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Traditional, One-pot Three-Component Synthesis and Antibacterial Evaluations of Some New Pyrazoline Derivatives

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Abstract

This work synthesized a new series of pyrazoline compounds containing azo linkages via classical and one-pot three-component reactions. The progress started with 3-chloro,4-methyl aniline, which has been diazotized and coupled with 4-hydroxy acetophenone to form azo compound 1-(3-((3-chloro-4-methyl phenyl) diazenyl)-4-hydroxyphenyl) ethan-1-one (1) and converted to 1-(4-(benzyloxy)-3-((3-chloro-4-methyl phenyl) diazenyl) phenyl) ethan-1-one (2). The later azo compound was subjected to both classical and one-pot methods to give target pyrazoline derivatives. The structure of all newly obtained compounds was supported by spectral data (^{1}H-NMR, ^{13}CNMR, Dept.135, and FT-IR). Finally, some pyrazoline derivatives were estimated for their antibacterial activity against *Escherichia coli* as gram-negative and *Staphylococcus aureus* as gram-positive. The results showed significant activity against both types of bacteria.

Keywords: Bis-benzyloxy; One-pot synthesis; Pyrazoline; Antibacterial activity; Claisen- Schmidt condensation

Introduction

A one-pot synthesis is a synthetically helpful approach for enhancing the efficiency of chemical processes in which interacting molecules undergo successive chemical reactions in a single vessel [1]. This is very useful for organic chemists because it significantly shortens the reaction process by reducing the number of steps and avoiding lengthy separation processes and purification of chemical intermediates [2]. It also contributes to a green chemistry approach by saving time and solvents for purification processes and increasing the proportion of output products [3].

Because diazonium salt is extremely unstable, the only stable diazonium salts are aromatic diazonium salts [4,5]. Diazonium salts are prepared in ice-cold solutions and used immediately [6]. Aromatic azo compounds are frequently used in a wide range of applications. They're utilized as organic dyes [7,8], indicators [9], radical reaction initiators [10], medicinal [11], and drug delivery agents [12], Nonlinear optics [13], optical storage media [14], chemosensory [15], and photochemical switches [16]. The Claisen-Schmidt condensation reaction of substituted acetophenones and benzaldehydes yields chalcones [17,18]. Chalcones and their derivatives demonstrate a wide range of biological activities, including anti-inflammation [18], it has been studied as potential antitumor agents [19], and it is the central core for a variety of important biological compounds [20]. Also, chalcones undergo a cyclization reaction with phenyl hydrazine to yield pyrazolines in a typical pyrazoline derivative synthesis.

Pyrazolines are well-known and vital nitrogen-containing five-membered heterocyclic compounds [21], synthesis of pyrazolines has gained significant attention due to their promising biological activities [22] such as anticancer [23], antimicrobial [24], antidepressant, anti-diabetic [25], immunosuppressive [26], Antihistaminic activity [27], anti-inflammatory [25], and the pyrazoline compounds are intensively used in medicines and agricultural industry [28]. Following that, other synthetic techniques for pyrazoline synthesis were published, including a one-pot three-component condensation reaction. Because

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of their wide range of therapeutic applications, herein interested in designing and synthesizing a new class of pyrazoline compounds functionalized with various azo linkages and evaluating their biological activities in this study. Through a one-pot three-component condensation process of azo acetophenone, substituted benzaldehydes, and phenylhydrazine [29].

Materials and Methods

Experimental Notes

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, U.S.A., and Merck, made in Germany. Melting points were taken by using an (OptiMelt, Sunnyvale, CA, USA.). IR spectra were recorded by using (Perkin-Elmer FTIR spectrophotometer, Waltham, MA, USA), using KBr disc. All reactions were monitored using both (Silia plate glass and Silia plate Aluminum) on Backed TLC plates DC-Glasplatten-Kieselgel (Radnor, PA, U.S.A.). The mobile phase was a mixture of ethyl acetate with normal hexane (3:7). 1H-NMR, 13C-NMR and 13C-DEPT.135 spectra were recorded on a Bruker Ultra Shield (400MHz) with T.M.S. and CDCl3 as an internal reference and solvent, respectively.

Synthesis of the azo compound

Synthesis of azo compound 1-(3-((3-chloro-4-methylphenyl) diazenyl)-4-hydroxyphenyl) ethan-1-one (1):

Step 1: Preparation of diazonium salt [30], 3-chloro, 4- methyl aniline (14.16g, 0.1Mol) was dissolved in 80mL Hydrochloric acid (3M), and put the solution in an ice bath near 0°C. Quickly prepared 100mL NaNO2 (1M) and added it dropwise with stirring to form diazonium salt. The temperature of the solution was kept near 0°C.

Step 2: 4-hydroxy acetophenone (13.615g, 0.1Mol) was dissolved in aqueous NaOH 4% (20 ml aqueous NaOH 4% for 0.01Mol of phenol). And put the solution in an ice bath near 0°C. Also, it was added to the solution step1 dropwise with stirring quickly for 15 minutes to complete of reaction. After (45-60min.) was collected by vacuum filtration. The precipitate was washed with cold distilled water several times and recrystallized with absolute ethanol to give (Brown-yellow) azo. C14H13ClN2O2, percentage yield 70%, melting point (144-145oC). IR (cm-1) str. 3100-3500 (-OH), 1673 (C=O), 1605 (C=C), 1479 (N=N),1276 (C-O), 1165 (C-N). 1H- NMR (ppm) 13.1 (1H, S, OH), 8.53 (1H, d, Ar-H14), 8.03 (1H, s, Ar-H8), 7.92 (1H, d, Ar-H4),7.72 (1H, s, Ar-H10), 7.42 (1H, d, Ar-H13), 7.12 (1H, d, Ar-H5), 2.67 (3H, s, COCH3), 2.49 (3H, s, Ar-CH3). 13C- NMR (ppm) 195.9: C2, 157: C6, 148.79: C9, 140.33: C12, 134.7: C11, 133.25: C4, 131: C13, 129.9: C3, 122: C7, 121.7: C8,10, 118.8: C6,14, 26.36: C1, 20.38: Ar-CH3.

