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A Convenient green Synthesis and Characterization of Some Pyrazolone derivatives: Anticancer Activity

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Abstract

This study presents a convenient and eco-friendly method for synthesizing 5-aryl pyrazole-3-one derivatives through the oxidation of 4-arylidinepyrazolidinediones using chromium trioxide nanoparticles in an acidic medium. The reaction was facilitated by sonication in acetic acid, yielding the desired compounds in good to excellent yields. Additionally, N-substituted pyrazolone derivatives were synthesized via sonication with various alkylating agents in the presence of the ionic liquid [DABCO-EtOH][AcO], achieving remarkable yields. The synthesized compounds were characterized using elemental analysis, and extensive spectral techniques, including 1D and 2D NMR. The biological evaluation of selected derivatives against three tumor cell lines—HepG-2, MCF-7, and A-549—demonstrated significant cytotoxic activity, with compounds 7c, 12, and 16 exhibiting promising potential as anticancer agents. The biological evaluation of selected derivatives against three tumor cell lines HepG-2, MCF-7, and A-549 demonstrated significant cytotoxic activity. The IC50 values of the tested compounds revealed that compound 7c exhibited IC50 values of 9.57 µg/ml, 12.92 µg/ml, and 14.16 µg/ml against HepG-2, MCF-7, and A-549 cell lines, respectively. Compound 12 demonstrated significantly lower IC50 values of 2.59 µg/ml, 4.42 µg/ml, and 2.93 µg/ml against the same cell lines, indicating potent cytotoxic activity. Additionally, compound 16 showed IC50 values of 9.55 µg/ml, 13.33 µg/ml, and 10.54 µg/ml against HepG-2, MCF-7, and A-549 cells, respectively. These results suggest that compounds 7c, 12, and 16 have promising potential as anticancer agents, particularly due to their lower IC50 values compared to the standard drug, doxorubicin. This research underscores the advantages of green chemistry in pharmaceutical synthesis, highlighting its effectiveness in producing biologically active compounds.

Keywords: Green; Sonosynthesis; Investigation; 2D NMR; Pyrazolone

1. Introduction

The present chemical industries [1] have been assessed using the "triple bottom line feature of sustainable chemistry." Sustainable chemical manufacturing[2, 3] is becoming more and more popular as a way to lessen the environmental impact of pharmaceutical synthesis. The manufacture of sustainable chemistry requires the use of eco-friendly reagents and solvents, such as water, when it is available[4-6], limited waste output, and atom economy. Water is now used more often as a green solvent as a result.[7-9]Green synthesis presents several advantages over traditional methods, primarily focusing on environmental sustainability and safety. One of the key benefits is the reduction of hazardous chemicals and solvents, which minimizes toxic waste and environmental pollution. [10]This approach also enhances safety for researchers, as it employs non-toxic and biodegradable materials, unlike traditional methods that often involve dangerous substances. Additionally, green synthesis can be more cost-effective by utilizing renewable resources, reducing the need for expensive raw materials and hazardous waste disposal.[11] It typically operates under milder conditions, leading to lower energy consumption compared to traditional processes that may require extensive heating. Furthermore, the products of green synthesis are generally more biocompatible, making them suitable for pharmaceutical and biomedical applications. This method encourages innovation in sustainable practices, fostering a shift towards industrial processes that align with global environmental goals, thus promoting a more sustainable future.[12]The pyrazole moiety is regarded as a versatile nucleus[13-15]; antimicrobial[16], antifungal[17], antitubercular[18], anti-inflammatory[19], anticonvulsant[20], anticancer [21-24],antiviral[25], inhibitory of angiotensin-converting enzymes (ACE), neuroprotective, antagonist of cholecystokinin-1 receptor, and ER ligand activity [26-28]have all been reported in its analogue derivatives. Derivatives of pyrazole have been regarded as essential elements in the search for antimicrobial drugs[29]. As we go forward with developing eco-friendly synthetic facility technologies, we will continue to focus on the important advantages of rate acceleration in green solvents[30-37]. Herein describes a straightforward and easy process for making pyrazole derivatives in excellent yields, which has the advantage of green chemistry rather than the reported conventional methods and is anticipated to have intriguing biological activity especially anticancer applications

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2. Experimental (Materials and Methods)

