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3-Acyl-5-hydroxybenzofuran derivatives as potential anti-estrogen breast cancer agents: A combined experimental and theoretical investigation

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ABSTRACT

We first report the application of 3-acyl-5-hydroxybenzofurans as a scaffold to develop potential drugs for breast cancer. Seven novel derivative compounds were synthesized by using a microwave-assisted synthesis method. Those compounds exhibited different antiproliferation against human breast cancer MCF-7 cells, with the best activity of IC_{50} = 43.08 μM for compound **1**. A Quantum Mechanics Polarized Ligand Docking (QPLD) study was carried out to investigate the binding interactions between these compounds and estrogen receptor alpha (ERα). The simulation results showed that the trend of receptor–ligand binding interactions was same as that of their antiproliferative activities. A detailed analysis indicated that compound **1** possesses the highest Van der Waals and hydrogen bond interactions compared to the other six compounds and better inhibitors are achievable by enhancing the hydrogen bond interactions. Based on these results, we addressed that 3-acyl-5-hydroxybenzofuran is an attractive scaffold for designing drugs against breast cancer.

Cancer is accounted for 13% of all worldwide deaths in 2007 and it is projected to continue rising, with an estimated 12 million deaths in 2030.[1] Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer deaths among women which is after lung cancer, accounting for 23% of total cancer cases in women, and 14% of cancer deaths.[2]

Estrogen receptor (ER) and aromatase are two major targets in the treatment of breast cancer. CYP19 enzyme is one of the important targets in breast cancer treatment and estrogen biosynthesis.[3] It is a crucial aromatase catalyzing the final formation step of estrogens from corresponding androgen precursors such as testosterone and androstenedione.[4] By inhibiting CYP19 enzyme, the estrogen production can be blocked and consequently the breast cancer cells are prevented from proliferation. The ER subtype ERα which is predominantly expressed in breast cancer cells is another important target. Different from the mechanism of CYP19 enzyme, ERα antagonists directly block the active site of ERα to prevent any estrogen from binding to it as well as to stop the function of hormone. In breast cancer cell, estrogen activates ERα by binding to its active site, which induces conformational changes that allow co-activators to attach on the complex. The estrogen–ERα complex further binds to the estrogen response element (ERE) on DNA to activate the target genes regulating transcription. As a result, several effects are stimulated, for instance, cell proliferation in the breast.[5] As a ligand inducible nuclear receptor, ERα plays a critical physiological role as mediator of the actions of the estrogen hormones.[6] Therefore, it is an attractive pharmaceutical target for the development of novel therapeutic agents for the treatment of breast cancer.

The link between estrogen and breast cancer suggested that 'antiestrogenic strategies'[7] might have potential as therapeutic agents.[8] In fact, many existing chemotherapies are ERα antagonists. Tamoxifen[9] is well known to remain the first line therapy for estrogen receptor positive breast cancer since the 1980s,[10,11] and it has been used successfully by more than 10 million patients.[12] However, the extensive evaluation of tamoxifen treatment revealed small but significant side effects such as endometrial cancer, blood clots and the development of acquired resistance.[13] More than that, many current breast cancer chemotherapy treatments are often associated with severe side effects, and according to statistics, up to approximate 50% of patients with ER-positive tumors either initially do not respond or become resistant to these drugs.[14] Thus contributing to a significant obstacle towards breast cancer treatment. Hence, there is a great need for the improvement of existing therapies and the development of new ones for the prevention and treatment of breast cancer.

