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Synthesis and biological evaluation of 2,3-diaryl isoquinolinone derivatives as anti-breast cancer agents targeting ERα and VEGFR-2

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ABSTRACT

The estrogen receptor α is recognized as important pharmaceutical target for breast cancer therapy, and vascular endothelial growth factor receptors (VEGFRs) play important roles in tumor angiogenesis including breast cancer. A series of 2,3-diaryl isoquinolinone derivatives were designed and synthesized targeting both estrogen receptor α (ERα) and VEGFR-2. Bioactivity evaluation showed that compounds **7c**, **7d** and **7f** exhibited significant anti-proliferative and anti-angiogenesis activities via ERα and VEGFR-2 dependent mechanisms.

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Breast cancer (BC) is the most common malignancy and the leading cause of cancer death in women worldwide.[1] Estrogen receptor α (ERα), a member of the large superfamily of nuclear receptors, is overexpressed and predominantly involved in more than 70% breast cancer patients.[2] ERα is responsible for estrogen-induced proliferation in breast cancer. In this case, tumors depend on estrogens for their survival and endocrine therapy is currently used to inhibit ER signaling by competitively binding to ER with anti-estrogens or estrogen deprivation. Selective estrogen receptor modulators (SERMs) are non-steroidal agents which act as anti-estrogens in breast tissue and are widely used in the treatment and prevention of ER positive breast cancer.[3] Despite their great benefits in treating BC, SERMs may cause negative side effects due to their estrogenic activity in other tissues. For example, stimulation in the uterus would increase risk of endometrial cancer. Another common side effect in endocrine therapy is drug resistance. Whatever the endocrine treatment used, resistance may occur. This is especially true with Tamoxifen which is never given more than 5 years. Although the molecular mechanism of resistance is incompletely clear, it had been proved that the activation of Ras/Raf-1/Mitogen-activated protein kinase (MAPK) signal pathway is involved in Tamoxifen resistance.[4] The MAPK pathway can phosphorylate and activate ERα in a ligand-independent manner, resulting in transcription of estrogen-regulated genes and cell proliferation.[5] Study also showed that MAPK pathway collaborates with ERα in exerting direct genomic actions in breast cancer cells through extracellular signal-regulated kinase 2 (ERK2), a downstream effector.[6]

Angiogenesis plays an important role in both local tumor growth and distant metastasis in many cancers as well as breast cancer.[7] Vascular endothelial growth factor receptor-2 (VEGFR-2, or kinase insert domain receptor, KDR) is a member of the receptor tyrosine kinase (RTK) family and is proposed to function as a dominant receptor of VEGF/VEGFR signaling in the angiogenesis pathway.[8] The Ras/MAPK pathway which is very important in promoting cell proliferation, is also activated in VEGF/VEGFR signal transduction and plays important role in promoting tumor angiogenesis.[9,10] VEGFR-2 inhibitors were reported in treating breast cancer but are not sufficient when used as monotherapy.[11,12] But a combination of Tamoxifen and a low dose of a VEGFR-2 inhibitor, Brivanib alaninate, was reported not only to maximize therapeutic efficacy but also to retard SERM resistant tumour growth.[13]

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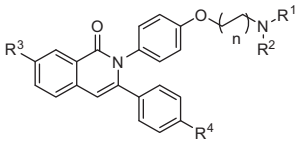
In the research to find novel and effective anti-breast cancer agents, the cross-talk between ER and receptor tyrosine kinase signaling aroused our interests. Based on the above facts, VEGFR-2 inhibitors can not only inhibit angiogenesis in breast cancer but may also retard SERMs resistance in BC through inhibition of Ras/MAPK pathway. So we hypothesized that if a compound could serve as an anti-estrogen as well as a VEGFR-2 inhibitor, there should be better effect in treating BC. Through review of literature, we found that VEGFR-2 inhibitors with scaffold of indol-2-one, such as Sunitinib and YM231146, bear some structural similarities with SERMs (Fig. 1). All of them contain aromatic scaffold and flexible side chain with tertiary amine substituent in the end. Based on these findings, we proposed to design and synthesize a series of compounds with characteristics of both SERMs and VEGFR-2 inhibitors. It was expected for these compounds with dual targets to gain more satisfactory effects for anti-breast cancer with fewer side effects. A series of 2,3-diaryl isoquinolinone derivatives with flexible basic side chains were synthesized and evaluated, the carbonyl of isoquinolinone was expected to function as in indol-2-one, the length of the side chain was also taken into consideration to evaluate its influence on activities (Table 1).

