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Design, synthesis and biological evaluation of novel triaryl (*Z*)-olefins as tamoxifen analogues

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ABSTRACT

Tamoxifen (TAM) is used for the treatment and prevention of estrogen receptor positive breast cancer. However, the limited activity, toxicity and the development of resistance raised the current need for new potent nontoxic antiestrogen. Six novel TAM analogues **5a**–**f** were synthesized using McMurry olefination reaction. Replacement of the dimethylamino group in TAM by piperidino, piperazino or *N*-methyl piperazino, substituting the phenyl ring with florine atom at *p*-position and changing the ethyl group by methyl, afforded compounds showing comparable activity to TAM (**1**). Compounds **5c** and **5e** showed significant increase in antiproliferative activity in two breast cancer cell lines (MCF-7 and MDA-MB-231) compared to tamoxifen, while other compounds showed similar activity. The increased anticancer activity of compounds **5c** and **5e** was attributed to their ability to induce ER-independent cell death.

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Breast cancer is the most leading cause of death and the most frequent cancer in women. Estrogens are the most known stimulator of breast cancer cell growth so antiestrogens are considered good candidates for the treatment.[1,2] Tamoxifen (TAM, **1**), the nonsteroidal antiestrogenic drug is one of the most extensively used drugs to treat hormone-responsive human breast cancers since 1970.[3–6] The mode of action of TAM (**1**) in cancer therapy and in preventing breast cancer in high risk women is believed to be partially through competing with estrogens for binding to estrogen receptors.[7] High concentrations of TAM activates caspases and trigger apoptotic cell death independently of its estrogen receptors (ER) binding activity.[8,9] Both ER-dependent and ER-independent pathways for tamoxifen-induced programmed cell death are critical for successful therapy. The accumulative risk-benefit assessment of TAM therapy and comparative studies with other new types of drugs established its efficacy and safety.[10] Therefore, the development of new tamoxifen—type drugs are significantly required.

The aminoalkyloxy moiety present in TAM ($OCH_2CH_2NMe_2$, Fig. 1) plays a major role in determining receptor binding affinity.[11] Decreasing the basicity of the protonated amino group (cationic site) is believed to diminish the binding interaction with Asp 351 (anionic carboxylate site) on the estrogen receptors.[12] Although replacement of the $-CH_2CH_3$ substituent in tamoxifen (**1**) by a $-CH_3$ substituent does not change the antiproliferative data for MCF-7 human breast cancer cells.[13,14] Accordingly, it was anticipated that replacement of the dimethylamino group of aminoalkyloxy moiety with piperidino, piperazino and *N*-methylpiperazino to elevate the basic characters of the amino group may provide a new tamoxifen analogs of potential high antiproliferative activity. Accordingly, here we describe the synthesis of novel TAM analogs, molecular modeling studies, and their antiproliferative effect on MCF-7 and MDA-MB-231 human breast cancer cells.

The synthetic pathways adopted for the preparation of the desired new compounds are illustrated in Scheme 1. The (*Z*)-1-[4-(2-chloroethoxy)phenyl]-1,2-diphenylprop-1-ene (**4a**) was synthesized using a McMurry olefination reaction by Zn–$TiCl_4$ catalzyed reductive cross-coupling of 4-(2-chloroethoxy)benzophenone (**2**) with acetophenone (**3a**) in 38% yield. The **4a** product was the sole

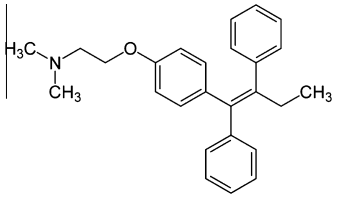

Figure 1. Chemical structure of Tamoxifen (**1**).

Abbreviations: TAM, tamoxifen; ER, estrogen receptor; MOE, molecular operating environment; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; DMEM/F12, Dulbecco's modified Eagle medium nutrient mixture F-12; FBS, fetal bovine serum.

