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Synthesis and in-vitro antibacterial activity of some *bis*-5-(thiophen-2-yl)-carbothioamide-pyrazoline derivatives

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ABSTRACT

Five new compounds, *bis*-pyrazoline derivatives (**2a**-**e**) with antibacterial properties, built around the alkyl chains of varying lengths were prepared via reaction of various 1-ω-alkoxy-*bis*-chalcones with *N*-substituted thiosemicarbazide in ethanolic NaOH solution. The antibacterial activity of these compounds were evaluated by the disk diffusion method against two Gram-positive and two Gram-negative bacteria and the minimum inhibitory concentration were determined. The structures of these compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, ESI mass spectrometry and their purities were also confirmed by elemental analyses. The formation and stereochemical features of the compounds, **2a**-**e**, are found to be independent of the internal spacer length. The results showed that compounds **2a** and **2e** are better antibacterial agent compared to Gentamicin and Tetracycline.

1. Introduction

In the modern society, incidences of water and food-borne gastroenteritis in both industrialized as well as in non-industrialized countries are increasing. These diseases caused by *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Listeria monocytogenes* and *Staphylococcus aureus* are new enteric pathogens in humans. One million annual cases of enteric disease, attributed to water and food borne bacterial infections cause loss of productivity and medical expenses [1].

In developing countries 76 million people and in developing regions, 3 million people die each year worldwide and that comprise of primarily children aged <12 years (WHO) [2]. One of the most difficult phenotypes to detect is one with decreased susceptibility to β-lactams. They may cause bacterial gastroenteritis, sepsis and bacteraemia in infants with multiple medical problems and in immune compromised hosts, especially those with malignant or hepatobiliary diseases [3]. Resistance of several pathogenic bacteria to anti-micro bacterial agents is an emerging problem that has prompted laboratory researchers to think testing of bacteria that were resistant to antimicrobial agent.

The heterocyclic ring system is found in a number of compounds showing analgesic [4] and anti-inflammatory activity [5-7]. On the other hand, several *bis*-pyrazoline derivatives are well known for their pronounced antidepressant and anticonvulsant activities [8-10].

The *bis*-pyrazoline ring is also an important building block in medicinal chemistry and has led to the discovery of a number of derivatives endowed with anti-inflammatory, antitubercular, antitumour and antidiabetic [11-16] significances of *bis*-pyrazolines [17,18]. Furthermore, the goal of this work is to synthesize a series of new *bis*-pyrazolines containing two systematically pyrazoline ring for the study of their structure-activity relationships and evaluated their antibacterial activity against strain of *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Listeria monocytogenes* and *Staphylococcus aureus,* responsible for intestinal diseases. In our recent studies, the ongoing research in our laboratory showed that *bis*-pyrazoline derivatives **2a** and **2e** are good candidate for these studies [16]. As part of our continuous efforts in this area, a series of some new *bis*-[3-(2-oxy-phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide]-pyrazoline derivatives have been synthesized according to Scheme 1 and evaluated for their antibacterial activities by using disk diffusion method.

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 precoated aluminium 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 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 spectrometer in $CDCl_3$. The following abbreviations were used to indicate the peak multiplicity s-singlet, d-doublet, t-triplet, m-multiplet. The mass spectra have been scanned on the Waters Micromass Q-T of Micro (ESI) spectrometer. Anhydrous sodium sulphate was used as a drying agent for the organic phase.

