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Development of Some Novel Coumarin-Chalcone Compounds as Anti-proliferative Agents

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Authors' contributions

This work was carried out in collaboration among all authors. All authors did the literature survey from standard databases, carried out the synthesis, conducted the compound characterization, performed the anti-cancer activity and wrote this manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Cancer is the world's second leading cause of death and morbidity, behind only heart failure, which claimed the lives of 18.2 million people in 2020. While massive initiatives to establish newer leads and innovative chemotherapeutic methods for combating different types of cancer, continues to be a major concern around the world. As a result, identifying cell-cycle inhibitors and apoptotic triggers to fight cancer cells is an appealing method for finding and developing new anti-tumor drugs.
Materials and Methods: The present study involves the rational development and characterization (both physicochemical and spectroscopy) of coumarin-chalcone compounds (A_1–A_{10}) and their anti-proliferative potentials against cancer lines of breast cancer origin (MDA-MB468, MDA-MB231, and MCF-7) and non-cancer breast epithelial cell (184B5).
Results: The compound A_2 exhibited the highest anti-proliferative activity against the cell line MDA-MB-231 as indicated by the GI_{50} value of 10.06 µM, the compound A_6 exhibited the highest anti-proliferative activity against the cell line MDA-MB-468 as indicated by the GI_{50} value of 17.54 µM, the compound A_1 exhibited the highest anti-proliferative activity against the cell line MCF-7 as

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indicated by the GI_{50} value of 25.86 µM, and the compound A_6 exhibited the highest anti-proliferative activity against the cell line 184B5 as indicated by the GI_{50} value of 23.26 µM.
Conclusion: Furthermore, the research urges medicinal chemists to choose chalcone prototypes with well-defined pathways and SARs while developing more powerful inhibitors. Furthermore, it opens up new pathways for the discovery of anti-cancer derivatives using low molecular weight ligands.

Keywords: Chalcone; coumarin; anti-cancer; synthesis; cell lines; breast cancer.

1. INTRODUCTION

Cancer is the world's second leading cause of death and morbidity, behind only heart failure, which claimed the lives of 18.2 million people in 2020 [1]. While massive initiatives to establish newer leads and innovative chemotherapeutic methods for combating different types of cancer, continues to be a major concern around the world [2]. The quest for new or unexplored types of substances to combat cancer cells has piqued the interest of scientists all over the world. As a result, identifying cell-cycle inhibitors and apoptotic triggers to fight cancer cells is an appealing method for finding and developing new anti-tumor drugs [3].

Recently, many heterocyclic and non-heterocyclic scaffolds have been investigated for antiproliferative activity against various tumor cell lines [4]. Among them chalcones is one of the most promising classes of compounds that exhibit anticancer activities along with other pharmacological activities. Chalcones are structurally simple compounds of the flavonoid family and are present in a variety of plant species [5]. Chemically, these are 1,3-diphenyl-2- propen-1-one and have reported a wide range of biological activities, including antileishmanial, antiinflammatory, antimitotic, modulation of P-glycoprotein-mediated multidrug resistance, and antimalarial activities, etc [6].

Chalcone, also known as (*E*)-1,3-diphenyl-2-propene-1-one, is a source of flavonoids and isoflavonoids, as well as an open-chain intermediate in the synthesis of flavones by aurones. They can be found in nature in a variety of conjugated ways with a benzylideneacetophenone scaffold, which connects the two aromatic nuclei with a three-carbon, unsaturated carbonyl bridge [6]. Kostanecki was the first to coin the concept after synthesizing a sequence of natural chromophoric compounds. Chalcone and its variants can be produced using a Claisen-Schmidt reaction between benzaldehyde and acetophenone and a 40 percent sodium hydroxide solution as a catalyst [7,8]. Thanks to its basic chemistry, simplicity of synthesis, a large amount of replaceable hydrogen to yield a large number of derivatives, and variety of promising biological activities such as anti-diabetic, anti-neoplastic, anti-hypertensive, anti-retroviral, anti-inflammatory, anti-histaminic, anti-oxidant, anti-malarial, etc., the chemistry of chalcones has remained a curiosity among researchers in the twenty-first century [9].

The present study involves the rational development and characterization (both physicochemical and spectroscopy) of coumarin-chalcone compounds (A_1–A_{10}) and their anti-proliferative potentials against cancer lines of breast cancer origin (MDA-MB468, MDA-MB231, and MCF7) and non-cancer breast epithelial cell origin (184B5) [10].