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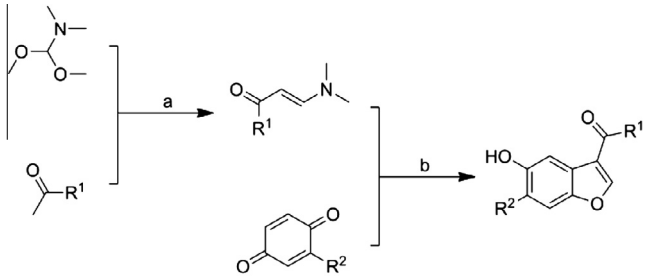
The significance of the hydroxylated benzofuran scaffold in medicinal chemistry is widely known.[15] Many of those compounds are serving as anticancer agent,[16] antifungal agents,[17] and anti-inflammatory agents.[18] For instance, 5-hydroxybenzofurans, are well-known on account of their reported inhibition of human leukocyte 5-lipoxygenase inhibitors.

Herein, we first report the application of 3-acyl-5-hydroxybenzofurans as a scaffold to develop potential drugs for breast cancer. Seven derivative compounds were synthesized (Scheme 1) by using a microwave-assisted synthesis method. The antiproliferative effects of these derivatives were evaluated on the human breast cancer MCF-7 cells using the MTT colorimetric assay.[19] The results demonstrated different concentrations in antiproliferative assays against the cell line with the best activity of IC_{50} values below 50 μM. To rationalize the observed ERα binding selectivity of the benzofuran products, a detailed Quantum Mechanics Polarized Ligand Docking (QPLD) study[20] was undertaken involving calculation of receptor–ligand interactions between those compounds and estrogen receptor alpha (ERα). The trends of receptor–ligand binding interactions and antiproliferative activity are presented into good linear relationship. We further analyzed the interactions between the seven compounds and ERα and found out that compound **1** possesses the highest Van der Waals and hydrogen bond interactions compared to the other six compounds. Better inhibitors can be achievable by enhancing the hydrogen bond interactions. We addressed that 3-acyl-5-hydroxybenzofuran is an attractive scaffold for designing drugs against breast cancer.

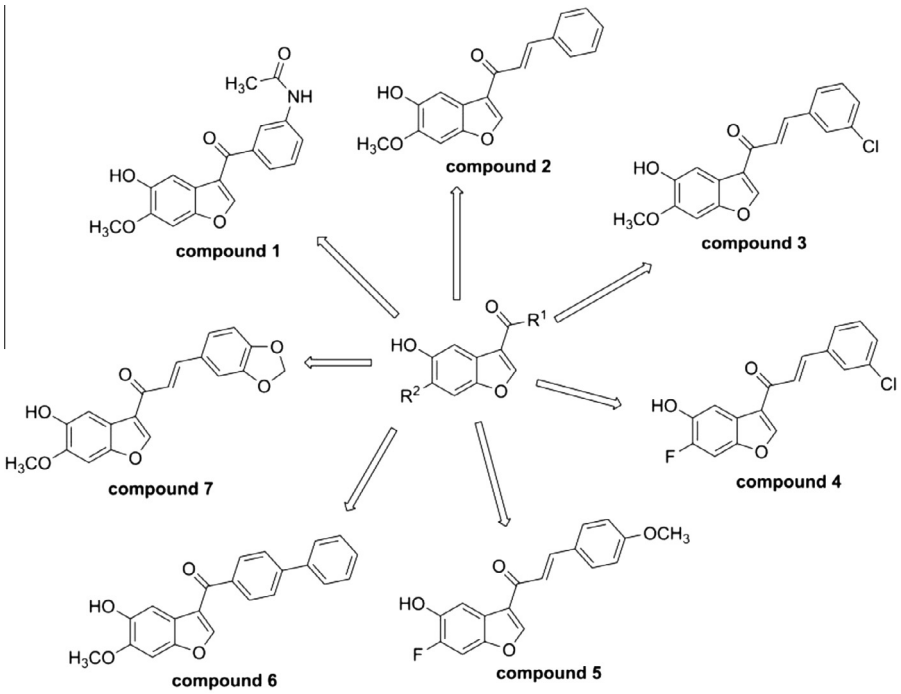
The synthetic route is displayed in Scheme 2. We used the synthetic method reported by our laboratory shortly before.[21] The starting materials are different ketones and quinines. A mixture of 1,1-dimethoxy-*N*,*N*-dimethylmethanamine, ketone and DMF first reacted under microwave irradiation. After the reaction mixture was cooling and analyzed by TLC, the quinine was added into the system. Then the system was exposure to microwave irradiation again. We finally got the desired compounds after cooling, separating, extracting, drying, filtering, and purifying. The detailed synthetic process can be found in the Supplementary data.



