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Design And Synthesis Of Coumarin Derivatives Association With Pyrazoline For Anticancer Activity

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ABSTRACT:

3-Acetyl-2H-chromen-2one (I) was created via Knoevenagel condensation of salicyladehyde with ethylacetoacetate in the presence of pipridine. Pyrazoline derivatives are utilised as possible therapeutic agents. 3-[(2E)-3-substituted-prop-2-enoyl] in succession Claisen-Schmidt condensation of 3-acetyl coumarin with aromatic aldehydes produced -2H-chromen-2-one derivatives (II a-h). When 3-substituted cinnamoylcoumarin was treated with hydrazine hydrate while ethanol was present, [5-substitutedphenyl] was produced.5 dihydro-1H-pyrazol-3-yl [-4, 5]III a-h, or -2H-chromen-2-one. Both conventional and microwave-assisted methods were used to synthesise the title chemical. FT-IR, 1H & 13C NMR, LC-MS, and elemental analysis were used to characterise all of the synthesised compounds. The in vitro MTT assay was used to screen for anticancer activity against the Hos, HT 29, G 361, A 549, and DU 145 cell lines. It was discovered that all of the compounds had superior activity against the human skin cancer cell line G 361 and the bone cancer cell line Hos.

KEY WORDS: *3-Acetyl coumarin, pyrazoline, anticancer, cell line*

1. INTRODUCTION

Cancer has become a major danger to human health and has shown an upward trend in occurrence with rising living standards [1,2]. Conventional treatments, such as surgery and radiation therapy, are quite effective in treating some types of cancer, but they have little effect on aggressive tumours. Chemotherapy, a primary multimodal cancer treatment method, is hindered by the significant toxicities of many anticancer drugs, which significantly lowers patients' quality of life [3, 4]. Consequently, it is now crucial for medicinal chemistry to produce anticancer medications with minimal toxicity and great selectivity. Because of their diverse range of pharmacological activity, heterocyclic molecules with nitrogen and oxygen have drawn a lot of attention [5]. In drug research, coumarins—natural, semi-synthetic, and synthetic—occupy a significant position. New synthetic pathways for the production of coumarin derivatives were sparked by their usefulness. [6] Furthermore, owing

of their many diverse properties, including antimalarial [7], anticonvulsant [8], anti-inflammatory [9], antioxidant [10, cytotoxic [11], anti-HIV [12], and antibacterial properties [13], coumarins have gained a unique position in the heterocyclic sector. Pyrazolines are very helpful in organic chemistry and have been instrumental in the development of heterocyclic chemistry theory. This class has received a lot of attention because of the intriguing biological activity of several substituted pyrazolines. As antimalerial [14], anticonvulsant [15], depressive [16], anti-epeleptic [17], antidiabetic [18], antioxidant [19], anticancer [20], antibacterial [21], and antitubercular medicines [22], the pyrazolines can be used with success. Thus, we found it interesting to synthesize new heterocyclic compound bearing both a coumarinyl moiety and a pyrazoline as possible anticancer agents.

MATERIALS AND METHODS:

Instrumentation and Materials: Melting points (mp) are uncorrected and were measured using a typical Boetius apparatus. Using the KBr disc approach, the IR spectra were captured in a Perkin-Elmer BXF1 FT-IR spectrophotometer. Using tetramethylsilane (TMS) as an internal standard (chemical shifts in ppm), 1H and 13C NMR spectra were acquired in the appropriate solvent on a Bruker AMX 400 and 100 MHz, respectively. The Agilent HPLC 1100 series was used to record the LC-MS [API/ESI-MS (80 eV)] spectra. The Carlo Erba 1108 elemental analyser was used to record the elemental analyses of the synthesised compounds, and the results were within ± 0.4% of the theoretical values. Using protective filters and a UV (254 nm) chamber, analytical TLC was carried out on Silica Gel F 254 plates (Merck). The National Centre for Cell Science (NCCS), located in Pune, India, provided the cell lines Hos (bone cancer), HT 29 (colon cancer), G 361 (human skin cancer), A 549 (lung cancer), and DU 145 (prostate cancer).

