Research Paper

Synthesis, Spectral Characterization, and *In Vitro* Anticancer Evaluation of Novel Flavanone-Arylhydrazone Derivatives

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Flavanones represent an important class of naturally inspired scaffolds with diverse pharmacological activities, including notable anticancer potential. Structural modification of the flavanone core offers opportunities to enhance biological performance and identify new lead molecules. In the present study, a series of nine flavanone-arylhydrazone derivatives was synthesised through the condensation of substituted flavanones with corresponding phenylhydrazines under reflux conditions. The synthesised compounds were obtained in good yields and were structurally confirmed using infrared spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry, which collectively verified the integrity of the hydrazone linkage and aromatic substitutions. The derivatives were evaluated for *in vitro* cytotoxicity against two human breast cancer cell lines, michigan cancer foundation-7 (hormone-dependent) and MDA-MB-231 (triple-negative), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Tamoxifen served as the reference standard. All compounds exhibited dose-dependent cytotoxicity, with variable responses across both cell lines. Among them, compounds 3 and 1 demonstrated the highest activity, with IC_{50} values of 33.54±2.2 µM and 36.60±2.4 µM in Michigan cancer foundation-7 cells and 41.08±2.6 µM and 44.21±2.7 µM in MDA-MB-231 cells, respectively. Structure-activity analysis indicated that derivatives bearing electron-withdrawing substituents, such as nitro and halogens, displayed comparatively enhanced potency. The findings emphasise the possible use of flavanone–arylyhydrazone hybrids as promising anticancer candidates. Compounds 3 and 1, in particular, may serve as lead molecules for further mechanistic investigations and optimisation studies aimed at developing new therapeutic agents for breast cancer.

Key words: Flavanone, hydrazone, breast neoplasms, Michigan cancer foundation-7 Cells, MDA-MB-231 cells

Flavanones constitute an important subclass of naturally occurring flavonoids widely distributed in fruits, vegetables, and medicinal plants. These compounds are recognised for their broad therapeutic relevance, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities[1-3]. Their structural diversity and ability to interact with multiple cellular targets make them valuable scaffolds in modern drug discovery[4]. Despite this promise, many natural flavonoids suffer from limitations such as suboptimal potency, metabolic instability, and restricted bioavailability, which necessitate chemical modification to improve their pharmacological performance[5].

The development of new anticancer agents remains a global priority, particularly for breast cancer, one of the leading causes of cancer-related mortality among women[6]. Among breast cancer subtypes, hormone-responsive (Michigan Cancer Foundation-7 (MCF-7)) and triple-negative (MDA-MB-231) cells represent clinically challenging models due to differences in receptor expression, signalling pathways, and therapeutic responsiveness[7]. Identifying small molecules capable of exerting cytotoxic effects against both subtypes is therefore essential for expanding treatment options.

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Hydrazone-based derivatives have gained considerable attention in medicinal chemistry owing to their ease of synthesis and versatile biological activities[8]. The hydrazone functional group is known to enhance molecular binding affinity, improve lipophilicity, and enable additional interactions with intracellular targets[9]. Incorporating hydrazone moieties into flavanone frameworks has emerged as a promising strategy to develop hybrid molecules with superior anticancer potential[10]. Previous studies report that introducing electron-withdrawing groups such as nitro or halogens on aromatic rings can further enhance cytotoxic and pharmacodynamics properties[11].

However, the design and systematic evaluation of flavanone-arylyhydrazone hybrids remain insufficiently explored, particularly in the context of breast cancer models[10, 12]. Therefore, there is a strong rationale to synthesise new derivatives, characterise their structural features and assess their biological activity to identify potential lead candidates[13,14].

