Bis(4,5-dihydropyrazole) Derivatives: Synthesis, Characterization and Antimicrobial-Antioxidant Evaluations

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In the present work, five new bispyrazolines **3(a-e)** have been prepared from the cyclization reaction of bischalcones **2(a-e)** with phenyl hydrazine under alcoholic medium. The bischalcones **2(a-e)** were synthesized by using the alkylation of chalcone **1** with appropriate alkylating agents. The structures of the newly prepared products were confirmed by IR, ^1H and ^{13}C NMR and ESI-MS. The final products were screened for their antimicrobial and antioxidant properties. The *ortho*-xylene linked bispyrazoline showed significant antimicrobial action while the bischalcones proved themselves significant antioxidant.

Keywords: Bischalcone, Phenylhydrazine, Bispyrazolines, Biological activities.

INTRODUCTION

Chalcones either natural or synthetic are associated with various biological activities such as antiangiogenic [1,2], cytotoxicity [3,4] and cholesterol-lowering activity [5]. These are used in agrochemicals, pharmaceuticals, perfumes [6] and as liquid crystalline polymer units [7]. The presence of a reactive, unsaturated keto moiety in chalcones is responsible for their biological behaviour, which may be altered depending on the type and position of substituent on the rings [8]. It is also an important precursor for the synthesis of a large number of bioactive molecules, primarily the nitrogen containing heterocyclics [9]. Invasive Fungal Infections (IFIs) are found in patients especially with weak immune function [10]. Azoles are termed as antifungal agents and ketoconazole, an imidazole derivative used as antifungal agent.

Pyrazoles are the structural isomers of imidazole and pyrazolines are the reduced forms of the pyrazoles. Pyrazole COX-2 inhibitor has highlighted the importance of these heterocycles in pharmaceutical chemistry. These heterocycles containing physiological activities such as antimicrobial [11], antiamebic [12], antidepressant [13], antinociceptive [14], anticancer [15] and antiinflammatory [16]. Pyrazolines are highly active insecticide towards coleopteran and lepidopteran insects [17] and exhibited fungicidal and plant growth

regulatory activities [18]. Pyrazole scaffold is also found in drugs such as celecoxib [19], sildenafil [20] and rimonabant [21]. Pyrazolines substituted with different functional groups can be considered as cyclic benzyldiazine moieties, endowed with MAO inhibitory activity [22]. Synthesis of pyrazoline compounds from α,β -unsaturated carbonyl compounds (chalcones) is done by the cyclization with hydrazine hydrate or substituted hydrazine. It is one of the most commonly used method for the synthesis of pyrazoline compounds. Recently, new catalysts are used for the synthesis such as glacial acetic acid under heating or ultrasound irradiation, K_2CO_3 mediated microwave irradiation [23], pyridine in refluxing ethanol [24] Amberlyst [25] in toluene [26], hot propionic acid [27], triethanolamine [28] and ethanolic sodium hydroxide [29] or sodium acetate [30]. The presence of pyrazole cores in medicinally active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic compounds. Bispyrazolines are the heterocycles in which two pyrazoline moieties are joined through the carbon chains of varying lengths and structures. In continuation of our research work [31-36] upon the bisheterocyclic compounds, we report herein the synthesis, characterization, antimicrobial and antioxidant studies of five new aromatic and rigid chain linked *bis*(4,5-dihydropyrazole) derivatives. These bispyrazolines were synthesized through the cyclization of bischalcones.

EXPERIMENTAL

All the chemicals were purchased from S.D. Fine Chem. Ltd. and E. Merck. The melting points of the synthesized heterocycles were determined through the open capillary. A Perkin Elmer RXIFT Infrared spectrophotometer was used for scanning the Infrared (IR) spectra through KBr pellets and a 400 MHz Bruker spectrophotometer was used to record ^1H and ^{13}C NMR spectra in $\text{CDCl}_3/\text{DMSO}-d_6$ solvent. The progress of reaction and after completion the purity of compounds was checked by using thin layer chromatography.

Synthesis of (2Z)-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (1): A mixture of 4-hydroxyacetophenone (1.36 g, 0.01 mol), anisaldehyde (1.36 g, 0.01 mol), and NaOH (5.0 g, 0.024 mol) was dissolved in ethanol (20 mL) and stirred for 6 h at 0°C . Reaction mixture was kept overnight in refrigerator and resulting mass then put into acidic ice. A yellow solid was separated out which was recrystallized from methanol to yield a pure compound **1** (Scheme-I). Yellow solid: yield: 81 %; m.p.: $162\text{--}164^\circ\text{C}$; IR (KBr, ν_{max} , cm^{-1}): 3280 (OH), 3094 (arom. C-H), 1653 (C=O) and 1258, 1077 (C-O); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 12.89 (1H, s, OH), 7.59 (1H, d, $J_{\text{trans}} = 15.6$ Hz, H-3), 7.53 (2H, d, $J_o = 8.0$ Hz, H-2', 6'), 7.48 (1H, d, $J_{\text{trans}} = 15.6$ Hz, H-2), 7.21 (2H, d, $J_o = 8.0$ Hz, H-2'', 6''), 7.01 (2H, d, $J_o = 8.3$ Hz, H-3', 5'), 6.93 (2H, d, $J_o = 8.6$ Hz, H-3'', 5''), 3.82 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 193.72 (C=O), 163.55 (C-4'), 158.53 (C-4''), 131.81 (C-3), 129.77 (C-1'), 129.61 (C-1''), 128.70 (C-2'', 6''), 120.03 (C-2', 6'), 118.92 (C-2), 118.78 (C-3', 5'), 118.55 (C-3'', 5''), 53.55 (OCH_3); ESI-MS: m/z 255 (M+1, 25 %), 254 (M, 100 %); Anal. Calcd. (%) for $\text{C}_{16}\text{H}_{14}\text{O}_3$: C, 75.57; H, 5.55; Found: C, 75.26; H, 5.52.