Synthesis of benzyloxy compound

1-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl) phenyl) ethan-1-one (2):

A mixture of azo dye compound (1) (10.1g,0.035Mol), Benzyl bromide (6.84g, 0.04Mol), and anhydrous potassium carbonate (6.2g, 0.045Mol) were mixed together with 100mL of ethanol in an R.B.F. 250mL. It was refluxed with stirring for (6hrs). The solution was cooled and poured into crushed ice. The precipitate of compound (2) was filtered by vacuum filtration, so washed several times with cold distilled water. After that, it was dried and recrystallized with pure ethanol to give (Brown-red) product, C22H19ClN2O2, a percentage yield of 88% and melting point (132-133oC). IR (cm-1) str. 1675 (C=O),1597 (C=C), 1478 (N=N), 1275 (C-O), 1175 (C-N). 1H-NMR (ppm) 8.08 (1H, s, Ar-H7), 8.06 (1H, d, Ar-H16), 7.98 (1H, s, Ar-H20), 7.81 (1H, d, Ar-H13), 7.56 (1H, d, Ar-H4), 7.28-7.44 (5H, m, Ar-H10,11,12,13,14), 7.19 (1H, d, Ar-H17), 5.42 (2H, s, Ar-CH2-O), 2.63 (3H, s, COCH3), 2.48 (3H, Ar-CH3).13C-NMR (ppm) 196.79: C=O, 159.56: C5, 151.99: C15, 141.84: C18, 139.42: C9, 136.2: C19, 135.2: C3, 132.28: C17, 131.3: C2,128.71: C11,13, 128.15: C12, 126.93: C10,14, 122.74: C6,122.48: C20, 117.76: C16, 114.58: C4, 71.2: C8, 26.52: C22, 20.25: C21.

Synthesis of chalcone derivatives

1-(4-(benzyloxy)-3-(-(3-chloro-4-methylphenyl) diazenyl) phenyl)-3-phenylprop-2-en-1-one (3a and 3b):

A mixture of compound (2) (0.76g, 0.002Mol), 0.5ml ethanolic NaOH 6%, and (0.002Mol) of substituted benzaldehydes were mixed with 20ml of ethanol 100% in the 50ml R.B.F. The mixture refluxed with stirring for (30-45min.) and heating (70-80 °C) until the reaction was completed, monitored by TLC, and changed colour [31]. After that, the product was poured into a beaker, condensed for 10 mL and cooled. The precipitated filtered off [1] and washed with cold distilled water, recrystallized with ethanol. The six products of chalcone derivatives containing (bis-benzyloxy) were obtained with different colors and percentage yields.

-1-(4-(benzyloxy)-3-(-(3-chloro-4-methylphenyl) diazenyl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (3a):

C30H25ClN2O3. m.p. (143-144oC), % yield (88.9%), color (yellow), Rf (0.6), (IR (cm-1) str. 1660 (C=O), 1603 (C=C),1497 (N=N), 1266 (C-O), 1168 (C-N). 1H-NMR (ppm) 8.34 (1H, d, Ar-H3), 8.16 (1H, d, Ar-H16), 8.0 (1H, s, Ar-H7), 7.8 (1H, d, Cβ-H), 7.64 (1H, s, Ar-H20), 7.6 (2H, d, Ar-H24,27), 7.54 (1H, d, Cα-H), 7.49 (1H, d, Ar-H4), 7.28-7.43(5H, m, Ar-H), 6.98 (1H, d, Ar-H17), 6.96 (2H, d, Ar-H24,26), 5.54 (2H, s, Ar- CH2-O), 3.88 (3H, s, O-CH3), 2.49 (3H, s, Ar-CH3). 13C-NMR (ppm) 188.5: C=O, 161.7: C5, 159.3: C25, 152.0: C15, 144.5: Cβ, 141.2: C18, 139.35: C9, 136.3: C19, 135.2: C3, 132.7:C2, 131.6: C17, 131.3: C23,27, 130.3: C11,13, 128.7: C12, 128.1: C12, 127.7: C10,14, 126.96: C6, 122.8: C7, 122.4: C20, 119.21: C16, 119.1: Cα, 117.58: C4, 114.44: C24, 114.4: C26, 71.36: C8, 55.86: C28, 20.25: C21.

