RESEARCH ARTICLE

Development of Unique Pyrazoline Analogues for Screening Anticancer and Anti-inflammatory Studies

Susmita Basakand, Ishwar Bhat K*

**Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be university) Deralakatte, Mangalore, Karnataka, India.*

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ABSTRACT

Cancer is a major threat to human society in the world, which results in a maximum probability of death. In the present scenario, chemotherapy is the main cancer treatment, but still, it has its limitations. Hence, cytotoxic and chemotherapy medicines are the main focus of research for medicinal chemists. So, it is the need of the hour to explore the possibility of effective and safe anticancer drugs. The current research involves the synthesis of pyrazoline derivatives (PYR1-PYR8), which are novel cytotoxic agents developed from recently formulated chalcones (CHL1-CHL8). Title compounds were prepared by subjecting the chalcones with by hydrazine hydrate in ethanolic acetic acid. The purified synthesized compounds were characterized by IR, ^{1}H-NMR, and mass spectroscopic evaluation. Using *in-vitro* models, cytotoxicity evaluations were conducted on cell lines associated with lung cancer by MTT and SRB methods using doxorubicin as standard, which revealed differing levels of cytotoxic effects among the compounds. The egg albumin denaturation and protein denaturation methods were used to access *in-vitro* anti-inflammatory activity. Compound PYR4 and PYR6 showed significant anti-inflammatory efficacy in relation to diclofenac sodium as the reference drug. Compound PYR3, PYR4, and PYR6 displayed noteworthy anticancer activity matched to doxorubicin, which is a reference drug. The current research asserts that the newly developed pyrazoline derivatives exhibit high levels of cytotoxicity and anti-inflammatory effects. However, their preclinical and clinical significance needs a thorough evaluation.

Keywords: Pyrazoline, Chalcones, Cytotoxic activity, Anti-inflammatory Activity.

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INTRODUCTION

Anticancer and chemotherapy medicines are the main objective of medicinal chemists due to the elevated mortality rates associated with cancer among emerging illnesses.[1] Malignancies are treated by chemotherapy, but it has certain limitations like lack of sensitivity, selectivity and major adverse effects. Also, it has resistance to multiple drugs.[2] In the recent past a number of heterocyclic compounds are gaining prominence because of their useful pharmacological and biological activities.[3] Continuous search of new pharmacophores against cytotoxicity is the need of the current scenario. Literature suggests pyrazoline derivatives as prominent synthons and pharmacophores attributed to their broad range of chemotherapeutic and biological applications. Pyrazoline and its derivatives are the important class of five-membered nitrogen heterocycles. Synthesis of pyrazoline has been reported by treating nucleophiles like hydrazine hydrate on chalcones in the presence of acetic acid. Pyrazoline derivatives serve as foundational structures for creating novel drug components with a wide range of pharmacological properties like antifungal[4], antibacterial[5], anti-inflammatory,[6] antioxidant,[7] anticonvulsant,[8] antidepressant,[9] antiviral,[10] anticancer,[11] antidiabetic,[12] antitubercular,[13] analgesic[14] and antimalarial.[15]

Numerous drugs on the market contain a variety of substituted pyrazoline derivatives like axitinib to treat severe aplastic anemia, ibrutinib for chronic lymphocytic leukemia, oxyphenbutazone (anti-inflammatory), aminopyrine (anti-inflammatory), antipyrine (antipyretic), novalgin (analgesic) and celecoxib (COX-2 inhibitor).

Chalcones are formed by condensation of simple or substituted aromatic aldehydes with simple or substituted aromatic ketones in the presence of alkali. In medicinal chemistry, they are a well-known class of compounds with

**Author for Correspondence: bhatishwark@nitte.edu.in*

a wide range of biological activities, including antiviral,[16] anticancer,[17] antioxidant,[18] analgesic,[19] antimalarial,[20] and antitubercular.[21] They are crucial intermediates in the synthesis of key heterocyclic drug moieties, including benzodiazepines, isoxazolines, pyrimidines, and pyrazolines. They are also helpful in clarifying the structural makeup of natural products.[22] Pyrazoline and chalcones are important pharmacophores in medicinal chemistry and drug research because of their complementary bioactivities. In light of the aforementioned results, our goal was to create and investigate a new series of unique pyrazoline derivatives and use *in-vitro* models to test them for cytotoxic and anti-inflammatory properties.

