254 thin layer plates procured from Merck (Germany). All melting points that were measured with a capillary apparatus are uncorrected. All the compounds were routinely checked by IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analyses. IR spectra were recorded in KBr on a Perkin–Elmer model 1620 FTIR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature using a Brucker spectrospin DPX-400 MHz spectrophotometer in $CDCl_3$ and DMSO. The following abbreviations were used to indicate the peak multiplicity s–singlet, d–doublet, t–triplet, m–multiplet. S1-MS mass spectra were recorded on a JEOL SX102 mass spectrometer using Argon/Xenon (6 kV, 10 mB) gas. Column chromatography was performed on silica gel (Merck). Anhydrous sodium sulfate was used as a drying agent for the organic phase.

2.2. General procedure for the synthesis of (2E)-l-(2-hydroxyphenyl)-3-(furan-2-yl) prop-2-en-1-one (*1*)

A solution of *O*-hydroxy acetophenone (1 equiv) and furfuraldehyde (1 equiv) in methanolic solution of NaOH (30%) was stirred for 8 h at room temperature. After completion of reaction, the solution was poured into ice cold water of pH-2 (pH adjusted by HCl). The solid was separated and dissolved in $CHCl_3$, washed with saturated solution of $NaHCO_3$ and evaporated to dryness. The residue was purified by column chromatography using $CHCl_3$ as eluent. The compound was recrystallized in ethanol (Husain et al., 2008).

Yellow needle; yield: 95%; m.p. 84 °C. Anal. Calc. for $C_{13}H_{10}O_3$: C, 72.89; H, 4.67. Found: C, 72.85; H, 4.64%. IR (KBr) ν_{max} (cm^{-1}): 1634 (C═O), 2949 (O–H); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 12.7 (1H, s, –OH), 8.02 (1H,d, J_{trans} = 15.4 Hz, H-3), 7.72(1H, d, J_{trans} = 15.4 Hz, H-2), 7.34 (1H, d{dd}, $J_{p,m,o}$ = 1.6 Hz, 3.5 Hz, 8.1 Hz, Ar-H), 7.25 (1H,m, Ar-H), 7.13 (1H, m, Ar-H), 7.02 (1H, dd, J = 1.5 Hz, 3.4 Hz, Ar-H), 7.31 (1H, t, J = 3.2 Hz, Ar-H), 6.92 (1H, m, Ar-H), 6.74 (1H, t, J = 8.3 Hz, Ar-H); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 192.4 (C═O), 142.6 (C═C), 137.8 (C═C), 152.8, 150.7, 148.6, 138.2, 136.2, 129.4, 128.7, 127.3, 125.5, 123.4, (Ar-C); GC–MS *m*/*z* (rel. int.%): 215, calc. 214.

2.3. General procedure for the synthesis (2E)-1-[2-(prop-2-en-1-yloxy)phenyl]-3-(furan-2-yl) prop-2-en-1-one (*1a*)

A suspension of l-(2-hydroxyphenyl)-3-(furan-2-yl) prop-2-en-1-one (**1**) (2.0 g, 0.005 mol), allyl bromide (1.5 ml, 0.0041 mol), freshly ignited K_2CO_3 (1.0 g) and tetrabutylammonium iodide as PTC (1.0 g) in dry acetone (25 ml) was heated at reflux for 1 h with stirring. In the absence of PTC, reaction occurred in 70–80 h and yields of the alkoxy chalcones. Use of PTC in these reactions, not only decreased the reaction time up to 4–5 h but also improved the yield of 1-[2-(prop-2-en-1-yloxy)phenyl]-3-(thiophen-2-yl) prop-2-en-1-one derivatives up to 80–85%. The products thus obtained were purified by passing through silica-gel column (60–120 mesh) and further crystallized from methanol to afford **1a** (Kumar and Yusuf, 2006).

Light brown; yield: 85%; m.p. 82 °C. Anal. Calc. for $C_{16}H_{14}O_3$: C, 75.59; H, 5.51. Found: C, 75.56; H, 5.48%. IR (KBr) ν_{max} (cm^{-1}): 1655 (C═O), 1598 (CH═CH); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 7.61 (1H, d, J_{trans} = 15.2 Hz, H-3), 7.52 (1H, d, J_{trans} = 15.3 Hz, H-2), 7.45 (1H, m, d{dd}, $J_{p,m,o}$ = 0.7 Hz, 1.8 Hz, 7.5 Hz, Ar-H), 7.01(2H, t, Ar-H), 6.82 (1H, d, Ar-H), 6.51 (IH, m, Ar-H),6.43 (1H, m, Ar-H), 6.02 (1H,m,Ar-H), 5.45 (2H, q, J = 6.7 Hz, –CH_2), 5.24 (1H, dd, J_{vic} = 6.2 Hz, –CH), 4.64 (2H, dd, J_{vic} = 5.1 Hz, –CH_2); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 192.2 (C═O), 154.5, 153.6, 144.6 (C═C), 141.5 (C═C), 132.5, 131.4, 130.8 128.8, 126.7, 125.8, 120.8 122.6 (Ar-C), 68.5 (CH_2), 62.4 (CH_2), 42.3 (CH); MS; *m*/*z* (M^+) 255.