1-(4-(benzyloxy)-3-(-(3-chloro-4-methylphenyl) diazenyl)phenyl)-3-(3-nitrophenyl)prop-2-en-1-one (3b):

C29H22ClN3O4, m.p. (146-147oC), % yield (98%), color (Brown-Yellow), Rf (0.55), IR (cm-1) Str. 1665 (C=O), 1606 (C=C), 1494 (N=N), 1249 (C-O), 1170 (C-N). 1H-NMR (ppm) 7.26 (1H, CHCl3), 8.55 (1H, s, Ar-H22), 8.35 (1H, d, Ar-H3), 8.29 (1H, d, Ar-H25), 8.19 (1H, d, Ar-H16), 7.98 (1H, d, Cβ-H), 7.97 (1H, d, Ar-H27), 7.87 (2H, s, Ar-H7,20), 7.85 (1H, dd, Ar-H26), 7.68 (1H, d, Cα-H), 7.55 (1H, d, Ar-H4), 7.28-7.43 (5H, m, Ar-H10,11,12,13,14), 7.28 (1H, d, Ar-H17), 5,47 (2H, s, Ar-CH2-O), 2.50 (3H, s, Ar-CH3). 13C-NMR (ppm) (77.0: CHCl3) 187.8: C=O, 159.8: C5, 151.96: C15, 148.73: C24, 141.52: Cβ, 139.59: C18, 136.71: C22, 136.1: C9, 135.26: C19, 134.37: C27, 132.85: C3, 131.37: C17, 130.67: C2, 130.05: C26, 128.76: C11,13, 128.2: C12, 126.95: C10,14, 124.67: C7, 124.15: C20, 123.8: C6, 122.68: C25, 122.51: C23, 117.78: Cα,16, 114.8: C4, 71.33: C8, 20.3: C21.13C-Dept.135 (ppm) 141.52: Cβ, 134.37: C27, 132.85: C3, 131.37: C17,130.05: C26, 128.76: C11,13, 128.22: C12, 126.95: C10,14, 124.67: C7, 124.15: C20, 122.68: C25, 122.51: C23, 117.78: Cα,16, 114.81: C4, 71.34: C8, 20.3: C21.

Synthesis of new series of pyrazoline derivatives with phenylhydrazine (4a-i):

Method (A) One-pot technique

The pyrazoline derivatives were prepared are (4a-i), a mixture of compound (2) (0.002Mol, 0.76g), substituted benzaldehyde (0.002Mol), 0.5mL ethanolic NaOH (6%), and phenylhydrazine (0.002Mol, 0.22g) was mixed together in 20 mL ethanol and refluxed with stirring for the appropriate time (6hrs.), until completion the reaction which was monitored by TLC, and colour change. After that, the product was poured into a beaker, condensed to 10 mL and cooled after 15min. The solid product was separated by suction filtration, washed several times with cold water and purified with a mixture of Xylene: cold ethanol (1: 10)[32].

3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl)phenyl)-5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4a):

C36H31ClN4O2, m.p. (131-132oC), %yield (99%), Rf (0.6), color (Red-Brown), IR (cm-1) str. 1597 (C=N), 1497 (N=N), 1249 (C-O), 1175 (C-N). 1H-NMR (ppm) 7.97 (1H, d, Ar-H16), 7.94 (1H, s, Ar-H7), 7.86 (1H, d, Ar-H3), 7.79 (1H, 2, Ar-H20), 7.53 (1H, d, Ar-H4), 7.28- 7.48 (5H, m, Ar-H10,11,12,13,14), 7.29 (1H, d, Ar-H17), 7. 25 (2H, d, Ar-H23,27), 7.19 (2H, d, Ar-H30,32), 7.10 (1H, d, Ar-H31), 6.88 (2H, d, Ar-H24,26), 6.80 (2H, d, Ar-H29,33), 5.40 (2H, s, Ar-CH2-O), 5.25 (1H, dd, N-CH34), 3.86, 3.79 (2H, dd, H-C35-H), 3.79 (3H, s, O-CH3), 2.48 (3H, s, Ar-CH3). 13C-NMR (ppm) 158.9: C25, 156.64: C5, 152.14: C1,15, 146.11: C28, 139.05: C18, 136.78: C9, 135.15: C22, 134.6: C19, 131.26: C3,17, 129.76: C30,32, 128.89: C11,13, 127.98: C12, 127.3: C10,14, 126.41: C2,23,27,122.79: C6,20,122.2: C7, 119.0: C16,31, 115.87: C29,33, 113.36: C4, 114.5: C24,26, 71.76: C8, 64.07: C34, 55.29: C36, 43.79: C35, 20.22: C21.

3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl)phenyl)-5-(3-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4b):

C35H28ClN5O3, m.p. (139-140oC), % yield (66%), Rf (0.8), color (Black-Brown), IR (cm-1) str. 1597 (C=N), 1495 (N=N), 1239 (C-O), 1173 (C-N).

3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl)phenyl)-1-phenyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazole (4c):

C36H31ClN4O, m.p. (133-134oC), %yield (73%), Rf (0.79), color (Red), IR (cm-1) Str. 1598 (C=N), 1494 (N=N), 1255 (C-O), 1169 (C-N).

3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl)diazenyl)phenyl)-5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4d):

C35H28Cl2N4O, M.P. (117-118oC), % yield (79%), Rf (0.78), color (Black-Brown), IR (cm-1) str. 1599 (C=N), 1496 (N=N), 1239 (C-O), 1173 (C-N).

3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl)phenyl)-5-(4-(benzyloxy)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4e):