MATERIALS AND METHODS

For this investigation, Sigma-Aldrich provided all of the chemicals and reagents. Using the proper solvents and a digital melting point device to measure the uncorrected melting point, the products were purified through recrystallization. All the intermediates and final compounds were purified chromatography method. As a solvent system, CH_3OCH_3 and $CH_3COOC_2H_5$ (1:5) was used. Utilizing infrared spectra recorded with an Alpha Bruker FTIR spectrometer in the 400 to 4000 cm^{-1} wavelength range, the title compounds were characterized. The Avance-2 equipment was employed for capturing proton nuclear magnetic resonance spectra operating at 300 MHz. Tetramethyl silane (TMS) served as the internal standard, and the solvent utilized was DMSO-d6. Chemical shifts were denoted in parts per million (ppm) relative to TMS to facilitate comparison. Additionally, the SYNAPT-G2 liquid chromatography (LC-MS) spectrometer was utilized for recording the mass spectra.

Chalcone Derivatives Synthesis using Novel ketones

Above said compounds were set with minimal deviation from conventional protocol.[23] 20% KOH (4 mL) was added to a combination of substituted 1-(2-phenylhydrazinylidene) propane-2-one (0.01 mol) and aromatic aldehyde (0.01 mol). Stirring was done until the reaction was completed, and then it was examined by acidifying with dilute hydrochloric acid and removing a few drops of the solution. The conclusion of the reaction was indicated by precipitation. After that, 5% hydrochloric acid was mixed to the reaction sample and it was placed in crushed ice. Subsequently, the product underwent filtration, washing, and ethanol recrystallization. Figure 1, Tables 1 and 2 provide the synthesis scheme, physical data, and spectral data of the chalcone intermediates (CHL 1-8).

Synthesis of Novel Pyrazoline Derivatives

Pyrazoline derivatives (PYR 1-8) were prepared as per standard procedure with minor modification.[24] 0.01M ethanolic solution of desired chalcone was refluxed with 0.01M hydrazine hydrate in the presence of acetic acid at elevated temp for 6 hours. The resulting product was cooled and put into cold water (ice cold), separated the solid mass by filtration and recrystallized with acetone. The purity of the final compound and intermediates were checked by thin layer chromatography (TLC) using CH_3COCH_3 and $CH_3COOC_2H_5$ (1:5) as solvent systems. IR, 1HNMR and mass spectra studies characterized all the intermediates and final products. Synthesis scheme, physical data and spectral data of pyrazoline derivatives(PYR1-8) are given in Figure 2, Tables 1 and 2.

In-vitro Cytotoxic Activity

MTT and SRB assay methods.[25,26] performed the cytotoxic assay of the final fabricated compounds. The MTT assay is a colorimetric method for assaying cell metabolic activity. The technique depends uponalive cells transforming into formazan crystals by MTT for evaluation of mitochondrial activity.

The cytotoxic effects were evaluated using the NCIH-460 and NCIH-522 lung cancer cell lines. Various concentrations (4–102 µg/mL) of specifically created molecules were introduced to cells and cultivated at 37°C for one day in a 5% CO_2 atmosphere. After 24 hours of incubation period, it was confirmed the purple formazan crystals which were solubilized. Subsequently, An introduction of 10 µL of MTT reagent occurred, followed by the measurement of absorbance at 550 and 600 nm using a microplate reader. The SRB assay operates by leveraging the ability of the sulforhodamine B protein-dye to bind, in a pH-dependent manner, to the basic amino acid component within cells treated with trichloroacetic acid. For cell proliferation cytotoxicity evaluation, the following procedure was adopted. 100 µL of cell suspension was filled in each well of a 96-well microtiter plate. This was placed in a 37℃ incubator in an atmosphere of 5% CO_2. Different concentrations of the prepared samples were introduced into the plate. However, this complex can dissociate under basic conditions. SRB protein assay was used to evaluate the cell proliferation cytotoxicity. 100 ɯL of the cell suspensions were seeded in 96 well microtiter plates and incubated in at humidified atmosphere at 37℃ in 4.5 to 5% CO_2. 24 hours' incubation was done using various concentrations of prepared samples. SRB protein assay performed according to standard protocol. At 565 nm, optical density was measured by using an ELISA microplate reader. Utilizing Microsoft Excel, the IC_{50} values were computed. Doxorubicin served as the standard drug in both methodologies. and were given in Table 3

$$\%\text{cell viability} = \frac{\text{O. D of test}}{\text{O.D of control}} \times 100 \text{ (O.D = optical Density)}$$

