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Synthesis and *in-vitro* antibacterial activity of some alkoxy based *N*-substituted-5-(furan-2-yl)-phenyl-*bis*-pyrazolines

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ABSTRACT

Bis-pyrazoline darivatives (**2a-e**) built around the alkyl chains of varying length were synthesized in good yield by refluxing *bis*-chalcones (**1a-e**) with phenyl hydrazine in CH_3COOH and ethanol. The structures of these compounds were elucidated by IR, ^1H NMR, ^{13}C NMR, Mass (ESI) spectrometries and their purities were confirmed by elemental analyses. The antibacterial activity of these compounds were evaluated by the disc diffusion assay against two Gram-positive and two Gram-negative bacteria and then the minimum inhibitory concentration of compounds were determined. The compounds 1,4-*bis*[1-(2-oxyphenyl)-5-(furan-2-yl)-4,5-dihydro-1*H*-pyrazole] butane (**2a**) and 1,10-*bis*[1-(2-oxyphenyl)-5-(furan-2-yl)-4,5-dihydro-1*H*-pyrazole]decane (**2e**) are better antibacterial agent as compared to Tetracycline and Gentamicin.

1. Introduction

Drinking water involving of emerging pathogens *Aeromonas hydrophila, Yersinia enterocolitica*, *Listeria monocytogenes* and *Staphylococcus aureus* are important source of human gastrointestinal infections [1]. It is speculated that resistance to multiple antibiotics in pathogens isolate may be mediated by several co-inducible enzymes under the selection pressure of certain widely prescribed antibiotics. Due to the production of multiple inducible, the resistance to β-lactam antibiotics chromosomally encoded β-lactames [2]. Resistance to the third generation cephalosporin is known to be associated with the derepression of the chromosomal enzymes [3]. Tetracycline resistance is most commonly mediated either by active efflux of tetracycline from the cell or by ribosomal protection, and in rare cases, through direct inactivation of the antibiotic or by mutations in the 16S r-RNA that prevent biding tetracycline to the ribosome [4]. The effective treatment for gastrointestinal is metronidazole; however, lengthy treatment or high doses often cause side effects such as headache, nausea, vomiting, dry mouth, metallic taste, dizziness, and neurological complications [5-7]. The chemistry of cyclised heterocyclic systems especially containing pyrazole moiety has been largely investigated due to their effective use in pharmacological areas [8-16]. Chalconoids group are the chalcone compounds which are the open chain molecule having two aromatic ring liked by the carbon fragment, exhibit a wide spectrum of beneficial biological activity anti-inflammatory, anti-invasive and optical properties [17]. The development for the synthesis of five member heterocyclic from readily available reagents is one of the major challenges in organic synthesis. Among five membered heterocycles, pyrazoline and imidazole are represents great importance in biological activities like, antidepressant [18], anticonvulsant [19], antimicrobial [20], analgesics [21] and antitumor [22]. In fact, pyrazoline derivative is now widely used in the market as anti-inflammatory [23], analgesics [24], antibacterial [25], antifungal [26], antituberculosis [27], anticonvolusant [28] and potential anticytokine agents [29,30]. Recently, some attention has also been focused upon the reactions of hydroxyl substituted chalcones can be O-alkylated under the basic medium with a suitable alkylating agent to give bifunctional *bis*-chalcones molecules which are formed by liking two chalcone moieties together through the carbon chains of varying length and structures. By keeping this aspect in view the present researchers are focused upon the transformations of *bis*-chalcones **1a-e** to *bis*-pyrazolines **2a-e** built around the alkyl chains consisting of four to ten methylene groups and their increasing importance in pharmaceutical and biological field. In this paper, we have to synthesize some novel series of *bis*-pyrazoline derivative from alkoxy based *bis*-chalcones as good anti-bacterial agents.

2. Experimental

All the chemicals were purchased from Aldrich Chemical Company (U.S.A) and were used without further purification. The reactions were monitored by TLC plates were coated with silica gel suspended in MeOH-$CHCl_3$ and iodine vapors used as visualizing agent. Percolated aluminum silica gel 60F 254 thin layer plates procured from Merck (Germany). All melting points were measured with a capillary apparatus and are uncorrected. All the compounds were routinely checked by IR, ^1H NMR, ^{13}C NMR, mass spectrometry and elemental analyses. IR spectra were recorded in KBr on a Perkin-Elmer model 1620 FTIR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded at ambient temperature using a Bruker 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. The

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mass spectra have been scanned on the Waters Micromass Q-T of Micro (ESI) spectrometer. Anhydrous sodium sulfate was used as a drying agent for the organic phase.