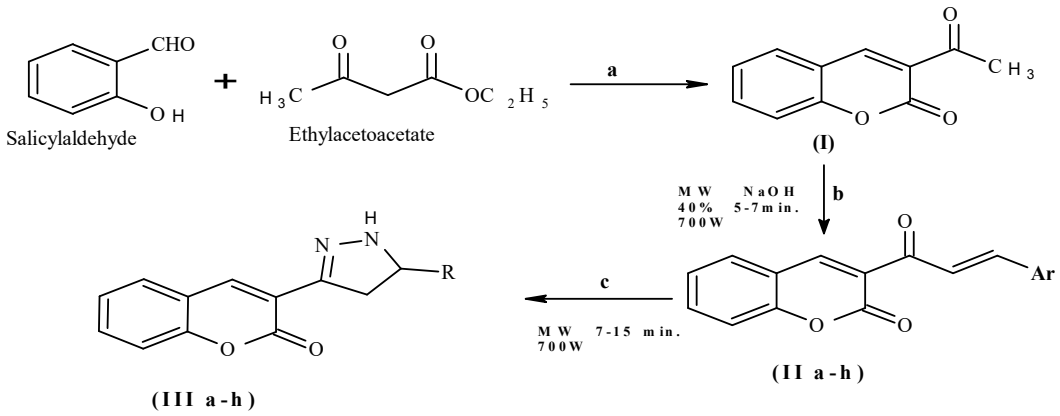
Preparation of 3-Acetyl coumarin 14 (I): 2 ml of piperidine was quickly stirred into a mixture of salicylaldehyde (1.8 g, 0.02 mol) and ethylacetoacetate (2.5 g, 0.02 mol). The yellowish material that had separated after 20 minutes was filtered out and given an ethanol wash. It melted at 120°C (lit mp 120-122°C), was recrystallised from ethanol, and yielded 83.55%. [23]

General procedure for the synthesis of 3-[(2E)-3-substituted-prop-2-enoyl]-2H-chromen-2-one (IIa-h): Ten millilitres of n-Butanol were heated to dissolve a combination of 3-Acetyl-2H-chromen-2-one (I) (0.01mol) and (0.012 mol) of the corresponding aromatic aldehydes. Then, 0.3 millilitres of glacial acetic acid and the same amount of piperidine were added. After four hours of refluxing the reaction mixture, the solvent was extracted using a vacuum. Twenty millilitres of ethanol were used to triturate the residue until a precipitate formed. The precipitate was then filtered out and recrystallised using an appropriate solvent. Table 1 shows the physical characteristics of the produced compounds (IIa-h). [24]

Microwave assisted General procedure (Solution phase MWI) for the synthesis of 3-[(2E)-3-substituted-prop-2-enoyl]-2H-chromen-2-one (IIa-h): Erlenmeyer's flask was filled with a mixture of 3-Acetyl-2H-chromen-2-one (I) (0.01 mol) and substituted aromatic aldehydes (0.012 mol) in ethanol (10 ml) and NaOH (4 ml, 40%). A 700W microwave oven was used to irradiate the reaction mixture for 5–7 minutes. After the reaction (TLC) was finished, the reaction mixture was allowed to cool to room temperature and was acidified using diluted HCL. Following separation, filtering, cold water washing, drying, and recrystallisation from ethanol, the product was obtained. In a similar manner, every other chemical in the (IIa-h) series was created. Table 1 lists their physical constants. [25]

General procedure for the 3-[5-substitutedphenyl]-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (III a-h): For three hours, 10 millilitres of 0.01 mol chalcone and hydrazine hydrate (0.02 mol) refluxed in an ethanolic solution. After pouring the reaction mixture onto crushed ice, it was agitated. The resulting solid is filtered, washed with water, and crystallised using the proper solvents to produce the corresponding (III a-h). Table 2 provides a summary of the synthesised compounds' physico-chemical and spectrum data (IIIa-h). [26]

Microwave assisted General procedure 16 (Solution phase MWI) for the synthesis of the 3-[5-substitutedphenyl]-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (III a-h): Erlenmeyer's flask was filled with a solution of substituted chalcone (0.01mol), hydrazine hydrate (0.02mol), and acetic acid (4 ml) in 10 ml of methanol. For seven to fifteen minutes, the reaction mixture was exposed to radiation at 700W in a microwave oven. After the reaction (TLC) was finished, the reaction mixture was cooled to room temperature and then covered with ice water. After being separated, the product was filtered, cleaned with cold water, dried, and recrystallised using an appropriate solvent. [27]



Scheme 1:

*Reagents and conditions: (a) Piperidine, stirr, rt, 20 min; (b) Ar-CHO, Piperidine / n- Butanol, reflux, 4hr; (c) Hydrazine hydrate, ethanol.

