

SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SUBSTITUTED PYRAZOLINES FROM 2-ACETYLFUORENE BASED CHALCONE

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ABSTRACT

Objective: The present work is based on the above pyrazoline derivative form heterocyclic substituted chalcone.

Methods: The elemental analysis, IR, ¹H and ¹³C NMR and mass spectrum analyses were used to characterise the structures of the obtained compounds.

Results: The scheme-1 and scheme-2 describe the synthesis of chalcone (thiophene-fluorenyl derivative) and synthesis of substituted pyrazolines respectively. Chalcone has been synthesized from 2-Acetylfluorene, which had been reacted with phenylhydrazine, 4-substituted phenylhydrazine (4-Chloro and 4-Nitro) and 2,4-Dinitrophenylhydrazine to form the substituted pyrazolines (CP01-CP04).

Conclusion: The antimicrobial and antioxidant properties were evaluated on those newly synthesised compounds.

Keywords: Heterocyclic compounds, Chalcones, Pyrazoline antimicrobial and Antioxidant activity

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INTRODUCTION

Many of the new drugs are coming in the field of medicine for every day. The new drugs are used as medicine for various ailments. These are obtained directly from natural sources or from a laboratory preparation. Most of the medicines contain organic compounds. For example, penicillin and its derivatives are naturally derived from fungi and laboratory synthetic method and which are used worldwide as an antibiotic. The various medicinal organic compounds are found naturally and this paper describing chalcones and their derivatives.

Chalcones (trans-1,3-diaryl-2-propen-1-one) or α , β -unsaturated ketones are open-chain organic compounds [1]. These are directly linked to the two aryl groups with a ketoethylenic group (Ph-CO-CH=CH-Ph). In naturally, these are known as flavonoids [2], which are important for the pigmentation of plant flowers [3]. Chalcones are prepared in the laboratory by the Claisen-Schmidt condensation method [4]. The presence of α , β -unsaturated carbonyl group in the chalcones so exhibit higher biological activities [5]. These can be found as precursors in a variety of heterocyclic compounds [6]. Chalcones have been used to synthesize heterocyclic compounds of Isoxazoles [7], 2-Aminopyrimidines [8], Thiazines [9], Oxazines [10], Pyrazoline [11], N-Acyl pyrazoline [12], 2-Amino-3-cyanopyridines [13], 3-Cyanopyridines [14], Barbitones [15], 1,4-Diazepine [16], 1,4-Benzothiazepines [17] and Benzo[1,5]thiazepine [18].

Pyrazolines are 5-membered heterocyclic compounds (fig. 1) [19]. Two nitrogen atoms and three carbon atoms combine to form a molecule in pyrazoline and the nitrogens are located at 1 and 2 position [20]. Pyrazolines are obtained from chalcones by reacting with hydrazine or phenylhydrazines in the presence of acetic acid [21].

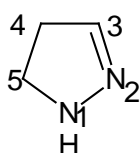


Fig. 1: General structure of pyrazoline

Derivatives of pyrazoline are subject to several studies due to their vast spectrum of potential physiological role. They are known to have antimicrobial [22], antibacterial [23], antifungal [24], antiviral [25], antioxidant [26], antitumour [27], antihistaminic [28], antiproliferative [29], anti-inflammatory [30], analgesic [31], antidepressant [32], fungicides [33], anticancer [34] and anti-tubercular activities [35]. They are used as a starting material for the synthesis of new drugs. They serve as the building blocks for a variety of new medications.

In this paper, we aimed at the synthesis, characterization and biological analysis of pyrazoline derivatives in the context of recent literature on novel heterocyclic research and development in the wake of the enumerated findings. The IR, ¹H and ¹³CNMR spectrum data and elemental analyses were used to confirm the structures of compounds. The spectral data and biological activities of the title compounds are summarized and the results are given in the discussion section.

MATERIALS AND METHODS

The chemicals shows in this experiment were sourced Sigma Aldrich and were directly used in the preparation part without purification. The compounds were prepared through the reporting method [11] and this method is described in Schemes 1 and 2. The reaction completion was verified and confirmed by thin-layer chromatography. Open capillary tube techniques were used to determine the melting point of synthesized compounds. The IR spectra of the compounds were scanned on a Shimadzu IR spectrophotometer using a KBr disk and values are expressed in cm⁻¹. On the Bruker (400MHz) spectrometer, the NMR (¹H and ¹³C) spectra were recorded on the DMSO-D₆ solvent using the TMS as the internal standard (chemical changes at, ppm). The calculated values of C, H, N and S of the synthesized compounds correspond to the actual results.