Synthesis of (2E,2E')-3,3'-(4,4'-(1,2-phenylenebis(methylene)))(oxy)bis(4,1-phenylene)bis[(4-methoxy)-3-phenylprop-2-en-1-one] (2a): A mixture of chalcone **1** (2.54 g, 0.01 mol), K_2CO_3 (2.0 g) with α, α' -dibromo-*o*-xylene (1.32 g, 0.005 mol) and tetrabutylammonium iodide (1.0 g) was dissolved in acetone and refluxed for 4 h with continuous stirring. Thin layer chromatography was used to observe the completion of reaction. On completion a colourless solid mass was separated from acetonic part and poured in to acidic ice to provide a crude solid. It was further recrystallized using methanol to yield a pure compound **2a** (Scheme-I). Off white solid: Yield: 72 %; m.p.: $130\text{--}132^\circ\text{C}$; IR (KBr, ν_{max} , cm^{-1}): 3070 (aromatic C-H), 1711 (C=O) and 1257, 1030 (C-O); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.78 (2H, d, $J_{\text{trans}} = 15.7$ Hz, H-3), 7.60 (2H, dd, $J = 5.1, 2.0$ Hz, H-5'''), 7.54 (2H, dd, $J = 6.0, 2.8$ Hz, H-6'''), 7.42 (2H, d, $J_{\text{trans}} = 15.7$ Hz, H-2), 7.04 (4H, d, $J = 2.2$ Hz, H-2', 6'), 7.02 (4H, dd, $J = 2.5, 8.0$ Hz, H-2'', 6''), 6.94 (4H, dd, $J = 4.5, 2.0$ Hz, H-3', 5'), 6.85 (4H, d, $J = 6.9, 2.0$ Hz, 8.3 Hz, H-3'', 5''), 5.48 (4H, s, OCH_2) 3.55 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 188.75 (C=O), 134.93 (C-4'), 134.54 (C-4''), 131.75 (C-3), 130.77 (C-1'''), 130.68 (C-1'), 130.16 (C-1''), 129.95 (C-2'', 6''), 129.30 (C-2', 6'), 128.88 (C-4''', 5'''), 128.74 (C-3''', 6'''), 127.75 (C-3', 5'), 122.32 (C-3'', 5''), 119.43 (C-2), 68.26 (OCH_2), 64.16 (OCH_3); ESI-MS: m/z 611 (M+1, 55 %), 610 (M, 100 %); Anal. calcd. (%) for $\text{C}_{40}\text{H}_{34}\text{O}_6$: C, 78.67; H, 5.61; Found: C, 78.35; H, 5.58.

Synthesis of (2E,2E')-3,3'-(4,4'-(1,4-phenylenebis(methylene)))(oxy)bis(4,1-phenylene)bis[(4-methoxy)-3-phenylprop-2-en-1-one] (2b): The bischalcones **2b** was synthesized from the reaction of chalcone **1** (2.54 g, 0.01 mol), with α, α' -dibromo-*p*-xylene (1.32 g, 0.005 mol) under the similar conditions as described for compound **2a**. Creamish solid: yield: 78 %; m.p.: $152\text{--}154^\circ\text{C}$; IR (KBr, ν_{max} , cm^{-1}): 3098 (arom. C-H), 1720 (C=O) and 1261, 1032 (C-O); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.01 (2H, d, $J_{\text{trans}} = 17.4$ Hz, H-3), 7.98 (4H, d, $J_o = 7.8$ Hz, H-2', 6'), 7.62 (4H, d, $J = 7.4$ Hz, H-2'', 6''), 7.52 (2H, d, $J_{\text{trans}} = 17.4$ Hz, H-2), 7.14 (4H, d, $J_o = 6.8$ Hz, H-2''', 3''', 5''', 6'''), 6.98 (4H, dd, $J_o = 8.8$ Hz, H-3', 5'), 6.75 (4H, d, $J_o = 8.3$ Hz, H-3'', 5''), 5.35 (4H, s, OCH_2) 3.75 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 192.16 (C=O), 145.91 (C-4'), 140.34 (C-4''), 134.77 (C-3), 133.76 (C-1'''), 132.18 (C-1'), 131.96 (C-1''), 131.24 (C-2'', 6''), 129.93 (C-2', 6'), 127.52 (C-2''', 3''', 5''', 6'''), 121.77 (C-3', 5'), 120.91 (C-3'', 5''), 120.69 (C-2), 64.43 (OCH_2), 58.96 (OCH_3); ESI-MS: m/z 634 (M+Na+1, 15 %), 611 (M+1, 75 %), 610 (M, 100 %); Anal. Calcd. (%) for $\text{C}_{40}\text{H}_{34}\text{O}_6$: C, 78.67; H, 5.61; Found: C, 78.38; H, 5.60.