2.1. Synthesis of chalcone

2.1.1. Synthesis of (E)-3-(furan-2-yl)-1-(2-hydroxyphenyl) prop-2-en-1-one (1)

A suspension of O-hydroxy acetophenone (5 g, 0.0004 mol) and furfural aldehyde (4.5 g, 0.0004 mol) in ethanolic solution of NaOH (30%) was stirred for 8 hrs at room temperature. After the completion of reaction, the reaction mixture was poured into acidic ice water pH = 2 (adjusted by HCl) to produce a solid compound which was filtered under suction and washed with H_2O. The solid was filtered recrystallized from CH_3OH:$CDCl_3$ (3:1) to obtain a pure pure chalcone, **1**, [31] (Scheme 1).

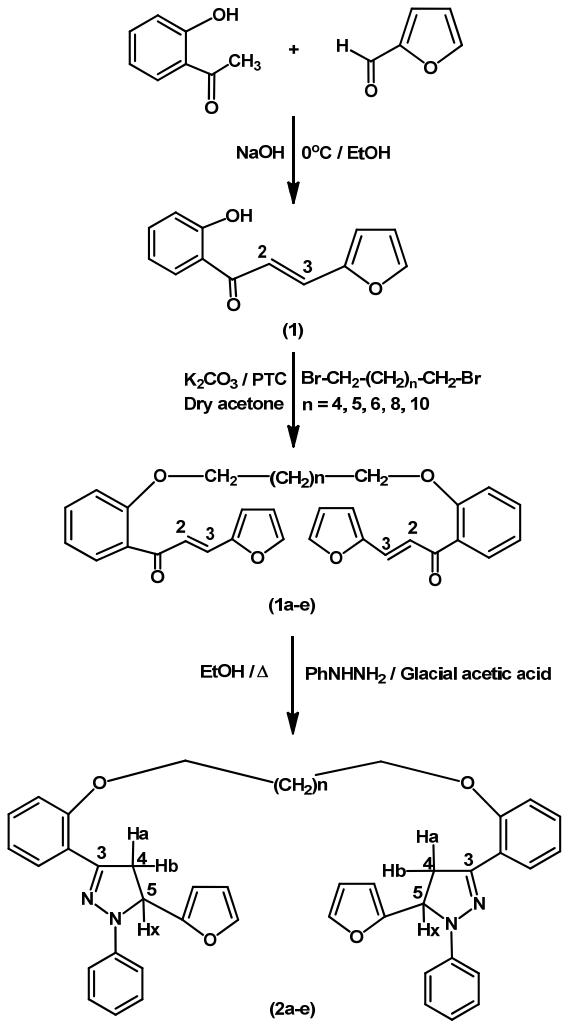
(E)-3-(furan-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**1**): Yellow needle. Yield: 95%. M.p.: 84 °C. IR (KBr, υ_{max}, cm^{-1}): 1634 (C=O), 2949 (O-H). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 12.7 (1H, s, -OH), 8.02 (1H, d, J_{trans} = 15.4 Hz, H-3), 7.7 (1H, t, J_{trans} = 15.1 Hz, H-2), 7.34 (1H, d{dd}, J_{pmo} = 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, Ar-H), 7.3 (1H, t, Ar-H), 6.9 (1H, m, Ar-H), 6.7 (1H, t, 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*): 215 [M$^+$]. Anal. calcd. for $C_{13}H_{10}O_3$: C, 72.89; H, 4.67. Found: C, 72.85; H, 4.64%.

2.1.2. General procedure for the synthesis of bis-chalcone (1a-e)

A suspension of furan chalcone (2.0 g, 0.008 mol), **1**, with suitable α-ω-di-bromo alkane (1,4-dibromobutane, 1,5-dibromopentane, 1,6-dibrpmohexane, 1,8-dibromooctane and 1,10-dibromodecane) (0.0050 mol), anhydrous K_2CO_3 (1.0 g) and phase transfer catalysts (PTC) (Tetra butyl ammonium iodide) (1.0 g) in dry acetone was refluxed with stirring for 8 hrs. at room temperature. The progress of reaction was monitored by thin layer chromatography (TLC). After the completion of reaction, the reaction mixture was turned white was pour into acidic ice water to ~pH = 2 (adjusted by HCl). The precipitated solid was filtered and recrystallized from CH_3OH:$CHCl_3$ (3:1) to obtain pure solids (Scheme 1).

