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Facile Synthesis of Aurones using Amberlyst-15 as a Reusable Catalyst and their Biological Evaluation

H. Sudhakar[1], Naveen Mulakayala[2]*

[1]Department of Polymer Science & Technology, Sri Krishnadevaraya University, Anantapuramu - 515 003, Andhra Pradesh, India. [2]Clearsynth Labs Ltd., Research Centre, Hyderabad - 500 076, Telengana, India.

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ABSTRACT

Amberlyst-15 mediated novel method for the synthesis of aurones was reporte. Condensation of an aldehyde with benzofuran-3(2H)-one using Amberlyst IR-15 resin in aqueous ethanol was well demonstrated to get good yields. The synthesized compounds 3e and 3f were most active against the inhibition of breast cancer cell lines MDA-MB-468 and MCF-7 in vitro compared with 5-fluorouracil.

Key words: *Aurones, Amberlyst IR-120 resin, In vitro evaluation, MCF-7, MDA-MB-468.*

1. INTRODUCTION

Aurones are an important class of natural products in flavanoid family that are found in many plants [1,2]. Fruits and flowers are the general sources for flavanoids along with several flavones, isoflavones, and chalcones (Figure 1). From past several years, flavones and chalcones have been well studied for various diseases, but the biological activity of aurones has not been extensively studied. Aurones are mainly found in the use of anti-cancer [3], antimalarial [4], and in microbial infections [5].

Chalcones are the main source for the synthesis of aurones in plants by oxidation, cyclization and rearrangement with the help of an enzyme aureusidin synthase [6-8]. Aurones showed a range of pharmacological activities [9] including anti-cancer, antifeedant, and anti-parasitic activities via modulating a variety of molecular targets such as G_2/M cell-cycle arrest,arresting the cell cycle in G_0/G_1 phase and displayed apoptosis-inducing effect on Hep-2 cells [10], inhibition of human sphingosine kinase [11], inhibition of P-glycoprotein (P-gp) related transport [12] high-affinity binding to cytosolic domain of P-gp [13] modulation of ABCG2 [14] activities, etc. Several literature reports are present in the literature for the synthesis of aurones [15-26].

2. EXPERIMENTAL

2.1. Materials and Methods

Unless stated otherwise, reactions were performed under a nitrogen atmosphere using oven-dried glassware. Reactions were monitored by thin-layer chromatography on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate, dichloromethane.^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were determined in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6 solution using 400 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to experimental tetramethylsilane (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. Mass spectrum (MS) spectra were obtained on a mass spectrometer.

2.2. General Procedure for Condensation of Coumaranone and Aryl Aldehydes

To benzofuran-3(2H)-one (1 mmol) was added the aryl aldehyde (1 mmol) in 2 ml of aqueous ethanol (50%) in a round bottom flask. To this Amberlyst-15 (10 mol%) was added and stirred at 50°C for the specified time (Table 1). The reaction mixture was then filtered (to separate the resin) and diluted with water (10 ml) and extracted with methylene chloride (3 × 10 ml). The organic layer was evaporated under vacuum and recrystallized from ethanol to afford the product in spectroscopically pure.

*Corresponding Author:
E-mail: naveen071280@gmail.com

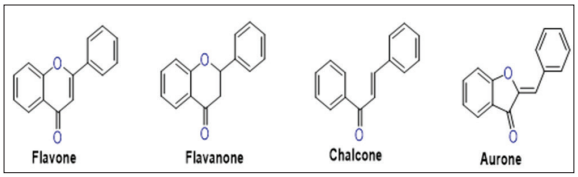


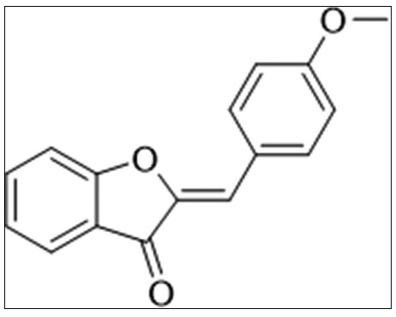
Figure 1: Some flavonoid sub-classes.

Table 1: Synthesis of aurones (3a-o).