2. MATERIALS AND METHODS

2.1 Instrumentation

Sigma-Aldrich® and Merck® provided chemicals that were used in this synthesis. The other solvents and reagents were purchased from industrial suppliers and were of analytical grade. The melting points were determined using the Perfit melting point apparatus and are unadjusted. On a Win IR FTS 135 instrument, infrared spectra were captured in KBr disks. The JEOL-JMS-DX 303 instrument was used to collect mass spectra. Merck® silica gel G coated TLC plates were used for thin-layer chromatography.

2.2 Synthesis of Target Compounds

2.2.1 Synthesis of 3-acetyl-4-hydroxy-2*H*-chromen-2-one

4-hydroxy coumarin (0.01 M) was combined with 15 mL glacial acetic acid, and then 60 mL phosphorous oxychloride was steadily applied to

the mixture and heated for 1.5 hours. The reaction mixture was then cooled and dumped onto crushed ice to extract the solid substance, which was then cleaned with water and crystallized from absolute alcohol (Scheme 1).

2.2.2 Synthesis of coumarin-Chalcones

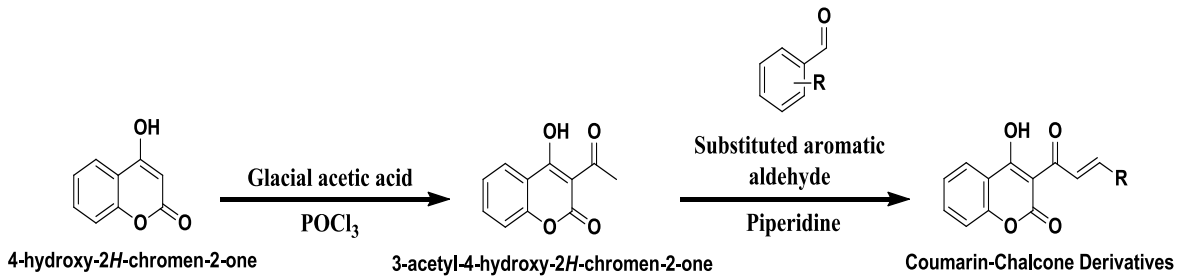
In 6 ml of chloroform, 4-hydroxy-3-acetyl coumarin (0.031 M) and the substituted aromatic aldehyde (0.03 M) were dissolved. The reaction mixture was refluxed for 3 hours with a catalytic volume of piperidine (0.001 M). The chloroform was removed from the substance, which was then washed with methanol (Scheme 1).

2.2.3 Anti-cancer screening

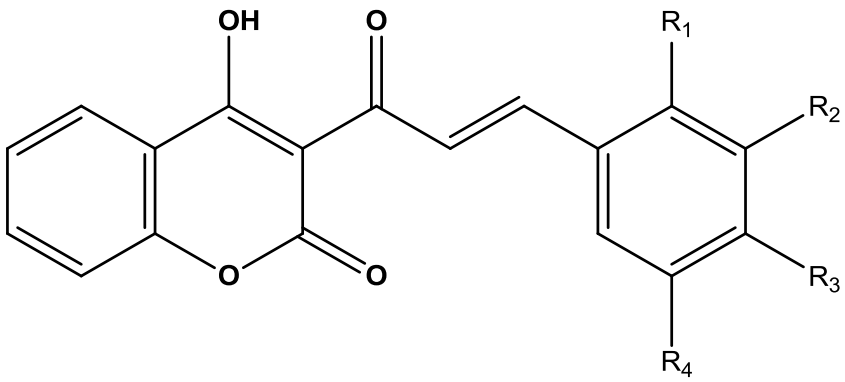
The cytotoxic effects of all the compounds synthesized were tested on three breast cancer cell lines, MDA-MB468, MDA-MB231, and MCF7, as well as one non-cancer breast epithelial cell line, 184B5. This Anti-cancer activity assay was done according to National Cancer Institute protocol. The results were stated in terms of growth inhibition (GI50 IM) values. The MCF-7 cell lines were grown in medium (RPMI 1640) comprising fetal bovine serum (10%) and L-glutamine (2 mM). Cells were injected into 96 well microtiter plates and incubated for 24 temperature 37 C, CO2 (5%), air (95%) and relative humidity (100%). Stock solutions were prepared in DMF with concentration of 10 ml/ml. The growth inhibitory assays were confirmed at 10-fold serial dilutions. Plates were incubated at standard conditions for 48 h and assay was terminated by the cold TCA. Addition Cells monolayer were fixed with cold TCA (50 ml, 30% (w/v)) and incubated at 4 C for 60 min. Discarded the supernatant, washed the plates and air dried. Prepared the solution of Sulforhodamine B (50 ml) in acetic acid (1%) and poured into wells, then incubated the plates at room temperature for 25 min. After staining, recovered unbound dye and removed residual dye by washing with acetic acid and plates air dried. The protein bound dye afterward eluted by dissolving in trizma base (10 mM) and absorbance was noted via Elisa plate reader at 540 nm with reference wavelength (690 nm) [11-12].