All solvents and reagents used for the synthesis of target compounds were of commercial grade without further purification before use. The time required for the completion of each reaction was monitored by TLC. The uncorrected melting points were determined using a Gallenkamp electronic melting point device. An Elementar Vario EI device was used to conduct elemental analysis. A Shimadzu 470 infrared spectrophotometer was used to record infrared spectra. Using TMS as an internal standard, a ^{1}H NMR LA 400 (Jeol) was used to measure the ^{1}H and ^{13}C NMR spectra at 400 and 100 MHz. The Hewlett-Packard model MS 5988 Spectrometer was used to record mass spectrometric studies utilizing the JOEL-JMS 600 and the direct inlet technique at 70 eV. Chromium trioxide nanoparticles were synthesized by the reported solvothermal method[38]. [DABCO-EtOH] [AcO] was synthesized by reported method [39]. The in vitro cytotoxicity activities of compounds **7a**, **7c**, **7d**, **12**, **16**, and **18** were evaluated using the MTT assay against three tumor cell lines: HepG-2 (Hepatocellular carcinoma), MCF-7 (Breast carcinoma), and A-549 (Lung carcinoma). The assays were conducted in the presence of doxorubicin as a standard reference drug.

*2.1. Synthesis of 5-aryl-1-phenylpyrazole-3-one **(7a-d)***

A 50 mL round flask was charged with a solution of 4-arylidene-1-phenyl-3,5-pyrazolidinedione **(1a-d)** (0.01mol) dissolved in glacial acetic acid (30 ml) and nano chromium trioxide (0.01 mol) dissolved in 5 ml of water. The reaction vessel had been immersed in the ultrasonic bath and sonicated at room temperature for 60 minutes. After filtration, the resulting filtrate was diluted with water to give a precipitate which was collected, crystallized from the proper solvent, and identified as 5-aryl-1-phenylpyrazole-3-one **(7a-d).** The results are summarized in Tables **2** and **3**.

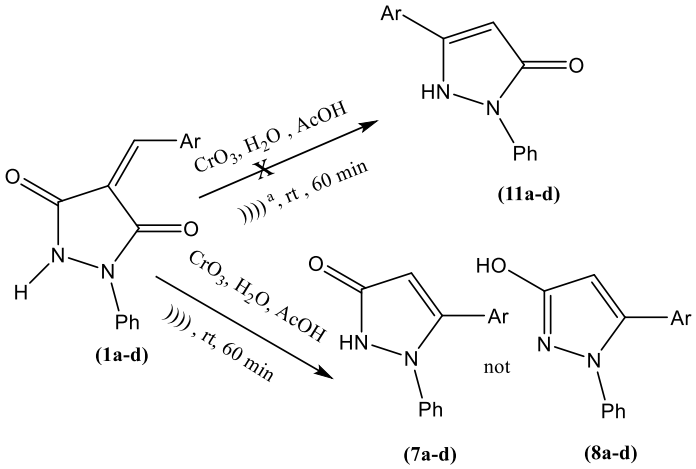
2.2. Reaction of 4-(p-methoxybenzylidene)-1-phenyl-3,5-pyrazolidinedione(1c) with ethyl iodide, methyl iodide, acetyl chloride, and benzoyl chloride

A 50 mL round flask had been charged with suspension of 4-arylidene-1-phenyl-3,5-pyrazolidinedione (1c) (0.002 mol) in ethanol (20 ml) , [DABCO-EtOH] [AcO](3 mol %) and the proper agent (ethyl iodide, methyl iodide, acetyl chloride and benzoyl chloride) (0.0025 mol). The reaction vessel had been immersed in the ultrasonic bath and sonicated at 40 0C for 2 hours. After filtration, the resulting filtrate was diluted with water to give a precipitate which was collected and recrystallized from the proper solvent and identified as N-substituted pyrazolone derivatives (12,14,16 and 18 respectively). The results are summarized in Tables **2** and **3**. Under vacuum, the catalyst was extracted from the aqueous layer, cleaned with n-hexane, and then utilized again for the subsequent reactions.