Bovine Serum Albumin Method

The synthesized compounds' anti-inflammatory properties were assessed *in-vitro* using the protein denaturation method with bovine serum albumin, following the standardized bovine serum albumin protocol.[27] Each test and control solution comprised 0.40 mL of a 5% w/v aqueous solution of bovine serum albumin and 0.05 mL of the test substance, totaling 0.5 mL. Additionally, the control, also totalling 0.50 mL, was prepared with 0.450 mL of distilled water and 0.05 mL of the test substance. For the standard solution (0.5 mL), 0.05 mL of diclofenac sodium and 0.45 mL of BSA (5% w/v aqueous

solution) were mixed, and using 1N HCl the pH was adjusted to 6.3.

Various sample concentrations (10, 20, 30, 40, 50 µg/mL) underwent incubation for 25 minutes at 37°C, followed by heating for 3 minutes at 57°C. Phosphate buffer (2.5 mL) was added to each solution after cooling. Subsequently, the absorbance of the resulting solutions was measured at 416 nm with a UV-visible spectrophotometer. The percentage scavenging of protein denaturation by bovine serum albumin is presented in Table 4. The calculation was performed utilizing a formula given below

% scavenging = 100 × [optical density test / optical density of control - 1] (Equation 1)

Egg Albumin Denaturation Assay

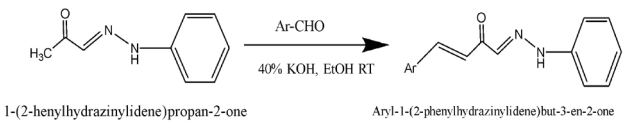
The compounds were screened according to the standard procedure outlined in reference.[27] The reaction mixture, totaling 5 mL, comprised 0.20 mL of egg albumin derived from eggs, 2.80 mL of phosphate-buffered saline at pH 6.4, and 2 mL of test compounds at concentrations ranging from 10 to 50 µg/mL. The control used is double-distilled H_2O. This mixture was then incubated at 300 K in a BOD incubator for 15 minutes. Subsequently, optical density was read at 660 nm, with the solvent used as the blank. Diclofenac sodium, at concentrations of 10, 20, 30, 40, and 50 µg/mL, was employed as the standard drug, and absorbance measurements were carried out in a similar manner. The recorded values representing the percentage of inhibition of protein denaturation by egg albumin are presented in Table 5. The calculation was performed using Equation 1.

RESULTS AND DISCUSSION

Synthesis of substituted pyrazoline derivatives using hydrazine hydrate in the presence of ethanol and acetic acid with various chalcones was the main aim of the present study, which is given in Scheme 2, and the physical information of the products is enclosed in Table 1. Chemical characterizations of prepared products were performed using FTIR, ^{1}H-NMR, and MS studies, which are given in Table 2.

The confirmation of functional group conversions in various chalcones and pyrazoline derivatives was achieved through analysis of bending and stretching frequencies in IR spectroscopy. The stretching frequency of the amine (N-H) was consistently observed within the range of 3210 to 3370 cm^{-1} across all compounds. Additionally, a stretching band in the region of 1600 to 1690 cm^{-1} was detected for the (C=N) group in all compounds, while aromatic C-H stretching occurred within the range of 3000 to 3100 cm^{-1}. The stretching of aromatic C=C bonds was observed within the region of 1515 to 1570 cm^{-1}. Moreover, low-intensity stretching bands of carbon and halogen-containing phenyl groups were observed in the region of 780 to 900 cm^{-1} for compounds CHL3, CHL4, CHL6, PYR3, PYR4, and PYR6.