In vitro anticancer activity using MTT assay: In DMEM media supplemented with 10% foetal bovine serum (FBS), 100 µg/mL penicillin, 200 µg/mL streptomycin, and 2 mM L-glutamine, the adherent Hos, HT 29, G 361, A 549, and DU 145 cell lines were cultivated. The culture was then kept in a humidified environment with 5% CO2. A stock solution containing 10 mg/mL of dimethyl sulfoxide was made. [28] To obtain the necessary concentration, different dilutions were prepared using sterile water from the stock mentioned above. The whole cell lines were planted in 100 µL of DMEM supplemented with 10% foetal bovine serum at a density of 1x104 cells per well (the number of cells was established using the trypan blue exclusion dye method). Following a 12-hour seeding period, fresh DMEM supplemented with 10% FBS was used to replace the above medium. Three separate additions of 10 µL of the sample from the above stock solutions were made to each well, resulting in final concentrations of 200, 100, 50, and 10 µg/mL. For 48 hours, the aforementioned cells were cultured at 37°C with 5% CO_2. [29]

Following a 48-hour incubation period, 100 µL of fresh DMEM without FBS was added to the aforesaid media. Ten microlitres of MTT (5 mg dissolved in 1 millilitre of PBS) were then added, and the mixture was incubated for three hours at 37 °C with 5% CO2. Using a multichannel pipette, the aforementioned media was removed after three hours of incubation. Next, 200 µL of DMSO was added to each well, and the wells were once more incubated for fifteen minutes at 37 °C. [30] After adding DMSO for one hour, the plate was finally measured at 570 nm using a spectrophotometer (Spectra Max, Molecular Devices). The number of cells used directly correlates with the absorbance. The test samples' validity was checked with the DMSO control after the values were averaged. Tables 3 and 4 display the findings and statistical evaluation of the compounds' anticancer efficacy. [31-33]

RESULTS AND DISCUSSION

3-Acetyl coumarin was synthesized by Knoevenagel condensation of salicylaldehyde with ethyl acetoacetate. Claisen-Schmidt condensation of 3-Acetyl coumarin with aromatic aldehydes gave 3-substituted cinnamoyl

coumarin. Treatment of 3-substituted cinnamoyl coumarin with hydrazine hydrate in ethanol gave 3-(5-substitutedphenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one Scheme-I. A silica gel G was used for thin-layer chromatography (TLC) analysis to determine the purity of the produced chemicals. IR and 1H-NMR spectral analyses were used to characterise the structure of the synthesised substances.

Table 1: Physicochemical data of 3-[(2*E*)-3-substituted-prop-2-enoyl]-2*H*-chromen-2-one derivatives (II a-h)

Compound	R	Yield (%)		M. P. (^{0}C)	Rf [b]
		Conventional	Microwave		
II a	-H	78	84	170	0.56
II b	4-OMe	55	85	155	0.58
II c	4-Cl	89	89	203	0.56
II d	4-NMe_2	78	90	214	0.58
II e	3-NO_2	67	91	221	0.62
II f	4-Me	63	86	176	0.56
II g	2-NO_2	54	84	146	0.61
II h	4-OH	64	85	198	0.66

[b]Hexane: Ethyl acetate (6:4) as a mobile phase

3-[(2E)-3-Phenylprop-2-enoyl]-2H-chromen-2-one IIa: IR (KBr cm-1): 1731 (Lactone of coumarin), 1657 (α, β-unsaturated ketone); 1HNMR (CDCl3): δ 8.61 (s, 1H, 4th proton of coumarin), δ 7.83 (d, 1H, =CH-Ar), δ 7.43-7.78 (m, 9H, Ar-H), δ 7.41 (d, 1H, =CH-CO).

3-[(2E)-3-(4-Methoxyphenyl) prop-2-enoyl]-2H-chromen-2-one IIb: IR (KBr cm-1): 1732 (Lactone of coumarin), 1654 (α, β-unsaturated ketone); 1HNMR (CDCl3): δ 8.58 (s, 1H, 4th proton of coumarin), δ 7.85 (d, 1H, =CH-Ar), δ 6.95-7.84 (m, 8H, Ar-H), δ 6.92 (d, 1H, =CH-CO), δ 3.86 (s, 3H, -OCH3).

