A novel synthesis of aurones: Their \textit{in vitro} anticancer activity against breast cancer cell lines and effect on cell cycle, apoptosis and mitochondrial membrane potential

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Abstract

A novel method for the synthesis of aurones from benzofuran-3(2H)-one by condensation with aromatic aldehydes is described. The anticancer activity of the synthesized compounds was evaluated by MTT assay and among the series of 15 compounds, (Z)-2-(4-methylbenzylidene) benzofuran-3(2H)-one (3e) and (Z)-2-(4-chlorobenzylidene) benzofuran-3(2H)-one (3f) were found to be most promising inhibitors against two breast cancer cell lines MDA-MB-231 and MCF-7. Further, the mechanism of action of these two compounds (3e and 3f) was studied by evaluating their effect on the cell cycle phase distribution, apoptosis induction by Hoechst staining as well as mitochondrial membrane potential loss using flow cytometry. The two compounds exhibited a time-dependent cell cycle arrest at G0/G1 phase. The two compounds induced a potent apoptotic effect in MCF-7 cancer cells showing chromatin condensation or dense staining fragmentation called apoptotic bodies, which implied an early apoptotic event. These two compounds also induced a potent loss in mitochondrial membrane potential.

Introduction

Aurones constitute an important class of natural products in the family of flavanoid that are found in many plants (Haudecoeur and Boumendjel, 2012; Boumendjel, 2003). Fruits and flowers are the common sources for flavanoids along with several flavones, isoflavones and chalcones (Figure 1). From the past several years flavones and chalcones have been well studied for treatment against various diseases, but the biological activity of aurones has not been extensively studied. Aurones are mainly used as anti-cancer (Lawrence et al., 2003), antimalarial (Kayser et al., 2001) and anti-microbial (Bandgar et al., 2010) agents.

![Figure 1: Some flavonoid sub-classes](image-url)
In plants aurones are synthesized from chalcones by oxidation, cyclisation and rearrangement involving the enzyme aureusidin synthase (Nakayama, 2002; Nakayama et al., 2001; Nakayama, 2000). Aurones exhibit a range of pharmacological activities (Boumendjel, 2003) including anti-cancer, anti-feedant and antiparasitic activities via modulation of a variety of molecular targets such as G2/M phase cell-cycle arrest, arresting the cell cycle in G0/G1 phase and displayed apoptosis-inducing effect on Hep-2 cells (Huang et al., 2007), inhibition of Human Sphingosine Kinase (French et al., 2003), inhibition of P-gp related transport (Vaclavikova et al., 2006), high-affinity binding to cytosolic domain of p-glycoprotein (Boumendjel et al., 2002), modulation of ABCG2 activities (Sim et al., 2011), etc.

Literature reveals that several methods have been reported for the synthesis of aurones. The most commonly used method for the synthesis of aurones involves the aldol condensation of benzofuran-3(2H)-ones with aromatic aldehyde. This reaction can be carried out by using alumina (Varma and Varma, 1992; Loser et al., 2004; Morimoto et al., 2007), HCl (Feuerstein and Kostanecki, 1898), EDDA (Manjulatha et al., 2012), and deep eutectic solvent (Hawkins and Handy, 2013). However, considering the importance of aurones, we devised a simple, green, and economically viable method for their synthesis. Where many of the reported methods are effective for the preparation of target compounds, but some of them suffer from disadvantages like harsh reaction conditions and low product yields.

| Table I: Optimisation of the catalyst for the preparation of aurone (3d) |
|---|
| Entry | Catalyst (10 mol%) | Time (hour) | Yield (%) |
| 1 | Silica gel | 6 | 44 |
| 2 | Silica sulphuric acid | 6 | 52 |
| 3 | Amberlite IR-120 resin | 0.25 | 96 |
| 4 | Amberlyst-15 | 2 | 56 |
| 5 | Nafion-NR50 | 2 | 70 |
| 6 | Dowex-50 | 2 | 79 |