The synthetic route of target 2-(4-alykoxylphenyl)-isoquinolinone derivatives is shown in Scheme 1. The key intermediate, 4-methoxyhomophthalic acid (**3**), was prepared from the commercially available 3-methoxybenzoic acid and chloral hydrate according to literature procedure in high yields.[14] In this procedure, *m*-methoxybenzoic acid was condensed with chloral hydrate to obtain the lactone **1**. This lactone was then reduced by zinc in acetic acid to give dichlorovinyl derivative **2**, and hydrolyzation of **2** in concentrated sulfuric acid finally gave **3**. The 4-methoxyhomophthalic acid was then condensed with anisoyl chloride by heating the intimate mixture of the two at 200 °C to obtain compound **4**. Reflux of compound **4** and *p*-aminophenol in acetic acid for 4 h produced compound **5**. The nucleophilic substitution reaction between compound **5** and various alkyl chlorides in acetone afforded compounds **6a**–**h** in the presence of K_2CO_3. Compounds **7a**–**f** were obtained by demethylation in acetic acid and 40% aqueous hydrobromide acid from compounds **6a**–**h**. The structures of synthesized compounds have been confirmed by IR, NMR and mass spectrometry.

The binding affinities of synthesized compounds with ERα were initially assessed by following a fluorescence polarization procedure[15] with Tamoxifen as the positive control. The results are displayed in Table 2. Most of the target compounds showed good binding affinities with ERα compared with Tamoxifen which

Table 1
Structures of synthesized compounds



Compound	R^3	R^4	*n*	R^1, R^2
6a	CH_3O	CH_3O	2	$-CH_3$, $-CH_3$
6b	CH_3O	CH_3O	2	$-(CH_2)_4-$
6c	CH_3O	CH_3O	2	$-(CH_2)_5-$
6d	CH_3O	CH_3O	2	$-CH_2CH_2N(CH_3)CH_2CH_2-$
6e	CH_3O	CH_3O	2	$-CH_2CH_2OCH_2CH_2-$
6f	CH_3O	CH_3O	2	$-CH_2CH_3$, $-CH_2CH_3$
6g	CH_3O	CH_3O	3	$-(CH_2)_4-$
6h	CH_3O	CH_3O	3	$-(CH_2)_5-$
7a	OH	OH	2	$-CH_3$, $-CH_3$
7b	OH	OH	2	$-(CH_2)_4-$
7c	OH	OH	2	$-(CH_2)_5-$
7d	OH	OH	2	$-CH_2CH_2N(CH_3)CH_2CH_2-$
7e	OH	OH	3	$-(CH_2)_4-$
7f	OH	OH	3	$-(CH_2)_5-$

indicates that the skeleton of 2,3-diaryl isoquinolinone could favorably mimic that of extradiol. The dihydroxyl compounds **7a**–**f** possessed apparently better affinities with ERα than dimethoxyl compounds **6a**–**h**. It was supposed that the dihydroxyl group had formed more hydrogen bonds with related amino acids than dimethoxyl group in the ligand binding domain of ERα. Among the dihydroxyl compounds, **7b**, **7e** and **7f** all held the inhibition rate of more than 95% which were very close to that of Tamoxifen. The IC_{50} of **7b**, **7e** and **7f** valued at 3.1 μM, 2.3 μM and 1.3 μM respectively, while the positive control Tamoxifen valued at 1.9 μM.

