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Research Article

# Synthesis, *in Vitro, and in Vivo* Biological Evaluation and Molecular Docking Analysis of Novel 3-(3-oxo-substitutedphenyl-3-)4-(2-(piperidinyl)ethoxy)phenyl) propyl)-2H-chromen-2-one Derivatives as Anti-breast Cancer Agents

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**The analogs of coumarin–chalcones have been reported to exhibit antineoplastic, anti-allergic, anti-hepatoprotective, and estrogenic activity. Herein, we have reported 3-(3-oxo-substitutedphenyl-3-)4-(2-(piperidinyl)ethoxy)phenyl)propyl)-2H-chromen-2-one derivatives as a new class of compounds that exhibit selectivity for ER-α binding along with antiproliferative and cytotoxic activity on human breast cancer cell line. The active compounds which show prominent activity against estrogen receptor-alpha-positive (ER+) human breast cancer cell lines MCF-7 and Zr-75-1 are subjected to *in vivo* screening. The Glide XP docking was performed for designed scaffold to optimize its structural requirement for ER-α inhibition.**

**Key words:** breast cancer, Coumarin–chalcone, docking, estrogen receptor

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Cancer is a notably complex, widespread, and lethal disease that are projected to continue rising, with an estimated 13.1 million deaths in 2030 (1). Cancer can affect almost every tissue lineage in the human body and poses great challenges to medical science. Most cancers were characterized by uncontrolled cell proliferation, lack of cell differentiation, and loss of contact inhibition, which confers upon the tumor cell, a capability to invade local tissues, and metastasize. The non-selectivity and acute toxicity of many anticancer agents beside the development of cellular drug resistance have been the major deterrent in their usage for treating human cancer, prompting the search for new antitumor agents with improved tumor selectivity, efficiency, and safety[a].

Among all cancers, breast cancer is the most common type of cancer affecting more than one million women and accounting for the highest mortality worldwide[b]. Even though large number of breast cancers express estrogen receptor (ER) and respond to therapy with hormones or aromatase inhibitors, there is a group of patients (12–17%) who do not respond to such treatment due to lack of ER. These are known as triple-negative breast cancers (TNBC) which represent a highly aggressive subtype of breast cancer that is difficult to treat. ER ligands that show differential responses within different tissues have been more accurately described as selective estrogen receptor modulators (SERMs). The tamoxifen (TAM) and raloxifene act as an ER antagonist in breast tissue from differential activation of multiple estrogen responsive pathways (1–3).

Epidemiological and animal studies have demonstrated that plant-derived dietary constituents of food play an important role in the prevention of disease. A number of food components that inhibit the initiation and progression of cancer or otherwise influence the potential for disease outcome have been identified (4). The beneficial effects of these dietary compounds have been attributed partly to the presence of numerous chalcone analogs with antioxidant and free radical scavenging properties. The natural and synthetic analogs of chalcones and coumarins have been reported to exhibit antineoplastic, antibacterial, antifungal, antimalarial, antiviral, antitubercular, anti-allergic, anti-inflammatory, antihepatoprotective, and estrogenic activity. The natural flavonoid and isoflavonoid compounds also have potent antitumor activities (4–6).

Herein, we have designed and synthesized coumarin–chalcone analogs (Figure 1, Scheme 1). The synthesized derivatives ware screen for their anticancer activity on human breast cancer cell line and animal model.



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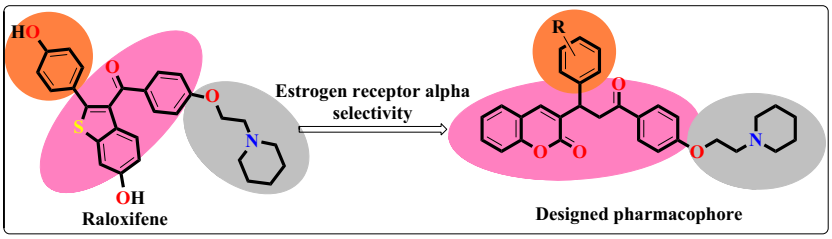



**Figure 1:** Designed pharmacophore.

## Experimental Section

### General

Melting points were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra of all synthesized compound in KBr were recorded using a (JASCO FT-IR 4000) spectrophotometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded on Bruker Avance (300 MHz) Spectrometer in $CDCl_3$ solutions, with TMS as an internal reference. Mass spectra were recorded on a Varian Inc., 410 Prostar Binary LC with 500 MS IT PDA Detectors. All the reagents and solvents used were of analytical grade.

### General procedure for synthesis of substituted p-hydroxy chalcone (3)

An equimolar mixture of 4-hydroxy acetophenone, substituted benzaldehydes, and KOH (2 mmol) was stirred in PEG-400 (15 mL) at 40 °C for 2–3 h. After the completion of the reaction (monitored by TLC), the crude mixture was worked up in ice-cold water (100 mL). The resultant product was separated out and recrystallized from absolute ethanol (7).

### General procedure for synthesis of substituted [(4-(2-piperidine-1-yl)ethoxy)] chalcone

A mixture of substituted chalcone (0.625 mmol), anhydrous $K_2CO_3$ (3.12 mmol), 1-(2-chloroethyl) piperidine (0.9 3 mmol), and dry acetone (10 mL) was refluxed for 24 h. $K_2CO_3$ was filtered off, and acetone was distilled out. The residue was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, brine and dried over anhydrous $Na_2SO_4$. The precipitate was recrystallized from absolute ethanol (7).

### General procedure for synthesis of substituted [3-(3-oxo-1-phenyl-3-)4-(2-(piperidinyl)ethoxy)phenyl) propyl)-2H-chromen-2-one

To the stirred solution of coumarin (0.46 mmol) in dry THF (10 mL), NaH (60%) was added and washed with n-hexane (0.6 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min. A solution of substituted chalcone (1.0 mmol) in dry THF (10 mL) was added drop wise at 0 °C, and the mixture was warmed to room temperature for 20 min. The resulting mixture was refluxed for 24 h. The mixture was quenched by adding of ice-cold water (20 mL) and was dried and recrystalized by methanol.