2.1. Synthesis of chalcone

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

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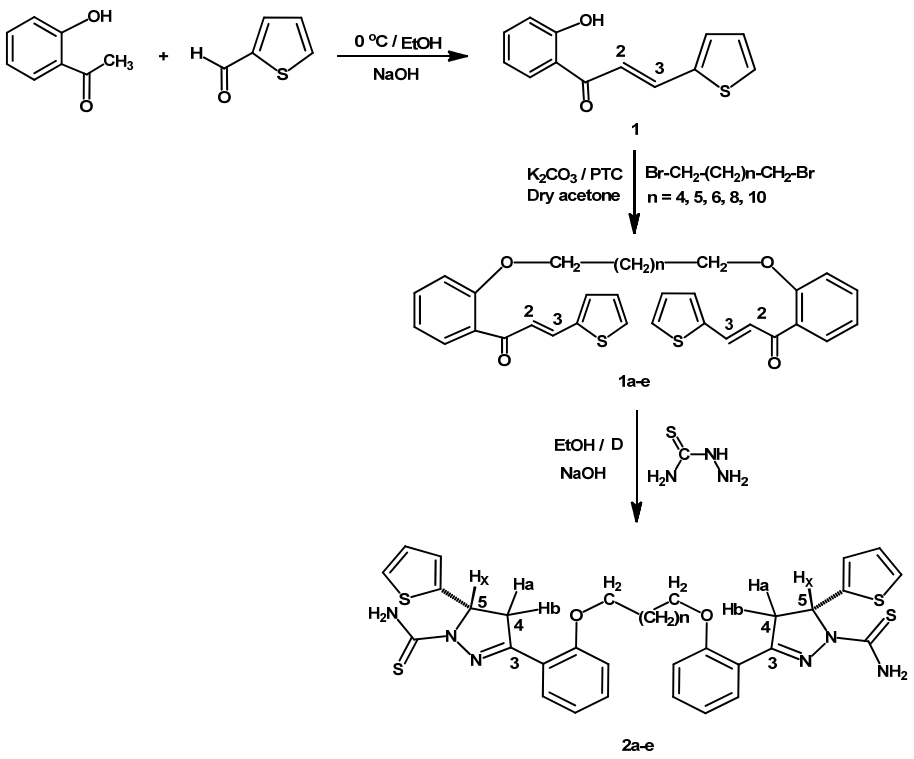

Scheme 1

A suspension of *o*-hydroxy acetophenone (1 mmol) and thiophene carboxaldehyde (1 mmol) in ethanolic solution of NaOH (30%) was stirred for 8 hr 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:$CHCl_3$ (3:1) to obtain a pure chalcone, **1**, [19] (Scheme 1). Color: Yellow, needles. Yield: 95%. M.p.: 86 °C. IR (KBr, ν_{max}, cm^{-1}): 1636 (C=O), 2952 (OH). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 12.86 (1H, s, -OH), 8.04 (1H, d, J_{trans} = 15.4 Hz, H-3), 7.82 (1H, d, J_{trans} = 15.4 Hz, H-2), 7.49 (1H, d{dd}, $J_{p,m,o}$ = 1.4 Hz, 3.6 Hz, 7.1 Hz, Ar-H), 7.45 (1H, m, Ar-H), 7.43 (1H, m, Ar-H), 7.01 (1H, dd, $J_{m,o}$ = 3.6 Hz, 7.1 Hz, Ar-H), 7.34 (1H, t, Ar-H), 7.10 (1H, t, Ar-H), 6.82 (1H, t, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.4 (C=O), 154.7, 149.6, 148.4, 143.8 (C=C), 138.7 (C=C), 133.5, 128.8, 127.5, 127.8, 125.8, 124.7, 122.6 (Ar-C). GC-MS (*m/z*, %): 231 (68) $[M+1]^+$. Anal. calcd. for $C_{13}H_{10}O_2S$: C, 67.82; H, 4.34. Found: C, 67.78; H, 4.30%.

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

A suspension of thiophen chalcone (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) tetrabutylammonium iodide (1.0 g) in dry acetone was refluxed with stirring for 8 hr 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 in CH_3OH:$CHCl_3$ (3:1) mixture (Scheme 1).