Results and Discussion

2.3. Synthesis

Green chemistry synthesis is characterized by its straightforward technique and quick, simple product isolation and purification. Because of these benefits, we developed an easy-to-use, environmentally friendly approach to the synthesis of 5-aryl pyrazole-3-one derivatives (**7a-d**) and some N-substituted pyrazolone derivatives (**12b**, **14b, 16b, and 18b**) in good to excellent yields. The oxidation process trial to get 5-aryl pyrazole-3-one derivatives by using selenium dioxide failed. Chromium trioxide [40] is a powerful oxidizing agent that has been used in various chemical reactions. When discussing its use as an oxidizing agent on chalcones (**1a-d**), it can facilitate the oxidation to form 5-arylpyrazole-3-one derivatives in moderate yield under conventional conditions[41].When used in nanoparticle form, its reactivity and surface area are enhanced, which can lead to improved efficiency and selectivity in oxidation reactions. And thus, the use of CrO_3 nanoparticle[42, 43] in acidic medium for oxidation of **1a-d** under ultrasonic conditions improved the catalytic activity and selectivity of the oxidation process to afford **7a-d** in excellent yields not the isomers **8a-d** or **11a-d** (Scheme **1**).



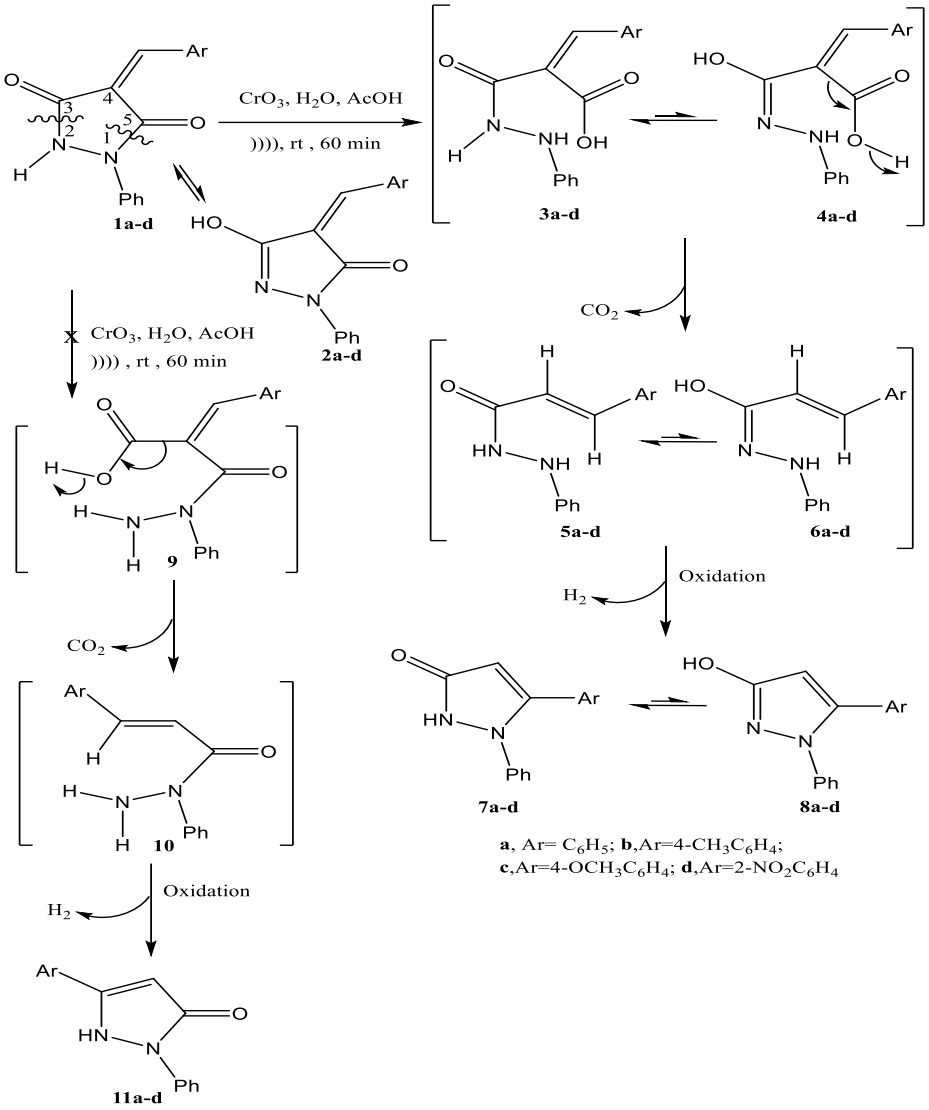
Ar: **7a**= C_6H_5, **7b**=4-$CH_3C_6H_4$,**7c**=4-$OCH_3C_6H_4$; **7d**=2-$NO_2C_6H_4$

a)))): ultrasonic irradiation (intensity of 80%)

Scheme 1. Formation of compounds **7a-d**.