Entry	Aurones					Time (min)/yield[a]
	R	R_1	R_2	R_3	R_4	
3a	H	H	OMe	H	H	20 min/94
3b	H	H	H	NO_2	H	30 min/90
3c	H	H	NO_2	H	H	28 min/92
3d	H	H	Br	H	H	15 min/96
3e	H	Me	H	H	OH	25 min/91
3f	H	H	OMe	H	OH	22 min/93
3g	H	H	Me	H	H	22 min/95
3h	H	H	Cl	H	H	15 min/97
3i	H	H	H	OMe	H	20 min/95
3j	H	H	H	H	OMe	16 min/94
3k	H	H	H	OMe	OMe	22 min/92
3l	H	H	F	H	H	15 min/95
3m	H	H	CN	H	H	25 min/90
3n	4,6-OMe	H	H	H	H	23 min/95
3o	4,6-OMe	H	H	H	H	30 min/90

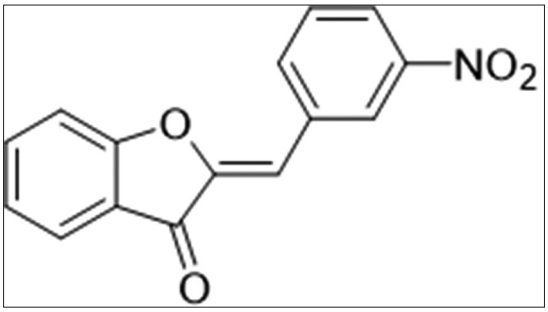
[a]All the reactions were carried out using compound 1a or 1b (1 mmol) and an appropriate aldehyde (1 mmol.) in the presence of Amberlyst-15 (10 mol%) at 50°C, [b]Isolated yield

2.2.1. (Z)-2-(4-methoxybenzylidene)benzofuran-3(2H) -one (3a)



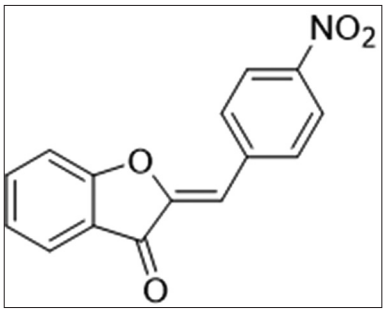
M.P. 137-139°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, *J*=8.8 Hz, 2H), 7.82 (dd, *J*=7.68 Hz, 1.47 Hz, 1H), 7.63 (ddd, *J*=7.69, 7.33, 1.47 Hz, 1H), 7.32 (d, *J*=7.69 Hz, 1H), 7.21 (ddd, *J*=7.69, 7.33, 0.74 Hz, 1H), 6.97 (d, *J*=8.78 Hz, 2H), 6.88 (s, 1H), 3.81 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 184.7, 166.5, 161.8, 144.8, 137.1, 134.4, 125.7, 124.6, 123.6, 121.6, 114.1, 113.3, 112.6, 55.9; MS (ESI) m/z = 253 $[M+H]^+$.

2.2.2. (Z)-2-(3-nitrobenzylidene)benzofuran-3(2H)-one (3b)



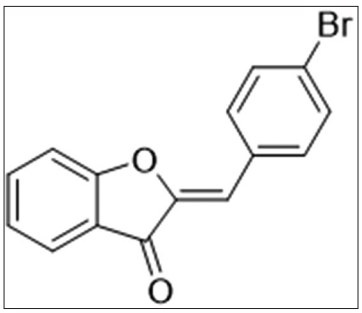
M.P. 161-164°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s,1H), 8.25 (d, *J*=8.1 Hz, 1H), 8.16 (d, *J*=7.6 Hz, 1H), 7.83 (d, *J*=8.4 Hz, 1H), 7.71 (t, *J*=8.6 Hz, 1H), 7.64 (t, *J*=8.1 Hz, 1H), 7.39 (d, *J*=8 Hz, 1H), 7.25 (t, *J*=7.8 Hz, 1H), 6.84 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$): δ 185.6. 167.3, 149.6, 148.9, 138.6, 137.8, 134.9, 130.8, 126.6, 125.9, 126.1, 125.1, 122.0, 114.2, 110.7; MS (ESI) m/z = 268 $[M+H]^+$.