Synthesis of chalcone

Synthesis of 3-(5-Bromofuran-2-yl)-1-(4-nitrophenyl)prop-2-en-1-one (C01)

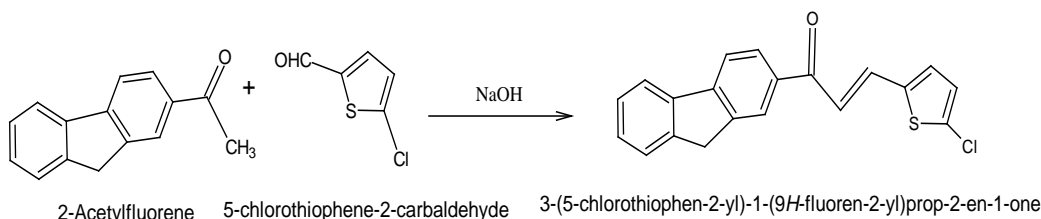
The ethanolic NaOH solution of 2-Acetylfluorene (0.01 mol) was added to 5-Chlorothiophene-2-carbaldehyde (0.01 ml) and the

mixture was stirred by mechanical stirring for approximately 3 h, and was allowed to cool for 24 h. The Thin layer chromatography technique was used for checking purity. The cold mixture is transferred into an HCl acid and crushed ice solution. The solid compound was then filtered through water and recrystallized with ethanol.

3-(5-chlorothiophen-2-yl)-1-(9H-fluoren-2-yl)prop-2-en-1-one

C₂₀H₁₃ClSO; m. p 192 °C; IR (KBr, ν_{max} , cm⁻¹): 3042 (Aromatic C-H str), 2933, 2841 (Aliphatic C-H str), 1692 (C=O str) 1578 (Aromatic C-C str) and 1465 (Aliphatic C-H str); ¹H NMR (400 MHz, CDCl₃, δ

(ppm)): 8.21 (d, 1H, $J=15.2$ Hz), 7.99 (d, 1H, $J=1.2$ Hz, C_{1''}-H) 7.81 (d, 1H, $J=7.2$ Hz, C_{4''}-H), 7.80-7.77 (m, 1H, C_{3''}-H), 7.66-7.64 (m, 1H, C_{5''}-H), 7.52 (d, 1H, $J=14.8$ Hz), 7.39-7.23 (m, 3H, C_{6''}-H-C_{8''}-H), 6.81 (d, 1H, $J=7.6$ Hz, C_{4'}-H), 6.76 (d, 1H, $J=7.6$ Hz, C_{3'}-H), 3.74 (s, 2H, C_{9''}-H); ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 191.08 (C1), 151.87 (C2''), 144.38 (C10'''), 142.12 (C14'''), 139.65 (C12'''), 139.34 (C11'''), 138.67 (C5''), 137.66 (C2'''), 126.12 (C6'''), 126.17 (C3), 125.68 (C6'''), 125.24 (C8'''), 125.17 (C3'''), 124.47 (C1'''), 123.13 (C3''), 122.68 (C4'''), 122.19 (C5'''), 121.45 (C2), 114.17 (C4''), 35.37 (C9'''); MS(EI): m/z 320 [M⁺]; Elemental analysis-calcd: C, 71.33; H, 3.86; S, 9.51 (%); found: C, 71.31; H, 3.88; S, 9.49 (%).



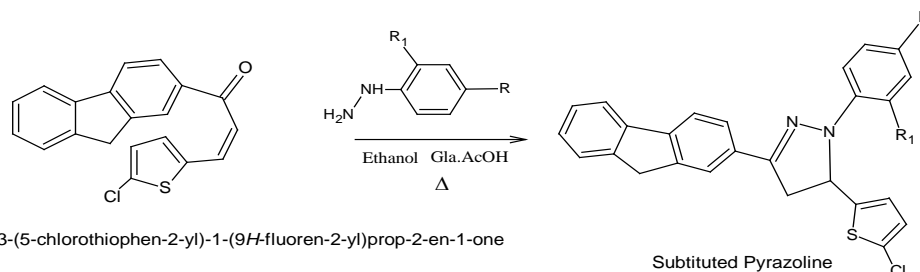
Scheme 1: Method for synthesis of chalcone