The starting compounds (**1a–d)** only lose one carbon monoxide molecule, according to elemental and mass spectrometric examination of the oxidation products that are produced. The amidic carbonyl groups (1630-1670 cm^{-1}) are present in the isolated compounds, according to their infrared absorption spectra. An olefinic proton was detected at an δ value of around 5.80-

5.88 ppm by ^{1}H NMR spectrum analysis. By using the following suggested reaction mechanism (Scheme **2**), we were able to use these analytical and spectral data to propose 5-aryl-1-phenyl-4-pyrazolin-3-ones (**7a-d)** as the reaction products rather than **8a-d** or **11a-d**.



Scheme 2. Proposed reaction mechanism for the formation of **8a-d**.

The production of **7a-d** depends on oxidative cleavage of compounds (**1a-b)** to give the α, β-unsaturated carboxylic acids **3a-d** and/or **4a-d** tautomer which are easily decarboxylated to give the hydrazides **5a-d** and/or hydrazones **6a-d** tautomers. **7a-d** and/or **8a-d** are produced by aromatizing molecules **5a-d** and/or **6a-d** by dehydrogenating them with chromium trioxide (Scheme 2). Since the N (1)–C (5) bond in compounds (**1a–d)** is the one that is easiest to break, the production of compounds **7a–d** is predicated on its cleavage[44]. The tautomeric forms of **1a-d** with the double bond between N (2)–C (3) to the tautomer **2a-d** make it impossible to cleave the N (2)–C (3) bond to get **11a-d**. By comparing derivative (**7a-d)** to a genuine sample that was prepared using a recognized technique, its authenticity was verified[45]. The physical and chemical characteristics of compound **11a-d**, which is well-known in the literature[46-50], are different from those of derivatives **7a-d**. Therefore, in contrast to the methods described in the literature[45], the oxidation process of CrO_3 nanoparticles constitutes a generic and practical method to produce **7-a-d** from readily available compounds. ^{1}HNMR and ^{13}CNMR revealed the 3-pyrazolone derivatives **7a-d** not the enolic form **8a-d**. ^{1}HNMR revealed that in addition to NH protons at δ values of ~9.93-12.16, there were alkene protons at δ values of ~5.80-5.88 ppm.^{13}CNMR showed an amidic carbonyl carbon at δ values of ~161.54-162.82 ppm. Moreover, intensive 2D NMR measurements were done in DMSO-d_6 for **7c.** COSY (Homonuclear Correlation Spectroscopy) measurements on compound (**7c)** showed that in the corresponding structure of **7c** the OCH_3 group is directly bonded by a quaternary carbon atom, i.e. there are no neighboring protons for the OCH_3 group and hence appear as one contour as it is only correlated with itself. The olefinic proton is flanked between the carbonyl group and quaternary carbon atom, i.e. there are no neighboring protons for the olefinic proton and hence appears as one contour as it only correlates with itself. The aromatic protons on the phenyl and the *p*-methoxyphenyl groups gave eight contours that show the correlation of the aromatic protons with their neighbor protons and with themselves.

Table 1. Common fragments (cation or radical cation) in the mass spectra of 7a-d derivatives under electron impact (70 ev).

Compound	M (R.I%)	M-H (R.I%)	M-H_2O (R.I%)	M-CHO (R.I%)	$ArNC_6H_5$ (R.I%)	$C_7H_6N_2O$ (R.I%)	$C_6H_5N_2O$ (R.I%)
7a	236 (100)	235 (40.2)	218 (1.8)	207 (23.8)	180 (11.8)	-	133 (4.3)
7b	250 (98.7)	249 (40.4)	-	221 (17.10)	-	135 (5.4)	133 (3.1)
7c	266 (100)	265 (18.2)	248 (6.3)	-	210 (6.3)	135 (4.3)	133 (8.8)
7d	281 (10.9)	-	-	-	-	135 (5.2)	133 (7.9)
Compound	C_7H_5NO (R.I%)	$C_6H_8N_2$ (R.I%)	$C_6H_6N_2$ (R.I%)	C_6H_5CO (R.I%)	C_6H_5CN (R.I%)	C_6H_5NH (R.I%)	C_6H_5 (R.I%)
7a	119 (1.1)	108 (1.7)	106 (1.8)	105 (6.4)	103 (13.2)	92 (3.0)	77 (53.9)
7b	119 (11.9)	108 (4.7)	106 (3.5)	105 (16.6)	103 (12.2)	92 (16.3)	77 (100)
7c	119 (5.2)	-	106 (4.0) (6.3)	105 (5.8)	92 (10.0)	135 (4.3)	77 (28.8)
7d	119 (3.3)	108 (5.9)	106 (6.4)	105 (19.0)	92 (5.6)	135 (5.2)	77 (38.3)

Table 2. Melting points, yields, and elemental analyses of all synthesized compounds.

