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RESEARCH PAPER

A stepwise synthetic approach and antibacterial assessment of some new pyrazoline derivatives containing azo and benzyloxy moieties

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A B S T R A C T:

This study has designed an efficient stepwise synthesis to produce some new pyrazoline derivatives that contains azo and benzyloxy linkages. The Synthesis route was initiated by diazotizing p-aminocetophenone with 2,6-dichlorophenol to give azo compound (1), which was then reacted with benzyl bromide producing the efficacious core material azo-benzyloxy acetophenone (2). Moreover, compound (2) was treated with a series of substituted benzaldehydes to form intermediate chalcone derivatives (3a-j) which were then treated with phenylhydrazine via Michael type addition reaction to form azo-benzyloxy pyrazoline molecules (4a-j). Ultimately, the structures of the synthesized compounds were revealed using spectroscopic techniques: FT-IR, ^{1}H-NMR, ^{13}C-NMR, and ^{13}C-DEPT-135 spectra. Antimicrobial activities were screened for synthezied Azo-chalcones and Pyrazolines against S.aureus as gram-positive and E.Coli as gram-negative in comparison to a standard antibiotic drug Azithromycine.

KEY WORDS: Diazotization, Benzylation, Chalcones, Pyrazoline, Antibacterial.
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1.INTRODUCTION :

During the past few decades, diseases have become more prevalent in society due to pollution and the resistance of microorganisms and bacteria to existing medicines. The synthesis of heterocyclic compounds is one of the most crucial solutions to these problems. Since they are safer, have higher antibacterial activity, and are less dangerous compared to those already on the markets. Azo compounds are crucial in the creation of new organic compounds (Hawaiz *et al* , 2014). They contain a (-N=N-) group linked to two aromatic rings.

More than 60% of the synthesized azo dyes are produced by an electrophilic aromatic substitution reaction between diazonium salts with a coupling agent (Mallikarjuna and Keshavayya, 2020). Azo--chalcone, azo-imine, and azo-pyrazoline derivatives are produced by using azo-linkage as a precursor.(Kmal and Behget, 2018).

Chalcones are α-β unsaturated carbonyl system joined with two aromatic rings. (Aboelnaga *et al.*, 2021). Chalcones are existed in the nature (as flavonoids family) (Ahmad *et al.*, 2016; Ngameni *et al.*, 2021), and can be synthesized by several methods such as, *Claisen-Schmidt condensation* reaction between aromatic ketones and aromatic aldehydes which catalyzed by base or acid, and take place under solvent-free condition (Khorsheed *et al.*, 2020). Chalcones use as a starting material in the synthesis of pyridines, thiazepines, isoxazoles, pyrazoles, and pyrazoline

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derivatives (Bonakdar *et al.*, 2020). Additionally, they display a variety of biological activities such as antioxidants, antifungals, and antimicrobial actions (A. Ebraheem, 2013).

The heterocyclic ring compounds usually contain two or more different elements, oxygen (o), nitrogen (n), and sulfur (s) (Abd El-Sattar *et al.*, 2018; Asad *et al.*, 2021). Pyrazolines are one of the most important rings as heterocyclic compounds, consist of a five-membered ring with two connected nitrogen atoms and an endo cyclic double bond (Jain *et al.*, 2013). Pyrazolines were synthesized via various methods such as cyclization of chalcone derivatives with hydrazine hydrate, phenyl hydrazine, thiosemicarbazide, and thiourea, in dimethylsulfoxide or dimethylformamide, also by using ultrasonic irradiation, and one-pot methods (Trilleras *et al.*, 2013). According to recent reviews, pyrazoline derivatives possess the activities against bacteria (Jainey and Bhat, 2012), inflammation (Yusuf and Jain, 2014), fungi (Rostom *et al.*, 2011), and oxidant (Hayat *et al.*, 2010).

2. MATERIALS AND METHODS

2.1. Experimental Notes:

Electro-thermal melting point apparatus used to determine melting points. Using KBr disc to record infrared spectra with FT-IR spectrometer SHIMADZU, Mod IR Affinity-1. ^{1}H-NMR, ^{13}C-NMR, and^{13}C-DEPT-135 spectra were recorded in Iran on a Bruker (400MHz) with TMS as an internal reference using Chloroform as solvent.

2.2. Preparation of Staring Materials:

2.2.1.Synthesis of 1-(4-((3,5-dichloro-4 -hydroxyphenyl)diazenyl)phenyl)ethan-1-one (1):(Hawaiz, et al 2014)

Step.1 Diazonium salt solution preparation

p-Aminoacetophenone (6.75gm, 50 mmol) was dissolved in HCl (3M, 42 mL) under heating, then the solution was cooled down in an ice bath to (0 °C). Freshly prepared $NaNO_2$ (3.5 gm, 50 mL) solution was added slowly to the solution in which the temperature controlled below (10 C°).

Step .2 Coupling reaction

2, 6-dichlorophenol (8.15gm, 50mmol) was dissolved in NaOH (100mL, %4). The solution was cooled down in an ice bath with continuous stirring and added slowly to the diazonium salt solution (step 1), with in gently stirring for 20 minutes at (0°C) until precipitation appear. The solid azo dye compound (1) was collected by vacuum filtrations, washed several times with water, dried, and recrystallized from absolute ethanol to give (red-orange) crystals: Mp. 168-170 °C, RF: 0.75, yield : 97%, FT-IR(cm^{-1}): 3178(OH), 1672(C=O), 1598(C=C), 1267(C-O).
^{1}H-NMR (δ/ ppm): 2.70 (s, 3H, CH_3), 6.31 (s, 1H, OH), 7.96 (d, 2H, Ar-H $_{3, 5}$), 7.99 (s, 2H, H $_{8, 12}$), 8.13 (d, 2H, $H_{2, 6}$). **^{13}C-NMR (δ/ ppm):** 26.90 (CH_3), 121.89 ($C_{9, 11}$), 123.01 ($C_{8,12}$), 123.59 ($C_{3,5}$), 129.46 ($C_{2,6}$), 138.60 (C_1), 146.03 (C_7), 150.49 (C_{10}), 154.51 (C_4), 197.48 (C=O).**^{13}C-DEPT-135:** 26.90(Ar-CH_3), 123.01($C_{8,12}$), 123.59($C_{3,5}$), 129.45($C_{2,6}$).

