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-o-alkoxy-bis-chalcones with N-substituted thiosemicarbazide in ethanolic NaOH solution. The antibacterial activity of these compounds was 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, 1H-NMR, 13C-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-microbial 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-1H-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 (USA) and were used without further purification. The reactions were monitored by precoated aluminium silica gel 60F254 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, 1H NMR, 13C NMR, mass spectrometry and elemental analyses. IR spectra were recorded in KBr on a Perkin-Elmer model 1620 FTIR spectrophotometer. 1H NMR and 13C NMR spectra were recorded at ambient temperature using a Bruker spectropin DPX-400 MHz spectrometer in CDCl3. 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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A suspension of o-hydroxy acetophenone (1 mol) 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 H2O. The solid was filtered recrystallized from CH3OH:CHCl3 (3:1) to obtain a pure chalcone, 1, [19] (Scheme 1). Color: Yellow, needles. Yield: 95%. M.p.: 86 °C. IR (KBr, νmax cm−1): 1636 (C=O), 2952 (OH). 1H NMR (400 MHz, CDCl3, δ ppm): 2.1.2. H2O. The solid was filtered under suction and washed with CH3OH:CHCl3 (3:1) to obtain a solid compound which was filtered under suction and washed with H2O. The solid was recrystallized from CH3OH:CHCl3 (3:1). Yield: 85%. M.p.: 110 °C. IR (KBr, νmax cm−1): 1662 (C=O), 1595 (C=C), 1510, 1484, 1438 (C=C), 1387 (C=C), 1335, 1288, 1275, 127.8, 125.8, 124.7, 122.6 (Ar-C). GC-MS (m/z, %): 231 [M+1]: Anal. calcd. for C31H28O4S2: C, 70.03; H, 5.05. Found: C, 70.01; H, 5.01%. (2E,2’E)-1,1’-(pentane-1,6-diylbis(oxy))bis(2,1-phenylene) bis-(3-(thiophen-2-yl)prop-2-en-1-one) (1a): Color: Light brown. Yield: 80%. M.p.: 115 °C. IR (KBr, νmax cm−1): 1610 (C=O), 1595 (C=C). 1H NMR (400 MHz, CDCl3, δ ppm): 7.77 (2H, d, Jtrans = 15.4 Hz, H-3), 7.66 (2H, d, Jtrans = 15.4 Hz, H-2), 7.45 (2H, dd, Jtrans = 1.7 Hz, 8.3 Hz, Ar-H), 7.01 (2H, m, Ar-H), 6.87 (2H, d, Jtrans = 8.3 Hz, Ar-H), 4.01 (4H, t, Jtrans = 6.7 Hz, -CH2), 2.01 (4H, q, Jtrans = 6.7 Hz, -CH2). 13C NMR (100 MHz, CDCl3, 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, 125.2 (Ar-C), 78.2 (OCH3), 68.7 (CH2). GC-MS (m/z, %): 515 (100 %). Anal. calcd. for C48H40O8S2: C, 70.03; H, 5.03. Found: C, 70.01; H, 5.01%.

(2E,2’E)-1,1’-(hexane-1,6-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, νmax cm−1): 1610 (C=O), 1595 (C=C). 1H NMR (400 MHz, CDCl3, δ ppm): 7.77 (2H, d, Jtrans = 15.4 Hz, H-3), 7.66 (2H, d, Jtrans = 15.4 Hz, H-2), 7.45 (2H, dd, Jtrans = 1.7 Hz, 8.3 Hz, Ar-H), 7.27 (4H, m, Ar-H), 7.03 (2H, t, Jf = 3.4 Hz, Ar-H), 7.19 (2H, dd, Jtrans = 1.2 Hz, 8.4 Hz, Ar-H), 6.96 (2H, t, Ar-H), 6.87 (2H, d, Jf = 8.2 Hz, Ar-H), 3.92 (4H, t, Jf = 6.1 Hz, -CH2), 1.80 (4H, m, Jf = 6.7 Hz, -CH2), 1.63 (2H, m, -CH2). 13C NMR (100 MHz, CDCl3, δ, ppm): 191.4 (C=O), 145.5 (C=C), 140.2 (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 (OCH3), 68.5 (CH2). GC-MS (m/z, %): 515 (100 %). Anal. calcd. for C60H48O8S4: C, 70.45; H, 5.30. Found: C, 70.41; H, 5.27%.