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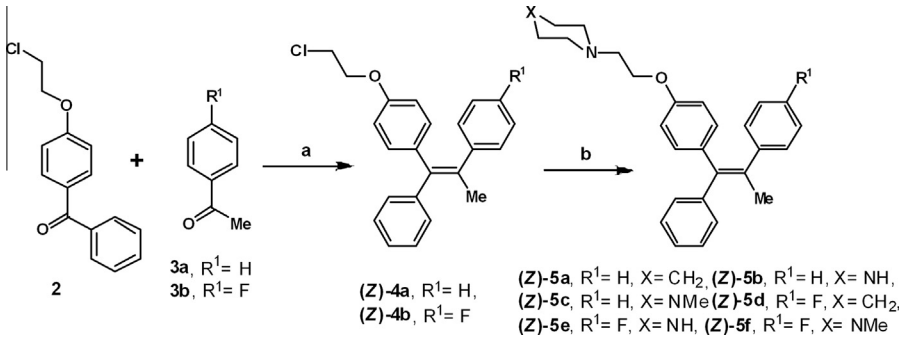

Scheme 1. Reagents and conditions: (a) Zn, $TiCl_4$, THF, reflux 3.5 h; (b) piperidine, piperazine or *N*-methylpiperazine, EtOH, reflux 24 h.

stereoisomer obtained after silica gel column chromatography and recrystallization from *n*-hexane. A similar cross-coupling reaction of the benzophenone analog **2** with 4-floroacetophenone (**3b**) afforded the (*Z*)-1-[4-(2-chloroethoxy)phenyl]-1-(4-florophenyl)-2-phenylprop-1-ene (**4b**) (40%). Subsequent reaction of the chloroethoxy **4a** and **4b** with piperidine, piperazine or *N*-methylpiperazine in EtOH at reflux afforded the target compounds **5a**–**f** in high yields (72–85%). Stereoisomer assignments were made based on ^{1}H NMR chemical shifts from published information.[15–19] 4-(2-Chloroethoxy)benzophenone (**2**) was prepared according to the reported procedure.[20]

The rational for the design of the new tamoxifen analogs (**5a**–**f**) was based on the expectation that replacement of a dimethyl amino moiety in TAM (**1**) by other secondary amine moieties such as piperidino, piperazino and *N*-methylpiperazino would furnish novel tamoxifen analogs with high antiproliferative activity. Changing the ethyl group with methyl one and substituting the *para* position of the phenyl ring with florine atom were also performed to study its impact on activity. Docking experiments for the prepared compounds **5a**–**f** in the ligand binding domain (LBD), derived from the structure of ERα crystallized with OH-Tam, showed that all compounds showed score energy less than or closely similar to tamoxifen (Table 1), docking tamoxifen into ERα presented in Figure 2. Furthermore, compounds **5c** and **5e** showed a hydrogen bonding to Asp-351 amino acid (Figs. 3 and 4). The antiproliferative effect of increasing concentrations of the synthesized compounds compared to tamoxifen in the presence and absence of estradiol on MCF-7 and MDA-MB-231 cells was estimated using MTT assay. Compounds **5c** and **5e** showed about twofold increase in the antiproliferative activity compared to tamoxifen (Table 2), while other compounds like **5a** and **5f** showed similar activities like tamoxifen. To figure out if the increased antiproliferative activity of test compounds is ER-dependent or not, the antiproliferative activity was estimated in an ER-negative breast cancer cell line, MDA-MB-231 cells. In addition, the ability of these compounds to antagonize estradiol-induced cell growth in MCF-7 cells was tested. Both compounds **5c** and **5e** showed similar potency in antagonizing estradiol-induced breast cancer cell growth and

Table 1
Docking energy score results. Tamoxifen (TAM) was docked in the active site of ERα receptor (3ERT.pdb) with $S = -30.1209$ kcal/mol (Fig. 2), the novel six tamoxifen analogs were docked against the same receptor using the same method

Compounds	Docking energy score (kcal/mol)
5a	−31.8890
5b	−31.7390
5c	−31.0242
5d	−31.1929
5e	−28.9745
5f	−31.4630
TAM	−30.1209