(2E,2'E)-1,1'-((butane-1,4-diylbis(oxy))bis(2,1-phenylene)) bis(3-(thiophen-2-yl)prop-2-en-1-one) (**1a**): Color: Light brown. Yield: 85%. M.p.: 110 °C. IR (KBr, ν_{max}, cm^{-1}): 1662 (C=O), 1615 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.74 (2H, d, J_{trans} = 15.7 Hz, H-3), 7.64 (2H, d{dd}, $J_{p,m,o}$ = 0.6 Hz, 1.7 Hz, 8.3 Hz, Ar-H), 7.42 (2H, d, J_{trans} = 15.7 Hz, H-2), 7.24 (6H, m, Ar-H), 7.04 (2H, dd, $J_{m,o}$ = 1.7 Hz, 8.3 Hz, Ar-H), 7.01 (2H, m, Ar-H), 6.87 (2H, d, J_o = 8.3 Hz, Ar-H), 4.01 (4H, t, J_{vic} = 6.7 Hz, -CH_2), 2.01 (4H, q, J_{vic} = 6.7 Hz, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.1 (C=O), 144.6 (C=C), 139.4 (C=C), 156.8, 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*, %): 515 (100) (M^+). Anal. calcd. for $C_{30}H_{26}O_4S_2$: C, 70.03; H, 5.05. Found: C, 70.01; H, 5.01%.

(2E,2'E)-1,1'-((pentane-1,5-diylbis(oxy))bis(2,1-phenylene)) bis(3-(thiophen-2-yl)prop-2-en-1-one) (**1b**): Color: Light brown. Yield: 80%. M.p.: 115 °C. IR (KBr, ν_{max}, cm^{-1}): 1610 (C= O), 1595 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.77 (2H, d, J_{trans} = 15.4 Hz, H-3), 7.66 (2H ,d, J_{trans} = 15.4 Hz, H-2), 7.45 (2H, d{dd}, $J_{p,m,o}$ = 0.7 Hz, 1.8 Hz, 8.4 Hz, Ar-H), 7.27 (4H, m, Ar-H), 7.03 (2H, t, J = 3.4 Hz, Ar-H), 7.19 (2H, dd, $J_{m,o}$ = 1.2 Hz, 8.4 Hz, Ar-H), 6.96 (2H, t, Ar-H), 6.87 (2H, d, J_o = 8.2 Hz, Ar-H), 3.92 (4H, t, J_{vic} = 6.1 Hz, -CH_2), 1.80 (4H ,m, J_{vic} = 6.7 Hz, -CH_2), 1.63 (2H, m, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.6 (C=O), 145.7 (C=C), 138.5 (C=C), 155.7, 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*, %): 529 (100) (M^+). Anal. calcd. for $C_{31}H_{28}O_4S_2$: C, 70.45; H, 5.30. Found: C, 70.41; H, 5.27%.

(2E,2'E)-1,1'-((hexane-1,6-diylbis(oxy))bis(2,1-phenylene)) bis(3-(thiophen-2-yl)prop-2-en-1-one) (**1c**): Color: Light brown. Yield: 83%. M.p.: 120 °C. IR (KBr, ν_{max}, cm^{-1}): 1667 (C=O), 1545 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.92 (2H, d, J_{trans} = 15.6 Hz, H-3), 7.52 (2H, d, J_{trans} = 15.6 Hz, H-2), 7.42 (2H, d{dd}, $J_{p,m,o}$ = 0.6 Hz, 1.5 Hz, 8.2 Hz, Ar-H), 7.26 (2H, m, Ar-H), 7.04 (2H, dd, $J_{m,o}$ = 1.5 Hz, 8.2 Hz, Ar-H), 7.02 (4H, m, Ar-H), 6.75 (2H, d, J_o = 8.4 Hz, Ar-H), 6.23 (2H, m, Ar-H), 4.06 (4H, t, J_{vic} = 6.5 Hz, -CH_2), 2.07 (4H, q, J_{vic} = 6.2 Hz, -CH_2), 1.92 (4H, m, -CH_2). ^{13}C NMR (100 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*, %): 543 (28.1) (M^+). Anal. calcd. for $C_{32}H_{30}O_4S_2$: C, 70.84; H, 5.53. Found: C, 70.80; H, 5.50%.