In this study, a series of nine flavanone-arylhydrazone derivatives was synthesised through condensation reactions between substituted flavanones and phenylhydrazines. The synthesised molecules were characterised using Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (NMR), and mass spectrometry to confirm structural integrity. Their anticancer potential was evaluated *in vitro* against MCF-7 and MDA-MB-231 breast cancer cell lines using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Furthermore, structure-activity relationships were examined to understand the contribution of various substituents to cytotoxic activity. The findings give fresh perspectives on the therapeutic relevance of flavanone–hydrazone hybrids and identify promising candidates for future development.

MATERIALS AND METHODS

Chemicals and instrumentation:

All chemicals and reagents used in this study were obtained from certified suppliers, such as Merck, Central Drug House (CDH) Pvt. Ltd. (India), Sigma-Aldrich, Loba Chemie, and Fischer Scientific (India), and were used without further purification. Melting points were measured with an open capillary method and are reported uncorrected. Thin-Layer Chromatography (TLC) was carried out on silica gel G-coated glass plates using a mobile phase of ethyl acetate, formic acid, and water (8:1:1). Spots were visualised using iodine vapour. Infrared (IR) spectra were recorded on a Shimadzu IR Affinity-1 FTIR spectrophotometer with the DRS-8000A accessory and are reported in cm^{-1}. Proton (^1H) and carbon (^13C) NMR spectra were recorded in $CDCl_3$ with Tetramethylsilane (TMS) as an internal standard using a Bruker Avance 500 MHz spectrometer. Mass spectra were acquired using a Waters Q-TOF (ESI–MS) system. All spectral analyses, including NMR and mass spectrometry, were performed at the Sophisticated Analytical Instrument Facility (SAIF), Panjab University, Chandigarh, India.

Synthesis of substituted flavanone, hesperitin, and hesperidin arylhydrazones: Substituted arylhydrazones of flavanone were synthesised *via* a condensation reaction with substituted phenylhydrazines. Equimolar quantities (5 mmol) of each flavanone and the respective substituted phenylhydrazine were dissolved in ethanol (50 ml) with a catalytic amount of acetic acid (0.5 ml). The reaction mixtures were refluxed for appropriate durations, 6 h to 17 h for flavanone derivatives. After cooling to ambient temperature, the obtained precipitates were isolated by vacuum filtration and purified by rinsing with ethanol (Table 1).

TABLE 1: PHYSICOCHEMICAL PROPERTIES OF SYNTHESISED COMPOUNDS

S. No.	R	Molecular formula (Molecular weight)	Percentage Yield	M.P.*	R_f Value (Ethyl acetate: Formic acid: Water) (8:1:1)
1	2,4-NO_2	$C_{21}H_{16}N_4O_5$ -404.38	78.99	197-199	0.77
2	4-Cl	$C_{21}H_{17}ClN_2O$ -348.83	73.13	241-243	0.59
3	4- NO_2	$C_{21}H_{17}N_3O_3$ -359.38	85.23	238-242	0.74

4	4-Phenyl	$C_{22}H_20N_2O$ -328.41	80.18	115-120	0.82
5	3-NO	$C_{21}H_{17}N_3O_3$ -359.38	65	217-218	0.68
6	4-F	$C_{21}H_{17}FN_2O$ -332.37	63	258-259	0.81
7	4-Br	$C_{21}H_{17}BrN_4O_5$ -393.28	78.99	197-199	0.77
8	3-OCH_3	$C_{22}H_20N_2O_2$ -344.41	73.13 %	150-151	0.59
9	2-CH_3	$C_{22}H_20N_2O$ -328.16	69.00 %	165-168	0.78

Characterisation data of synthesised flavanone derivatives:

Compound 1: IR (KBr) ν (cm^{-1}): 3010 (Ar=C–H stretching), 1610 (C=O stretching), 1589 (C-C stretching), 1592, 1462, 1419 (Ar C=C stretching), 1190 (C-O-C stretching), 1506 (NO_2 stretching); ^{1}H NMR (DMSO-d_6) δ in ppm: 2.77-2.84 (m, ^{1}H, 3-H), 3.35-3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.44 (t, 2H, J=7.2 Hz, 3′-H and 5′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 5-H), 8.11 (d, 2H, J=8.0 Hz, 3″-H and 5″-H), 10.31 (s, ^{1}H, NH); Mass (ESI-MS): m/z 403 (M+1); Elemental analysis: C, 62.37; H, 3.99; N, 13.86; O, 19.78.

