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ORIGINAL ARTICLE

# Design, synthesis and biological activity against estrogen receptor-dependent breast cancer of furo[1] benzofuran derivatives

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Furo[1]benzofuran derivatives

**Abstract** The docking study on a series of furo[1]benzofuran derivatives with ERα has been demonstrated. The synthesis and characterization of a series of furo[1]benzofuran derivatives were described. All the target compounds were conducted to in vitro for the inhibitory activities against human breast cancer strains T-47D, MCF-7 and toxicity against human liver normal cell strains HL7702 via MTT assay. Most of the target compounds possessed anti-estrogen receptor-dependent breast cancer activities with weak toxicity to healthy cell strains. The preliminary structure–activity relationships were discussed.



## 1. Introduction

Breast cancer, one of the most common malignant tumors, has threatened many females' health for many years (D'Souza et al., 2022). Modern biology studies showed that it is a kind of all-over body disease that is related to estrogen (Jordan, 2003). More studies demonstrated that estrogen plays a key role in the development and progression of tumor through estrogen receptor (ER) signaling pathways and estrogen metabolic products (Chargari et al., 2009). ER, a member of the steroid hormone nuclear receptor protein superfamily, has three subtypes; ERα、ERβ and ERγ (Ogawa and Parhar, 2020; Poutiainen et al., 2010; Matsushima et al., 2007; Durrant et al., 2015; Ho et al., 2018). Owing to the big difference of amino acid sequence homology in ER functions, these three subtypes also have different capacities on the combination of various ligands (Gu et al., 2022). ERα is mainly expressed in breast, central nervous system, cardiovascular system, the

*Abbreviations*: DMSO, dimethyl sulfoxide; EI, electronic impact; ER, estrogen receptor; ESI, electrospray ionization; IR, infrared spectra; LGA, Lamarckian genetic algorithm; MS, mass spectra; MTT, meth ylthiazolyldiphenyltetrazolium bromide; NMR, nuclear magnetic resonance spectra; PDB, protein data bank; SERMs, selective estrogen receptor modulators; TBAB, tetrabutylammonium bromide

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bone, and especially in uterus; ERβ has a higher expression in cardiovascular system, central nervous system, immune system, gastrointestinal system, uterus, kidney, lungs and the bone (Gu et al., 2022; Wang et al., 2014); ERγ is a third subtype receptor of ERs and highly expressed in adult human and mouse tissues, such as the brain, skeletal muscle, heart, kidney, pancreas, and placenta (He et al., 2022; Hong et al., 1999; Heard et al., 2000). It is reported that the expression of ERα and ERβ has been founded in human and mammal breast cancer tissues and strains while ERγ's expression hasn't.

The ideal anti-breast cancer drugs should have no side effects on systems without estrogen, and tissues with estrogen, such as bone and cardiovascular system, and selective estrogen receptor modulators (SERMs) are such kinds of drugs (Kuiper et al., 1997). Raloxifene, the second-generation drug of SERMs, can reduce the proliferation of breast cancer cells and endometrial cancer cells with a therapeutic dose (Lopes-Costa et al., 2010). But the results of clinical trials for raloxifene are not striking as expected (the effective rate is only 33 %) with low bioavailability (Gradishar et al., 2000). There are also some side effects including hot flashes, leg cramps, and an increased risk of thromboembolic disease and other cardiovascular events such as stroke (Salem et al., 2021; Sauter, 2018). It's of theoretical and practical to develop a novel chemical structure of SERMs, which are more efficient, safer and have specificity in tissue.

Benzofuran compounds have strong biological activities in the treatment of Alzheimer's disease, osteoporosis, arrhythmia, Parkinson's disease and tumors (Zeng et al., 2021; Giordano et al., 2017). For example, fruquintinib (**1,** Fig. 1) with benzofuran was discovered and developed by Hutchison MediPharma for the treatment of solid tumors (Zeng et al., 2021). Giordano et al. (Giordano et al., 2017) reported that a series of methyl benzofuran-2-acetate derivatives (**2,** Fig. 1) showed antiproliferative and pro-apoptotic activities against breast cancer cells (Fig. 1). Atta et al. (Atta et al., 2010) synthesized a series of compounds containing benzofuran and benzopyrone structures, and tested these compounds for inhibition of growth of human breast cancer cells (MCF-7). Compound **3** (Fig. 1) had the best cytotoxic activity ($IC_{50}$ value is 5.56 μg/mL − 20.8 μg/mL). In this study, we use raloxifene (**4**) as a lead compound, and replace the benzothiophene ring in the raloxifene structure with a 2-arylcarbonyl-3-methylfuro[1]benzofuran scaffold based on the principle of scaffold hopping (Woo et al., 2022) and bioisosterism (Chen et al., 2022; El-Shershaby et al., 2021). Then, a novel series of furo[1]benzofuran derivatives were designed as the target compounds (Fig. 2) according to the reported structure–activity relationship of raloxifene and active sites of ERα. Nine target compounds were synthesized and evaluated in biological assays for the inhibition of human breast cancer strains T-47D and MCF-7. Herein, we report the design, synthesis and biological evaluation of these target compounds.

## 2. Results and discussion

### 2.1. Docking studies

To further investigate the structure-binding affinity correlations and how those ligands **5a-5i** oriented, the docking simulations were performed based on the available inhibitor co-crystalized crystallographic structure (PDB code: 2OUZ (Vajdos et al., 2007) of ERα via AutoDock 4.0 (Huey et al., 2007). In order to obtain the better results from docking pro-

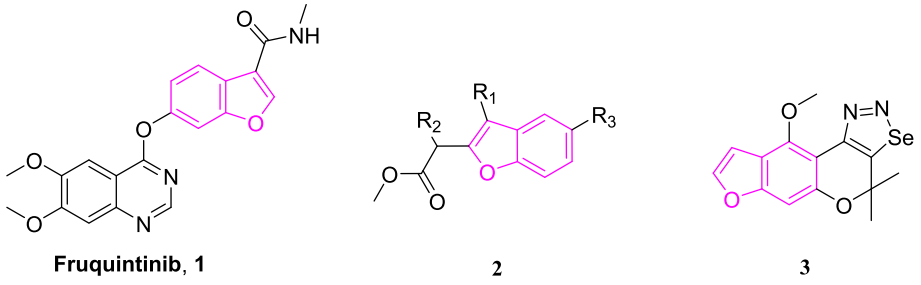



**Fig. 1** The compounds containing benzofuran scaffold.

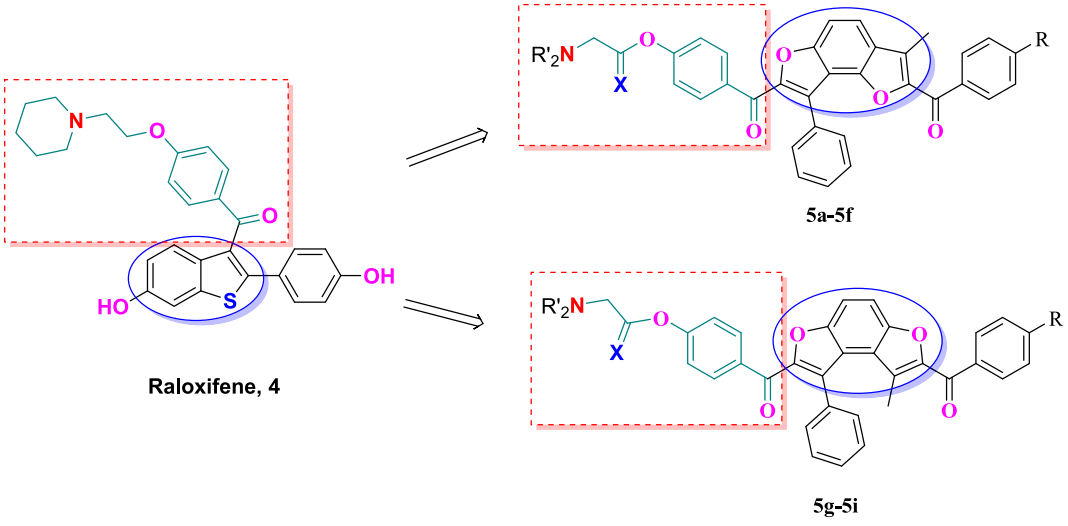



**Fig. 2** The design mentality of target compounds.

tocols, water molecules were excluded. The polar hydrogen atoms of the enzymes were added, the non-polar hydrogen atoms were merged and Gasteiger charges were assigned. For all ligands, the non-polar hydrogen atoms were merged, and the Gasteiger charges were assigned. The grid box dimensions were 20 Å × 20 Å × 20 Å around the active site and the grid spacing was set to 0.375 Å. Docking was performed using the empirical free energy function together with the Lamarckian genetic algorithm (LGA). The LGA protocol applied a population size of 150 individuals, while 250,000 energy evaluations were used for the 20 LGA runs. In addition, the maximum number of evaluations was set to 27,000. Discovery Studio 3.5 software was used for visualization of protein–ligand interactions (Accelrys Software Inc, 2012).

Autodock binding affinities revealed that the binding free energies (ΔGb) of **5a-5i** are −10.38, −11.59, −11.70, −12.33, −9.02, −9.88, −11.65, −11.40 and −12.56 kcal/mol, respectively. The calculations with the flexible docking protocol placed all target compounds correctly into the binding pocket as presented in Fig. 3. The docking simulation revealed that ERα (2OUZ) and compounds **5a**, **5f** and **5i** interacted through cation-π interaction, π-π aromatic interaction and hydrogen bonding which are depicted as sticks in Figs. 4, 5 and 6. Both the compounds **5a** and **5f** formed hydrophobic interactions with residues, no other interactions could find except a weak hydrogen bonding between **5f** and THR347 with the distance of 3.8 Å, shown in Figs. 4 and 5. For the compound **5i**, more H-bond interactions with TYR526, LYS529, VAL533, LEU536, TYR537 and cation-π interactions with LYS529 were found, shown in Fig. 6, which might be the reason for its increased inhibitory potency.