Compound	M.P. ºC (Yield %)	Color (Solvent of crystallization)	Mol. Formula (M. W.)	Analysis (Calcd. /Found %)		
				C	H	N
7a	250-252 (90.83)	Yellow amorphous (Benzene)	C12H12N2O5 (236.27)	76.25 76.10	5.11 5.07	11.85 10.78
7b	252-254 (91.86)	Yellow amorphous (Ethanol)	C16H14N2O (250.29)	76.78 76.77	5.63 5.24	11.19 10.91
7c	225-227 (92.39)	Yellow amorphous (Benzene)	C16H14N2O2 (266.29)	72.16 71.85	5.29 4.78	10.52 10.35
7d	273-275 (90.82)	Brown amorphous (Ethanol)	C15H11N3O3 (281.26)	64.05 64.21	3.91 3.89	14.94 14.65
12	145-147 (88.34)	Orange flakes (petroleum ether 40-60 oC)	C19H15N3O3 (322.13)	70.79 69.95	5.63 5.43	8.69 9.56
14	142-144 (90.35)	Orange flakes (petroleum ether 40-60 oC)	C18H16N2O3 (308.12)	70.12 70.05	5.23 5.12	9.09 8.80
16	170-172 (87.5)	Yellow amorphous (Chloroform/petroleum ether 40-60 oC, 1:1)	C19H16N2O4 (336.11)	67.85 67.44	4.80 4.45	8.33 8.80
18	140 (90.0)	Orange amorphous (Tetrahydrofuran)	C24H18N2O4 (398.13)	72.35 72.12	4.55 4.26	7.03 7.01

The CHSHF (^{13}C Detected Heteronuclear Shift Correlation 2D NMR) spectrum of **7c** shows seven contours characteristics for seven H-C connectivity which includes O$\underline{CH_3}$ protons, olefinic proton directly bonded to the olefinic carbon atom(C4) and five H-C connectivity characteristics for the phenyl and the *p*-methoxyphenyl groups. All compounds **(7a-d)** have been examined by mass spectrometric analysis. Each compound gave a fragmentation pattern agreeing with its suggested structure as listed in (Table **1)**.

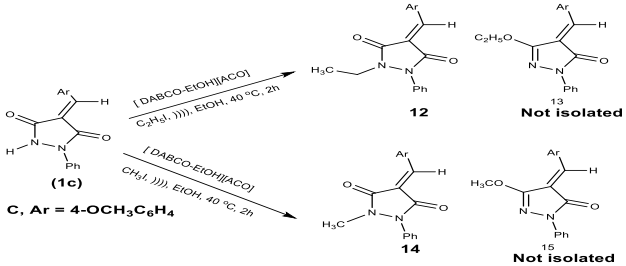
[DABCO-EtOH][AcO],[37, 38] (a deep eutectic solvent or DES) is used in the alkylation of protonated nitrogen (NH). It is crucial, as it acts both as a green solvent and a catalytic system that promotes the alkylation, acylation and benzoylation of NH efficiently. It is a green solvent that offers a more sustainable alternative to traditional solvents, minimizing environmental impact, where it is biodegradable and less toxic compared to conventional organic solvents. In organic synthesis, [DABCO-EtOH][AcO] (a complex of 1,4-diazabicyclo [2.2.2]octane (DABCO), ethanol, and acetate) is used as a mild and efficient catalyst for the alkylation of protonated nitrogen (NH). This reagent complex facilitates nucleophilic substitution reactions, enhancing the reaction between protonated nitrogen (NH) and alkyl halides or alkylating agents. It allows the reaction to occur under mild conditions, reducing the need for harsh reagents or high temperatures. It can also act as a phase-transfer catalyst, ensuring that both hydrophilic and hydrophobic reactants remain in solution, leading to an efficient alkylation reaction. [DABCO-EtOH][AcO] was prepared according to the previously reported method [39] . The behavior of 4-arylidine derivative (**1c**) towards alkylation, acylation, and benzoylation reagents was investigated in the presence of ionic liquid, [DABCO-EtOH][AcO]. This study aims to find the site of alkylation, acylation and benzoylation, besides improving the reaction yield