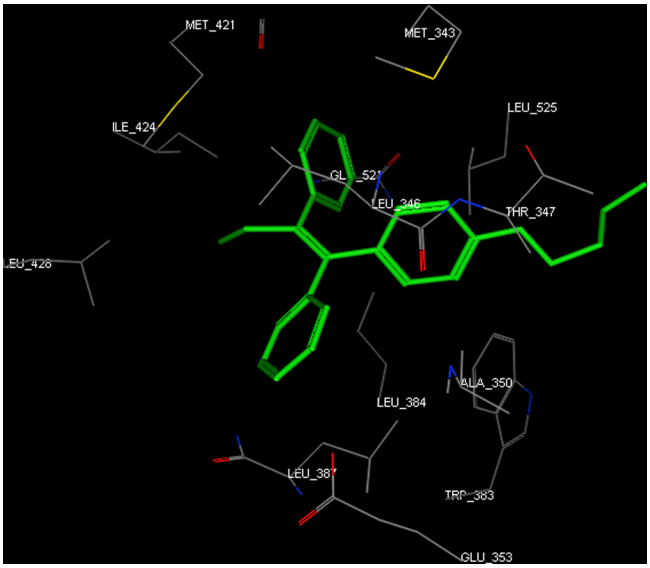

Figure 2. Docking of Tamoxifen in the active site of ERα (3ERT), $S = -30.12$ kcal/mol.

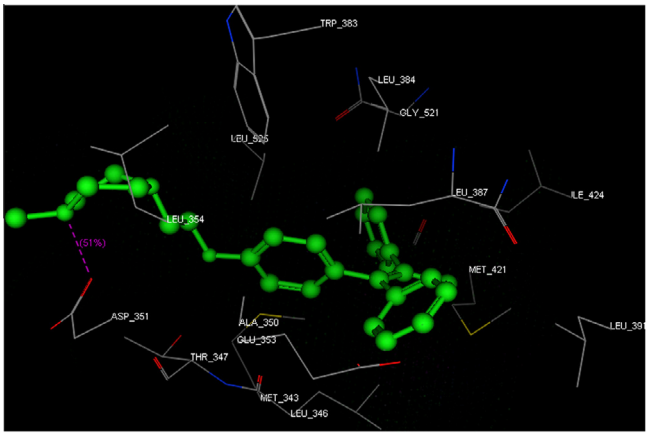

Figure 3. Docking of **5c** (green colored) in the active site of 3ERT ($S = -31.02$ kcal/mol) exhibiting interaction with Asp-351, hydrogen bond 2.34 Å.

inhibiting the proliferation of MDA-MB-231 cells which indicates that the increased anticancer activities is ER-independent (Table 2, Fig. 5). To explain the increased anticancer activity of compounds **5c** and **5e**, their ability to induce ER-independent cell death was tested. Both compounds showed relatively high ability to trigger classic caspase-dependent apoptosis as indicated by increased caspase 3/7 activities in MCF-7 treated cells compared to TAM (Fig. 6).

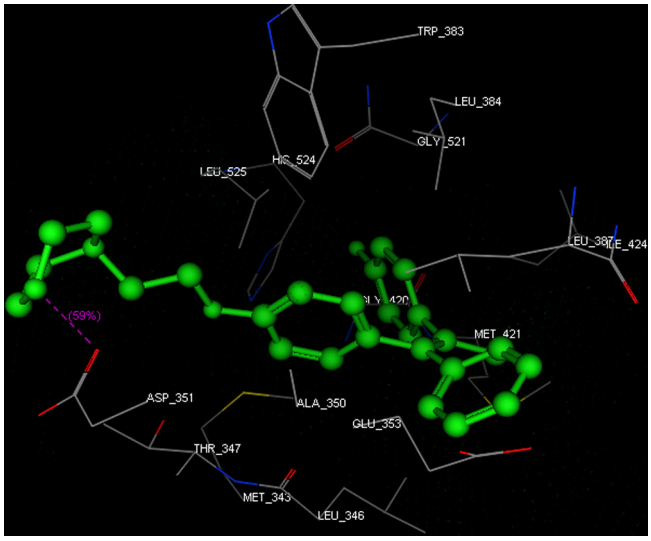

Figure 4. Docking of **5e** (green colored) in the active site of 3ERT ($S = -28.97$ kcal/mol) exhibiting interaction with Asp-351, hydrogen bond 2.35 Å.