Compound 2: IR (KBr) ν (cm^{-1}): 3012 (Ar=C–H stretching), 1667 (C=O stretching), 1598 (C-C stretching), 1567, 1485, 1443 (Ar C=C stretching), 1178 (C-O-C stretching), 1506 (NO_2 stretching); ^{1}H NMR (Dimethyl Sulfoxide (DMSO)-d6) δ in ppm: 2.75-2.84 (m, ^{1}H, 3-H), 3.35-3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 5-H), 8.11 (d, 2H, J=8.0 Hz, 3″-H and 5″-H), 10.21 (s, ^{1}H, NH); Mass (ESI-MS): m/z 347 (M+1); Elemental analysis: C, 72.31; H, 4.91; Cl, 10.16; N, 8.03; O, 4.59.

Compound 3: IR (KBr) ν (cm^{-1}): 3065 (Ar=C-H stretching), 2840 (C-H stretching), 1658 (C=O stretching), 1600 (C-C stretching), 1559, 1458, 1420 (Ar C=C stretching), 1196 (C-O-C stretching), 1124 (C-O stretching), 757 (C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ in ppm: 2.77-2.84 (m, ^{1}H, 3-H), 3.35-3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.44 (t, 2H, J=7.2 Hz, 3′-H and 5′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 5-H), 8.11 (d, 2H, J=8.0 Hz, 3″-H and 5″-H), 10.31 (s, ^{1}H, NH); Mass (ESI-MS): m/z 358 (M+1); Elemental analysis: C, 70.18; H, 4.77; N, 11.69; O, 13.

Compound 4: IR (KBr) ν (cm^{-1}): 3200 (O-H stretching), 3117 (Ar=C-H stretching), 1670 (C=O stretching), 1612 (C-C stretching), 1576, 1456, 1426 (Ar C=C stretching), 1488 (N-O asymmetric stretching), 1369 (N-O symmetric stretching), 1167 (C-O-C stretching); ^{1}H NMR (DMSO-d_6) δ in ppm: 2.77-2.84 (m, ^{1}H, 3-H), 3.35-3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.44 (t, 2H, J=7.2 Hz, 3′-H and 5′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 5-H), 8.11 (d, 2H, J=8.0 Hz, 3″-H and 5″-H), 10.31 (s, ^{1}H, NH); Mass (ESI-MS): m/e 328.16 (100.0 %), 329.16 (24.8 %), 330.16 (3.2 %); Elemental analysis: C, 80.46; H, 6.14; N, 8.53; O, 4.87.

Compound 5: IR (KBr) ν (cm^{-1}): 3342 (O-H stretching), 3038 (Ar=C-H stretching), 2846 (C-H stretching), 1669 (C=O stretching), 1587 (C-C stretching), 1553, 1460, 1432 (Ar C=C stretching),

1214 (C-O stretching), 1117 (C-O-C stretching), 1499 (NO_2 stretching); ^{1}H NMR (DMSO-d_6) δ in ppm: 2.77-2.84 (m, ^{1}H, 3-H), 3.35-3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.44 (t, 2H, J=7.2 Hz, 3′-H and 5′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 5-H), 8.11 (d, 2H, J=8.0 Hz, 3″-H and 5″-H), 10.31 (s, ^{1}H, NH); Mass (ESI-MS): m/e 328.16 (100.0 %), 329.16 (24.8 %), 330.16 (3.2 %); Elemental analysis: C, 80.46; H, 6.14; N, 8.53; O, 4.87; m/z=358 (M+1); C, 70.18; H, 4.77; N, 11.69; O, 13.36.