Synthesis of (2E,2E')-3,3'-(4,4'-(1,3-phenylenebis(methylene)))(oxy)bis(4,1-phenylene)bis[(4-methoxy)-3-phenylprop-2-en-1-one] (2c): The bischalcones **2c** was prepared from the reaction of chalcone **1** (2.54 g, 0.01 mol), with α, α' -dibromo-*m*-xylene (1.32 g, 0.005 mol) under the same protocol as given for compound **2a**. Yellow solid: yield: 59 %; m.p.: $142\text{--}144^\circ\text{C}$; IR (KBr, ν_{max} , cm^{-1}): 3100 (arom. C-H), 1717 (C=O) and 1258, 1029 (C-O); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.96 (2H, d, $J_{\text{trans}} = 16.8$ Hz, H-3), 7.93 (4H, d, $J_o = 8.0$ Hz, H-2', 6'), 7.78 (4H, d, $J = 7.9$ Hz, H-2'', 6''), 7.56 (1H, dd, $J = 6.8, 2.4$ Hz, H-5'''), 7.49 (2H, d, $J_{\text{trans}} = 16.8$ Hz, H-2), 7.34 (2H, d, $J_o = 6.9$ Hz, H-4''', 6'''), 7.21 (1H, s, H-2'''), 6.84 (4H, d, $J_o = 7.4$ Hz, H-3', 5'), 6.79 (4H, d, $J_o = 7.6$ Hz, H-3'', 5''), 5.25 (4H, s, OCH_2) 3.81 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 191.86 (C=O), 140.31 (C-4'), 137.74 (C-4''), 135.57 (C-3), 131.48 (C-1'), 130.79 (C-1''), 129.88 (C-2'', 6''), 129.33 (C-2', 6'), 129.11 (C-1''', 3''') 127.97 (C-5'''), 127.12 (C-4''', 6'''), 124.87 (C-2'''), 120.88 (C-3', 5'), 119.98 (C-3'', 5''), 119.87 (C-2), 62.54 (OCH_2), 57.32 (OCH_3); ESI-MS: m/z 611 (M+1, 25 %), 610 (M, 100 %); Anal. Calcd. (%) for $\text{C}_{40}\text{H}_{34}\text{O}_6$: C, 78.67; H, 5.61; Found: C, 78.69; H, 5.62.

Synthesis of (2E,2E')-3,3'-(4,4'-(Z)-but-2-ene-(bis(methylene)))(oxy)bis(4,1-phenylene)bis[(4-methoxy)-3-phenylprop-2-en-1-one] (2d): The bischalcones **2d** was obtained from the reaction of chalcone **1** (2.54 g, 0.01 mol), with *trans*-1,4-dibromo-2-butene (1.06 g, 0.005 mol) under the similar conditions as mentioned for compound **2a**. Off white solid: yield: 72 %; m.p.: $126\text{--}128^\circ\text{C}$; IR (KBr, ν_{max} , cm^{-1}): 3111 (arom. C-H), 1721 (C=O) and 1251, 1031 (C-O); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.12 (4H, d, $J_o = 7.3$ Hz, H-2'', 6''), 7.91 (4H, d, $J_o = 7.0$ Hz, H-2', 6'), 7.80 (2H, d, $J_{\text{trans}} = 15.5$ Hz, H-3), 7.72 (2H, d, $J_{\text{trans}} = 15.5$ Hz, H-2), 7.07 (4H, d, $J_o = 7.4$ Hz, H-3', 5'), 7.02 (4H, d, $J_o = 7.3$ Hz, H-3'', 5''), 6.10 (2H, t, $J_{\text{vic}} = 5.2$ Hz, $\text{CH}_2\text{CH=}$), 5.14 (4H, d, $J_{\text{vic}} = 5.2$ Hz, OCH_2) 3.84 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 187.14 (C=O), 139.01 (C-4), 135.14 (C-4''), 130.59 (C-3), 129.19 ($\text{CH}_2\text{CH=}$), 128.86 (C-1'), 128.29 (C-1''), 128.08 (C-2'', 6''), 127.00 (C-2', 6'), 121.81 (C-2), 116.86 (C-3', 5'), 115.98 (C-

a. NaOH, EtOH, 0 °C; b. anhydrous K₂CO₃, dry acetone, PTC, BrCH₂(X)CH₂Br, c. PhNHNH₂, dry ethanol, AcOH, Δ

Scheme-I

3'', 5''), 67.33 (OCH₂), 55.19 (OCH₃); ESI-MS: *m/z* 561 (M+1, 10 %), 560 (M, 90 %); Anal. Calcd. (%) for C₃₆H₃₂O₆: C, 77.12; H, 5.75; Found: C, 76.81; H, 5.72.