Method for synthesis of substituted pyrazoline derivative

1-(4-chlorophenyl)-5-(5-chlorothiophen-2-yl)-3-(9H-fluoren-2-yl)-4,5-dihydro-1H-pyrazole

A 25 ml of glacial acetic acid mixed to phenylhydrazine (0.005 mol) and 3-(5-chlorothiophen-2-yl)-1-(9H-fluoren-2-yl)prop-2-en-1-one

(0.005 mol). Then the reacting mixture was heated for 4 h. The thin layer chromatography technique was used for checking purity and then the mixture is transferred into an ice-cold water solution (100 ml). The resulting solid compound was then filtered through water and recrystallized with ethanol. Other compounds CP02-CP04 were also prepared using the same method.



Scheme 2: Method for synthesis of pyrazoline derivatives

Table 1: Pyrazoline derivatives

Radical	CP01	CP02	CP03	CP04
R	Cl	Br	NO ₂	NO ₂
R ₁	H	H	H	NO ₂

5-(5-chlorothiophen-2-yl)-3-(9H-fluoren-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazole

C₂₆H₁₉ClN₂S; m. p 187 °C; IR (KBr, ν_{max} , cm⁻¹): 3049 (Aromatic C-H str), 2936, 2842 (Aliphatic C-H str), 1702 (C=O str) 1631 (Aromatic C-C str) and 1458 (Aliphatic C-H str); ¹H NMR (400 MHz, CDCl₃, δ (ppm)): 7.91 (d, 1H, $J=1.2$ Hz, C_{1''}-H) 7.88 (d, 1H, $J=7.6$ Hz, C_{4''}-H), 7.76-7.41 (m, 1H, C_{3''}-H), 7.73-7.71 (m, 1H, C_{5''}-H), 7.56-7.52 (m, 2H, C_{3'}-H and C_{5'}-H), 7.47-7.37 (m, 2H, C_{6''}-H and C_{8''}-H) 7.39 (m, 2H, C_{2'}-H and C_{6'}-H), 7.33-7.29 (m, 1H, C_{7''}-H), 7.29-7.25 (m, 1H, C_{4'}-H) 6.84 (d, 1H, $J=7.6$ Hz, C_{4'}-H), 6.75 (d, 1H, $J=7.6$ Hz, C_{3'}-H), 5.12 (dd, 1H, $J=18, 8.8$ Hz Py-H_a), 3.76 (s, 2H, C_{9''}-H) 3.90 (dd, 1H, $J=12.4, 8.8$ Hz, Py-H_a) 3.15 (dd, 1H, $J=12.4, 8.8$ Hz, Py-H_b); ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 154.34 (C1), 144.28 (C10'''), 142.45 (C13'''), 142.20 (C1'), 139.73 (C11'''), 139.62 (C12'''), 136.94 (C2'''), 136.35 (C2''), 135.36 (C3'''), 135.12 (C5''), 133.44 (C4''), 129.23 (C3' and C5'), 126.12 (C7'''), 125.43 (C6'''), 125.35 (C8'''), 124.44 (C4'''), 124.22 (C4'), 123.92 (C1'''), 122.04 (C5'''), 119.32 (C3'''), 117.63 (C2' and C6'), 76.72 (C3), 38.06 (C2), 35.36 (C9'''); MS (EI): m/z 426 [M⁺]; Elemental analysis-calcd: C, 73.16; H, 4.45; N, 6.56; S, 8.31 (%); found: C, 73.13; H, 4.48; N, 6.56; S, 8.30 (%).

1-(4-chlorophenyl)-5-(5-chlorothiophen-2-yl)-3-(9H-fluoren-2-yl)-4,5-dihydro-1H-pyrazole