(2E,2'E)-1,1'-((octane-1,8-diylbis(oxy))bis(2,1-phenylene)) bis(3-(thiophen-2-yl)prop-2-en-1-one) (**1d**): Color: Light yellow. Yield: 87%. M.p.: 122 °C. IR (KBr, ν_{max}, cm^{-1}): 1646 (C=O), 1584 (C=C). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.72 (2H, d, J_{trans} = 15.2 Hz, H-3), 7.51 (4H, dd, J = 5.0 Hz, 8.2 Hz, Ar-H), 7.45 (2H, d, J_{trans} = 15.2 Hz, H-2), 7.06 (2H, t, J = 3.5 Hz, Ar-H), 6.9 (2H, d{dd}, $J_{p,m,o}$ = 0.8 Hz, 3.0 Hz, 8.4 Hz, Ar-H), 6.8 (2H, d, J_o = 8.4 Hz, Ar-H), 6.7 (2H, dd, $J_{m,o}$ = 3.4 Hz, 8.6 Hz, Ar-H), 6.3 (2H, d, $J_{m,o}$ = 3.2 Hz, 8.7 Hz, Ar-H), 4.06 (4H, t, J_{vic} = 6.2 Hz, -CH_2), 2.02 (4H, q, J_{vic} = 6.4 Hz, -CH_2), 1.8 (4H, q, J_{vic} = 6.5 Hz, -CH_2), 1.6 (4H, q, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.1 (C=O), 144.6 (C=C), 139.4 (C=C) 156.4, 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*, %): 571 (38.5) (M^+). Anal. calcd. for $C_{34}H_{34}O_4S_2$: C, 71.57; H, 5.96. Found: C, 70.54; H, 5.92%.

(2E,2'E)-1,1'-((decane-1,10-diylbis(oxy))bis(2,1-phenylene)) bis(3-(thiophen-2-yl)prop-2-en-1-one) (**1e**): Color: Light yellow. Yield: 85%. M.p.: 125 °C. IR (KBr, ν_{max}, cm^{-1}): 1655(C=O), 1598 (C=C). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.65 (2H, d, J_{trans} = 15.4 Hz, H-3), 7.41 (4H, dd, J = 1.8 Hz, Ar-H), 7.34 (2H, d, J_{trans} = 15.4 Hz, H-2), 7.02 (2H, t, J = 3.2 Hz, Ar-H), 6.91 (2H, d{dd}, $J_{p,m,o}$ = 0.8 Hz, 3.5 Hz, 8.2 Hz, Ar-H), 6.84 (2H, d, J_o= 8.7 Hz, Ar-H), 6.62 (2H, dd, $J_{m,o}$ = 3.5, 8.2 Hz, Ar-H), 6.46 (2H, d, $J_{m,o}$ = 2.5 Hz, 8.2 Hz, Ar-H), 4.05 (4H, t, J_{vic} = 2.6 Hz, -CH_2), 2.0 (4H, q, J_{vic} = 6.4 Hz, -CH_2), 1.8 (4H, q, J_{vic} = 7.1 Hz, -CH_2), 1.7 (4H, m, -CH_2), 1.6 (4H, m, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.7 (C=O), 145.6 (C=C), 138.9 (C=C) 157.9, 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*, %): 599 (26.2) (M^+). Anal. calcd. for $C_{36}H_{38}O_4S_2$: C, 72.24, H, 6.35. Found: C, 72.20, H, 6.31%.

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

Bis-pyrazoline **2a-e** was obtained from the reaction of **1a-e** (0.002 mol), thiosemicarbazide (0.00175 mol) and NaOH (0.002 mol) in dry ethanol (25 mL) was refluxed for 12 hr. The progress of reaction was monitored by TLC. After the completion of reaction, the reaction mixture was pour into acidic ice water to pH = 2 (adjusted by HCl) to obtained precipitated solid was filter and in crystallized from CH_3OH to yield *bis*-pyrazolines **2a-e** [20].