### 3-(3-oxo-1-phenyl-3-(4-(2-(piperidin-1-yl)ethoxy) phenyl)propyl)-2H-chromen-2-one (BI-1)

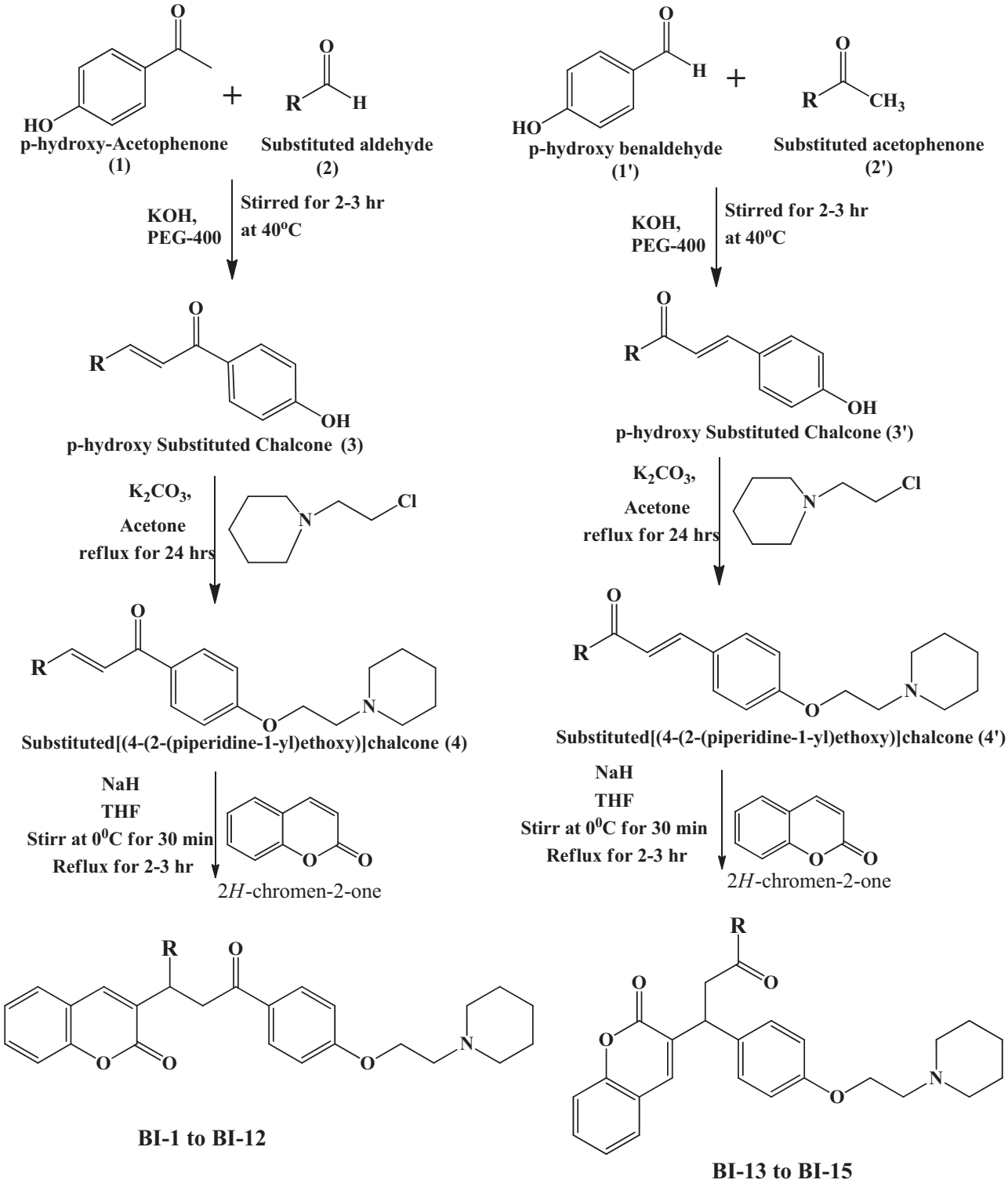
% Yield: 54.86; MW: 481.23; MF: $C_{31}H_{31}NO_4$; MP: 128–130 °C; IR (KBr): 678 (Ar-H), 1183 (C-O), 1341 (C-N), 1533 (C=C), 1724 (C=O) $cm^{-1}$; $^{1}H$ NMR (DMSO, 400 MHz): $\delta$ = 7.7–7.4 (m, 14H, Ar-H), 4.3 (t, 2H, $H_{11'}$), 4.1 (t, 1H, $H_{1'}$), 3.3 (d, 2H, $H_{2'}$), 2.7 (t, 2H, $H_{12'}$), 2.3–1.6 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 201.5 (C3′, C=O), 165.2 (C7′), 161.1 (C2, C=O), 154.6 (C10), 143.8 (C1″), 140.1 (C4), 129.8–129.4 (C5′, C6′, C8′, C9′), 128.2–126.9 (Ar-C), 120.3 (C5), 116.4 (C9), 69.2 (C11′), 58.6 (C12′), 57.2–57.0 (C14′, C18′), 42.3 (C2′), 28.5 (C1′), 25.9 (C15′, C17′), 23.8 (C16′); MS: *m/z* = 482.2 [M + 1].

### 3-(1-(4-chlorophenyl)-3-oxo-3-(4-(2-(piperidin-1-yl) ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-2)

% Yield: 60.34; MW: 515.19; MF: $C_{31}H_{30}ClNO_4$; MP: 142–144 °C; IR (KBr): 623 (Ar-H), 751 (C-Cl), 1174 (C-O), 1309 (C-N), 1541 (C=C), 1737 (C=O) $cm^{-1}$; $^{1}H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.2 (m, 13H, Ar-H), 4.1 (t, 2H, $H_{11'}$), 3.9 (t, 1H, $H_{1'}$), 3.3 (d, 2H, $H_{2'}$), 2.6 (t, 2H, $H_{12'}$), 2.2–1.7 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 199.3 (C3′, C=O), 164.8 (C7′), 160.2 (C2, C=O), 153.7 (C10), 144.2 (C1″), 139.9 (C4), 132.5 (C4″, C-Cl), 129.6–129.3 (C5′, C6′, C8′, C9′), 128.1–126.5 (Ar-C), 121.4 (C5), 114.8 (C9), 67.6 (C11′), 59.8 (C12′), 57.3–57.1 (C14′, C18′), 44.3 (C2′), 29.9 (C1′), 24.2 (C15′, C17′), 21.3 (C16′); MS: *m/z* = 517.2 [M + 2].

