International Research Journal of Multidisciplinary Scope (IRJMS), 2025; 6(2): 960-972 IRJMS

Original Article | ISSN (O): 2582-631X DOI: 10.47857/irjms.2025.v06i02.03636

Novel Coumarin Chalcone Derivatives: Synthesis and Anticancer Evaluation

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Abstract

Breast cancer is the most frequently diagnosed disease which affects women 's worldwide. Aggressiveness of cancer cells has thwarted several scientific approaches, preventing their invasion and spread to nearby tissues despite scientists' unrelenting attempts. In this study, a series of coumarin chalcone hybrids were designed and synthesised by Vilsmeier-Haack, nucleophilic aromatic substitution and Claisen condensation reaction. Characterization of derivatives done by spectral studies such as IR, NMR and Mass spectrometry. The purpose of this work was assessing the cytotoxic effects of recently synthesised coumarin chalcone compounds on breast cancer cell lines (MCF-7). Swiss ADME web tool and computational data from computer-assisted software were used to compare the results. According to molecular docking all the produced molecules met Lipinski's rule. Additionally, findings from cytotoxicity tests showed that substances 5h and 5i displayed significant efficacy against MCF-7 cells with GI_{50} (9.8 and 9.6µg/ml respectively), according this study, the compounds mentioned above had more antitumor effects on MCF-7 cell lines (human breast cancer) than standard drug Imatinib (IG_{50} = 9.4µg/ml). GraphPad prism was used for data analysis. Consequently, it is possible to see compounds 5a, 5e, 5h and 5i as potential agent against MCF-7. It is concluded that the potential of synthesised hybrids could be more effective in aggressive breast cancer (MCF-7).

Keywords: ADMET, Anticancer, Imatinib, MCF-7, Molecular Docking.

Introduction

Cancer from non-communicable diseases is greatest reason for premature deaths in world (1). Over 19 million new cases reported worldwide in 2020 which leading to millions of deaths. Even with advancement in diagnoses and treatment of malignant carcinoma. Breast cancer is still in leading causes of death. With almost six lakh deaths annually, breast cancer is still furious for females. Furthermore, greater than 28 million additional incidents of cancer and greater than 16 million demises are predicted to occur globally by 2040 (2, 3). The phrase, Cancer is not unknown to the great majority of people in the world whether they are laypeople or medical experts. More often, it was found that breast cancer in women was more diagnosed than lung cancer (4, 5). Worldwide, 1 in 20 women will develop breast cancer, and 1 in 8 in under developed countries (6). Both modifiable and non-modifiable factors like age, race, lifestyle, sex, genetic makeup, food and hormone replacement treatment (7). On the basis of various biochemical processes which includes control of cell division and cell growth, cancer cells differ from normal cells. The proliferation index of cells in cancer is larger than normal cells. Hence blocking proliferative pathway is thought to be an effective tactic for preventing cell division through cell cycle arrest or causing cell death through apoptosis in order to defeat the illness. Many novel antineoplastic agents have been identified. Even though, two common approaches for breast cancer are chemotherapy and surgery, there is still need to develop innovative targeted cancer treatments that prevent cancer cells from toxicities. This is because significant drawbacks like multidrug resistance and systemic toxicity of conventional chemotherapeutic agents (6, 8). Due to toxicity and resistance downsides of classic cytotoxic treatments multi-acting medicines and combination anticancer therapies are chosen medicinally. For potential anticancer strategy, a more potent and targeted chemotherapeutic drug is still required (9-11). Protein kinases are known to be interesting targets for anticancer agents because of their significant role in oncogenesis. Clinical practice has authorised a number of kinase

(Received 27th December 2024; Accepted 15th April 2025; Published 30th April 2025)

inhibitors (12). Tyrosine kinase belongs to larger class of enzymes known as Protein kinases which attach phosphates to amino acid like serine and threonine. Tyrosine kinase is highly relevant for cancer (11). For cell survival and proliferation, PI3K/AKT signalling pathway is necessary (13). Control of intrinsic apoptosis is linked with the PI3K/AKT pathway as it can modulate activity of members of the Bcl-2 family (14). Therefore, cell cycle arrest may result through inhibition of PI3K/AKT pathway (15). Thus, blocking the PI3K/AKT pathway is a promising target for treatment of human cancers (16). Furthermore, in breast cancer, disruption of this signalling axis is primarily responsible for resistance to hormone therapy, targeted therapy and antineoplastic chemotherapy medicines (8, 17). Naturally occurring scaffolds like coumarin and chalcone ring have been shown to have strong anticancer effects through interfering with multiple cellular processes. According to various studies, inclusion of coumarin in hybridization structures results to potential cancer treatment due to its ability to kill tumour cells (18). A study reported the anticancer activity of coumarin chalcone derivatives against cancer cells with potential effects in terms of IC_{50} 0.1μM (19). The synthesised coumarin chalcone compounds were tested against cancer cells showed cytotoxic effect same as standard vinblastine (20). Our research work inspired to develop new anticancer agents by utilising these scaffolds. Coumarins shown their anti-tumour activity through inhibition of protein kinases (21). Furthermore, studies have demonstrated that coumarins can impede growth of cancerous cells through stopping cell cycles at the phases G0/G1 and G2/M alongwith inhibiting P-glycoprotein (P-gp) in cancer cells (21-23). Some naturally occurring compounds have similar structural similarities and exhibits different pharmacological activities, including antioxidant, antidiabetic, antibacterial, antitubercular. Both coumarin and chalcone used as medicinal agent as of their therapeutic potential like anti-inflammatory, anti-hyperlipidemic, antibacterial etc. That's why these compounds have drawn significant attention to the synthesis of number of chalco-coumarin derivatives in recent years. Number of anticancer drugs based on molecular hybridization have been discovered in previous 10 to 15 years (24). The development of novel drugs to treat a variety of complex disorders depends on molecular hybridization approach (25). This research study focused at evaluation of potent anticancer activity of coumarin chalcones in MCF-7 cell lines (breast cancer cell lines).