*All the reactions were carried out using compound 1a or 1b (1 mmol) and an appropriate aldehyde (1 mmol) in the presence of amberlite IR-120 resin (10 mol %) at 50°C.*

Alternatively use of acidic resin either in stoichiometric or in catalytic quantity has attained importance in organic synthesis due to several advantages such as operational ease, non-hazardous, low cost, ease of isolation and reusability after completion of the reaction. For example, amberlite-IR 120 resin has emerged as an important heterogeneous catalyst for chemical transformations (Tewari et al., 2003; Akagawa et al., 2007; Park et al., 2007; Bhattacharya and Rana, 2008; Mulakayala et al., 2012). Due to our continuous interest in the development of biologically important molecules for the inhibition of cancer, we now report the use of amberlite IR-120 resin as a green and expedient catalyst for the synthesis of aurones. Further, the synthesized aurone compounds were evaluated for their anti-cancer activity against two breast cancer cell lines MDA-MB-231 and MCF-7 in vitro. The effect of the compounds was also evaluated on cell cycle phase distribution, apoptosis as well as on mitochondrial membrane potential loss in MCF-7 cells.

### Materials and Methods

**Chemistry:** Condensation of benzofuranone 1 with aromatic aldehydes 2 in presence of amberlite IR-120 resin in aqueous EtOH at 50°C resulted in the formation of aurones 3 in excellent yields in a short period of time.

Initially the reaction of benzofuran-3(2H)-one 1a with p-bromobenzaldehyde was carried out in the presence of various solid acid catalysts like silica sulphuric acid (SSA), silica gel, amberlite IR-120 resin, amberlyst-15, nafion-NR50 and dowex-50 (Table I) to yield 3d. Among the catalysts screened silica gel, SSA, amberlyst-15 gave poor yields, nafion-NR50 and dowex-50 gave good yields and amberlite IR-120 resin led to excellent yields of 3d.

Following the above optimized reaction conditions a variety of aurones were synthesized by using different aromatic aldehydes containing different substituents mainly from electron withdrawing and also electron donating groups (Table II). The key starting materials for this study were benzofuran-3(2H)-one 1a and 4, 6-dimethoxybenzofuran-3(2H)-one 1b, which were synthesized from the corresponding phenols [14]. Aldol type condensation of compound 1a or 1b with substituted benzaldehydes 2 (Figure 2) in presence of amberlite IR-120 resin in aqueous ethanol at 50°C provided the corresponding products (3a-3o) in excellent yields (Table II).

Most of the reactions completed within 15-60 min depending on the nature of aldehydes employed affording the desired products in excellent yields. No product formation was observed when the reaction time was increased up to 48 hours in the absence of amberlite IR-120 resin. Therefore Ambelite IR-120 resin catalyzed reaction was found to be advantageous in the present synthesis of aurones. All the synthesized compounds (3a-o) were characterized by spectral (NMR, IR and MS) data analysis.

Recyclability of the amberlite IR-120 catalyst was also examined. For this purpose, the catalyst used for the conversion of 1a to 3d was recovered from the reaction mixture by filtration, dried and reused for the same reaction. This procedure was repeated for four times and results are summarized in Table III. It is evident from the Table that the catalyst can be recycled...
successfully for several times without significant loss of its catalytic activities.

**Experimental:** All the reactions were performed under nitrogen atmosphere using dry solvents and oven dried glassware unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualized with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate, dichloromethane. ¹H NMR and ¹³C NMR spectra were recorded in CDCl₃ or DMSO-d₆ solution by using 400 MHz spectrometer. Proton chemical shifts (δ) are relative to experimental tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer.