Synthesis of (2*E*,2'*E*)-3,3'-(4,4'-(*Z*)-but-2-yne-(bis(methylene))(oxy)bis(4,1-phenylene)bis[(4-methoxy)-3-phenylprop-2-en-1-one] (2e): The bischalcones **2e** was prepared on treatment of chalcone **1** (2.54 g, 0.01 mol), with 1,4-dichloro-2-butyne (0.64 g, 0.005 mol) under the same reaction condition as given for compound **2a**. Brown solid: yield: 58 %; m.p.: 140-142 °C; IR (KBr, ν_{max}, cm⁻¹): 3104 (arom. C-H), 1737 (C=O) and 1249, 1029 (C-O); ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.97 (4H, d, *J*_o = 8.7 Hz, H-2'', 6''), 7.89 (4H, d, *J*_o = 8.4 Hz, H-2', 6'), 7.80 (2H, d, *J*_{trans} = 15.4 Hz, H-3), 7.32 (2H, d, *J*_{trans} = 15.4 Hz, H-2), 7.01 (4H, d, *J*_o = 7.8 Hz, H-3', 5'), 6.96 (4H, d, *J*_o = 7.7 Hz, H-3'', 5''), 5.06 (4H, s, OCH₂), 3.87 (3H, s, OCH₃); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 190.56 (C=O), 145.11 (C-4'), 137.21 (C-4''), 130.54 (C-3), 129.25 (C-1'), 128.29 (C-1''), 128.08 (C-2'', 6''), 126.44 (C-2', 6'), 119.17 (C-2), 117.70 (C-3', 5'), 114.49 (C-3'', 5''), 88.48 (C≡C) 65.49 (OCH₂), 55.43 (OCH₃); ESI-MS: *m/z* 559 (M+1, 100 %), 558 (M, 75 %); Anal. Calcd. (%) for C₃₆H₃₂O₆: C, 77.12; H, 5.75; Found: C, 76.81; H, 5.72.

Synthesis of 1,2-bis[(4-(1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)(4-methoxy)-2-phenyl)phenoxy methyl]benzene] (3a): A mixture of bischalcone **2a** (0.6 g, 0.001 mol) and phenyl hydrazine (0.22 g, 0.002 mol) in dry EtOH (25.0 mL) was refluxed for 4 h in 100 mL round bottom flask using glacial acetic acid as catalyst. The progress and completion of reaction

was confirmed with the help of TLC plates. The resulting reaction mixture was concentrated and cooled in an ice bath to yield a crude solid. The crude product was further crystallized from a mixture of methanol and chloroform to provide pure product **3a** (Scheme-I). Brown solid: yield: 68 %; m.p.: 116-118 °C; IR (KBr, ν_{max}, cm⁻¹): 3034 (arom. C-H), 2944, 2872 (methylene C-H), 1598 (C=N); ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.92 (2H, d, *J*_o = 8.8 Hz, H-4''', 5'''), 7.82 (2H, d, H-3''', 6'''), 7.79 (4H, d, *J* = 3.7 Hz, H-2', 6'), 7.64 (4H, d, *J* = 3.3 Hz, H-2'', 6''), 7.53 (4H, d, *J* = 3.2 Hz, H-2''', 6'''), 7.38 (6H, m, H-3''', 4''', 5'''), 7.00 (4H, d, *J*_o = 8.9 Hz, H-3', 5'), 6.82 (4H, d, *J*_o = 7.0 Hz, H-3'', 5''), 5.87 (2H, dd, *J*_{XA} = 3.0 Hz, *J*_{XM} = 11.0 Hz, H-X), 5.28 (4H, s, OCH₂), 3.81 (2H, dd, *J*_{MX} = 11.9 Hz, *J*_{MA} = 16.8 Hz, H-M), 3.10 (2H, dd, *J*_{AX} = 3.2 Hz, *J*_{AM} = 17.8 Hz, H-A), 3.77 (3H, s, OCH₃); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 153.74 (C-4'), 143.39 (C-4''), 140.48 (C-3), 137.11 (C-1'), 135.02 (C-1''), 134.66 (C-1'''), 131.41 (C-2'', 6''), 128.98 (C-2', 6'), 125.91 (C-3''', 5'''), 124.66 (C-4'''), 120.73 (C-2''', 6'''), 114.81 (C-3', 5'), 113.60 (C-3'', 5''), 67.27 (OCH₂), 63.11 (OCH₃), 55.92 (C-5), 43.45 (C-4); ESI-MS: *m/z* 813 (M+Na, 14 %), 790 (M, 100 %); Anal. Calcd. (%) for C₅₂H₄₆N₄O₄: C, 78.96.17; H, 5.86, N, 7.08; Found: C, 78.64. H, 5.83, N, 7.05.