2.2.2. Synthesis of 1-(4-((4-(benzyloxy)-3,5-dichlorophenyl)diazenyl)phenyl)ethan-1-one (2): (Marlin *et al..*, 2017)

Compound (1) (10gm, 32.36 mmol), benzyl bromide (11gm, 64.31mmol), and anhydrous K_2CO_3 (5.5gm, 43mmol), in ethanol (30mL, 99%) was refluxed for 12hrs, then the solution cooled down and poured into crushed ice. The precipitate was filtered off, washed several times with cold water, dried, and recrystallized from absolute ethanol to give light-orange crystals of compound (2). Yield: 94.96%, Mp.148-150 °C, RF: 0.8, FT-IR (cm^{-1}): 1680(C=O), 1598(C=C), 1259(C-O).

^{1}H-NMR (δ/ ppm): 2.71 (s, 3H, $COCH_3$), 5.18 (s, 2H, OCH_2), 7.40-7.65 (m, 5H, Ar-$H_{15,16,17,18,19}$), 7.96 (d, 2H, Ar-H $H_{3, 5}$), 7.99 (s, 2H, Ar-$H_{8, 12}$), 8.13 (d, 2H, $H_{2, 6}$). **^{13}C-NMR (δ/ ppm):** 26.92 (CH_3), 75.27 (OCH_2, C_{13}), 123.17 ($C_{8, 12}$), 123.82 ($C_{3, 5}$), 128.59 ($C_{2, 6}$), 128.63 ($C_{15, 19}$), 128.67 (C_{17}), 129.46 ($C_{16, 18}$), 130.62 ($C_{9, 11}$), 135.88 (C_{14}), 138.90 (C_1), 148.65 (C_7), 153.90 (C_{10}), 154.45 (C_4), 197.38 (C=O). **^{13}C-DEPT-135**: 26.92(Ar-CH_3), -75.27(OCH_2, C_{13}), 123.17($C_{8, 12}$), 123.82($C_{3, 5}$), 128.57($C_{2, 6}$), 128.63($C_{15, 19}$), 128.67(C_{17}), 129.46($C_{16, 18}$).

2.3. Synthesis of chalcones: 1-(4-(4-benzyloxy-3,5-di chlorophenyl)diazenyl) phenyl)-3-(substituted phenyl)prop-2-en-1-one (3a-ȷ):(Mahmoud, 2019)

A combination of compound **(2)** 0.8gm (2 mmol), and substituted benzaldehydes (4mmol) was dissolved in absolute ethanol (50 mL), then slowly added alcoholic sodium hydroxide 4% (4mL) and stirred for (20-30 minutes), until the reaction completed which was monitored by color changing, the formation of precipitate and by TLC using n-hexane: ethyl acetate (70:30 as eluent) .Then the mixture was directly filtered off, dried, and washed several times with water and ethanol. The product was recrystallized from toluene to give (yellow-brown) colored products. The physical properties are outlined in Table (I).

3b: ^{1}H-NMR (δ/ ppm): 2.44 (s, 3H, Ar-CH_3-C_{26}), 5.21 (s, 2H,-O-CH_2-C_{13}), 6.89-8.20 (m, 17H,Ar-H and 2H CH-α and CH-β). **^{13}C-NMR (δ/ ppm):** 21.61 (CH_3), 75.22 (O-CH2-C_{13}), 120.81 (CHα), 123.22 ($C_{8,12}$), 123.81 ($C_{3,5}$), 128.58 (C_{17}), 128.63 ($C_{15,19}$), 128.65 ($C_{2,6,22,24}$), 129.59 ($C_{16,18}$), 129.81 ($C_{21,25}$), 130.61 ($C_{9,11}$), 131.99 (C_{20}), 135.90 (C_{14}), 140,45 (C_{23}), 141.47 (C_1), 145.68 (Cβ), 148.71 (C_7), 153.45 (C_{10}), 154.20 (C_4), 189.9 (C=O). **^{13}C-DEPT-135:** 21.61 (CH_3), -75.22(O-CH_2-C_{13}), 120.81(CHα), 123.22($C_{8,\ 12}$), 123.81($C_{3,5}$), 128.58(C_{17}), 128.63($C_{15,19}$), 128.65($C_{2,6,22,24}$), 129.59($C_{16,18}$), 129.81($C_{21,25}$), 145.68(CHβ).

3c: ^{1}H-NMR (δ/ ppm): 3.90 (s, 3H,–O-CH_3-C_{26}), 5.18 (s, 2H,-O-CH_2-C_{13}), 6.99-8.13 (m, 15H, Ar-H and 2H CH-α and CH-β). **^{13}C-NMR (δ/ ppm):** 55.48 (OCH_3), 75.27 (O-CH_2-C_{13}), 114.51 ($C_{22,24}$), 119.48 (CH_α), 123.21 ($C_{8,12}$), 123.81 ($C_{3,5}$), 127.45 ($C_{9,11}$), 128.59 ($C_{2,6}$), 128.63 ($C_{15,19}$), 128.66 (C_{17}), 129.53 ($C_{16,18}$), 130.45 ($C_{21,25}$), 130.61 (C_{14}), 135.90 (C_1), 145.46 (C_β), 148.71 (C_7), 153.42 (C_{10}), 154.14 (C_4), 161.93 (C_{23}), 189.83 (C=O). **^{13}C-DEPT-135**: 55.48(CH_3), -75.27(OCH_2-C_{13}), 114.51($C_{22,\ 24}$), 119.48(CH_α), 123.21($C_{8,12}$), 123.81($C_{3,5}$), 128.59($C_{2,6}$), 128.63($C_{15,19}$), 128.66(C_{17}), 129.53($C_{16,18}$), 130.45($C_{21,25}$), 145.46(CH_β).

3d: ^{1}H-NMR (δ/ ppm): 4.93 (s, 2H, -O-CH_2-C_{13}), 6.91-7.95 (m,17H,Ar-H and 2H of CH-α and CH-β).**^{13}C-NMR (δ/ ppm):** 75.27 (O-CH_2-C_{13}), 116.37 ($C_{22,24}$), 121.51 (Cα), 123.26 ($C_{8,12}$), 123.83 ($C_{3,5}$), 128.58 ($C_{2,6}$), 128.62 ($C_{15,19}$), 128.66 (C_{17}), 129.60 ($C_{16,18}$), 130.48 ($C_{21,25}$), 130.63 ($C_{9,11}$), 131 (C_{20}), 135.89 (C_{14}), 140.16 (C_1), 144.20 (Cβ), 148.68 (C_7), 153.51 (C_{10}), 154.29 (C_4), 162.99 (C_{23}), 189.47 (C=O). **^{13}C-DEPT-135:** -75.27(OCH_2-C_{13}), 116.37($C_{22,\ 24}$), 121.51(Cα), 123.26($C_{8,12}$), 123.83($C_{3,5}$),128.58($C_{2,6}$), 128.62($C_{15,19}$), 128.66(C_{17}), 129.60($C_{16,18}$), 130.48($C_{21,25}$), 145.1(Cβ).