2.3.1. (2E)-1-[2-(Benzyloxy)phenyl]-3-(furan-2-yl)prop-2-en-1-one (*1b*)

A suspension of **1** (2.0 g, 0.001 mol), benzyl chloride (1.5 g, 0.004 mol), under the similar conditions was used for **1a** and further crystallized from methanol to afford **1b**.

Dark brown; yield: 85%; m.p. 85 °C. Anal. Calc. for $C_{20}H_{16}O_3$: C, 78.94; H, 5.26. Found: C, 78.90; H, 5.23%. IR

(KBr) ν_{max} (cm^{-1}): 1603 (C═O), 1552 (CH═CH); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 7.62 (1H, d, J_{trans} = 15.7 Hz, H-3), 7.54 (1H, d{dd}, $J_{p,m,o}$ = 0.9 Hz, 1.4 Hz, 7.8 Hz, Ar-H), 7.44 (4H, m, Ar-H), 7.43 (1H, d, J_{trans} = 15.6 Hz, H-2), 7.31 (3H, m, Ar-H), 7.05 (1H, m, Ar-H), 7.03 (2H, m, Ar-H), 6.72 (1H, d, J_o = 7.9 Hz, Ar-H), 5.18 (2H, s, –CH_2); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 191.4(C═O), 154.1, 153.5, 143.8 (C═C), 141.3, 132.6, 130.9, 130.0, 129.6, 129.6, 128.1, 127.5, 125.8, 122.7, 122.5, 120.4, 121.2, 121.2, 112.7(Ar-C), 77.42 (CH_2); MS; m/z (M^+) 305.

2.3.2. (2E)-1-[2-(Ethaloxy)phenyl]-3-(furan-2-yl)prop-2-en-1-one (1c)

A suspension of (**1**) (2.0 g, 0.01 mol), bromoethyl acetate (1.2 g, 0.004 mol), under the similar conditions was used for **1a** and further crystallized from methanol to afford **1c**.

Light brown; m.p. 87 °C; yield: 85%. Anal. Calc. for $C_{17}H_{16}O_5$: C, 68.00; H, 5.33. Found: C, 67.96; H, 5.30%. IR (KBr) ν_{max} (cm^{-1}): 2980, 2933 (methylene C–H), 1600, 1754 (C═O), 1549(CHCH); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm:, 7.56 (1H, d{dd}, $J_{p,m,o}$ = 0.8 Hz, 1.7 Hz, 7.8 Hz, Ar-H), 7.45 (1H, d, J_{trans} = 15.2 Hz, H-2), 7.38 (1H, d, J_{trans} = 15.3 Hz, H-3), 7.24 (2H, dd, J = 3.3 Hz, Ar-H), 6.93 (1H, d, Ar-H), 7.07 (1H, m, Ar-H), 7.02(1H, m, Ar-H), 6.82 (1H, m, Ar-H), 4.24 (2H, q, J_{vic} = 6.9 Hz, –OCH_2), 1.34(3H, t, J_{vic} = 7.2 Hz, –CH_3), 5.18(2H, s, –CH_2); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 191.2 (2C═O), 155.8, 154.2, 143.6 (C═C), 142.5, 132.5, 131.7, 130.3, 128.5, 126.6, 125.4, 120.2 114.6(Ar-C), 72.4 (OCH_2), 68.5(CH_2), 55.4(CH_3); MS; m/z (M^+)301.

2.3.3. (2E)-1-[2-(Naphthalen-2-ylmethoxy)phenyl]-3-(furan-2-yl)prop-2-en-1-one (1d)

A suspension of (**1**) (2.0 g, 0.01 mol), 1-chloromethyl-napthalene (1.4 g, 0.004 mol), under the similar conditions was used for **1a** and further crystallized from methanol to afford **1d**.