### 3-(1-(4-fluorophenyl)-3-oxo-3-(4-(2-(piperidin-1-yl) ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-3)

% Yield: 58.76; MW: 499.22; MF: $C_{31}H_{30}FNO_4$; MP: 112–114 °C; IR (KBr): 682 (Ar-H), 1028 (C-F), 1216 (C-O), 1289 (C-N), 1520 (C=C), 1721 (C=O) $cm^{-1}$; $^{1}H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.3 (m, 13H, Ar-H), 4.2 (t, 2H, $H_{11'}$), 3.8 (t, 1H, $H_{1'}$), 3.1 (d, 2H, $H_{2'}$), 2.7 (t, 2H, $H_{12'}$), 2.3–1.8 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 189.8 (C3′, C=O), 163.7 (C7′), 161.5 (C4″, C-F), 157.8 (C2, C=O), 152.3 (C10), 142.4 (C1″), 138.6 (C4), 129.5–129.3 (C5′, C6′, C8′, C9′), 127.8–126.4 (Ar-C), 120.9 (C5), 112.1 (C9), 66.7 (C11′), 60.1 (C12′), 57.2–57.0 (C14′, C18′), 43.7 (C2′), 30.1 (C1′), 23.8 (C15′, C17′), 21.5 (C16′); MS: *m/z* = 501.2 [M + 1].




**Scheme 1:** Synthesis of designed compounds.

### 3-(1-(2,3-dichlorophenyl)-3-oxo-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-4)

% Yield: 68.48; MW: 549.15; MF: $C_{31}H_{29}Cl_2NO_4$; MP: 127–129 °C; IR (KBr): 629 (Ar-H), 746 (C-Cl), 1169 (C-O), 1311 (C-N), 1474 (C=C), 1722 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.7–7.1 (m, 12H, Ar-H), 4.4 (t, 2H, $H_{11'}$), 3.9 (t, 1H, $H_{1'}$), 3.3 (d, 2H, $H_{2'}$), 2.8 (t, 2H, $H_{12'}$), 2.4–1.9 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 194.3 (C3′, C=O), 165.8 (C7′), 160.2 (C2, C=O), 153.7

(C10), 144.2 (C1″), 139.9 (C4), 133.6 (C3″, C-Cl), 131.4 (C2″, C-Cl), 129.2–128.7 (C5′, C6′, C8′, C9′), 128.0–126.4 (Ar-C), 120.9 (C5), 113.5 (C9), 66.7 (C11′), 60.6 (C12′), 57.5–57.3 (C14′, C18′), 43.8 (C2′), 29.5 (C1′), 23.9 (C15′, C17′), 20.3 (C16′); MS: *m/z* = 551.1 [M + 2].

### 3-(1-(2,3-dimethoxyphenyl)-3-oxo-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-5)

% Yield: 62.44; MW: 541.25; MF: $C_{33}H_{35}NO_6$; MP: 152–154 °C; IR (KBr): 662 (Ar-H), 1182 (C-O), 1296 (C-N), 1527 (C=C), 1719 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.9–7.5 (m, 12H, Ar-H), 4.3 (t, 2H, $H_{11'}$), 4.1 (t, 1H, $H_{1'}$), 3.9 (s, 6H, $OCH_3$), 3.5 (d, 2H, $H_{2'}$), 2.8 (t, 2H, $H_{12'}$), 2.4–1.8 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 198.5 (C3′, C=O), 167.3 (C7′), 162.4 (C2, C=O), 156.8 (C10), 151.2 (C2″, C-O), 146.4 (C3″, C-O), 143.7 (C1″), 141.2 (C4), 129.8–129.5 (C5′, C6′, C8′, C9′), 128.2–127.1 (Ar-C), 121.1 (C5), 114.8 (C9), 67.3 (C11′), 61.2 ($OCH_3$), 58.6 (C12′), 57.2-57.0 (C14′, C18′), 53.4 ($OCH_3$), 43.5 (C2′), 29.6 (C1′), 24.9 (C15′, C17′), 21.8 (C16′); MS: *m/z* = 542.3 [M + 1].

### 3-(1-(2,4-dichlorophenyl)-3-oxo-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-6)

% Yield: 54.90; MW: 549.15; MF: $C_{31}H_{29}Cl_2NO_4$; MP: 139–141 °C; IR (KBr): 628 (Ar-H), 786 (C-Cl), 1163 (C-O), 1291 (C-N), 1524 (C=C), 1747 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.2 (m, 12H, Ar-H), 4.3 (t, 2H, $H_{11'}$), 3.6 (t, 1H, $H_{1'}$), 3.1 (d, 2H, $H_{2'}$), 2.9 (t, 2H, $H_{12'}$), 2.3–1.9 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 197.3 (C3′, C=O), 167.3 (C7′), 161.5 (C2, C=O), 155.2 (C10), 144.8 (C1″), 138.1 (C4), 132.8 (C4″, C-Cl), 132.0 (C2″, C-Cl), 129.2–128.5 (C5′, C6′, C8′, C9′), 127.6–126.2 (Ar-C), 121.2 (C5), 116.5 (C9), 68.9 (C11′), 61.5 (C12′), 57.6–57.4 (C14′, C18′), 44.2 (C2′), 30.4 (C1′), 23.8 (C15′, C17′), 20.1 (C16′); MS: *m/z* = 551.1 [M + 2].