3e: ^{1}H-NMR (δ/ ppm): 4.93 (s, 2H, -O-CH_2-C_{13}), 7.04-7.95 (m, 17H, Ar-H and 2H of CH-α and CH-β). **^{13}C-NMR (δ/ ppm):** 75.27 (O-CH_2-C_{13}), 122.19 (Cα), 123.27 ($C_{8,12}$), 123.84 ($C_{3,5}$), 128.58 ($C_{2,6}$), 128,62 ($C_{15,19}$), 128,66 (C_{17}), 129.30 ($C_{22,24}$), 129.62 ($C_{16,18}$), 129.72 ($C_{21,25}$), 130.64 ($C_{9,11}$), 133.21 (C_{20}), 135.88 (C_{14}), 136.76 (C_{23}), 140 (C_1), 144 (Cβ), 148.69 (C_7), 153.52 (C_{10}), 154.34 (C_4), 189.40 (C=O). **^{13}C-DEPT-135:** -75.27(OCH_2-C_{13}), 122.19(Cα), 123.27($C_{8,\ 12}$), 123.84($C_{\ 3,5}$), 128.58($C_{2,6}$), 128.62($C_{15,19}$), 128.66(C_{17}), 129.30($C_{22,24}$), 129.62($C_{16,18}$), 129.72($C_{21,25}$), 144(Cβ).

3f: ^{1}H-NMR (δ/ ppm): 4.99 (s, H, -O-CH_2-C_{13}), 5.05 (s, 2H, -O-CH_2-C_{26}), 6.90-8.08 (m, 21H, Ar-H and 2H of CH-α and CH-β). **^{13}C-NMR (δ/ ppm):** 69.1 (OCH_2-C_{13}), 75.23 (OCH_2-C_{26}), 115.33 ($C_{22,24}$), 119.71(CHα), 123.22 ($C_{8,12}$), 123.81 ($C_{3,5}$), 127.88 ($C_{9,11}$), 128.59 ($C_{2,6}$), 128.63 ($C_{15,19}$), 128.67 (C_{17}), 128.82 ($C_{28,32}$), 128.91 ($C_{29,31}$), 129.54 ($C_{16,18}$), 130.47 ($C_{21,25}$), 130.60 (C_{20}), 134(C_{30}), 134.84 (C_{27}), 135.90 (C_{14}), 140.55 (C_1), 145.23 (CHβ), 148.70 (C_7), 153.44 (C_{10}), 154.15 (C_{23}), 160.75 (C_4), 189.63 (C=O). **^{13}C-DEPT-135:** -69.10(C_{13}), -75.23(C_{26}), 115.33($C_{22,24}$), 119.71(Cα), 123.22($C_{8,12}$), 123.81($C_{3,5}$), 128.59($C_{2,6}$), 128.63($C_{15,19}$), 128.67(C_{17}), 128.82($C_{28,32}$), 128.91($C_{29,31}$), 129.54($C_{16,18}$), 130.47($C_{21,25}$), 145.23(Cβ).

Table I: Some physical properties and IR data for the synthesized Chalcones (3a-j).

Prod.	**R**	**C=O str. cm^{-1}**	**C=C str. cm^{-1}**	**Yield %**	**M.P./°C**	**RF**
3a	H	1655	1606	90	168-170	0.93
3b	4-CH_3	1657	1608	70	182-183	0.7
3c	4-OCH_3	1652	1595	81.52	145-147	0.70
3d	4-F	1654	1600	83	178-180	0.97
3e	4-Cl	1654	1600	81.6	165-	0.96

					167	
3f	4-(p-Cl-Bz)	1656	1595	80	190-191	0.77
3g	2-Cl	1657	1594	82	183-185	0.82
3h	4-NO_2	1660	1606	86	159-161	0.96
3i	4-Bz	1655	1599	85	152-154	0.94
3j	2-F	1652	1596	85	155-157	0.85

Note: Bz = benzyloxy compound.

2.4. Synthesis of 3-(4-((4-(benzyloxy)-3,5-dichlorophenyl)diazenyl)phenyl)-5-aryl-1-phenyl-2-pyrazolines(4a-j):

(Guo *et al.*, 2008)

The mixture of chalcone derivatives (3a-j) (1mmol) with phenyl hydrazine 0.21 gm (2 mmole), were dissolved in absolute ethanol (30 mL) and slowly added alcoholic sodium hydroxide 4% (2mL) as a catalyst , then refluxed for (9 hrs.). The completion of the reaction was monitored by TLC using n-hexane: ethylacetate (70:30 as eluent)), and in treating with H_2SO_4 give a green color. The precipitate was dried and recrystallized from toluene. The physical properties of the prepared azo-pyrazoline (4a-j) are summarized in table. 2

4c: ^{1}H-NMR (δ/ ppm): 3.19 (dd, 1H, CH_2-Ha), 3.87 (dd, 1H, CH_2-Hb), 3.88 (s, 3H –OCH_3 C_{23}), 5.16 (s, 2H,-O-CH_2-C_{13}), 5.35 (dd, 1H, CH-Hx), 6.85-7.97 (m, 20H, Ar-H). **^{13}C-NMR (δ/ ppm:** 43.25 (CH_2 of pyra.), 55.31 (OCH_3), 64.16 (CH of pyra.), 75.27 (O-CH_2- C_{13}), 113.64 ($C_{22,24}$). 114.57 ($C_{27,31}$), 119.65 (C_{29}), 123.49 ($_{8,12}$), 123.59 ($C_{3,5}$), 126.32 ($C_{21,25}$), 127.0 ($C_{16,18}$), 128.57 ($C_{2,6}$), 128.61 ($C_{15,19}$), 128.63 (C_{17}), 128.99 ($C_{28,30}$), 130.46 (C9,11), 134.22 (C_{20}), 136 (C_{14}), 136.13

(C_1), 144.13 (C_{26}), 145.45 (C_7), 148.96 (C_{10}), 151.63 (C_4), 152.78 (C=N), 159.08 (C_{23}). **^{13}C-DEPT-135**: -43.33(CH_2.pyra.), 55.31(OCH_3), 64.16(CH pyra.), -75.17(OCH_2), 113.63($C_{22,24}$), 114.57($C_{27,31}$), 119.65(C_{29}), 123.49($C_{8,12}$), 123.59($C_{3,5}$), 126.32($C_{21,25}$), 127($C_{16,18}$), 128.57($C_{2,6}$), 128.61($C_{15,19}$), 128.63(C_{17}), 128.99($C_{28,30}$).