Once the binding affinity of our compounds with ERα was confirmed, we conducted the VEGFR-2 kinase inhibition assay. The results are shown in Table 2. From the results, we can see that synthesized compounds exhibited from moderate to strong inhibitory activities compared with Sunitinib. When R^3 and R^4 were changed from methoxyl to hydroxyl (**6a** and **7a**, **6c** and **7c**, **6d** and **7d**), there was an increase in inhibitory activity except for compounds **6b** and **7b** where a decrease in inhibition was observed. There was also an increase in inhibition when the length of side chain was increased by one carbon atom (**6c** and **6g**, **6d** and **6h**, **7c** and **7f**). But a slight decrease was also observed for **7b** and

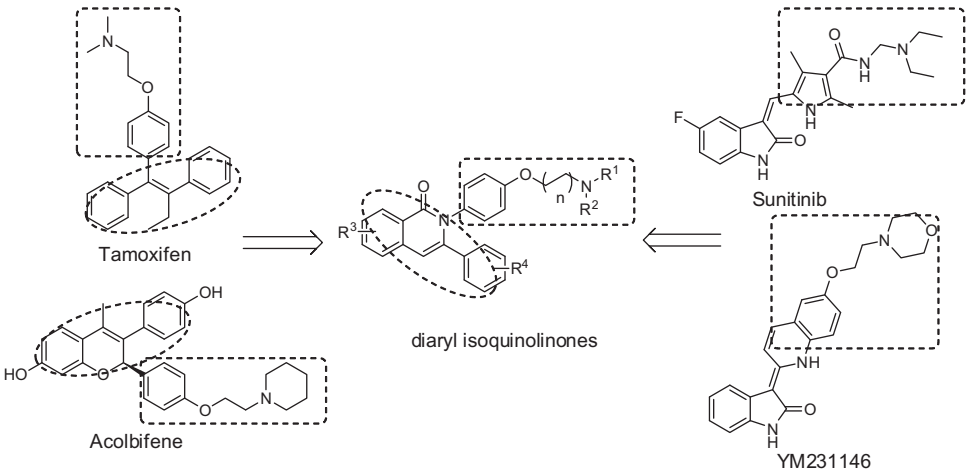
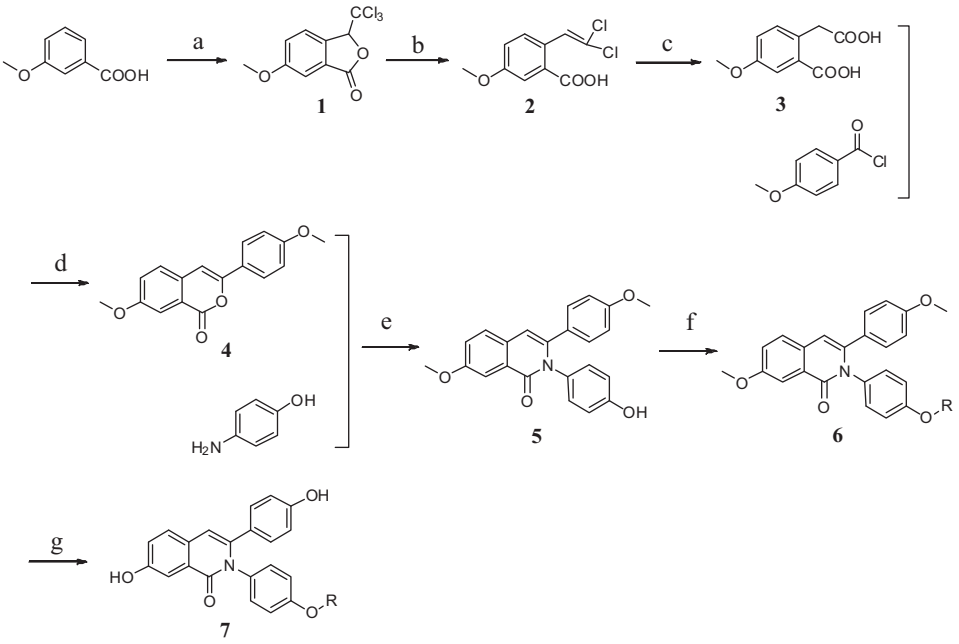