C₂₆H₁₈Cl₂N₂S; m. p 199 °C; IR (KBr, ν_{max} , cm⁻¹): 3047 (Aromatic C-H str), 2949, 2852 (Aliphatic C-H str), 1692 (C=O str) 1635 (Aromatic C-C str) and 1432 (Aliphatic C-H str); ¹H NMR (400 MHz, CDCl₃, δ (ppm)): 7.90 (d, 1H, $J=1.6$ Hz, C_{1''}-H), 7.87 (d, 1H, $J=7.2$ Hz, C_{4''}-H), 7.75-7.71 (m, 2H, C_{3''}-H and C_{5''}-H), 7.55 (d, 2H, $J=7.2$ Hz, C_{3'}-H and C_{5'}-H), 7.46-7.37 (m, 2H, C_{6''}-H and C_{8''}-H), 7.34 (d, 2H, $J=7.6$ Hz, C_{2'}-H and C_{6'}-H), 7.31-7.29 (m, 1H, C_{7''}-H), 6.84 (d, 1H, $J=6.8$ Hz, C_{4'}-H), 6.75 (d, 1H, $J=7.6$ Hz C_{3'}-H), 5.12 (dd, 1H, $J=18.8, 8.4$ Hz Py-H_a), 3.78 (s, 2H, C_{9''}-H) 3.92 (dd, 1H, $J=12.8, 9.2$ Hz, Py-H_a), 3.11 (dd, 1H, $J=12.4, 8.8$ Hz, Py-H_b); ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 154.62 (C1), 144.31 (C10'''), 142.13 (C13'''), 140.67 (C1'), 139.68 (C12'''), 139.63 (C11'''), 136.92 (C2'''), 136.33 (C2''), 135.33 (C3'''), 135.09 (C5''), 133.41 (C4''), 129.69 (C4'), 129.01 (C3' and C5'), 126.16 (C7'''), 125.46 (C6'''), 125.32 (C8'''), 124.41 (C4'''), 123.94 (C1'''), 122.09 (C5'''), 119.31 (C3'''), 118.67 (C2' and C6'), 76.76 (C3), 38.12 (C2), 35.31 (C9'''); MS (EI): m/z 461 [M⁺]; Elemental analysis-calcd: C, 67.69; H, 3.9; N, 6.07; S, 6.94 (%); found: C, 67.67; H, 3.93; N, 6.07; S, 6.95 (%).

5-(5-chlorothiophen-2-yl)-3-(9H-fluoren-2-yl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole

C₂₆H₁₈ClN₃O₂S; m. p 201 °C; IR (KBr, ν_{max}, cm⁻¹): 3032 (Aromatic C-H str), 2945, 2843 (Aliphatic C-H str), 1701 (C=O str) 1638 (Aromatic C-C str) and 1443 (Aliphatic C-H str); ¹H NMR (400 MHz, CDCl₃, δ (ppm)): 8.3 (d, 2H, J=7.6Hz, C₃H and C₅H), 7.90 (d, 1H, J=1.2Hz, C₁H), 7.88 (d, 1H, J=7.6Hz, C₄H), 7.75-7.73 (m, 1H, C₃H), 7.71 (d, 1H, J=1.2Hz, C₅H), 7.63 (d, 2H, J=7.6Hz, C₂H and C₆H), 7.46-7.37 (m, 2H, C₆H and C₈H), 7.33-7.29 (m, 1H, C₇H), 6.91 (d, 1H, J=7.6Hz, C₃H), 6.78 (d, 1H, J=7.2Hz, C₄H), 5.13 (dd, 1H, J=18.4, 8 Hz Py-H_α), 3.78 (s, 2H, C₉H), 3.93 (dd, 1H, J=12.8, 9.2 Hz, Py-H_β), 3.20 (dd, 1H, J=12.4, 9.2 Hz, Py-H_γ); ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 154.45 (C1), 145.33 (C1'), 144.26 (C10'''), 142.32 (C13'''), 141.98 (C4'), 139.77 (C11'''), 139.65 (C12'''), 136.95 (C2'''), 136.36 (C2''), 135.39 (C3'''), 135.16 (C5'''), 133.49 (C4''), 126.09 (C7'''), 125.72 (C3' and C5'), 125.47 (C6'''), 125.37 (C8'''), 124.39 (C4'''), 123.96 (C1'''), 122.12 (C5'''), 119.34 (3'''), 118.91 (C2' and C6'), 76.74 (C3), 38.08 (C2), 35.29 (C9'''); MS(EI): m/z 471 [M+]; Elemental analysis-calcd: C, 66.17; H, 3.81; N, 8.9; S, 6.78 (%); found: C, 66.16; H, 3.84; N, 8.9; S, 6.77 (%).