2.2.3. (Z)-2-(4-nitrobenzylidene)benzofuran-3(2H)-one (3c)



M.P. 163-167°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (d, *J*=8.7 Hz, 2H); 8.06 (d, *J*=9.1 Hz, 2H), 7.84 (d, *J*=6.9 Hz, 1H), 7.67 (t, *J*=8.7 Hz, 1H), 7.35 (d, *J*=8.1 Hz, 1H),7.26 (t, *J*=7.5 Hz, 1H), 6.85 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$): 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 $[M+H]^+$.

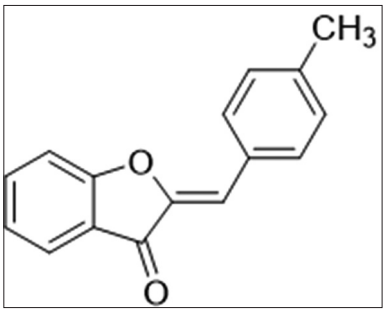
2.2.4. (Z)-2-(4-bromobenzylidene)benzofuran-3(2H)-one (3d)



M.P. 152-155°C.;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J*=8.4 Hz, 1H), 7.79 (d, *J*=8.5 Hz, 2H), 7.65

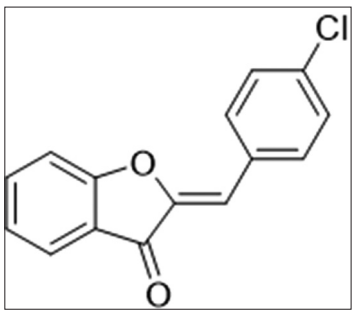
(t, *J*=8.5 Hz, 1H), 7.59 (d, *J*=8.4 Hz, 2H), 7.36 (d, *J*=8.1 Hz, 1H), 7.25 (t, *J*=7.5 Hz, 1H), 6.81 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$): δ 185.8, 166.9, 147.9, 137.9, 133.1 (2C), 132.7 (2C), 131.3, 125.6, 125.1, 124.7, 121.9, 112.7, 111.7. MS (ESI) m/z = 300/302.

2.2.5. (Z)-2-(4-methylbenzylidene)benzofuran-3(2H)-one (3e)



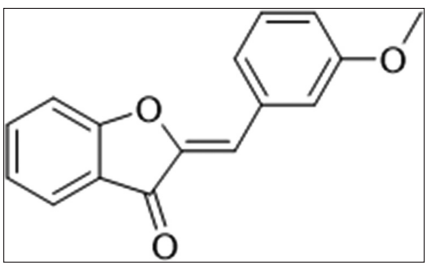
M.P. 98-100°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82-7.78 (m, 2H), 7.63 (dd, *J*=7.69, 7.33, 1.46 Hz, 1H), 7.32-7.17 (m, 4H), 6.87 (s, 1H), 2.39 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 185.7, 166.8, 147.1, 141.6, 136.8, 132.6, 129.8, 129.1, 127.1, 124.8, 123.2, 121.7, 114.2, 113.2, 22.6; MS (ESI) m/z = 237 $(M+H)^+$.

2.2.6. (Z)-2-(4-chlorobenzylidene)benzofuran-3(2H)-one (3f)



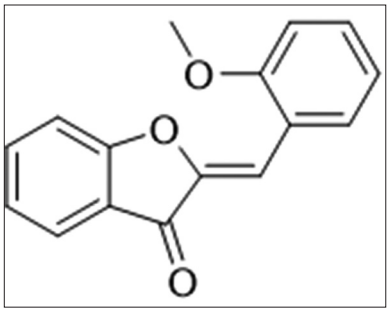
M.P. 155-159°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (dd, *J*=8.43, 1.84 Hz, 2H), 7.83-7.80 (m, 1H), 7.68 (ddd, *J*=8.42, 7.32, 1.46 Hz, 1H), 7.43 (dd, *J*=8.79, 1.83 Hz, 2H) 7.34 (d, *J*=8.06 Hz, 1H), 7.27-7.22 (m, 1H), 6.84 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$): δ 184.4, 166.3, 147.6, 138.2, 136.5, 132.6, 130.8, 129.2, 124.8, 123.7, 121.5, 113.0, 111.6; MS (ESI) m/z = 257/259.