(2E,2’E)-1,1’-(butane-1,4-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, νmax cm−1): 1667 (C=O), 1545 (C=C). 1H NMR (400 MHz, CDCl3, δ ppm): 7.92 ( 2H, d, Jtrans = 15.6 Hz, H-3), 7.52 (2H, d, Jtrans = 15.6 Hz, H-2), 7.42 (2H, dd, Jtrans = 0.6 Hz, 1.5 Hz, 8.2 Hz, Ar-H), 7.26 (2H, m, Ar-H), 7.04 (2H, d, Jf = 1.5 Hz, 8.2 Hz, Ar-H), 7.02 (4H, m, Ar-H), 6.75 (2H, d, Jf = 8.4 Hz, Ar-H), 6.23 (2H, m, Ar-H), 4.06 (4H, t, Jf = 6.5 Hz, -CH2). 13C NMR (100 MHz, CDCl3, δ, ppm): 191.2 (C=O), 145.5 (C=C), 140.2

![Scheme 1](image1.png)
(C=C), 148.8, 147.2, 137.8, 136.5, 133.7, 132.8, 129.1, 128.2, 125.5, 124.2 (Ar), 77.8 (OCH3), 68.6 (CH2), 50.3 (CH), 48.0 (CH3). GC-MS (m/z, %): 543 (28.1) (M+). Anal. calc. for C17H18O3S2C: 708.4, 153.7. Found: C, 70.80, H, 5.50%.

(2E,E)–11–[(octane–1,8-diylbis(oxo))bis(2,1-phenylene)] bis[3-(thiophen–2-yl)prop–2–en–1–one] (14): Color: Light yellow. Yield: 88%. Mp.: 122–123 °C. IR (KBr, νmax, cm−1): 1646 (C=O) (1584 (C=C). 1H NMR (400 MHz, CDCl3, δ, ppm): 7.21 (2H, d, J = 6.8 Hz, Ar–H), 7.17 (2H, d, J = 6.8 Hz, Ar–H), 7.13 (2H, d, J = 6.8 Hz, Ar–H), 7.10 (2H, d, J = 6.8 Hz, Ar–H), 7.05 (2H, d, J = 6.8 Hz, Ar–H), 6.95 (2H, d, J = 6.8 Hz, Ar–H), 6.92 (2H, d, J = 6.8 Hz, Ar–H), 6.87 (2H, d, J = 6.8 Hz, Ar–H), 6.78 (2H, d, J = 6.8 Hz, Ar–H), 6.73 (2H, d, J = 6.8 Hz, Ar–H), 6.68 (2H, d, J = 6.8 Hz, Ar–H), 6.61 (2H, d, J = 6.8 Hz, Ar–H), 6.55 (2H, d, J = 6.8 Hz, Ar–H), 6.48 (2H, d, J = 6.8 Hz, Ar–H), 6.42 (2H, d, J = 6.8 Hz, Ar–H), 6.35 (2H, d, J = 6.8 Hz, Ar–H). GC–MS: (m/z, %): 767 (26%) (M+). Anal. calc. for C21H15O3S2 (1H, 1H, 5.91). Found: C, 70.45, H, 5.92%.

Bis-pyrazine 2a–e was obtained from the reaction of 1a–e (0.002 mol), thiocarbamizide (0.00175 mol) and NaN3 (0.002 mol) in dry ethanol (25 mL) was refluxed for 12 hr. The progress of the reaction was monitored by TLC. After the completion of reaction, the mixture was poured into acidic ice water to pH = 2 (adjusted by HCl) to obtained precipitated solid was filter and crystallized in hexane to yield bis-pyrazines 2a–e [20].

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

Bis-pyrazine 2a–e was obtained from the reaction of 1a–e (0.002 mol), thiocarbamizide (0.00175 mol) and NaN3 (0.002 mol) in dry ethanol (25 mL) was refluxed for 12 hr. The progress of the reaction was monitored by TLC. After the completion of reaction, the mixture was poured into acidic ice water to pH = 2 (adjusted by HCl) to obtained precipitated solid was filter and crystallized in hexane to yield bis-pyrazines 2a–e [20].

Yield: 95%. Mp.: 237–238 °C. IR (KBr, νmax, cm−1): 3404 (NH), 3122 (Ar–H), 1556 (C=N) 1096 (C=O). 1H NMR (400 MHz, CDCl3, δ, ppm): 8.36 (4H, s, NH), 7.25 (2H, d, J = 6.2 Hz, 8.1 Hz, Ar-H), 7.08 (4H, m, Ar-H), 7.04 (4H, s, Ar-H), 6.82 (2H, d, J = 8.1 Hz, Ar-H), 6.62 (2H, d, J = 7.9 Hz, Ar-H), 5.32 (3H, s, J = 6.9 Hz, J = 6.1 Hz), 5.19 (3H, s, J = 6.9 Hz, J = 6.1 Hz), 4.52 (2H, q, ax = 6.7 Hz, CH3), 2.25 (4H, q, ax = 5.4 Hz -CH3). 13C NMR (100 MHz, CDCl3, δ, ppm): 176.6 (C=O), 175.1 (C=N), 148.3, 146.3, 142.4, 127.8, 122.7, 124.1, 121.4, 121.4, 114.5 (Ar–C), 123.9 (CH). GC–MS (m/z, %): 661 (30.4) (M+). Anal. calc. for C17H17O3S2N2C: 581.88, H, 4.84, N, 12.72. Found: C, 58.15; H, 4.81; N, 12.68%.

