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Graphical Abstract

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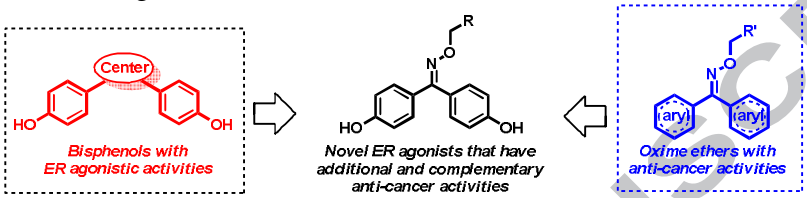

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ABSTRACT

In this study, a series of bis(4-hydroxy)benzophenone oxime ether derivatives such as **12c**, **12e** and **12h** were identified as novel estrogen receptor (ER) agonists that have additional and complementary anti-proliferative activities *via* ER-independent mechanism in cancer cells. These compounds are expected to overcome the therapeutic limitation of existing ER agonists such as estradiol and tamoxifen, which have been known to induce the proliferation of cancer cells.

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Estrogens, endogenous agonists of estrogen receptors (ERs) known as members of the nuclear receptor gene family[1], play crucial roles in diverse physiological processes including reproductive events, depending on the target organs in the reproductive tract, liver, bone, and cardiovascular system.[2,3] Lots of women at postmenopausal stage suffer from postmenopausal syndrome of which symptoms are hot flashes, depression, osteoporosis, atherosclerosis, acute myocardial infarction and other cardiovascular diseases, caused by low estrogen level. To alleviate the symptoms, ER agonists such as estrogens and selective ER modulators (SERMs) have been used for past decades, respectively (Figure 1). Estradiol (**1**) is one of the estrogens and has been used as a representative agent for hormone replacement therapy (HRT) when estrogen deficiency occurs. Recently, its use has been significantly decreased since the report from Women's Health Initiative (WHI) that HRT might be beneficial in patients with postmenopausal syndrome but rather possible to increase the risk of breast cancer due to its ER agonistic activity in breast tissue.[4]

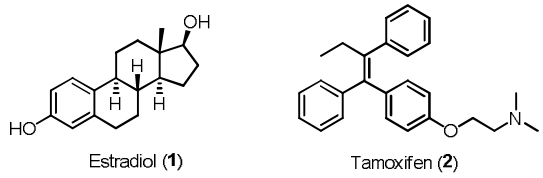


Figure 1. Structures of estradiol (**1**) and tamoxifen (**2**)

SERMs including tamoxifen (**2**) have been also used as therapeutic agents for the treatment of postmenopausal syndrome. They can act as agonists in the majority of tissues for relieving the symptom but antagonists of the ERs in breast tissue for lowering the risk of hormone-dependent ERα positive breast cancer.[5] However, it has been known that tamoxifen (**2**), sometimes used as anti-cancer agents for ER-(+) breast cancer, might be linked to an increased risk of endometrial cancer in some women, due to its partial ER agonistic activity in endometrial tissues.[6]

Thus, discovery of novel ER agonists that can not only alleviate postmenopausal syndrome but also lower the risk of cancers remains necessary to overcome such the therapeutic limitation of existing ER agonists such as estradiol (**1**) and tamoxifen (**2**). Considering that ER-mediated signaling intrinsically increases the risk of cancers by inducing the proliferation of cancer cells in some tissues such as breast and endometrial tissues, it was assumed that the ER agonists having additional and complementary anti-cancer activities *via* ER-independent mechanism, neutralizing their ER-associated cancer cell proliferation, might be desired for efficacious and safe treatment of postmenopausal syndrome and its related diseases.

It has been well known that many ER agonists including daidzein (**3**), and bisphenol A (**4**) are generally made up of a rigid center and two phenol moieties, which form hydrogen bondings with Glu353, Arg394 and His524 in the human ER ligand binding domain (LBD). This interaction is crucial for the activation of ER signaling.[7] Recently, some rigid oxime ether (**5**), (**6**) and (**7**), derived from biaryl ketones have been reported to display anti-proliferative activities in cancer cells, regardless of their various substituents on biaryl moieties or alkyl substituents on oxime ethers.[8] Based on the insights gained from those reports, we envisaged that bis(4-hydroxy)benzophenone oxime ether derivatives comprised of a rigid oxime ether in a center and two phenol moieties as terminal aryl groups could be novel scaffolds which not only have ER agonistic activities but also additional and complementary anti-cancer activities. Thus, it is distinguished from existing ER agonists such as estradiol (**1**) and tamoxifen (**2**), which have been known to induce the proliferation of cancer cells. Herein, we report the identification of novel ER agonists that have additional and complementary anti-proliferative activities *via* ER-independent mechanism in cancer cells. (Figure 2)