2.3 Statistical Analysis

The mean was used to express the outcomes of the tests. The magnitude of the variations between the control and treated groups was determined using an ANOVA accompanied by a Dunnett's t-test, with $P < 0.05$ found meaningful.



Scheme 1. Overall synthetic scheme



Proposed structure

Table 1. FT-IR characterization data

Compound code	FT-IR Frequencies of various functional groups (cm^{-1})						
	Ar-H	C-H	C=O (Chalcone)	C=C (α-Pyrone)	C-O	C=C (Ring)	Others
A_1	3006 (str)	2836 (str)	1520 (s, str)	1613 (s, str)	1136 (str)	1434(str)	-
A_2	2944 (str)	2836	1720 (str)	1619 (str)	1287(str)	1497(str)	-
A_3	2943 (str)	-	1605(str)	-	1287(str)	-	-
A_4	3072(str)	-	1612(str)	-	1249(str)	1498(str)	-
A_5	3013, 2971(str)	2837(str)	1711(str)	1597(str)	-	-	-
A_6	3100(str)	-	1718(str)	1612(str)	1317(str)	1503(str)	-
A_7	3154(str)	2827(str)	1563(str)	1608(str)	1107	-	C-Cl 1178(str)
A_8	3099(str)	-	1502(str)	1611(str)	1231(str)	1502(str)	C-Cl 1096,130(str)
A_9	3043(str)	2839(str)	1582(str)	1608(str)	1263(str)	1452(str)	Ar-NH_2 1582(str)
A_{10}	-	2934(str)	1612(str)	-	1153(str)	-	C-Cl 1110(str)

Table 2. Physicochemical and mass spectral data of synthesized compounds

Compound	Molecular Formula	Molecular Mass	Melting Point (°C)	% Yield	R_f value	C logP	Molecular ion	Product ion
A_1	$C_{20}H_{16}O_6$ Br	352.34	217.1	69.0	0.115	3.60	352.34	160,201,336
A_2	$C_{21}H_{18}O_6Cl_2$	382.36	215.2	62.0	0.86	3.78	382.36	160,132,231
A_3	$C_{19}H_{14}O_6Cl_2$	338.0	190.1	54.8	0.175	3.13	338.0	186,61,255
A_4	$C_{21}H_{18}O_6ClBr$	382.36	152.3	36.8	0.152	3.78	382.36,402	81,191,240
A_5	$C_{21}NH_{18}O_6Cl$	322.08	192.2	65.0	0.164	3.87	322.08	61,186,202
A_6	$C_{21}NH_{18}O_6Br$	292.07	187.3	81.7	0.175	3.95	292.07,411	146,202
A_7	$C_{18}H_{11}NO_6$	326.73	197.2	63.8	0.142	4.66	326.73	182,186
A_8	$C_{18}H_{13}NO_4$	360.2	210·0	62.2	0.125	5.25	360.2	117,169,238
A_9	$C_{21}H_{18}O_7$	322.30	248.3	33.1	0.142	3.45	322.30	161,220
A_{10}	$C_{19}H_{14}O_6$	282.05	186.2	69.2	0.154	3.12	282.05	102,141

Table 3. Anti-proliferative potentials of coumarin-chalcone derivatives

Compounds	R1	R2	R3	R4	GI_{50} [a,b] (μM)			
					MDA-MB231	MDA-MB468	MCF-7	184B5
A_1	H	H	Br	H	22.11±0.21	41.08±0.52	25.86±0.25	47.41±0.69
A_2	Cl	Cl	H	H	10.06±2.01	25.34±0.88	30.72±1.75	40.09±1.01
A_3	H	Cl	Cl	H	68.95±0.87	44.94±0.65	84.60±1.32	39.19±0.85
A_4	Cl	H	Br	H	51.33±0.92	37.89±0.72	57.83±1.01	65.09±0.98
A_5	Cl	NO_2	H	H	58.75±0.78	110.82±1.33	63.45±0.99	152.08±2.01
A_6	H	H	NO_2	Br	11.51±1.97	17.54±1.69	40.72±1.87	23.26±1.36
A_7	H	H	$N(CH3)_2$	H	62.60±0.85	58.48±0.48	75.43±1.36	75.88±0.98
A_8	Cl	OCH_3	OCH_3	H	106.33±1.98	119.25±1.81	78.27±1.48	110.22±1.23
A_9	OCH_3	OCH_3	OCH_3	Cl	111.51±1.97	117.54±1.69	90.72±1.87	123.26±1.36
A_{10}	H	OCH_3	OCH_3	OCH_3	43.64±0.65	74.41±1.24	64.66±1.23	60.36±1.58
Cisplatin					23.65±0.23	31.02±0.45	25.77±0.38	25.54±0.35