Light brown; m.p. 94 °C; yield: 88%. Anal. Calc. for $C_{24}H_{18}O_3$: C, 81.35; H, 5.08. Found: C, 81.31; H, 5.04%. IR (KBr) ν_{max} (cm^{-1}): 1633 (C═O), 1589(CH═CH); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 8.01 (1H, dd, J = 7.8 Hz, Ar-H), 7.80 (1H, d, J_o = 7.8 Hz, Ar-H), 7.91 (1H,d, J_{trans} = 15.7 Hz, H-3), 7.54 (1H, dd, J = 1.0, 1.7 Hz, Ar-H), 7.47 (1H, d, J_{trans} = 15.7 Hz, H-2), 7.31 (2H, d, J = 3.4 Hz, Ar-H), 7.24 (4H, m, Ar-H), 7.05 (2H, m, Ar-H), 7.09 (1H, d{dd}, $J_{p,m,o}$ = 0.9 Hz, 1.6 Hz, 7.8 Hz, Ar-H), 6.81 (2H, m, Ar-H), 5.49 (2H, s, –CH_2); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 192.3 (C═O), 154.7, 153.3, 142.6 (C═C), 138.7 (C═C), 142.6, 132.5, 131.4, 130.3, 129.4, 128.8, 128.8, 127.5, 126.6, 126.6, 125.3, 124.5, 122.6, 121.5, 120.4, 118.5, 114.3, 114.3 (Ar–C), 74.04(CH_2); m/z (M^+) 355.

2.3.4. (5R)-5-(Furan-2-yl)-1-phenyl-3-[2-(prop-2-en-1-yloxy)phenyl]-4,5-dihydro-1H-pyrazole (2a)

A mixture of -1-[2-(prop-2-en-1-yloxy)phenyl]-3-(thiophen-2-yl) prop-2-en-1-one (**1a**) (0.5 g, 0.0015 mol) was refluxed with phenyl-hydrazine (0.800 ml, 0.004 mol) in dry EtOH (30 ml) and catalytic amount of glacial acetic acid for at 80 °C for 8 h. The progress of reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and the residue obtained was purified by column chromatography (20:80, diethyl ether–petroleum ether) to obtain a solid that was crystallized from EtOH to yield pyrazolines **2a**.

Dark brown; m.p. 138 °C; yield: 86%. Anal. Calc. for $C_{22}H_{20}O_2$ $N_{2:}$ C, 76.74, H, 5.81, N, 8.13. Found; C, 76.70; H, 5.78; N, 8.09%. IR (KBr) ν_{max} (cm^{-1}): 3308 (N–H), 1585 (C═N), 1483 (N–N); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 7.52 (5H, m, Ar-H), 7.02 (2H, m, Ar-H), 7.32 (1H, d{dd}, $J_{p,m,o}$ = 0.7 Hz, 1.8 Hz, 7.5 Hz, Ar-H), 6.7 (1H, m, Ar-H), 6.8 (1H, d, Ar-H), 6.02 (2H, m, Ar-H), 5.32 (2H, t, J = 6.7 Hz, -CH_2), 4.52 (1H, dd, –CH), 4.64 (2H, dd, J_{vic} = 6.1 Hz, –CH_2); 5.25 (1Hx, dd, J_{xa} = 6.7 Hz, J_{xb} = 11.6 Hz), 3.52 ($1H_a$, dd, J_{ax} = 6.7 Hz, J_{ab} = 16.7 Hz), 3.24 ($1H_b$, dd, J_{bx} = 11.6 Hz, J_{ba} = 16.7 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 156.7 (C═N), 156.2, 155.2, 154.4, 144.2, 136.4, 131.5, 130.9, 129.8, 129.8, 128.2, 127.5, 125.5, 123.2, 123.2, 121.8, 116.7 (Ar-C), 77.3 (pyr. ring, C-5), 76.70 (CH_2), 70.01 (CH_2), 67.01 (pyr. ring, C-4), 46.1(CH); MS; m/z (M^+) 345.

2.3.5. (5R)-5-(Furan-2-yl)-1-phenyl-3-[2-(benzyloxy)phenyl]-4,5-dihydro-1H-pyrazole (2b)

Pyrazoline **2b** was obtained from the reaction of **1b** (0.5 g, 0.0015 mol) with phenyl-hydrazine (0.430 g, 0.00398 mol) under the similar conditions as used for **2a** and further crystallized from ethanol to afford **2b**.