For characterization, ^{1}H-NMR spectroscopy was employed for all intermediates and final compounds. Aromatic protons were consistently observed as multiples in the region of δ 7.1



Where,

Ar=phenyl for CHL1

Ar=4-dimethylaminophenyl for CHL2

Ar= 4-bromo phenyl for CHL3

Ar= 2-Chloro phenyl for CHL4

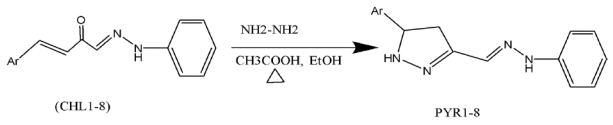
Ar =4-hydroxy phenyl for CHL5

Ar= 4-fluoro phenyl for CHL6

Ar= 2-methoxy phenyl for CHL7

Ar= pyrrole for CHL8

Figure 1: Chalcone intermediates (CHL1-8) scheme for synthesis.



Where,

Ar=phenyl for PYR 1

Ar=4-dimethylaminophenyl for -PYR -2

Ar= 4-bromo phenyl for PYR -3

Ar= 2-Chloro phenyl for PYR -4

Ar =4-hydroxy phenyl for PYR -5

Ar= 4-fluoro phenyl for PYR -6

Ar= 2-methoxy phenyl for PYR -7

Ar= pyrrole for PYR -8

Figure 2: Pyrazoline derivatives (PYR1-8) scheme of synthesis

Table 1: Information of compounds

Compound	*Molecular formula*	*Melting point (°C)*	*Mol. weight*	*Yield (%)*
CHL-1	$C_{16}H_{14}N_2O$	126-128	250.29	72
CHL-2	$C_{18}H_{19}N_3O$	165-167	293.85	55
CHL-3	$C_{16}H_{13}BrN_2O$	212-214	329.18	68
CHL-4	$C_{16}H_{13}ClN_2O$	140-142	283.7	81
CHL-5	$C_{16}H_{14}N_2O_2$	165-167	266.29	58
CHL-6	$C_{16}H_{13}FN_2O_2$	147-149	268.28	68
CHL-7	$C_{17}H_{16}N_2O_2$	175-177	280.37	62
CHL-8	$C_{14}H_{13}N_3O$	136-138	239.27	64
PYR1	$C_{16}H_{16}N_4$	144-146	264.3	70
PYR2	$C_{18}H_{21}N_5$	218-220	307.38	59
PYR3	$C_6H_{15}BrN_4$	183-185	343.21	68
PYR4	$C_{16}H_{15}ClN_4$	164-166	298.7	79
PYR5	$C_{16}H_{16}N_4O$	179-181	280.3	59
PYR6	$C_{16}H_{15}FN_4$	186-188	282.3	69
PYR7	$C_{17}H_{18}N_4O$	218-220	294.3	64
PYR8	$C_{14}H_{15}N_5$	209-211	253.29	72