2.2.7. (Z)-2-(3-methoxybenzylidene)benzofuran-3(2H)-one (3g)



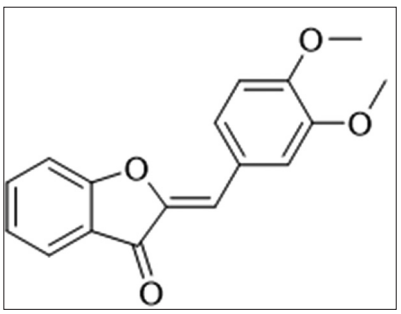
M.P. 120-121°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (ddd, *J*=7.68, 1.46, 0.73 Hz, 1H), 7.62 (ddd, *J*=7.33, 1.46 Hz, 1H), 7.51-7.50 (m, 2H), 7.38 (d, *J*=8.42 Hz, 1H), 7.33 (dd, *J*=8.42, 0.73 Hz, 1H), 7.23 (dd, *J*=7.33 Hz, 1H), 6.97 (dd, *J*=8.42, 2.56 Hz, 1H), 6.87 (s, 1H), 3.88 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): 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 = 253 $[M+H]^+$.

2.2.8. (Z)-2-(2-methoxybenzylidene)benzofuran-3(2H)-one (3h)



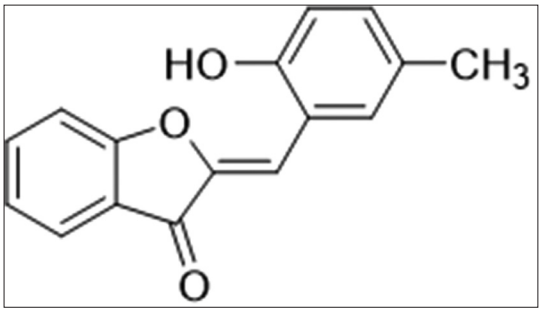
M.P. 168-171°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (dd, *J*=7.9, 1.7 Hz, 1H), 7.83 (ddd, *J*=7.7, 1.5, 0.7 Hz, 1H), 7.61 (ddd, *J*=8.4, 7.3, 1.5 Hz, 1H), 7.49 (s, 1H), 7.37 (ddd, *J*=8.4, 7.3, 1.2 Hz, 1H), 7.34 (dt, *J*=8.2, 0.7 Hz, 1H), 7.22 (ddd, *J*=7.7, 7.3, 0.7 Hz, 1H), 7.09 (dddd, *J*=7.8, 7.5, 1.1, 0.6 Hz, 1H), 6.94 (dd, *J*=8.4, 1.1 Hz, 1H);^{13}C NMR (100 MHz, $CDCl_3$): δ 185.7, 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 $[M+H]^+$.

2.2.9. (Z)-2-(3,4-dimethoxybenzylidene)benzofuran-3(2H)-one (3i)



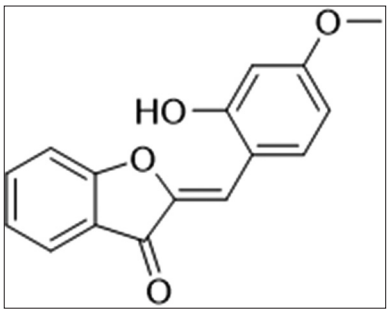
M.P. 125-127°C;^{1}H NMR (400 MHz,$CDCl_3$): δ 7.76 (d, *J*=7.45 Hz, 1H), 7.57 (t, *J*=8.55 Hz, 1H), 7.46 (d, *J*=1.7 Hz, 1H), 7.40 (d, *J*=10.3 Hz, 1H), 7.25 (d, *J*=8.6 Hz, 1H), 7.16 (t, *J*=7.4 Hz, 1H), 6.87 (d, *J*=8.6Hz, 1H), 6.79 (s, 1H), 3.90 (s, 3H), 3.88 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 185.3, 166.6, 150.2, 148.9, 145.6, 136.1, 125.8, 125.2, 124.4, 123.3, 121.8, 113.7, 112.7, 111.3; MS (ESI) m/z = 283 $[M+H]^+$.