### 2.2. Chemistry

In general, the target compounds furo[2,3-e][1]benzofuran and furo[3,2-e][1]benzofuran derivatives (**5a-5i**) were synthesized via eight steps as described in Scheme 1 and Scheme 2. 2′,4′-Dihydroxyacetophenone or 2′,5′-dihydroxyacetophenone was chosen as the starting material. As known, the hydroxyl of 4′ position or 5′ position in acetophenone moiety has stronger reaction activity than 2′-hydroxyl which has intermolecular hydrogen bond. Hence, 4′-hydroxyl or 5′-hydroxyl was protected by benzyl chloride in dry acetone under the catalyzing of potassium iodide so as to give 2′-hydroxy-4′-benzyloxyacetophenone (**6a**) and 2′-hydroxy-5′-benzyloxyacetophenone (**6b**). Then, 2-aroyl-3-methyl-6-hydroxybenzofuran (**8a-8b**) and 2-aroyl-3-methyl-5-hydroxybenzofuran (**8c**) were obtained after cyclizing with 2-bromoacetophenone derivatives and deprotecting hydroxyl group. In dichloromethane solution, the compounds **9a-9c** were synthesized by esterification reaction of **8a-8c** with benzoyl chloride in the presence of triethylamine. The compounds **10a-10c** were obtained by Fries rearrangement reaction of **9a-9c** under anhydrous aluminum chloride. Similar to the second step cyclization reaction, **11a-11c** were synthesized with **10a-10c** and 2-chloro-4′-methoxyacetophenone, then using anhydrous aluminum chloride as demethylation reagent to obtain the target parent compounds **12a-12c**. The target compounds **5a-5i** could be obtained by ordinary Williamson reaction.

Furthermore, it is worthy to mention that after the Fries rearrangement reaction, the aroyl group is rearranged to the 7-position of **10a-10b** and the 4-position of **10c**, instead of the 5-position of **10a-10b** and the 6-position of **10c**. This result has been confirmed by $^{1}$H NMR and $^{1}$H–$^{1}$H COSY spectra, as shown in Fig. 7 and Table 1. During the Fries rearrangement from 2-benzoyl-3-methyl-6-benzoyloxybenzofuran, there were two active sites at the 5 and 7 positions under the condition that 6-hydroxyl of benzofuran ring had two adjacent hydrogens, as a result the products should be isomers A and B. If Fries rearrangement occurred in the 7-position, there should be two singlet peaks in $^{1}$H NMR spectra for no coupling between 4-position and 7-position hydrogen. But in fact, there

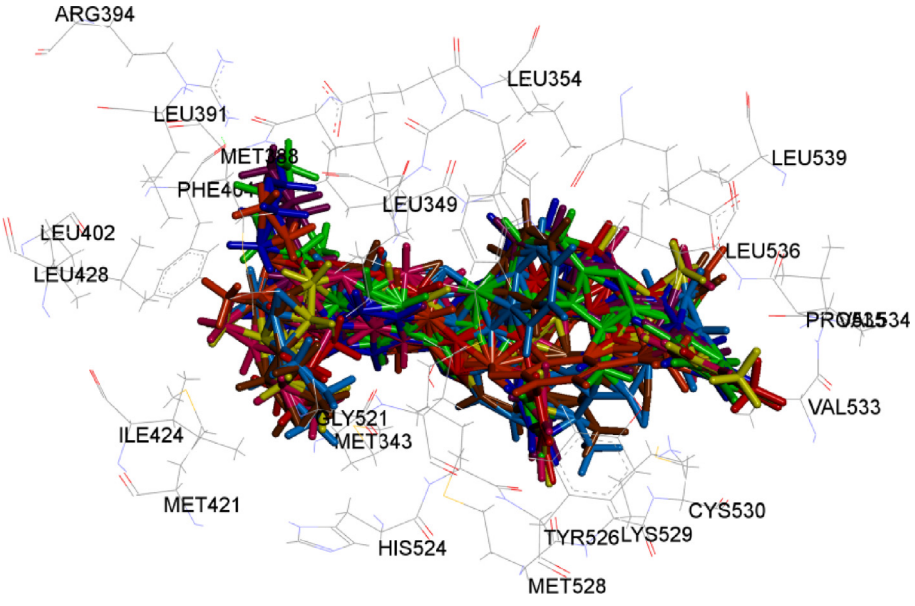



**Fig. 3** The binding models of target compounds **5a** (pink), **5b** (brown), **5c** (sky blue), **5d** (orange), **5e** (yellow), **5f** (red), **5g** (blue), **5h** (purple) and **5i** (green) with ERα (PDB code: 2OUZ).

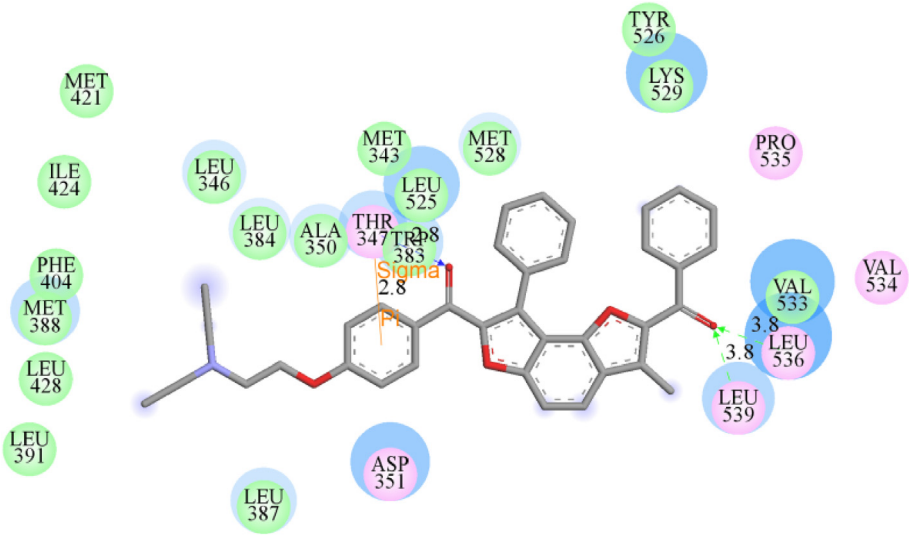



**Fig. 4** Docking model of **5a** in the active site of ERα (2OUZ).

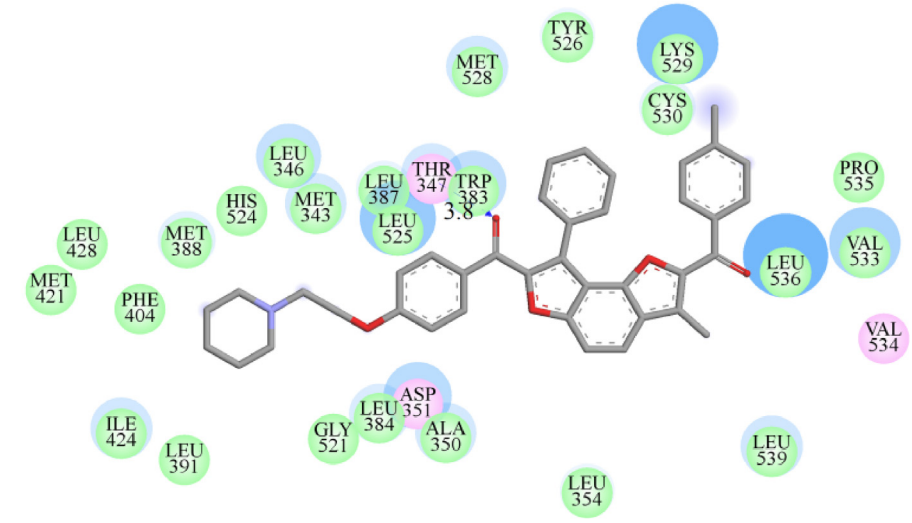



**Fig. 5** Docking model of **5f** in the active site of ERα (2OUZ).

was only one doublet peak at 7.82 ppm but not two singlet peaks in $^{1}$H NMR spectra, which was ascribed to H-4. Another on doublet peak, H-5, was further confirmed by 2D-H NMR spectra and the overlap chemical shifts in the range of 7.11–7.15 ppm were assigned to H-5 and H-22. Accordingly, we speculated that Fries rearrangement occurred selectively in 7 position of benzofuran ring to give the product of 2,7-dibenzoyl-3-methyl-6-hydroxybenzofuran (A).

### *2.3. Inhibition of human mammary cancer*

All the target compounds were tested for inhibition of human breast cancer strain T-47D and toxicity of human liver normal cell strain HL7702 by means of MTT assay, in comparison to tamoxifen and the results were illustrated in the Table 2. Human breast cancer strain T-47D was an estrogen-positive breast cancer cell, which was similar with the cancer strain MCF-7 or ZR75. All the target compounds exhibited weak cytotoxic effects on HL7702 with $IC_{50}$ > 100 μM.

According to the result of preliminary screening, the compounds **5d** and **5i**, which $IC_{50}$ values were similar to tamoxifen, were selected for further biological activity study on human breast cancer strain MCF-7. Concentration set was further refined, in which different concentrations had been set up. Calculate the cellular proliferation inhibition rate and the accurate $IC_{50}$ values, specific result of biological activity shown in Table 3.

Compared with **5d** and **5i**, which possessed the same substituting groups, the activities of 7-benzoyl-8-methylfuro[3,2-e][1]benzofuran derivatives were generally stronger than corresponding 2-benzoyl-3-methylfuro[2,3-e][1]benzofuran derivatives, which may be due to 7-benzoyl-8-methylfuro[3,2-e][1]benzofuran derivatives have more active binding site with ERα such as H-bond interactions and cation-π interactions. Compounds **5a-5d** and **5g-5i**, which had no groups at the para position on the 7-benzoyl or 2-benzoyl, showed higher human breast cancer inhibitory activities. Compounds **5e** and 5**f** with electron donating groups, such as the methyl group at the para

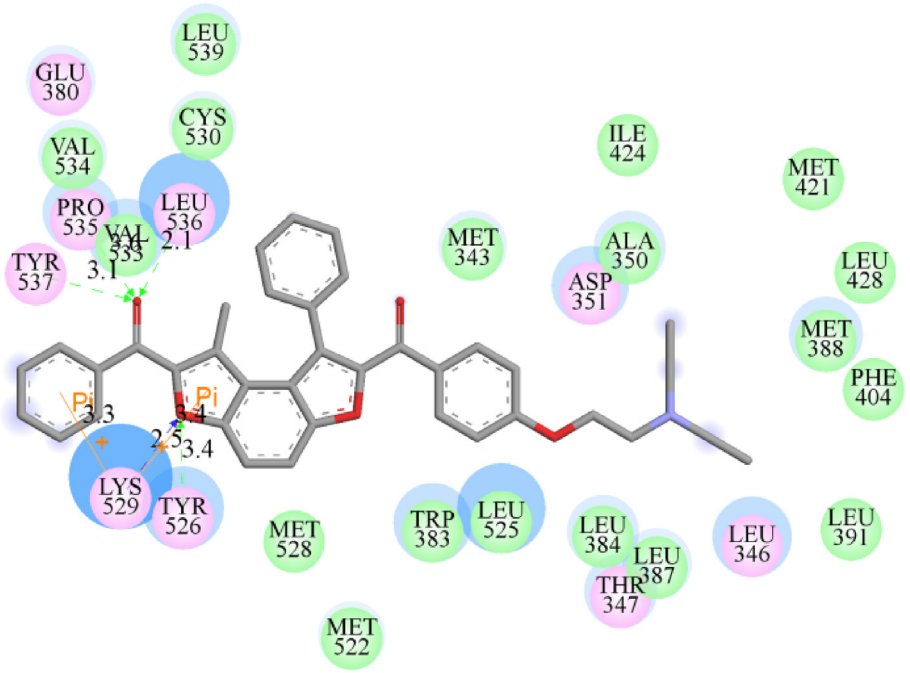



**Fig. 6** Docking model of **5i** in the active site of ERα (2OUZ).

position on the 7-benzoyl or 2-benzoyl, showed decreasing inhibitory activities, which suggested that electron donating group in these compounds could inhibit them to access to the receptor-binding site; Compared with compounds 5**a** and 5**d**, which had the same scaffold and substituent R, **5d** with alkyl amine group (R'), showed stronger human breast cancer inhibitory activities than **5a** with amide. Meanwhile, compounds which possessed diethylamino group showed stronger activities than other amino groups (piperidyl or morpholinyl) no matter they were alkoxide or acyloxy compounds, for example, the order of activities from high to low was **5a**, **5b** and **5c**.