Synthesis of 1,4-bis[(4-(1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-(4-methoxy)-2-phenyl)phenoxy methyl]benzene] (3b): The bispyrazoline **3b** was synthesized through refluxing of a mixture of bischalcone **2b** (0.6 g, 0.001 mol), phenyl hydrazine (0.22 g, 0.002 mol) and glacial acetic acid (5.0 mL) in dry EtOH (25.0 mL) under the similar reaction condition as

described above for compound **3a**. Brown solid: yield: 72 %; m.p.: 124-126 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3034 (arom. C-H), 2944, 2872 (methylene C-H), 1592 (C=N); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.33 (4H, d, $J_o = 7.8$ Hz, H-2''', 3''', 5''', 6'''), 7.19 (4H, d, $J_o = 6.9$ Hz, H-2', 6'), 7.04 (4H, d, $J = 3.6$ Hz, H-2'', 6''), 7.03 (4H, d, $J_o = 7.2$ Hz, H-2'', 6''), 6.98 (6H, m, H-3''', 4''', 5'''), 6.93 (4H, d, $J_o = 8.2$ Hz, H-3', 5'), 6.62 (4H, d, $J_o = 7.8$ Hz, H-3'', 5''), 5.98 (2H, dd, $J_{\text{XA}} = 2.6$ Hz, $J_{\text{XM}} = 11.7$ Hz, H-X), 5.71 (4H, s, OCH_2), 3.78 (2H, dd, $J_{\text{MX}} = 11.3$ Hz, $J_{\text{MA}} = 17.3$ Hz, H-M), 3.24 (2H, dd, $J_{\text{AX}} = 2.9$ Hz, $J_{\text{AM}} = 16.9$ Hz, H-A), 3.53 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 149.94 (C-4'), 145.09 (C-4''), 139.71 (C-3), 137.20 (C-1'), 134.63 (C-1''), 130.23 (C-1'''), 123.11 (C-2'', 6''), 121.93 (C-2', 6'), 118.99 (C-3''', 5'''), 116.86 (C-4'''), 112.88 (C-2''', 6'''), 109.55 (C-3', 5'), 104.36 (C-3'', 5''), 68.17 (OCH_2), 64.21 (OCH_3), 54.99 (C-5), 44.85 (C-4); ESI-MS: m/z 814 (M+Na+1, 35%), 790 (M, 100%); Anal. Calcd. (%) for $\text{C}_{52}\text{H}_{46}\text{N}_4\text{O}_4$: C, 78.96.17; H, 5.86, N, 7.08; Found: C, 78.68; H, 5.84; N, 7.06.

Synthesis of 1,3-bis[(4-(1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-(4-methoxy)-2-phenyl)phenoxy methyl]benzene] (3c): The compound **3c** was prepared by treating a mixture of bischalcone **2c** (0.6 g, 0.001 mol), phenyl hydrazine (0.22 g, 0.002 mol), and glacial acetic acid (5.0 mL) in dry EtOH (25.0 mL) under the same conditions as described previously for compound **3a**. Yellow solid: yield: 63 %; m.p.: 110-112 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3015 (arom. C-H), 2951, 2862 (methylene C-H), 1591 (C=N); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.92 (4H, d, $J_o = 7.3$ Hz, H-2', 6'), 7.53 (1H, dd, $J = 3.6, 6.2$ Hz, H-5'''), 7.40 (2H, d, $J = 3.8$ Hz, H-4''', 6'''), 7.35 (1H, s, H-2'''), 7.22 (6H, m, H-3''', 4''', 5'''), 7.08 (4H, d, $J_o = 7.6$ Hz, H-2'', 6''), 6.99 (4H, d, $J_o = 6.9$ Hz, H-2''', 6'''), 6.94 (4H, d, $J_o = 8.3$ Hz, H-3', 5'), 6.92 (4H, d, $J_o = 8.0$ Hz, H-3'', 5''), 5.91 (2H, dd, $J_{\text{XA}} = 3.9$ Hz, $J_{\text{XM}} = 12.3$ Hz, H-X), 5.87 (4H, s, OCH_2), 3.75 (2H, dd, $J_{\text{MX}} = 11.8$ Hz, $J_{\text{MA}} = 17.7$ Hz, H-M), 3.28 (2H, dd, $J_{\text{AX}} = 3.3$ Hz, $J_{\text{AM}} = 17.3$ Hz, H-A), 3.61 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 154.91 (C-4'), 151.06 (C-4''), 145.78 (C-3), 139.98 (C-1'), 137.55 (C-1''), 132.43 (C-1'''), 127.19 (C-2'', 6''), 125.13 (C-2', 6'), 120.90 (C-3''', 5'''), 115.86 (C-4'''), 110.81 (C-2''', 6'''), 108.15 (C-3', 5'), 106.66 (C-3'', 5''), 69.19 (OCH_2), 66.28 (OCH_3), 52.98 (C-5), 43.95 (C-4); ESI-MS: m/z 791 (M+1, 15 %), 790 (M, 100 %); Anal. Calcd. (%) for $\text{C}_{52}\text{H}_{46}\text{N}_4\text{O}_4$: C, 78.96.17; H, 5.86, N, 7.08; Found: C, 78.70; H, 5.87; N, 7.07.