2.2.10. (Z)-2-(2-hydroxy-5-methylbenzylidene) benzofuran-3(2H)-one (3j)



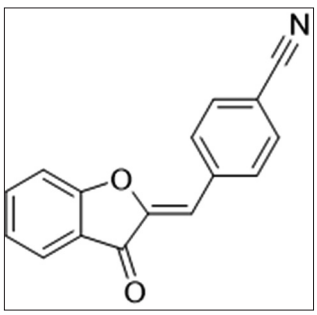
M.P. 178-180°C;^{1}H NMR (400 MHz, DMSO-d_6): δ 9.7 (s, 1H), 7.98 (d, *J*=1.6 Hz, 1H), 7.78 (s, 1H), 7.74 (dd, *J*=8.0, 1.0 Hz, 1H), 7.70 (dd, *J*=6.8 Hz, 1.2 Hz, 1H), 7.42 (d, *J*=8.4 Hz, 1H), 7.40 (s, 1H), 7.39 (s,1H), 7.07 (dd, *J*=8.6 Hz, 2.0 Hz, 1H), 2.35 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6): 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, 115.5, 113.4, 106.6, 20.4; MS (ESI) m/z = 253 $[M+H]^+$.

2.2.11. (Z)-2-(2-hydroxy-4-methoxybenzylidene) benzofuran-3(2H)-one (3k)



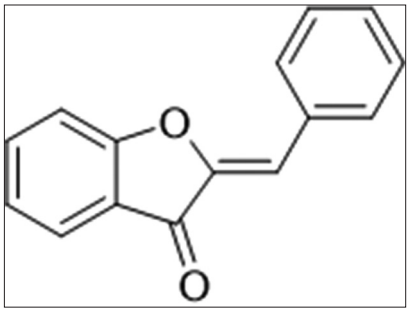
M.P. 171-174°C;^{1}H NMR (400 MHz, DMSO-d_6): δ 8.19 (d, *J*=1.6 Hz, 1H), 7.82-7.73 (m, 2H), 7.22 (d, *J*=7.6 Hz, 2H), 7.23 (t, *J*=7.6 Hz, 1H), 6.53-6.51 (d, *J*=7.6 Hz, 2H), 3.81 (s, 3H).^{13}C NMR (400 MHz, DMSO-d_6): δ 182.4, 164.1, 161.8, 158.5, 143.3, 136.1, 131.9, 123.4, 122.7, 121.4, 120.8, 111.4, 111.2, 105.5, 99.7, 54.5; MS (ESI) m/z = 269 $[M+H]^+$.

2.2.12. (Z)-4-((3-oxobenzofuran-2(3H)-ylidene) methyl)benzonitrile (3l)



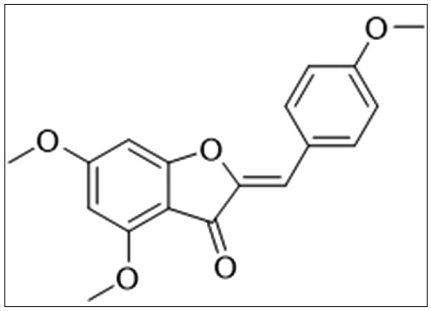
M.P. 180-183°C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J*=8.55 Hz, 2H); 7.79 (d, *J*=7.45, 1H), 7.65-7.70 (m, 3H), 7.32 (d, *J*=8.6 Hz, 1H), 7.24 (t, *J*=6.85 Hz, 1H), 6.79 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$): δ 185.7, 167.4, 149.1, 138.4, 137.3, 133.6 (2H), 132.1 (2H), 126.4, 125.4, 122.2, 119.5, 114.1, 112.8, 110.9; MS (ESI) m/z = 248 $[M+H]^+$.

2.2.13. (Z)-2-benzylidenebenzofuran-3(2H)-one (3m)



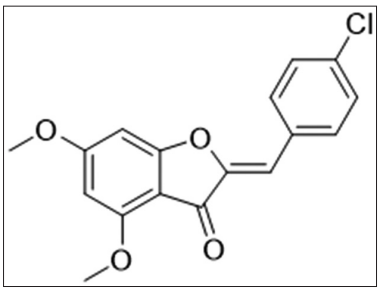
M.P. 99-100°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (dd, *J*=7.0, 1.8 Hz, 2H), 7.84 (ddd, *J*=7.8, 1.5, 0.8 Hz, 1H), 7.68 (t, *J*=8.3 Hz, 1H), 7.51-7.42 (m, 3H),7.36 (d, *J*=8.3, 1.2 Hz, 1H), 7.24 (td, *J*=7.5, 1.6 Hz, 1H), 6.91 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$): δ 186.5, 168.6, 147.8, 137.4, 133.4, 132.9, 129.9, 128.7, 124.8, 123.6, 121.3, 113.9, 113.2; MS (ESI) *m/z* =245 (M+Na).