## 3. Materials and methods

### *3.1. Chemistry*

Melting points were determined on a X-4 digital melting point apparatus and were uncorrected. Infrared spectra (IR) were recorded on KBr disks with a Bruker IFS55-IR spectrometer. $^1$H NMR spectra were recorded on Bruker ARX-300, ARX-400 or ARX-600 MHz NMR spectrometers in $CDCl_3$ or DMSO $d_6$. The mass spectra (MS) were obtained by electronic impact (EI) at 70 eV in an Agilent spectrometer (with direct insertion probe) or by electrospray ionization (ESI) in a Waters spectrometer.

All commercial reagents and solvents were of analytical grade and used without further purification. 2-chloro-4′-meth oxyacetophenone, 2-chloro-4′-methylacetophenone, 4-chloroacetylmorpholine, 1-acetylpiperidine, *N*,*N*-diethyl-2-chloroacetamide, 4-(2-chloroethyl)morpholine hydrochloride, 1-(2-chloroethyl)piperidine hydrochloride and 2-chloro-*N*,*N*-diethylethanamine hydrochloride were synthesized by the method of literatures (Gurjar et al., 1990; Lewin et al., 1995; Jaen et al., 1991; Ohtaka et al., 1998).

#### *3.1.1. General procedure for preparing benzyloxyacetophenone derivatives (**6**)*

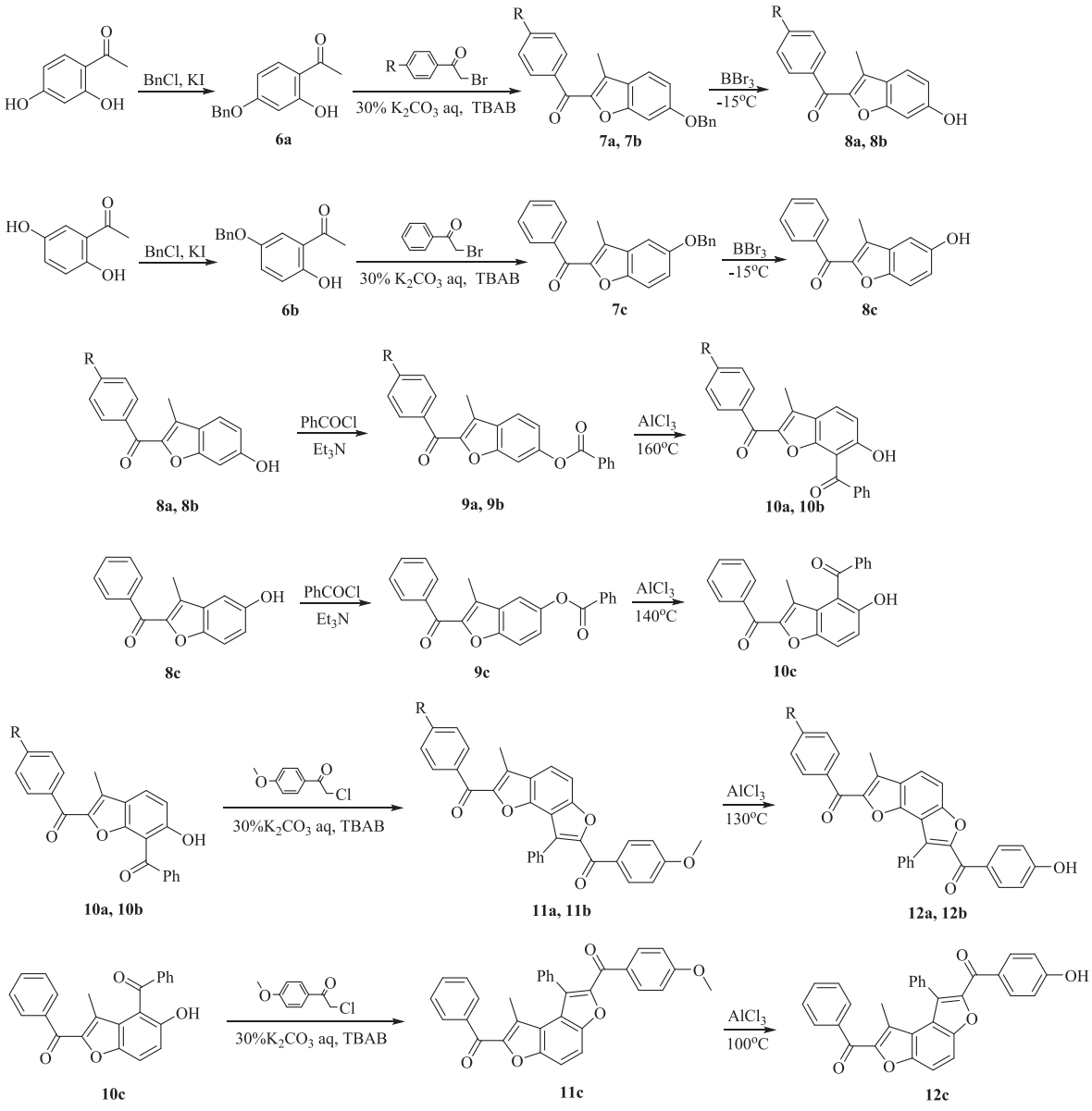
In a 250 ml round-bottomed flask were placed 2′,4′-dihydroxya cetophenone (15.20 g, 0.1 mol) or 2′,5′-dihydroxyacetophe none (15.20 g, 0.1 mol), benzyl chloride (13.23 g, 0.105 mol), potassium iodide (1.66 g, 0.01 mol), anhydrous potassium carbonate (138.20 g, 1 mol) and 100 ml dry acetone. The mixture was refluxed for 5 h at 56–60 °C in oil bath. After reaction was finished, the reaction liquid was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to obtain the crude. The solid was recrystallized from 100 ml ethanol to obtain **6.**

**2′-hydroxy-4′-benzyloxyacetophenone (6a).** A white powder (20.62 g), yield 85.2 %, m.p: 101.6–103.4 °C (101–103 °C, lit (Yates et al., 2009). EI-MS: 243.0 ($[M + H]^+$), 264.9 ($[M + Na]^+$); IR: 3030.4, 2940.4, 1622.9, 1569.9, 1507.3, 1494.2, 1466.2, 1452.7, 1430.5, 1388.2, 1364.6, 1247.1, 1183.2, 1132.7; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 12.75 (1H, s), 7.64 (1H, d, $J$ = 8.4 Hz), 7.44–7.32 (5H, m), 6.55–6.48 (2H, m), 5.09 (2H, s), 2.56 (3H, s).

**2′-hydroxy-5′-benzyloxyacetophenone (6b).** A bright yellow needly crystal (19.53 g), yield 80.7 %, m.p: 60.5–63.2 °C (69.5–70 °C, lit (Baltzly et al., 1950). EI-MS: 243.0 ($[M + H]^+$), 265.0 ($[M + Na]^+$); IR: 3068.2, 2929.8, 1643.7, 1619.0, 1583.2, 1492.9, 1469.9, 1384.3, 1341.5, 1297.2, 1220.5, 1199.5; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 11.90 (1H, s), 7.47–7.34 (5H, m), 7.25 (1H, d, $J$ = 3.0 Hz), 7.18 (1H, dd, $J$ = 9.0, 3.0 Hz), 6.93 (1H, d, $J$ = 9.0 Hz), 5.04 (2H, s), 2.58 (3H, s).

#### *3.1.2. General procedure for preparing 2-aroyl-3-methyl-5-benzyloxybenzofuran derivatives or 2-aroyl-3-methyl-6-benzyloxybenzofuran derivatives (**7**)*

A mixture of **6** (0.1 mol), 2-chloroacetophenone derivatives (0.11 mol), TBAB (16.12 g, 0.05 mol), 50 ml 30 % aqueous



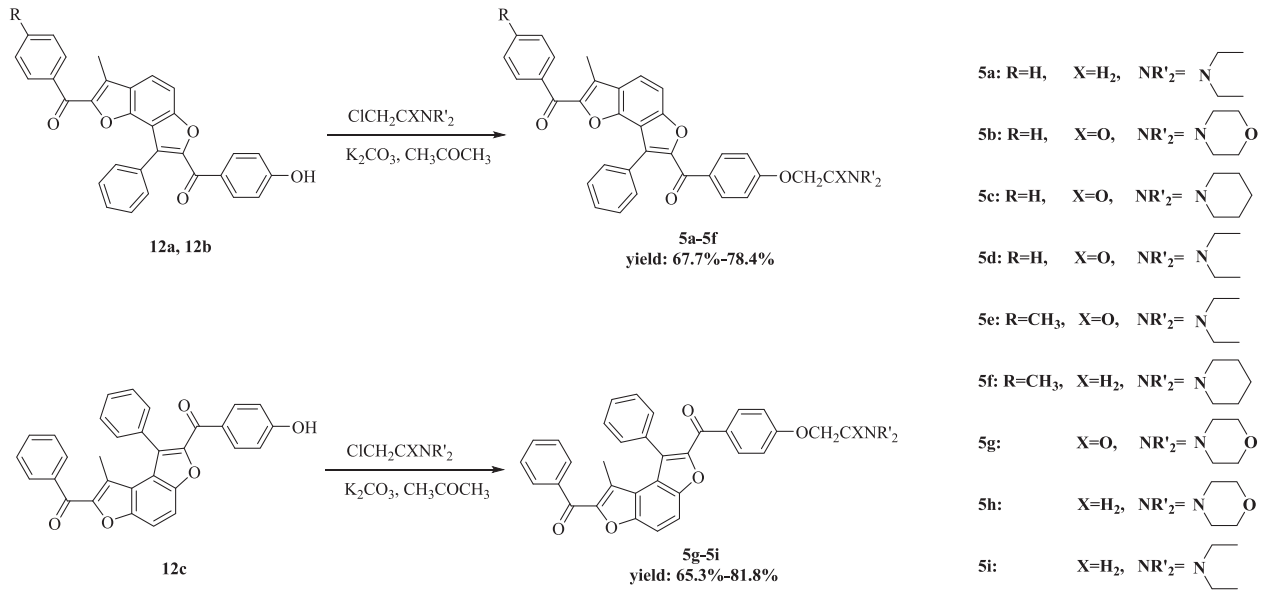

**Scheme 1** The synthetic route of parentfuro[1]benzofuran derivatives.

potassium carbonate and 100 ml dichloromethane was refluxed at 40–45 °C in oil bath for 12 h. Then the organic phase was separated, washed with 1 mol/L hydrochloric acid (30 ml × 3) and water (30 ml), dried over $Mg_2SO_4$, and concentrated under reduced pressure to obtain solid. The solid was recrystallized from 80 ml ethanol to obtain crystal **7**.