**General procedure for the preparation of compounds (3a-o):** To a solution of benzofuran-3(2H)-one (1 mmol) in 2 mL of aqueous ethanol (50%) in a round bottom flask

![Figure 2: Synthesis of aurones using amberlite IR-120 resin in aqueous ethanol](image)
was added the aromatic aldehyde (1 mmol). To the reaction mixture was added amberlite IR-120 resin (10 mol%) and stirred at 50°C for the specified time (Table II). The reaction mixture was then filtered (to separate the resin), diluted with water (10 mL) and extracted with methylene chloride (3 X 10 mL). The organic layer was evaporated under vacuum and recrystallized from ethanol to afford the pure desired product in excellent yield.

(Z)-2-(4-methoxybenzylidene) benzofuran-3(2H)-one (3a): M.P. 137–139°C; 1H NMR (400 MHz, CDCl3): δ 7.91 (d, J = 8 Hz, 2H), 7.82 (dd, J = 7 Hz, 1H, 1H), 7.63 (dd, J = 8, 7, 1H, 1H), 7.32 (d, J = 8 Hz, 1H), 7.21 (dd, J = 8, 7, 1H, 1H), 6.97 (d, J = 8Hz, 2H), 6.88 (s, 1H), 6.81 (s, 1H), 132.8, 126.6, 125.9, 132.7, 121.9, 112.7, 111.7. MS (ESI) m/z = 300/302.

(Z)-2-(4-methylbenzylidene) benzofuran-3(2H)-one (3e): M.P. 98–100°C; 1H NMR (400 MHz, CDCl3): δ 7.82–7.78 (m, 2H), 7.63 (dd, J = 8, 7, 1H, 1H), 7.32–7.17 (m, 4H), 6.87 (s, 1H), 2.39 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 185.7, 166.8, 147.1, 141.6, 136.8, 132.6, 129.8, 129.1, 127.1, 148.4, 147.6, 138.2, 136.5, 132.6, 130.8, 129.2, 124.8, 123.7, 121.5, 113.0, 111.6; MS (ESI) m/z = 237 [M+H]⁺.

(Z)-2-(4-chlorobenzylidene) benzofuran-3(2H)-one (3f): M.P. 155–159°C; 1H NMR (400 MHz, CDCl3): δ 7.86 (dd, J = 8, 2 Hz, 2H), 7.83–7.80 (m, 1H), 7.68 (ddd, J = 8, 7, 1H, 1H), 7.43 (dd, J = 8, 2 Hz, 2H), 7.34 (d, J = 8 Hz, 1H, 1H), 7.27–7.22 (m, 1H), 6.84 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 206.8, 183.8, 165.40, 149.4, 139.6, 138.3, 132.6 (2C), 126.1, 125.2, 124.8 (2C), 103.7, 114.1, 109.5; MS (ESI) m/z = 268 [M+H]⁺.

(Z)-2-(4-methoxybenzylidene) benzofuran-3(2H)-one (3c): M.P. 163–167°C; 1H NMR (400 MHz, CDCl3): δ 8.29 (d, J = 8 Hz, 2H), 8.06 (d, J = 9 Hz, 2H), 7.84 (d, J = 7 Hz, 1H), 7.67 (t, J = 9 Hz, 1H), 7.35 (d, J = 8 Hz, 1H), 7.26 (t, J = 8 Hz, 1H), 6.85 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 206.8, 183.8, 165.40, 149.4, 139.6, 138.3, 132.6 (2C), 126.1, 125.2, 124.8 (2C), 103.7, 114.1, 109.5; MS (ESI) m/z = 268 [M+H]⁺.

(Z)-2-(4-bromobenzylidene) benzofuran-3(2H)-one (3d): M.P. 152–155°C; 1H NMR (400 MHz, CDCl3): δ 7.82 (d, J = 8 Hz, 1H), 7.79 (d, J = 8 Hz, 2H), 7.65 (t, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 2H), 4.36 (d, J = 8 Hz, 1H), 7.25 (t, J = 8 Hz, 1H), 6.81 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 185.8, 166.9, 147.9, 137.9, 133.1 (2C), 132.7 (2C), 131.3, 125.6, 125.1, 124.7, 121.9, 112.7, 111.7. MS (ESI) m/z = 300/302.