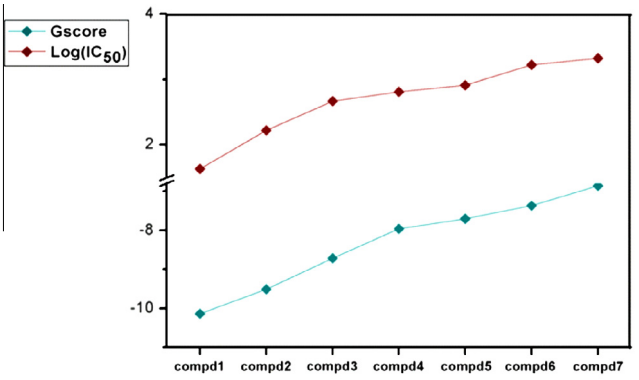
Scheme 2. Synthesis routines of compounds. Reagents and conditions: (a) microwave irradiation, DMF, 190 °C, 30 min; (b) microwave irradiation, DMF, acetic acid, 60 °C, 30 min.

We evaluated the activity against breast cancer in vitro.[22] The antiproliferative activities of all compounds on MCF-7 human mammary carcinomas cell line are in micromolar range. Here we used tamoxifen as a positive control and its IC_{50} value measured in this test was 12.35 μM. The IC_{50} values are presented in Figure 1. The general order of antiproliferative activities is as follows: compound **1** > **2** > **3** > **4** > **5** > **6** > **7**. Compound **1** shows much better activity than the others, and its IC_{50} value was comparable to that of tamoxifen.

Schrödinger Suite 2010 containing Maestro 9.1,[23] Glide 5.6,[24] and QM-Polarized Ligand Docking[25] was the principal software used in this simulation. The crystal structure of the complex (PDB code: 1A52)[26] formed by 17β-estradiol (E2) and the human estrogen receptor α (ERα) ligand binding domain (hERαLBD) was selected for the calculation.[27–29] The detailed simulation process can also be found in the Supplementary data. The best Gscore values (Fig. 1) obtained from the Quantum Mechanics Polarized Ligand Docking[30,31] were −10.138 kcal/mol for compound **1**, −9.512 kcal/mol for compound **2**, −8.715 kcal/mol for compound **3**, −7.961 kcal/mol for compound **4**, −7.706 kcal/mol for compound **5**, −7.370 kcal/mol for compound **6** and −6.862 kcal/mol for compound **7**, respectively. The numerical order of the above



Scheme 1. Core structure of 3-acyl-5-hydrogenbenzofuran and chemical structures of 3-acyl-5-hydrogenbenzofuran derivatives synthesized in this project.



	compd1	compd2	compd3	compd4	compd5	compd6	compd7
Gscore (kal/mol)	-10.138	-9.512	-8.715	7.961	-7.706	-7.370	-6.862
IC_{50} (μM)	43.08	167.18	463.57	643.67	813.69	1662.91	2106.83
$Log(IC_{50})$	1.634	2.223	2.666	2.809	2.910	3.221	3.324

Figure 1. Linear relation comparison between Gscores of QPLD simulation and $\log(IC_{50})$ of the seven compounds.

Gscore values is completely the same as that of the IC_{50} values of the seven compounds against the ERα in vitro: compound **7** > **6** > **5** > **4** > **3** > **2** >**1** (Gscores are negative and a low value indicates a good result).