3-[(2E)-3-(4-Chlorophenyl) prop-2-enoyl]-2H-chromen-2-one IIc: IR (KBr cm-1): 1716 (Lactone of coumarin), 1662 (α, β-unsaturated ketone); 1HNMR (CDCl3): δ 8.60 (s, 1H, 4th proton of coumarin), δ 7.92 (d, 1H, =CH-Ar), δ 7.83 (d, 1H, =CH-CO), δ 7.34-7.69 (m, 8H, Ar-H).

3-{(2E)-3-[4-(Dimethylamino) phenyl] prop-2-enoyl}-2H-chromen-2-one IId: IR (KBr cm-1): 1734 (Lactone of coumarin), 1657 (α, β-unsaturated ketone); 1HNMR (CDCl3): δ 8.55 (s, 1H, 4th proton of coumarin), δ 7.85 (d, 1H, =CH-Ar), δ 7.75 (d, 1H, =CH-CO), δ 6.67-7.75 (m, 8H, Ar-H), δ 3.05 [s, 6H, -N(CH3)2].

3-[(2E)-3-(3-Nitrophenyl) prop-2-enoyl]-2H-chromen-2-one IIe: IR (KBr cm-1): 1712 (Lactone of coumarin), 1661 (α, β-unsaturated ketone); 1HNMR (CDCl3): δ 8.64 (s, 1H, 4th proton of coumarin), δ 8.49 (d, 1H, =CH-Ar), δ 8.27 (d, 1H, =CH-CO), δ 7.38-8.00 (m, 8H, Ar-H).

3-[(2E)-3-(4-Methylphenyl) prop-2-enoyl]-2H-chromen-2-one IIf: IR (KBr cm-1): 1731 (Lactone of coumarin), 1657 (α, β-unsaturated ketone); 1HNMR (CDCl3): δ 8.57 (s, 1H, 4th proton of coumarin), δ 7.93 (d, 1H, =CH-Ar), δ 7.59 (d, 1H, =CH-CO), δ 7.21-7.87 (m, 8H, Ar-H), δ 2.39 (s, 3H, -CH3).

3-[(2E)-3-(2-Nitrophenyl) prop-2-enoyl]-2H-chromen-2-one IIg: IR (KBr cm-1): 1712 (Lactone of coumarin), 1661 (α, β-unsaturated ketone); 1HNMR (CDCl3): δ 8.64 (s, 1H, 4th proton of coumarin), δ 8.49 (d, 1H, =CH-Ar), δ 8.27 (d, 1H, =CH-CO), δ 7.38-8.00 (m, 8H, Ar-H).

3-[(2E)-3-(4-Hydroxyphenyl) prop-2-enoyl]-2H-chromen-2-one IIh: IR (KBr cm-1): 1729 (Lactone of coumarin), 1657 (α, β-unsaturated ketone); 1HNMR (CDCl3): δ 9.63 (s, 1H, -OH), δ 8.55 (s, 1H, 4th proton of

coumarin), δ 7.81 (d, 1H, =CH-Ar), δ 7.73 (d, 1H, =CH-CO), δ 6.89-7.72 (m, 8H, Ar-H).

Similarly, all other compounds of the series were synthesized by using different aromatic aldehydes (III a-h). Their physical constants, yield are recorded in Table 2.

Table 2: Physicochemical data of 3-[5-substitutedphenyl]-4, 5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one (III a-h)

Compound	R	Yield (%)		M. P. (^{0}C)	Rf
		Conventional	Microwave		
III a	-H	78	88	181	0.32
III b	4-OMe	65	90	221	0.27
III c	4-Cl	67	85	145	0.32
III d	4-NMe_2	68	87	142	0.31
III e	3-NO_2	69	84	113	0.29
III f	4-Me	75	87	113	0.34
III g	2-NO_2	69	88	127	0.35
III h	4-OH	72	89	215	0.44

Hexane: Ethyl acetate (6:4) as a mobile phase

3-(5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one IIIa: IR (KBr cm-1): 3440 (NH), 2915 (CH2), 1596 (lactone of Coumarin), 1523 (C=C); 1HNMR (CDCl3): δ 2.77 (dd, 1H, 4-Ht), δ 3.33 (dd, 1H, 4-Hc), δ 6.88 (dd, 1H, 5-H of pyrazoline), δ 7.21 -7.84 (m, 9H, Ar-H), δ 7.65 (s, 1H, 4-H of coumarin), δ 9.02 (s, H, NH).