(S)-3-(2-(4-(2-((R)-1-carbamothioyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)butoxy)phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2a**): Color: Light brown. Yield: 95%. M.p.: 237 °C. IR (KBr, ν_{max}, cm^{-1}): 3404 (NH), 3212 (Ar-H), 1556 (C=N), 1096(C=S). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.36 (4H, s, -NH_2), 7.25 (2H, dd, $J_{p,m,o}$ = 0.6 Hz, 3.2 Hz, 8.1 Hz, Ar-H), 7.08 (4H, m, Ar-H), 7.04 (4H, s, Ar-H), 6.82 (2H, d, J_o = 8.1 Hz, Ar-H), 6.25 (2H, t, J_o = 7.9 Hz, Ar-H), 5.33 (2H, dd, J_{xa} = 6.9 Hz, J_{Xb} = 11.8 Hz, H_x), 3.65 (2H, dd, J_{ab} = 17.2 Hz, J_{ax}= 6.4 Hz, H_a), 3.52 (2H, dd, J_{ba} = 17.2 Hz, J_{bx} = 11.8 Hz, H_b), 4.02 (4H, t, J_{vic} = 6.7 Hz, CH_2), 2.25 (4H, q, J_{vic} = 5.4 Hz, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 176.6 (C=S), 157.1 (C=N), 148.3, 146.9, 142.4, 127.8, 125.4, 124.2, 123.1, 121.4, 120.8, 114.7 (Ar-C), 76.13 (pyr. ring, C-4), 68.09 (OCH_2), 62.56 (CH_2), 47.02 (pyr. ring, C-5). GC-MS (*m/z*, %): 661 (30.4) (M^+). Anal. calcd. for $C_{32}H_{32}O_2S_4N_6$: C, 58.18, H, 4.84, N, 12.72. Found: C, 58.15; H, 4.81; N, 12.68%.

(S)-3-(2-((5-(2-((R)-1-carbamothioyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)pentyl)oxy)phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2b**): Color: Light yellow. Yield: 94%. M.p.: 244 °C. IR (KBr, ν_{max}, cm^{-1}): 3394 (NH), 3283 (Ar-H), 1596 (C=N), 1378 (C=S). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.05 (4H, s, -NH_2), 7.04 (4H, m, Ar-H), 7.45 (2H, d{dd}, $J_{p,m,o}$ = 0.8 Hz, 3.1 Hz, 6.7Hz, Ar-H), 7.23 (2H, t, J = 3.6 Hz, Ar-H), 7.25 (4H, dd, J = 0.9 Hz, 3.4 Hz, Ar-H), 6.8 (2H, d, J = 8.1 Hz, Ar-H), 5.32 (2H, dd, J_{xa} = 6.5 Hz, J_{Xb} = 11.2 Hz, H_x), 3.52 (2H, dd, J_{ab} = 16.8 Hz, J_{ax} = 6.5 Hz, H_a), 3.21 (2H, dd, J_{ba} = 16.8 Hz, J_{bx} = 11.2 Hz, H_b), 4.04 (4H, m, J = 6.5 Hz, -CH_2), 2.08 (4H ,t, J = 6.1 Hz, -CH_2), 1.64 (2H, m, J = 6.1 Hz, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 177.8 (C=S), 157.3 (C=N), 147.2, 145.2, 143.8, 128.3, 125.8, 124.6, 122.6, 121.7, 119.2, 116.3, (Ar-C), 77.15 (pyra. ring, C-4), 67.46 (OCH_2), 64.76 (CH_2), 48.34 (pyr. ring, C-5). GC-MS (*m/z*, %): 675 (26.4) (M^+). Anal. calcd. for $C_{33}H_{34}O_2S_4N_6$: C, 58.75, H, 5.04, N, 12.46. Found: C, 58.71; H, 5.01; N, 12.42%.