Table 2: Spectral values of various chalcones and pyrazoline derivatives

Compound	*FTIR (cm^{-1})*	*^{1}HNMR(DMSO- d6,PPM) δ*	*Mass(m/z)*
CHL1	3342 (N-H), 3062 (C-H), 1545(C=N), 1512 (C=C), 1643 (C=O)	6.4,6.6 (s, Ali-H, 2H), 6.9 (s, N=C-H,1H), 7.4-7.8 (m, Ar-H, 9H), 9.8 (s, NH, 1H)	250.2 (M+), 251.1 (M+1)
CHL2	3368 (N-H), 3065(C-H), 1654(C=O), 1518 (C=N), 1484 (C=C)	3.5 (s, $N(CH_3)_2$, 6H), 6.3,6.4 (s, Ali-H, 2H), 6.5 (s, N=C-H, 1H), 7.2-7.6 (m, Ar-H, 9H), 10.2 (s, NH, 1H)	293.1 (M+)
CHL3	3359(N-H), 3042(C-H), 1648(C=O), 1523 (C=N), 1469 (C=C), 810 (C-Br)	6.2-6.3 (s, Ali-H, 2H), 6.6 (S, N=C-H, 1H), 7.1-7.6 (m, Ar-H, 9H), 9.9 (S, NH, 1H)	329.1 (M+)
CHL4	3382 (N-H), 3058 (C-H), 1653 (C=O), 1528 (C=N), 1449 (C=C), 853(C-Cl)	6.1,6.2 (s, Ali-H, 2H), 6.7 (s, N=C-H, 1H), 7.2-7.5 (m, Ar-H, 9H), 9.7(s, NH, 1H)	283.2 (M+), 285.1 (M+2)
CHL5	3359(N-H), 3079(C-H), 1672 (C=O), 1506(C=N), 1479 (C=C), 3410 (O-H)	6.7,6.5 (s, Ali-H, 2H), 6.9 (s, N=C-H, 1H), 7.2-7.7 (m, Ar-H, 9H), 10.3(s, NH, 1H)	266.2 (M+)
CHL6	3365 (N-H), 3056 (C-H), 1688 (C=O), 1514 (C=N), 1465 (C=C), 810 (C-F)	6.2,6.4 (s, Ali-H, 2H), 6.8 (s, N=C-H, 1H), 7.1-7.6 (m, Ar-H, 9H), 10.4(s, NH, 1H)	268.2 (M+)
CHL7	3379 (N-H), 3042 (C-H), 1694 (C=O), 1508 (C=N), 1472 (C=C)	6.1,6.3 (s, Ali-H, 2H), 6.9 (s, N=C-H, 1H), 7.3-7.8 (m, Ar-H, 9H), 9.9 (s, NH, 1H), 3.8 (s, OCH3, 3H)	280.2 (M+)
CHL8	3326 (N-H), 3062 (C-H), 1686 (C=O), 1561 (C=N), 1524 (C=C)	6.1,6.2 (s, Ali-H, 2H), 6.8 (s, N=C-H, 1H), 7.2-7.8 (m, Ar-H, 7H), 9.8 (s, NH, 1H), 10.4 (m, pyrrole, 1H)	239.2 (M+)
PYR1	3217(N-H),3097(C-H),1586(C=N), 1541(C=C)	8.9(s, 2×NH, 2H), 7.2-8.1(m, Ar-H, 1H), 3.3(dd,1H,Hα),3.7(dd,1H,Hβ), 3.8(dd,1H, Hγ)	264.3(M+)
PYR2	3204(N-H),3025(C-H),1565(C=N), 1529(C=C), 2835(aliphatic C-H)	8.7(s, 2×NH, 2H), 7.1-7.9(m, Ar-H, 9H), 6.9(s, N=C-H, 1H),3.1S,2×CH_3, 6H), 3.2(dd,1H,Hα),3.4(dd,1H,Hβ), 3.6(dd,1H, Hγ)	307.3(M+)
PYR3	3284(N-H), 3018(C-H),1518(C=N), 1545(C=C),910(C-Br)	8.8(s,2×NH, 2H), 7.0-7.7(m, Ar-H, 9H), 7.2(s, N=C-H,1H), 3.1(dd, 1H, Hα),3.4(dd, 1H, Hβ), 3.7(dd,1H, Hγ)	343.2(M+)
PYR4	3290(N-H), 3026(C-H),1535(C=N), 1560(C=C),840(C-Cl)	8.6(s, 2×NH, 2H), 7.1-7.7(m, Ar-H, 9H),7.3(s, N=C-H, 1H), 3.3(dd, 1H, Hα),3.5(dd, 1H, Hβ), 3.8(dd,1H, Hγ)	298.7(M+) 300.6(M+2)
PYR5	3405(O-H), 3285(N-H),3040(C-H)1528(C=N), 1542(C=C)	8.9(s, 2×NH, 2H), 7-7.6(m, Ar-H, 9H),7.2(s, N=C-H, 1H), 3.2(dd, 1H, Hα),3.4(dd, 1H, Hβ), 3.5(dd,1H, Hγ)	280.3(M+)
PYR6	3215(N-H), 3042(C-H),1549(C=N), 1569(C=C),795(C-Br)	8.7(s, 2×NH, 2H), 7-7.7(m, Ar-H, 9H),7.1(s, N=C-H, 1H), 3.3(dd, 1H, Hα),3.5(dd, 1H, Hβ), 3.7(dd,1H, Hγ)	282.3(M+)
PYR7	3324(N-H), 3080(C-H),1549(C=N), 1528(C=C)	8.8(s, 2×NH, 2H), 7.4-7.9(m, Ar-H, 9H),7.4(s, N=C-H, 1H), 3.7(s, OCH_3, 3H), 3.2(dd, 1H, Hα),3.5(dd, 1H, Hβ), 3.6(dd,1H, Hγ)	294.3(M+)
PYR8	3341(N-H), 3064 (C-H),1565(C=N), 1510(C=C),3342(N-H of pyrrole)	8.6(s, 2×NH, 2H), 7-7.9(m, Ar-H, 8H),7.5(s, N=C-H, 5H),8.7(s, NH of pyrrole, 1H) 3.3(dd, 1H, Hα),3.5(dd, 1H, Hβ), 3.7(dd,1H, Hγ)	253.2(M+)