Methodology

Docking Studies

Using Chem Sketch software around 200 chemical structures of coumarin chalcone moiety were created experimentally, 3D optimized and docked with Phosphoinositol-3-kinase (PI3K) by using Autdock Vina software to obtain protein- ligand interactions (26). The ligands with structural diversity and conformational stability were docked with crystal structure of tyrosine kinase PI3K (PDB ID: 5jhb) taken from the RSCB (27). In docking process tyrosine kinase is used like receptor. Coumarin chalcone derivatives docks perpendicular and create a network of H- bonds with PI3K residues' active site. Inhibition induced by hydrogen bonding of active site with hydroxyl group of coumarin ring. Docking results were compared with standard drug i.e. Imatinib, a popular PI3K inhibitor according to their lowest binding energy (28). Ten compounds from docked molecules were identified to be near minimum binding energy of reference drug (-8.1Kcal/mol).

Chemistry

Evaluate purity of synthesised compounds by using TLC on pre-coated silica gel G glass plates (Merck) and results identified by exposing at λ 254 nm under UV lamp. Open capillary tubes were used for melting point determination. FT-IR spectra obtained by using pellet technique of KBr disk and in cm^{-1} υ_{max} is expressed on Bruker spectrophotometer. NMR spectra were recorded on JNM-ECZ600R/S1 spectrometer in $CDCl_3$ as solvent at 600 MHz for ^{1}HNMR and 100 MHz for ^{13}CNMR by using TMS as internal standard. Parts per million (ppm) was used to express chemical shifts (δ). Terms singlet(s), doublet(d), triplet(t), multiplets(m) refer to spin multiplicities. Applied Biosystems 3200 Q-Trap Spectrometer used to get mass spectra.

Synthesis

Compounds 2 and 3 were synthesised by following scheme as given in literature (28). Series of derivatives was synthesised as per scheme 1 given in Figure 1. Obtained characteristics of compounds

like colour, yield, melting point, R_f value and structure given in Table 1.

General method of synthesis of 3-((E)-3-(4-((Z)-benzylideneamino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one (5a-j)

At room temperature, mixture of 4(a-j) (5mmol), acetic acid (20ml) and dimethylamine (5mmol) was shaken for 20 minutes. After that, above mixture poured into 30ml ice-cold water. Obtained product was filtered and cleaned with three volumes (20ml) of (1:1) acetic acid (aqueous) and kept dried. Recrystallization of obtained crude product done with 1,4-dioxane to get product 5(a-j) (29-31).

3-((1E,3E)-3-(4-(((Z)-4-hydroxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5a

Colour: Pale yellow; FTIR (KBr, cm^{-1}): 3422(-OH str), 3424 (-OH str), 1654 (-C=O str, chalcone), 1716 (-C=C str, coumarin), 1468 (aromatic, -C=C str), 3069 (-CH str, aromatic), 1638 (-C=C str, alkenyl), 1359 (-CH_3 str);^{1}HNMR ($CDCl_3$, 600MHz, δ): 6.8-7.98 (s,1H, Aromatic-H), 8.00 (s, 1H, Imine), 3.75 (s, 1H, OH), 1.25-1.30 (d,3H, CH_3); ^{13} CNMR ($CDCl_3$, δ, ppm): 67.1 (C-O-C), 119.9-146.1(C, Aromatic), 29.7 (CH_3), 127.5 (C=C), 171.62 (CO, coumarin); MS (ESI) m/z: 442.24 (M+2).

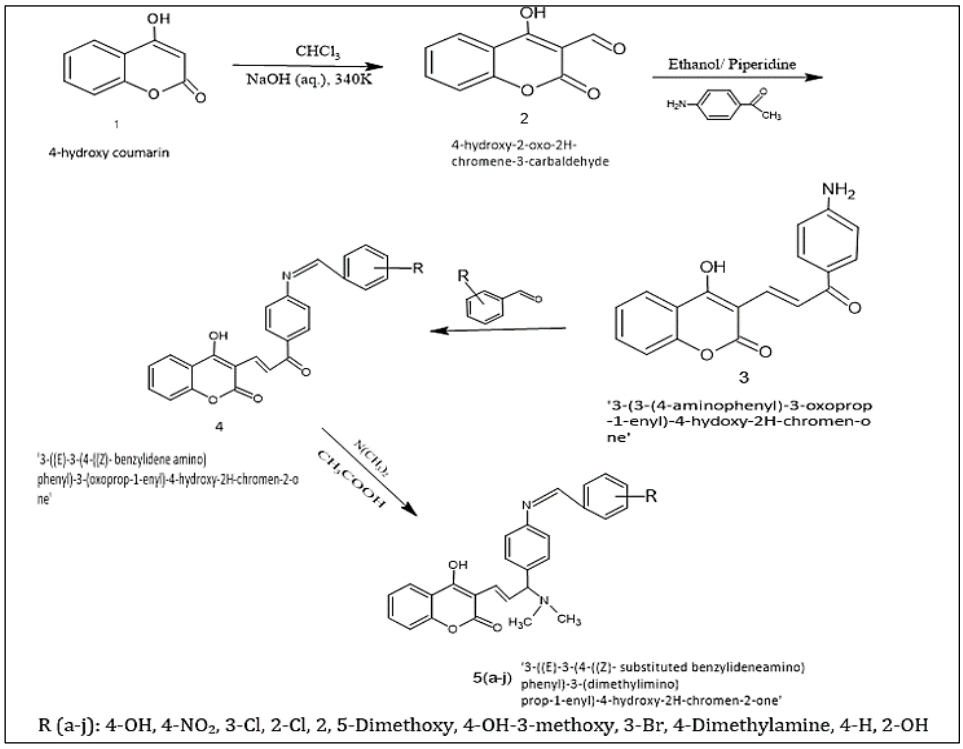


Figure 1: Synthetic Scheme of Coumarin Chalcone Derivatives 5(a-j)