**2-benzoyl-3-methyl-6-benzyloxybenzofuran (7a).** Bright yellow needly crystal (26.78 g), yield 78.3 %; m.p: 76.8–79.2 °C. EI-MS: 343.1 ($[M + H]^+$), 365.1 ($[M + Na]^+$); IR: 3060.5, 2923.0, 1692.36, 1628.2, 1598.7, 1563.3, 1500.9, 1447.7, 1383.0, 1268.5, 1167.9; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.06 (2H, d, $J$ = 7.2 Hz), 7.61–7.55 (2H, m), 7.54–7.50 (2H, m), 7.47 (2H, d, $J$ = 7.2 Hz), 7.43–7.39 (3H, m), 7.06 (2H, dd, $J$ = 10.6, 1.8 Hz), 5.14 (2H, s), 2.61 (3H, s).

**2-(4-methylbenzoyl)-3-methyl-6-benzyloxybenzofuran (7b).** Bright yellow needly crystal (20.76 g), yield 58.3 %, m.p: 124.2–125.8 °C. EI-MS: 357.1 ($[M + H]^+$), 379.0 ($[M + Na]^+$), 395.1 ($[M + K]^+$); IR: 3097.3, 2925.3, 1620.9, 1606.7, 1585.5, 1547.2, 1468.5, 1409.3, 1388.2, 1357.4, 1266.4, 1240.4, 1216.7, 1187.9, 1160.9, 1132.1; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.01 (2H, d, $J$ = 8.2 Hz), 7.56 (1H, d, $J$ = 8.5 Hz), 7.48–7.36 (5H, m), 7.33 (2H, d, $J$ = 8.1 Hz), 7.09–7.03 (2H, m), 5.13 (2H, s), 2.62 (3H, s), 2.46 (3H, s).

**2-benzoyl-3-methyl-5-benzyloxybenzofuran (7c).** A white powder (23.81 g), yield 69.6 %, m.p: 91.4–94.2 °C. EI-MS: 343.1 ($[M + H]^+$), 365.2 ($[M + Na]^+$), 381.1 ($[M + K]^+$); IR: 3070.63, 2916.3, 1736.00, 1688.78, 1635.6, 1597.0, 1560.4, 1498.5, 1470.9, 1448.1, 1385.6, 1368.7, 1216.8, 1201.7, 1183.6; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.09 (2H, d, $J$ = 7.2 Hz), 7.63–7.59 (1H, m), 7.55–7.48 (4H, m), 7.46–7.39 (3H, m), 7.37 (1H, d, $J$ = 7.2 Hz), 7.21 (1H, d, $J$ = 2.5 Hz), 7.17 (1H, dd, $J$ = 9.1, 2.5 Hz), 5.14 (2H, s), 2.62 (3H, s).




**Scheme 2** The synthesis of furo[2,3-e][1]benzofuran derivatives and furo[3,2-e][1]benzofuran derivatives.

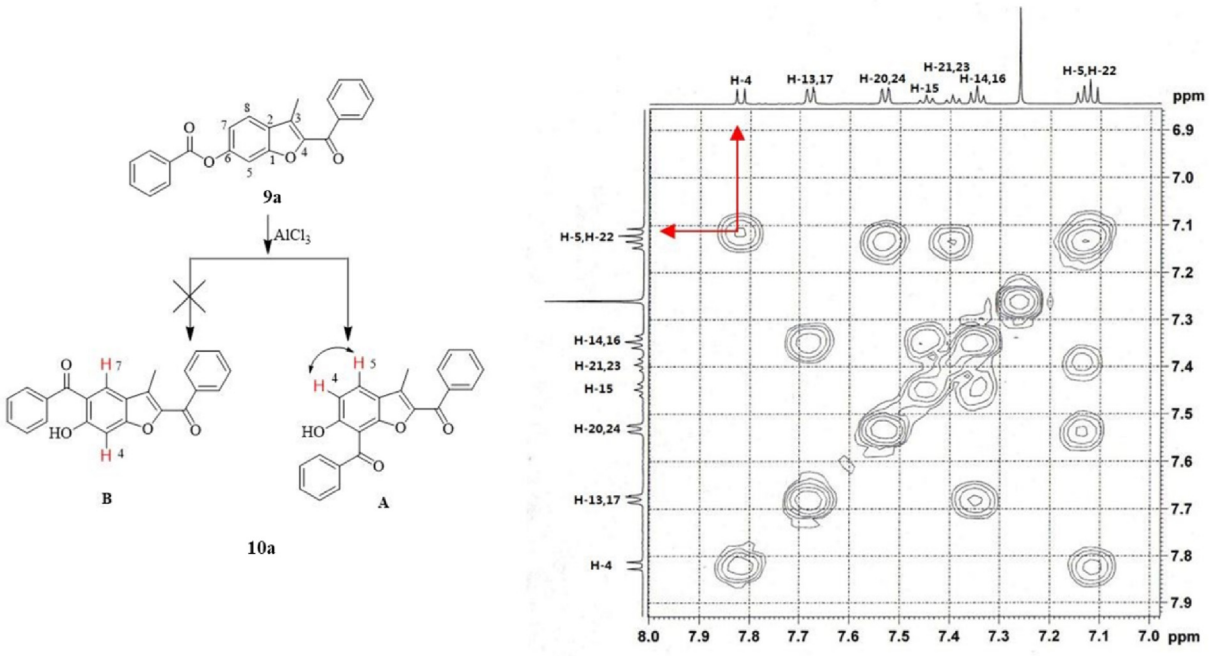



**Fig. 7** 2D-H NMR spectra of the compound **10a.**

*3.1.3. General procedure for preparing 2-aroyl-3-methyl-5-hydroxybenzofuran derivatives or 2-aroyl-3-methyl-6-hydroxybenzofuran derivatives (**8**)*

To a solution of **7** (0.01 mol) in 30 ml dichloromethane, was added dropwise slowly $BBr_3$ (0.02 mol) in −15 °C ice bath. The reaction continued for 10 min. After the reaction was finished, poured the mixture and 30 ml water into 125 ml separating funnel. The organic phase was separated and the water phase was extracted with 20 ml ethyl acetate. Combine organic phase, dried over $Mg_2SO_4$ and concentrated under reduced pressure to obtain crude. The crude was washed with 10 ml petroleum ether and 3 ml dichloromethane to obtain the solid **8**.

**2-benzoyl-3-methyl-6-hydroxybenzofuran (8a).** Yellow powder (2.37 g), yield 94.0 %, m.p: 150.8–153.0 °C. EI-MS: 253.0 ($[M + H]^+$), 274.8 ($[M + Na]^+$), 290.8 ($[M + K]^+$); IR: 3228.8, 2923.2, 1612.6, 1549.1, 1501.2, 1474.3, 1446.7, 1377.1, 1353.5, 1311.9, 1242.3, 1224.1, 1162.2; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 8.07–8.02 (2H, m), 7.63–7.47 (4H, m), 6.99 (1H, d, $J$ = 2.1 Hz), 6.90 (1H, dd, $J$ = 8.5, 2.2 Hz), 2.59 (3H, s).

**2-(4-methylbenzoyl)-3-methyl-6-hydroxybenzofuran (8b).** Yellow powder (2.02 g), yield 75.9 %, m.p: 162.2–163.8 °C. EI-MS: 267.0 ($[M + H]^+$), 289.0 ($[M + Na]^+$),305.1 ($[M + K]^+$); IR: 3331.6, 2922.1, 1605.5, 1540.7, 1500.2, 1438.8, 1376.6, 1350.8, 1304.4, 1239.6, 1211.3, 1187.4, 1128.2;

**Table 1** $^{1}H$ NMR and $^{13}C$ NMR spectral data of the compound **10a.**

| Carbon No. | Chemical shift, δ $CDCl_3$ | | 2D-H NMR (cross singal) |
|---|---|---|---|
| | $^{1}H$ | $^{13}C$ | |
| 2 | — | 151.7 | / |
| 3 | — | 128.995 | / |
| 4 | 7.820 | 119.6 | H-5 |
| 5 | 7.108–7.148 | 115.8 | H-4 |
| 6 | — | 159.6 | / |
| 7 | — | 106.3 | / |
| 8 | — | 116.8 | / |
| 9 | — | 161.3 | / |
| 10 | 2.67 | 14.3 | / |
| 11 | — | 191.5 | / |
| 12 | — | 139.0 | / |
| 13,17 | 7.675 | 128.7 | H-14, 16 |
| 14,16 | 7.347 | 127.9 | H-15, H-13, 17 |
| 15 | 7.449 | 132.4 | H-14, 16 |
| 18 | — | 198.3 | / |
| 19 | — | 139.6 | / |
| 20,24 | 7.532 | 129.0 | H-22 |
| 21,23 | 7.396 | 128.6 | H-22 |
| 22 | 7.108–7.148 | 132.8 | H-21, 23, H-20, 24 |

$^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.97 (2H, d, $J$ = 8.2 Hz), 7.53 (1H, d, $J$ = 8.5 Hz), 7.32 (2H, d, $J$ = 8.0 Hz), 6.99 (1H, d, $J$ = 2.0 Hz), 6.89 (1H, dd, $J$ = 8.5, 2.2 Hz), 2.59 (3H, s), 2.45 (3H, s).

**2-benzoyl-3-methyl-5-hydroxybenzofuran (8c).** Yellow powder (2.20 g), yield 87.0 %, m.p: 164.0–165.8 °C. EI-MS: 253.1 ($[M + H]^+$), 274.8 ($[M + Na]^+$); IR: 3279.4, 2918.1, 1737.26, 1623.5, 1599.6, 1579.5, 1552.7, 1446.3, 1377.6, 1307.5, 1237.6, 1245.8, 1204.8, 1181.2; $^{1}H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.10–8.05 (2H, m), 7.63–7.48 (3H, m), 7.40 (1H, d, $J$ = 8.8 Hz), 7.07–7.01 (2H, m), 2.58 (3H, s).