5-(5-chlorothiophen-2-yl)-1-(2,4-dinitrophenyl)-3-(9H-fluoren-2-yl)-4,5-dihydro-1H-pyrazole

C₂₆H₁₇ClN₄O₄S; m. p 206 °C; IR (KBr, ν_{max}, cm⁻¹): 3032 (Aromatic C-H str), 2921, 2836 (Aliphatic C-H str), 1697 (C=O str) 1621 (Aromatic C-C str) and 1449 (Aliphatic C-H str); ¹H NMR (400 MHz, CDCl₃, δ (ppm)): 9.28 (d, 1H, J=1.6Hz, C₃H), 8.78 (dd, 1H, J=7.2, 1.2Hz, C₅H), 7.92 (d, 1H, J=1.6Hz, C₁H), 7.93 (d, 1H, J=8Hz, C₄H), 7.08 (d, 1H, J=1.6Hz, C₃H), 7.74-7.72 (m, 1H, C₅H), 7.83 (d, 1H, J=7.2Hz, C₆H), 7.42-7.30 (m, 2H, C₆H and C₇H), 7.47-7.44 (m, 1H, C₈H), 6.79 (d, 1H, J=7.6Hz, C₃H), 6.84 (d, 1H, J=2.8Hz, C₄H), 5.11 (dd, 1H, J=29.6, 17.2Hz Py-H_α), 3.77 (s, 2H, C₉H), 3.94 (dd, 1H, J=12.4, 4.8Hz, Py-H_β), 3.16 (dd, 1H, J=12.4, 5.2 Hz, Py-H_γ); ¹³C NMR (100 MHz, CDCl₃, δ (ppm)): 154.27 (C1), 144.34 (C10'''), 142.23 (C13'''), 139.69 (C11'''), 139.67 (C12'''), 138.70 (C2'), 138.13 (C1'), 138.01 (C4'), 136.96 (C2'''), 136.38 (C2''), 135.37 (C3'''), 135.07 (C5'''), 133.45 (C4''), 129.97 (C5'), 126.13 (C7'''), 125.45 (C6'''), 125.39 (C8'''), 124.43 (C4'''), 123.94 (C1'''), 123.88 (C3'), 122.15 (C5'''), 121.46 (C6'), 119.36 (3'''), 76.22 (C3), 38.04 (C2), 35.44 (C9'''); MS(EI): m/z 516 [M+]; Elemental analysis-calcd: C, 60.41; H, 3.29; N, 10.84; S, 6.19 (%); found: C, 60.4; H, 3.31; N, 10.83; S, 6.2 (%).

Method of biological activity

Antimicrobial activity

The Kirby-Bauer disc diffusion method [36] of *in vitro* antimicrobial activity was used to evaluate all the synthesized compounds.

The antibacterial and antifungal activity of the compounds was examined on *B. subtilis*, *S. aureus*, *S. typhi*, *E. coli*, and *Candida albicans*. Synthetic and standard compounds for antibacterial and antifungal activities have been prepared in DMSO various concentrations. Antibacterial and antifungal activity was measured after 24h at 35–37 °C and 48h at 25 °C respectively. *Ciprofloxacin* and *Flucanazole* were used as standard compounds for bacteria and fungi, respectively. Table 2 relates the antimicrobial activity of synthesized compounds to that of standard drugs.

Antioxidant activity

The DPPH technique was used to determine the antioxidant activity of the compounds. [37]. Table 3 shows the antioxidant values of the compounds. Test solutions were prepared at different concentrations by adding DPPH (0.1 mmol, 2 ml) solution with 2 ml of synthesized or standard compounds. Then the solution mixture was kept in the dark and incubation of 37 °C at 20 min. The solution absorbance have been measured at 517 nm. AA and BHA were used as positive controls. The antioxidant values of the compounds were calculated using the formula below. Inhibition (%) = (blank OD-sample OD/blank OD)×100.