3-((1E,3E)-3-(4-(((Z)-4-nitrobenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5b

Colour: Yellow; FTIR (KBr, cm^{-1}): 3422(-OH str), 1656 (-C=O str, chalcone), 1714 (-C=C str, coumarin), 1468 (aromatic, -C=C str), 3114 (-CH str, aromatic), 1356 (-CH_3 str), 1685 (-C=C str, alkenyl),868 (-NO_2); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 6.7-7.9 (s,1H, Ar -H), 8.01 (s, H, imine), 9.18 (No_2), 4.9 (s,1H, OH), 7.4-7.7 (m, 12H, Ar), 1.7-1.9 (s, CH_3); ^{13}CNMR (CDCl3, δ, ppm): 133.2, 136.8, 151.2 (C, Aromatic), 178.60 (CO, coumarin), 129.3 (C=C), 25.9 (CH_3), 26.01 (CH_3); MS IESI) m/z: 471.08 (M+2)

3-((1E,3E)-3-(4-(((Z)-3-chlroxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5c

Colour: Pale yellow; FTIR (KBr, cm^{-1}): 3420 (-OH str), 1654 (-C=O str, chalcone), 1712 (-C=C str, coumarin), 1465 (aromatic, -C=C str), 3066 (-CH str, aromatic), 1359 (-CH_3 str), 1688 (-C=C str, alkenyl), 755 (-C-Cl, str); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 7.15-7.18 (s, 1H, Ar), 8.06 (s, imine), 3.78 (s, OH), 7.2-7.9 (s, 12H, Ar), 2.09 (s, CH_3); ^{13}CNMR (CDCl3, δ, ppm): 24.8 (CH3), 26.1 (CH3), 181.4 (CO, coumarin), 103.1 (C, Aromatic), 115.7-133.04 (C, Aromatic), 67.3 (C-O-C), 152.7 (C=C); MS (ESI) m/z: 460.21 (M+2)

3-((1E,3E)-3-(4-(((Z)-2-chloroxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5d

Colour: Dark red; FTIR (KBr, cm^{-1}): 3420 (-OH str), 1656 (-C=O str, chalcone), 1717 (-C=C str, coumarin), 1464 (aromatic, -C=C str), 3114 (-CH str, aromatic), 1685 (-C=C str, alkenyl), 1356 ($-CH_3$ str), 763 (-C-Cl, str); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 7.1-7.4 (m, 1H, Ar) ,8.06 (d, H, imine), 3.78 (s, H, OH), 7.6-7.9 (s, 12H,Ar), 1.98 (s, CH_3); ^{13}CNMR ($CDCl_3$, δ, ppm): 22.5 (CH3), 26.4 (CH_3), 103.1 (C, Aromatic), 115.7-129.0 (C, Aromatic), 168.5 (CO, coumarin), 151.4 (C=C); MS (ESI) m/z: 460.29(M+2)

3-((1E,3E)-3-(4-(((Z)-2,5 dimethoxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5e

Colour: Dark yellow; FTIR (KBr, cm^{-1}): 3424 (-OH str), 1654 (-C=O str, chalcone), 1714 (-C=C str, coumarin), 1469 (aromatic, -C=C str), 3066 (-CH str, aromatic), 1358 ($-CH_3$ str), 1688 (-C=C str, alkenyl), 1134 ($-OCH_3$); ^{1}HNMR($CDCl_3$, 600MHz, δ): 7.2-7.4 (m, 1H,Ar) , 8.08-8.09 (s, H, imine), 3.70 (H, OH), 7.10-7.46 (m, 12H,Ar), 2.84 (s,OCH_3), 3.4-3.7 (s, OCH_3), 1.38 (s,CH_3); ^{13}CNMR($CDCl_3$, δ): 55.5 (OCH_3), 56.5(OCH_3), 115.4 (-CH), 168.1,171.2 (CO, coumarin), 103.4, 110.8, 112.54, 116.6, 120.4, 131.1, 131.4 (C, Aromatic), 33.3 (CH_3), 35.7 (CH_3); MS (ESI) m/z: 484.74 (M+)

3-((1E,3E)-3-(4-(((Z)-benzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one 5f

Colour: Yellow; FTIR (KBr, cm^{-1}): 3426(-OH str), 1654 (-C=O str, chalcone), 1712 (-C=C str, coumarin), 1468 (aromatic, -C=C str), 3068 (-CH str, aromatic), 1356 ($-CH_3$ str), 1632 (-C=C str, alkenyl); ^{1} HNMR ($CDCl_3$, 600MHz, δ): 3.15 (s, H, OH), 1.6-1.8 (s, CH_3), 7.3-7.4 (m,12H,Ar), 10.18 (H, Imine); ^{13}CNMR($CDCL_3$, δ, ppm): 22.3 (CH3), 22.5 (CH3), 196.5 (CO, coumarin), 113.7- 130.8 (C, Aromatic), 147.4 (C=-phenyl); MS (ESI) m/z: 426.44 (M+2)

3-((1E,3E)-3-(4-(((Z)-3-bromoxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5g