### 3-(1-(2,6-dichlorophenyl)-3-oxo-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-7)

% Yield: 65.96; MW: 549.15; MF: $C_{31}H_{29}Cl_2NO_4$; MP: 144–146 °C; IR (KBr): 651 (Ar-H), 773 (C-Cl), 1176 (C-O), 1288 (C-N), 1536 (C=C), 1739 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.3 (m, 12H, Ar-H), 4.4 (t, 2H, $H_{11'}$), 3.6 (t, 1H, $H_{1'}$), 3.0 (d, 2H, $H_{2'}$), 2.8 (t, 2H, $H_{12'}$), 2.3–1.7 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 196.8 (C3′, C=O), 165.2 (C7′), 161.4 (C2, C=O), 153.7 (C10), 142.6 (C1″), 138.1 (C4), 133.4 (C6″, C-Cl), 131.9 (C2″, C-Cl), 129.3–128.5 (C5′, C6′, C8′, C9′), 127.5–126.2 (Ar-C), 120.7 (C5), 112.1 (C9), 66.9 (C11′), 61.2 (C12′), 57.6–57.4 (C14′, C18′), 41.3 (C2′), 32.5 (C1′), 24.2 (C15′, C17′), 20.3 (C16′); MS: *m/z* = 551.1 [M + 2].

### 3-(1-(3-methoxyphenyl)-3-oxo-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-8)

% Yield: 72.38; MW: 511.24; MF: $C_{32}H_{33}NO_5$; MP: 114–116 °C; IR (KBr): 664 (Ar-H), 1166 (C-O), 1297 (C-N), 1534 (C=C), 1725 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.1 (m, 13H, Ar-H), 4.4 (t, 2H, $H_{11'}$), 4.1 (t, 1H, $H_{1'}$), 3.8 (s, 3H, $OCH_3$), 3.5 (d, 2H, $H_{2'}$), 2.9 (t, 2H, $H_{12'}$), 2.3–1.8 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 199.7 (C3′, C=O), 168.1 (C7′), 161.6 (C2, C=O), 157.2 (C10), 147.1 (C3″, C-O), 144.3 (C1″), 140.9 (C4), 129.8–129.3 (C5′, C6′, C8′, C9′), 128.3–127.4 (Ar-C), 121.2 (C5), 111.7 (C9), 63.2 (C11′), 60.4 ($OCH_3$), 56.5 (C12′), 57.1–56.9 (C14′, C18′), 42.9 (C2′), 30.2 (C1′), 24.5 (C15′, C17′), 21.6 (C16′); MS: *m/z* = 512.3 [M + 1].

### 3-(3-oxo-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-1-(2,3,4-trimethoxyphenyl)propyl)-2H-chromen-2-one (BI-9)

% Yield: 75.88; MW: 571.26; MF: $C_{34}H_{37}NO_7$; MP: 162–164 °C; IR (KBr): 644 (Ar-H), 1167 (C-O), 1307 (C-N), 1498 (C=C), 1727 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 8.1–7.4 (m, 11H, Ar-H), 4.3 (t, 2H, $H_{11'}$), 4.0 (t, 1H, $H_{1'}$), 3.9 (s, 9H, $OCH_3$), 3.4 (d, 2H, $H_{2'}$), 2.8 (t, 2H, $H_{12'}$), 2.4–1.9 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 192.5 (C3′, C=O), 167.4 (C7′), 162.4 (C2, C=O), 156.9 (C10), 152.3 (C2″, C-O), 147.5 (C3″, C-O), 146.9 (C4″, C-O), 143.5 (C1″), 141.3 (C4), 129.9–129.6 (C5′, C6′, C8′, C9′), 128.2–127.3 (Ar-C), 122.4 (C5), 114.7 (C9), 67.8 (C11′), 61.6 ($OCH_3$), 58.5 (C12′), 57.2–57.0 (C14′, C18′), 53.7 ($OCH_3$), 43.5 (C2′), 29.6 (C1′), 24.9 (C15′, C17′), 21.8 (C16′); MS: *m/z* = 572.3 [M + 1].

### 3-(1-(furan-2-yl)-3-oxo-3-(4-(2-(piperidin-1-yl) ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-10)

% Yield: 62.24; MW: 471.20; MF: $C_{29}H_{29}NO_5$; MP: 130–132 °C; IR (KBr): 673 (Ar-H), 1172 (C-O), 1289 (C-N), 1519 (C=C), 1731 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.7–7.4 (m, 10H, Ar-H), 6.1–6.0 (m, 2H, furan ring), 4.3 (t, 2H, $H_{11'}$), 4.0 (t, 1H, $H_{1'}$), 3.4 (d, 2H, $H_{2'}$), 2.7 (t, 2H, $H_{12'}$), 2.2–1.6 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 188.2 (C3′, C=O), 165.2 (C7′), 161.1 (C2, C=O), 154.6 (C10), 149.8 (C1″), 140.1 (C4), 129.8–129.5 (C5′, C6′, C8′, C9′), 128.2–126.9 (Ar-C), 120.3 (C5), 116.4 (C9), 109.4, 105.6 (C-furan), 69.7 (C11′), 58.4 (C12′), 57.2-57.0 (C14′, C18′), 42.3 (C2′), 28.5 (C1′), 25.9 (C15′, C17′), 23.8 (C16′); MS: *m/z* = 472.2 [M + 1].

### 3-(3-oxo-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-1-(p-tolyl)propyl)-2H-chromen-2-one (BI-11)

% Yield: 76.74; MW: 495.24; MF: $C_{32}H_{33}NO_4$; MP: 164–166 °C; IR (KBr): 668 (Ar-H), 1182 (C-O), 1311 (C-N), 1517 (C=C), 1724 (C=O), 2912 (C-H) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.1 (m, 13H, Ar-H), 4.4 (t, 2H,

$H_{11'}$), 4.1 (t, 1H, $H_{1'}$), 3.5 (d, 2H, $H_{2'}$), 2.9 (t, 2H, $H_{12'}$), 2.4 (s, 3H, $CH_3$), 2.3–1.8 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 198.6 (C3′, C=O), 167.2 (C7′), 162.2 (C2, C=O), 155.4 (C10), 148.2 (C3″, C-O), 145.1 (C1″), 141.7 (C4), 136.3 (C4″), 129.5–129.2 (C5′, C6′, C8′, C9′), 128.2–127.1 (Ar-C), 121.4 (C5), 113.8 (C9), 65.9 (C11′), 57.3–57.0 (C14′, C18′), 56.5 (C12′), 43.2 (C2′), 31.4 (C1′), 25.3 (C15′, C17′), 21.1 (C16′), 20.3 ($CH_3$); MS: *m/z* = 496.2 [M + 1].