4d: ^{1}H-NMR (δ/ ppm): 3.19 (dd, IH, CH_2-Ha), 3.86 (dd, IH, CH_2-Hb), 5.16 (s, 2H, OCH_2-C_{13}), 5.39 (dd, IH, CHx), 6.79-7.96 (m, 20H, Ar-H). **^{13}C-NMR (δ/ ppm):** 42.90 (CH_2.pyra.), 63.86 (CH.pyra.), 75.29 (OCH_2.C_{13}), 113.6 ($C_{22,24}$), 116.($C_{27,31}$), 119.5 (C_{29}), 123.5 ($C_{8,12}$), 123.59 ($C_{3,5}$), 126.36 ($C_{28,30}$), 127.5 ($C_{21,25}$), 128.56 ($C_{2,6}$), 128.61 ($C_{15,19,9,11}$), 128.88 (C_{17}), 129 ($C_{16,18}$), 130.46 (C_1), 135.8 (C_{14}), 135.99 (C_{20}), 143.96 (C_{26}), 145.45 (C_7), 148.94 (C=N), 151.73 (C_4), 152.8 (C_{10}), 160.9 (C_{23}). **^{13}C-DEPT-135:** -42.906(CH_2.pyra.), 63.86(CH.pyra.), -75.29(OCH_2.C_{13}), 113.6($C_{22,24}$), 116.($C_{27,31}$), 119.5(C_{29}), 123.5($C_{8,12}$), 123.59($C_{3,5}$), 126.36($C_{28,30}$), 127.5($C_{21,25}$), 128.56($C_{2,6}$), 128.61($C_{15,19,9,11}$), 128.88(C_{17}), 129($C_{16,18}$).

4e: ^{1}H-NMR (δ/ ppm): 3.14 (dd, 1H, CH_2-Ha), 3.92 (dd, 1H, CH_2-Hb), 5.16 (s, 2H, -O-CH_2-C_{13}), 5.36 (dd, 1H, CH-Hx), 6.79-7.96 (m, 20H, Ar-H). **^{13}C-NMR (δ/ ppm):** 43.05 (CH_2 of pyra), 64 (CH of pyra), 75.27 (O-CH_2-C_{13}), 113.61 ($C_{27,31}$), 119.94 (C_{29}), 123.51 ($C_{8,12}$), 123.60 ($C_{3,5}$), 126.38 (C_{17}), 127.29 ($C_{21,25}$), 128.57 ($C_{2,6,15,19}$), 128.62 ($C_{22,24}$), 129.($C_{16,18}$) 129.47 ($C_{28,30}$) 130.47 ($C_{9,11}$), 133.54 (C_{23}), 135.78 (C_{14}), 136 (C_1), 140.65 (C_{20}), 143.92 (C_{26}), 145.48 (C_7), 148.93 (C=N), 151.78 (C_4), 152.85 (C_{10}). **^{13}C-DEPT-135:** -43.05(CH_2 pyra.), 64(CH pyra), -75.27(OCH_2, C_{13}), 113.60($C_{27,31}$), 119.94(C_{29}), 123.51($C_{8,12}$), 123.60($C_{3,5}$), 126.38(C_{17}), 127.29($C_{21,25}$), 128.58($C_{2,6,15,19}$), 128.62($C_{22,24}$), 129.($C_{16,18}$), 129.47($C_{28,30}$).

4f- 1H-NMR (δ/ ppm): 3.09 (dd, 1H, CH_2-Ha), 3.79 (dd, 1H, CH_2-Hb), 4.93 (s, 2H, -O-CH_2-C_{13}), 5.07 (s, 2H, -O-CH_2-C_{26}), 5.26 (dd, 1H, CH-Hx), 6.76-7.87 (m, 24H, Ar-H). **^{13}C-NMR (δ/ ppm):** 43.32 (CH_2 of pyra.), 64.10 (CH of pyra.), 69.26 (O-CH_2- C_{13}), 75.27 (O-CH_2-C_{26}), 113.62 ($C_{22,24}$), 115.46 ($C_{34,38}$), 119.67 (C_{36}), 123.49 ($C_{8,12}$), 123.59 ($C_{3,5}$), 126.33 ($C_{28,32}$), 127.1 ($C_{21,25}$), 128.57 ($C_{2,6}$), 128.63 ($C_{15,17,19}$), 128.81 ($C_{16,18,29,31}$), 129.01 ($C_{35,37}$),130.46 ($C_{9,11}$), 133.83 (C_{30}), 134.74 (C_{27}), 135.34 (C_{20}), 136.01(C_{14}), 136.07 (C_1), 144.10 (C_{33}), 145.45 (C_7), 148.95(C=N), 151.64 (C_4), 152.80 (C_{10}), 158.05 (C_{23}). **^{13}C-DEPT-135:** -43.22(CH_2 of pyra.), 64.10(CH of pyra.), -69.19(O-CH_2- C_{13}), -75.27(O-CH_2-C_{26}), 113.62($C_{22,24}$), 115.46($C_{34,38}$), 119.67(C_{36}), 123.49($C_{8,12}$), 123.59($C_{3,5}$), 126.33($C_{28,32}$), 127.1($C_{21,25}$), 128.57($C_{2,6}$),128.63($C_{15,17,19}$), 128.81($C_{16,18,29,31}$), 129.01($C_{35,37}$).

Table2: Some physical properties and IR data for the synthesized Azo-Pyrazolines.

Prod.	**R**	**C=N str. cm-1**	**Yield %**	**M.P./°C**	**RF**
4a	H	1595	92	62-64	0.75
4b	4-CH_3	1596	85	116-118	0.85
4c	4-OCH_3	1594	82	83-85	0.82
4d	4-F	1597	80	91-93	0.82
4e	4-Cl	1597	80	88-90	O.80
4f	4-(p-Cl-Bz)	1597	70	91-93	0.7
4g	2-Cl	1595	81.52	116-118	0.70
4h	4-NO_2	1595	95.95	61-63	0.78
4i	4-Bz	1597	83	59-62	0.80
4j	2-F	1597	80	125- 127	0.77

Note: Bz = benzyloxy compound.

2.5 Antibacterial Activity

In vitro antibacterial activities of the pyrazoline and chalcone compounds in different concentrations (200, 400, 600, 800, and 1000 ppm in DMSO) were evaluated using the well diffusion method on Mueller-Hinton agar (MHA). The inhibition zones were reported in millimeters (mm). S.aureus (ATCC 25923) and E. coli (ATCC 25922) were used as references, MHA agar plates were inoculated with bacterial strain under aseptic conditions, and wells (diameter = 8mm) were filled with 50 µl of the test samples and incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones were measured. Azithromycin was used as a standard drug for antibacterial activity as shown in (Table 3, 4).