Colour: Pale yellow; FTIR (KBr, cm^{-1}): 3422(-OH str), 1654 (-C=O str, chalcone), 1712 (-C=C str, coumarin), 1460 (aromatic, -C=C str), 3065 (-CH str, aromatic), 1359 ($-CH_3$ str), 1622 (-C=C str, alkenyl),639 (C-Br, str); ^{1}HNMR (CDCl3, 600MHz, δ): 1.67(s,CH_3), 3.17 (H,OH), 7.3 (s,12H,Ar), 9.30 (s, H, imine); ^{13}CNMR($CDCl_3$, δ, ppm): 22.4 (CH3), 26.5 (CH3), 119.9-133.2 (C, Aromatic), 196.7 (CO, coumarin), 141.8 (CH), ; MS (ESI) m/z: 503.02 (M+)

3-((1E,3E)-3-(4-(((Z)-4-dimethylaminebenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5h

Colour: Red; FTIR (KBr, cm^{-1}): 3424 (-OH str), 1654 (-C=O str, chalcone), 1716 (-C=C str, coumarin), 1468 (aromatic, -C=C str), 3066 (-CH str, aromatic), 1358 ($-CH_3$ str), 1625 (-C=C str, alkenyl); ^{1} HNMR ($CDCl_3$, 600MHz, δ): 7.2-7.9 (m,1H,Ar), 3.82 (H,OH),1.25-1.60 (d, 3H, CH_3), 2.81-2.90 (m,CH_3), 8.05 (H, imine); ^{13}CNMR($CDCl_3$, δ, ppm): 168.52(CO, coumarin), 112.8-132.5(C, Aromatic), 67.18 (C-O-C), 115.6, 116.6 (CH), 152.6 (C=C); MS (ESI) m/z: 425.03 (M+1)

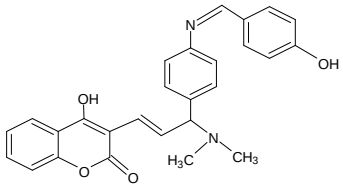
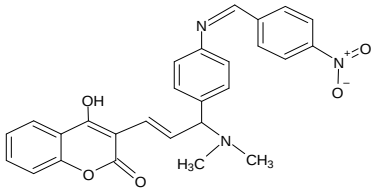
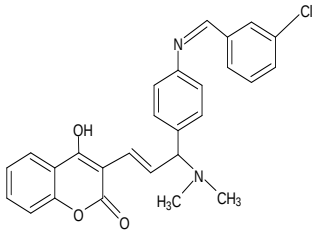
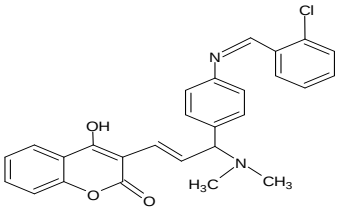
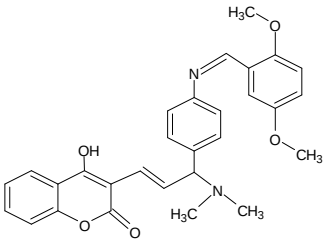
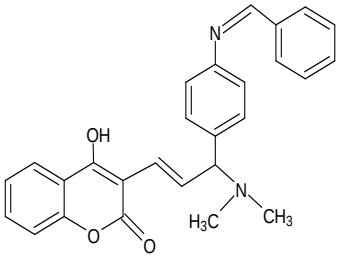
3-((1E,3E)-3-(4-(((Z)-4-hydroxy-3-methoxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5i

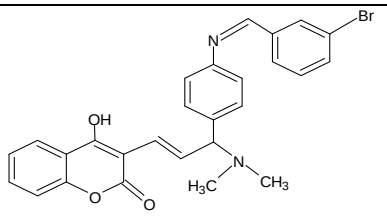
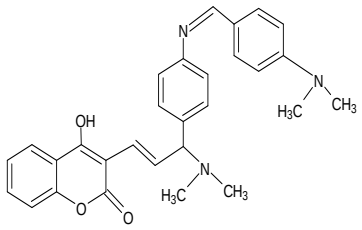
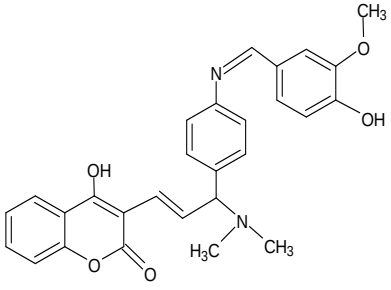
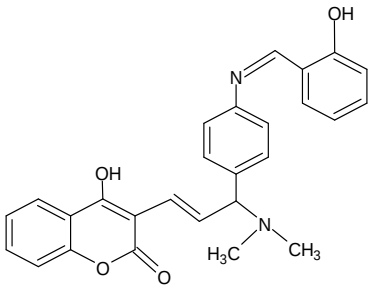
Colour: Pale yellow; FTIR (KBr, cm^{-1}): 3424(-OH str), 3426 (-OH str), 1656 (-C=O str, chalcone), 1718 (-C=C str, coumarin), 1465 (aromatic, -C=C str), 3068 (-CH str, aromatic), 1354 ($-CH_3$ str), 1688 (-C=C str, alkenyl), 1358 ($-CH_3$ str), 1130 ($-OCH_3$); ^{1} HNMR ($CDCl_3$, 600MHz, δ): 4.05 (H,OH), 2.45 (s,CH_3), 3.15 (s, OCH_3), 7.2-7.6 (H, Ar), 11.03 (H, imine); ^{13}CNMR($CDCl_3$, δ, ppm): 55.8 (OCH_3), 27.4 (CH_3), 27.7 (CH_3), 114.6, 119.6, 122.01, 122.8, 131.6, 133.4 (C, Aromatic), 167.2 (CO, coumarin); MS (ESI) m/z: 471.81 (M+1)