### 3-(1-(2-hydroxyphenyl)-3-oxo-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BI-12)

% Yield: 70.68; MW: 497.22; MF: $C_{31}H_{31}NO_5$; MP: 158–160 °C; IR (KBr): 653 (Ar-H), 1171 (C-O), 1296 (C-N), 1526 (C=C), 1737 (C=O), 3245 (O-H) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.6–7.0 (m, 13H, Ar-H), 5.1 (s, 1H, OH), 4.3 (t, 2H, $H_{11'}$), 4.1 (t, 1H, $H_{1'}$), 3.5 (d, 2H, $H_{2'}$), 2.8 (t, 2H, $H_{12'}$), 2.4–2.0 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 190.4 (C3′, C=O), 163.7 (C7′), 161.6 (C2, C=O), 153.1 (C10), 147.0 (C3″, C-O), 144.9 (C1″), 140.5 (C4), 132.8 (C2″, C-O), 129.7–129.3 (C5′, C6′, C8′, C9′), 128.4–127.2 (Ar-C), 121.6 (C5), 116.2 (C9), 66.4 (C11′), 58.8 (C12′), 57.4–57.2 (C14′, C18′), 45.6 (C2′), 30.9 (C1′), 24.8 (C15′, C17′), 22.7 (C16′); MS: *m/z* = 498.2 [M + 1].

### 3-(3-oxo-3-phenyl-1-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BII-1)

% Yield: 61.74; MW: 481.23; MF: $C_{31}H_{31}NO_4$; MP: 128–130 °C; IR (KBr): 681 (Ar-H), 1189 (C-O), 1339 (C-N), 1541 (C=C), 1730 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.4 (m, 14H, Ar-H), 4.4 (t, 2H, $H_{11'}$), 4.2 (t, 1H, $H_{1'}$), 3.2 (d, 2H, $H_{2'}$), 2.7 (t, 2H, $H_{12'}$), 2.4–1.6 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 202.8 (C3′, C=O), 166.1 (C7′), 163.4 (C2, C=O), 154.7 (C10), 143.2 (C1″), 141.0 (C4), 129.9–129.4 (C5′, C6′, C8′, C9′), 128.2–126.9 (Ar-C), 120.3 (C5), 116.4 (C9), 69.2 (C11′), 58.6 (C12′), 57.2–57.0 (C14′, C18′), 42.3 (C2′), 27.9 (C1′), 25.9 (C15′, C17′), 23.8 (C16′); MS: *m/z* = 482.2 [M + 1].

### 3-(3-(4-chlorophenyl)-3-oxo-1-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BII-2)

% Yield: 74.96; MW: 515.19; MF: $C_{31}H_{30}ClNO_4$; MP: 142–144 °C; IR (KBr): 625 (Ar-H), 756 (C-Cl), 1174 (C-O), 1311 (C-N), 1545 (C=C), 1734 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.2 (m, 13H, Ar-H), 4.2 (t, 2H, $H_{11'}$), 3.9 (t, 1H, $H_{1'}$), 3.4 (d, 2H, $H_{2'}$), 2.6 (t, 2H, $H_{12'}$), 2.2–1.7 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 199.9 (C3′, C=O), 165.1 (C7′), 160.6 (C2, C=O), 154.7 (C10), 143.7 (C1″), 139.8 (C4), 132.5 (C4″, C-Cl), 129.6–129.3 (C5′, C6′, C8′, C9′), 128.1–126.5 (Ar-C), 121.4 (C5), 114.8 (C9), 67.6 (C11′), 59.8 (C12′), 57.3–57.1 (C14′, C18′), 44.3 (C2′), 30.2 (C1′), 24.5 (C15′, C17′), 22.4 (C16′); MS: *m/z* = 517.2 [M + 2].

### 3-(3-(4-methoxyphenyl)-3-oxo-1-(4-(2-(piperidin-1-yl)ethoxy)phenyl)propyl)-2H-chromen-2-one (BII-3)

% Yield: 77.34; MW: 511.24; MF: $C_{32}H_{33}NO_5$; MP: 114–116 °C; IR (KBr): 673 (Ar-H), 1188 (C-O), 1319 (C-N), 1535 (C=C), 1742 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO, 400 MHz): $\delta$ = 7.8–7.0 (m, 13H, Ar-H), 4.3 (t, 2H, $H_{11'}$), 4.0 (t, 1H, $H_{1'}$), 3.7 (s, 3H, $OCH_3$), 3.4 (d, 2H, $H_{2'}$), 2.9 (t, 2H, $H_{12'}$), 2.3–1.8 (m, 10H, piperidine); $^{13}C$ NMR (DMSO, 100 MHz): $\delta$ = 200.3 (C3′, C=O), 169.0 (C7′), 162.8 (C2, C=O), 157.3 (C10), 147.5 (C4″, C-O), 144.4 (C1″), 140.8 (C4), 129.8–129.3 (C5′, C6′, C8′, C9′), 128.3–127.4 (Ar-C), 121.9 (C5), 113.7 (C9), 64.6 (C11′), 60.4 ($OCH_3$), 56.5 (C12′), 57.1–56.9 (C14′, C18′), 44.8 (C2′), 31.3 (C1′), 25.1 (C15′, C17′), 22.7 (C16′); MS: *m/z* = 512.3 [M + 1].

## *Anticancer screening*

### *In vitro* screening

*In vitro* testing is performed using SRB assay protocols (8), each drug is tested at four dose levels ($1 \times 10^{-7}$ M, $1 \times 10^{-6}$ M, $1 \times 10^{-5}$ M, and $1 \times 10^{-4}$ M, or 10, 20, 40, and 80 $\mu$g/mL). Appropriate positive controls are run in each experiment, and each experiment is repeated thrice. Results are given in terms of $GI_{50}$, TGI, and $LC_{50}$ values. The compounds were tested for their cytotoxic assay using MCF-7 and Zr-75-1 estrogen receptor-positive cancer cell lines.

### *In vivo* screening

The active compounds which show prominent activity against MCF-7 and Zr-75-1 cancer cell line are subjected for *in vivo* screening.

Female virgin Sprague Dawley rats were obtained from Wockhardt Pvt Ltd (Aurangabad) at 35 days of age. Rats were housed at 4 per cage and maintained at (25 ± 2) °C under 12-h dark/light cycle with access to standard diet and water *ab libitum*. Animals were experimented with prior approval from the institutional ethics committee.