(Z)-2-(4-hydroxybenzylidene) benzofuran-3(2H)-one (3g): M.P. 120–121°C; 1H NMR (400 MHz, CDCl3): δ 7.83 (dd, J = 8, 2, 1H, 1H), 7.62 (ddd, J = 8, 2, 1H, 1H), 7.51–7.50 (m, 2H), 7.38 (d, J = 8 Hz, 1H), 7.33 (dd, J = 8, 2 Hz, 2H), 6.87 (s, 1H), 3.88 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 186.3, 168.2, 159.5, 148.5, 137.2, 134.8, 129.7, 125.2, 124.9, 124.8, 123.7, 117.7, 116.1, 113.6, 113.2, 55.6; MS (ESI) m/z = 257/259.
166.2, 158.7, 147.4, 146.6, 138.1, 132.4, 132.6, 124.8, 123.5, 121.1, 120.9, 112.7, 110.6, 107.1, 55.9; MS (ESI) m/z = 253 [M+H]+.

(Z)-2-(3,4-dimethoxybenzylidene)benzofuran-3(2H)-one (3i): M.P. 125-127 °C; 1H NMR (400 MHz, CDCl3): δ 7.50 (d, J = 8 Hz, 1H), 7.81 (t, J = 8 Hz, 1H), 7.52 (d, J = 2 Hz, 1H), 4.19 (s, 2H), 3.91 (s, 3H), 3.88 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 186.5, 168.6, 147.8, 137.4, 133.4, 132.9, 129.9, 128.7, 124.8, 123.6, 121.3, 113.9, 113.2; MS (ESI) m/z = 245 [M+Na]+.

(Z)-2-(3-hydroxy-4-methoxybenzylidene)benzofuran-3(2H)-one (3k): M.P. 178-180 °C; 1H NMR (400 MHz, DMSO-d6): δ 9.7 (s, 1H), 7.98 (d, J = 2 Hz, 1H), 7.78 (s, 1H), 7.74 (dd, J = 8, 1 Hz, 1H), 7.70 (dd, J = 7 Hz, 1H, 1H), 7.42 (d, J = 8 Hz, 1H), 7.40 (s, 1H), 7.39 (s, 1H), 7.07 (dd, J = 8 Hz, 2.0 Hz, 1H), 2.35 (s, 3H); 13C NMR (100 MHz, DMSO-d6): δ 183.7, 166.8, 155.7, 145.2, 137.5, 132.3, 131.1, 128.7, 124.5, 123.9, 121.4, 118.7, 115.3, 113.4, 106.6, 20.4; MS (ESI) m/z = 253 [M+H]+.

(Z)-2-(3-hydroxy-4-methoxybenzylidene)benzofuran-3(2H)-one (3l): M.P. 178-180 °C; 1H NMR (400 MHz, CDCl3): δ 7.97 (d, J = 8 Hz, 2H), 7.79 (d, J = 7 Hz, 1H), 7.65-7.70 (m, 3H), 7.32 (d, J = 8 Hz, 1H), 7.24 (t, J = 7 Hz, 1H, 1H), 6.79 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 185.7, 167.4, 149.1, 138.4, 137.3, 133.6 (2H), 132.1 (2H), 126.4, 125.4, 122.2, 119.5, 114.1, 112.8, 110.9; MS (ESI) m/z = 248 [M+Na]+.

(Z)-2-benzylidenebenzofuran-3(2H)-one (3m): M.P. 99-100 °C; 1H NMR (400 MHz, CDCl3): δ 7.94 (dd, J = 7, 2 Hz, 2H), 7.84 (ddd, J = 8, 2, 0.8 Hz, 1H), 7.68 (t, J = 8 Hz, 1H), 7.51-7.42 (m, 3H), 7.36 (d, J = 8 Hz, 1H), 7.24 (td, J = 8, 1 Hz, 1H), 6.91 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 186.5, 168.6, 147.8, 137.4, 133.4, 132.9, 129.9, 128.7, 124.8, 123.6, 121.3, 113.9, 113.2; MS (ESI) m/z = 245 [M+Na]+.