3-((1E,3E)-3-(4-(((Z)-2-hydroxybenzylidene) amino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one, 5j

Colour: Dark yellow; FTIR (KBr, cm^{-1}): 3420(-OH str), 3424 (-OH str), 1654 (-C=O str, chalcone), 1712 (-C=C str, coumarin), 1465 (aromatic, -C=C str), 3066 (-CH str, aromatic), 1684 (-C=C str, alkenyl), 1359 ($-CH_3$ str); ^{1}HNMR ($CDCl_3$, 600MHz, δ): 4.25 (H,OH), 2.45 (s, CH_3), 7.24-7.79 (12H,Ar), 9.82 (H, imine), 3.70 (s, H, OH) ; ^{13}CNMR ($CDCl_3$, δ, ppm): 131.4, 133.04, 141.4 (C, Aromatic), 26.8 (CH_3), 24.7 (CH_3), 178.4 (CO, coumarin) ; MS (ESI) m/z: 442.53 (M+2)

Table 1: Physical Characteristics of Compounds 5(a-j)

Compound	Structure	Melting point (^{0}C)	Yield (%)	Rf value
5a		179-182^{0}C	69	0.71
5b		185-189^{0}C	61	0.84
5c		159-161^{0}C	59	0.79
5d		149-152^{0}C	71	0.78
5e		191-196^{0}C	66	0.76
5f		167-171^{0}C	52	0.69

5g		163-168^{0}C	80	0.62
5h		173-178^{0}C	73	0.89
5i		175-178^{0}C	66	0.86
5j		153-155^{0}C	66	0.82

Anticancer Activity

Cell Culture: MCF-7 cancer cell lines (human breast) purchased from NCCS, Pune. DMEM medium was used to culture the cell lines and added 10% FBS (Sigma Aldrich, Germany) as well as 1% antibiotic solution (Penicillin/Streptomycin mixture) as per standard tissue culture protocol (30-32). In this screening experiment, 96 well micro titer plates with 100 µL plate density were used for cell inoculation. Then micro titar plates incubated at 37^{0}C under standard conditions such as 95% air, 100% relative humidity, 5% Co_2 for 24 hours before experimental drugs addition.

Antitumor Activity (SRB Assay): One 96-well plate (8000 cells per well) was secured with TCA (Trichloroacetic acid) after a period of 24 hours, to reflect cell population measurement during drug addiction. Prior to usage, the experimental drugs firstly dissolved in 100mg/ml DMSO then diluted with water (1mg/ml) and kept refrigerated. On the addition of drug, a sample of frozen concentrate (1mg/ml) was melted, diluted with complete medium and test sample to achieve concentrations of 100 µg/ml, 200 µg/ml, 400 µg/ml, 800 µg/ml and 1000 µg/ml. To achieve the final desired drug concentration of 10,20,40,80µg/ml, an amount of 10 µL of each of these drug dilutions was introduced in relevant micro-titer well which already contained 90 µL of medium. Plates were incubated for 48 hours under standard conditions following the addition of drug. The experiment was stopped by addition of TCA (cold). 30% w/v cold TCA (50 µL,10% TCA, final concentration) was gently or carefully filled to the cells and allowed to set in place for 60 minutes at 4^{0}C. Plates washed with tap water (5 times) repeatedly and allowed to air dry, after discarding the supernatant liquid layer. At room temperature, incubation of plates was done for 20 minutes following addition of 50 µL SRB (Sulphorhodamine B) solution (0.4% w/v

concentration) in acetic acid (1%). Plates were subjected to air dry after the staining process, the unbound dye was recovered and remaining dye was then washed with acetic acid (1%) for 5 times. Following eluting the bound stain with 10 mmol Tris base solution (pH= 10.5), at 510 nm absorbance was measured with reference wavelength of 690nm on Elisa plate reader (iMark, Biorad, USA). The mean absorbance of test well divided by mean absorbance of control wells × 100 represented as percent growth (percent cell viability). The GI_{50} was determined by using software Graph Pad Prism -6 and plotted a graph of %cell viability value versus concentration of each compound and compared against reference drug Imatinib. Results presented as Mean± SEM.

Results and Discussion

Such active compounds i.e. coumarin chalcone derivatives i.e. 3-((E)-3-(4-((Z)- substituted benzylideneamino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one (5a-5j) were synthesized on the basis of results of molecular docking and identification done by spectroscopic studies. Scheme 1 displays the derivatives synthesized by multistep reaction. As mentioned in scheme 1**,** 4-hydroxy coumarin [1] on reaction with chloroform and aqueous sodium hydroxide at 340K afforded 3-carbaldehyde substituted 4-hydroxy coumarin [2] which on refluxing with 4-aminoacetophenone in presence of piperidine/ethanol has yielded 3-(3-(4-aminophenyl)-3-oxoprop-1-enyl)-4-hydoxy-2H-chromen-one [3], On further refluxing treatment of [3] with different substituted benzaldehydes in presence of ethanol and acetic acid yielded 3-((E)-3-(4-((Z)- substituted benzylidene amino) phenyl)-3-(oxoprop-1-enyl)-4-hydroxy-2H-chromen-2-one (4a-4j), condensation of (4a-4j) one by one with dimethylamine in presence of acetic acid produced 3-((E)-3-(4-((Z)-benzylideneamino) phenyl)-3-(dimethylimino) prop-1-enyl)-4-hydroxy-2H-chromen-2-one (5a-5j). All the obtained derivatives were analysed by melting point and TLC techniques (provided in Table 1) and also characterised by spectroscopic studies. In NMR (^{1}H and $^{13}CNMR$), FTIR and in Mass spectrometry characteristic peaks were observed. Compounds 5a-5j exhibit a distinct and characteristic peak in FTIR spectra in region 1640 -1670 cm^{-1} due to chalcone stretching -C=O. In region 1460- 1480 cm^{-1} stretching vibration band for C=C aromatic is seen. The corresponding structures were also supported by the 1H-NMR and 13C-NMR spectrum data. All the derivatives provide satisfactory M+, M+1, M+2 peaks in Mass spectra.