C42H35ClN4O2, m.p. (99-100oC), %yield (60%), Rf (0.76), color (Black-Brown), IR (cm-1) str. 1597 (C=N), 1496 (N=N), 1243 (C-O), 1173 (C-N).1H-NMR (ppm) 7.99 (1H, d, Ar-H16), 7.97 (1H, s, Ar-H7), 7.95 (1H, d, Ar-H3), 7.88 (1H, s, Ar-H20), 7.8 (1H, d, Ar-H4), 7.29-7.55 (5H, m, Ar-H10,11,12,13,14), 7.27 (1H, d, Ar-H17), 7.18 (2H, d, Ar-H23,27), 7.12 (2H, dd, Ar-H30,32), 6.97 (2H, d, Ar-H24,26), 6.95 (1H, dd, Ar-H31), 6.82 (2H, d, Ar-H29,33), 5.4 (4H, s, -CH2-O), 5.05 (1H, dd, N-C34-H), 3.18,3.14 (2H, dd, H-C35-H), 2.49 (3H, s, Ar-CH3). 13C-NMR (ppm) 158.24: C25, 156.66: C5, 152.13: C1, 146.14: C15, 144.88: C28, 142.29: C18, 139.0: C9, 136.92: C37, 136.79: C19,22, 131.3: C3,17, 129.8: C30,32, 128.94: C11,13, 128.6: C 39,41, 127.5: C12,40, 127.08: C10,14,38,42, 126.36: C2, 122.79: C23,27, 122.3: C6,20, 121.97: C7, 119.01: C16,31, 115.8: C29,33, 115.4: C4, 114.31: C24, 113.36: C26, 71.69: C8, 70.06: C36, 64.08: C34, 43,78: C35, 20.27: C21. 13C-Dept. 135 (ppm)131.3: C3,17, 129.8: C30,32, 128.9: C11,13, 128.6: C 39,41, 127.5: C12,40, 127.09: C10,14,38,42, 122.79: C23,27, 122.32: C20, 121.79: C7, 119.0: C16,31, 115.8: C29,33, 115.4: C4, 114.3: C24, 113.36: C26, 71.69: C8, 70.06: C36, 64.08: C34, 43.78: C35, 20.27: C21.

3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl)phenyl)-5-(3-((4-chlorobenzyl)oxy)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4f):

C42H34Cl2N4O2, m.p. (120-121oC), %yield (55%), Rf (0.7), color (Yellow-Orange), IR (cm-1) str. 1597 (C=N), 1495 (N=N), 1262 (C-O), 1157 (C-N).

(E)-4-(3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-N,N-dimethylaniline (4g):

C37H34ClN5O, m.p. (127-128oC), %yield (62%), Rf (0.6), color (Black-Brown), IR (cm-1) str. 1597 (C=N), 1489 (N=N), 1265 (C-O), 1157 (C-N).

3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl)phenyl)-5-(3-(benzyloxy)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4h):

C42H35ClN4O2, m.p. (107-108 oC), % yield (59%), Rf (0.7), color (Brown), IR (cm-1) str. 1596 (C=N), 1496 (N=N), 1260 (C-N), 1156 (C-O). 1H-NMR (ppm) 7.97 (1H, d, Ar-H16), 7.86 (1H, s, Ar-H7), 7.81 (1H, d, Ar-H3), 7.68 (1H, s, Ar-H20), 7.55 (1H, d, Ar-H4),7.28- 7.38 (10H, m, Ar-H10,11,12,13,14,38,39,40,41,42), 7.2 (2H, d, Ar-H17,26), 7.10 (2H, d, Ar-H30,32), 7.04 (1H, 3, Ar-H23), 6.97 (1H, m, Ar-H31), 6.95 (1H, d, Ar-H25), 6.90 (1H, d, Ar-H27), 6.82 (2H, d, Ar-H29,33), 5.41, 5.51 (4H, s, Ar-CH2-O), 5.03 (1H, dd, N-C34-H), 3.60, 3.87 (2H, dd, H-C35-H), 2.49 (3H, s, Ar-CH3).13C-NMR (ppm) 159.46: C24, 156.67: C5, 152.12: C1, 146.16: C15, 144.91: C22, 144.36: C28, 142.3: C18, 139.9: C9, 136.97: C37, 135.16: C19, 131.29: C3, 130.29: C17, 129.82: C26,30,32, 128.95: C11,13, 128.5: C 39,41, 127.66: C12,40, 127.06: C10,14,38,42, 126.26: C2, 122.78: C6, 122.31: C7,20, 119.09: C16,31, 118.39: C27, 115.81: C29, 114.37: C33, 113.87: C4, 113.31: C23, 112.26: C25, 71.6: C8, 70.01: C36, 64.59: C34, 43.72: C35, 20.26: C21. 13C-Dept. 135 (ppm) 131.29: C3, 130.29: C17, 129.82: C26,30,32, 128.95: C11,13, 128.5: C39,41, 127.66: C12,40, 127.06: C10.14.38,42, 122.31: C7,20, 119.09: C16.31, 118.39: C27, 115.81: C29, 114.37: C33, 113.87: C4, 113.31: C23, 112.26: C25, 71.06: C8, 70.01: C36, 64.59: C34, 43.72: C35, 20,26: C21.

3-(4-(benzyloxy)-3-((3-chloro-4-methylphenyl) diazenyl)phenyl)-5-(3-bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (4i):