Table 2
Antiproliferative effect of test compounds and tamoxifen on MCF-7 and MDA-MB-231 cells. The reported IC_{50} values are concentrations at which cells death measures 50% relative to DMSO control after 72 h exposure test compounds or tamoxifen (TAM) in 5% FBS-containing DMEM/F12 in 96-well plates. Cell viability was assessed by MTT assay. Differences among means were analyzed for statistical significance using one-way ANOVA followed by the Dunnett's test. Values are means ± SD ($n = 6$)

Compounds	IC_{50} (μM)	
	MCF-7	MDA-MB-231
5a	55.46 ± 4.21*	73.54 ± 5.03*
5b	>100	>100
5c	6.75 ± 1.34*	10.53 ± 1.98*
5d	16.55 ± 2.56*	42.97 ± 3.27*
5e	5.58 ± 1.76*	13.04 ± 2.40*
5f	86.28 ± 3.98*	>100
TAM	27.96 ± 2.64	64.85 ± 4.09

* $P < 0.01$as compared to tamoxifen (TAM)-treated cells.

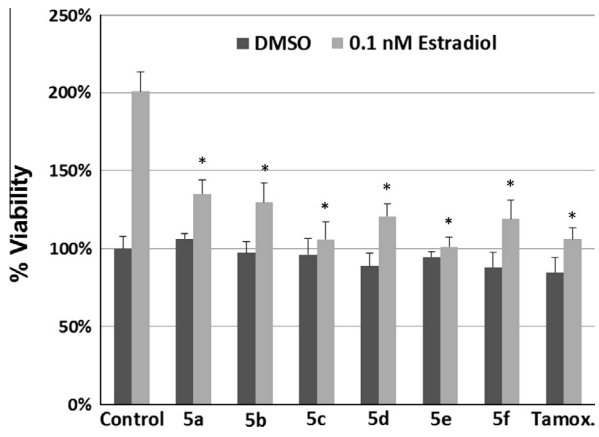

Figure 5. Antiproliferative effect of test compounds and tamoxifen at 5 μM concentration in the presence and absence of 1 nmol/L estradiol on MCF-7 cells. Cell proliferation is expressed as the percentage of the cells compared with the control wells. Differences among means were analyzed for statistical significance using one-way ANOVA followed by the Dunnett's test. Columns, mean of two experiments, each with six replicates; bars, SD. *$P < 0.01$ as compared to control (estradiol)-treated cells.

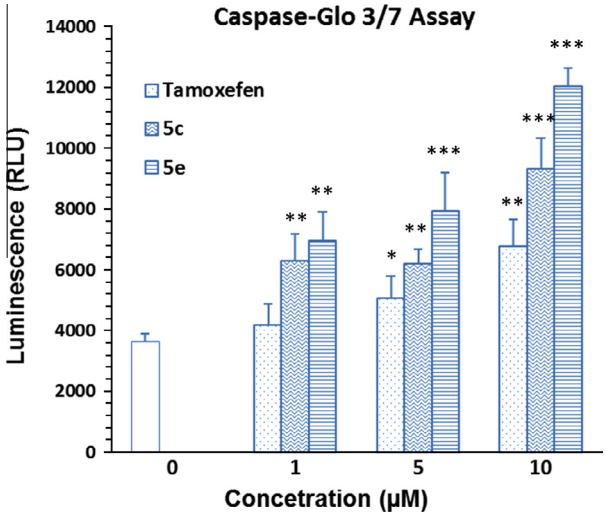

Figure 6. Compounds **5c**, **5e** and tamoxifen increase caspases activities in MCF-7 cells. Apoptosis was assessed by analysis of activation of caspase-3 and -7 using luminogenic substrate containing the DEVD sequence from Caspase-Glo™ 3/7 assay kit. Results are expressed as the mean of relative light unit (RLU). Differences among means were analyzed for statistical significance using one-way ANOVA followed by the Dunnett's test Columns, mean; bars, SD ($n = 6$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared to vehicle (DMSO)-treated cells.