Figure 1. Chemical structures of SERMs, VEGFR-2 inhibitors and designed compounds.



Scheme 1. Reagents and conditions: (a) Chloral hydrate, concd H_2SO_4, rt 12 h; (b) Zn, HOAc, rt 30 min; (c) Concd H_2SO_4, rt 30 min; (d) 200 °C, 6 h; (e) HOAc, reflux, 4 h; (f) $R(CH_2)_nCl$, K_2CO_3, acetone, reflux 12 h; (g) HOAc, 40% HBr, reflux, 24 h.

Table 2
Bioactivities of synthesized compounds

Compound	ERα Inh% (0.1 mg/ml)	MCF-7 IC_{50} (μM)	VEGFR-2 Inh% (0.1 mg/ml)
6a	63.12	N/A	66.07
6b	63.60	863	83.80
6c	72.83	322	65.30
6d	62.09	429	79.43
6e	64.84	N/A	51.19
6f	69.10	383	92.54
6g	57.57	479	98.46
6h	59.65	N/A	72.75
7a	88.57	18.9	79.43
7b	95.93	11.8	49.87
7c	87.14	3.63	84.83
7d	87.94	9.45	103.34
7e	95.70	12.7	48.33
7f	99.89	2.73	100.26
Tamoxifen	100.0	1.89	—
Sunitinib	—	—	100

N/A = not active, the IC_{50} is more than 1000 μM.

7e. Interestingly, **6b**, **7b** and **7e** all have a pyrrolidinyl group in the end of side chain while **7b** and **7e** hold the lowest inhibition rate which is less than 50%. Compound **6e** with morpholinyl group which is same with YM231146 had an inhibition rate of only 51.19% while **6f** with diethylamino group that is same with Sunitinib held the inhibition rate of 92.54%. There seems to be big influence on activity by different basic group. Among these compounds, **7d** and **7f** were shown to be the most potent. Their activities were at the same level with Sunitinib, the IC_{50} value of **7d** and **7f** was 1.9 μM and 1.4 μM respectively with Sunitinib valued at 1.03 μM.

The results in ERα binding and VEGFR-2 kinase inhibition tests have confirmed our idea of designing dual-targeted compounds. We then tested their anti-proliferative activities in vitro against MCF-7 breast cancer cells with Tamoxifen as the positive control.[16] The results are summarized in Table 2. Synthesized compounds demonstrated anti-proliferative activities from close to Tamoxifen to inactive. The activity differences (**7a**–**f** vs **6a**–**h**) in ERα binding affinity assay were also observed in this test. Dihydroxyl compounds led to notably better anti-proliferative activities than dimethoxyl compounds. It seemed that there is no significant influence on activity caused by different length of side chain.