under green chemistry conditions. FT-IR, ^{1}H NMR and ^{13}C NMR analysis of the reaction products prove that N-alkylation, N-acylation, and N-benzoylation occur. The following (**Schemes 3 & 4**) represent alkylation, acylation, and benzoylation of **1c.** Table 3. Represents the Spectral data of all synthesized compounds.^{1}H NMR spectrum of **12** in $CDCl_3$ shows the following signals which conform with its structure: a triplet at δ 1.55 (3H; CH_3, J = 6 HZ), a singlet at δ 3.9 (3H; OCH_3), a quartet at δ 4.55(2H; CH_2, J=6 HZ), and a multiplet centered at δ7.5 (10H,9 Ar-H and 1H, =CH).

Table 3. Spectral data of all synthesized compounds.

No.	Spectral data
7a	FT-IR: 3150 (NH),3050(SP2 CH), 1680 (amidic CO), 1590 (C=C). 1H NMR (DMSO-d6): 9.93(s, 1H, NH), 7.10-7.27 (m, 10H, ArH), 5.80(s,1H, =CH).13C NMR (DMSO-d6): 161.54(CO), 142.94 (=C), 139.66,130.02, 128.0,128.21, 127.84, 127.78 ,124.76, 124.06, 93.90 (=CH), MS: m/z 236 (M,+100 %).
7b	FT-IR: 3160 (NH), 3050(SP2 CH),2950 and 2850 (SP3 CH), 1660 (amidic CO), 1580 (olefinic double bond). 1H NMR (DMSO-d6): 10.15(s, 1H, NH), 7.08-7.7.31 (m, 9H, ArH), 5.87(s,1H, =CH), 2.26(s,3H, CH3).13C NMR(DMSO-6):161.65(CO),143.19(=C),139.96,137.74,129.06,128.78,128.16,126.40,124.46,93.99(=CH), 20.71(CH3). MS: m/z 236 (M,+100 %).MS: m/z 250 (M,+ 98.7%).
7c	FT-IR: 3220 (NH), 3050(SP2 CH),2950 and 2850 (SP3 CH), 1680 (amidic CO), 1600 (olefinic double bond). 1H NMR (DMSO-d6): 10.14(s,1H, NH),6.87-7.7.32(m,9H, ArH),5.88(s,1H, =CH),3.72(s,3H,OCH3). 13C NMR(DMSO-6):161.67(CO),143.07(=C),140.04,129.58,128.82,126.35,159.17,113.98,124.48, 122.73,93.79 (=CH), 55.13 (OCH3). MS: m/z 266 (M,+ 100%).
7d	FT-IR: 3150 (NH), 3050(SP2 CH),2950 and 2900 (SP3 CH), 1680 (amidic CO), 1590 (olefinic double bond). 1H NMR (DMSO-d6): 12.16(s, 1H, NH), 7.12-7.90 (m, 9H, ArH), 5.88(s,1H, =CH).13C NMR (DMSO-d6): 162.82(CO),154.88(=C),143.03,148.30,135.95,130.70,129.02,128.86,123.66,119.44,113.02,87.74(=CH). MS: m/z 281 (M,+ 10.9 %).
12	FT-IR: 3050 (SP2 CH), 2900 and 2800 (SP3 CH),1680 (CO),1615 (olefinic double bond).1H NMR (CDCl3): 3.9 (s,3H, OCH3), 4.1 (s,3H, NCH3), 7.50 (m, 10H, 9ArH and 1H, =CH). 13C NMR(CDCl3):163.87 (CO), 162.72(CO),160.56,146.16,138.91,136.82, 128.75, 126.14 (=C),124.13, 118.76, 117.01 (=CH), 114.42, 55.17 (N-CH3) ,55.05(OCH3). MS: m/z 308 (M,+ 100 %).
14	FT-IR: 3050 (SP2 CH),2900 and 2850 (SP3 CH),1680 (CO),1620 (olefinic double bond). 1H NMR (CDCl3): 1.55(t,3H, CH3, J = 6 HZ), 3.9 (s,3H, OCH3), 4.55(q,2H, CH2, J=6HZ) 7.50 (m, 10H, 9ArH and 1H, =CH). 13C NMR (CDCl3): 164.50(CO), 164.97(CO), 160.56,146.16, 138.91, 136.82, 128.75, 125.97 (=CH) ,124.13, 118.76, 118.29(=C),114.42, 55.17 (N-CH3), 55.05 (OCH3). MS: m/z 308 (M+ , 100 %).
16	FT-IR: 3040(SP2 CH), 2900 and 2850(SP3 CH),1710(CO),1580(olefinic double bond).1H NMR (CDCl3): 2.65 (s,3H, COCH3),3.9 (s,3H, OCH3),7.8 (m, 10H, 9ArH and 1H, =CH). 13C NMR (CDCl3): 166.32(CO), 165.85 (CO), 163.81(CO), 155.59,155.165, 138.56, 128.78, 126.76(=C), 125.61, 122.28, 121.76, 114.75, 112.75(=CH), 55.77 (OCH3), 25.29 (CH3). MS: m/z 336 (M, + 3.6 %).
18	FT-IR: 3040 (SP2 CH), 2900 and 2850 (SP3 CH),1730 (CO),1620 (olefinic double bond). 1H NMR (DMSO-d6):3.9 (s,3H, OCH3),7.5 (m, 15H, 14ArH and 1H, =CH). 13C NMR (DMSO-d6): 167.28(CO), 163.91(CO), 163.87(CO),161.94,135.94,132.84, 131.79,130.72, 129.23,128.57,125.51, 124.97, 119.34(=C), 118.79, 114.51, 113.63(=CH), 55.69 (OCH3). MS: m/z 398 (M+ , 8.3 %).