(2E,2'E)-1,1'-((butane-1,4-diylbis(oxy))bis(2,1-phenylene)) bis(3-(furan-2-yl)prop-2-en-1-one) (**1a**): Brown light. Yield: 85%. M.p.: 116 °C. IR (KBr, υ_{max}, cm^{-1}): 3110 (Ar-H), 1600 (C=O), 1238, 1021 (C-O), 1548 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.64 (2H, dd, Ar-H), 7.36 (2H dd, J_{trans} = 15.2 Hz, H-2), 7.4 (4H, t, Ar-H), 7.2 (2H, dd, J_{trans} = 15.4 Hz, H-3), 7.03 (2H, d{dd}, J_{pmo} = 0.8 Hz, 1.04 Hz, 8.3 Hz, Ar-H), 6.8 (2H, d, Ar-H), 6.6 (2H, dd, Ar-H), 6.4 (2H, q, J_p = 1.8 Hz, Ar-H), 4.02 (4H, t, J_{vis} = 6.2 Hz, -CH_2), 2.01(4H, q, J = 5.9 Hz, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 192.1 (C=O), 144.6 (C=C), 139.4 (C=C), 149.8, 148.8, 147.2, 138.2, 132.7, 129.6, 128.4, 128.1, 125.7, 123.2 (Ar-C), 78.2 (OCH_2), 68.7 (CH_2). GC-MS (*m/z*): 483 [M$^+$]. Anal. calcd. for $C_{30}H_{26}O_6$: C, 74.68; H, 5.39; Found: C, 74.65; H, 5.35%.

(2E,2'E)-1,1'-((pentane-1,5-diylbis(oxy))bis(2,1-phenylene)) bis(3-(furan-2-yl)prop-2-en-1-one) (**1b**): Brown light. Yield: 85%. M.p.: 122 °C. IR (KBr, υ_{max}, cm^{-1}): 3110 (Ar-H), 1610 (C=O), 1652 (C=C), 1548 (CH = CH). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.02 (2H, d, Ar-H), 7.67 (2H, d, J_{trans} = 15.8 Hz, H-2), 7.58 (4H, d, Ar-H), 7.25 (2H, d, Ar-H), 7.05 (2H, d{dd}, J_{pmo} = 0.8 Hz, 1.0 Hz, 7.6 Hz, Ar-H), 7.42 (2H, d, J_{trans} = 15.8 Hz, H-3), 6.92 (4H, d, Ar-H), 4.04 (4H, t, J_{vic} = 6.3 Hz, OCH_2), 1.86 (4H, quintet, J_{vic} = 6.3 Hz, -CH_2), 1.67 (2H, quintet, J_{vic} = 6.3 Hz, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 191.8 (C=O), 145.7 (C=C), 138.5 (C=C), 148.7, 147.6, 147.5, 136.3, 133.5, 129.8, 128.7, 127.2, 125.4, 123.7 (Ar-C), 77.2 (OCH_2), 68.5 (CH_2), 47.09 (CH_2). GC-MS (*m/z*): 497 [M$^+$]. Anal. calcd. for $C_{31}H_{28}O_6$: C, 75.00; H, 5.64; Found: C, 74.96; H, 5.60%.



Scheme 1

(2E,2'E)-1,1'-((hexane-1,6-diylbis(oxy))bis(2,1-phenylene)) bis(3-(furan-2-yl)prop-2-en-1-one) (**1c**): Dark Brown. Yield: 75%. M.p.: 108 °C. IR (KBr, υ_{max}, cm^{-1}): 3105 (Ar-H), 1602 (C=O), 1545 (C=C), 1257, 1210, 1006 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.82 (2H, dd. J_{trans} = 15.2 Hz, H-2), 6.83 (2H, d, Ar-H), 7.52 (2H, m, Ar-H), 7.45 (2H, d{dd}, J = 1.5 Hz, 1.8 Hz, 8.2 Hz, Ar-H), 7.26 (2H, m, Ar-H), 7.04 (2H, m, Ar-H), 7.02 (4H, m, Ar-H), 6.95 (2H, dd, J_{trans} = 15.2 Hz, H-3), 4.05 (4H, t, J_{vis} = 6.5 Hz, -CH_2), 2.06 (4H, q, J_{vis} = 6.2 Hz, -CH_2), 2.02 (2H, q, -CH_2), 1.82 (2H, m, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 192.2 (C=O), 145.5 (C=C), 140.2 (C=C), 148.8, 147.2, 137.8, 136.5, 133.7, 132.8, 129.1, 128.2, 125.5, 124.2 (Ar-C), 77.8 (-OCH_2), 68.6 (-CH_2), 56.24 (-CH_2), 48.08 (-CH_2). GC-MS (*m/z*): 511 [M$^+$]. Anal. calcd. for $C_{32}H_{30}O_6$. C, 55.29; H, 5.88; Found: C, 55.26; H, 5.84%.