3-[5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one IIIb: IR (KBr cm-1): 3436 (NH), 2919 (CH2), 1592 (lactone of Coumarin), 1492 (C=C); 1HNMR (CDCl3): δ 2.77 (dd, 1H, 4-Ht), δ 3.35 (dd, 1H, 4-Hc), δ 3.75 (s, 3-H, OCH3), δ 6.88 (dd, 1H, 5-H of pyrazoline), δ 7.24 -7.84 (m, 8H, Ar-H), δ 7.65 (s, 1H, 4-H of coumarin), δ 9.02 (s, H, NH).

3-[5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one IIIc: IR (KBr cm-1): 3436 (NH), 2919 (CH2), 1677 (lactone of Coumarin), 1565 (C=C); 1HNMR (CDCl3): δ 2.78 (dd, 1H, 4-Ht), δ 3.34 (dd, 1H, 4-Hc), δ 6.88 (dd, 1H, 5-H of pyrazoline), δ 7.32 - 7.84 (m, 8H, Ar-H), δ 7.65 (s, 1H, 4-H of coumarin), δ 9.02 (s, H, NH).

3-{5-[4-(dimethylamino)phenyl]-4,5-dihydro-1H-pyrazol-3-yl}-2H-chromen-2-one IIId: IR (KBr cm-1): 3350 (NH), 2965 (CH2), 1700 (lactone of Coumarin), 1550 (C=C); 1HNMR (CDCl3): δ 2.76 (dd, 1H, 4-Ht), δ 3.02 (s, 6H, -NMe2), δ 3.35 (dd, 1H, 4-Hc), δ 6.54 (dd, 1H, 5-H of pyrazoline), δ 6.77-7.84 (m, 8H, Ar-H), δ 7.65 (s, 1H, 4-H of coumarin), δ 9.02 (s, H, NH).

3-[5-(3-nitrophenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one IIIe: IR (KBr cm-1): 3440 (NH), 2923 (CH2), 1697 (lactone of Coumarin), 1627 (C=C); 1HNMR (CDCl3): δ 2.80 (dd, 1H, 4-Ht), 3.36 (dd, 1H, 4-Hc), 6.94 (dd, 1H, 5-H of pyrazoline),7.31 – 8.394 (m, 8H, Ar-H), 8.40 (s, 1H, 4-H of coumarin), 9.02 (s, H, NH).

3-[5-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one IIIf : IR (KBr cm-1): 3436 (NH), 2919 (CH2), 1677 (lactone of Coumarin), 1573 (C=C); 1HNMR (CDCl3): δ 2.21 (s, 3H, -CH3), δ 2.77 (dd, 1H, 4-Ht), δ 3.33 (dd, 1H, 4-Hc), δ 6.88 (dd, 1H, 5-H of pyrazoline), δ 7.05 -7.84 (m, 8H, Ar-H), δ 7.65 (s, 1H, 4-H of coumarin), δ 9.02 (s, H, NH).

3-[5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one IIIh: 1HNMR (CDCl3): δ 2.77 (dd, 1H, 4-Ht), δ 3.34 (dd, 1H, 4-Hc), δ 6.73 (dd, 1H, 5-H of pyrazoline), δ 7.16-7.84 (m, 8H, Ar-H), δ 7.65 (s,

1H, 4-H of coumarin), δ 9.02 (s, 2H, NH2), δ 9.18 (s, 1H, -OH).

The structure of the title compounds was confirmed by the IR spectra of compounds (II a-h), which showed the characteristic peaks of C=O of α-pyrone and C=O of ketone at 1735.01 and 1656.10, respectively, and C=O of α-pyrone, C=N, and C=C of pyrazoline at 1720-1730, 1585-1600, and 1533-1545 cm-1, respectively. Furthermore, a thorough 1H-NMR analysis of the compounds allowed for the determination of their structure. Three doublet-doublets between δ 3.27 and 5.63 of -CH2 and -CH of pyrazoline and a multiplet between δ 6.85 and 8.42 are typical peaks of aromatic protons in the spectrum of 3-[5-substitutedphenyl] in the 1H-NMR spectra of compounds (III a-h).Dihydro-1H-pyrazol-3-yl [4,5]Structures are confirmed using -2H-chromen-2-one.

Anticancer activity Using the MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, the anticancer activity of all the synthesised compounds (IIa-h) and (IIIa-h) was assessed against a variety of human cancer cell lines, including Hos (bone cancer), HT 29 (colon cancer), G 361 (skin cancer), A 549 (lung cancer), and DU 145 (prostate cancer). Methotrexate and doxorubicin served as reference substances.