(S)-3-(2-((6-(2-((R)-1-carbamothioyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)hexyl)oxy)phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2c**): Color: Light yellow. Yield: 95%. M.p.: 245-250 °C. IR (KBr, ν_{max}, cm^{-1}): 3228 (Ar-H), 3260 (NH), 1535 (C=N), 1368 (C=S). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.18 (4H, s, -NH_2), 7.42 (4H, dd, J = 1.2 Hz, 3.0 Hz, Ar-H), 7.25 (4H, d{dd}, $J_{p,m,o}$ = 0.7 Hz, 3.2 Hz, 8.4 Hz, Ar-H), 7.21 (2H, t, J = 0.8 Hz, 3.4 Hz, Ar-H), 6.91 (2H, m, Ar-H), 6.83 (2H, d, J = 8.2 Hz, Ar-H), 5.36 (2H, dd, J_{xa} = 6.4 Hz, J_{Xb} = 11.7 Hz, H_x), 3.52 (2H, dd, J_{ab} = 16.4 Hz, J_{ax} = 6.4 Hz, H_a), 3.2 (2H, dd, J_{ba} = 16.4 Hz, J_{bx} = 11.7 Hz, H_b), 2.08 (4H, t, J_{vic} = 5.9 Hz, -CH_2), 1.94 (4H, q, J_{vic} = 6.5 Hz, -CH_2), 1.52 (4H, m, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 178.5 (C=S), 157.4 (C=N), 147.4, 145.7, 143.5, 126.4, 125.8, 124.2, 123.8, 121.6, 118.8, 115.3 (Ar-C), 77.43 (pyr. ring, C-4), 68.25 (OCH_2), 65.82 (CH_2), 63.76 (CH_2), 48.21 (pyr. ring, C-5). GC-MS (*m/z*, %): 689 (100) (M^+). Anal. calcd. for $C_{34}H_{36}O_2S_4N_6$: C, 59.30, H, 5.23, S, 18.60, N, 12.20. Found; C, 59.26; H, 5.20; S, 18.56; N, 12.16%.

(S)-3-(2-((8-(2-((R)-1-carbamothioyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)octyl)oxy)phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2d**): Color: Light yellow. Yield: 92%. M.p.: 238 °C. IR (KBr, ν_{max}, cm^{-1}): 3364 (NH), 3211 (Ar-H), 1528 (C=N), 1357 (C=S). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.07 (4H, s, -NH_2), 7. 52 (4H, dd, $J_{p,m,o}$ = 1.0 Hz, 3.4 Hz, 8.7 Hz, Ar-H), 7.35 (2H, t, Ar-H), 7.04 (4H, m, Ar-H), 6.92 (2H, m, Ar-H), 6.84 (2H, dd, Ar-H), 5.34 (2H, dd, J_{xa} = 6.7 Hz, J_{Xb} = 11.2 Hz, H_x), 3.62 (2H, dd, J_{ab} = 16.7 Hz, J_{ax} = 6.7 Hz, H_a), 3.54 (2H, dd, J_{ba} = 16.7 Hz, J_{bx} = 11.2 Hz, H_b), 4.05 (4H, t, J = 6.5 Hz, -CH_2), 2.03 (4H, m, J = 6.4 Hz, -CH_2), 1.92 (4H, m, J = 6.5 Hz, -CH_2), 1.71 (4H, m, J = 6.3 Hz, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 178.2 (C=S), 157.6 (C=N), 147.4, 145.7, 143.6, 126.7, 125.6, 124.5, 123.6, 122.5, 120.9, 113.9 (Ar-C), 78.23 (pyr. ring, C-4), 67.03 (OCH_2), 65.24 (CH_2), 64.78 (CH_2), 63.45 (CH_2), 48.22 (pyr. ring, C-5). GC-MS (*m/z*, %): 717 (80.4) (M^+). Anal. calcd. for $C_{36}H_{40}O_2S_4N_6$: C, 60.33, H, 5.58, N, 11.73. Found: C, 60.30; H, 5.54; N, 11.70%.