Blackish brown, m.p. 125 °C; yield: 85%. Anal. Calc. for $C_{26}H_{22}O_2N_2$: C, 79.18, H, 5.58; N, 7.10. Found: C, 79.15; H, 5.54; N, 7.06%. IR (KBr) ν_{max} (cm^{-1}): 3317 (N–H), 1596 (C═N), 1493 (N–N); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 6.82 (1H, d, J_o = 7.8 Hz, Ar-H), 7.34 (1H, d{dd}, $J_{p,m,o}$ = 0.9 Hz, 1.4 Hz, 7.8 Hz, Ar-H), 7.25 (3H, m, Ar-H), 7.21 (5H, m, Ar-H), 7.12 (5H, m, Ar-H), 7.04 (2H, m, Ar-H), 5.32 (2H, s, –CH_2), 5.23 (1Hx, dd, J_{xa} = 6.5 Hz, J_{xb} = 11.2 Hz), 3.82 ($1H_a$, dd, J_{ax} = 6.5 Hz, J_{ab} = 17.1 Hz), 3.52 ($1H_b$, dd, J_{bx} = 11.2 Hz, J_{ba} = 17.1 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 156.1 (C═N), 153.4, 152.3, 142.3, 142.2, 133.2, 130.1, 129.6, 129.6, 128.2, 128.2, 127.8, 127.2, 126.2, 126.2 125.2, 124.7, 124.7, 118.2, 116.2, 115.2, 114.6, 114.6 (Ar-C), 76.21 (pyr. ring, C-5), 67.23 (pyr. ring, C-4), 64.02 (CH_2); MS; m/z (M^+) 395.

2.3.6. (5R)-5-(Furan-2-yl)-1-phenyl-3-[2-(ethyloxy)phenyl]-4,5-dihydro-1H-pyrazole (2c)

Pyrazoline **2b** was obtained from the reaction of **1c** (0.5 g, 0.0015 mol) with phenyl-hydrazine (0.430 g, 0.00398 mol) under the similar conditions as used for **2a** and further crystallized from ethanol to afford **2c**.

Radish brown; m.p. 127 °C; yield: 92%. Anal. Calc. for $C_{23}H_{22}N_2O_4$: C, 70.76; H, 5.64; N, 7.17. Found: C, 70.72; H, 5.61; N, 7.14%. IR (KBr) ν_{max} (cm^{-1}): 2878, 2889 (methylene C–H), 3369 (N–H), 1755 (C═O), 1587 (C═N) 1457 (N–N); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 6.87 (1H, d, Ar-H), 7.06 (2H, m, Ar-H), 7.35 (1H, d{dd}, $J_{p,m,o}$ = 0.8 Hz, 1.7 Hz, 7.8 Hz, Ar-H), 7.23 (3H, m, Ar-H), 7.81 (5H, m, Ar-H), 5.25 (1Hx, dd, J_{xa} = 6.5 Hz, J_{xb} = 11.8 Hz), 4.61 (2H, s, –CH_2),4.01 (2H, q, J_{vic} = 6.4 Hz, –OCH_2), 3.82 ($1H_a$, dd, J_{ax} = 6.5 Hz, J_{ab} = 16.6 Hz), 3.42 ($1H_b$, dd, J_{bx} = 11.8 Hz, J_{ba} = 16.6 Hz), 1.25(3H, t, J_{vic} = 6.9 Hz,-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 190.5 (C═O), 156.6 (C═N), 155.6, 155.2, 154.2, 144.4, 136.7, 131.2, 130.6, 129.4, 129.4,

128.4, 127.6, 125.6, 123.4, 123.4, 122.8 114.7 (Ar-C), 76.70 (pyr. ring, C-5), 72.2 (OCH_2), 67.6 (CH_2), 67.01 (pyr. ring, C-4), 55.2 (CH_3); MS; *m*/*z* (M^+) 391.

*2.3.7. (5R)-5-(Furan-2-yl)-1-phenyl-3-[2-(naphthalen-2-ylmethoxy prop-2-en-1-yloxy)phenyl]-4,5-dihydro-1H-pyrazole (**2d**)*

Pyrazoline **2d** was obtained from the reaction of **1d** (0.5 g, 0.0015 mol) with phenyl-hydrazine (0.430 g, 0.00398 mol) under the similar conditions as used for **2a** and further crystallized from ethanol to afford **2d**.

Radish brown; m.p. 158 °C; yield: 94%, Anal. Calc. for $C_{30}H_{24}N_2O_2$: C, 81.08; H, 5.40; N, 6.30. Found: C, 81.04; H, 5.37; N, 6.25%. IR (KBr) ν_{max} (cm^{-1}): 3314 (N–H), 1586 (C═N), 1445 (N–N); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 8.02 (2H, m, Ar-H), 7.52 (1H, d{dd}, $J_{p,m,o}$ = 0.8 Hz, 1.9 Hz, 7.6 Hz, Ar-H), 7.49 (5H, m, Ar-H), 7.10 (7H, m, Ar-H), 6.82 (4H, m Ar-H), 5.23 (1Hx, dd, J_{xa} = 6.7 Hz, J_{xb} = 11.8 Hz), 5.04 (2H, s, –CH_2), 3.62 (1H_a, dd, J_{ax} = 6.7 Hz, J_{ab} = 16.2 Hz), 3.23 (1H_b, dd, J_{bx} = 11.8 Hz, J_{ba} = 16.2 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm: 156.7(C═N), 155.2, 154.3 142.6, 144.2, 135.2, 131.9, 130.7, 129.2, 129.2, 128.7, 128.7, 127.6, 126.5, 126.4, 126.4, 125.6, 124.3, 124.3, 123.8, 123.7, 122.6, 121.4, 121.4, 120.4, 118.2, 115.5 (Ar-C), 74.6 (pyr. ring, C-5), 71.2 (CH_2), 68.3 (pyr. ring, C-4); *m*/*z* (M^+) 445.