(2E,2'E)-1,1'-((octane-1,8-diylbis(oxy))bis(2,1-phenylene)) bis(3-(furan-2-yl)prop-2-en-1-one) (**1d**): Light brown. Yield: 75%. M.p.: 102 °C. IR (KBr, υ_{max}, cm^{-1}): 3107 (Ar-H), 1607 (C=O), 1547 (C=C), 1210, 1006 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.65 (2H, dd, J_{trans} = 15.2 Hz, H-2), 7.45 (4H, m, Ar-H), 7.40 (2H, dd. J_{trans} = 15.2 Hz, H-3), 7.3 (2H, d, Ar-H), 7.02 (2H, dt, Ar-H), 6.45 (2H, d{dd}, J_{pmo} = 1.8 Hz, 3.8 Hz, 6.8 Hz, Ar-H), 6.97 (2H, dd, Ar-H), 6.63 (2H, d, Ar-H), 4.06 (4H, t, J_{vic} = 6.2 Hz, -CH_2), 1.74 (4H, q, J_{vic} = 5.8 Hz, -CH_2), 1.5 (4H, q, J_{vic} = 6.9 Hz, -CH_2), 1.3

(4H, q, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 192.4 (C=O), 144.6 (C=C), 139.4 (C=C) 148.4, 147.4, 138.6, 135.4, 132.7, 129.6, 128.4, 128.1, 125.7, 123.2 (Ar-C), 76.2 (-OCH_2), 67.6 (-CH_2), 58.26 (-CH_2), 48.03 (-CH_2). GC-MS (*m/z*): 539 [M$^+$]. Anal. calcd. for $C_{34}H_{34}O_6$: C, 75.83; H, 6.31; Found: C, 75.80; H, 6.28%.

(2E,2'E)-1,1'-((decane-1,10-diylbis(oxy))bis(2,1-phenylene)) bis(3-(furan-2-yl)prop-2-en-1-one) (**1e**): Light Brown. Yield: 78%. M.p.: 120 °C. IR (KBr, υ$_{max}$, cm^{-1}): 3112 (Ar-H), 1655(C=O), 1552 (C=C), 1235, 1017 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.66 (2H, dd, J_{trans} = 7.3 Hz, H-3), 7.42 (4H, m, Ar-H), 7.39 (2H, dd. J_{trans} = 15.2 Hz, H-2), 7.03 (2H, dd, *J* = 0.8 Hz, 3.0 Hz, 8.2 Hz, Ar-H), 6.9 (2H, d, Ar-H), 6.73 (2H, t, Ar-H), 6.63 (2H, d, Ar-H), 6.42 (2H, d, Ar-H), 4.05 (4H, t, *J* = 6.2 Hz, -CH_2), 1.80 (4H, q, *J* = 6.4 Hz, -CH_2), 1.74 (4H, q, -CH_2), 1.60 (4H, m, -CH_2), 1.41 (4H, m, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 191.7 (C=O), 145.6 (C=C), 138.9 (C=C) 149.3, 148.2, 137.8, 136.4, 133.9, 129.7, 128.9, 127.6, 124.8, 123.5 (Ar-C), 76.2 (-OCH_2), 67.6 (-CH_2), 52.46 (-CH_2), 58.26 (-CH_2), 48.03 (-CH_2). GC-MS (*m/z*): 567 [M$^+$]. Anal. calcd. for $C_{36}H_{38}O_6$: C, 76.32; H, 6.71; Found: C, 76.29; H, 6.68%.