New TAM analogs **5a–f** were designed for evaluation as antiproliferative agents. Compounds **5c**, **5d** and **5e** analogues exhibited better activity than tamoxifen, whoever, **5a**, **5b** and **5f** analogues were less active than tamoxifen. The structure–activity data acquired indicate that (i) some compounds exhibit better antiproliferative activity than tamoxifen when tested against MCF-7 and MDA-MB-231 human breast cancer cells, (ii) replacement of a dimethyl amino moiety in TAM (**1**) by more basic moieties such as piperazino in **5c** or *N*-methylpiperazino in **5e** can be used as a tool to prepare novel tamoxifen analogs with high antiproliferative activity, (iii) addition of the florine atom on the *p*-position of the phenyl ring resulted in more active analogues than tamoxifen **5c** and **5e** except for compound **5f** was less active than tamoxifen, (iv) docking studies revealed that all the synthesized analogues have low docking score energy with ERα. Moreover, compounds **5c** and **5e** exhibited a hydrogen bond interaction with Asp-831 amino acid, (v) pharmacological screening revealed the ability of some of the synthesized analogues to target breast cancer cells not only via ER receptor binding but also via ER receptor-independent mechanisms.

Titanium tetrachloride (0.99 mL, 9 mmol) was added drop wise to a stirred suspension of Zn powder (1.18 g, 18 mmol) in dry THF (15 mL) under an argon atmosphere at −10 °C, and this mixture was heated under reflux for 1.5 h to produce the titanium reagent. A cooled suspension of this titanium reagent was added to a solution of 4-(2-chloroethoxy)benzophenone (**2**, 0.78 g, 3.0 mmol) andacetophenone (**3a**, 0.36 g, 3.0 mmol) in THF (20 mL) at 0 °C, and the reaction was allowed to proceed at reflux for 2 h. After cooling to 25 °C, the reaction mixture was poured into a 10% aqueous K_2CO_3 solution (45 mL), this mixture was stirred vigorously for 5 min, and the dispersed insoluble material was removed by vacuum filtration. The organic fraction was separated, the aqueous layer was extracted with EtOAc (3 × 25 mL), and the combined organic fractions were dried (Na_2SO_4). Removal of the solvent in vacuum afforded a residue which was purified by silica gel column chromatography using EtOAc–hexane (1:4, v/v) as eluent followed by recrystallization of the product from *n*-hexane to give (*Z*)-**4a** as white crystals. Compound **4b** was synthesized using the same procedure described for the preparation of **4a**, by reaction of

(4-chloroethoxyphenyl)benzophenone (**2**) with the 4-floroacetophenone (**3b**). The product obtained as white crystals.

A mixture of **4a** or **4b** (2 mmol) and an appropriate secondary amine (100 mmol) in absolute ethanol (30 ml) was refluxed for 24 h, the solvent was removed under vacuum and the residue was crystallized from ethanol to produce the pure compounds **5a**–**f** as white crystals. The effect of test agents on cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay in six replicates as previously reported.[21] Caspase-3/7 and caspase-8 activities in MCF-7 cells treated with test agents were measured using a Caspase-Glo assay kit as mentioned before[22] and according to the manufacturer's instructions. Results were verified by docking study. [21-25]