Yield: 94%. Mp.: 224–244 °C. IR (KBr, νmax, cm−1): 3394 (NH), 3283 (Ar–H), 1596 (C=N), 1378 (C=O). 1H NMR (400 MHz, CDCl3, δ, ppm): 8.05 (4H, s, NH), 7.04 (4H, m, Ar–H), 7.23 (2H, J = 0.9 Hz, Ar–H), 0.72 (4H, d, J = 8.1 Hz, Ar–H), 5.32 (2H, d, J = 6.5 Hz, J = 11.2 Hz, H), 3.52 (2H, dd, J = 16.8 Hz, J = 6.5 Hz, H), 3.21 (2H, dd, J = 16.8 Hz, J = 11.2 Hz), 4.04 (4H, m, J = 6.5 Hz, -CH2), 2.08 (4H, t, J = 6.1 Hz, -CH2), 1.64 (2H, m, J = 6.1 Hz, -CH2). 13C NMR (100 MHz, CDCl3, δ, ppm): 177.8 (C=O), 157.3 (C=N), 147.2, 145.2, 143.8, 128.3, 125.8, 124.6, 122.7, 119.2, 116.3, 137.1, 77.15 (pyr. ring–C, 4), 67.64 (OCH2), 64.76 (CH2), 48.34 (pyr. ring–C, 5). GC–MS (m/z, %): 675 (26%) (M+). Anal. calc. for C17H13S2O3N2: 507.75, H, 5.04, N, 12.46. Found: C, 50.71; H, 5.01; N, 12.42%.

In vitro antibacterial activities

In vitro antibacterial activities of bis-pyrazine 2a–e derivatives were carried out using the culture of Aeromonas hydrophila (MTCC 646), Versinia enterococitica (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 DMSO 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.

<table>
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<tr>
<th>Compounds</th>
<th>Corresponding effect on microorganisms</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A. hydrophila</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>24.2±0.4</td>
</tr>
<tr>
<td>2a</td>
<td>24.2±0.4</td>
</tr>
<tr>
<td>2b</td>
<td>16.5±0.4</td>
</tr>
<tr>
<td>2c</td>
<td>12.5±0.4</td>
</tr>
<tr>
<td>2d</td>
<td>14.2±0.4</td>
</tr>
<tr>
<td>2e</td>
<td>21.5±0.4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>13</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>21</td>
</tr>
<tr>
<td>Sterotopes (S050)</td>
<td>-</td>
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</table>

3. Results and discussion

In this present work, a series of five new bis[4-(2-oxo-phenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbo-thioamide]-pyrazoline derivatives, 2a-e, were synthesized starting from bis[2E]-1-(2-oxo-phenyl)-3-(thiophen-2-yl)prop-2-en-1-one, 1a-e, and new bis-pyrazoline derivatives were synthesized in satisfactory yields (62-98%) 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 νC=S stretching at 1528-1597 cm⁻¹ and νC=N stretching vibration at 1480-1514 cm⁻¹, respectively. IR bands provides significant indications for the formation of the cyclized bis-thiosemiacarbonyl pyrazoline, 2a-e, νC=O stretching at 1085-1378 cm⁻¹ and also additional sharp bands exhibited the NH stretching at 3260-3404 cm⁻¹, which also confirm the formation of desired bis-pyrazoline compounds.

The 1H NMR spectra signals corresponding to the double hydrogens (H-2 and 3) at 8 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 Ho proton of bis-pyrazoline ring were observed as doublet of doublet at 8 5.52-5.36 ppm (1H, dd, Jaa = 6.4-6.9 Hz, Jab = 11.2-11.8 Hz) and 3.25-3.72 ppm (1H, dd, Jaa = 6.4-6.9 Hz, Jbb = 16.4-17.4 Hz) describes the trans relationship between Hx and Hb and Ha 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 C8 (Ha and Hb) protons compared with the C9 (H-x) protons of the pyrazoline ring can be assumed due to its structure Scheme 1. The NH proton of thiocarboxamyl group was seen at 8 0.8-3.6 ppm generally broad bands. The protons belonging to the aromatic ring and the other cyclic groups were observed with the expected ppm.

Finally, 13C NMR spectra of all compounds were recorded in CDC13 and spectral signs are in good agreement with the probable structures. The carbon of C6 O and C6 C displayed signal at 189.7-193.2 and 138.9-145.7 ppm in 1a-e. The C5 and C5 carbon of bis-pyrazolines 2a-e resonated at 77.13-78.23 and 47.92-48.34 ppm respectively. The compounds 2a-e showed two signal at 176.2-178.5 ppm and 155.8-157.4 ppm assigned to C=O and C=N, respectively. The downfield resonance of former as compared to C-4 could be attributed to its benzylc 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 benzylc 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⁺) 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.

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).

The results of the antibacterial activity test are summarized in Table 1. The compounds show varying degrees of inhibition against the tested microorganisms, with the bis-pyrazolines 2a-e demonstrating excellent inhibitory activity compared to the standard drugs. This indicates the potential of these compounds as novel antimicrobial agents.
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 enteroxolitica, 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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