3. Results and discussion

The present study describes, the synthesis and antibacterial evaluation of some pyrazoline derivatives **2a**–**2d** that were tested for in vitro antibacterial activity using the disc-diffusion method by measuring and the diameter of the zone of inhibition measured in mm. It was found that all the compounds were screened in vitro for their antibacterial activity against a variety of Gram-positive, Gram-negative bacterial strains, such as *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes* and *S. aureus* emerging pathogens responsible for gastrointestinal. Looking at the structure–activity relationship, compounds **2a**, **2b**, **2c** and **2d** exhibit significant activities while **2b** and **2d** exhibit good activity as compared to standard known antibiotics, such as Gentamicin (10 mcg) and Tetracycline (30 mcg) that were used for comparison purposes and the antibacterial screening data are recorded in Tables 1 and 2. On the basis of the above observations, modification will be done to improve antibacterial activity.

Assignment of selected characteristic IR bands provides significant indications for the formation of the cyclized pyrazoline analogues of the phenyl hydrazine **2a**–**2d**. In starting material alkoxy chalcones, **1a**–**1d** (C═O) and (CH═CH), functional absorbed in the expected region; (C═O) at 1603–1754 cm^{-1} and (CH═CH) at 1552–1598 cm^{-1}, respectively. The IR spectra of the compounds showed ν(C═N) stretching at 1585–1596 cm^{-1} and ν(N–N) stretching vibration at 1445–1493 cm^{-1}, respectively, which also confirm the formation of desired pyrazoline compounds.

The ^{1}H NMR spectra (400 MHz, $CDCl_3$), the signals corresponding to the double hydrogens (H-2 and 3) at δ 7.43–7.52, and 7.38–7.91 alkoxy chalcones **1a**–**1d** were found missing altogether which indicates the involvement of the enone moiety during the cyclization reactions and the coupling value of J = 15.2–15.7 Hz between theses hydrogens describes the *trans* geometry around the C-2 and C-3 double bonds. The aromatic protons were located at δ 6.7–7.9, respectively. The downfield resonance of the H-3 as compared to H-2 could be ascribed to the electron deficient nature of the β-carbon in the enone moiety. The major feature of this spectrum was the signals of the pyrazoline **2a**–**2d** ring proton (H-x) and (H-a and b) which were found in the region at δ 5.23–5.25 (1H, dd, J_{xa} = 6.5–6.7 Hz, J_{xb} = 11.2–11.8 Hz) and 3.23–3.82 ppm (1H, dd, J_{ax} = 6.5–6.7 Hz, J_{ab} = 16.1–17.1 Hz) describes the *trans* relationship between H_X and H_b & H_a are geminally placed at C-4,which clearly describes the inter-relationship between the H-x, b and a. The proposed expression **2a**–**2d** the strong deshielding of the C_5 (H-a and b) protons compared with the C_4 (H-x) protons of the pyrazoline ring can be assumed due to its structure Fig. 2. The protons belonging to the aromatic ring and the other cyclic groups were observed with the expected chemical shift and integral values.

Finally, ^{13}C NMR (400 MHz, $CDCl_3$) spectra of all compounds were recorded in DMSO and spectral signals are in good agreement with the probable structures. The carbon of C═O and C═C displayed signals at 191.2–191 and 141.5–144.6 ppm in **1a**–**1d**. The methylene groups for **1c** and **2c** were resonating at δ 55.2–55.4 ppm (CH_3) and δ 72.2–72.4 ppm (OCH_2); the downfield resonance of the former suggests their placement near an electronegative oxygen atom. The signals are due to the aromatic carbons and the carbon at 1-N substituted aliphatic group. The C_4 and C_5 carbon of pyrazoline resonated at 42.3–68.3 and 62.4–77.4 ppm, respectively. The downfield resonance of former as compared to C-4 could be attributed to its benzylic nature and proximity to nitrogen atom. The other resonates were showed at their usual position in Section 2.