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23. Spectral data for compounds **4a**, **4b** and **5a**–**f**. Compound **4a**: (*Z*)-1-[4-(2-chloroethoxy)phenyl]-1,2-diphenyl-prop-1-ene. 38% yield; mp 128–130 °C; IR: 3053 (CH aromatic), 2909 (CH aliphatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.12 (s, 3H, CH_3), 3.74 (t, J = 5.7 Hz, 2H, OCH_2CH_2Cl), 4.11 (t, J = 5.7 Hz, 2H, OCH_2CH_2Cl), 6.56 (dd, J = 6.6, 1.8 Hz, 2H, chloroethoxyphenyl H-3, H-5), 6.79 (dd, J = 6.6, 1.8 Hz, 2H, chloroethoxyphenyl H-2, H-6), 7.16–7.38 (m, 10H, phenyl hydrogens); ^{13}C NMR ($CDCl_3$): δ 23.3, 41.8, 67.7, 113.6, 126.0, 126.5, 127.8, 128.0, 129.2, 130.8, 131.9, 135.0, 136.2, 138.5, 143.6, 144.1, 156.1; MS m/z (ES^+) 349.1, $C_{23}H_{22}ClO$ (M+H) requires 348.86.
Compound **4b**: (*Z*)-1-[4-(2-chloroethoxy)phenyl]-1-(4-florophenyl)-2-phenylprop-1-ene. 40% yield; mp 125–127 °C; IR: 3050 (CH aromatic), 2918 (CH aliphatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3), 3.75 (t, J = 5.7 Hz, 2H, OCH_2CH_2Cl), 4.12 (t, J = 5.7 Hz, 2H, OCH_2CH_2Cl), 6.58 (dd, J = 6.6, 1.8 Hz, 2H, chloroethoxyphenyl H-3, H-5), 6.77 (dd, J = 6.6, 1.8 Hz, 2H, chloroethoxyphenyl H-2, H-6), 6.84–6.89 (m, 2H, fluorophenyl H-3, H-5); 7.08–7.13 (m, 2H, fluorophenyl H-2, H-6); 7.21–7.38 (m, 5H, phenyl hydrogens); ^{13}C NMR ($CDCl_3$): δ 23.3, 41.8, 67.9, 113.6, 114.9, 126.6, 128.1, 129.8, 130.8, 131.9, 133.9, 136.1, 139.0, 140.0, 143.5, 156.2, 162.8; MS m/z (ES^+) 367.1, $C_{23}H_{21}ClFO$ (M+H) requires 367.86.
Compound **5a**: (*Z*)-1-{2-[4-(1,2-diphenyl-propenyl)-phenoxy]-ethyl}piperidine. 78% yield; mp 132–133 °C; IR: 3056 (CH aromatic), 2937 (CH aliphatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34–1.43 (m, 2H, piperidinyl H-4), 1.56–1.64 (m, 4H, piperidinyl H-3, H-5), 2.13 (s, 3H, CH_3), 2.46–2.48 (m, 4H, piperidinyl H-2, H-6), 2.71 (t, J = 6.1 Hz, 2H, OCH_2CH_2N), 4.00 (t, J = 6.1 Hz, 2H, OCH_2CH_2N), 6.58 (dd, J = 6.6, 1.8 Hz, 2H, ethoxyphenyl H-3, H-5), 6.80 (dd, J = 6.6, 1.8 Hz, 2H, ethoxyphenyl H-2, H-6), 7.10–7.32 (m, 10H, phenyl hydrogens); ^{13}C NMR ($CDCl_3$): δ 23.1, 23.7, 25.2, 54.9, 57.7, 65.1, 113.8, 126.2, 126.8, 127.8, 128.1, 129.2, 130.9, 131.9, 135.0, 136.3, 138.7, 143.6, 144.2, 156.0; MS m/z (ES^+) 398.1, $C_{28}H_{32}NO$ (M+H) requires 398.55. Anal. Calcd. for $C_{28}H_{31}NO$ (397.55): C, 84.59; H, 7.86; N, 3.52. Found: C, 84.67; H, 7.94; N, 3.81.
Compound **5b**: (*Z*)-1-{2-[4-(1,2-diphenyl-propenyl)-phenoxy]-ethyl}piperazine. 72% yield; mp 134–136 °C; IR: 3130 (NH), 3044 (CH aromatic), 2939 (CH aliphatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.13 (s, 3H, CH_3), 2.74 (t, J = 4.6 Hz, 4H, piperazinyl H-3, H-5), 2.78 (t, J = 6.1 Hz, 2H, OCH_2CH_2N), 3.09 (t, J = 4.6 Hz, 4H, piperazinyl H-2, H-6), 3.98 (t, J = 6.1 Hz, 2H, OCH_2CH_2N), 6.56 (dd, J = 6.6, 1.8 Hz, 2H, ethoxyphenyl H-3, H-5), 6.80 (dd, J = 6.6, 1.8 Hz, 2H, ethoxyphenyl H-2, H-6), 7.12–7.38 (m, 10H, phenyl hydrogens); ^{13}C NMR ($CDCl_3$): δ 23.1, 43.1, 53.9, 57.6, 67.6, 113.6, 126.2, 126.6, 127.9, 128.2, 129.5, 130.9, 131.4, 135.5, 136.3, 138.2, 143.2, 143.6, 156.0; MS m/z (ES^+) 399.1, $C_{27}H_{31}N_2O$ (M+H) requires 399.55. Anal. Calcd. for $C_{27}H_{30}N_2O$ (398.54): C, 81.37; H, 7.59; N, 7.03. Found: C, 81.44; H, 7.63; N, 7.27.