*3.1.4. General procedure for preparing 2-aroyl-3-methyl-5-benzoyloxybenzofuran derivatives or 2-aroyl-3-methyl-6-benzoyloxybenzofuran derivatives (9)*

To a solution of **8** (0.01 mol) and triethylamine (1.21 g, 0.012 mol) in 50 ml dichloromethane was added benzoyl chloride (1.51 g, 0.012 mol). The reaction was stirred for 0.5 h at room temperature. The mixture was washed with 1 mol/L hydrochloric acid (30 ml) and water (30 ml), dried over $Mg_2SO_4$, and concentrated under reduced pressure to obtain crude. The crude was recrystallized from 20 ml absolute ethyl alcohol to obtain the solid **9.**

**2-benzoyl-3-methyl-6-benzoyloxybenzofuran (9a).** White powder (3.32 g), 93.2 % yield, m.p: 109.8–111.2 °C. EI-MS: 357.1 ($[M + H]^+$), 379.0 ($[M + Na]^+$), 395.1 ($[M + K]^+$); IR: 3068.0, 2921.3, 1735.4, 1640.2, 1598.5, 1559.8, 1488.0, 1450.8, 1345.6, 1178.4; $^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 8.24 (2H, dd, $J$ = 8.3, 1.1 Hz), 8.11–8.06 (2H, m), 7.74 (1H, d, $J$ = 8.5 Hz), 7.67–7.60 (2H, m), 7.56–7.51 (4H, m), 7.47 (1H, d, $J$ = 1.9 Hz), 7.23 (1H, dd, $J$ = 8.5, 2.0 Hz), 2.66 (3H, s).

**2-(4-methylbenzoyl)-3-methyl-6-benzoyloxybenzofuran (9b).** White powder (3.14 g), 84.8 % yield, m.p: 116.2–118.0 °C. EI-MS: 371.1 ($[M + H]^+$), 393.2 ($[M + Na]^+$), 409.1 ($[M + K]^+$); IR:2922.7, 1701.54, 1688.47, 1638.0, 1609.6, 1561.1, 1509.0, 1451.5, 1383.9, 1357.7, 1315.6, 1293.4, 1271.3, 1249.7, 1179.3, 1143.1, 1127.2; $^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 8.24 (2H, d, $J$ = 7.3 Hz), 8.01 (2H, d, $J$ = 8.2 Hz), 7.73 (1H, d, $J$ = 8.5 Hz), 7.67 (1H, t, $J$ = 7.4 Hz), 7.54 (2H, t, $J$ = 7.8 Hz), 7.47 (1H, d, $J$ = 2.0 Hz), 7.33 (2H, d, $J$ = 8.4 Hz), 7.22 (1H, dd, $J$ = 8.5, 1.9 Hz), 2.65 (3H, s), 2.46 (3H, s).

**2-benzoyl-3-methyl-5-benzoyloxybenzofuran (9c).** Yellow powder (2.80 g), 78.6 % yield, m.p: 110.8–113.0 °C. EI-MS: 357.1 ($[M + H]^+$), 379.1 ($[M + Na]^+$); IR: 3068.2, 1727.9, 1640.8, 1597.5, 1561.1, 1492.4, 1451.2, 1384.4, 1253.9, 1225.8, 1174.4; $^{1}H$ NMR (400 MHz, $CDCl_3$) $\delta$ 8.25 (2H, d, $J$ = 8.4 Hz), 8.09 (2H, d, $J$ = 8.4 Hz), 7.69–7.51 (8H, m), 7.33 (1H, dd, $J$ = 8.9, 2.3 Hz), 2.63 (3H, s).

**Table 2** Inhibition of human breast cancer strains T-47D by the target compounds.

| No. | Substituents | | | Inhibition (%) | $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| | R | X | $NR'_2$ | | |
| **5a** | H | $H_2$ |  | 58.43 ± 1.63 | 6.85 ± 1.63 |
| **5b** | H | O |  | 36.01 ± 1.54 | 11.11 ± 1.54 |
| **5c** | H | O |  | 38.05 ± 3.78 | 10.51 ± 3.78 |
| **5d** | H | O |  | 78.95 ± 4.46 | 5.07 ± 4.46 |
| **5e** | $CH_3$ | O |  | 2.64 ± 3.66 | 151.27 ± 3.66 |
| **5f** | $CH_3$ | $H_2$ |  | 2.20 ± 1.85 | 182.00 ± 1.85 |
| **5g** | —— | O |  | 39.86 ± 3.28 | 10.03 ± 3.28 |
| **5h** | —— | $H_2$ |  | 37.84 ± 3.93 | 10.57 ± 3.93 |
| **5i** | —— | $H_2$ |  | 98.17 ± 0.25 | 3.95 ± 0.25 |
| tamoxifen | | | | 64.51 ± 3.87 | 6.20 ± 3.87 |

**Table 3** Inhibition of human breast cancer strains MCF-7 of **5d** and **5i**.

| No. | Concentration (μM) | | | | | | | | $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|---|---|
| | 0.03 | 0.1 | 0.3 | 1 | 3 | 10 | 30 | 100 | |
| **5d** | 3.25 ± 2.42 | 5.96 ± 1.49 | 8.46 ± 1.33 | 18.24 ± 2.39 | 35.33 ± 1.84 | 81.38 ± 2.64 | 95.63 ± 1.84 | 98.65 ± 1.24 | 4.24 ± 1.78 |
| **5i** | 5.04 ± 2.02 | 8.45 ± 2.08 | 13.74 ± 1.36 | 20.25 ± 2.28 | 68.64 ± 1.28 | 89.36 ± 1.38 | 98.35 ± 1.46 | 98.67 ± 1.64 | 2.36 ± 1.24 |
| tamoxifen | 2.13 ± 4.09 | 4.86 ± 1.15 | 10.29 ± 1.15 | 15.86 ± 1.15 | 32.16 ± 1.32 | 75.69 ± 4.56 | 92.36 ± 3.65 | 98.15 ± 3.61 | 4.95 ± 2.48 |

*3.1.5. General procedure for preparing 2,7-diaroyl-3-methyl-6-hydroxybenzofuran derivatives or 2,4-diaroyl-3-methyl-5-hydroxybenzofuran derivatives (**10**)*

**9** (0.01 mol) and aluminium chloride anhydrous (5.28 g, 0.04 mol) were grounded rapidly in the mortar, then transferred the mixture to a 100 ml round-bottomed flask which top attached an air condenser and a drying tube filled with calcium chloride anhydrous. Keep the temperature at 165 or 140 °C in oil bath for 6 h, then cooled to room temperature. The mixture was added hydrochloric acid 5 ml and crushed ice 20 g, after the crushed ice melting, the mixture was filtered and the solid was washed with water to pH = 7. The crude was recrystallized from 10 ml absolute ethyl alcohol to obtain the solid **10**.

**2,7-dibenzoyl-3-methyl-6-hydroxybenzofuran (10a).** Yellow powder (2.95 g), 82.8 % yield, m.p: 170.2–172.5 °C. EI-MS: 357.0 ($[M + H]^+$), 378.8 ($[M + Na]^+$), 395.1 ($[M + K]^+$); IR: 3424.5, 2923.1, 1631.3, 1619.1, 1597.3, 1572.0, 1552.0, 1488.9, 1446.0, 1384.7, 1334.5, 1288.0, 1229.4,1210.1, 1186.1, 1171.0; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 12.15 (1H, s), 7.82 (1H, d, $J$ = 8.4 Hz), 7.68 (2H, dd, $J$ = 1.8, 8.4 Hz), 7.53 (2H, dd, $J$ = 1.2, 8.4 Hz), 7.45–7.43 (1H, m), 7.41–7.38 (1H, m), 7.36–7.33 (2H, m), 7.15–7.11 (3H, m), 2.67 (3H, s).

**2-(4-methylbenzoyl)-7-benzoyl-3-methyl-6-hydroxybenzo furan (10b).** Yellow powder (3.02 g), 81.5 % yield, m.p: 113.8–116.2 °C. EI-MS: 371.0 ($[M + H]^+$), 393.1 ($[M + Na]^+$), 409.1 ($[M + K]^+$); IR: 3060.0, 2919.7, 1627.1, 1604.6, 1549.2, 1491.2, 1446.4, 1386.3, 1339.6, 1289.2, 1235.8, 1184.3, 1166.6; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 12.14(1H, 1 s), 7.77 (1H, d, $J$ = 8.7 Hz), 7.66 (2H, d, $J$ = 8.3 Hz), 7.45 (1H, s), 7.42 (2H, d, $J$ = 8.1 Hz), 7.36–7.33 (2H,m), 7.06 (1H, d, $J$ = 8.7 Hz), 6.91 (2H, d, $J$ = 8.0 Hz), 2.64 (3H, s), 2.36 (3H, s).

**2,4-dibenzoyl-3-methyl-5-hydroxybenzofuran (10c).** Bright yellow powder (2.14 g), 60.0 % yield, m.p: 148.2–150.0 °C. EI-MS: 357.1 ($[M + H]^+$), 378.9 ($[M + Na]^+$), 395.2 ($[M + K]^+$); IR: 3262.8, 2923.3, 1619.4, 1597.5, 1577.5, 1544.8, 1465.8, 1448.0, 1375.6, 1352.6, 1241.4, 1197.4, 1178.5; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 9.70 (1H, s), 8.00 (2H, dd, $J$ = 1.2, 7.2 Hz), 7.72 (2H, dd, $J$ = 1.2, 7.2 Hz), 7.68 (1H, d, $J$ = 9.0 Hz), 7.62–7.60 (1H, m), 7.54–7.50 (3H, m), 7.48–7.46 (2H, m), 7.23 (1H, d, $J$ = 9.0 Hz), 2.59 (3H, s).

*3.1.6. General procedure for preparing furo[2,3-e][1]benzofuran derivatives or furo[3,2-e][1]benzofuran derivatives (**11**)*

A mixture of **10** (0.1 mol), 2-chloro-1-(4-methoxyphenyl)etha none (20.24 g, 0.11 mol), TBAB (5.06 g, 0.05 mol), 30 % aqueous potassium carbonate 50 ml and dichloromethane 100 ml was refluxed at 40–45 °C in oil bath for 12 h. After reaction was finished, the organic phase was separated, washed with 1 mol/L hydrochloric acid (30 ml × 3) and water (30 ml), dried over $Mg_2SO_4$, and concentrated under reduced pressure to obtain the crude. The crude was washed with ethyl acetate 5 ml to obtain the solid **11**.