Synthesis of (Z)-1,4-bis[(4-(1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-(4-methoxy)-2-phenyl)phenoxy]but-2-ene (3d): The compound **3d** was synthesized by treating a mixture of bischalcone **2d** (0.5 g, 0.001 mol) with phenyl hydrazine (0.22 g, 0.002 mol) under the similar reaction conditions as given above for compound **3a**. Brown solid: yield: 72 %; m.p.: 104-106 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3033 (arom. C-H), 2990, 2880 (methylene C-H), 1595 (C=N); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.80 (4H, d, $J_o = 8.4$ Hz, H-2', 6'), 7.48 (6H, m, H-3''', 4''', 5'''), 7.11 (4H, d, $J_o = 7.8$ Hz, H-2'', 6''), 7.01 (4H, d, $J_o = 7.1$ Hz, H-2''', 6'''), 6.98 (4H, d, $J_o = 8.8$ Hz, H-3', 5'), 6.94 (4H, d, $J_o = 8.3$ Hz, H-3'', 5''), 5.83 (2H, dd, $J_{\text{XA}} = 3.6$ Hz, $J_{\text{XM}} = 12.6$ Hz, H-X), 6.07 (2H, t, $J_{\text{vic}} = 6.4$ Hz, $\text{OCH}_2\text{CH}=\text{CH}$), 5.81 (4H, d, $J_{\text{vic}} = 6.4$ Hz, OCH_2), 3.71 (2H, dd, $J_{\text{MX}} = 11.3$ Hz,

$J_{\text{MA}} = 17.2$ Hz, H-M), 3.26 (2H, dd, $J_{\text{AX}} = 3.6$ Hz, $J_{\text{AM}} = 17.8$ Hz, H-A), 3.72 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 156.11 (C-4'), 147.63 (C-4''), 141.88 (C-3), 135.81 (C-1'), 133.15 (C-1''), 129.51 (C-1'''), 123.19 (C-2'', 6''), 121.33 (C-2', 6'), 119.35 (C-3''', 5'''), 117.66 (C-4'''), 111.32 (C-2''', 6'''), 109.95 (C-3', 5'), 108.16 (C-3'', 5''), 67.73 (OCH_2), 65.88 (OCH_3), 53.48 (C-5), 43.53 (C-4); ESI-MS: m/z 741 (M+1, 25 %), 740 (M, 100 %); Anal. Calcd. (%) for $\text{C}_{48}\text{H}_{44}\text{N}_4\text{O}_4$: C, 77.81; H, 5.99, N, 7.56; Found: C, 77.49; H, 5.96; N, 7.52.

Synthesis of (Z)-1,4-bis[(4-(1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-(4-methoxy)-2-phenyl)phenoxy]but-2-yne (3e): The bispyrazoline **3e** was prepared by refluxing a mixture of bischalcone **2e** (0.5 g, 0.001 mol) with phenyl hydrazine (0.22 g, 0.002 mol) under the similar reaction conditions as given above for compound **3a**. Brown solid: yield: 58 %; m.p.: 104-106 °C; IR (KBr, $\nu_{\max}$, cm^{-1}): 3022 (arom. C-H), 2989, 2873 (methylene C-H), 1594 (C=N); ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.68 (4H, d, $J_o = 7.6$ Hz, H-2', 6'), 7.52 (4H, m, H-3''', 5'''), 7.19 (4H, d, $J_o = 7.1$ Hz, H-2'', 6''), 7.01 (4H, d, $J_o = 6.9$ Hz, H-2''', 6'''), 6.99 (1H, t, $J_o = 6.8$ Hz, H-4'''), 6.93 (4H, d, $J_o = 8.0$ Hz, H-3', 5'), 6.91 (4H, d, $J_o = 8.7$ Hz, H-3'', 5''), 5.79 (2H, dd, $J_{\text{XA}} = 2.8$ Hz, $J_{\text{XM}} = 11.8$ Hz, H-X), 5.75 (4H, s, OCH_2), 3.73 (2H, dd, $J_{\text{MX}} = 11.7$ Hz, $J_{\text{MA}} = 16.9$ Hz, H-M), 3.29 (2H, dd, $J_{\text{AX}} = 2.8$ Hz, $J_{\text{AM}} = 16.7$ Hz, H-A), 3.69 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 148.11 (C-4'), 141.33 (C-4''), 139.73 (C-3), 134.88 (C-1'), 131.55 (C-1''), 128.59 (C-1'''), 122.89 (C-2'', 6''), 121.36 (C-2', 6'), 118.33 (C-3''', 5'''), 116.96 (C-4'''), 112.22 (C-2''', 6'''), 110.95 (C-3', 5'), 107.76 (C-3'', 5''), 88.48 (C $\equiv$ C), 69.77 (OCH_2), 66.81 (OCH_3), 53.78 (C-5), 43.77 (C-4); ESI-MS: m/z 761 (M+Na, 15 %), 740 (M, 85 %); Anal. Calcd. (%) for $\text{C}_{48}\text{H}_{44}\text{N}_4\text{O}_4$: C, 78.03; H, 5.73, N, 7.58; Found: C, 77.71; H, 5.70; N, 7.54.