Compound 6: IR (KBr) ν (cm^{-1}): 3342 (O-H stretching), 3038 (Ar=C-H stretching), 2846 (C-H stretching), 1669 (C=O stretching), 1587 (C-C stretching), 1553, 1460, 1432 (Ar C=C stretching), 1214 (C-O stretching), 1117 (C-O-C stretching), 1234 (C-F stretching); ^{1}H NMR (DMSO-d_6) δ in ppm: 2.77-2.84 (m, ^{1}H, 3-H), 3.35-3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.44 (t, 2H, J=7.2 Hz, 3′-H and 5′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 5-H), 8.11 (d, 2H, J=8.0 Hz, 3″-H and 5″-H), 10.31 (s, ^{1}H, NH); Mass (ESI-MS): m/z 331 (M+1); Elemental analysis: C, 75.89; H, 5.16; F, 5.72; N, 8.43; O, 4.81.

Compound 7: IR (KBr) ν (cm^{-1}): 3342 (O–H stretching), 3038 (Ar=C-H stretching), 2846 (C-H stretching), 1669 (C=O stretching), 1587 (C-C stretching), 1553, 1460, 1432 (Ar C=C stretching), 1214 (C-O stretching), 1117 (C-O-C stretching), 638 (C-Br stretching); ^{1}H NMR (DMSO-d_6) δ in ppm: 2.77-2.84 (m, ^{1}H, 3-H), 3.35-3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.44 (t, 2H, J=7.2 Hz, 3′-H and 5′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H); Mass (ESI-MS): m/z 391 (M+1); Elemental analysis: C, 64.13; H, 4.36; Br, 20.32; N, 7.12; O, 4.07.

Compound 8: IR (KBr) ν (cm^{-1}): 3342 (O-H stretching), 3038 (Ar=C-H stretching), 2846 (C-H stretching), 1669 (C=O stretching), 1587 (C-C stretching), 1553, 1460, 1432 (Ar C=C stretching), 1214 (C-O stretching), 1117 (C-O-C stretching), 2830 (OCH3 stretching); ^{1}H NMR (DMSO-d_6) δ in ppm: 2.66-2.84 (m, ^{1}H, 3-H), 3.35-3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.44 (t, 2H, J=7.2 Hz, 3′-H and 5′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 5-H), 8.11 (d, 2H, J=8.0 Hz, 3″-H and 5″-H), 10.31 (s, ^{1}H, NH); Mass (ESI-MS): m/z 342 (M+1); Elemental analysis: C, 76.72; H, 5.85; N, 8.13; O, 9.

Compound 9: IR (KBr) ν (cm^{-1}): 3342 (O-H stretching), 3038 (Ar=C-H stretching), 2846 (C-H stretching), 1669 (C=O stretching), 1587 (C-C stretching), 1553, 1460, 1432 (Ar C=C stretching), 1214 (C-O stretching), 1117 (C-O-C stretching), 2830 (C-CH3 stretching); ^{1}H NMR (DMSO-d_6) δ in ppm: 2.77-2.84 (m, ^{1}H, 3-H), 3.35–3.40 (m, ^{1}H, 3-H), 5.25 (dd, ^{1}H, J=12.0, 3.2 Hz, 2-H), 6.96 (d, ^{1}H, J=8.0 Hz, 8-H), 7.03 (t, ^{1}H, J=8.0 Hz, 7-H), 7.27 (t, ^{1}H, J=8.0 Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, ^{1}H, J=7.2 Hz, 4′-H), 7.44 (t, 2H, J=7.2 Hz, 3′-H and 5′-H), 7.56 (d, 2H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 2″-H and 6″-H), 8.07 (d, ^{1}H, J=8.0 Hz, 5-H), 8.11 (d, 2H, J=8.0 Hz, 3″-H and 5″-H), 10.31 (s, ^{1}H, NH); Mass (ESI-MS): m/z 327 (M+1); Elemental analysis: C, 80.46; H, 6.14; N, 8.53; O, 4.87.