2.1.3. General procedure for the synthesis of bis-pyrazolines (2a-e)

Bis-pyrazoline, **2a-e**, was obtained from the reaction of **1a-e** (0.00087 mol), phenyl hydrazine (0.75 mL, 0.00175 mol) and glacial acetic acid (5 mL) in dry ethanol (25 mL) was refluxed for 12 hrs. The progress of reaction was monitored by TLC. After completion the reaction, the reaction mixture was cooled in refrigerator, to obtain precipitated solid was filtered and in crystallized from CH_3OH to yield *bis*-pyrazolines **2a-e** [32] (Scheme 1).

(S)-3-(2-(4-(2-((R)-1-carbamothioyl-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)butoxy)phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2a**): Brown light. Yield: 87%. M.p.: 182 °C. IR (KBr, υ$_{max}$, cm^{-1}): 3051 (Ar-H), 1482 (N-N), 1596 (C = N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.45 (2H, t, Ar-H), 7.28 (10H, m, Ar-H), 7.18 (4H, m, Ar-H), 7.46 (2H, d{dd}, $J_{p,\,m,\,o}$ = 1.7 Hz, 3.2 Hz, 8.1 Hz,, Ar-H), 6.98 (4H, dd, Ar-H), 6.86 (2H, d, Ar-H), 5.23 (2Hx, dd, J_{xa} = 6.0 Hz, J_{xb} = 11.6 HZ), 3.78 (2H$_a$, dd, J_{ab} = 16.7 Hz, J_{ax} = 6.0 Hz), 3.52 (2H$_b$, dd, J_{ba} = 16.7 Hz, J_{bx} = 11.6 Hz), 4.05 (4H, t, J_{vic} = 6.8 Hz, -CH_2), 2.01 (4H, q, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 156.8 (C=N), 148.8, 147.2, 146.2, 144.1, 142.8, 130.1, 129.1, 126.8, 124.6, 124.8, 121.9, 120.5, 119.2, 113.7, 112.9 (Ar-C), 77.3 (pyra. ring C-4), 67.7 (-OCH_2), 60.4 (-CH_2), 47.09 (pyra. ring, C-5). GC-MS (*m/z*): 663 [M$^+$]. Anal. calcd. for $C_{42}H_{38}O_4N_4$: C, 76.13, H, 5.74, N, 8.45; Found: C, 76.09, H, 5.70, N, 8.41%.

(S)-3-(2-((5-(2-((R)-1-carbamothioyl-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)pentyl)oxy)phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2b**): Brown light. Yield: 88%. M.p.: 175 °C. IR (KBr, υ$_{max}$, cm^{-1}): 3051 (Ar-H), 1514 (N-N), 1586 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.72 (4H, d, Ar-H), 7.37 (2H, dd, $J_{p.o}$ = 1.1, 7.8 Hz, Ar-H), 7.30 (6H, m, Ar-H), 7.20 (4H, m, Ar-H), 7.11 (4H, m, Ar-H), 6.85 (4H, dd, Ar-H), 5.22 (2H$_x$, dd, J_{xa} = 7.1 Hz, J_{xb} = 11.7 Hz,), 3.95 (4H, t, J_{vic} = 6.3 Hz, -OCH_2), 3.80 (2H$_b$, dd, J_{bx} = 11.7 Hz, J_{ba} = 16.2 Hz), 3.11 (2Ha, dd, J_{ax} = 7.1 Hz, J_{ab} = 16.2 Hz), 1.82 (4H, q, J_{vic} = 6.3 Hz, -CH_2), 1.64 (2H, q, J_{vic} = 6.3 Hz, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 155.7 (C=N), 148.7, 147.6, 146.5, 145.3, 143.7, 130.2, 129.4, 125.7, 124.5, 124.9, 122.7, 121.7, 118.4, 114.8, 113.7 (Ar-C), 78.51 (-OCH_2), 67.48 (pyra. ring C-4), 66.87 (CH_2), 61.6 (CH_2), 48.08 (pyra. ring, C-5). GC-MS (*m/z*): 677 [M$^+$]. Anal. calcd. for $C_{43}H_{40}O_4N_4$: C, 76.33, H, 5.91, N, 8.28; Found: C, 76.30, H, 5.88, N, 8.24%.