It is obvious from the spectral data that a single geometric isomer i.e, Z-isomer was obtained in all the

(Z)-2-(3,4-dimethoxybenzylidene)benzofuran-3(2H)-one (3n): M.P. 178-180 °C; 1H NMR (400 MHz, CDCl3): δ 7.97 (d, J = 8 Hz, 2H), 7.79 (d, J = 7 Hz, 1H), 7.65-7.70 (m, 3H), 7.32 (d, J = 8 Hz, 1H), 7.24 (t, J = 7 Hz, 1H, 1H), 6.79 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 185.7, 167.4, 149.1, 138.4, 137.3, 133.6 (2H), 132.1 (2H), 126.4, 125.4, 122.2, 119.5, 114.1, 112.8, 110.9; MS (ESI) m/z = 248 [M+Na]+.

(Z)-2-benzylidenebenzofuran-3(2H)-one (3o): M.P. 178-180 °C; 1H NMR (400 MHz, CDCl3): δ 7.97 (d, J = 8 Hz, 2H), 7.79 (d, J = 7 Hz, 1H), 7.65-7.70 (m, 3H), 7.32 (d, J = 8 Hz, 1H), 7.24 (t, J = 7 Hz, 1H, 1H), 6.79 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 185.7, 167.4, 149.1, 138.4, 137.3, 133.6 (2H), 132.1 (2H), 126.4, 125.4, 122.2, 119.5, 114.1, 112.8, 110.9; MS (ESI) m/z = 248 [M+Na]+.

(Z)-2-(4-chlorobenzylidene)-4,6-dimethoxybenzofuran-3(2H)-one (3p): M.P. 172-174 °C; 1H NMR (400 MHz, CDCl3): δ 7.66 (d, J = 8 Hz, 2H), 7.27 (d, J = 8 Hz, 2H), 6.57 (s, 1H), 6.26 (d, J = 2 Hz, 1H, 1H), 6.01 (d, J = 2 Hz, 1H), 3.85 (s, 3H), 3.81 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 181.7, 169.7, 168.3, 159.2, 147.2, 135.2, 132.6, 131.1, 128.1, 109.7, 104.3, 94.4, 89.0, 56.2, 56.5; MS (ESI) m/z = 317 [M+H]+.

NMR (100 MHz, CDCl3): δ 181.7, 169.7, 168.3, 159.2, 147.2, 135.2, 132.6, 131.1, 128.1, 109.7, 104.3, 94.4, 89.0, 56.2, 56.5; MS (ESI) m/z = 317 [M+H]+.
reactions. The formation of Z-isomer is also supported by its thermodynamic stability compared to E-isomer. While the geometry of the double bond could be established on the basis of chemical shift (δ) value of the vinylic proton as well as carbon observed in the corresponding 1H and 13C NMR spectra (Venkateswarlu et al., 2007; Hastings and Heller, 1972).

Cell lines, growth medium and treatment conditions: Two metastatic breast cancer cell lines, including MDA-MB-231 and MCF-7 Were procured from Shanghai Institute of Cell Resource Center of Life Science (Shanghai, China). Cells were grown in Minimum Essential Medium (MEM) and Roswell Park Memorial Institute medium (RPMI) supplemented with 10%FCS and 1% penicillin. Penicillin was dissolved in PBS and sterilized by filtering through 0.2 μm filter in laminar air flow hood. Cells were cultured in CO2 incubator (New Brunswick, Galaxy 170R, eppendroff) with an internal atmosphere of 95% air and 5% CO2 gas and the cell lines were maintained at 37°C. The media was stored at low temperature (2-8°C) and the medium for cryopreservation contained 20%FCS and 10%DMSO in growth medium.