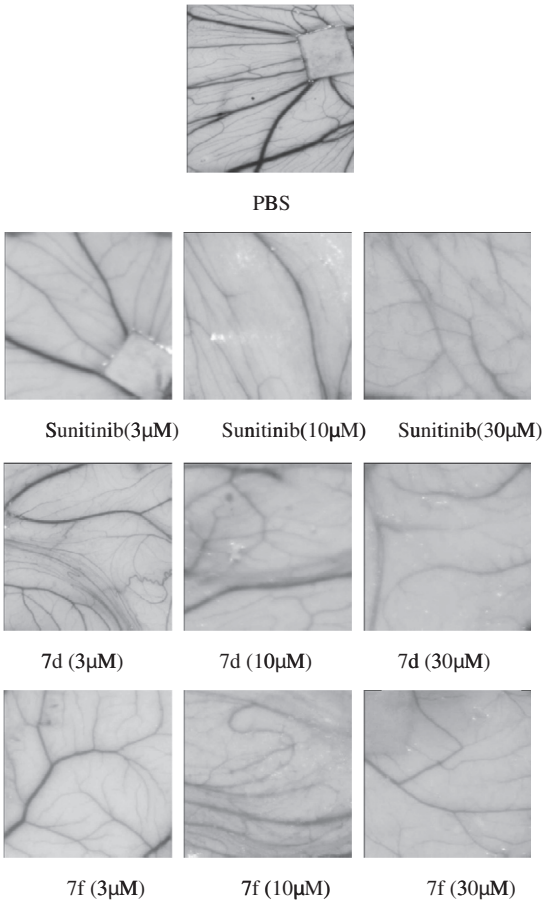


Figure 2. Results of CAM assay.

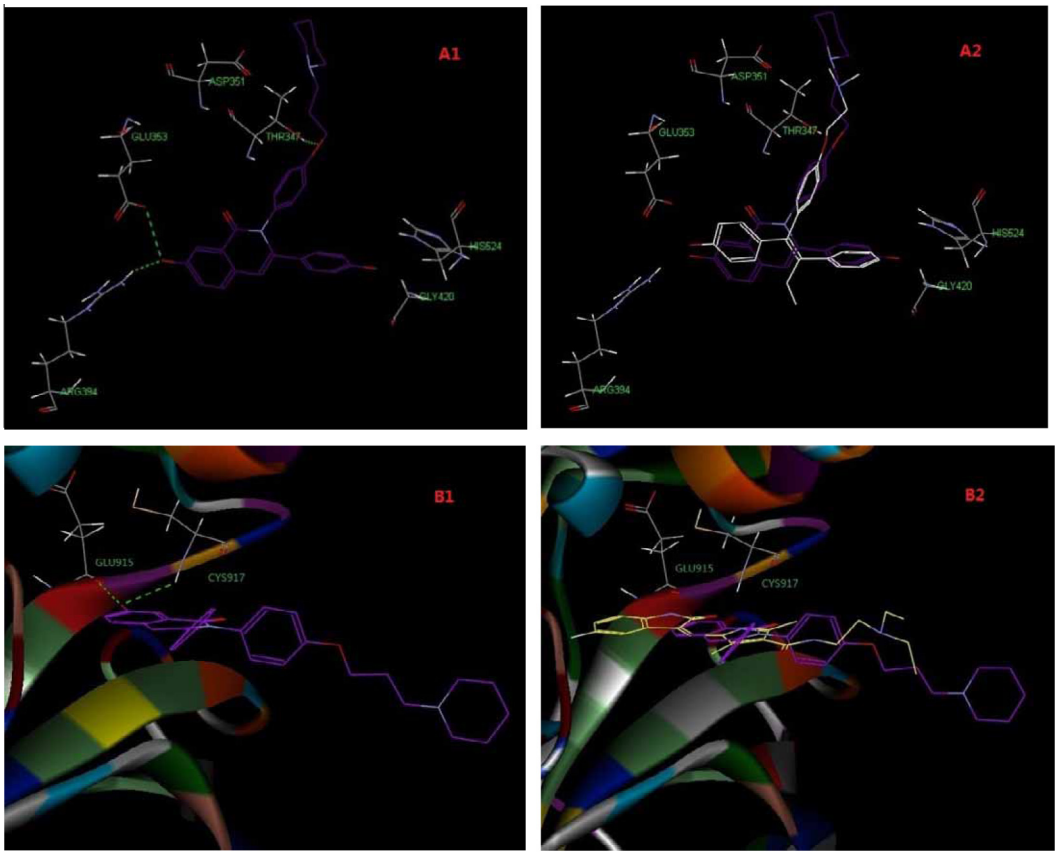


Figure 3. (A) A1: Docking poses of **7f** (purple) within LBD of ERα, A2: Superimposed poses of **7f** and 4-OH-Tamoxifen (white), PDB ID: 3ERT. (B) B1: Docking poses of **7f** (purple) within LBD of VEGFR-2, B2: Superimposed poses of **7f** and Sunitinib (yellow), PDB ID: 4AGD.