The MNU (methyl nitrosourea) was purchased from Sigma-Aldrich (USA). An aqueous solution at a concentration of 10 mg/mL was made by wetting the MNU powder with 3% acetic acid and then dissolving it in 0.9% NaCl solution; a fresh solution was prepared for each injection. Rats were given intraperitoneally (i.p.) 50 mg/kg of MNU on the 50th and 57th day. Animals were divided into different groups with six animals in each group. Group I (intact control) received 0.9% NaCl with 3% acetic acid *i.p.* Groups II–VI were introduced with MNU. After 60 days, animals were treated with synthesized compounds and tamoxifen (TAM) (5 mg/kg and 10 mg/kg in 1% Tween-80 by gavage once per day) for four weeks. Animals in intact control group and untreated MNU group were given vehicle (Tween-80) according to experimental protocol.

After completion of treatment, blood was collected from retro orbital puncture and analyzed for estrogen-level mea-

**Table 1:** Anticancer activity of designed compounds

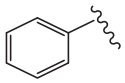
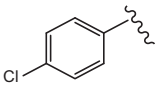
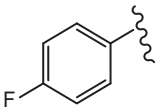
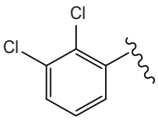
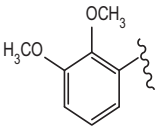
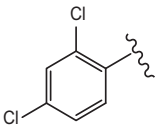
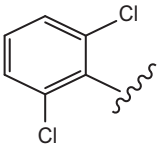
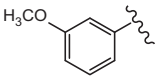
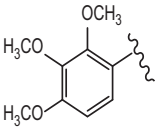
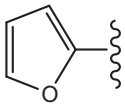
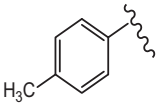
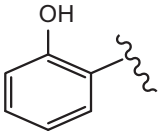
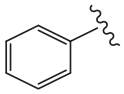
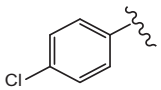
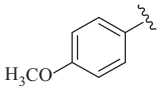
| | | Cell line MCF7 | | | Cell line Zr-75-1 | | |
|---|---|---|---|---|---|---|---|
| Comp code | R | LC50 | TGI | GI50 | LC50 | TGI | GI50 |
| BI-1 |  | >80 | 45.6 | <10 | >80 | 70.3 | 21.1 |
| BI-2 |  | 67.9 | 33.9 | <10 | 49.3 | 23.6 | <0.1 |
| BI-3 |  | >80 | 61.7 | 28.2 | >80 | >80 | 36.8 |
| BI-4 |  | >80 | 47.2 | <10 | >80 | 65.4 | 13.4 |
| BI-5 |  | >80 | >80 | 51.9 | >80 | >80 | >80 |
| BI-6 |  | 64.8 | 35.1 | <10 | 51.1 | 21.9 | <0.1 |
| BI-7 |  | 60.2 | 18.1 | <10 | >80 | 56.1 | 18.6 |
| BI-8 |  | >80 | 78.5 | 37.2 | >80 | >80 | >80 |
| BI-9 |  | 77.0 | 45.3 | 13.7 | >80 | 60.1 | 18.8 |
| BI-10 |  | >80 | 40.6 | <10 | >80 | 54.8 | 8.6 |
| BI-11 |  | >80 | 36.4 | <10 | 57.0 | 26.2 | <0.1 |

**Table 1:** continued

| Comp code | R | Cell line MCF7 | | | Cell line Zr-75-1 | | |
|---|---|---|---|---|---|---|---|
| | | LC50 | TGI | GI50 | LC50 | TGI | GI50 |
| BI-12 |  | 67.4 | 29.2 | <10 | 68.3 | 34.6 | 0.9 |
| BII-1 |  | 68.5 | 31.8 | <10 | >80 | 62.4 | 22.07 |
| BII-2 |  | >80 | 67.9 | 26.1 | >80 | >80 | 43.6 |
| BII-3 |  | 77.2 | 42.1 | <10 | 65.2 | 41.1 | 16.9 |
| TAM | – | 29.4 | 11.2 | <10 | 61.8 | 36.6 | <0.1 |
| ADR | – | 68.5 | 31.5 | <10 | 79.9 | 35.8 | <0.1 |

surement. Serum hormone value for estrogen was averaged from blood samples collected on 30th and 45th the day of treatment.

### *Docking study*

Docking procedure aims to identify the correct binding poses within the binding site of the protein. To locate the appropriate binding orientations and conformations of chalcone compounds interacting with human estrogen receptor-α (hER-α), molecular docking was performed with the GLIDE-5.8 program interfaced with MAESTRO-9.3 of Schrödinger 2012. The flexible docking method has been shown to be very efficient on numerous protein receptors. To better describe the possible binding modes of chalcone compounds with hERα, RAL was used. We examined the ability of our procedure in reproducing the binding position for RAL in PDB entry 1ERR, when docked in another X-ray structure (PDB: 1ERR) with a cocrystalized antagonist. The procedure was successful in reproducing the binding position for RAL. The crystal structures of hER-α (PDB entry code: 1ERR) was extracted from Brookhaven Protein Database (PDB http://www.rcsb.org/pdb). Prior to docking calculations, ligand preparation was performed with the LigPrep program using OPLS_2005 forcefield. Default parameters were used for docking. The docking poses are ranked according to Glide score.

## Results and Discussion

### *Chemistry*

The proposed derivatives were synthesized in three steps and their structures were verified by IR, $^{1}$H-NMR, $^{13}$C-NMR, and LC-MS spectroscopy. The first step in the synthetic route consisted of the Claisen–Schmidt condensation of 4′-hydroxyl acetophenone with substituted benzaldehydes and 4′-hydroxyl benzaldehydes with substituted acetophenone in basic conditions (10% aqueous KOH) to give α, β-unsaturated ketones (chalcones, Scheme 1) (7). The second step consisted of the condensation of hydroxyl group by amino side chain. The target compounds (**BI–1** to **BI–15**) were synthesized by Michael addition of coumarin to chalcone derivatives. The substitutions of target compounds are shown in Table 1.