Cell viability by MTT anticancer assay: Inhibition of cell proliferation of the compounds was measured by the MTT assay. Briefly, MDA-MB-231 and MCF-7 were plated in 96-well culture plates (1 × 104 cells/well) separately. After 24 hours incubation, cells were treated with different compounds (10 μM, eight wells per concentration), MTT solution (5 mg/mL) was then added to each well. After 4 hours incubation, the formazan precipitate was dissolved in dimethyl sulfoxide (100 μL) and then the absorbance was measured in an ELISA reader (Thermo Molecular Devices Co., Union City, USA) at 570 nm (Mosman, 1983). The cell viability ratio was calculated by the following formula:

\[
\text{Inhibitory ratio (\%)} = \left( \frac{\text{OD control} - \text{OD treated}}{\text{OD control}} \right) \times 100\%.
\]

Cytotoxicity was expressed as the concentration of the extract inhibiting cell growth by 50% (IC50 value).

Cell cycle analysis: MCF-7 Cells (5x104) were seeded in 60-mm dishes and subjected to various concentrations (4 and 5 μM concentration of 3f and 3e compounds respectively) for 24, 36 and 48 hours. Floating and adherent cells were collected by trypsinization and washed twice with PBS. Cells were incubated in 70% ethanol at -20°C overnight, treated with 10 μg/mL RNase A, then stained with 2.0 μg/mL of propidium iodide. Finally the stained cells were analyzed and studied by Flow cytometry at wavelength of 488 nm (FACS Calibur instrument (BD Biosciences, San Jose, CA, USA) equipped with Cell Quest 3.3 software).

Detection of cell apoptosis by Hoechst staining: Breast Cancer Cells grown on coverslips in 12-well plates were exposed to the synthesized compounds for 48 hours, then incubated with Hoechst 33258 (Hoechst Staining Kit, Beyotime, China). Fluorescence microscopy was used to observe cell shape captured from six random visual fields. The ratio of apoptotic cells to total cell number was calculated.

Measurement of mitochondrial membrane potential (ΔΨ m) loss: Mitochondrial membrane potential (ΔΨ m) was measured by rhodamine-123 (1 mM) dye. Rhodamine fluorescence can be used as a measure of membrane polarization in live cell assays within mitochondria. Briefly, 5x104 MCF-7 cells were treated with various concentrations (4 and 5 μM concentration of 3f and 3e compounds respectively) for 24, 36 and 48 hours and ΔΨm was measured by flow cytometry (FACS Calibur instrument). Rhodamine-123 (Rh-123) (2 mM) was added 1.5 hours before the termination of experiment. Cells were collected, washed in PBS and incubated with propidium iodide (10 μg/mL) for 15 min. The reduction in fluorescence intensity because of mitochondrial membrane potential loss was analyzed by the instrument. Mean fluorescence intensity was detected by FL1 channel of BD FACS Calibur (BD Bioscience).

Statistical analysis: All the data were analysed using analysis of variance (ANOVA), followed by Dunnett’s test for pair wise comparison. Mean values are presented with their deviation (SD). Statistical significance was defined as p<0.05 for all tests.

Results and Discussion

The anticancer activity of the synthesized compounds was evaluated by MTT cell viability assay. Among the synthesized compounds (3a-o), most of them exhibited moderate activity (30-50 μM) whereas the IC50 of 3e against MDA-MB-231 (10.2 μM), and MCF-7 (6.4 μM) was found to be comparable to that of 5-FU (14.2 μM and 7.9 μM respectively). Moreover, 3e and 3f display-