In order to reveal the relationship between docking calculation and antiproliferative activity, we conducted a diagram based on the data of Gscores of QPLD simulation[32] and IC_{50} values (Fig. 1). For the sake of reflecting the relationship more clearly in the pattern, here we adopted the logarithms of the IC_{50} values ($\log(IC_{50}) = \log_{10}{}^{(IC50)}$). This figure shows that the results of receptor–ligand binding interactions and antiproliferative activity are presented into a good linear relationship. The linear relationship can be written as a mathematical equation $IC_{50} = b_0 + b_1{}^*Gscore$, where $b_0 = 6.73$, $b_1 = 0.49$ with correlation $R^2 = 0.95$.

We further analyzed the interactions between the seven compounds and ERα. Figure 2 showed the simulation results of compounds **1**–**7** docked into the binding sites to ERα. The main binding modes in these complexes can be described as following. One major interaction is hydrogen bond. Compound **1** formed hydrogen bonds with the amino acid residues 5-OH/Leu346, N–H/Thr347 of ERα (Fig. 2A). Compounds **2** and **3** formed a hydrogen bond with 5-OH/Leu346 of ERα (Fig. 2B and C), respectively. Compound **6** formed a hydrogen bond with 5-OH/Glu353 (Fig. 2F) and compound **7** form a hydrogen bond with 5-OH/His524 (Fig. 2G). Another major interaction is π–π interaction. We got the 2-dimensional diagrams from the ligand interaction diagram module in Schrodinger Suite 2010. From the 2-dimensional diagrams of ligand–receptor interactions (Fig. 3), we found that the benzene rings of Phe 404, Trp383, His524 were able to coordinate the ligands position in the active site of ER α throught Van der Waals intercations. Compound **1** formed π–π conjugate interactions between the two rings of benzofuran skeleton and Phe404 (Fig. 3A). Compounds **2** and **6** formed π–π conjugate interactions between the two rings of benzofuran skeleton and Phe404, the ring of side chain and Trp383, respectively (Fig. 3B and F). Compound **3** formed π–π conjugate interactions between the six-membered ring of benzofuran skeleton and Phe404, the ring of side chain and Trp383, respectively (Fig. 3C). Compounds **4** and **5** formed π–π conjugate interactions between the five-membered ring of benzofuran skeleton and His524, the ring of side chain and Phe404, respectively (Fig. 3D and E).

Figure 4 displayed the results of QPLD. (GScore = a^*vdW + b^*Coul + Lipo + Hbond + Metal + Buryp + RotB + Site, $a = 0.065$, $b = 0.130$, here we only discussed the main items which are vdW, Coul and Hbond). Compound **1** possesses the lowest score of Van der Waals interations (a^*vdW = −2.320 kcal/mol) and hydrogen bond interactions (Hbond = −1.297 kcal/mol) compared to the other six compounds (Fig. 4), and thus contributed to its best QM calculation score (Gscore = −10.138 kcal/mol). Though compound **2** formed π–π conjugate interactions with Phe404 similar to compound **1**, but lacked the hydrogen bond with N–H (Fig. 3A and B). Therefore its hydrogen bond interaction (Hbond = −0.698 kcal/mol) was much weaker than compound **1** (Hbond = −1.297 kcal/mol). Similarly, its coulomb force with protein (b^*Coul = −0.236 kcal/mol) was also weaker than that of compound **1** (b^*Coul = −0.538 kcal/mol). Compounds **2** and **3** both formed hydrogen bonds with Leu346, but the hydrogen bond interaction of compound **2** was stronger than that of the latter (Hbond = −0.480 kcal/mol). They also shaped π–π conjugate interactions with Phe404 and Trp383, but Phe404 was merely affected the six-membered ring of benzofuran skeleton for compound **3** (Fig. 3C), thus brought about a weaker Van der Waals interaction (a^*vdW = −1.367 kcal/mol) compared to compound **2** (a^*vdW = −1.553 kcal/mol). This leads to a lower Gscore for compound 2 (Gscore = −9.512 kcal/mol) than that for compound **3**