(S)-3-(2-((10-(2-((R)-1-carbamothioyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenoxy)decyl)oxy)phenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2e**): Color: Light yellow. Yield: 94%. M.p.: 236 °C. IR (KBr, ν_{max}, cm^{-1}): 3142 (Ar-H), 3264 (NH), 1561(C=N), 1085 (C=S), 1155 (C-N). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.01 (4H, dd, -NH_2), 7.04 (4H, m, J = 2.3 Hz, Ar-H), 7.25 (2H, dd, $J_{p,m,o}$ = 1.0 Hz, 3.1 Hz, 7.8 Hz, Ar-H), 6.9 (6H, m, Ar-H), 6.8 (2H, d, J = 7.9 Hz, Ar-H), 5.24 (2H, dd, J_{xa} = 6.5 Hz, J_{Xb} = 11.5 Hz, H_x), 3.45 (2H, dd, J_{ab} = 17.4 Hz, J_{ax} = 6.5 Hz, H_a), 3.72 (2H, dd, J_{ba} = 17.4 Hz, J_{bx} = 11.5 Hz, H_b), 4.04 (4H, t, J_{vic} =4.5 Hz, -CH_2), 1.91 (4H, q, J = 7.1 Hz, -CH_2), 1.72 (4H, q, J_{vic} = 7.8 Hz, -CH_2), 1.72 (4H, m, -CH_2), 1.63 (4H, m, -CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 176.4 (C=S), 157.2 (C=N), 148.3, 146.4, 144.5, 127.3, 126.3, 125.8, 124.7, 123.4, 122.4, 114.6 (Ar-C), 77.23 (pyr. ring, C-4), 67.48 (OCH_2), 66.86 (CH_2), 65.89 (CH_2), 64.45 (CH_2), 63.23 (CH_2), 47.84 (pyr. ring, C-5). GC-MS (*m/z*, %): 661 (100) (M^+). Anal. calcd. for $C_{38}H_{44}O_2S_4N_6$: C, 61.29, H, 5.91, N, 11.29. Found: C, 61.25; H, 5.88; N, 11.25%.

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),

Table 1. Antibacterial activity of *bis*-pyrazoline derivatives, positive control (Gentamicin and Tetracycline) 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	24.5±0.4	25.2±0.4	23.4±0.4	22.6±0.4
2b	16.5±0.4	13.4±0.4	14.3±0.4	15.8±0.4
2c	12.5±0.4	13.5±0.4	15.5±0.4	14.5±0.4
2d	14.2±0.4	15.6±0.4	16.8±0.4	12.6±0.4
2e	21.5±0.4	23.4±0.4	22.5±0.4	24.7±0.4
Tetracycline	13	20	12	14
Gentamicin	21	-	-	17
DMSO	-	-	-	-

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 [21]. *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. 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 test are summarized in Table 1.

3. Results and discussion

In this present work, a series of five new *bis*[4-(2-oxy phenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbo thioamide]-pyrazoline derivatives, **2a**-**e**, were synthesized starting from *bis*[(2*E*)-1-(2-oxyphenyl)-3-(thiophen-2-yl)prop-2-en-1-one], **1a**-**e**, and new *bis*-pyrazoline derivatives were synthesized in satisfactory yields (62-80%) as illustrated in Scheme 1 and their structures were confirmed by spectral data.

IR spectra of started material *bis*-chalcone, **1a**-**e**, displayed intense absorptions at 1610-1667 cm^{-1} and 1545-1615 cm^{-1} due to C=O and C=C stretching, respectively. In addition, the IR spectra of the compounds afforded pyrazoline $\nu_{C=N}$ stretching at 1528-1597 cm^{-1} and $\nu_{(N-N)}$ stretching vibration at 1480-1514 cm^{-1}, respectively. IR bands provides significant indications for the formation of the cyclized *bis*-thiosemicarbomyl pyrazoline, **2a**-**e**, $\nu_{C=S}$ stretching at 1085-1378 cm^{-1} and also additional sharp bands exihibited the NH stretching at 3260-3404 cm^{-1}, which also confirm the formation of desired *bis*-pyrazoline compounds.