(S)-3-(2-((6-(2-((R)-1-carbamothioyl-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)hexyl)oxy)phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2c**): Dark brown. Yield: 85%. M.p.: 167 °C. IR (KBr, υ$_{max}$, cm^{-1}): 3030 (Ar-H), 1485 (N-N), 1590 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.30 (8H, m, Ar-H), 7.45 (2H, d{dd}, J_{pmo} = 1.2 Hz, 2.3 Hz, 8.3 Hz, Ar-H), 7.23 (4H, m, Ar-H), 7.14 (4H, m, Ar-H), 6.91 (2H, t, Ar-H), 6.8 (2H, t, Ar-H), 5.26 (2Hx, dd, J_{xa} = 6.4 Hz, J_{Xb} = 11.7 Hz), 3.79 (2H$_a$ dd, J_{ax} = 6.4 Hz, J_{ab} = 16.4 Hz), 3.53 (2H$_b$, dd, J_{ba} = 16.4 Hz, J_{bx} = 11.7 Hz), 4.01(4H, t, J_{vic} = 5.8 Hz, -CH_2), 3.92 (4H, q, J_{vic} = 6.2 Hz, -CH_2), 2.1 (4H, q, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 156.9 (C=N), 147.9, 147.2, 145.6, 144.7, 143.9, 131.4, 128.7, 125.6, 124.8, 123.6, 122.8, 121.9, 120.4, 114.5, 113.8 (Ar-C), 76.13 (pyra. ring, C-4), 68.09 (-OCH_2), 62.56 (CH_2), 61.8 (CH_2), 47.02 (pyra. ring, C-5). GC-MS (*m/z*): 691 [M$^+$]. Anal. calcd. for $C_{44}H_{42}O_4N_4$: C, 76.52; H, 6.08; N, 8.11; Found: C, 76.48, H, 6.04, N, 8.08%.

(S)-3-(2-((8-(2-((R)-1-carbamothioyl-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)octyl)oxy)phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2d**): Light brown. Yield: 88%. M.p.: 176 °C. IR (KBr, υ$_{max}$, cm^{-1}): 3030 (Ar-H), 1525 (N-N), 1602 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.62 (2H, dd, $J_{o.p.}$ = 1.0 Hz, 8.7 Hz, Ar-H), 7.32 (4H, m, Ar-H), 7.20 (2H, m, Ar-H), 7.10 (6H, m, Ar-H), 7.02 (4H, dt, *J* = 1.0 Hz, 8.4 Hz, Ar-H), 6.76 (4H, dt, $J_{p.o}$ = 1.0 Hz, 8.4 Hz, Ar-H), 6.68 (2H, td, *J* = 2.6 Hz, 4.8 Hz, Ar-H), 5.12 (2Hx, dd, J_{xa} = 7.2 Hz, J_{Xb} = 12.2 Hz), 3.81 (4H, t, J_{vic} = 6.4 Hz, - OCH_2), 3.70 (2Hb, dd, J_{bx} = 12.3 Hz, J_{ba} = 16.8 Hz), 3.02 (2Ha, dd, J_{ax} = 7.2 Hz, J_{ab} = 16.8 Hz), 1.66 (4H, q, J_{vic} = 6.0 Hz, -CH_2), 1.35 (4H, m, -CH_2), 1.28 (4H, m, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 157.3 (C = N), 148.5, 146.8, 146.4, 145.6, 144.7, 133.6, 127.9, 126.8, 125.9, 124.3, 123.9, 122.5, 121.6, 115.3, 114.6 (Ar-C), 78.23(pyra. ring, C-4), 77.24 (OCH_2), 67.54 (CH_2), 63.87 (CH_2), 62.32 (CH_2), 48.43 (pyra. ring, C-5). GC-MS (*m/z*): 719[M$^+$]. Anal. calcd. for $C_{46}H_{46}O_4N_4$: C, 76.88; H, 6.40; N, 7.79; Found: C, 76.84; H, 6.36; N, 7.75%.