Compound **5c**: (*Z*)-1-{2-[4-(1,2-diphenyl-propenyl)-phenoxy]-ethyl}-4-methylpiperazine. 85% yield; mp 115–117 °C; IR: 3054 (CH aromatic), 2936 (CH aliphatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.13 (s, 3H, CH_3), 2.37 (s, 3H, NC*H*3), 2.59–2.67 (m, 8H, piperazinyl H), 2.78 (t, J = 6.1 Hz, 2H, OCH_2CH_2N), 3.99 (t, J = 6.1 Hz, 2H, OCH_2CH_2N), 6.57 (d, J = 8.5 Hz, 2H, ethoxyphenyl H-3, H-5), 6.79 (d, J = 8.5 Hz, 2H, ethoxyphenyl H-2, H-6), 7.12–7.39 (m, 10H, phenyl hydrogens); ^{13}C NMR ($CDCl_3$): δ 23.4, 45.6, 53.0, 54.8, 57.0, 65.6, 113.5, 126.0, 126.5, 127.9, 128.0, 129.2, 130.0, 131.9, 134.8, 135.7, 138.7, 143.7, 144.2, 156.7; MS m/z (ES^+) 413.1, $C_{28}H_{32}N_2O$ (M+H) requires 413.57. Anal. Calcd. for $C_{28}H_{32}N_2O$ (412.57): C, 81.51; H, 7.82; N, 6.79. Found: C, 81.43; H, 7.76; N, 6.56.
Compound **5d**: (*Z*)-1-(2-{4-[2-(4-fluoro-phenyl)-1-phenyl-propenyl]-phenoxy}-ethyl)piperidine. 73% yield; mp 106–108 °C; IR: 3050 (CH aromatic), 2934 (CH aliphatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29–1.46 (m, 2H, piperidinyl H-4), 1.64–1.66 (m, 4H, piperidinyl H-3, H-5), 2.13 (s, 3H, CH_3), 2.56–2.58 (m, 4H, piperidinyl H-2, H-6), 2.80 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 4.06 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 6.58 (dd, J = 6.6, 2.4 Hz, 2H, ethoxyphenyl H-3, H-5), 6.77 (dd, J = 6.6, 2.4 Hz, 2H, ethoxyphenyl H-2, H-6), 6.84–6.90 (m, 2H, fluorophenyl H-3, H-5), 7.09–7.14 (m, 2H, fluorophenyl H-2, H-6), 7.24–7.38 (m, 5H, phenyl hydrogens); ^{13}C NMR ($CDCl_3$): δ 23.3, 23.9, 25.5, 54.9, 57.7, 65.3, 113.6, 114.6, 126.6, 128.1, 129.9, 130.8, 131.9, 133.7, 135.5, 139.1, 140.1, 143.6, 156.7, 162.7; MS m/z (ES^+) 416.1, $C_{28}H_{31}FNO$ (M+H) requires 416.54. Anal. Calcd. for $C_{28}H_{30}FNO$ (415.54): C, 80.93; H, 7.28; N, 3.37. Found: C, 80.84; H, 7.24; N, 3.35.
Compound **5e**: (*Z*)-1-(2-{4-[2-(4-fluoro-phenyl)-1-phenyl-propenyl]-phenoxy}-ethyl) piperazine. 74% yield; mp 115–117 °C; IR: 3133 (NH), 3055 (CH aromatic), 2942 (CH aliphatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.11 (s, 3H, CH_3), 2.61 (t, J = 4.5 Hz, 4H, piperazinyl H-3, H-5), 2.76 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 3.00 (t, J = 4.5 Hz, 4H, piperazinyl H-2, H-6), 4.00 (t, J = 5.5 Hz, 2H, OCH_2CH_2N), 6.59 (dd, J = 6.6, 1.8 Hz, 2H, ethoxyphenyl H-3, H-5), 6.78 (dd, J = 6.6, 1.8 Hz, 2H, ethoxyphenyl H-2, H-6), 6.84–6.91 (m, 2H, fluorophenyl H-3, H-5), 7.09–7.14 (m, 2H, fluorophenyl H-2, H-6), 7.22–7.36 (m, 5H, phenyl hydrogens); ^{13}C NMR ($CDCl_3$): δ 23.3, 45.5, 53.7, 57.6, 65.6, 113.6, 114.9, 126.6, 128.1, 129.9, 130.8, 131.9, 133.7, 135.5, 139.1, 140.1, 143.6, 156.8, 162.7; MS m/z (ES^+) 417.1, $C_{27}H_{30}FN_2O$ (M+H) requires 417.53. Anal. Calcd. for $C_{27}H_{29}FN_2O$ (416.53): C, 77.85; H, 7.02; N, 6.73. Found: C, 77.90; H, 7.13; N, 6.69.