Anticancer evaluation of the synthesised derivatives:

***In vitro* anti-breast cancer activity evaluation against MCF-7 and MDA-MB-231 cell lines:** The synthesised test compounds were thoroughly assessed for their *in vitro* anticancer activity against two breast cancer cell lines: MCF-7 (hormone-dependent) and MDA-MB-231 (triple-negative). These cell lines were chosen due to their clinical relevance and common use as models in anticancer research. Experiments were conducted in triplicate at each concentration to ensure reproducibility and statistical accuracy. A fluorescence-based detection method was applied to evaluate the cytotoxic potential of the compounds. This approach involved staining viable cells with fluorescent dyes, allowing quantification of cell viability after treatment. The technique is recognised for its sensitivity and capacity to deliver reliable results across diverse cell types. Tamoxifen, a well-established clinical anticancer drug, was employed as a positive control to benchmark the activity of the test compounds,

providing a reference for comparing their efficacy under identical experimental conditions.

The anticancer activity of the compounds was measured by determining the percentage of cell growth inhibition at different concentrations. In addition, the Half-Maximal Inhibitory Concentration (IC_{50}) values were calculated for each compound. The IC_{50} indicates the concentration needed to suppress 50 % of cell growth and serves as an important parameter for comparing the potency of various compounds.

Methodology: The viability of cells following treatment with three DLM concentrations (1 µM, 10 µM, and 100 µM) was assessed using the MTT assay. The relative cell survival was expressed as a percentage of the control cells. Cells (1×10^4) were plated in 96-well plates and treated with DLM for 6 and 18 h at 37° in a CO2 incubator. Ten microliters of MTT (5 mg/ml in Phosphate Buffer Solution (PBS)) was added 4 h before the end of the incubation, followed by centrifugation at 1200×g for 10 min. After discarding the supernatant, 100 µl of DMSO was added. Cell viability was determined using the CellTiter 96 Proliferation Assay Kit (Promega, Madison, WI, United States of America (USA) according to the manufacturer's instructions. Absorbance was measured with a Microplate Reader (Model 680, BIORAD) at 490 nm. The effect of different concentrations of the compound on cell viability was expressed as a percentage by comparing the absorbance of treated cells with that of cells maintained in culture medium alone. The percentage of cell growth inhibition and the IC_{50} values were determined using Prism software (GraphPad, San Diego, CA).

RESULTS AND DISCUSSION

A novel series of nine flavanone-based arylhydrazone derivatives was synthesised *via* a condensation reaction between substituted flavanones and substituted phenylhydrazines. The synthetic approach involved equimolar amounts of flavanone (5 mmol) and substituted phenylhydrazine in refluxing ethanol (50 ml) with a catalytic amount of acetic acid (0.5 ml) over 6-17 h. Upon completion and cooling to ambient temperatures, the desired products were isolated by vacuum filtration and purified through ethanol washing. The synthesised compounds exhibited moderate to excellent yields ranging from 63 % to 85 %. Structural characterisation was accomplished *via* different spectroscopic techniques. The IR spectra consistently showed a prominent absorption band around 1665-1610 cm^{-1}, corresponding to the C=O stretching of the flavanone core, while the characteristic N-H stretch appeared in the region of 3300-3400 cm^{-1}. ^1H NMR analysis revealed singlet peaks in the δ 10.21-10.31 ppm range, indicative of hydrazone N-H protons, and multiples between δ 6.9 and 8.1 ppm attributable to aromatic protons. Mass spectrometry data showed molecular ion peaks consistent with the expected molecular weights of each compound, thereby validating their successful synthesis. The combination of spectral features confirmed the integrity of the synthesised flavanone arylhydrazone scaffolds and their suitability for subsequent biological screening.