2.2.14. (Z)-4,6-dimethoxy-2-(4-methoxybenzylidene) benzofuran-3(2H)-one (3n)



M.P. 168-169°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (d, *J*=8.9 Hz, 2H), 6.94 (d, *J*=8.9 Hz, 2H), 6.73 (s, 1H), 6.36 (d, *J*=1.83 Hz, 1H), 6.11 (d, *J*=1.83 Hz, 1H), 3.95 (s, 3H), 3.89 (s, 3H), 3.84 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 184.5, 169.1, 168.5, 161.5, 159.3, 147.5, 132.8, 130.1, 126.3, 115.4, 114.2,110.1, 105.5, 93.4, 89.8, 56.8, 55.9, 55.4, 55.6; MS (ESI) m/z = 313 $[M+H]^+$.

2.2.15. (Z)-2-(4-chlorobenzylidene)-4,6-dimethoxybenzofuran-3(2H)-one (3o)



M.P. 172-174°C;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, *J*=8.43 Hz, 2H), 7.27 (d, *J*=8.42 Hz, 2H), 6.57 (s, 1H), 6.26 (d, *J*=1.84 Hz, 1H), 6.01 (d, *J*=1.83 Hz, 1H), 3.85 (s, 3H), 3.81 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): 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 $[M+H]^+$.

It is obvious from the spectral data that a single geometric isomer (Z) was obtained in all the cases, which is Z-isomer, thermodynamically more stable than E-isomer. While the geometry of the double bond could be established on the basis of chemical shift value of the vinylic proton as well as carbon observed in the corresponding ^{1}H and ^{13}C NMR spectra [26,27].

2.3. In Vitro Cytotoxicity Assay

The *in-vitro* cytotoxic activities of the synthesized compounds against two metastatic breast cancer cell lines, including MDA-MB-468 and MCF-7 were assayed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The cytotoxicity of these compounds was evaluated at the concentration of 10 µM using an MTT assay as reported earlier [28]. It is proved from the MTT assay results (Table 2) that compounds 3d, 3e, 3f and 3k showed better or comparable anti-proliferative properties than 5-fluorouricial (5-FU) which was used as a control for these experiments.

3. RESULT AND DISCUSSION

3.1. Chemistry

The synthesis starts from the condensation of benzofuranone 1 with aromatic aldehydes 2 in the presence of Amberlyst-15 in aqueous EtOH at 50°C led to aurones in excellent yields in shorter reaction times. (Scheme 1)

To identify the suitable catalyst, several solid acid catalysts such as silica sulphuric acid (SSA), Silica gel, Alumina, Amberlyst-15, Nafion-NR50, Dowex-50 were screened to synthesize aurone 3d (Table 2). Among the catalysts Silicagel, SSA, alumina gave lesser yields compared with Nafion-NR50, Dowex-50, and Amberlyst-15. Interestingly Amberlyst-15 was found to give good results than other catalysts.

Following the above reaction conditions a wide variety of aurones were synthesized by different aryl aldehydes containing different substituents mainly from electron withdrawing and also electron donating groups (Table 1).

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

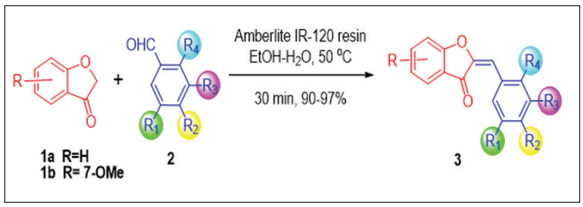
Table 2: Optimization of the catalyst for the preparation of Aurone (3d).

Entry	Catalyst (10 mol%)	Time	Yield (%)[a]
1	Silica gel	6 h	44
2	Silica sulphuric acid	6 h	52
3	Alumina	15 h	36
4	Amberlyst-15	20 min	96
5	Nafion-NR50	2 h	70
6	Dowex-50	2 h	79

[a]All the reactions were carried out using compound 1a or 1b (1 mmol) and an appropriate aldehyde (1 mmol) in the presence of Amberlyst-15 (10 mol%) at 50°C

Table 3: Recycle of the Amberlyst-15 catalyst for the preparation of aurone (3d).