RESULTS AND DISCUSSION

The scheme-1 and scheme-2 describe the synthesis of chalcone (thiophene-fluorenyl derivative) and synthesis of substituted pyrazolines, respectively. Chalcone has been synthesised from 2-Acetylfluorene, which had been reacted with phenylhydrazine, 4-substituted phenylhydrazine (4-Chloro and 4-Nitro) and 2,4-Dinitrophenylhydrazine to form the substituted pyrazolines (CP01-CP04). Spectroscopic methods such as IR, NMR and mass spectroscopy, including elemental analysis, were used to confirm the structures of all compounds and spectrum values have been compared with previous literature reports. The detailed spectrum data's are present in the individual compound title. The spectral characterization of 1-(4-chlorophenyl)-5-(5-chlorothiophen-2-yl)-3-(9H-fluoren-2-yl)-4,5-dihydro-1H-pyrazole is described as an example. The IR spectrum revealed 3047, 2949 and 1635 and 1432 cm⁻¹ values, respectively. It is obtained due to the compound which contains the characteristics of groups of aromatic and aliphatic CH, respectively. ¹H NMR spectrum exhibited four doublets at δ 7.90 (J=1.6Hz) ppm, δ 7.87 (J=7.2Hz) ppm, δ 7.34 (J=7.6Hz) ppm and δ 6.84 (J=7.2Hz) ppm each for one proton is assignable to C1''H, C4''H, C2'H, C6'H and C4''H respectively. A signals at δ 5.12, 3.92 and 3.11 ppm are attributed for the H_{XAB} protons in the pyrazole ring, respectively. The C9''H proton has been observed at singlet at 3.78 ppm. The ¹³C NMR spectral results are described below. The signal exhibited at δ 154.62 ppm due to the pyrazoline carbon (C1=N) and the carbons of C-3 and C-2 show δ 76.76, 38.12 ppm, respectively. Also, three types of carbon skeletons are joined in the pyrazoline ring system, namely as a fluorenyl ring, 4-chlorophenyl ring and thiophenyl ring. The values of the aromatic carbons of the all the rings correspond to the reported literary values. The methylene carbon, C9 is observed at 35.31 ppm. The mass spectrum of the molecular ion peak is reported at 461 [M⁺]. This value refers to the molecular weight of the compound. Hence, the above spectral data are compatible with the structure of desired product, 1-(4-chlorophenyl)-5-(5-chlorothiophen-2-yl)-3-(9H-fluoren-2-yl)-4,5-dihydro-1H-pyrazole. The Kirby-Bauer disc diffusion method was used to evaluate all synthesised compounds for *in vitro* antimicrobial activity. The inhibition zone was measured and compared against standards. Table 2 shows the results of antibacterial and antifungal activities. The newly synthesised compounds were found to have considerable antimicrobial action against selected microorganisms. Due to the dinitro substitution of the CP04 had the strongest antifungal activity compared to the other compounds. Table 3 shows the results of antioxidant activity of synthesised compounds at various concentrations. The CP04 is the most active of the compounds and has an IC50 value of 23.47µg/ml, whereas AA and BHA had IC50 values of 8.66 and 6.31µg/ml respectively.

Table 2: Antimicrobial activity of the synthesized compounds

Sample code	Zone of inhibition (mm) of synthesized compounds																			
	Antibacterial activity																Antifungal activity			
	<i>Bacillus subtilis</i>				<i>Staphylococcus aureus</i>				<i>Salmonella typhi</i>				<i>Escherichia coli</i>				<i>Candida albicans</i>			
100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	100 mcg	50 mcg	25 mcg	Std	
C01	8	7	4	17	7	3	-	13	6	4	-	19	9	5	-	19	8	5	3	24
CP01	10	7	2	24	8	6	4	17	5	3	-	17	11	7	4	15	21	15	11	25
CP02	10	7	4	15	9	6	5	16	7	5	3	16	10	6	-	18	18	12	8	17
CP03	12	8	6	16	13	6	5	24	8	7	4	19	9	5	-	16	16	10	6	15
CP04	16	9	4	22	19	8	5	21	11	9	3	16	12	8	6	18	23	14	8	26

Table 3: Antioxidant activity of synthesized compounds

Compound	Concentration ($\mu\text{g/ml}$)					IC ₅₀ ($\mu\text{g/ml}$)*
	20	40	60	80	100	
C01	67.32	71.64	75.79	79.56	81.91	76.27
CP01	58.85	64.05	69.16	71.68	75.57	27.02
CP02	62.52	69.37	76.10	81.51	85.91	26.17
CP03	45.66	52.86	65.11	70.71	78.24	29.87
CP04	48.24	55.49	66.89	72.12	79.38	23.47
BHT	57.81	64.76	71.71	81.86	94.08	6.31
AA	57.46	64.36	76.70	87.81	98.09	8.66

*Average of three independent determinations

CONCLUSION

The substituted pyrazoline derivative has been synthesized. The elemental and spectrum studies supported the compound structures. Antimicrobial activity has been reported in newly synthesized compounds against selected bacteria and fungi. The compound CP04 showed high antioxidant activity. Finally 5-(5-chlorothiophen-2-yl)-1-(2,4-dinitrophenyl)-3-(9H-fluoren-2-yl)-4,5-dihydro-1H-pyrazole is observed to have good antifungal and high antioxidant activity.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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