3. Results and Discussion

3.1. Chemistry

This study includes synthesizing new series of azo-pyrazoline (4a-j) which are (five-member heterocyclic) compounds in good yields from the cyclization of azo-benzyloxy-chalcone derivatives (3a-j) with phenyl hydrazine in alcoholic sodium hydroxide. Compounds (3a-j) were prepared by Claisen-Schmidt condensation reaction of different substituted aromatic aldehydes having electron withdrawing and donating groups in different positions, with azo-benzyloxy-acetophenone (2). The processes for preparation are illustrated in Scheme (1).

3.2 Spectroscopic characterization

The IR spectrum of compound **(1)** showed broad band at (3178 cm^{-1}) for the hydroxyl group and the band of the C=O str. appeared at (1672 cm^{-1}), this confirmed the structure of synthesized azo-linkage. The ^{1}H-NMR spectrum of comp**. (1)** showed two singlet signals at (2.70 ppm), and (6.31ppm) belonging to acetyl and hydroxyl proton respectively. A singlet at 7.99 for Ar-H $_{8, 12}$ and two doublets at 7.96 and 8.13 attributed to Ar-H $_{3, 5}$ and Ar-$H_{2, 6}$ are good evidence for the azo coupling process. ^{13} C-NMR spectrum shows ten singlet signals for ten different types of carbons. Also, the ^{13}C-DEPT-135 shows four upward signals for mono-protonated carbons in aromatic rings and the (CH_3) group and the disappearance of six non-protonated carbons. (Al-hamdani *et al*., 2010)

The strong evidence for benzylation of the hydroxyl group is the broad band of the hydroxyl group disappeared, and the peak of the C=O str. is shifted from (1672 cm^{-1}) to (1687 cm^{-1}) in IR spectrum for compound (2). The ^{1}H-NMR spectrum shows two singlet signals at (2.71, 5.18) ppm for the (-CH_3) group attached to carbonyl, and -OCH_2). Additional multiplet signals at 7.40-7.65 for five protons of phenyl ring support the benzylation reaction. ^{13}C-NMR showed fifteen singlet signals for fifteen types of carbons in different environment in the molecule. Also, the ^{13}C-DEPT-135 showed seven upward signals for mono-protonated carbons in aromatic rings and the (CH_3) group. Only one downward signal for a diprotonated carbon atom (-OCH_2-) group, along with the disappearance of seven non-protonated carbons. (Hawaiz and Samad, 2012)

The most powerful absorption band that indicates the formation of the conjugated enone system (chalcone) compound **(3).** Which is the shifting of the carbonyl absorption band to nearly (1654 cm^{-1}) it is a lower wavenumber than it appeared in compound **(2)** at (1687cm^{-1}) since the conjugation between (C=O, C=C bonds) will lead

to presence some signal bond character and lower the absorption band frequency. ^{1}H-NMR spectrum display that the resonance effect of the phenyl ring bounded to *β*- carbon atom cause to deshield doublet signal of *β*- proton to (7.77 -7.97)ppm. Nevertheless, it is difficult to separate certain chemical shift of the (*α*-proton) since it is overlapping with aromatic protons. ^{13}C-NMR spectrum shows more deshielded the characteristic peak for *β*- carbon atom at (144-145.68) ppm and *α*-carbon atom at (120-122.19) ppm. The ^{13}C-DEPT-135 shows mono protonated Carbone atoms upward for (C*α,* C*β,* and aromatic rings). Also, shows a characteristic downward signal around (-70) ppm related to the di-protonated carbon atom of the (-O-CH_2-) group. (Alkazmi *et al*., 2017)

Good evidence for producing pyrazoline derivatives (are newly imine system) formation, which have a band at 1596 cm-1 approximately and a disappearance of the carbonyl group absorption band for enone system at (1655 cm^{-1}) in the IR spectrum, Figure (3). ^{1}H-NMR spectrum proves 2-pyrazoline ring structure by the appearance of three (dd) signals for three different protons (Ha, Hb, Hx) attached to carbon atoms at (3.09, 3.79, 5.26) ppm as an (ABX) spin system belongs to two geminal and one vicinal proton Figure (4). ^{13} C-NMR spectrum shows two distinct signals for two carbons (-CH_2-, -CH-) at (43.32, 64.58) ppm respectively Figure (5). ^{13}C-DEPT-135 spectrum for compound (4f) shows three down ward peaks for (-CH_2-Pyra., -OCH_2-C_{13}, and -OCH_2-C_{26}) at (-43.22, -69.19, -75.25) ppm respectively, figure (6). (Hussain *et al*., 2013)

3.3 Biological evaluations

Pyrazoline and chalcone derivatives exhibit a broad spectrum of biological activity, according to this all of the synthesized pyrazoline and chalcone compounds had screened for their antimicrobial activity against *E.coli* as (gram negative bacteria) and *S.aureus* as (gram-positive bacteria). The results showed that all prepared compounds influence both bacteria's (gram positive and gram negative) with different activities. Furthermore, in all different concentrations, the pyrazoline compounds have higher activity than the synthesized Chalcones toward E.coli, as illustrated in table (3). In addition, at the concentration (1000ppm) of chalcone compounds had higher (NG) antimicrobial activity toward Ecoli (gram-negative) than the S.aureus (gram-positive), as shown in Fig. (1). Pyrazoline and chalcone compounds at concentration (1000ppm) except (3c) reach NG (non-growth of bacteria) against *E.coli* (gram-negative), as shown in table (3). Compound 3j had NG at (1000ppm) against both of the microorganisms, for instance in table (3, 4). The E.coli bacteria didn't have resistance to our pyrazoline products, in the case of compounds 4(a, b, d, e, h, i, j) show complete zone inhibition in their bacterial cultures, but these compounds show moderate to high activities against S.aureus (gram-positive) Fig (2). The rest of the studied compounds exhibited good to moderate activity (16-NG mm), (28-NG) against S.aureus (gram-positive) and E.coli (gram-negative), respectively.