C35H28BrClN4O, m.p. (104-105oC), %yield (78%), Rf (0.8), color (Brown), IR (cm-1) str. 1597 (C=N), 1497 (N=N), 1274 (C-O), 1160 (C-N). 1H-NMR (ppm) 7.97 (1H, d, Ar-H16), 7.94 (1H, s, Ar-H7), 7.85 (1H, d, Ar-H3), 7.81 (1H, s, Ar-H20), 7.53 (1H, d, Ar-H4), 7.41 (1H, d, Ar-H25), 7.29-7.42 (5H, m, Ar-H10,11,12,13,14), 7.29 (1H, d, Ar-H27), 7.23 (1H, dd, Ar-H26), 7.22 (1H, d, Ar-H17), 7.07 (2H, dd, Ar-H30,32), 6.85 (1H, m, Ar-H31), 6.62 (2H, d, Ar-H29,33), 5.4 (2H, s, Ar-CH2-O), 5.21 (1H, dd, N-C34-H), 3.17,3.88 (2H, dd, H-C35-H), 2.44 (3H, s, Ar-CH3). 13C-NMR (ppm) 156.78: C5, 152.1: C1,15, 146.17: C22, 144.9: C28, 139.14: C18, 136.7: C9, 135.16: C19, 131.3: C3,23, 130.8: C17, 129.8: C25,30,32, 129.0: C26, 128.6: C11,13, 128.02: C12, 127.05: C10,14, 125.96: C2, 124.51: C27, 123.24: C6, 122.82: C20, 122.29: C7,24, 119.37: C16,31, 115.8: C29,33, 114.39: C4, 71.67: C8, 64.0: C34, 43.67: C35, 20.27: C21.13C-Dept. 135 (ppm) 131.3: C3,23, 130.8: C17, 129.8: C25,30,32, 129.0: C26, 128.56: C11,13, 128.02: C12, 127.05: C10,14, 124.51: C27, 122.82: C20, 122.29: C7, 119.35: C16,31, 115.8: C29,33, 114.39: C4, 71.69: C8, 65.3: C34, 43.65: C35, 20.27: C21.

Method (B) Traditional technique

In the traditional method, chalcone derivatives (0.001 Mol), phenylhydrazine (0.11g, 0.001Mol), and

0.5mL of ethanolic NaOH 6% were mixed together in 15 mL ethanol and refluxed with stirring for (6hrs) until completion the reaction which was monitored by TLC, and colour change. The product was poured into a beaker, condensed to 10 mL and cooled after 15min. The cold solid product was separated by suction filtration, washed several times with cold water and recrystallized with ethanol [3] to give pyrazoline (4a).

Antimicrobial activity patterns of some of the synthesized pyrazoline derivatives against S. aureus as gram-positive and E. coli as gram-negative.

The antibacterial activity was performed on Escherichia coli as gram-negative and Staphylococcus aureus bacteria as gram-positive with different concentrations (200, 400, 600, 800 ppm) of pyrazoline derivatives (4a-i) were selected and prepared in (DMSO 99%) as solvent. Different methods for the preparation of the inoculum can be used [33]: (A) the Colony suspension method, (B) the Growth method, and (C) the Growth method using overnight cultures. The MIC. of the compounds used was calculated using a spectrophotometer at 600nm, and the following dilutions for each compound were ordered. The MIC for the synthesized target compounds that inhibited bacterial growth was calculated and compared to a control sample made up of 2mL of nutritional broth and 40μL of activated bacterial suspension, which was incubated at 37°C for 20 hours [34]. The optical density (OD) was reported the next day, as indicated in Table 1,2.

Results and Discussion

Spectroscopic Characterization:

The present work involves the synthesis of two new chalcones (3a and 3b) from the reaction of benzyloxy and substituted aldehyde, followed by their transformation to new pyrazoline derivatives, using two methods like one pot and traditional techniques. Only two pyrazolines (3a and 3b) were synthesized by the classical method. All the others are synthesized by the one-pot method because this method is more straightforward, requires less time consuming, in addition, has more yield, and has more accuracy.

The results of both methods are nearly the same and show that they produce relatively high yields. Synthesized compounds were confirmed on the basis of their spectral data (FT-IR, ^{1}H-NMR, ^{13}C-NMR, and ^{13}C-NMR Dept.135).

In azo compound (1) I.R. spectrum showed: a broad absorption band at (3421cm^{-1}) of the hydroxyl group and a strong carbonyl group absorption band at (1672cm^{-1}). ^{1}H-NMR spectrum refers to the present 13.15 ppm (s) of hydroxyl group and 2.49 ppm (s) of Ar-CH_3. ^{13}C-NMR showed a distinct signal for each type of carbon 26.36ppm for the methyl group of -$COCH_3$, 20.38ppm for Ar-CH_3 and thirteen singlet signals for aromatic and carbonyl groups.

While the IR spectrum of intermediate benzyloxy compound (2) shows a strong carbonyl group absorption band appearing at (1675 cm^{-1}). ^{1}H-NMR spectra at 13.15ppm (s) of hydroxyl group disappeared due to elimination of hydrogen atom with protecting group, although it is referring to the present 5.42ppm (s) of Ar-CH_2-O. ^{13}C-NMR a singlet peak at 71.2ppm of Ar-CH_2-O, 26.52ppm of CO-CH_3, and 20.25ppm of Ar-CH_3.