**2-benzoyl-3-methyl-8-phenyl-7-(4-methoxybenzoyl)furo[2,3-e][1]benzofuran (11a).** Yellow powder (37.32 g), 76.8 % yield, m.p: 189.0–190.2 °C. EI-MS: 487.0 ($[M + H]^+$), 509.0 ($[M + Na]^+$), 525.3 ($[M + K]^+$); IR: 3073.7, 2936.7, 1632.5, 1596.1, 1491.5, 1380.8, 1350.3, 1307.1, 1292.1, 1254.5, 1220.3, 1178.1, 1157.1; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.03–7.97 (2H, m), 7.96–7.91 (2H, m), 7.80 (1H, d, $J$ = 8.8 Hz), 7.68–7.60 (3H, m), 7.59–7.52 (1H, m), 7.34 (5H, m), 6.91–6.82 (2H, m), 3.85 (3H, s), 2.78 (3H, s).

**2-(4-methylbenzoyl)-3-methyl-8-phenyl-7-(4-methoxybenzoyl) furo[2,3-e][1]benzofuran (11b).** White powder (27.56 g), 55.1 % yield, m.p: 189.6–191.0 °C. EI-MS: 501.2 ($[M + H]^+$), 523.0 ($[M + Na]^+$); IR: 2922.4, 1648.5, 1629.1, 1597.3, 1549.8, 1492.7, 1420.2, 1348.1, 1295.4, 1252.5, 1220.0, 1178.7, 1156.2; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.95–7.89 (3H, m), 7.76 (1H, d, $J$ = 8.8 Hz), 7.66–7.61 (3H, m), 7.39–7.30 (3H, m), 7.15 (2H, d, $J$ = 8.1 Hz), 6.85 (2H, d, $J$ = 8.9 Hz), 3.84 (3H, s), 2.75 (3H, s), 2.45 (3H, s).

**1-phenyl-2-(4-methoxybenzoyl)-7-benzoyl-8-methylfuro[3,2-e][1]benzofuran (11c).** White powder (22.56 g), 46.4 % yield, m.p: 203.8–205.6 °C. EI-MS: 487.1 ($[M + H]^+$), 509.1 ($[M + Na]^+$), 525.3 ($[M + K]^+$); IR: 3083.2, 2931.2, 1640.2, 1599.7, 1555.3, 1510.8, 1489.5, 1447.1, 1388.0, 1308.4, 1280.1, 1265.0, 1245.6, 1171.5; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.06 (2H, d, $J$ = 8.8 Hz), 7.99 (2H, d, $J$ = 7.3 Hz), 7.72 (2H, dd, $J$ = 29.6, 9.1 Hz), 7.62–7.58 (1H, m), 7.51–7.45 (7H, m), 6.94 (2H, d, $J$ = 8.9 Hz), 3.88 (3H, s), 1.83 (3H, s).

*3.1.7. General procedure for preparing 7-(4-hyroxybenzoyl)furo[2,3-e][1]benzofuran derivatives or 2-(4-hyroxybenzoyl) furo[3,2-e][1]benzofuran (**12**)*

**11** (0.01 mol) and aluminium chloride anhydrous (7.91 g, 0.06 mol) were grounded rapidly in the mortar, then transferred the mixture to a 100 ml round-bottomed flask which top attached an air condenser and a drying tube filled with calcium chloride anhydrous. Keep the temperature at 130 or 100 °C in oil bath for 6 h and cooled to room temperature. The mixture was added hydrochloric acid 5 ml and crushed ice 20 g, after the crushed ice melting, the mixture was filtered and the solid was washed with water to pH = 7. The crude was recrystallized from 50 ml absolute ethyl alcohol to obtain the solid **12**.

**2-benzoyl-3-methyl-8-phenyl-7-(4-hydroxybenzoyl)furo[2,3-e][1]benzofuran (12a).** Bright yellow power (2.00 g), 42.3 % yield, m.p: 234.2–235.8 °C. EI-MS: 473.2 ($[M + H]^+$), 495.2 ($[M + Na]^+$), 511.2 ($[M + K]^+$); IR: 3387.0, 2925.1, 2854.6, 1736.2, 1719.7, 1544.8, 1490.8, 1373.7, 1289.4, 1167.5; $^1$H NMR (400 MHz, DMSO $d_6$): $\delta$ 10.51 (1H, s), 8.05 (1H, d, $J$ = 8.8 Hz), 7.96 (2H, d, $J$ = 7.4 Hz), 7.87 (1H, d, $J$ = 8.8 Hz), 7.76 (2H, d, $J$ = 8.6 Hz), 7.69–7.63 (3H, m), 7.53–7.49 (2H, m), 7.39–7.30 (3H, m), 6.78 (2H, d, $J$ = 8.7 Hz), 2.71(3H, s).

**2-(4-methylbenzoyl)-3-methyl-8-phenyl-7-(4-hydroxybenzoyl) furo[2,3-e][1]benzofuran (12b).** Bright yellow power (2.15 g), 44.2 % yield, m.p: 224.0–226.2 °C. EI-MS: 487.2 ($[M + H]^+$), 509.1 ($[M + Na]^+$); IR: 3355.3, 2923.1, 1736.1, 1603.5, 1547.4, 1445.4, 1348.6, 1285.9, 1159.4; $^1$H NMR (400 MHz, DMSO $d_6$): $\delta$ 10.51(1H, s), 7.97 (1H, d, $J$ = 8.8 Hz), 7.84–7.74 (5H, m), 7.60 (2H, d, $J$ = 7.3 Hz), 7.42–7.37 (1H, m), 7.33–7.31 (2H, m), 7.26 (2H, d, $J$ = 8.1 Hz), 6.78 (2H, d, $J$ = 8.6 Hz), 2.65(3H, s), 2.42 (3H, s).

**1-phenyl-2-(4-hyroxybenzoyl)-7-benzoyl-8-methylfuro[3,2-e][1]benzofuran (12c).** Bright yellow power (2.61 g), 55.2 % yield, m.p: 259.4–261.2 °C. EI-MS: 473.3 ($[M + H]^+$), 495.2 ($[M + Na]^+$); IR: 3367.7, 2926.5, 1638.6, 1600.9, 1577.6, 1488.9, 1386.2, 1279. 5, 1169.9; $^1$H NMR (400 MHz, DMSO $d_6$): $\delta$ 10.52 (1H, s), 7.99 (1H, d, $J$ = 9.0 Hz), 7.83–7.91 (5H, m), 7.69–7.65 (1H, m), 7.58–7.54 (2H, m), 7.46–7.48 (5H, m), 6.85 (2H, d, $J$ = 8.3 Hz), 1.68 (3H, s).

### *3.1.8. General procedure for preparing furo[3,2-e][1] benzofuran and furo[2,3-e][1]benzofuran derivatives (**5**)*

A mixture of **12** (2 mmol), 2-chloroacetamide or 2-chloroethanamine hydrochloride (2.2 mmol), anhydrous potassium carbonate (2.76 g, 20 mmol), potassium iodide (0.17 g,1 mmol) and dry acetone 50 ml was refluxed for 12 h, cooled to room temperature and filtered. The filtrate was consentrated under reduced pressure to obain the crude and purified by column chromatography to afford **5**.

**2-benzoyl-3-methyl-8-phenyl-7-{4-[(2-diethylamino)-2-oxoethoxy]benzoyl}furo[2,3-e][1]benzofuran (5a).**White crystal (0.85 g), 72.6 % yield, m.p:183.7–184.7 °C. EI-MS: 586.4 ($[M + H]^+$), 608.3 ($[M + Na]^+$), 624.4 ($[M + K]^+$); IR: 2930.1, 1448, 1378, 1596.2, 1576.2, 1644, 1252.7, 1166.9; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.05 (1H, d, $J$ = 8.8 Hz), 7.95 (2H, d, $J$ = 7.6 Hz), 7.88–7.82 (3H, m), 7.66 (3H, dd, $J$ = 17.0, 7.6 Hz), 7.51–7.49 (2H, m), 7.39–7.32 (3H, m), 6.97 (2H, d, $J$ = 8.4 Hz,), 4.09–4.06 (2H, m), 2.75–2.71 (2H, m), 2.70–2.68 (3H, m), 2.65–2.51 (4H, m), 1.09–1.05 (6H, m).$^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$ 184.09, 183.42, 163.11, 155.40, 148.31, 147.68, 137.40, 133.09, 132.53, 130.54, 130.48, 129.80, 129.41, 128.88, 128.68, 128.58, 128.49, 126.19, 125.23, 121.71, 114.72, 113.99, 110.04, 67.29, 51.59, 47.43, 12.35, 10.55.

**2-benzoyl-3-methyl-8-phenyl-7-{4-[2-(4-morpholinyl)-2-oxoethoxy]benzoyl}furo[2,3-e][1]benzofuran (5b).** Bright yellow powder (0.94 g), 78.4 % yield, m.p: 210.7–211.8 °C. EI-MS: 600.2 ($[M + H]^+$), 622.2 ($[M + Na]^+$), 638.2 ($[M + K]^+$); IR: 3058.7, 2920.8, 2853.5, 1669.2, 1637.4, 1598.8, 1443.6, 1382.8, 1251.6, 1115.2; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.00–7.92 (4H, m), 7.81 (1H, d, $J$ = 8.8 Hz), 7.69–7.51 (4H, m), 7.29–7.39 (5H, m), 6.91 (2H, d, $J$ = 8.9 Hz), 4.75 (2H, s), 3.66–3.58 (8H, m), 2.78 (3H, s). $^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$ 165.88, 162.67, 155.41, 147.75, 147.70, 137.41, 133.10, 132.37, 130.52, 130.48, 129.87, 129.81, 128.89, 128.75, 128.60, 128.49, 125.25, 121.77, 114.95, 110.09, 66.51, 66.45, 66.18, 45.08, 42.09, 10.56.

**2-benzoyl-3-methyl-8-phenyl-7-{4-[2-(1-piperidinyl)-2-oxoethoxy]benzoyl}furo[2,3-e][1]benzofuran (5c).** Bright yellow powder (0.87 g), 73.2 % yield, m.p: 190.1–192.4 °C. EI-MS: 598.2 ($[M + H]^+$), 620.2 ($[M + Na]^+$), 636.2 ($[M + K]^+$); IR: 2923.0, 2852.2, 1662.6, 1639.9, 1598.9, 1444.1, 1383.4, 1251.2, 1117.6; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.96 (4H, dd, $J$ = 19.3, 8.2 Hz), 7.81 (1H, d, $J$ = 8.9 Hz), 7.70–7.49 (4H, m), 7.41–7.27 (5H, m), 6.92 (2H, d, $J$ = 8.8 Hz), 4.73 (2H, s), 3.57 (2H, s), 3.49 (2H, s), 2.78 (3H, s), 1.49–1.75 (6H, m). $^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$ 184.19, 165.25, 162.78, 155.42, 147.73, 137.44, 133.12, 132.39, 130.53, 130.48, 129.82, 129.79, 128.91, 128.83, 128.75, 128.61, 128.50, 125.28, 121.79, 114.92, 110.13, 66.46, 45.55, 42.66, 39.46, 26.37, 25.73, 24.40, 10.58.