Cycle	Amberlyst-15 (mol%)	% yield
1	10	96
2	10	94
3	9	92
4	9	89



Scheme 1: Synthesis of Aurones using Amberlite IR-120 resin in aqueous ethanol.

Recyclability of the Amberlyst-15 catalyst was examined. For this reason, the catalyst used was recovered from the reaction of 1a to 3d via filtration, dried and reused for the same reaction. This procedure was repeated for 4 times, and results are summarized in Table 3. It is evident from Table 3 that the catalyst can be recycled successfully for several times without significant loss of its catalytic activities.

3.2. Biology

Among the synthesized compounds (3a-o) the inhibitory concentration (IC_{50}) of 3e against MDA-MB-468 is 10.3 µM, and against MCF-7 is 6.5 µM which is comparable to that of 5-FU (14.4 µM and 7.8 µM). The most of compounds show moderate activity (30-50 µM) against MDA-MB-468 and MCF-7, which is

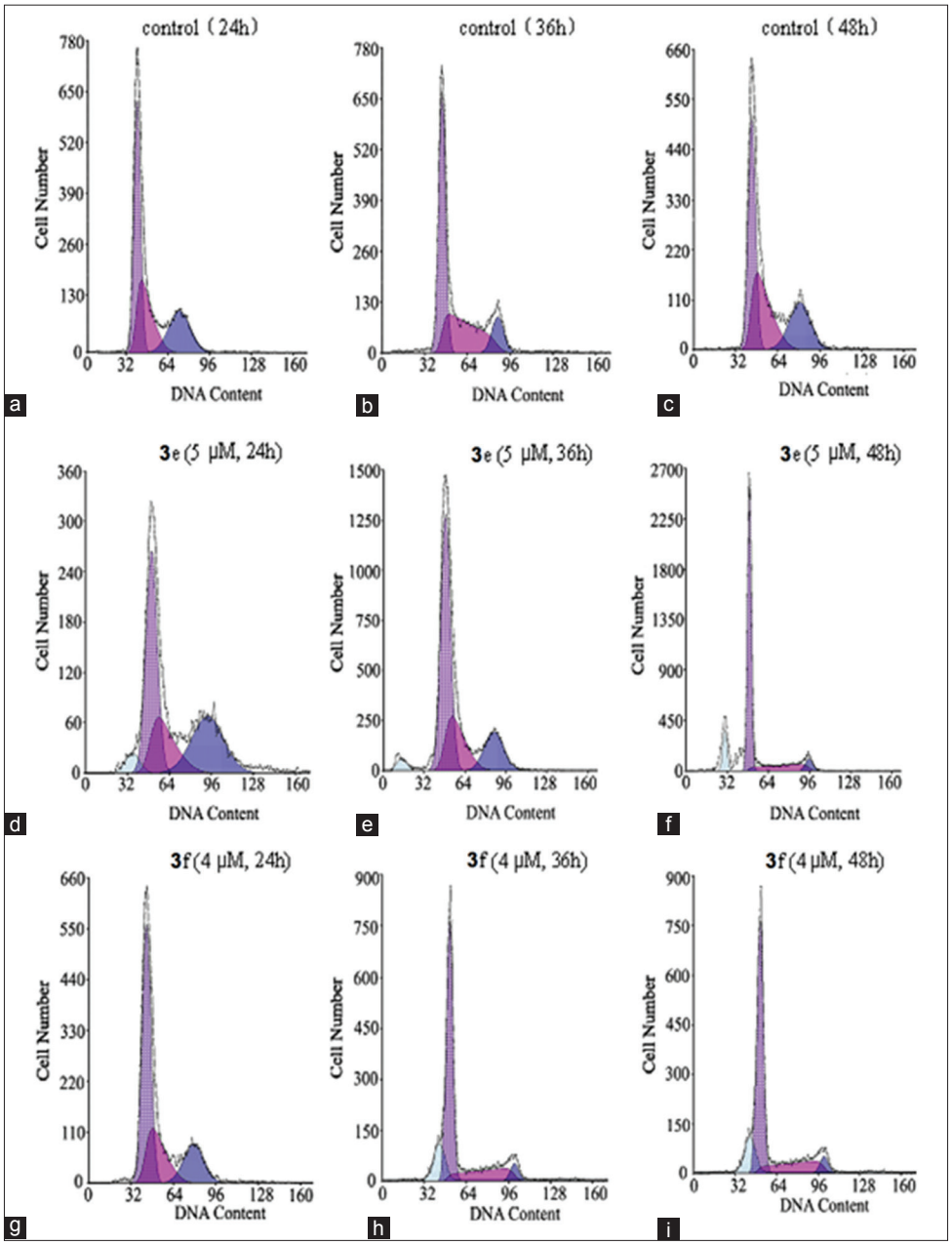


Figure 2: Compounds 3e and 3f induced apoptosis in MCF-7 cells.