| Table IV: Effects of 3e and 3f on cell cycle progression in MCF-7 cells |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Compound**    | **Control**     | **3e (5 μM)**   | **3f (4 μM)**   |
| Sub-Go | Go/G1 | G2/M | Sub-Go | Go/G1 | G2/M | Sub-Go | Go/G1 | G2/M |
| 24 hours | 0 | 46.15 | 29.35 | 24.45 | 4.70 | 44.88 | 22.99 | 32.15 | 0 | 55.90 | 25.01 | 19.09 |
| 36 hours | 0 | 46.71 | 39.19 | 14.33 | 4.97 | 58.74 | 23.88 | 17.50 | 4.58 | 63.06 | 18.48 | 18.55 |
| 48 hours | 0 | 41.98 | 33.63 | 24.42 | 18.23 | 75.88 | 17.86 | 6.35 | 16.45 | 73.70 | 20.88 | 5.40 |
ed higher activities against MDA-MB-231 and MCF-7 than 5-FU.

To understand the effect of the synthesized compounds on cell cycle progression, flow-activated cell sorting analysis was performed (Lee et al., 2002; Lawrence et al., 2000; Darzynkiewicz and Bednery, 2000). The effect of 3e and 3f on MCF-7 cell cycle phase distribution was assessed using flow cytometry. When the cells were grown to about 70% confluence in 6-well micro plates and treated with 3e and 3f at given concentrations (IC\textsubscript{50} concentration). After 24, 36, and 48 hours, cells were harvested by trypsinization, washed in PBS, and fixed in 70% ice cold (4ºC) ethanol overnight. As shown in Figure 3 and Table IV, compounds 3e and 3f arrest the cell cycle in G\textsubscript{0}/G\textsubscript{1} phase, raising the G\textsubscript{0}/G\textsubscript{1} peak from 42.0% to 75.9% (3e) and 73.7% (3f) after 48 hours treatment. Subsequently, cells accumulated in sub-G\textsubscript{0} phase at 18.23% (3e) and 16.45% (3f). Because the increase of cells in sub-G\textsubscript{0} phase generally indicates the increase of apoptotic cell death, 3e and 3f might display apoptosis-inducing effect on MCF-7 cells (Figure 3).

MCF-7 cells were exposed to different concentration (5 µm of 3e compound and 4 µm of 3f compound) of the synthesized compounds and apoptosis was evaluated after an incubation of 48 hours. The cells were stained and evaluated for nuclear shape under an inverted fluorescence microscope (Figure 4). Treated cells showed chromatin condensation and dense staining fragmentation called apoptotic bodies, which implied an early apoptotic event. It can be seen that 3f compound exhibited much more apoptosis effect as compared to 3e compound because the number of apoptotic bodies is much higher in 3f treated cells as compared to the 3e treated cells.

Several cationic dyes distribute electrophoretically into the mitochondrial matrix in response to the electric potential across the inner mitochondrial membrane.
Figure 4: Apoptosis induction by compounds 3e and 3f in MCF-7 cancer cells

Figure 5: Mitochondrial membrane potential loss induced by 3e and 3f synthesized compounds in MCF-7 breast cancer cell line
The accumulation takes place as a consequence of their charge and of their solubility in both the inner membrane lipids and the matrix aqueous space. For this reason, these dyes have been extensively used to measure the mitochondrial electric potential exploiting their spectroscopic properties. Among these dyes, rhodamine 123 (RH-123) is one of the mostly used probe. In our experiment, we also employed this dye. The effect of 3e and 3f compounds was further evaluated on the mitochondrial membrane potential loss in MCF-7 breast cancer cell line. As can be seen in Figure 5, both these compounds induced a substantial and time-dependent loss of mitochondrial membrane potential in this cell line. The number of cells with intact mitochondrial membrane potential decreased considerably while as the number of cells with decreased mitochondrial membrane potential increased.

In conclusion, a series of 15 aurone derivatives were synthesized and evaluated for their anticancer activity against MDAMB-231 and MCF-7 cancer cell lines. Among the synthesized compounds, 3e and 3f showed good anti-proliferative properties against the tested cancer cell lines compared with the standard.

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