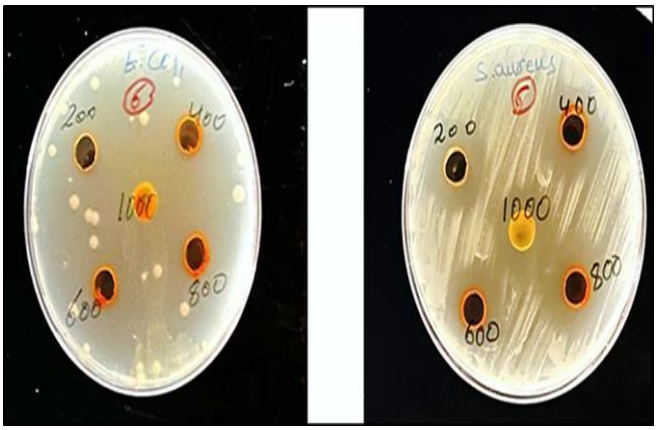


Figure 1: Inhibition zone of chalcone compounds at concentration (200, 400, 600, 800, and 1000ppm) on E. coli and S.aureus microorganism

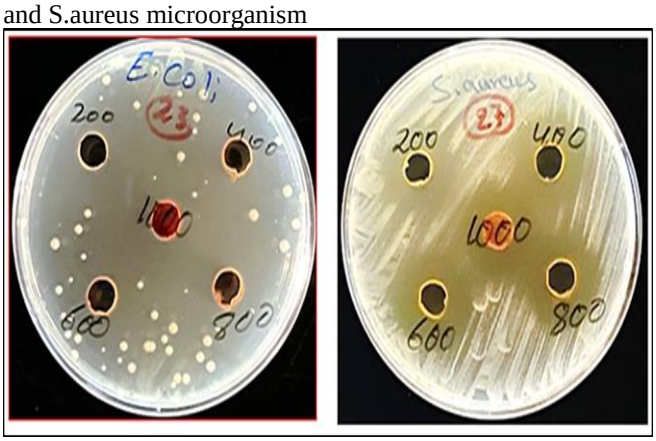


Figure 2: Inhibition zone of pyrazolin compounds at concentration (200, 400, 600, 800, and 1000ppm) on E. coli and S.aureus microorganism.

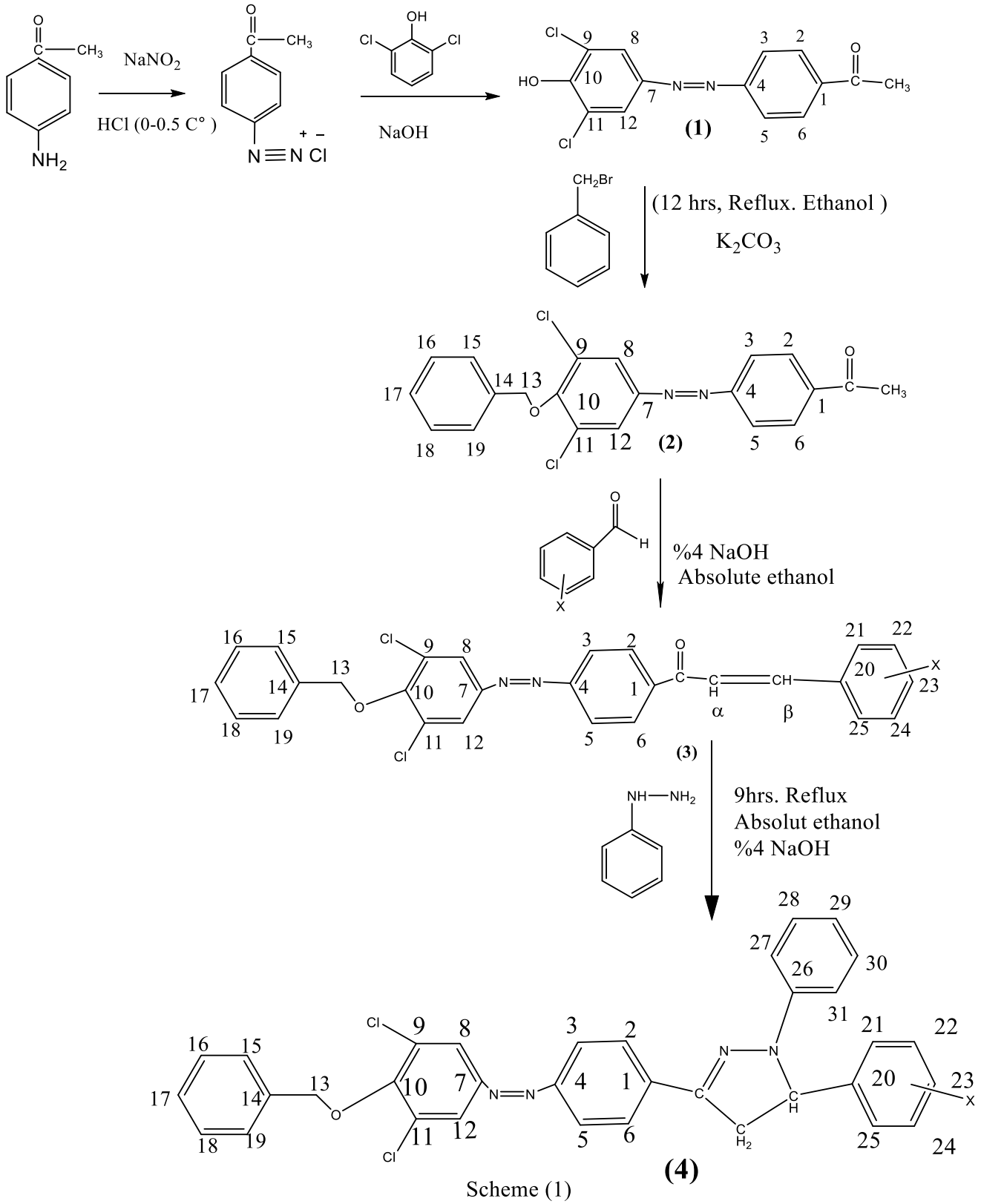
Table 3: Antimicrobial activity for the synthesized Azo-Chalcones and Pyrazolines against E.Coli bacteria.

No.	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm
3a	30	28	28	30	NG

4a	NG	NG	NG	NG	NG
3b	28	30	30	30	NG
4b	NG	NG	NG	NG	NG
3c	32	36	32	36	50
4c	30	30	32	32	NG
3d	30	30	32	32	NG
4d	NG	NG	NG	NG	NG
3e	30	32	38	36	NG
4e	NG	NG	NG	NG	NG
3f	30	30	28	26	NG
4f	28	30	34	34	NG
3g	30	30	32	32	NG
4g	30	28	32	30	NG
3h	24	26	32	30	NG
4h	NG	NG	NG	NG	NG
3i	30	32	30	32	NG
4i	NG	NG	NG	NG	NG
3j	30	30	34	34	NG
4j	NG	NG	NG	NG	NG

Table 4: Antimicrobial activity for the synthesized Azo-Chalcones and Pyrazolines against S.aureus.