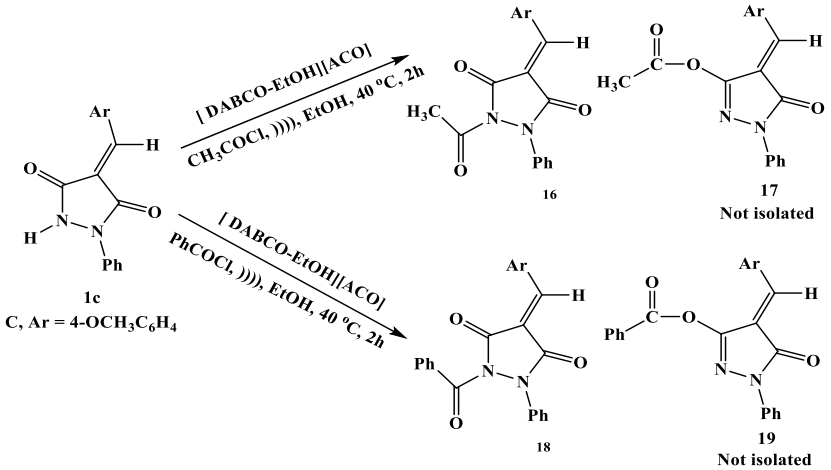
^{13}C NMR of **12** gave signals at δ: 164.503 (CO), 164.973 (CO), 125.972(=CH),163.753,145.958, 136.752, 128.605, 124.031, 123.531, 118.722, 114.237 (aromatic carbon atoms), 63.985(OCH_3), 55.514 (CH_3) and 39.763(CH_2). The combination of ^{1}H NMR and ^{13}C NMR proves the alkylation of N-2 which represents the dicarbonyl structure of **12**. ^{1}H NMR spectrum of **14** in $CDCl_3$ shows the following signals in conformity with its structure: a singlet at δ 3.9(3H; OCH_3), singlet at δ 4.1(3H; NCH_3) and a multiplet centered at δ 7.5 (10H,9 aromatic protons and 1H, =CH).^{13}C NMR of compound **14** in $CDCl_3$ gave conclusive proof about structure **14**. ^{13}C NMR shows signals at δ: 163.870 (CO), 162.723 (CO), 126.140 (=CH), 160.562, 146.164, 138.917, 136.825, 128.752, 124.134, 114.428, 118.766 (aromatic carbon atoms), 55.055 (NCH_3) and 55.176 (OCH_3). Acetylation of **1c** using acetyl chloride gave the N-acetyl derivative (**16)**. ^{1}H NMR in $CDCl_3$ gave the following structural signals: a singlet at δ 2.65(3H; $COCH_3$); a singlet at δ 3.9(3H; OCH_3) and a multiplet centered at 7.8(10H,9 aromatic protons and 1H, =CH).^{13}CNMR shows the presence of 166.326(CO) and 165.856(CO) signals which confirm the suggested structure with acylation at N-2.Benzoylation of **1c** using benzoyl chloride gave **18** which gave the following ^{1}H NMR signals which conform to structure **18 ,** a singlet at δ: 3.9 (3H, OCH_3) and a multiplet centered at δ:7.5(15H,14 aromatic protons and 1H,=CH). ^{13}C NMR shows the presence of 167.284(CO) and 163.872(CO) signals which confirm the suggested structure with benzoylation at N-2.