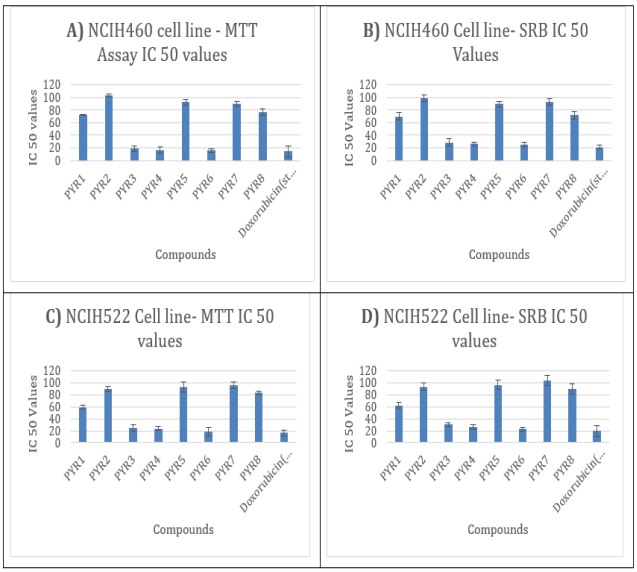


Figure 3: MTT and SRB assays on cell lines

Table 3: Cytotoxicity activity IC_{50} values of pyrazoline derivatives

Compound	*NCIH460*		*NCIH522*	
	MTT	*SRB*	*MTT*	*SRB*
PYR1	72.16 ± 1.28	69.76 ± 6.04	59.34 ± 3.19	62.57 ± 4.09
PYR2	102.37 ± 2.36	98.45 ± 5.39	89.37 ± 4.36	93.46 ± 6.17
PYR3	18.72 ± 4.26	28.35 ± 6.17	24.57 ± 5.37	30.18 ± 3.19
PYR4	16.53 ± 5.07	26.13 ± 3.15	23.46 ± 3.07	26.34 ± 4.16
PYR5	92.34 ± 4.17	89.36 ± 4.36	92.93 ± 8.06	96.34 ± 8.09
PYR6	15.69 ± 3.16	25.37 ± 3.62	18.65 ± 6.39	22.57 ± 3.06
PYR7	89.65 ± 4.17	93.16 ± 5.13	96.37 ± 5.32	103.57 ± 9.07
PYR8	76.49 ± 5.09	71.57 ± 6.19	82.75 ± 3.05	89.67 ± 9.06
Doxorubicin(std)	14.69 ± 8.78	21.14 ± 3.08	16.21 ± 4.07	19.52 ± 9.03

to 7.9 ppm across all compounds. In chalcone intermediates, two aliphatic protons appeared as singlets at δ 6.1 to 6.5 ppm. Additionally, one proton of the amine group was detected as a

Table 4: Anti-inflammatory activity of pyrazoline derivatives by bovine serum albumin method

Conc (µg/mL)	*Standard*	*PYR1*	*PYR2*	*PYR3*	*PYR4*	*PYR5*	*PYR6*	*PYR7*	*PYR8*
10	26	18	15	20	21	20	22	17	18
20	37	27	26	28	31	27	32	29	24
30	48	39	38	40	44	40	45	39	41
40	59	51	53	50	56	52	57	49	51
50	74	69	68	67	71.4	66	69	65	67