**2-benzoyl-3-methyl-8-phenyl-7-{4-[2-(1-diethylamino)ethoxy]benzoyl}furo[2,3-e][1]benzofuran (5d).** Yellow powder (0.82 g), 71.8 % yield, m.p: 185.1–187.0 °C. EI-MS: 572.1 ($[M + H]^+$); IR: 2966.4, 2924.5, 2803.8, 1635.5, 1596.5, 1549.6, 1448.5, 1371.5, 1254.4, 1165.7; $^1$H NMR (600 MHz, DMSO $d_6$) $\delta$ 8.08–7.91 (4H, m), 7.79 (1H, d, $J$ = 9 Hz), 7.67–7.53 (4H, m), 7.39–7.29 (5H, m), 6.91 (2H, d, $J$ = 7.6 Hz), 4.72 (2H, s), 3.42–3.47 (4H, m),2.78 (3H, s) 1.13–1.24 (6H, m). $^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$183.42, 166.06, 162.79, 155.40, 147.76, 147.70, 137.41, 133.10, 132.38, 130.53, 130.47, 129.81, 128.89, 128.74, 128.60, 128.49, 126.29, 125.24, 121.76, 114.90, 114.04, 110.09, 66.34, 14.58, 13.35, 10.56.

**2-(4-methylbenzoyl)-3-methyl-8-phenyl-7-{4-[(2-diethylamino)-2-oxoethoxy]benzoyl}furo[2,3-e][1]benzofuran (5e).** Yellow powder (0.87 g), 72.6 % yield, m.p: 200.4–202.4 °C. EI-MS: 600.1 ($[M + H]^+$) 622.0($[M + Na]^+$); IR: 2924.8, 1644.9, 1598.5, 1436.5, 1382.0, 1240.8, 1117.6; $^1$H NMR (600 MHz, DMSO $d_6$) $\delta$ 7.87 (2H d, $J$ = 8.7 Hz), 7.80 (1H, d, $J$ = 7.3 Hz), 7.74–7.67 (4H, m), 7.64 (1H, d, $J$ = 8.9 Hz), 7.59–7.56 (1H, m), 7.44 (2H, dd, $J$ = 14.3, 7.0 Hz), 7.38 (2H, d, $J$ = 7.5 Hz), 6.97 (2H, d, $J$ = 8.6 Hz), 2.42 (3H, s), 2.35–2.38 (4H, m), 1.16 (3H, t, $J$ = 6.9 Hz), 1.04 (3H, t, $J$ = 6.8 Hz). $^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$166.07, 162.79, 152.88, 146.44, 143.96, 136.46, 133.44, 132.39, 130.63, 129.78, 129.60, 129.30, 129.24, 128.94, 128.45, 125.80, 120.84, 114.92, 113.59, 109.33, 66.34, 40.95, 21.68, 14.58, 13.35.

**2-(4-methylbenzoyl)-3-methyl-8-phenyl-7-{4-[2-(1-piperidinyl) ethoxy]benzoyl}furo[2,3-e][1]benzofuran (5f).** Yellow powder (0.81 g), 67.7 % yield, m.p: 182.8–186.6 °C. EI-MS: 598.1 ($[M + H]^+$); IR: 2931.8, 2852.3, 1445.6, 1380.2, 1599.0, 1639.8, 1251.1, 1118.1; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 7.92 (4H, m), 7.79 (1H, d, $J$ = 8.8 Hz), 7.67–7.62 (3H, m), 7.39–7.378 (1H, m), 7.33–7.30 (2H, m), 7.16 (2H, d, $J$ = 7.9 Hz), 6.85 (2H, d, $J$ = 8.9 Hz,), 4.15–4.13 (2H, m), 2.75–2.78 (4H, m), 2.5–2.48 (4H, m), 2.45 (3H, s), 1.60–1.64 (7H, m). $^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$183.43, 163.06, 147.75, 147.58,

143.53, 132.52, 130.59, 130.48, 129.97, 129.46, 129.38, 128.70, 128.62, 128.11, 125.23, 121.69, 114.77, 114.03, 109.99, 66.51, 57.55, 54.82, 26.03, 24.37, 21.70, 10.55.

**1-phenyl-2-{4-[2-(4-morpholinyl)-2-oxoethoxy]benzoyl}-7-benzoyl-8-methylfuro[3,2-e][1]benzofuran (5 g).** Yellow powder (0.97 g), 81.5 % yield, m.p:160.4–162.2 °C. EI-MS: 600.2 ($[M + H]^+$); IR: 2921.4, 2852.7, 1734.2, 1674.0, 1599.0, 1573.3, 1444.8, 1386.1, 1275.0, 1173.3; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 8.06 (2H, d, $J$ = 8.9 Hz), 7.99 (2H, d, $J$ = 8.4 Hz), 7.76 (1H, d, $J$ = 9.1 Hz), 7.70 (1H, d, $J$ = 9.1 Hz), 7.61–7.59 (1H, m), 7.45–7.53 (7H, m), 7.00 (2H, d, $J$ = 8.9H), 4.78–4.77 (2H, m), 3.60–3.68 (8H, m), 1.83 (3H, s). $^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$185.52, 182.84, 165.92, 162.56, 151.97, 151.20, 137.88, 133.29, 132.27, 130.85, 130.05, 129.76, 128.95, 128.90, 128.57, 128.46, 126.75, 122.61, 114.97, 113.88, 113.57, 66.45, 66.16, 45.08, 42.09, 11.62.

**1-phenyl-2-{4-[2-(4-morpholinyl)ethoxy]benzoyl}-7-benzoyl-8-methylfuro[3,2-e][1]benzofuran (5 h).** Yellow powder (0.76 g), 65.3 % yield, m.p: 188.4–191.3 °C. EI-MS: 586.0 ($[M + H]^+$); IR: 3058.2, 2930.9, 2852.3, 1644.8, 1598.9, 1552.4, 1445.1, 1386.6, 1276.6, 1170.2; $^1$H NMR (600 MHz, $CDCl_3$)$\delta$ 8.03 (2H, d, $J$ = 8.9 Hz), 7.99 (2H, d, $J$ = 7.1 Hz), 7.76 (1H, d, $J$ = 9.1 Hz), 7.68 (1H, d, $J$ = 9.1 Hz,), 7.61–7.59 (1H, m), 7.44–7.52 (7H, m), 6.93 (2H, d, $J$ = 8.9 Hz), 4.19–4.17 (2H, m), 2.81–2.79 (2H, m), 2.52 (4H, s), 1.83 (3H, s), 1.61–1.64 (4H, m). $^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$185.47, 182.79, 162.95, 151.95, 151.16, 148.92, 137.87, 133.26, 132.42, 132.30, 130.88, 129.75, 129.65, 128.93, 128.87, 128.54, 122.60, 114.77, 113.83, 113.50, 57.59, 54.83, 26.04, 24.38, 11.64.

**1-phenyl-2-{4-[2-(1-diethylamino)ethoxy]benzoyl}-7-benzoyl-8-methylfuro[3,2-e][1]benzofuran (5i).** Bright yellow powder (0.78 g), 68.1 % yield, m.p: 180.2–180.6 °C. EI-MS: 572.1 ($[M + H]^+$); IR: 3057.9, 2966.9, 2929.8, 1642.2, 1599.0, 1554.4, 1446.5, 1386.6, 1277.6, 1170.8; $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 8.03 (2H, d, $J$ = 8.9 Hz), 7.99 (2H, d, $J$ = 7.2 Hz,), 7.76 (1H, d, $J$ = 9.1 Hz), 7.68 (1H, d, $J$ = 9.1 Hz), 7.61–7.59 (1H, m), 7.44–7.51 (7H, m), 6.93 (2H, d, $J$ = 8.9 Hz), 4.13–4.11 (2H, m), 2.90 (2H, s), 2.66–2.63 (4H, m), 1.83 (3H, s), 1.09–1.07 (6H, m). $^{13}$C NMR (101 MHz, DMSO $d_6$) $\delta$182.90, 163.03, 152.00, 151.20, 149.25, 148.96, 137.90, 133.48, 133.31, 132.47, 130.92, 129.77, 129.65, 128.97, 128.89, 128.57, 128.34, 126.74, 123.16, 122.64, 114.78, 113.89, 113.55, 67.29, 51.63, 47.44, 12.36, 11.66.

### *3.2. Screening assay*

The inhibitory potency against ER-dependent breast cancer and toxicity against human liver normal cell were evaluated by means of MTT assay (Mosmann, 1983; Naoum et al., 2007; Jin et al., 2020; Zhang et al., 2017). Human breast cancer strains T-47D; MCF-7 and human liver normal cell HL7702 (provided by Shanghai Cell Bank of Chinese Academy of Sciences) was used. The incubation time was 4 h, with protection from light. T-47D and MCF-7 were chosen as the ER-dependent breast cancer strains, HL7702 as the healthy cell strains and tamoxifen was used as positive control. In brief, human breast cancer strains T-47D, MCF-7 and HL7702 were seed onto 96-well plates with a density of 10,000 cells per well and incubated at 37 °C in a humidified atmosphere of 95 % air and 5 % $CO_2$ for 24 h. The medium in each well was replaced with 180 μl of culture medium containing the compound of choice at 8 μM concentration and cultured for a further 48 h. The incubation medium was then added 20 μl of 5 mg/ml MTT reagent and incubated for 4 h at room temperature in the dark. The incubation medium was then replaced with 150 μl DMSO. The absorbance intensity at 570 nm was recorded on a microplate reader. Each performed in thrice. Calculate the inhibition rate and $IC_{50}$ values.

## 4. Conclusions

In this article, raloxifene, the second-generation drug of SERMs, was used as a lead compound. We replace the benzothiophene ring in the raloxifene structure with a 2-arylcarbonyl-3-methylfuro[1]benzofuran scaffold based on the principle of scaffold hopping and bioisosterism. The target compounds; furo[2,3-e][1]benzofuran and furo[3,2-e][1]benzofuran derivatives (**5a-5i**) were synthesized from 2′,4′-dihydroxya cetophenone or 2′,5′-dihydroxyacetophenone in a higher overall yield via eight steps including substitution, cyclization, debenzylation, esterification, Fries rearrangement, cyclization, demethylation and Williamson reaction. All the target compounds were tested for inhibition of human breast cancer strains T-47D and MCF-7 by means of MTT test, in comparison to the tamoxifen. The compounds **5d** and **5i**, which $IC_{50}$ values were similar to tamoxifen, were expected to be potent SERMs.