Molecular Docking Studies

To determine the potencies of the developed hybrids based on binding affinities, molecular docking simulation was used as a prediction technique (33, 34). Results of docking shown in Table 2. Interaction of compound 5h, 5i and reference drug respectively with PI3K shown in Figures 2A, 2B, 2C. Compound 5h and 5i shown highest binding energy. Docking demonstrated the interaction of targeted molecule with receptor, termed as binding affinity which observed through different interactions like van der waals', carbon hydrogen bonding, pi sigma, hydrophobic π alkyl interaction, π-π interaction, electrostatic interactions like pi anion and pi cation etc. Binding pose with main active residues TRR A:463, LYS A:501, ILE A:487, ASN A:498, showed hydrogen bonding and hydrophobic interactions.

ADMET Profile and Drug-Likeness (In Silico)

All hybrids' ADMET profile and drug-likeness (in silico) were computed and are shown in Table 3. LRO5 was cited and the results were contrasted with ideal range (35, 36). Derivatives 5a, 5e, 5i, and 5h seem to have reasonable ADMET profile whereas only one compound 5g violet one rule of Lipinski i.e. molecular weight. No compound violet safety assessment profile like Brenk and PAINS indicate potential safety except 5b and 5d (violet Brenk profile).

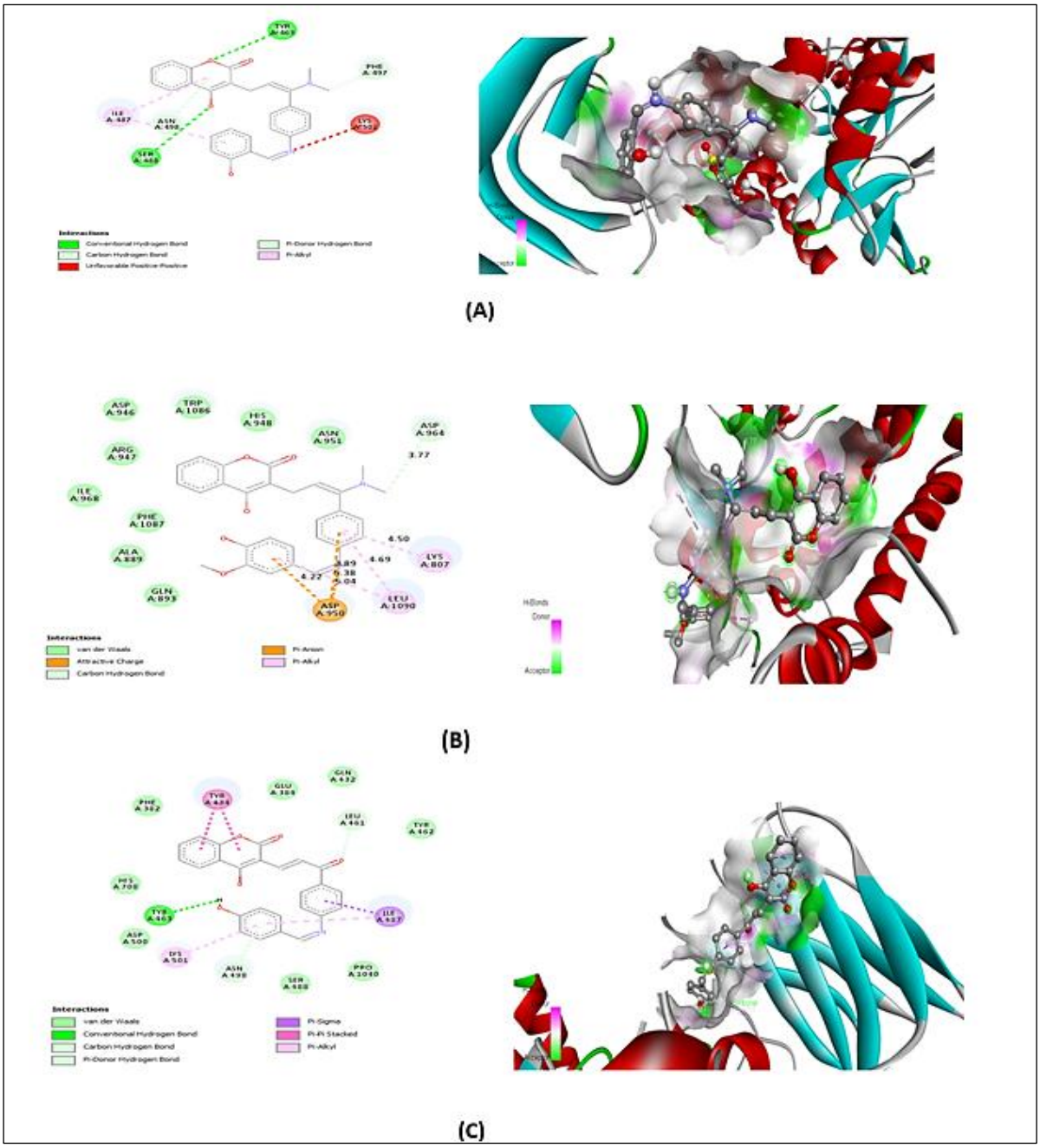


Figure 2: 2D and 3D Molecular Interaction of (A) 5h, (B) 5i and (C) Reference

Table 2: Docking Simulations of Derivatives

Compounds	Binding Energy (Kcal/mol)
5a	-7.9
5b	-6.8
5c	-7.2
5d	-7.3
5e	-7.9
5f	-7.2
5g	-7.1
5h	-8.5
5i	-8.0
5j	-7.9
Reference	-8.1