Characteristic peaks were observed in the mass spectra of all compounds, which followed the similar fragmentation pattern. The spectrum of the compound **2a** showed a molecular ion peak (M^+) at *m*/*z* 345. The characteristic peaks observed within the mass spectra of pyrazoline compounds are given in experimental section.

It may be concluded that this study describes the general method for the synthesis of some pyrazolines linked through the 5-aryl ring under normal conditions. These results show that compounds **2b** and **2d** are better antibacterial agents against *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes*, and *S. aureus* as compared to Gentamicin and Tetracycline. Thus the accumulation of the pyrazoline derivatives will better antibacterial agents for therapeutic use.

4. Conclusions

In the present studies, this research examined the antibacterial activities of the pyrazoline derivatives from alkoxy chalcones that were found to be more active among all pyrazoline compounds while **2b** and **2d** displayed as good antibacterial agents as compared to Gentamicin and Tetracycline. The molecular structure of these active compounds showed property of pyrazoline; these compounds might be more efficacious drugs against these bacteria and their biological effects which could be helpful in designing more potent antibacterial agents.

5. Pharmacology

All the synthesized compounds of bis-pyrazoline derivatives were evaluated for in vitro antibacterial activity by using disk diffusion method and the Minimum Inhibitory Concentration (MIC) as compared to antibiotics positive controls. The results of antibacterial activity and Minimum Inhibitory Concentration (MIC) are summarized in Tables 1 and 2 respectively.

5.1. Materials and method

Pure cultures *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes*, and *S. aureus* were grown in brain heart infusion broth for sensitivity testing. Mueller Hinton agar (Hi Media) and pyrazoline compounds **2a**–**2d** absolutely diluted (concentration of 40, 30, 20 and 10 mcg) were applied as described by Bauer et al. (1966). The strains were tested against the following antibiotics (Hi Media): Gentamicin 10 mcg and Tetracycline 30 mcg. These were enriched in BHIB for 6–8 h at 37 °C, the cultures were streaked on differential media agar plates using a cotton swab. With an antibiotic disc dispenser, the discs were placed on the agar surface. After 30 min of pre-diffusion time, the plates were incubated at 37 °C for 18–24 h, after incubation, the diameter of the inhibition zones were measured and compared to the interpretive chart of performance standards for antimicrobial disk susceptibility tests (Hi Media) and classified as resistant, intermediate or sensitive.

5.2. In vitro antibacterial activities

In vitro antibacterial activities of pyrazoline **2a**–**2d** derivatives were carried out using the culture of *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes*, and *S. aureus* by the disc-diffusion method using nutrient broth medium [contained (mcg/ml): beef extract 1 g; peptone 5 g; pH 7.0]. In the disc-diffusion method, sterile paper discs (0.5 mm) impregnated with compound dissolved in dimethylsulfoxide (DMSO) at a concentration of 100 mcg/mL were used. Gentamicin and Tetracycline were used as the standard drugs, where as DMSO poured disk was used as negative control. Then, the paper discs impregnated with the solution of the compounds were tested placed on the surface of the media inoculated with the microorganism. The plates were incubated at 18 h, at 37 °C. After incubation, the diameters of the inhibition zones were measured. The pyrazoline derivatives were further checked by MIC method and results are presented in Tables 1 and 2. The in vitro studies result showed that compounds **2b** and **2e** are of highest activity

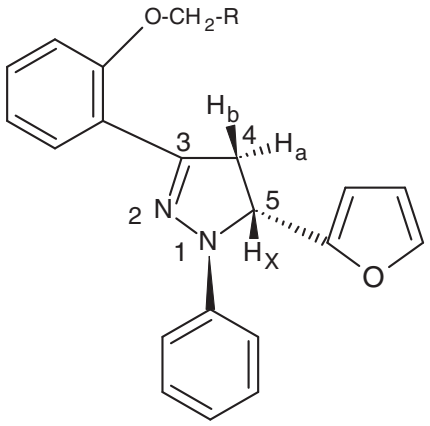

Figure 1 Schematic diagram indicating the ring conformation of compounds **2a**–**2d**.

Table 1 Antibacterial activity of pyrazoline derivatives, positive control (oflofloxine) and negative control (DMSO) measured by the halo zone test (Unit, mm).

Compounds	Corresponding effect on microorganisms			
	A. hydrophila	*Y. enterocolitica*	*L. monocytogenes*	*S. aureus*
2a	15.6	16.4	13.4	14.6
2b	**22.3**	**21.4**	**24.3**	**22.8**
2c	14.6	14.5	15.5	15.5
2d	**21.7**	**25.2**	**19.4**	**23.6**
Oflofloxine	25.1	13.3	12.2	16.2
Gentamicin	21.2	–	–	171
Tetracycline	13.4	20.3	12.2	14.4
DMSO	–	–	–	–

Table 2 Minimum Inhibition Concentration (MIC) of pyrazoline derivatives and standard drugs (Ampicillin, Gentamicin, Tetracycline) (Unit, mcg).