Compound **7f** was found to be the most active inhibitor with the IC_{50} value of 2.73 μM which was slightly greater than that of Tamoxifen (IC_{50} = 1.89 μM), following by **7c** with IC_{50} value of 3.63 μM. It was also observed that compound **6e** with morpholinyl in side chain had no activity against MCF-7 breast cancer cells while **7f** and **7c** with piperidyl group turned to be the most potent. Considering the role in ERα binding, basic group as an important bonding site made great contribution to the activity of the compound.

Based on the preliminary results of VEGFR-2 kinase inhibitory activity, compounds **7d** and **7f** were selected to perform chicken chorioallantoic membrane (CAM) assay to investigate their inhibition of angiogenesis in vivo. Test compounds and the positive control Sunitinib dissolved in DMSO were placed on sterile methyl cellulose filter papers at 3 μM, 10 μM and 30 μM with phosphate buffered saline (PBS) as the blank control. Results are shown in Figure 2. Compared with blank control group, compounds **7d** and **7f** could significantly inhibit angiogenesis. And the inhibitory ability was proportional to the concentration. Compared with Sunitinib group, **7d** and **7f** presented comparable inhibitory activity. Overall, compounds showed potential anti-angiogenesis activities in vivo.

To further rationalize the prospective activities of 2,3-diaryl isoquinolinone derivatives against ERα and VEGFR-2, molecular docking studies were performed using the Discovery Studio 2.5/CDOCKER protocol. The docking orientation and interactions of **7f** within the ligand binding domain (LBD) of ERα and VEGFR-2 are shown in Figure 3. In the docking study, the core skeleton of 2,3-diaryl isoquinolinone is favorably positioned similar to 4-hydroxytamoxifen (OHT). The 7-OH of isoquinolinone plays the role of 4-OH of OHT and forms hydrogen bonds with Glu353 and Arg394. The other OH group points toward His524, while the antiestrogenic side chain is projected toward Asp351 (Fig. 3, A1 and A2). In the binding pocket of VEGFR-2, the indol-2-one skeleton of Sunitinib is located into the binding pocket and the side chain stretches toward the edge of the pocket. The hydrogen bond interaction of 1-NH with Glu915 and 2-carbonyl with Cys917 play important role in stabilizing the binding mode. Compound **7f** could enter the binding pocket and form hydrogen bonds with Glu915 and Cys917 with its 7-OH but not 1-carbonyl which was out of our expectation (Fig. 3, B1 and B2). It was supposed that the 3-phenyl become a hindrance for compound to enter the pocket deeply. The side chain of **7f** also stretched toward the edge of the pocket like Sunitinib. Docking simulation suggested possible basis for the observed activities.

In conclusion, we have designed and synthesized a series of ‘-diaryl isoquinolinones targeting both ERα and VEGFR-2. These compounds were expected to perform more effective anti-ERα positive breast cancer effects through inhibition of ERα and VEGFR-2 simultaneously. Biological evaluation showed that most of the synthesized compounds exert ERα binding affinity and VEGFR-2 inhibition. Further investigation also showed they possessed good anti-proliferation effects against MCF-7 breast cancer cells and potential anti-angiogenesis effects in vivo. Compound **7f** was found to be the most potential dual inhibitor and deserved further study. This work may provide a new and potential route to develop effective drugs for 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.2014.03.042. These data include MOL files and InChiKeys of the most important compounds described in this article.

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