### *Anticancer screening*

#### *In vitro* screening

The target compounds were evaluated for anticancer activity against estrogen receptor-alpha-positive (ER+) human breast cancer cell lines MCF-7 and Zr-75-1 (8). The *in vitro* activity profile is shown in Table 1. The $GI_{50}$ concentration for each compound was calculated with reference to a control sample, which represents the concentration that results in a 50% decrease in cell growth/proliferation after 48-h incubation in the presence of drug. The total growth inhibition (TGI) is the concentration of test drug which signifies a cytostatic effect. The $LC_{50}$ is concentration of compound that produces 50% cytocidal effect. Tamoxifen and adriamycin were used as reference.

#### *In vivo* screening

The active compounds **BI–2**, **BI–6**, and **BI–11** which show prominent activity against MCF-7 and Zr-75-1 cancer cell

lines are subjected to *in vivo* screening. *In vivo* study was carried out using M-methyl-nitrosourea (MNU)-induced mammary carcinoma in female Sprague Dawley rats. MNU is most often used to model mammary tumor initiation and progression. The compounds which were shown prominent activity against MCF-7 and Zr-75-1 cancerous cell lines are also shows marked modulator activity on estrogen receptor (Table 2). All the treated groups show marked antagonistic activity on estrogen receptor which results in decrease level of estrogen to normal range as compared to group II.

## SAR analysis

The SAR analysis suggested that the all compounds showed moderate to good anticancer activity. Among the synthesized derivatives, **BI–2**, **BI–6**, **BI–7**, **BI–10**, and **BI–11** show good antiproliferative activity as compared to standard tamoxifen. The compounds **BI–2**, **BI–6**, and **BI–11** showed potent cytotoxic activity on both cell lines. This indicates the substitution of chloro or methyl group on para position of substituted phenyl ring increases activity. Whereas substitution of methoxy group on substituted phenyl ring decreases activity. The structural difference between raloxifene (RAL) and synthesized compounds showed that the presence of hydrophobic thiophene ring in RAL is responsible for strong ER-α binding. The modification at propenone linkage of chalcone by any hydrophobic ring and hydrogen bond donor or acceptor group may cause increase in antiproliferative activity in comparison with RAL.

## Molecular docking analysis

To explore the main interactions with the target hER-α receptor of the chalcone derivatives, we performed molecular docking studies. These results indicated an acceptable reliability of the parameters specified in Maestro-Glide in reproducing the binding mode for this compounds (9).

After successful reproduction of the binding mode of RAL, the docking method was used to search for the binding modes of the whole data set. All the compounds were successfully docked into the binding pocket of ER-α, and their binding interactions are tabulated in Table 3. In this research, to illustrate the interaction mechanism, the attention has been focused on protein–ligand interactions of the dichloro-substituted compounds **BI–4** and **BI–6**, and the furan-substituted compound **BI–10**. The chemical struc-

**Table 2:** Estrogen levels (pg/mL) of animals on day 30 and 45

| | | Estrogen level (mean ± SE) | |
|---|---|---|---|
| Group | Compound no. | On 30th day | On 45th day |
| I | Control | 9.9 ± 1.22 | 9.7 ± 1.36 |
| II | MNU | 18.96 ± 2.09 | 20.77 ± 1.98 |
| III | **BI-2** | 11.16 ± 1.42 | 11.08 ± 1.22 |
| IV | **BI-6** | 11.18 ± 1.09 | 10.74 ± 1.76 |
| V | **BI-11** | 12.91 ± 1.62 | 13.12 ± 1.37 |
| VI | TAM | 11.06 ± 1.18 | 11.28 ± 1.69 |

**Table 3:** Binding interaction of compounds with estrogen receptor-α

| Compound ID | Docking score | No. of H-bonds | Hydrogen bond details |
|---|---|---|---|
| **BI-1** | −7.768 | 1 | $NH^+$-ASP 351 (2.108 Å) |
| **BI-2** | −8.058 | 0 | – |
| **BI-3** | −8.399 | 1 | $NH^+$-ASP 351 (1.729 Å) |
| **BI-4** | −9.401 | 1 | $NH^+$ASP 351 (1.646 Å) |
| **BI-5** | −8.493 | 2 | $NH^+$-ASP 351 (1.867 Å) |
| **BI-6** | −9.031 | 1 | $NH^+$-ASP 351 (2.168 Å) |
| **BI-7** | −7.972 | 0 | – |
| **BI-8** | −4.622 | 1 | $NH^+$ GLU380 (1.674 Å) |
| **BI-9** | −8.133 | 0 | – |
| **BI-10** | −8.850 | 1 | $NH^+$-ASP 351 (1.664 Å) |
| **BI-11** | −8.199 | 0 | – |
| **B1-12** | −6.271 | 1 | OH-TRH 347 (2.005 Å) |
| Tamoxifene | −9.028 | 1 | $NH^+$-ASP 351 (1.936 Å) |
| 4-OH Tamoxifen | −9.321 | 2 | $NH^+$-ASP 351 (1.645 Å)<br>OH-HID 524 (2.437 Å) |
| Raloxifene | −11.99 | 4 | OH-GLU 353 (1.596 Å)<br>OH-ARG394 (1.887 Å)<br>OH-HID 524 (2.411 Å)<br>$NH^+$-ASP 351 (1.646 Å) |