Table 3: In vitro cytotoxic activity of compound (IIa-h) against various human cancer cell lines by MTT assay

S. No.	Compound	R	IC_{50} (µM)				
			Bone cancer (Hos)	Colon cancer (HT 29)	Human skin cancer (G 361)	Lung cancer (A 549)	Prostate cancer (DU 145)
1	IIa	-H	122.76	189.43	167.65	163.32	150.54
2	IIb	4-OMe	50.78	52.63	76.95	76.16	49.11
3	IIc	4-Cl	118.14	98.97	114.27	186.59	178.24
4	IId	4-NMe_2	122.65	139.76	126.54	*a*	167.09
5	IIe	3-NO_2	131.14	*a*	148.73	*a*	*a*
6	IIf	4-Me	153.35	102.19	160.98	149.43	118.97
7	IIg	2-NO_2	112.13	134.65	*a*	113.56	111.45
8	IIh	4-OH	112.27	125.53	147.56	119.74	120.11
9	Doxorubicin		4.32	0.08	6.54	5.76	0.22
10	Methotrexate		2.5	10.87	2.13	0.35	0.31

[a] Inactive (IC_{50} values > 200 µM); IC_{50} values mean of three experiments in replicate.

O-methyl at the C-4 position on ring-B of pyrazoline may be the reason why IIb, out of all the derivatives, had substantial activity against all the cell lines, with IC50 values ranging from 51-77 µM and 78-102 µM, respectively. When tested against Hos cell lines, all of the compounds showed detectable activity. With IC50 values of 50.78 µM and 49.11 µM, respectively, chemical IIb was found to be the lead chemical with strong cytotoxic action against all cell lines, particularly against Hos and DU 145 cancer cells.

Table 4: In vitro cytotoxic activity of compound (IIIa-h) against various human cancer cell lines by MTT assay

S. No.	Compound	R	IC_{50} (µM)				
			Bone cancer (Hos)	Colon cancer (HT 29)	Human skin cancer (G 361)	Lung cancer (A 549)	Prostate cancer (DU 145)
1	IIIa	-H	45.75	47.63	46.48	64.34	45.26

2	IIIb	4-OMe	2.38	9.71	3.31	11.88	16.09
3	IIIc	4-Cl	6.43	98.75	44.67	76.54	36.11
4	IIId	4-NMe_2	21.67	35.56	40.65	49.98	45.78
5	IIIe	3-NO_2	38.86	56.11	45.23	56.43	39.11
6	IIIf	4-Me	37.06	96.36	55.56	58.13	75.32
7	IIIg	2-NO_2	4.38	9.30	6.86	10.65	12.56
8	IIIh	4-OH	18.44	17.65	19.67	18.58	20.54
9	Doxorubicin		0.32	5.64	0.22	6.98	6.76
10	Methotrexate		0.54	3.12	9.46	3.04	1.03

IC_{50} values mean of three experiments in replicate.

The MTT assay method was used to assess the anticancer activity of the eight synthesised derivatives (IIIa-h) against the five cell lines stated above. Table 4 provides a summary of the IC_{50} values. IC_{50} values for compounds IIIb, IIIc, and IIIg ranged from 2 to 16 µM, 4 to 27 µM, and 5 to 14 µM, respectively, and shown strong anticancer activity against all tested cancer cell lines. With $IC5_0$ values of 2.38 µM, 4.04 µM, and 4.38 µM, respectively, compound IIIb, IIIc, and IIIg demonstrated maximum activity against Hos cancer cells. In contrast, compound IIIb and IIIg demonstrated moderate activity against G 361 cancer cells, with IC50 values of 3.31 µM and 6.12 µM, respectively. The remaining derivatives showed minimal levels of cytotoxicity (IC50 values greater than 25 µM), and they were all determined to be effective against every cancer cell that was tested.

CONCLUSION

Based on the data, it can be said that pyrazoline has greater anticancer activity than both chalcones and coumarin, which are cyclized from the same chalcones. Following the conversion of chalcones to their corresponding pyridopyrimidines, anticancer activity was significantly increased. This conclusion showed that the pyrazoline nucleus enhances anticancer activity and that pyrazoline and coumarin are superior lead molecules as anticancer medicines, particularly when it comes to Hos and G 361 cell lines. To clarify the precise mechanism of action for their therapeutic potential as anticancer drugs, more research is necessary.