Table 3: ADMET Profile of Derivatives (Predicted)

Compounds	Mol wt.	TPSA	HBD	HBA	Log P	Log S	No. of heavy atoms	RB	MR	PAINS	BRENk
5a	440.49	86.27	2	6	2.84	-5.44	33	6	132.12	0	0
5b	469.49	111.86	1	7	3.28	-5.65	35	7	138.92	0	3
5c	458.94	66.04	1	5	2.85	-6.18	33	6	135.11	0	0
5d	458.94	66.04	1	5	2.85	-6.18	33	6	135.11	0	3
5e	484.54	84.50	1	7	2.70	-5.73	36	8	143.08	0	0
5f	424.49	66.04	1	5	3.38	-5.58	32	6	130.10	0	0
5g	503.39	66.04	1	5	3.14	-6.49	33	6	137.80	0	0
5h	424.49	66.04	1	5	2.38	-5.58	32	6	130.10	0	0
5i	470.52	95.50	2	7	2.50	-5.51	35	7	138.61	0	0
5j	440.49	86.27	2	6	2.84	-5.44	33	6	132.12	0	0

Anticancer Activity (*In vitro*)

All freshly synthesised coumarin chalcone derivatives were tested for antitumour action (in vitro) against MCF 7 cancer cell lines through Sulphorodamine B assay using Imatinib as reference drug (37, 38). SRB assay method provided permanently stable and non- destructive colorimetric end point. Therefore, it is a sensitive and suitable assay method to determine the percentage of growth inhibition. At five distinct concentrations i.e. 10, 20, 40, 80 µg/ml, relative cell viability in percentage was evaluated and compared with standard. Each compound's GI_{50} determined and compared against standard. The anticancer activity results displayed in Table 4. Anticancer profile of compounds 5a, 5e, 5h, 5i and standard (Imatinib) shown in Figures 3A, 3B, 3C, 3D, 3E. The graph plotted between % control growth versus concentration of derivatives showed in Figure 4. GI_{50} value correlation of derivatives shown in Figure 5. Results shown that synthesised compounds 5h, 5e, 5i and 5a have potential GI_{50} values less than 10µg/ml means they are comparable to standard drug Imatinib.

Compounds 5i and 5h hydroxyl groups and dimethyl-amino groups at para position as substituents respectively exhibited exhibited the activity less than 10 µg/ml. As well as derivative 5a and 5e exhibited strong cytotoxic activity against tested cell lines. 5c, 5d and 5j showed medium cytotoxic profile for MCF-7 cancerous cell lines. Derivative 5f, 5g and 5b exhibited less anticancer actions for MCF-7 cell lines.

Aforementioned group's electron releasing property raises the parent molecule's electron density which boosts the compound's potency for anticancer activity. In addition, combination of coumarin and chalcone rings increases the therapeutic values.

Table 4: Results for Anticancer Activity of Compounds

	Human Breast Cancer Cell lines (MCF-7) *In vitro* **cytotoxicity (% Control growth) Drug concentration (µg/ml)**				**GI_{50} Value (µg/ml)**
Compound no.	10	20	40	80	
5a	11.2	1.3	-15.6	-26.2	9.8
5b	55.6	41.5	25.7	14.5	30.4
5c	55.6	32.5	17.9	9.8	27.7
5d	55.8	33.8	18.9	9.2	27.4
5e	25.8	3.6	-10.5	-20.9	9.6
5f	60.2	43.9	16.8	9.6	35.4
5g	25.8	10.5	-10.6	-15.9	15.1
5h	39.7	22.3	11.1	2.2	18.1
5i	25.7	2.5	-14.6	-25.2	15.1
5j	50.2	35.8	24.8	13.8	20.5
Std.	0.8	-12.6	-35.8	-61.4	9.4