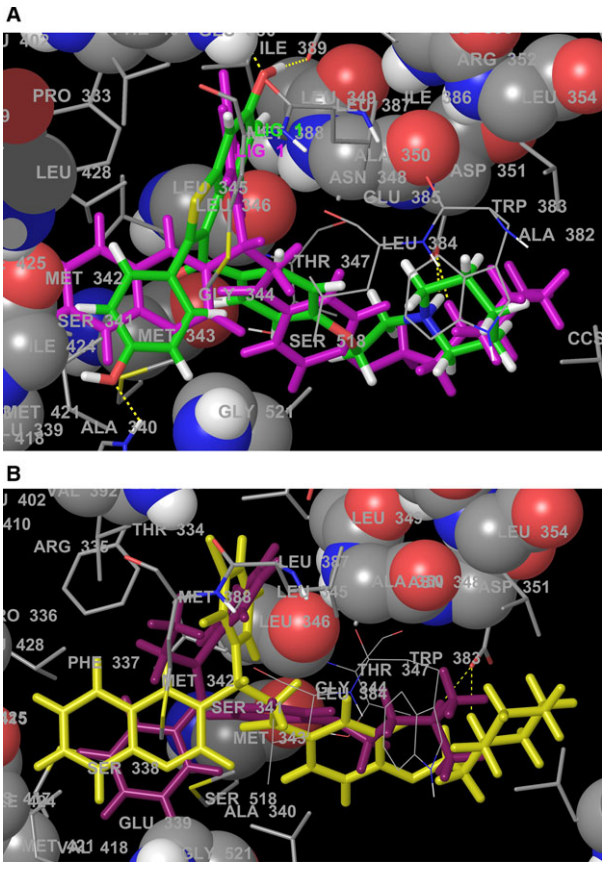



**Figure 2:** Docking pose of compounds (A) **BI–4** (purple) with raloxifene (green) and (B) **BI–7** (yellow) with tamoxifen (maroon).

tures and binding modes of **BI–4** with standard RAL and **BI–7** with standard TAM are displayed in Figure 2. It can be seen that **BI–4** and **BI–7** are located at the active site of the receptor. Specifically, the tertiary nitrogen group (piperidine ring) acted as a hydrogen bond donor and formed a hydrogen bond with the oxygen atom of Asp351 same as that of RAL as show in Figure 3. Compounds **BI–5** and **BI–7** form $\pi$–$\pi$ stacking interaction with Phe404, whereas compound **BI–5** forms $\pi$–$\pi$ stacking interaction with Hid525.

In summary, we have prepared substituted chalcones and coumarins (**BI–1** to **BI–15**), and evaluated for their anticancer activity using MCF-7 and Zr-75-1 cell line culture. *In vivo* study shows the prominent estrogen receptor modulator activity. Molecular docking suggested that multiple hydrophobic and hydrogen bond interactions are two predominant factors that affect the binding process. The decomposition of binding free energy to each residue revealed that the most favorable contributions came from Asp351, Phe404, Phe425, Leu354, Met388, Met421, and His524. The results of this study may conclude that further structural modification is required for the development of new therapeutic agent.

## Conclusion

It can be concluded that the 3-(1-(4-substituted phenyl)-3-(3-oxo-1,3-diphenylpropyl)-2H-chromen-2-one derivatives designed are a new class of estrogen receptor modulators endowed with significant antitumor activity on MCF-7 and Zr-75-1 cell line culture. Several compounds are interesting as they display favorable inhibitory potency on estrogen receptor. Molecular docking suggested that multiple hydrophobic and hydrogen bond interactions are two predominant factors that affect the binding process. The decomposition of binding free energy to each residue revealed that the most favorable contributions came from Asp351, Phe404, Phe425, Leu354, Met388, Met421, and His524. The results of this study may conclude that further structural modification is required for the development of new therapeutic agent.

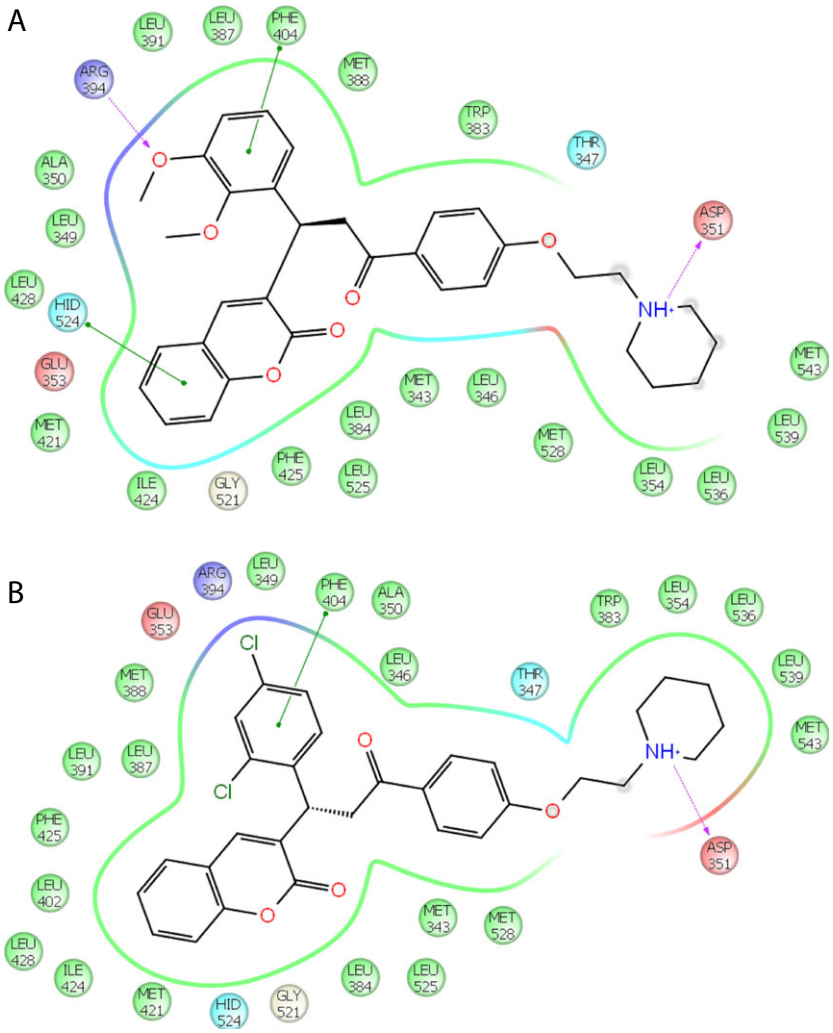



**Figure 3:** Ligand interaction diagram of (A) compound **BI–5** and (B) compound **BI–6**.

## Acknowledgments

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## Notes

[a]Cancer Trends Progress Report – 2009/2010 Update, 2012. WHO Website: http://www.who.int/cancer/en and http://progressreport.cancer.gov.

[b]Ferlay J., Soerjomataram I., Ervik M., Dikshit R., Eser S., Mathers C., Rebelo M., Parkin D.M., Forman D., Bray F. (2012) GLOBOCAN 2012v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11.