RESULTS AND DISCUSSION

The bispyrazolines **3(a-e)** were synthesized in the present study. The compound **1** was synthesized through the condensation reaction of 4-hydroxyacetophenone with anisaldehyde. The O-alkylation of chalcone **1** with appropriate aromatic and rigid linker using acetone as solvent in the presence of K_2CO_3 and tetrabutylammonium iodide provided bischalcones **2(a-e)**. The cyclization of bischalcones with phenyl hydrazine in acidic medium led to the formation of bispyrazolines **3(a-e)**. The phase transfer catalysis (PTC) used in these synthesis was highly helpful to improve the yield of bischalcones. The structures of prepared compounds **2(a-e)** and **3(a-e)** were analyzed on the basis of their various spectral data (IR, ^1H & ^{13}C NMR and ESI-MS). The IR spectra of compounds **2(a-e)** showed carbonyl (C=O) and aromatic (C-H) stretching absorptions at 1737-1711 and 1602-1594 cm^{-1} , respectively. The proton spectra of bischalcones exhibited the doublets at δ 8.01-7.78 and 7.52-7.32 could be assigned to H-3 and H-2 protons and these protons are trans to each other confirmed through coupling value of 17.4-15.4 Hz. The H-2', 6' and H-3', 5' could produce suitable doublet which were easily centered at δ 7.98-7.89 ($J_o = 8.4$ -7.8 Hz) and 7.07-6.84 ($J_o = 8.8$ -7.4 Hz), respectively. The protons belonging to intermediate chain (OCH_2) generated appropriate singlet at 5.48-5.06. In ^{13}C NMR spectra of compounds **2(a-e)**, OCH_2 groups were resonating at δ 68.26-62.54 which suggest their

placement near an electronegative oxygen atom. The signals of carbon atom C-2 and C-3 found to be placed at δ 121.81-119.17 and 139.77-130.54, respectively while carbonyl carbon atoms resonated at the most downfield positions at δ 192.16-187.14. IR spectra of bispyrazolines **3(a-e)** did not reveal any absorption in the region of 1737-1711 cm^{-1} , which confirmed the absence of C=O group and the major bands were observed in the region at 1598-1591 cm^{-1} due to C=N group. In ^1H NMR spectra of compounds **3(a-e)** signals corresponding to H-2 and H-3 at δ 8.01-7.78 and 7.52-7.32 present in compounds **2(a-e)** were found to be absent which confirmed the involvement of enone moiety during the cyclization reactions. The pyrazoline ring protons H-X, H-M and H-A generated well defined dd at δ 5.98-5.79 (2H, dd), 3.81-3.71 (2H, dd) and 3.29-3.10 (2H, dd), respectively.

The stereochemical features of bispyrazolines **3(a-e)** were studied from the considerations of coupling constants (J) of H-X, H-M and H-A. The vicinal coupling constant 12.7-11.6 Hz, between H-X and H-M confirmed that these hydrogens are *cis*- to each other, whereas H-X and H-A are *trans*- to each other with coupling value of $J_{\text{XA}} = 2.6$ -3.9 Hz and $J_{\text{MA}} = 17.8$ -16.9 Hz. H-M and H-A are geminally placed at C-4 and the phenyl groups placed at N-1 and C-2 are *trans* to each other and avoid any intramolecular repulsion.

The remaining aromatic protons of compounds **3(a-e)** were absorbed at appropriate positions. ^{13}C NMR spectra of compounds **3(a-e)** did not exhibit any signals in the carbonyl group region which proved that these group reacted during the transformation of chalcone moiety. The bispyrazoline ring carbon atoms C-3, C-4 and C-5 were resonating at δ 145.78-139.71, 44.45-43.45 and 55.92-52.98, respectively. The remaining aromatic and OCH_2 carbon group carbon atoms were characterized through their respective signals at appropriate δ values.

Antimicrobial activity: The bischalcones **2(a-e)** and bispyrazolines **3(a-e)** were tested against bacterial and fungal species namely *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Aspergillus janus*, *Fusarium oxysporum*, *Penicillium glabrum*, *Aspergillus niger*, *Aspergillus sclerotum*, respectively to evaluate their antibacterial and antifungal activities. Through serial dilution technique the minimum inhibitory concentrations of the intermediate **2(a-e)** and final product **3(a-e)** were determined. Minimum inhibitory concentration (MIC) is that minimum concentration which is required to prevent the growth of both bacterial and fungal strains [37]. The standard