No.	200	400	600	800	1000
	ppm	ppm	ppm	ppm	ppm
3a	20	20	20	20	20
4a	20	20	20	20	20
3b	26	24	24	24	24
4b	22	24	24	26	28
3c	22	18	20	18	20
4c	16	18	18	20	20
3d	20	26	26	28	20
4d	24	24	20	24	28
3e	21	20	24	24	22
4e	20	22	25	25	20
3f	20	20	18	18	18
4f	18	18	20	20	28
3g	20	20	22	22	22
4g	20	22	22	22	24
3h	20	22	24	22	24
4h	20	22	22	24	26
3i	28	26	30	26	30
4i	26	24	26	24	----------
3j	24	26	30	30	NG
4j	28	24	24	20	24



Scheme (1)

X=H, 2-Cl, 2F, 4-Cl, 4F, OCH_3, CH_3, 4-NO_2, 4-$PhOCH_2$, 4-$ClPhOCH_2$.

Scheme 1: A stepwise synthetic process of some new pyrazoline derivatives containing azo and benzyloxy moieties.

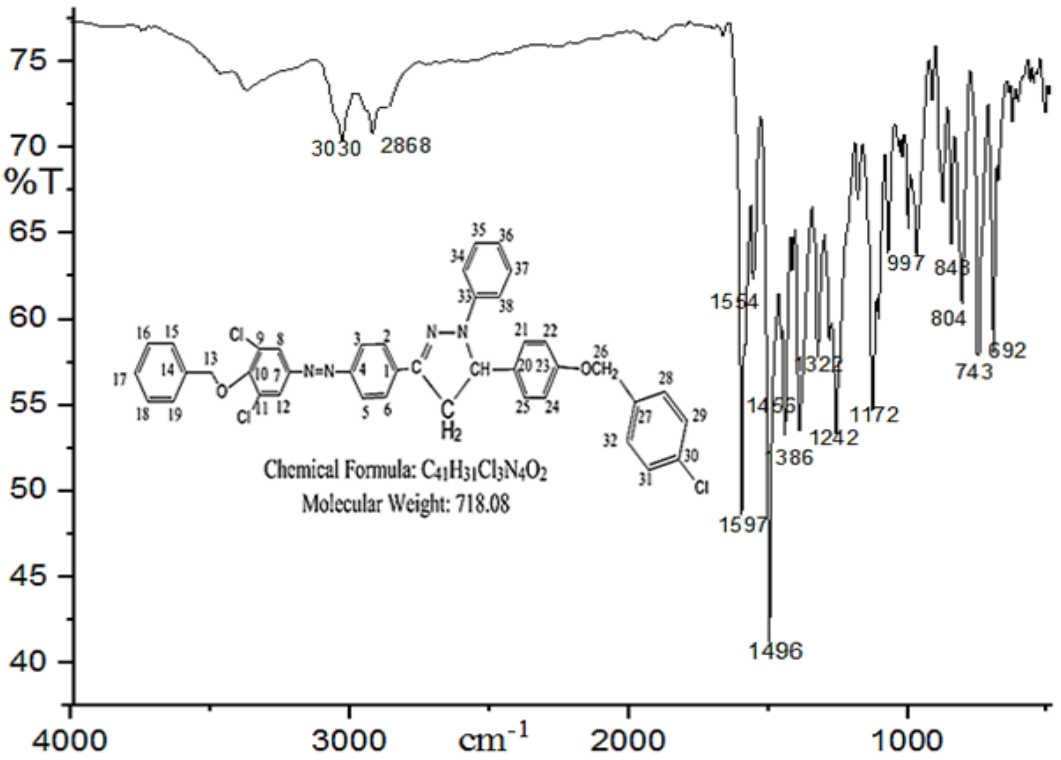


Figure 3: IR spectrum for compound (4f).

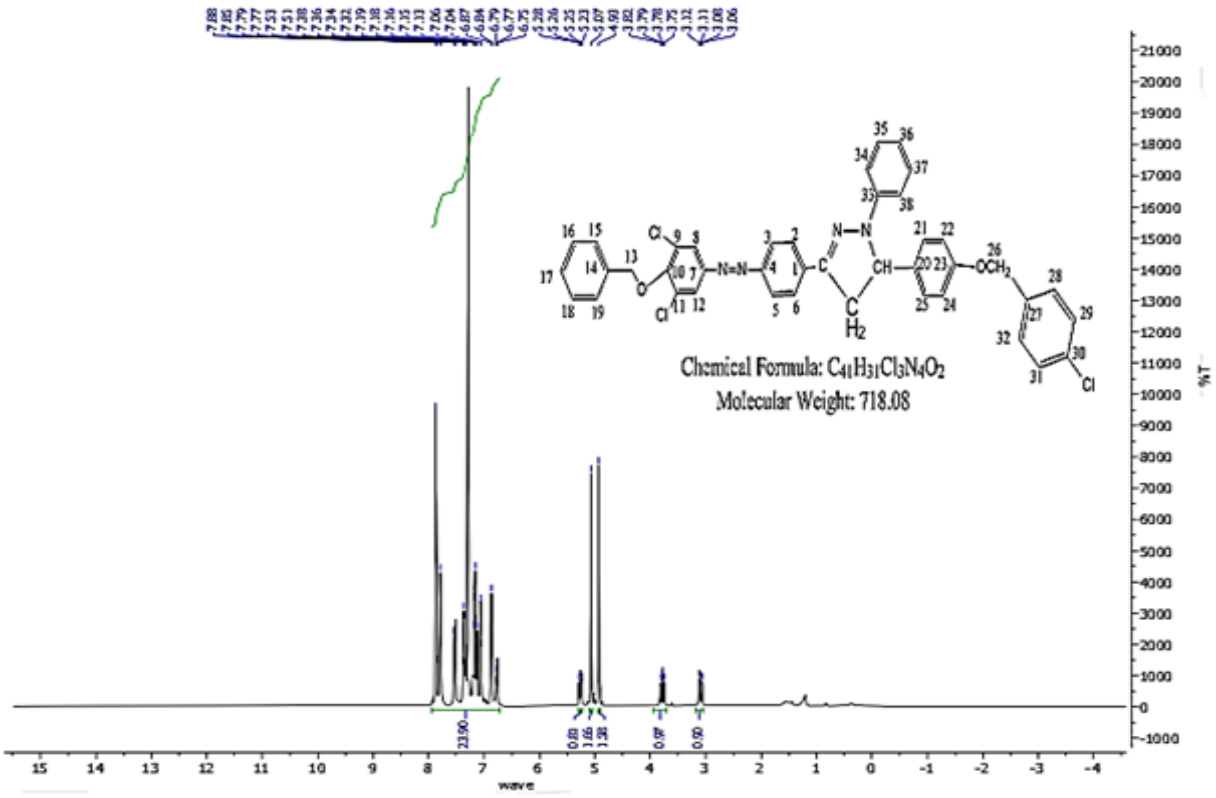


Figure 4: ^{1}H-NMR spectrum for compound (4f).