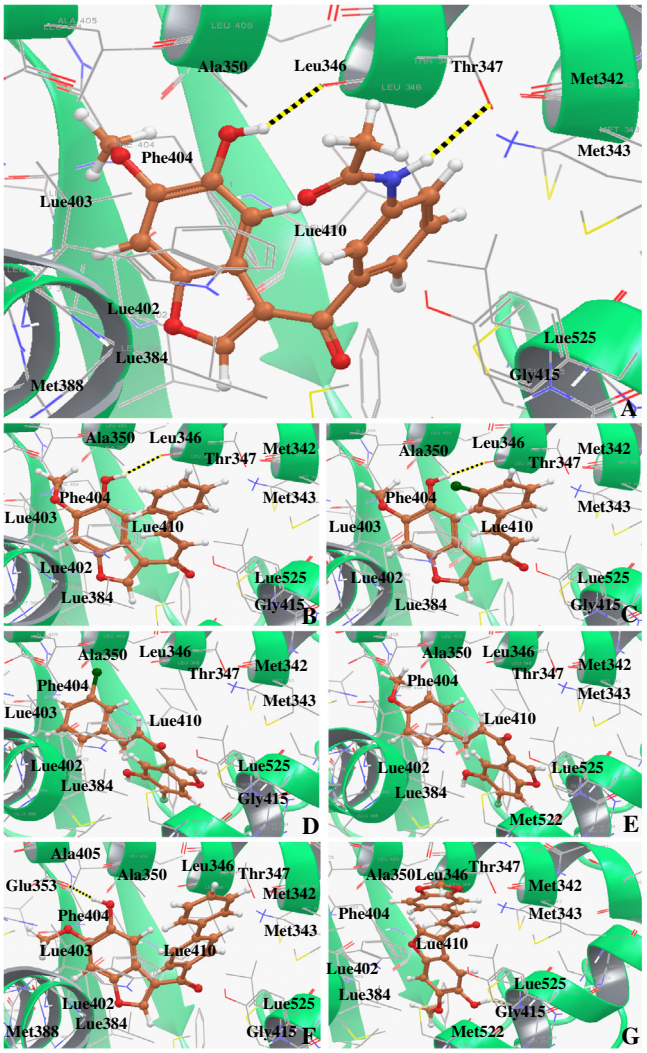


Figure 2. Schematic 3-dimensional diagrams of compound **1**–**7** in ERα active site. Hydrogen bonds are indicated by yellow dotted lines between ligand and protein. Binding site of ligands to Erα. (A) compound **1**. (B) compound **2**. (C) compound **3** (D) compound **4** (E) compound **5** (F) compound **6** (G) compound **7**. (The yellow dashed lines show the formation of the H-bonds. Active site residues are represented by wires and colored by element. Atoms in the ligand are colored as follows: O, red; C, brown; H, white; N, blue; F, bright green; Cl, dark green.)