REFERENCES

1. Murata T, Shimada M, Sakakibara S, Yoshino T, Masuda T, Shintani T, Sato H, Koriyama Y, Fukushima K, Nunami N, Yamauchi M, Fuchikami K, Komura H, Watanabe A, Ziegelbauer KB, Bacon KB and Lowinger TB: Synthesis and structure-activity relationships of novel IKK-beta inhibitors. Part 3: Orally active anti-inflammatory agents. Bioorganic and Medicinal Chemistry Letters 2004; 14:4019-4022.
2. Dobaria AV, Patel JR and Parekh HH: Synthesis and antimicrobial screening of cyanopyridines. Journal of the Indian Chemical Society 2002; 79:772-773.
3. Popat KH, Kachhadia VV, Nimavat KS and Joshi HS: Synthesis of some new cyano pyrans and cyanopyridines and their biological activities. Journal of the Indian Chemical Society 2004; 81:157-159.
4. Sharma U, Ahmed S and Boruah RC: A facile synthesis of annelated pyridines from β formyl enamides under microwave irradiation. Tetrahedron Letters 2000; 41:3493-3495.
5. Xiang-shan Wang, Zhao-sen Zeng, Da-qing Shi, Xian-yong Wei and Zhi-min Zong: KF- Alumina catalyzed one-pot synthesis of pyrido[2,3-d]pyrimidine derivatives. Synthetic Communications 2004; 34:4331-4338.
6. Mahadevan KM, Arunakumar DB, Prakash GK, Kumaraswamy MN, Nandeshwarappa BP, Shrigara BS: Microwave assisted facile synthesis of amino pyrimidines bearing benzofuran and investigation of their antimicrobial activity. Indian J Chem 2006; Vol.45B:1699-1703.

7. Akhtar W, Faraz Khan M, Verma G, Shaquiquzzaman M, Akhter M, Marella A, Parmar S, Khatoon R, Mumtaz Alam M: Coumarin-Pyrazoline Derivatives: Their One-Pot Microwave Assisted Synthesis and Antimalarial Activity. Journal of Pharmaceutical and Medicinal Chemistry 2017; 3 (1): 5-9
8. Bhat MA, Siddiqui N, Khan SA: Synthesis of novel thioureido derivatives of sulfonamides and thiosemicarbazido derivatives of coumarin as potential anticonvulsant and analgesic agents. Indian Journal of Pharmaceutical Sciences 2006; 68(1): 120-124.
9. Hai-Ning Lv, Shu Wang, Ke-Wu Zeng, Jun Li, Xiao-Yu Guo, Daneel Ferreira, Jordan K. Zjawiony, Peng-Fei Tu, and Yong Jiang: Anti-inflammatory Coumarin and Benzocoumarin Derivatives from Murraya alat. Journal of Natural Product 2015; 78 (2): 279–285
10. Ahn JH, Kim HM, Jung SH, Kang SK, Kim KR, Rhee SD: Synthesis and DP-IV inhibition of cyano-pyrazoline derivatives as potent anti-diabetic agents. Bioorganic and Medicinal Chemistry Letters 2004; 14; 4461-4465.
11. Vishvakrama P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014 Jun 24:47-55.
12. Vishvakarma P. Design and development of montelukast sodium fast dissolving films for better therapeutic efficacy. Journal of the Chilean Chemical Society. 2018 Jun;63(2):3988-93.
13. Vishvakarma P, Mandal S, Verma A. A review on current aspects of nutraceuticals and dietary supplements. International Journal of Pharma Professional's Research (IJPPR). 2023;14(1):78-91.
14. Prabhakar V, Agarwal S, Chauhan R, Sharma S. Fast dissolving tablets: an overview. International Journal of Pharmaceutical Sciences: Review and Research. 2012;16(1):17.
15. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: an analysis of the Medicinal properties of the plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.
16. Tengli Ak, Badami S, Prashantha Kumar BR, Dongre SH, Ravi S, Ananda T: Microwave assiated synthesis of pyrazoline derivatives and their antiangiogenic and antioxidant activities. Indian Journal of Heterocyclic Chemistry 2007; 16: 333-336.
17. Muhammed Karabacak, Mehlika Dilek Altıntop, Halil İbrahim Çiftçi, Ryoko Koga, Masami Otsuka, Mikako Fujita and Ahmet Özdemir: Synthesis and Evaluation of New Pyrazoline Derivatives as Potential Anticancer Agents. Molecules 2015; 20: 19066-19084.
18. Aftab Ahmad, Asif Husain, Shah Alam Khan, Mohd. Mujeeb, Anil Bhandari: Synthesis, antimicrobial and antitubercular activities of some novel pyrazoline derivatives. Journal of Saudi Chemical Society 2016; 20: 577–584.
19. Abdullah Sulaiman Al-Ayed and Naceur Hamdi: A New and Efficient Method for the Synthesis of Novel 3-Acetyl Coumarins Oxadiazoles Derivatives with Expected Biological Activity. Molecules 2014; 19: 911-924.
20. Gridnev YS, Saraf AS, Pogrebnyak AV, Oganesyan ET: Synthesis and study of structure-antiallergic activity relationship in a series of 4-R-3-cinnamoylcoumarin derivatives. Pharmaceutical Chemistry Journal 1995; 29(11):31-34.
21. Nikalje AP, Malhotra P, Pattan S: Microwave assisted synthesis and anti-inflammatory activity of 3, 5-diaryl substituted-2-pyrazolines. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(2):21-26.
22. Victory P, Cirujeda J and Vidal-Ferran A: Two step synthesis of pyrido[2,3-d]pyrimidines from acyclic precursors. Cyclization of 2-cyanamino-4,6-diphenyl pyridine-3-carbonitrile by hydrogen halides. Tetrahedron 1995; 51:10253-10258.
23. Wenyan Mo, Guihong Liao, Tao Wang and Hongwu He: Facile synthesis and biological activities of novel fluorine-containing pyrido[4,3-d]pyrimidines. Journal of Fluorine Chemistry 2008; 129:519-523.
24. Forsch RA, Queener SF and Rosowsky A: Preliminary in vitro studies on two potent, water-soluble trimethoprim analogues with exceptional species selectivity against dihydrofolate reductase from Pneumocystis carinii and Mycobacterium avium. Bioorganic and Medicinal Chemistry Letters 2004; 14:1811-1815.