**Supplementary Materials:** The following are available online, Figure S1-S90: IR, $^1$H NMR, and ESI-MS of the target compounds **5a-5i**, respectively.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2022.104227.

## References

Accelrys Software Inc., Discovery studio modeling environment, Release 3.5, San Diego: Accelrys Software Inc.; **2012**.

Atta, S.M., Farrag, D.S., Sweed, A.M., Abdel-Rahman, A.H., 2010. Preparation of new polycyclic compounds derived from benzofu-

rans and furochromones. An approach to novel 1,2,3-thia-, and selena-diazolofurochromones of anticipated antitumor activities. Eur. J. Med. Chem. 45 (11), 4920–4927.
Baltzly, R., Buck, J.S., Ide, W.S., 1950. Amines related to 2,5-dimethoxyphenethylamine. V. 2,5-Dihydroxy and 2-methoxy-5-hydroxy derivatives. J. Am. Chem. Soc. 72, 382–384.
Chargari, C., Toillon, R.A., Macdermed, D., Castadot, P., Magné, N., 2009. Concurrent hormone and radiation therapy in patients with breast cancer: what is the rationale? Lancet Oncol. 10 (1), 53–60.
Chen, Z., Li, J., Yang, H., He, Y., Shi, Q., Chang, Q., Liu, R., Huang, X., Li, Y., 2022. Discovery of novel benzimidazole derivatives as potent p300 bromodomain inhibitors with anti-proliferative activity in multiple cancer cells. Bioorg. Med. Chem. 66, 116784.
D'Souza, M.S., O'Mahony, J., Karkada, S.N., 2022. Effectiveness and meaningfulness of breast cancer survivorship and peer support for improving the quality of life of immigrant women: A mixed methods systematic review protocol. Clin. Epidemiol. Global Health 10, 100678.
Durrant, J.D., Carlson, K.E., Martin, T.A., Offutt, T.L., Mayne, C. G., Katzenellenbogen, J.A., Amaro, R.E., 2015. Neural-network scoring functions identify structurally novel estrogen-receptor ligands. J. Chem. Inf. Model. 55 (9), 1953–1961.
El-Shershaby, M.H., Ghiaty, A., Bayoumi, A.H., Al-Karmalawy, A. A., Husseiny, E.M., El-Zoghbi, M.S., Abulkhair, H.S., 2021. From triazolophthalazines to triazoloquinazolines: a bioisosterism-guided approach toward the identification of novel PCAF inhibitors with potential anticancer activity. Bioorg. Med. Chem. 42, 116266.
Giordano, C., Rovito, D., Barone, I., Mancuso, R., Bonofiglio, D., Giordano, F., Catalano, S., Gabriele, B., Ando, S., 2017. Benzofuran-2-acetic ester derivatives induce apoptosis in breast cancer cells by upregulating p21Cip/WAF1 gene expression in p53-independent manner. DNA Repair 51, 20–30.
Gradishar, W., Glusman, J., Lu, Y., Vogel, C., Cohen, F.J., Jr, S.G. W., 2000. Effects of high dose raloxifene in selected patients with advanced breast carcinoma. Cancer 88 (9), 2047–2053.
Gu, Y., Zhang, N., Zhu, S., Lu, S., Jiang, H., Zhou, H., 2022. Estradiol reduced 5-HT reuptake by downregulating the gene expression of Plasma Membrane Monoamine Transporter (PMAT, Slc29a4) through estrogen receptor β and the MAPK/ERK signaling pathway. Eur. J. Pharmacol. 924, 174939.
Gurjar, M.K., Joshi, S.V., Sastry, B.S., Rao, A.V.R., 1990. A new route to (±)-metoprolol. Synth. Commun. 20 (22), 3489–3496.
He, S., Xiao, H., Luo, S., Li, X., Zhang, J.-D., Ren, X.-M., Yang, Y., Xie, X.-D., Zhou, Y.-Y., Yin, Y.-L., Luo, L., Cao, L.-Y., 2022. Benzotriazole ultraviolet stabilizers promote breast cancer cell proliferation via activating estrogen-related receptors α and γ at human-relevant levels. Environ. Sci. Technol. 56 (4), 2466–2475.
Heard, D.J., Norby, P.L., Holloway, J., Vissing, H., 2000. Human ERRγ, a third member of the estrogen receptor-related receptor (ERR) subfamily of orphan nuclear receptors: tissue-specific isoforms are expressed during development and in the adult. Mol. Endocrinol. 14 (3), 382–392.
Ho, M.F., Correia, C., Ingle, J.N., Kaddurah-Daouk, R., Wang, L., Kaufmann, S.H., Weinshilboum, R.M., 2018. Ketamine and ketamine metabolites as novel estrogen receptor ligands: Induction of cytochrome P450 and AMPA glutamate receptor gene expression. Biochem. Pharmacol. 152, 279–292.
Hong, H., Yang, L., Stallcup, M.R., 1999. Hormone-independent transcriptional activation and coactivator binding by novel orphan nuclear receptor ERR3. J. Biol. Chem. 274 (32), 22618–22626.
Huey, R., Morris, G.M., Olson, A.J., Goodsell, D.S., 2007. A semiempirical free energy force field with charge-based desolvation. J. Comput. Chem. 28 (6), 1145–1152.
Jaen, J.C., Wise, L.D., Heffner, T.G., Pugsley, T.A., Meltzer, L.T., 1991. Dopamine autoreceptor agonists as potential antipsychotics. 2. (Aminoalkoxy)-4H-1-benzopyran-4-ones. J. Med. Chem. 34 (1), 248–256..
Jin, L.P., Xie, Q., Huang, E.F., Wang, L., Zhang, B.Q., Hu, J.S., Wan, D.C.C., Jin, Z., Hu, C., 2020. Design, synthesis, and biological activity of a novel series of benzofuran derivatives against oestrogen receptor-dependent breast cancer cell lines. Bioorg. Chem. 95, 103566.
Jordan, V.C., 2003. Antiestrogens and selective estrogen receptor modulators as multifunctional medicines. 1. Receptor interactions. J. Med. Chem. 46 (6), 883–908.
Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J.A., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138 (3), 863–870.
Lewin, G., Rolland, Y., Privat, S., Breugnot, C., Lenaers, A., Vilaine, J.P., Baltaze, J.P., Poisson, J., 1995. Synthesis and evaluation of 3',5'-di-tert-butyl-4'-hydroxyflavones as potential inhibitors of low density lipoprotein (LDL) oxidation. J. Nat. Prod. 58 (12), 1840–1847.
Lopes-Costa, P.V., dos Santos, A.R., dos Santos, L.G., da Silva, B.B., 2010. Evaluation of Ki-67 and Bcl-2 antigen expression in breast carcinomas of women treated with raloxifene. Cell Prolif. 43 (2), 124–129.
Matsushima, A., Kakuta, Y., Teramoto, T., Koshiba, T., Liu, X., Okada, H., Tokunaga, T., Kawabata, S., Kimura, M., Shimohigashi, Y., 2007. Structural evidence for endocrine disruptor bisphenol A binding to human nuclear receptor ERR gamma. J. Biochem. 142 (4), 517–524.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.
Naoum, F., Kasiotis, K.M., Magiatis, P., Haroutounian, S.A., 2007. Synthesis of novel nitro-substituted triaryl pyrazole derivatives as potential estrogen receptor ligands. Molecules 12, 1259–1273.
Ogawa, S., Parhar, I.S., 2020. Single-cell gene profiling reveals social status-dependent modulation of nuclear hormone receptors in GnRH neurons in a male cichlid fish. Int. J. Mol. Sci. 21 (8), 2724.
Ohtaka, H., Yoshida, K., Suzuki, K., Shimohara, K., Tajima, S., Ito, K., Benzylpiperazine derivatives. VIII., 1998. Syntheses, antiulcer and cytoprotective activities of 1-(aminocarbonylalkyl)-4-benzylpiperazine derivatives and related compounds. Chem. Pharm. Bull. 36 (10), 3948–3954.
Poutiainen, P.K., Venäläinen, T.A., Peräkylä, M., Matilainen, J.M., Väisänen, S., Honkakoski, P., Laatikainen, R., Pulkkinen, J.T., 2010. Synthesis and biological evaluation of phenolic 4,5-dihydroisoxazoles and 3-hydroxy ketones as estrogen receptor alpha and beta agonists. Bioorg. Med. Chem. 18 (10), 3437–3447.
Salem, H.F., Gamal, A., Saeed, H., Tulbah, A.S., 2021. The impact of improving dermal permeation on the efficacy and targeting of liposome nanoparticles as a potential treatment for breast cancer. Pharmaceutics 13 (10), 1633.
Sauter, E.R., 2018. Breast cancer prevention: Current approaches and future directions. European Journal of Breast Health 14 (2), 64–71.
Vajdos, F.F., Hoth, L.R., Geoghegan, K.F., Simons, S.P., LeMotte, P. K., Danley, D.E., Ammirati, M.J., Pandit, J., 2007. The 2.0 a crystal structure of the ERalpha ligand-binding domain complexed with lasofoxifene. Protein Sci. 16, 897–905.
Wang, X., Sun, R., Huang, Y., Yan, Y., Gao, M., Wang, D.N., Koirala, D., Li, D.W., Hu, C., 2014. Development of thieno [3',2':5,6]thiopyrano[4,3-c]pyrazole-3-carboxamide derivatives as the estrogen receptor ligands: synthesis, characterization and biological activity. Med. Chem. 10 (8), 836–842.

Woo, J., Christian, A.H., Burgess, S.A., Jiang, Y., Mansoor, U.F., Levin, M.D., 2022. Scaffold hopping by net photochemical carbon deletion of azaarenes. Science 376 (6592), 527–532.

Yates, M.H., Koenig, T.M., Kallman, N.J., Ley, C.P., Mitchell, D., 2009. An Efficient Synthesis of a Multipotent Eicosanoid Pathway Modulator. Org. Process Res. Dev. 13 (2), 268–275.

Zeng, H., Peng, F., Wang, J., Meng, R., Zhang, J., 2021. Effects of Fruquintinib on the pluripotency maintenance and differentiation potential of mouse embryonic stem cells. Cell. Reprogramming 23 (3), 180–190.

Zhang, L., Deng, X.S., Zhang, C., Meng, G.P., Wu, J.F., Li, X.S., Zhao, Q.C., Hu, C., 2017. Design, synthesis and cytotoxic evaluation of a novel series of benzo[*d*]thiazole-2-carboxamide derivatives as potential EGFR inhibitors. Med. Chem. Res. 26 (9), 2180–2189.