Table 4: Effects of 3e and 3f on cell cycle progression in MCF-7 cells.

Compound	Control				3e (5 μM)				3f (4 μM)			
	Sub-G_0	G_0/G_1	S	G_2/M	Sub-G_0	G_0/G_1	S	G_2/M	Sub-G_0	G_0/G_1	S	G_2/M
24 h	0	46.15	29.35	24.45	4.70	44.88	22.99	32.15	0	55.90	25.01	19.09
36 h	0	46.71	39.19	14.33	4.97	58.74	23.88	17.50	5.58	63.06	18.48	18.55
48 h	0	41.98	33.63	24.42	18.23	75.88	17.86	6.35	16.45	73.70	20.88	5.40

comparable to 5-FU. Within the synthesized aurones, compounds 3e and 3f showed the most promising antitumor activities against the two tested tumor cell lines. Moreover, 3e and 3f displayed higher activities against MDA-MB-468 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 [29-31]. The effect of 3e and 3f on MCF-7 cell cycle phase distribution was assessed using flow cytometry.

As shown in Figure 2 and Table 3, compounds 3e and 3f arrest the cell cyclein G_0/G_1 phase, raising the G_0/G_1 peak from 41.98% to 75.88% (3e) and 73.70% (3f) after 48 h treatment. Subsequently, cells accumulated in sub-G_0 phase at 18.23% (3e) and 16.45% (3f). Because the increase of cells in sub-G_0 phase generally informed the increase of apoptotic cell death, 3e and 3f might display apoptosis-inducing effect on MCF-7 cells (Figure 2).

When the cells were grown to about 70% confluence in 6-well microplates and treated with 3e and 3f at given concentrations (IC_{50} concentration). After 24, 36, and 48 h, cells were harvested by trypsinization, washed in PBS, and fixed in 70% ice cold (4°C) ethanol overnight. They were washed with PBS, incubated with R-Nase (100 µg/mL final concentration) at 37°C for 30 min, stained with propidium iodide (50 µg/mL final concentration), and analyzed by flow cytometry (Beckman Coulter).

The most promising compounds 3e and 3f were tested against MCF-7 cell lines at the 50%-inhibiting concentration according to the MTT assay. Figure 2 and Table 3 show the results after 24 h, 36 h and 48 h. As shown in Figure 2 and Table 4, compounds 3e and 3f arrest the cell cyclein G_0/G_1 phase, raising the G_0/G_1 peak from 41.98% to 75.88% (3e) and 73.70% (3f) after 48 h treatment. Subsequently, cells accumulated in sub-G_0 phase at 18.23% (3e) and 16.45% (3f). Because the increase of cells in sub-G_0 phase generally informed the increase of apoptotic cell death, 3e and 3f might display apoptosis-inducing effect on MCF-7 cells (Figure 2).

5. CONCLUSION

Various aurone derivatives have been explored as new and potential inhibitors of breast cancer cell lines. Synthesis of these compounds was carried out by reacting benzofuran-3(2H)-one with a range of benzaldehydes in the presence of a reusable catalyst Amberlyst-15. Compound 3e and 3f showed good anti-proliferative properties against two cancer cell lines, i.e., MDAMB-231 and MCF-7 than the standard. In general, our study suggests that the aurone framework will give an idea to find novel inhibitors for breast cancer.

6. ACKNOWLEDGMENT

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****Bibliographical Sketch***

Naveen Mulakayala earned his Ph.D. in chemistry from Sri Krishnadevaraya University, Anantapur, India. In 2008, he joined Dr. Reddy's Institute of Life Sciences, Hyderabad as a research associate with Dr. Manojit Pal and became research scientist in 2010. Naveen joined in AAP Pharma technologies as a Senior Research Scientist and then moved to Clearsynth Labs as a Principle Scientist.