Compounds (MIC)	(MIC) Corresponding effect on microganisms (Unit, mcg)			
	A. hydrophila	*Y. enterocolitica*	*L. monocytogenes*	*S. aureus*
2a	10	10	10	10
2b	30	30	30	30
2c	40	40	40	40
2d	20	20	20	20
Gentamicin	10	10	10	10
Tetracycline	30	30	30	30

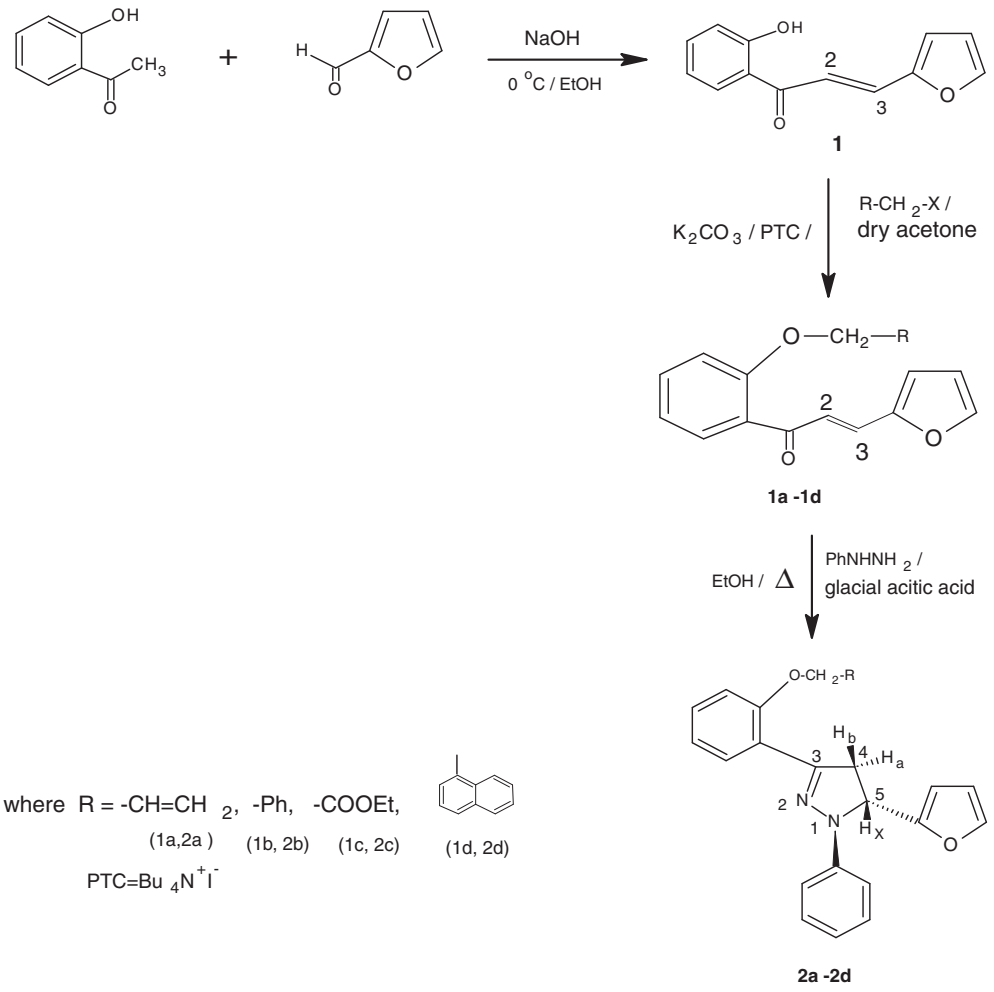

Figure 2 Schematic diagram indicating the synthesis of compounds **2a**–**2d**.

against *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes*, *S. aureus* among all the pyrazolines when compared to antibiotics. The susceptibility of the bacteria to the test compounds were determined by the formation of an inhibitory zone after 48 h of incubation at 37 °C. The molecular structure of these active compounds showed enhanced activity. The distinct differences in the antibacterial property of these compounds further justify the purpose of this study. The importance of such work lies in the possibility that the new compounds might be more efficacious drugs against bacteria for which a thorough investigation regarding the structure–activity relationship, toxicity and their biological effects which could be helpful in designing more potent antibacterial agents for therapeutic use (Fig. 1).