The synthesised compounds (1-9) were evaluated for *in vitro* anti-breast cancer activity using whole-cell models of MCF-7 (hormone-dependent) and MDA-MB-231 (triple-negative). The assays measured the percentage of viable cells following treatment with the test compounds, and the results were compared to untreated controls to assess cytotoxic effectiveness. Tamoxifen, a widely utilised standard anti-breast cancer medication, served as the positive control to evaluate the efficacy of the synthesised compounds. The evaluation was performed over a wide concentration range (0.5 to 300 µg/ml) to provide a detailed understanding of the dose-response relationship for each compound. Activity was quantified by determining cell viability at each concentration, and results were expressed as the percentage of cell growth inhibition relative to the control. Graphs depicting % growth inhibition *vs*. compound concentration were plotted for both MCF-7 and MDA-MB-231 cell lines (fig. 1 and fig. 2).

Comparison with standard tamoxifen showed that it had the lowest IC_{50} values of 11.46±0.2 µM (MCF-7) and 27.13±0.4 µM (MDA-MB-231), indicating strong cytotoxic activity (Table 2). In contrast, all tested compounds exhibited higher IC_{50} values, reflecting lower potency. Nevertheless, certain compounds demonstrated notable activity, particularly 3, 1, 5, 6, and 9, with IC_{50} values below 75 µM in at least one cell line. Among these, compound 3 (33.54±2.2 µM) showed the highest cytotoxicity in MCF-7 cells, followed by 1 (36.6±2.4 µM), 9 (65.45±4.5 µM), 5 (60.42±4.3 µM), and 6 (72.11±3.2 µM). These compounds had significantly lower IC_{50} values than

the others, indicating a stronger inhibitory effect on estrogen receptor-positive breast cancer cells. For the triple-negative MDA-MB-231 cell line, compounds 3 (41.08±2.6 µM), 1 (44.21±2.7 µM), 5 (89.53±3.6 µM), and 6 (59.98±4.2 µM) showed substantial cytotoxic activity.

In this study, the synthesised compounds showed different levels of cytotoxicity against MCF-7 and MDA-MB-231 cell lines derived from breast cancer, suggesting potential differences in their mechanisms of action, receptor interactions, and cellular uptake. Notably, 3, 1, 5, and 6 demonstrated comparable cytotoxicity across both cell lines, indicating their potential as broad-spectrum anticancer agents.

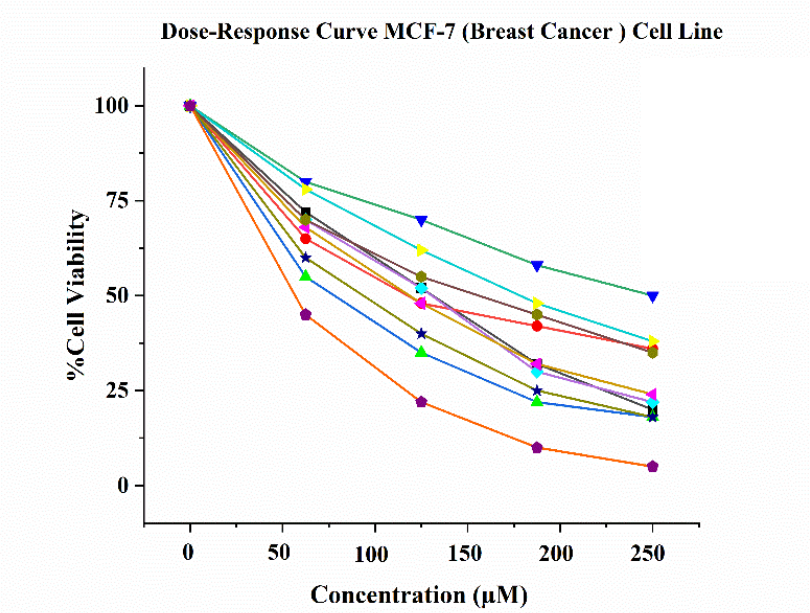

Fig. 1: Effect of the compounds 1-9 and Tamoxifen against MCF-7 cell line viability. The plot represents the data in terms of % cell viability *vs.* concentration
Note: (—■—): RS1; (—●—): RS2; (—▲—): RS3; (—▼—): RS4; (—◆—): RS5; (—◀—): RS6; (—▶—): RS7; (—●—): RS8; (—★—): RS9 and (—⬟—): Tamoxifen