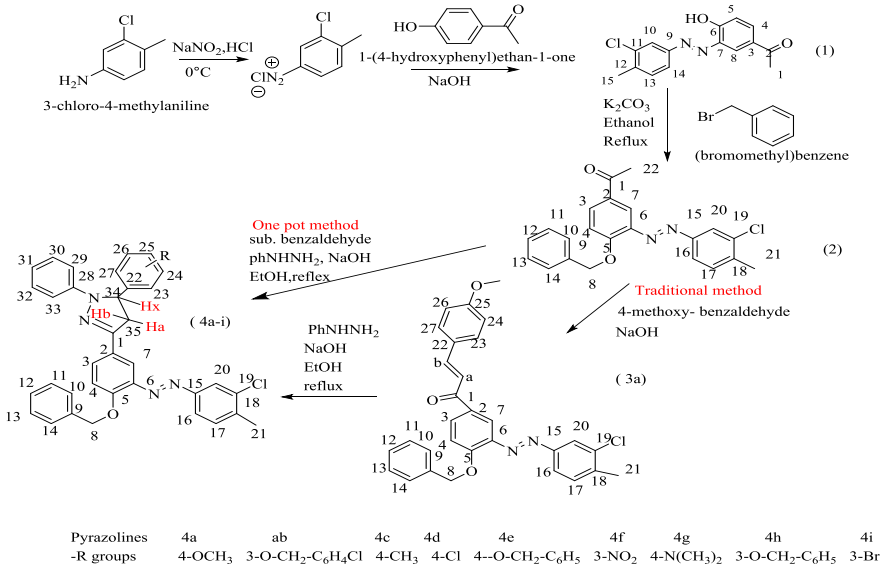
Although in Chalcone compounds (3b) carbonyl group absorption band shift around (1650-1660cm^{-1}), strong band of carbon-carbon double bond appear around (1590-1605cm^{-1}), which leads to present enolate form between them (Figure 1). The ^{1}H-NMR spectra of Chalcone derivatives (3a, 3b) show signals of protons attached to (Cβ, Cα) carbon atoms; For 3a are 7.84 ppm (d), 7.54 ppm (d), and for 3b are 7.98 ppm (d), 7.68 ppm (d) (Figure 2). ^{13}C-NMR spectra of Chalcone derivative (3a,3b) show signals; for 3a are (Cα: 119.2ppm, Cβ: 144.5ppm), and for 3b are (Cα:117.78ppm, Cβ:141.52 ppm) (Figure 3), sometimes Cβ resonance is lower than Cα.

The pyrazoline derivatives, the disappearance of carbonyl band at (1660-1710cm^{-1}) (Figure 4), and a new absorption band of (C=N) appeared around (1496-1497cm^{-1}), it is good evidence for the formation of five-membered ring heterocyclic compound. Also, the ^{1}H-NMR spectra of the pyrazoline derivative ring show signals of proton attached to (C_{34}, C_{35}) carbon atoms in pyrazolines (**4a, 4e**) which appeared three signals; For 4a are 5.25ppm (dd), 3.86ppm (dd), 3.79ppm (dd), and for 4e are 5.40 ppm (dd), 3.18 ppm (dd), 3.14 ppm (dd), one of them vicinal and the another two are geminals (Figure 5). The ^{13}C-NMR spectra of the pyrazoline ring of (4a, 4e) show two signals: for 4a are (C_{34}: 60.07ppm, C_{35}: 43.79ppm), and for 4e are (C_{34}: 63.08 ppm, C_{35}: 43.78 ppm) (Figure 6), these two signals were downward if compared with signals of the same carbon atoms in chalcone derivatives. It is another evidence of the formation of five-membered ring heterocyclic compounds. DEPT-135 spectra were used to distinguish between primary, secondary, tertiary, and quaternary carbons.

Antimicrobial activity

Because they compromise the ability to assess the concentration of the tested antibacterial agent in the broth medium microdilution, dilution procedures are the best appropriate approach for estimating M.I.C. values. To quantify the antimicrobial effects of bacteria and microorganisms in vitro, both broth and agar dilution methods can be used. The minimum concentration of the tested antimicrobial agent that stops the observable growth of the tested microorganisms is known as the MIC value. The

antibacterial evaluation for some of the synthesized target compounds was carried out using by microtiter method. The findings of this study revealed that tested compounds (4a-i) had increased biological activity at 200 µg/mL against both *S. aureus* and *E. Coli* as gram-positive and gram-negative bacteria, while they are less active in 400 µg/mL and 600 µg/Ml, reach N.G. at 800 µg/mL (Table 1,2). As a result, the produced chemicals have been found to have high biological activity, especially (4i) for both bacteria



Scheme 1. Synthesis route of Pyrazoline derivatives (4a-i).

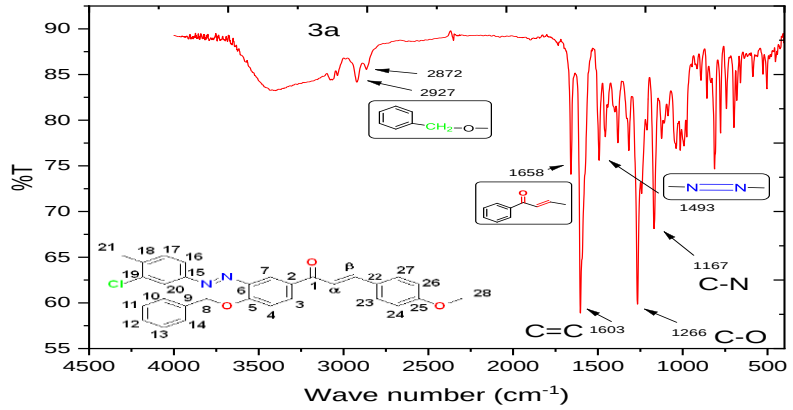


Figure 1. IR Spectrum of Chalcone derivative (3a).

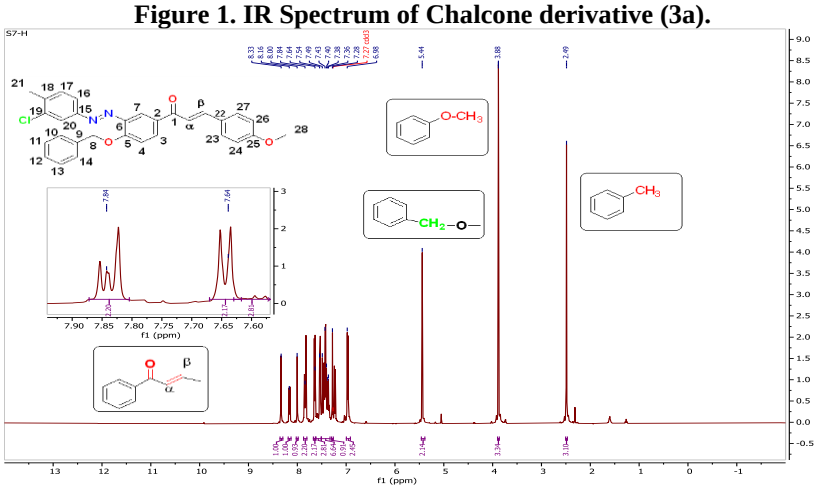


Figure 2. ^{1}H-NMR of Chalcone derivative (3a).