Compound **5f**: (*Z*)-1-(2-{4-[2-(4-fluoro-phenyl)-1-phenyl-propenyl]-phenoxy}-ethyl)-4-methylpiperazine. 82% yield; mp 127–129 °C; IR: 3047 (CH aromatic), 2937 (CH aliphatic) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.09 (s, 3H, CH_3), 2.38 (s, 3H, NC*H*3), 2.52–2.75 (m, 8H, piperazinyl H), 2.78 (t, J = 6.1 Hz, 2H, OCH_2CH_2N), 4.00 (t, J = 6.1 Hz, 2H, OCH_2CH_2N), 6.57 (dd, J = 6.6, 1.8 Hz, 2H, ethoxyphenyl H-3, H-5), 6.76 (dd, J = 6.6, 1.8 Hz, 2H, ethoxyphenyl H-2, H-6), 6.83–6.89 (m, 2H, fluorophenyl H-3, H-5), 7.08–7.13 (m, 2H, fluorophenyl H-2, H-6), 7.20–7.37 (m, 5H, phenyl hydrogens); ^{13}C NMR ($CDCl_3$): δ 23.3, 45.7, 53.1, 54.8, 57.0, 65.7, 113.6, 114.9, 126.6, 128.1, 129.9, 130.8, 131.9, 133.7, 135.5, 139.1, 140.1, 143.6, 156.8, 162.7; MS m/z (ES^+) 431.2, $C_{28}H_{32}FN_2O$ (M+H) requires 431.56. Anal. Calcd. for $C_{28}H_{31}FN_2O$ (430.56): C, 78.11; H, 7.26; N, 6.51. Found: C, 77.99; H, 7.23; N, 6.38.
24. Docking studies were achieved by Molecular Operating Environment (MOE, Version 2005.06, Chemical Computing Group Inc., Montreal, Quebec, Canada) using 3ERT.pdb file, which is a co-crystallized 4-hydroxytamoxifen with the human ER-alpha. The top score docking energy value for each ligand was recorded after performing 100 docking interactions for each one of these ligands.
25. MCF-7 human breast cancer cells were obtained from the American Type Culture Collection (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium/F12 medium (DMEM/F-12, Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco). All cells were cultured at 37 °C in a humidified incubator containing 5% CO_2. Cells were seeded in 96-well flat-bottomed plates for 24 h, and treated with test agents in 5% FBS-supplemented DMEM/F-12 for 72 h in the presence and absence of 0.1 nM estradiol. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. After treatment, cells were incubated in the same medium containing 0.5 mg/ml MTT at 37 °C for 2 h. Reduced MTT was solubilized in DMSO (200 μL/well) for determination of absorbance at 570 nm using a microplate reader. Cells were plated at 1×10^4 (100 μl/well) into clear bottom, opaque wall 96-well tissue culture plates and incubated for 24 h. The medium was removed and the cells were then treated with test compounds for 72 h. Caspase-3/7 activity were then calculated according to the instructions included in the kit. The activity of caspase-3 and -7 was measured indirectly by assessing the product of its reaction with luminogenic substrate containing the DEVD sequence. Results are expressed as the mean of relative light unit (RLU). The luminescence of plates was read using an illuminometer.