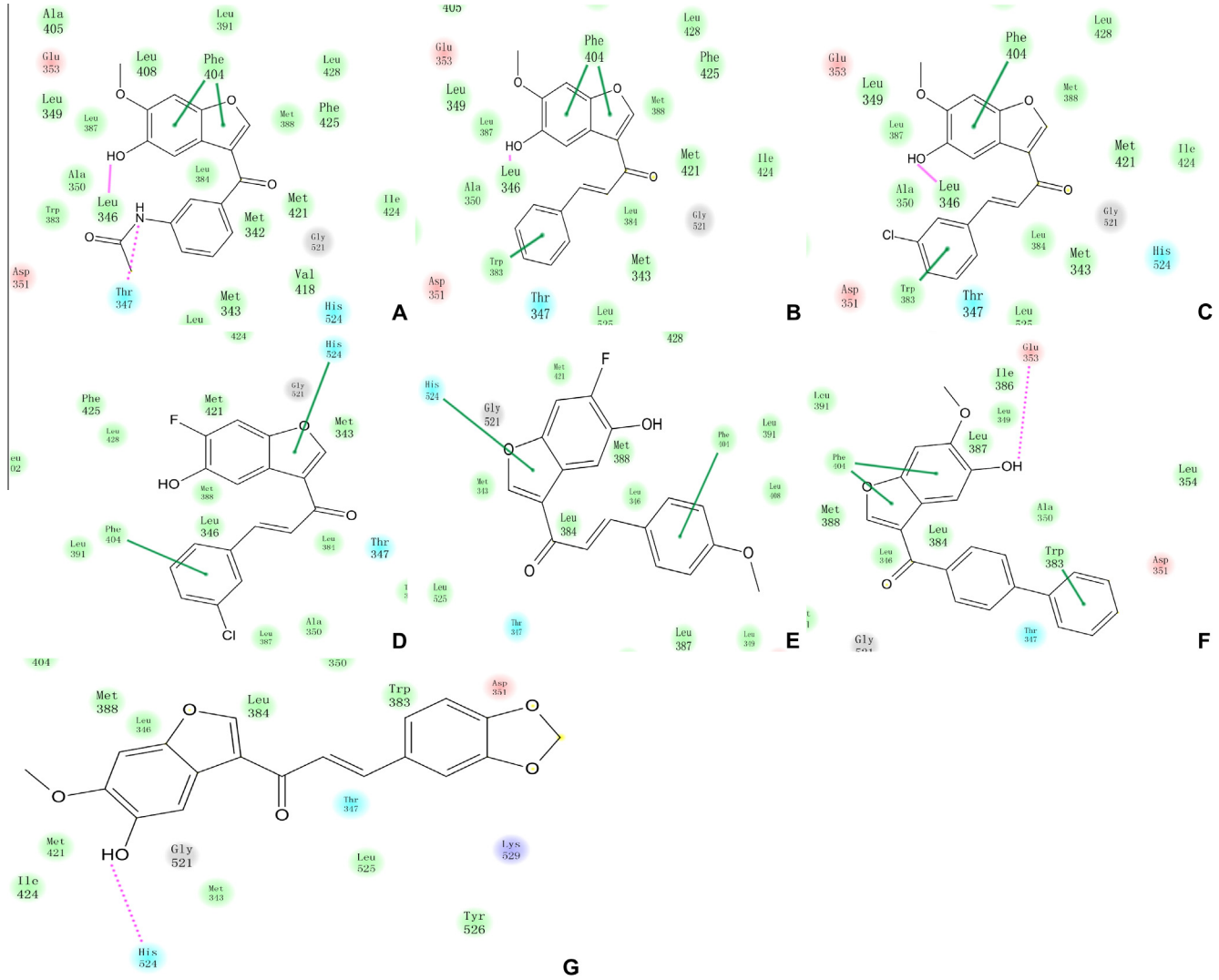
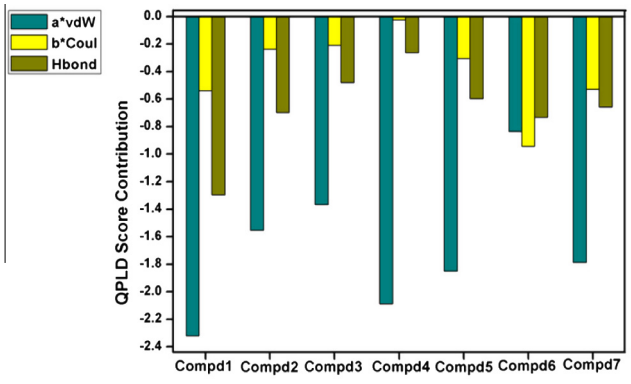


Figure 3. Schematic 2-dimensional diagrams of ligand–receptor interactions of compound **1**–**7** and ERα. (A) compound **1** (B) compound **2** (C) compound **3** (D) compound **4** (E) compound **5** (F) compound **6** (G) compound **7** (hydrogen-bonds are indicated by red lines. Aromatic π–π interactions are displayed by green lines. Green residues are hydrophobic contacts, cyan residues are polar contacts, blue residues are positively charged contacts, and red residues are negatively charged contacts. The residue font size indicates the distance (larger letters are closer).