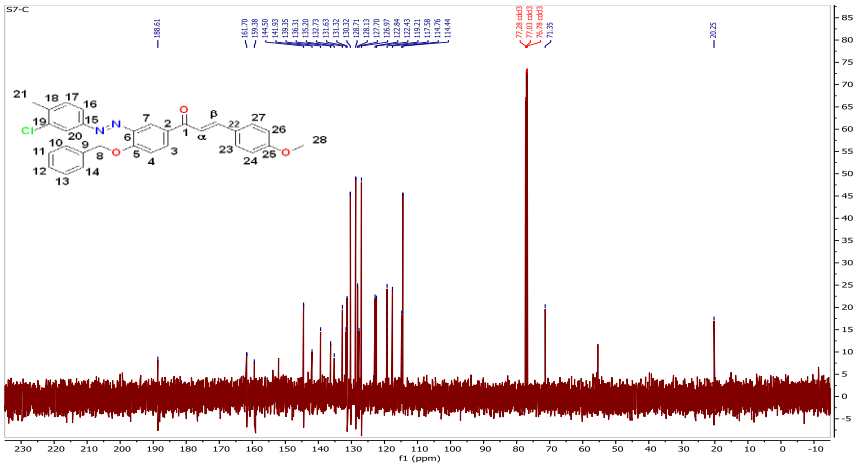


Figure 3. ^{13}C-NMR of Chalcone derivative (3a).

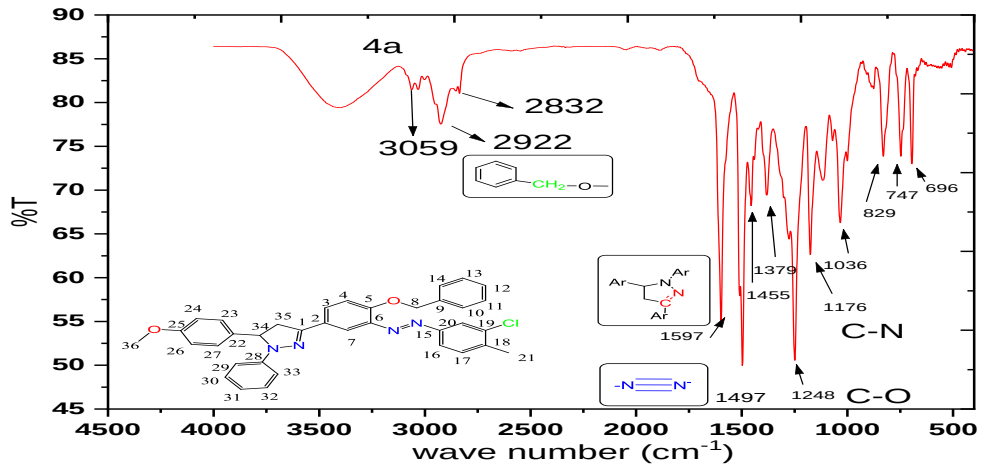


Figure 4. I.R. spectrum of Pyrazoline derivative (4a).

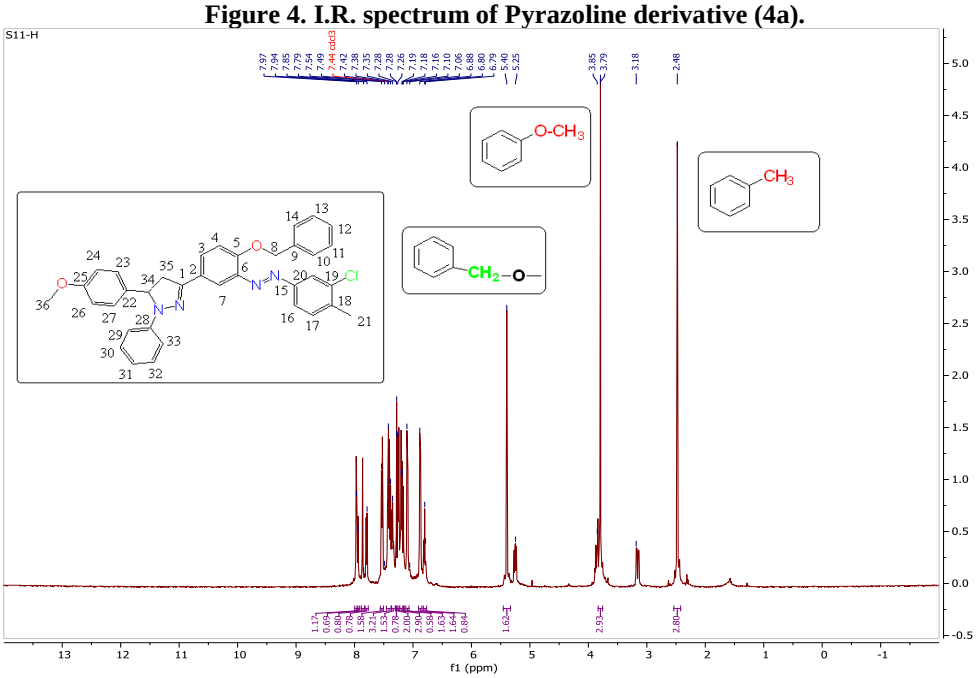


Figure 5. ^{1}H-NMR of Pyrazoline derivatives (4a).