(S)-3-(2-((10-(2-((R)-1-carbamothioyl-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)decyl)oxy)phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2e**): Light brown. Yield: 85%. M.p.: 184 °C. IR (KBr, υ$_{max}$, cm^{-1}): 3034 (Ar-H), 1493 (N-N), 1598 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.33 (4H, m, Ar-H), 7.19 (2H, d, Ar-H), 7.15 (10H, m Ar-H), 7.08 (2H, d{dd}, $J_{p,\,m,\,o}$ = 1.0 Hz, 2.2 Hz, 8. Hz, Ar-H), 6.99 (4H, m, Ar-H), 6.72 (2H, m, Ar-H), 6.28 (2H, m, Ar-H), 5.94 (2Hx, dd, J_{xa} = 6.5 Hz, J_{xb} = 11.0 Hz), 3.54 (2H$_a$, dd, J_{ab} = 16.1 Hz, J_{ax} = 6.5 Hz), 3.32 (2H$_b$, dd, J_{ba} = 16.1 Hz, J_{bx} = 11.0 Hz), 4.72 (4H, t, J_{vic} = 5.3 Hz, -CH_2), 4.62 (6H, q, J_{vic} = 5.8 Hz, -CH_2), 2.52 (10H, q, J_{vic} = 6.4 Hz, -CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 157.0 (C=N), 147.5, 146.8, 145.3, 144.5, 143.8, 132.9, 129.4, 125.2, 124.4, 123.9, 122.1, 121.5, 119.6, 115.3, 114.2 (Ar-C), 77.43 (pyra. ring, C-4), 67.56 (-OCH_2), 65.78 (CH_2), 64.12, (CH_2), 62.32 (CH_2), 46.76 (pyra. ring, C-5). GC-MS (*m/z*): 747 [M$^+$]. Anal. calcd. for $C_{48}H_{50}O_4N_4$: C, 77.21; H, 6.70; N, 7.50; Found: C, 77.18; H, 6.66; N, 7.46%.

2.2. In-vitro antibacterial activities

In vitro antibacterial activities of *bis*-pyrazoline **2a-e** derivatives were carried out using the culture of *Aeromonas hydrophila* (MTCC 646), *Yersinia enterocolitica* (MTCC 3099), *Listeria monocytogenes* (MTCC 657), and *Staphylococcus aureus* (MTCC 96) by the disc diffusion method. Gentamicin and Tetracycline were used as the standard drugs, whereas DMSO poured disk was used as negative control. DMSO did not show inhibition against the tested organisms. Pure cultures were grown in brain heart infusion broth for sensitivity testing. Mueller Hinton agar (HiMedia) and *bis*-pyrazoline compounds **2a-e** absolutely diluted (concentration of 40, 30, 20 and 10 µg/mL) were applied as described by Bauer *et al*. 1966 [33]. *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes*, and *S. aureus* strain were tested against the following antibiotics (HiMedia): Tetracycline 30 µg and Gentamicin 10 µg. After enrichment in brain heart infusion broth for 6-8 hrs at 37 °C, the cultures were streaked on Mueller Hinton agar plates using a cotton swab. The antibiotic discs and prepared compound discs were placed on the agar surface.

Table 1. Antibacterial activity of *bis*-pyrazoline derivatives, positive control (Tetracycline and Gentamicin) and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

Compounds	Minimum Inhibitory Concentration, mm			
	A. hydrophila	*Y. enterocolitica*	*L. monocytogenes*	*S. aureus*
2a	24.5 ± 0.4	22.4 ± 0.5	21.3 ± 0.2	25.8 ± 0.4
2b	14.5 ± 0.5	15.5 ± 0.2	18.4 ± 0.4	14.6 ± 0.3
2c	19.5 ± 0.2	18.5 ± 0.5	16.5 ± 0.3	15.5 ± 0.2
2d	15.2 ± 0.4	17.6 ± 0.4	15.8 ± 0.2	14.6 ± 0.3
2e	25.2 ± 0.6	23.6 ± 0.3	24.2 ± 0.4	22.7 ± 0.2
Tetracycline	13	20	12	14
Gentamicin	11	16	15	14
DMSO	-	-	-	-

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 (HiMedia) and classified as resistant, intermediate or sensitive. The results of antibacterial activity and Minimum Inhibitory Concentration (MIC) are summarized in Table 1.

3. Results and discussion

The novel *bis*-pyrazolines (**2a-e**) were prepared in three steps, starting from the Claisen-Schmidt reaction of O-hydroxy acetophenone with furfuraldehyde in the presence of NaOH (50%) to give 95% yield (**1**). The starting *bis* chalcones (**1a-e**) were obtained in good yields from the O-alkylation of furan-chalcones with suitable α-ω-di-bromo alkane (1,4-dibromobutane, 1,5-dibromopentane, 1,6-dibrpmohexane, 1,8-dibromooctane and 1,10-dibromodecane, respectively) in the presence of K_2CO_3/PTC/dry acetone (Scheme 1). The cyclization of latter with phenyl hydrazine under alcoholic conditions led to the formation of new compounds (**2a-e**) which were crystallized from CH_3OH to give pure compounds in moderate yields. All the compounds are insoluble in water but soluble in organic solvents. The chemical structure of these compounds (**1**, **1a-e** and **2a-e**) were established by rigorous analysis of their elemental analysis IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data.