The ^{1}H NMR spectra signals corresponding to the double hydrogens (H-2 and 3) at δ 7.34-7.66 ppm, and 7.65-7.92 ppm *bis*-chalcones, **1a**-**e**, were found missing altogether which indicates the involvement of the enone moiety during the cyclization reactions and coupling value of J = 15.2-15.7 Hz between these hydrogens describes the *trans* geometry around the C-2 and C-3 double bond. 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 and H_b proton of *bis*-pyrazoline ring were observed as doublet of doublet at δ 5.24-5.36 ppm (1H, dd, J_{Xa} = 6.4-6.9 Hz, J_{Xb} = 11.2-11.8 Hz) and 3.25-3.72 ppm (1H, dd, J_{ax} = 6.4-6.9 Hz, J_{ab} = 16.4-17.4 Hz) describes the *trans* relationship between H_X and H_b and 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**-**e**, the strong deshielding of the C_5 (Ha and Hb) protons compared with the C_4 (H-x) protons of the pyrazoline ring can be assumed due to its structure Scheme 1. The NH proton of thiocarbamoyl group was seen at 8.01-8.36 ppm generally broad bands. The protons belonging to the aromatic ring and the other cyclic groups were observed with the expected ppm.

Finally, ^{13}C NMR spectra of all compounds were recorded in $CDCl_3$ and spectral signals are in good agreement with the probable structures. The carbon of C=O and C=C displayed signal at 189.7-193.2 and 138.9-145.7 ppm in **1a**-**e**. The C_4 and C_5 carbon of *bis*-pyrazolines **2a**-**e** resonated at 77.13-78.23 and 47.02-48.34 ppm, respectively. The compounds **2a**-**e** showed two signal at 176.2-178.5 ppm and 155.8-157.4 ppm assigned to C=S and C=N, 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 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 facile 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-3, 4 and 5) belonging to *bis*-pyrazoline ring resulted resonances at δ 156.10, 77.15 and 48.34, 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*-pyrazolene compounds are given in experimental section.

Selected compounds **2a**, **2b**, **2c**, **2d**, and **2e** showed similar results when tested against Gram-negative and Gram-positive strain Gram-negative bacterial strains, like *A. hydrophila*, *L. monocytogenes*, *Y. enterocolitica* and *S. aureus* new emerging pathogens responsible for gastrointestinal. Gentamicin (10 mg) and Tetracycline (30 mg) were taken as the standard drugs and DMSO was used as a blank. The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. Among all, compounds of the first series where *bis*-pyrazoline **2a**-**e** were found to be active in the MIC test which is an indicative of their ability to prevent seizure spread. On the other hand, *bis*-pyrazolines **2a** and **2e** showed excellent inhibitory activity (inhibitory zone 21.5±0.4 to 24.5±0.4 mm) as compared to Gentamicin (10 mg) and Tetracycline (30 mg), were used for comparison purposes. The results of the compounds of preliminary antibacterial testing are shown in Table 1. According to structure-activity relationships, it can be concluded that *bis*-pyrazolines and carbothioamide moieties are essential for the antibacterial activity.

4. Conclusion

According to structure-activity relationships, a methodology for the synthesis of new class of different aromatic *bis*-pyrazoline molecules, **2a-e**, has been developed highlighted by the cyclization of a thiosemicarbazide and evaluated for antibacterial activity against *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Listeria monocytogenes* and *Staphylococcus aureus.* The route allows for the synthesis of bicyclic pyrazolones scaffolds in moderate to excellent yield. Compounds **2a-e** showed significant antibacterial activity while **2a** and **2e** were found good antibacterial activity than their respective drug. It can be concluded that this class of compounds certainly holds great promise towards good active leads in medicinal chemistry. Further development of this methodology towards the synthesis of other heterocycles is being investigated.

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