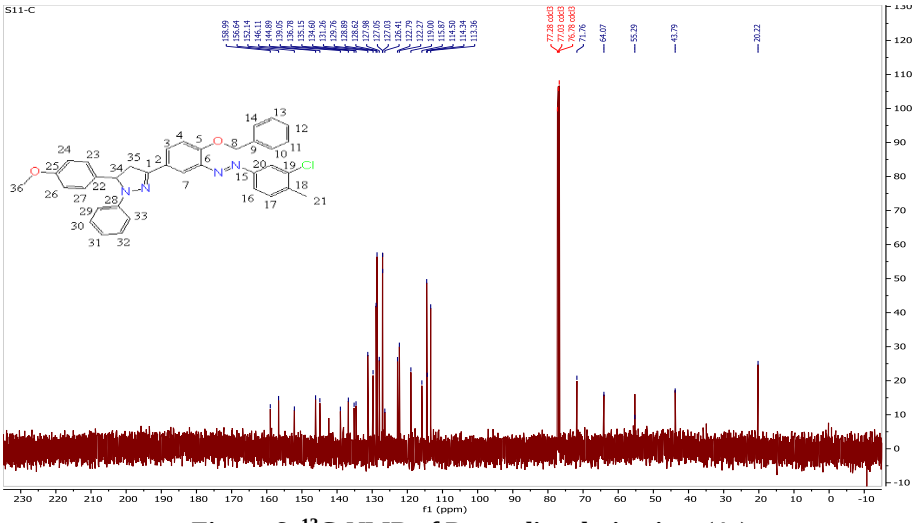


Figure 6. ^{13}C-NMR of Pyrazoline derivatives (4a).

Table 1. Positive control OD=0.982 for synthesized pyrazoline (4a-i), with deferent concentration against *E. coli* as gram-negative. Std.= Metronidazole

Concentration	4a	4b	4c	4d	4e	4f	4g	4h	4i	Std.
200	1.884	1.173	1.396	1.423	1.048	1.902	1.482	1.123	0.791	1.374
400	1.232	0.426	NG	NG	0.037	0.926	NG	0.159	0.311	0.018
600	0.242	0.08	NG	NG	NG	0.159	NG	0.003	0.742	NG
800	NG	NG	NG	NG	NG	NG	NG	NG	0.134	NG

Table 2. Positive control OD=1.026 for synthesized pyrazolines(4a-i), with deferent concentration against S. aureus as gram-positive. Std.= Metronidazole

Concentration	4a	4b	4c	4d	4e	4f	4g	4h	4i	Std.
200	1.803	0.908	1.314	1.013	1.247	1.534	0.861	1.285	0.987	1.536
400	1.07	0.396	NG	0.314	0.104	0.708	NG	0.473	0.393	0.01
600	0.239	NG	NG	NG	NG	0.247	NG	NG	0.978	NG
800	NG	NG	NG	NG	NG	NG	NG	NG	0.158	NG

NG: no Bacterial growth. The MIC is the minimum availability of antibacterial agent that totally stops the growth of any organism. The concentration of pyrazoline derivatives (µg/mL) (conc. vs abs.)

Conclusions

The results obtained in this research show that one-pot multi-component synthesis is very useful for the synthesis of pyrazoline derivatives because it is: Less time consumed, low cost, low waste, more yield, and more accuracy. The Claisen-Schmidt condensation reaction between benzyloxy compound (2) substituted benzaldehydes to the preparation of chalcones and their transformation to pyrazolines was carried out successfully. The newly synthesized pyrazoline showed a remarkable inhibition effect on both grams of positive and negative Bacteria.

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Conflict of Interest

There is no conflict of interest

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التخليق ودراسة النشاطات المضادة للبكتريا لبعض المشتقات بايرازولين الجديدة من خلال الطريقة الكلاسيكية وتفاعل ثلاث مكونات في قارورة واحدة

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في هذا العمل ، تم تصنيع سلسلة جديدة من مركبات البيرازولين التي تحتوي على رابطة الآزو من خلال تفاعلات كلاسيكية وتفاعلات القدر الواحد مكونة من ثلاثة مكونات. بدأ التفاعل بـ 3-كلورو ، 4-ميثيل أنيلين الذي تم ديازوتيزته ومقترن بـ 4-هيدروكسي أسيتوفينون لتكوين مركب آزو 1-((3-كلورو-4-ميثيل فينيل) ديازينيل) -4-هيدروكسي فينيل) إيثان -1-ئيثانول (1) وتحول بـ 1- (4- (بنزيلوكسي) -3 - ((3-كلورو-4-ميثيل فينيل) ديازينيل) فينيل) إيثان-1-وان (2). تم تعريض مركب الازو المحضر لكل من الطريقة الكلاسيكية وطريقة القدر الواحد لإعطاء جزيئات البيرازولين المستهدفة. تم دعم بنية جميع المركبات الجديدة التي تم الحصول عليها بواسطة البيانات الطيفية (^{1}H-NMR و ^{13}CNMR و Dept.135 و FT-IR). أخيرًا ، قدرت مشتقات البيرازولين نشاطها المضاد للبكتيريا ضد *Escherichia coli* باعتبارها سالبة الجرام و *Staphylococcus aureus* موجبة الجرام ، وأظهرت النتائج نشاطًا معنويًا ضد كلا النوعين من البكتيريا.