*std = Diclofenac sodium

Table 5: Anti-inflammatory activity data of pyrazoline derivatives by egg albumin denaturation method (%inhibition)

Conc (µg/mL)	*Standard*	*PYR1*	*PYR2*	*PYR3*	*PYR4*	*PYR5*	*PYR6*	*PYR7*	*PYR8*
10	28	19	21	22	23	20	24	18	22
20	39	26	28	30	32	27	34	29	28
30	48	37	38	36	41	37	44	35	36
40	62	45	47	44	53	50	54	49	46
50	73	67	66	67	69.9	65	68.3	64	66

*std = Diclofenac sodium

singlet at δ 8.9 to 11.2 ppm in all compounds, and one proton of the N=C-H group was observed as a singlet at δ 6.5 to 7.2 ppm. Signals of protons in the pyrazoline ring were observed as Hα, Hβ, and Hγ, appearing as doublets of doublets integrating for three protons in the regions of δ 2.9 to 3.1, 3.2 to 3.5, and 3.7 to 3.8, respectively. In CHL8 and PYR8, the NH proton of the pyrazole moiety was observed at δ 8.6 and 10.2 ppm, respectively.

The three methoxy group protons were detected as a singlet at approximately δ 3.6 ppm in CHL7 and 3.5 ppm in PYR7, in line with typical values. Molecular ion peaks were observed for intermediate chalcones at 250.2, 293.1, 329.1, 283.2, 266.2, 268.2, 280.2, and 239.2, respectively for CHL1-8 and CHL2, consistent with calculated masses. In CHL4, an M+2 peak was seen due to the presence of a chlorine isotope. Molecular ion peaks of pyrazoline derivatives were observed at 264.2, 307.4, 343.1, 332.2, 280.3, 282.1, 294.3, 253.2, respectively for PYR1-8, respectively, exhibited mass values consistent with the calculated mass for all pyrazoline derivatives. In PYR4, the presence of a chlorine isotope resulted in the observation of the M+2 peak. Standard literature references corroborated the characterization of all compounds.[28,29] The physical data for compounds (CHL1-8) and (PYR1-8) are documented in Table 1.

In-vitro anticancer evaluations of pyrazoline derivatives were conducted utilizing the above methods on NCIH-460 and NCIH-522 cell lines of lung cancer, with doxorubicin serving as the standard drug. Cytotoxic activity of PYR3, PYR4 and PYR6 revealed that the presence of electron-withdrawing groups in the benzene ring induces a good cytotoxic effect, which is represented in Table 3 and Figure 3(A, B, C, D)

The capacity of pyrazoline to hinder protein denaturation, which constitutes the mechanism of its reducing inflammation property, was explored using the bovine serum albumin method and the egg albumin method. In BSA denaturation method, all the tested compounds showed inhibition in the increasing order of concentration. PYR4 and PYR6 showed moderate anti-inflammatory activity against diclofenac sodium as a standard drug. Compound PYR4 and PYR6 showed inhibition of 71.4 and 69% at 50 µg/mL, respectively, which may be due to the presence of strong electron-withdrawing groups like chloro and fluoro in the benzene ring of pyrazoline. The rest of the tested compounds showed moderate activity. In the egg albumin denaturation method, compounds PYR4 and PYR6 showed 69.9 and 68.3% inhibition at 50 µg/mL, respectively. The rest of the tested compounds showed moderate activity which are represented in Tables 4 and 5.

CONCLUSION

A number of pyrazoline derivatives were synthesized from novel chalcones in the current study. Good yields were obtained from every compound that was synthesized. Spectra data were used to characterize each of the title compounds after they underwent screening for both *in-vitro* cytotoxic and anti-inflammatory activity. Our investigation's findings indicate that PYR3, PYR4, and PYR6 are good cytotoxic agents and that PYR4 and PYR6 are useful anti-inflammatory agents. Nevertheless, additional screening is necessary to determine the preclinical and clinical significance of these compounds**.**

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