drugs used were amoxicillin and fluconazole and all the tubes were introduced with the bacterial and fungal strains. Nutrient broth as food for bacterial strain and malt extract as food for fungal strain were added to each tube for their successive growth. Different dilutions of the concerned bisheterocycles at the concentration 100, 50, 25, 12.5, 6.25, 3.12, 1.56 $\mu\text{g/mL}$ were introduced against above said microorganisms. Table-1 represented the MIC values of bischalcones **2(a-e)** and bispyrazolines **3(a-e)**. The results in Table-1 indicated that compounds **2(a-e)** exhibited the moderate antimicrobial action against the tested strains. The compounds **2a** exhibited good activity against bacterial strains *Escherichia coli* and *Bacillus subtilis* and against fungal strain *Penicillium glabrum* at the MIC of 12.5 $\mu\text{g/mL}$. Similarly, compound **2d** revealed its potent behaviour against bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa* and fungal strains *Aspergillus janus*, *Penicillium glabrum* and *Aspergillus niger* at the similar MIC values. The bischalcones **2b** and **2c** exhibited the MIC value of 25 $\mu\text{g/mL}$ against *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus Janus* and *Penicillium glabrum*. In Table-1, bispyranopyrazole **3a** showed a significant antimicrobial action against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Penicillium glabrum* at the MIC of 6.25 $\mu\text{g/mL}$ and excellent result against *Klebsiella pneumoniae* at the MIC of 3.12 $\mu\text{g/mL}$, which is equivalent to the standard drug fluconazole. The compounds **3b** and **3c** were found to be moderately active at the MIC value of 12.5 $\mu\text{g/mL}$ while compound **3d** is found to be active *Klebsiella pneumoniae*, *Penicillium glabrum* and *Aspergillus niger* at the MIC of 6.25 $\mu\text{g/mL}$. It is evaluated from the data that the bispyrazolines **3(a-e)** are found to be more biological significant as compare to the corresponding bischalcones **3(a-e)**.

Antioxidant activity: The bisheterocycles **2(a-e)** and **3(a-e)** were also screened for their antioxidant evaluation. The free radical scavenging activity of the prepared bischalcones and bispyrazolines were measured in terms of radical scavenging ability to become as diamagnetic compound [38,39]. Three different concentrations 50, 75 and 100 $\mu\text{g/mL}$ of the sample solutions were prepared and 1.5 mL solution of both DPPH (1 mM) and prepared compounds **2(a-e)** and **3(a-e)** was mixed. At room temperature the sample solutions were left for 30 min for incubation in the dark. The absorbance of the blank solution and prepared samples was noticed at 517 nm in UV spectrophotometer. The antioxidant capacity [40] was calculated by the following equation:

TABLE-1
MIC ($\mu\text{g/mL}$) DATA OF BISCHALCONES **2(a-e)** AND BISPYRAZOLINES **3(a-e)**

		Compounds										Amoxicillin	Fluconazole
		2a	3a	2b	3b	2c	3c	2d	3d	2e	3e		
Gram-negative	<i>E. coli</i>	12.5	6.25	25	25	25	12.5	25	12.5	25	25	3.12	3.12
	<i>K. pneumoniae</i>	25	3.12	50	25	50	25	25	6.25	50	25	3.12	3.12
	<i>P. aeruginosa</i>	25	6.25	25	12.5	25	12.5	12.5	12.5	25	12.5	3.12	3.12
Gram-positive	<i>S. aureus</i>	25	12.5	50	25	50	12.5	25	25	25	50	3.12	3.12
	<i>B. subtilis</i>	12.5	12.5	50	12.5	50	12.5	25	12.5	50	12.5	3.12	3.12
Fungi	<i>A. janus</i>	50	25	25	25	25	25	12.5	6.25	12.5	25	3.12	3.12
	<i>P. glabrum</i>	12.5	6.25	25	12.5	25	25	12.5	6.25	25	25	3.12	3.12
	<i>A. niger</i>	25	25	50	25	50	12.5	12.5	25	50	12.5	3.12	3.12
	<i>F. oxysporum</i>	25	12.5	50	12.5	50	25	50	12.5	25	25	3.12	3.12
	<i>A. sclerotium</i>	50	12.5	50	12.5	50	12.5	25	12.5	25	12.5	3.12	3.12

TABLE-2
PERCENTAGE SCAVENGING AT DIFFERENT CONCENTRATIONS OF BISCHALCONES **2(a-e)** AND BISPYRAZOLINES **3(a-e)**

Conc. (µg/mL)	Compounds										Ascorbic acid
	2a	3a	2b	3b	2c	3c	2d	3d	2e	3e	
50	38.3	42.3	37.6	38.3	35.9	39.6	41.8	41.8	40.5	40.5	53.8
75	42.1	51.1	40.8	43.4	39.3	47.3	48.1	49.9	45.7	48.7	67.5
100	49.9	63.9	44.3	48.6	42.6	51.3	51.9	57.9	48.5	53.5	75.4

$$\text{Inhibition (\%)} = \frac{A_b - A_{ss}}{A_b} \times 100$$

where, A_b is the absorbance of the blank solution; A_{ss} is the absorbance of the sample solutions of different solutions.

The radical scavenging abilities of bischalcones **2(a-e)** and bispyrazolines **3(a-e)** have been represented in Table-2. Thus, it is clear that the synthesized bisheterocycles exhibited moderate to significant radical scavenging activity. The compound **3a** could prove itself a potential antioxidant as compared to other bispyrazoline derivatives.

Conclusion

In this study, we have synthesized the bispyrazoline linked through aromatic and rigid chain linkers. The *o*-xylene linked bispyrazoline showed significant antimicrobial action while the bischalcones proved themselves significant antioxidant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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