[a] GI_{50} values were calculated from Sigmoidal dose response curves (variable slope), which were generated with GraphPad Prism V. 4.02 (GraphPad Software Inc.). [b] Values are the mean of triplicates of at least two independent experiments

3. RESULTS AND DISCUSSION

3.1 Chemistry

All the compounds show Ar-H, C-H, C=O (Chalcone),C=C (α-Pyrone), C-O, and C=C (Ring) stretching between 3000-3100 cm^{-1}, 2800-2900 cm^{-1}, 1500-1600 cm^{-1}, 1600-1700 cm^{-1}, 1100-1300 cm^{-1}, and 1450-1600 cm^{-1}, respectively that confirm the synthesis of 4-hydroxy-3-acetyl coumarin nucleus (Table 1). The absorption band between 3004-3150 cm^{-1} (stretching) confirmed the presence of aromatic ring in all compounds, bands between 1505-1750 cm^{-1} showed the presence of C=O (chalcone) and were found in all compounds. The absorption band lies between 2800-2900 cm^{-1} which signifies the presence of the C-H group. The absorption band between 1400-1500 cm^{-1} confirmed the presence of (Ar) C=C in all compounds and the absorption band between 1100-1200 cm^{-1} due to compounds having C-O bonding in their structure.

The mass spectra of all the compounds showed that the molecular ion peak of the compound was as similar as was anticipated. Finally, the structure of compounds was confirmed by FT-IR spectroscopy and mass spectra. All the band frequencies for the molecular weight of synthesized compounds were matching (within limits of variation) with those reported (Table 2).

3.2 Anti-cancer Activity

Compound A_6 had the strongest anti-proliferative efficacy against all cell lines, as shown by the GI_{50} values, in the SRB assay (Table 3). The compound A_2 exhibited the highest anti-proliferative activity against the cell line MDA-MB-231 as indicated by the GI_{50} value of 10.06 µM, the compound A_6 exhibited the highest anti-proliferative activity against the cell line MDA-MB-468 as indicated by the GI_{50} value of 17.54 µM, the compound A_1 exhibited the highest anti-proliferative activity against the cell line MCF-7 as indicated by the GI_{50} value of 25.86 µM, and the compound A_6 exhibited the highest anti-proliferative activity against the cell line 184B5 as indicated by the GI_{50} value of 23.26 µM. As opposed to the traditional anti-cancer medication cisplatin, both of the compounds had higher GI_{50} levels. The substituents and their locations played an important role in exhibiting anti-proliferative behavior by modulating different unknown goals, according to Structure-Activity Relationships (SARs) and logical chalcone scaffold architecture. The electron-withdrawing substituents such as Br results in an improvement in anti-cancer capacity as well as boosts the anti-cancer behavior due to an increase in the log P-value. A compound's lipophilicity (or hydrophobicity) is a critical physical property that influences bilipid membrane permeation, dissolution rate, compound bioavailability, and drug interaction with biological systems [12,13,14]. It was also discovered that analogs with an electron-withdrawing group in the R_4 role had higher antiproliferative behavior than analogs with other positions.

4. CONCLUSION

The research discovered that coumarin-chalcone compounds had the ability to be effective anti-proliferative agents. The thesis highlighted the important function and locations of substitution on the system's phenyl moiety. According to the GI_{50} values, compound A_6 had the most anti-proliferative effect against all cell lines. It is reasonable to conclude that cytotoxic effects of all of the synthesized derivatives were mediated by various pathways, none of which were evaluated in this study. Furthermore, the research urges medicinal chemists to choose chalcone prototypes with well-defined pathways and SARs while developing more powerful inhibitors. Furthermore, it opens up new pathways for the discovery of anti-cancer derivatives using low molecular weight ligands.

DISCLEMAR

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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