	Compd1	Compd2	Compd3	Compd4	Compd5	Compd6	Compd7
Gscore	-10.138	-9.512	-8.715	-7.961	-7.706	-7.370	-6.862
vdW	-35.697	-23.895	-21.031	-32.104	-28.462	-12.846	-27.489
a*vdW	-2.320	-1.553	-1.367	-2.087	-1.850	-0.835	-1.787
Coul	-4.142	-1.816	-1.604	-0.191	-2.347	-7.263	-4.079
b*Coul	-0.538	-0.236	-0.209	-0.025	-0.305	-0.944	-0.530
Hbond	-1.297	-0.698	-0.480	-0.264	-0.598	-0.733	-0.659

Figure 4. Part of Quantum mechanism calculation results. Final selection of top docking scores was ranked based on GScore.

(Gscore = −8.715 kcal/mol). Despite the π–π conjugate interactions with Phe 404 and His524, compound **4** did not show any strong hydrogen bonds (Fig. 3D). Hence compound **4** (Gscore = −7.961 kcal/mol) was weaker interaction (Hbond = −0.264 kcal/mol) and coulombian force (*b**Coul = −0.025 kcal/mol) to the receptor. The ligand–receptor ligand than compound **3** because of its poor hydrogen bond interactions for compound **4** and **5** are very similar (Fig. 3D and F). They both shaped π–π conjugate interactions between the five-membered ring of benzofuran skeleton and His524, the benzene ring and Phe404, but the distance between compound and His524 was much shorter for compound **4**. So it has a better Van der Waals interaction (*a**vdW = −2.087 kcal/mol) compared to compound **5** (*a**vdW = −1.850 kcal/mol). This leads to a weaker ligand–receptor interaction for compound **5** (Gscore = −7.706 kcal/mol). Regardless of the strong coulombian force (*b**Coul = −0.944 kcal/mol) and slightly stronger hydrogen bond interactions (Hbond = −0.733 kcal/mol), compound **6** did not gain a considerable QM docking score (Gscore = −7.370 kcal/mol) on account of the considerably poor Van der Waals interactions (*a*∗vdW = −0.835 kcal/mol). Although compound **6** formed more π–π conjugate interactions with ERα than other compounds, the binding affinities were weaker, which is due to farther distance

between compound **6** and the amino acids. Compound **7** did not show any apparent Van der Waals intercations in the 2-dimensional diagram (Fig. 3G), in spite of the hydrogen bond between His524 and the benzofuran skeleton, it gained a comparative poor QM calculation score (Gscore = −6.862 kcal/mol). From discussion above, we found that the hydrogen bonds and Van der Waals interactions are key factors of ligand–receptor interactions. Thus we proposed that a better candidate can be achieved with the methyl of N-methylacetamide replaced by chlorine or bromine, which enhances the hydrogen bond interactions.

In summary, we synthesized seven compounds as drug candidates against breast cancer based on the core structure of 3-acyl-5-hydroxybenzofuran. We evaluated their antiproliferative activities in vitro and they showed different antiproliferation against human breast cancer MCF-7 cell with the best activity of IC_{50} = 43.08 μM for compound **1**. In order to clarify the mechanism of the receptor–ligand binding interactions and their differential inhibitory activity against ERα, we carried out a Quantum Mechanics Polarized Ligand Docking (QPLD) study for docking these seven compounds in ERα receptor. The simulation results show that the receptor–ligand binding interactions are presented into linear correlation with the results of antiproliferative activities. We further analyzed the interactions between the seven compounds and ERα, found out that compound **1** possesses the highest Van der Waals and hydrogen bond interactions compared to the other six compounds. Based on these results, we addressed that 3-acyl-5-hydroxybenzofuran is an attractive scaffold for designing drugs against breast cancer.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.022.

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