Scheme 3. Formation of compounds **12** and **14**.

2.4. *Biological Evaluation (Cytotoxicity activities against three tumor cell lines for compounds **7a**, **7c**,**7d**, **12**,**16**, and **18**)*

The in vitro cytotoxicity activities of compounds **7a**, **7c**, **7d**, **12**, **16**, and **18** were evaluated using the MTT assay against three tumor cell lines: HepG-2 (Hepatocellular carcinoma), MCF-7 (Breast carcinoma), and A-549 (Lung carcinoma). The assays were conducted in the presence of doxorubicin as a standard reference drug. The results were quantified by determining the half-maximal inhibitory concentration (IC50) for each compound. The IC50 values were calculated to assess the efficacy of the tested compounds in inhibiting cell viability. The findings revealed that compound **(7c)** exhibited IC50 values of 9.57 µg/ml, 12.92 µg/ml, and 14.16 µg/ml against HepG-2, MCF-7, and A-549 cell lines, respectively. Compound (**12)** demonstrated significantly lower IC50 values of 2.59 µg/ml, 4.42 µg/ml, and 2.93 µg/ml against the same cell lines, indicating potent cytotoxic activity. Additionally, compound **(16)** showed IC50 values of 9.55 µg/ml, 13.33 µg/ml, and 10.54 µg/ml against HepG-2, MCF-7, and A-549 cells, respectively. These results suggest that compounds (**7c**, **12**, and **16)** have promising potential as anticancer agents, particularly due to their lower IC50 values compared to the standard drug, doxorubicin.



Scheme 4. Formation of compounds **16** and **18**.

Table 4. IC50 values for compounds **7a**, **7c**,**7d**, **12**,**16** and **18** Doxorubicin.

	IC_{50} values (µg /ml)		
	HepG-2 CELL LINE	**MCF-7 CELL LINE**	**A-549 CELL LINE**
7a	35.89± 2.03	77.34 ± 3.12	56.78 ± 2.35
7c	9.57 ± 0.27	12.92 ± 0.82	14.16 ± 0.93
7d	22.73 ± 0.59	30.31 ± 1.25	25.94 ± 0.72
12	2.59 ± 0.31	4.42 ± 0.28	2.93 ± 0.09
16	9.55 ± 0.63	13.33 ± 1.74	10.54 ± 0.58
18	22.12 ± 0.84	26.8 ± 0.92	58.19 ± 2.45
#	0.75 ± 0.11	1.02 ± 0.14	1.27 ± 0.35

Doxorubicin Reference Drug.

3. Conclusions

This study successfully demonstrates a convenient and eco-friendly synthesis of various pyrazolone derivatives utilizing chromium trioxide nanoparticles as an oxidizing agent. Moreover, the protonated nitrogen (NH) pyrazolone derivative was subjected to alkylation, acylation, and benzoylation to find the site of substitution besides improving the reaction yield using a sustainable catalyst ([DABCO-EtOH][Ac) under green chemistry conditions. The synthesized compounds were characterized using elemental analysis and extensive spectral techniques, confirming their structures. Notably, the in vitro cytotoxicity assessments revealed that several derivatives, particularly compounds **7c**, **12**, and **16**, exhibited significant anticancer activity against HepG-2, MCF-7, and A-549 cell lines, with IC50 values indicating their potential as effective anti-tumor agents. Emphasizing the potential of compound **12** as a potent alternative to traditional chemotherapeutic agents. These findings highlight the promise of pyrazolone derivatives in cancer therapy and underscore the advantages of green chemistry in pharmaceutical synthesis, paving the way for the development of environmentally friendly and biologically active compounds. Further investigation into the mechanisms of action and broader biological activity of these derivatives is warranted.

4. Conflicts of interest

There are no conflicts of interest to be declared.

5. Formatting of funding sources

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