25. Gangjee A, Adair O and Queener SF: Pneumocystis carinii and Toxoplasma gondii dihydrofolate reductase inhibitors and antitumor agents: synthesis and biological activities of 2,4-diamino-5-methyl-6-[(monosubstituted anilino)methyl]pyrido[2,3-d]pyrimidines. Journal of Medicinal Chemistry 1999; 42:2447-2455.
26. Assaraf YG, IIan Ifergan, Kadry WN and Pinter RY: Computer modeling of antifolate inhibition of folate metabolism using hybrid functional petri nets. Journal of Theoretical Biology 2006; 240:637-647.
27. Gmeiner WH: Novel chemical strategies for thymidylate synthase inhibition. Current Medicinal Chemistry 2005; 12:191-202.
28. Edmondson JM, Armstrong IS and Martinez AO: A rapid and simple MTT-based spetrophotometric assay for determining drug sensitivity in monolayer cultures. Journal of Tissue Culture Methods 1988; 11:15-17.
29. Jignesh PR and Kishor RD: Synthesis and antimicrobial activity of new triazolopyridinyl phenothiazines. Arkivoc 2005; Xiii:21-28.
30. Abdel-Rahman BA, El-Gazzar and Hafez HN: Synthesis of 4-substituted pyrido[2,3-d] pyrimidin-4(1H)-one as analgesic and anti-inflammatory agents. Bioorganic and Medicinal Chemistry Letters 2009; 19:3392-3397.
31. Gholap AR, Kiran ST, Shirazi F, Kumari R, Manoj Kumar B, Deshpande MV and Srinivasan KV: Synthesis and evaluation of antifungal properties of a series of the novel 2-amino-5-oxo-4-phenyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile and its analogues. Bioorganic and Medicinal Chemistry 2007; 15:6705-6715.
32. Divyesh CM, Manish PP and Ranjan GP: An efficient one-pot synthesis and in vitro antimicrobial activity of new pyridine derivatives bearing the tetrazoloquinoline nucleus. Arkivoc 2009; Xiv:64-74.
33. Baldwin JJ, Engelhardt EL, Hirschmann R, Ponticello GS, Atkinson JG, Wasson BK, Sweet CS and Scriabine A: Heterocyclic analogs of the antihypertensive beta adrenergic blocking agent (S)-2-[3-(tert-butylamino)-2-hydroxypropoxy]-3-cyanopyridine. Journal of Medicinal Chemistry 1980; 23:65-70.