IR spectra of starting material *bis*-chalcone, **1a-e**, displayed intense absorptions at 1600-1655 cm^{-1} and 1552-1652 cm^{-1} due to C=O and C=C stretching, respectively. IR bands provide significant indications for the formation of the cyclized *bis*-pyrazoline analogues of the *bis*-pyrazoline, **2a-e**. In addition, the IR spectra of the compounds showed $\nu_{C=N}$ stretching at 1590-1602 cm^{-1} and $\nu_{(N-N)}$ stretching vibration at 1482-1525 cm^{-1}, which also confirm the formation of desired *bis*-pyrazoline compounds.

In the ^{1}H NMR spectra, 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 the compounds **2a-e**, Hx and Ha & H_b proton of *bis*-pyrazoline ring were observed as doublet of doublet at δ, 5.12-5.94 ppm (1H, dd, J_{xa} = 6.0-7.2 Hz, J_{Xb} = 11.0-12.2 Hz) and 3.32-3.95 ppm (2H, dd, J_{ax} = 6.0-7.2 Hz, J_{ab} = 16.1-16.8 Hz), respectively, which clearly describes the inter-relationship between the Hx, Hb and Ha. The strong deshielding of the C_5 (Ha and Hb) protons compared with the C_4 (Hx) protons of the *bis*-pyrazoline ring can be assumed due to its structure (Scheme 1). The protons belonging to the aromatic ring and the other cyclic groups were observed with the expected chemical shift and integral values.

^{13}C NMR spectra of the compounds **2a-e** were recorded in DMSO and spectral signals are in good agreement with the probable structures. The C_4 and C_5 carbon of *bis*-pyrazoline resonated at 67.48-78.23 and 46.76-48.08 ppm, respectively. The carbon of C=O and C=C displayed signal at 189.7-193.2 ppm and 137.8-145.7 ppm in the all compounds. The compounds, **2a-e**, showed two signal at 155.7-157.6 ppm assigned to C=N. The signals due to the aromatic carbons and the carbon at 1-*N* substituted aliphatic group. The other resonates were showed at their usual position in the experimental section. Encouraged by these cyclization reactions, it was considered to be of major interest to extend this study on the *bis*-chalcones, **1a-e**, in order to investigate the effect of lengthy methylene chains upon the formation and the stereo chemical features of the *bis*-pyrazoline rings. The carbon atoms (C-4 and 5) belonging to *bis*-pyrazoline ring resulted resonances at δ, 67.48 and 46.78 ppm, respectively. The downfield resonance of former as compared to C-4 could be attributed to its benzylic nature and proximity to the nitrogen atom. The carbon atoms due to phenyl rings present at the N-1, C-3 and 5, were observed at the expected positions in the aromatic region.

Characteristic peak were observed in the mass spectra of compounds molecular ion peak ($M^{+\cdot}$) were observed. The characteristics peaks observed within the mass spectra of *bis*-pyrazoline compounds are given in experimental section.

All the synthesized compounds **2a-e** were evaluated for *in vitro* antibacterial activity by using disc-diffusion method and the diameter of zone of inhibition was measured in mm. It was found that all the compounds **2a-e** were screened *in vitro* for their antibacterial activity against a variety of Gram-positive and Gram-negative bacterial strains, like *A. hydrophila*, *L. monocytogenes*, *Y. enterocolitica* and *S. aureus*. Gentamicin (10 mg) and Tetracycline (30 mg) were taken as the standard drugs and DMSO was used as a blank. The in vitro studies result showed that the compounds **2a** (10 mg) and **2e** (30 mg) are highest activity against *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes*, and *S. aureses* 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 in their biological effects which could be helpful in designing more potent antibacterial agents for therapeutic use. On the basis of above observations, modification will be done to improve antibacterial activity.

4. Conclusions

It may be concluded that this research involves the synthesis of *bis*-pyrazoline derivatives (**2a-e**) of *bis*-chalcones (**1a-e**). Compounds **2a** and **2e** showed highest activity against *A. hydrophila*, *Y. enterocolitica*, *L. monocytogenes*, *S. aureses* and better antibacterial agents than the respective standard drugs. Thus the accumulation of the *bis*-pyrazoline derivatives will better antibacterial agents as compared to Gentamicin and Tetracycline. The molecular structure of these active compounds showed property of the 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 for therapeutic use.

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