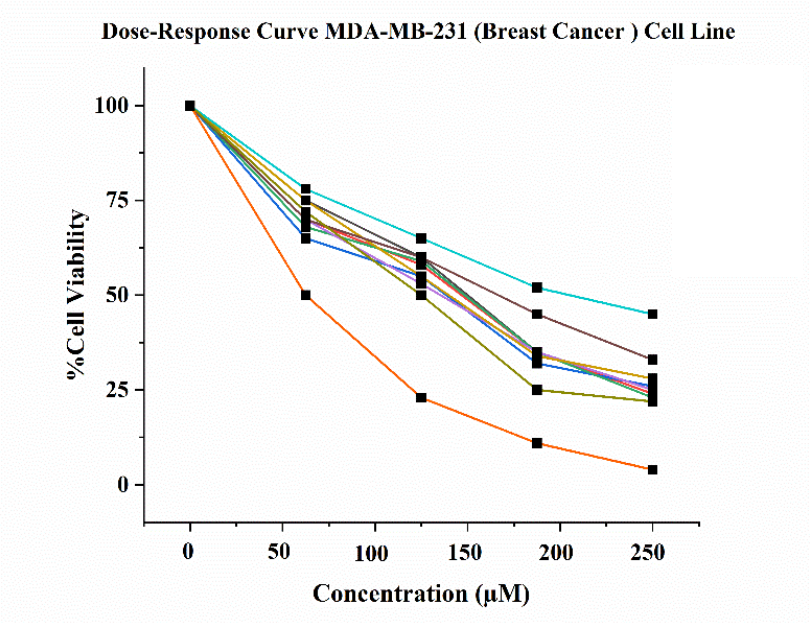

Fig. 2: Effect of the compounds 1-9 and Tamoxifen on MDA-MB-231 cell line viability. The plot represents the data in terms of % cell viability *vs.* concentration
Note: (—■—): RS1; (—■—): RS2; (—■—): RS3; (—■—): RS4; (—■—): RS5; (—■—): RS6; (—■—): RS7; (—■—): RS8; (—■—): RS9 and (—■—): Tamoxifen

TABLE 2: IC_{50} OF COMPOUNDS AND TAMOXIFEN AGAINST MCF-7 AND MDA-MB-231 CELL LINES

Sr. no	Molecule	IC_{50}	
		MCF-7	MDA-MB-231
1	1	36.6±2.4 µM	44.21±2.7 µM
2	2	145.1±5.4 µM	125.02±8.4 µM
3	3	33.54±2.2 µM	41.08±2.6 µM
4	4	206.18±9.75 µM	241.13±5.85 µM
5	5	60.42±4.3 µM	89.53±3.6 µM
6	6	72.11±3.2 µM	59.98±4.2 µM
7	7	259.80±7.99 µM	159.76±9.74 µM
8	8	173.40±5.78 µM	294.84±9.04 µM
9	9	65.45±4.5 µM	195.69±5.49 µM
10	Tamoxifen	11.46±0.2 µM	27.13±0.4 µM

The structure-activity relationship analysis offers explanations for the molecular features contributing to cytotoxicity. Compounds bearing electron-withdrawing functional groups, such as halogens, nitro, and carbonyl moieties, appeared to enhance anticancer activity, likely due to their influence on electron density and molecular interactions with cellular targets. Furthermore, the presence of hydrophobic moieties may play a crucial role in membrane permeability, particularly in MDA-MB-231 cells, which lack estrogen receptor expression and rely on alternative pathways for drug uptake. The SAR analysis suggests that compounds with electron-withdrawing functional groups (e.g., halogens, nitro, carbonyl) might contribute to enhanced activity. The presence of hydrophobic moieties could influence membrane permeability. This was especially observed in the triple-negative MDA-MB-231 cell line. The observed differences in potency between ER-positive and triple-negative breast cancer cells could be due to variations in estrogen receptor interactions, apoptotic signalling, and drug efflux processes. These results emphasise the significance of molecular modifications in optimising the cytotoxic potential of these compounds and warrant further investigation into their mechanistic pathways and therapeutic applicability.