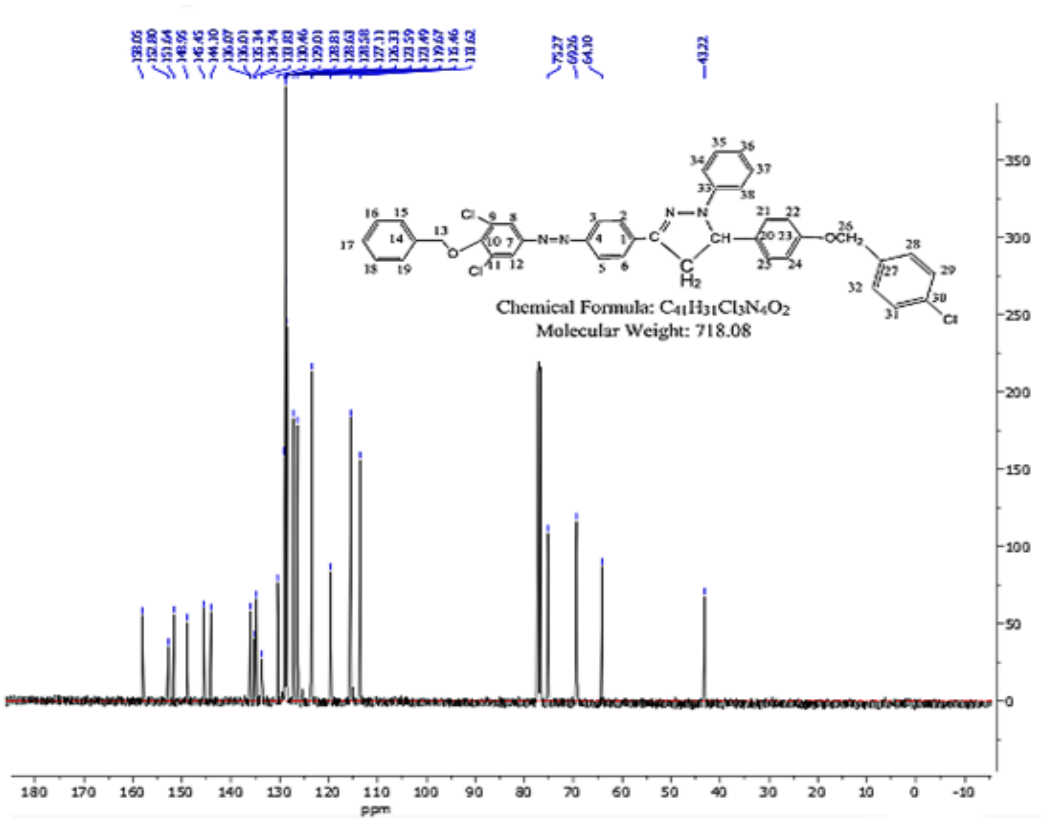


Figure 5:[13]C-NMR spectrum for compound (4f).

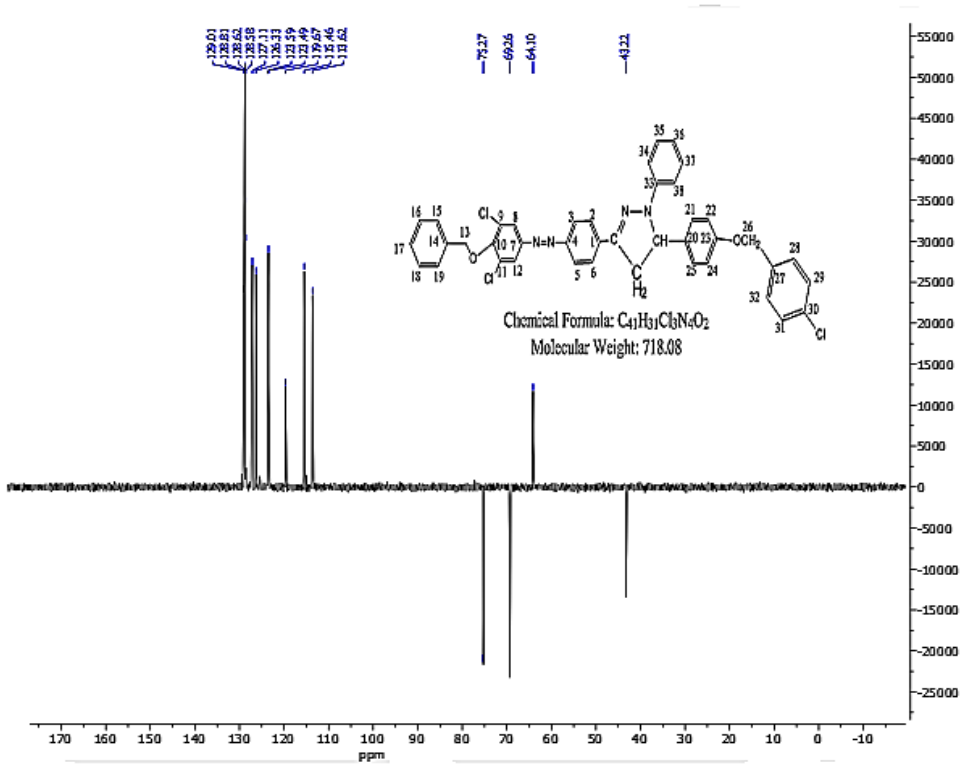


Figure 6: [13]C-DEPT-135 spectrum for compound (4f).

4 Conclusions

From the results obtained in this study, it was concluded that p-aminoacetophenone has an advantage due to having two active groups (amino and acetyl) with in its structure, making it a valuable synthon for stepwise reactions to produce new biologically active compounds. It has been able to synthesize intermediate Chalcones and their corresponding pyrazoline derivatives in high yields and reasonable reaction times. A biological screening study conducted in vitro showed that certain newly synthesized compounds have good activity against both types of microorganisms S.aureus as gram-positive and E.Coli as gram-negative**.**

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Conflict of Interest

There is no conflict of interest.

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