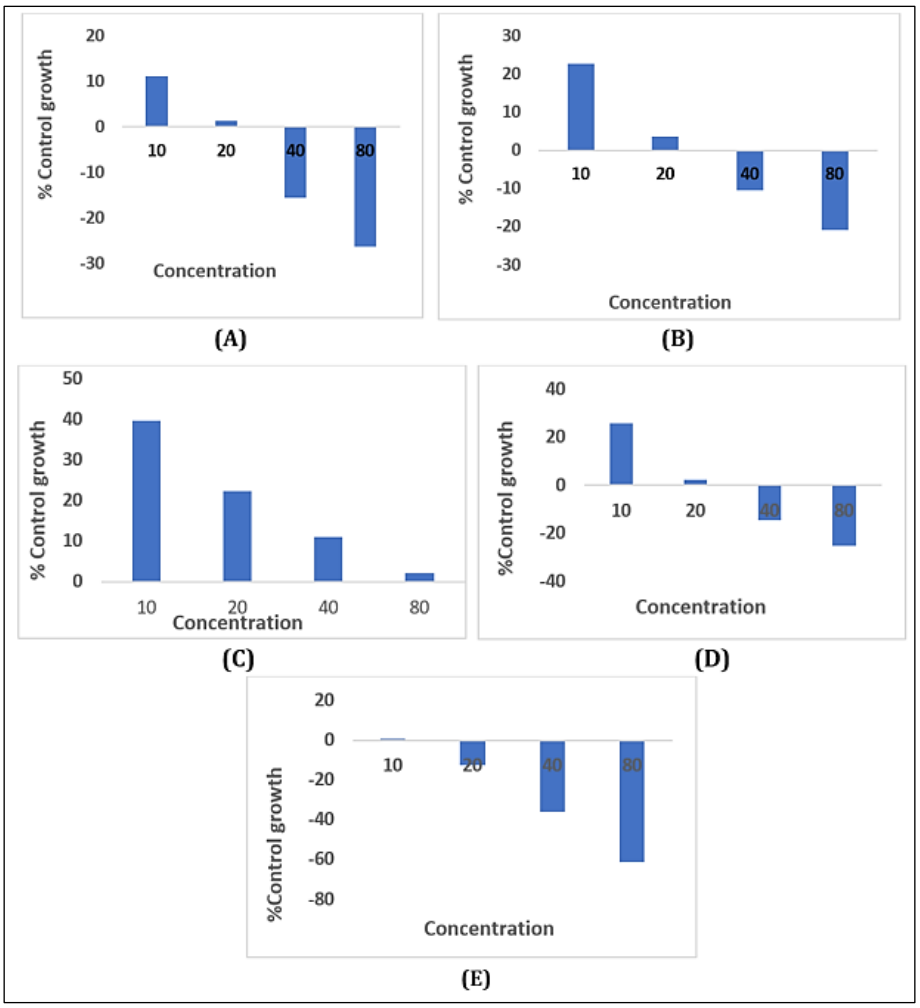


Figure 3: Anticancer Profile of Compounds (A) 5a, (B) 5e, (C) 5h, (D) 5i and (E) Standard (Imatinib)

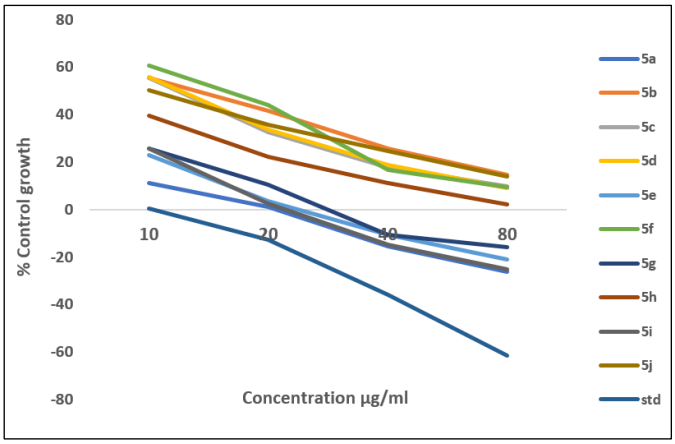


Figure 4: Percentage (%) Control Growth versus Drug Concentration (Anticancer Activity)

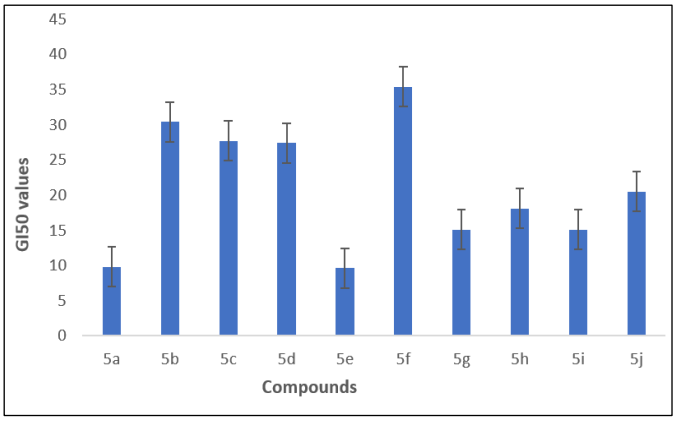


Figure 5: Graphical Representation of GI50 Values for Derivatives

Conclusion

In conclusion, we developed PI3K inhibitors by combining coumarin and chalcone moieties with aromatic substitutions. Hydrogen attached with oxygen atom involved in hydrogen bonding with active site of Phosphoinositide 3 Kinase. The potency is enhanced by the presence of electron releasing group. Percentage cell viability or percent control growth and GI_{50} value were used to assess the strength of synthesised derivatives. In this study, derivatives 5a, 5e, 5h and 5i proved as most significant compounds as these showed excellent efficacy against (MCF-7) cancer cell lines. Derivatives exhibited dose-dependent inhibition of breast cancer cell growth and proliferation, according to SRB assay. Our research shows that hybrid derivatives of coumarin chalcones may have an impact by producing cell damage and apoptotic body formation. These findings suggest that these cytotoxic actions may have anticancer importance for future investigations. These derivatives could have reasonable value for development as therapeutic agents by conducting *in vivo* studies.

Abbreviations

DMSO: Dimethyl sulfoxide, GI: Growth Inhibition, LRO5: Lipinski's rule of five.

Acknowledgment

The authors are highly thankful to Jagannath university, Jaipur's Department of pharmacy, Faculty of Medical/Para Medical and Allied Sciences, Rajasthan- 302022 and SGT university, Gurugram, Haryana-122005 for providing necessary facilities to conduct the study.

Author Contributions

Sumita Kumara: Completed all the evaluation parameters, Navgeet Kaur: Data Compilation, Amit Sharma: Manuscript Evaluation, Sonia Yadav: Manuscript Evaluation.

Conflict of Interest

The authors have no conflict of interest.

Ethics Approval

Not applicable.

Funding

None.

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