The present study aimed to develop flavanone-arylhydrazone hybrids and evaluate their anticancer potential against two representative breast cancer cell lines, MCF-7 and MDA-MB-231. Flavonoids, including flavanones, are well recognised for their ability to modulate multiple intracellular pathways associated with cancer progression, which makes them attractive scaffolds for anticancer drug design[15,16]. The synthesised compounds displayed distinct cytotoxic profiles, highlighting the influence of structural modifications on biological activity. These findings are consistent with earlier reports demonstrating that strategic functionalization of flavonoid frameworks significantly enhances anticancer efficacy[17].

Cytotoxic screening revealed that compounds 3, 1, 5, and 6 exhibited comparatively higher activity than the other synthesised molecules, with IC_{50} values below 50 µM in at least one of the tested cell lines[18]. Substituent effects played a notable role in modulating cytotoxic potency. Electron-withdrawing groups such as nitro, chloro and fluoro substituents have been shown to increase molecular electrophilicity and strengthen interactions with cellular targets[19]. This trend was also observed in our findings, as nitro-bearing compounds (3 and 5) showed enhanced potency, possibly due to their ability to induce oxidative stress or facilitate better membrane penetration[20].

Differential sensitivity between MCF-7 and MDA-MB-231 cells further supports the complexity of their underlying signalling mechanisms. MCF-7 cells, which are estrogen receptor-positive, often respond more readily to small-molecule inhibitors, whereas MDA-MB-231 cells exhibit aggressive, drug-

resistant characteristics typical of triple-negative breast cancer[21,22]. Nevertheless, compounds 3 and 1 showed comparable activity in both cell lines, suggesting receptor-independent mechanisms, potentially involving mitochondrial disruption or modulation of redox homeostasis[23].

Although Tamoxifen displayed the highest potency, its clinical limitations, including endocrine resistance and long-term adverse effects, highlight the need for alternative chemotypes with improved safety and broader activity profiles. While the synthesised derivatives did not surpass Tamoxifen's potency, several compounds demonstrated sufficient cytotoxic activity to justify further lead optimisation. The flavanone-arylyhydrazone framework offers flexibility for introducing additional substituents that may improve metabolic stability, target binding and cellular uptake[24, 25].

Taken together, the present findings provide a strong foundation for continued investigation. The observed structure-activity relationships indicate that future work should focus on fine-tuning electron-withdrawing substituents, optimising hydrophobic domains and exploring heterocyclic substitutions within the arylhydrazone region. Additionally, mechanistic studies including apoptosis assays, molecular docking and *in vivo* validation would help define the biological pathways involved and support the development of these derivatives as potential anticancer agents[26].

In conclusion, nine flavanone-arylhydrazone derivatives were synthesised and evaluated for their *in vitro* cytotoxic potential against MCF-7 (hormone-dependent) and MDA-MB-231 (triple-negative) breast cancer cell lines. Several derivatives demonstrated notable cytotoxicity, particularly compounds 3, 1, 5 and 6, which exhibited IC_{50} values below 50 µM in at least one of the tested models. Although Tamoxifen remained the most potent reference agent, the activity profiles of compounds 3 and 1 indicate their potential as promising lead candidates. The overall findings highlight the relevance of structural modifications on the flavanone scaffold for improving anticancer efficacy. Further mechanistic studies, structural optimisation and preclinical evaluation are warranted to advance these molecules toward potential therapeutic development.

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Conflict of interests:

The authors declared no conflict of interests.

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