Acknowledgments

Author is highly thankful to Rajiv Gandhi fellowship (UGC), N. Delhi, India for the generous grant. The necessary facilities and some financial assistance providing by Prof. Baldev Singh, Head, Department of Chemistry, Punjabi University, Patiala, has also been highly acknowledged.

References

Alauddin, M., Smith, M.M., 1962. Neuromuscular blocking properties of stereoisomeric androstane-3,17-bisquaternary ammonium salts. J. Pharm. Phamacol. 14, 325–349.

Amnerkar, N.D., Bhusari, K.P., 2010. Synthesis and antimicrobial studies of new series of pyrazoline bearing *Bis*-heterocycles *via*1,3-dipolar cycloaddition reactions. Eur. J. Med. Chem. 45, 149–159.

Andotra, C.S., Khajuria, J., Singh, G.B., Singh, S.J., 1993. ChemInform abstract: synthesis and biocidal activity of some substituted pyrazolines and pyrimidines. Indian Chem. Soc. 70, 266–267.

Aubagnac, J.L., Elguero, J., Jacquier, R., 1969. Synthesis of 1,2-diphenylpyrazolidin-4-ol and its derivatives. Bull. Soc. Chim. Fr., 3292

Aysel Gursoy, S., Demirayak, Gultaze capen, et al, 2000. Synthesis and preliminary evaluation of new 5-pyrazolinone derivatives as analgesic agents. Euro. J. Med. Chem. 35, 359–364.

Bhatnagar, I., George, M.V., 1968. An efficient oxidation of 2-pyrazolines and isoxazolines by bis-bromine-1,4-diazabicyclo[2.2.2]octane complex (DABCO-Br_2). Tetrahedron 24, 1293.

Bilgin, A.A., Palaska, E., Sunal, R., Gümüsel, B., 1994. Some 1,3,5-triphenyl-2-pyrazolines with antidepressant activities. Pharmazie 49, 67.

Brzozwskim, Z., Czewski, F.S., Gdaniec, M., 2000. Synthesis, structural characterization and antitumor activity of novel 2,4-diamino-1,3,5-triazine derivatives. Euro. J. Med. Chem. 35, 1053.

Datta, D.V., Singh, S.A., Chhutani, P.N., 1974. Treatment of amebic liver abscess with emetine hydrochloride, niridazole, and metronidazole. Am. J. Trop. Med. Hyg. 23, 586–589.

Goňi-Urriza, M., Cabdepuy, M., Arpin, C., Raymond, N., Caumette, P., Quentin, C., 2000a. Impact of an urban effluent on antibiotic resistance of riverine *Enterobacteriaceae* and *Aeromonas* spp. Appl. Environ. Microbiol. 66, 125–132.

Goňi-Urriza, M., Pineau, L., Capdepuy, M., Roques, C., Caumette, P., Quentin, C., 2000b. Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. J. Antimicrob. Chemother. 46, 297–301.

Husain, K., Abid, M., Azam, A., 2008. Novel Pd(II) complexes of 1-N-substituted 3-phenyl-2-pyrazoline derivatives and evaluation of antiamoebic activity. Eur. J. Med. Chem. 43, 393–403.

Jeong, Tae-Sook, Kim, Kyung Soon, An, So-Jin, Cho, Kyung-Hyun, Lee, Sangku, Lee, Woo Song, 2004. Novel 3,5-diaryl pyrazolines as human Acyl-CoA:cholesterol acyltransferase inhibitors. Bioorg. Med. Chem. Lett. 14, 2715.

Kumar, R., Yusuf, M., 2006. Chromones and bischromones: an account of photoinduced reactions. ARKIVOC ix, 239–264.

Mishriky, N., Asaad, F.M., Ibrahim, Y.A., Girgis, A.S., 1996. ChemInform abstract: new 2-pyrazolines of anticipated molluscicidal activity. Pharmazie 51, 544–548.

Ozdemir, Z., Kandilie, H., Burak, Bulent, G., 2007. Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. Euro. J. Med.Chem. 42, 373.

Prasad, Y., Rajendra, Rao, Laxmana, L., Prasoona, K., Murali, Kumar, P., Ravi, 2005. Preliminary anticancer activity of some prop-2-eneamido, thiazole and 1-acetyl-pyrazolin derivatives of aminobenzothiazoles. Bioorg. Med. Chem. Lett. 15, 5030.

Sammour, A.E.A., 1964. The reactions of flavanone with substituted hydrazines. Tetrahedron 20, 1067.

World Health Organization (WHO), 2006. Guidelines for drinking-water quality: First Addendum to third edition, vol. 1. Geneva.

Vivekanandhan, G., Savithamani, K., Hatha, A.A.M., Lakshmanaperumalsamy, P., 2002. Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. Int. J. Food Microbiol. 76, 165–168.