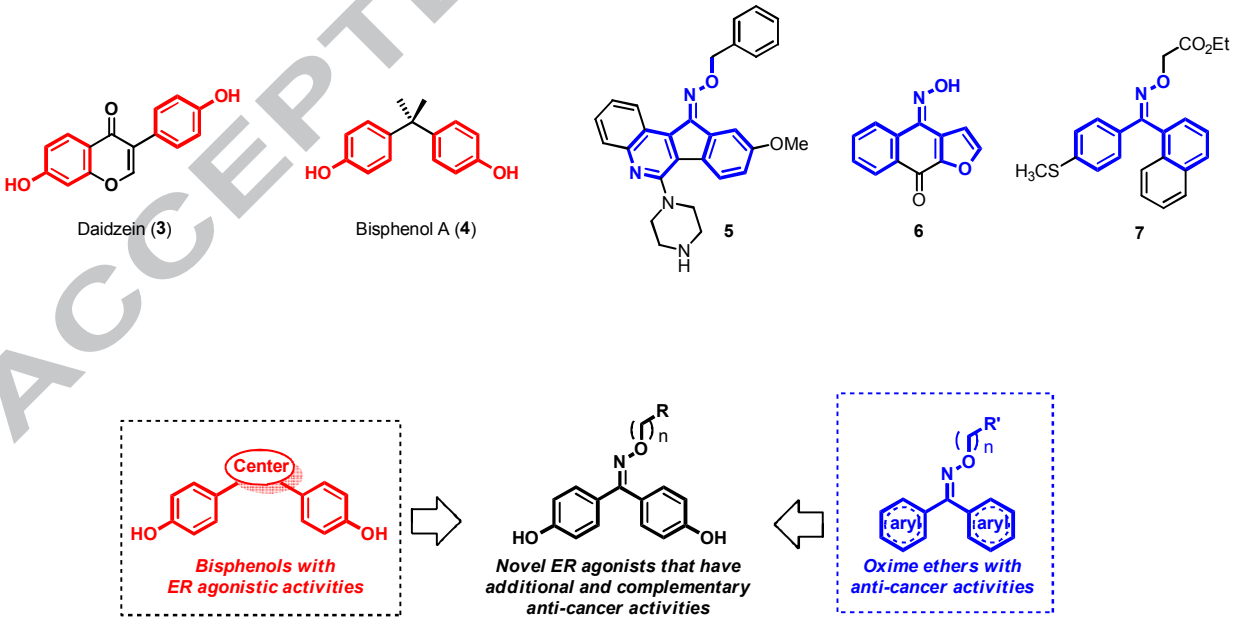
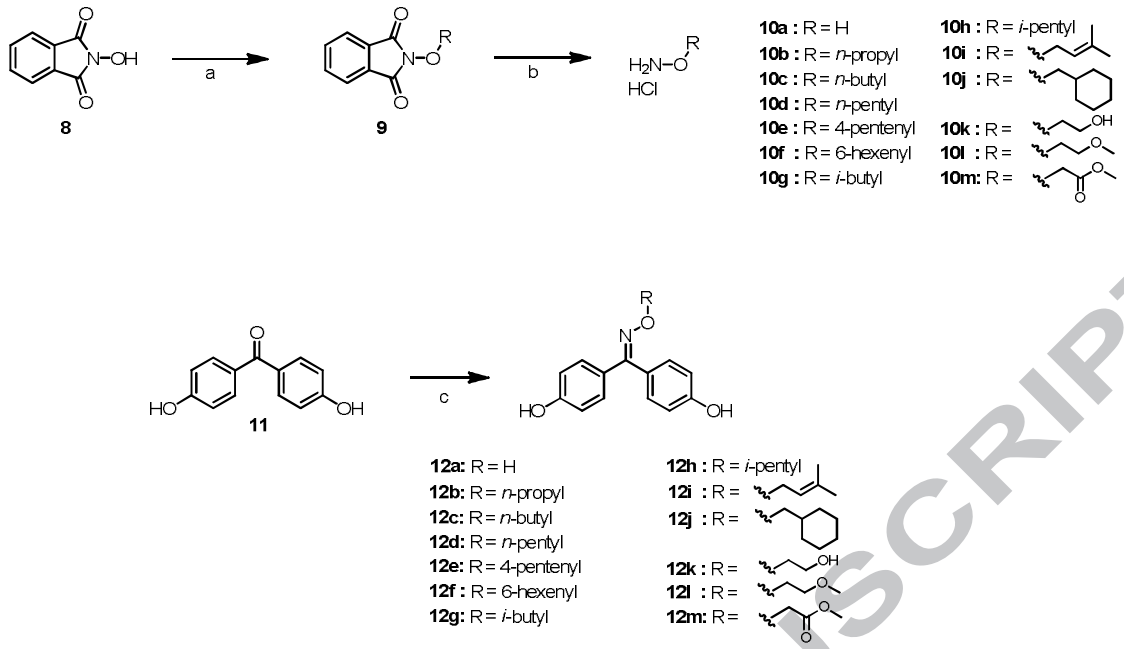


Figure 2. Design of novel estrogen receptor (ER) agonists that have additional and complementary anti-cancer activities



Scheme 1. Synthesis of compounds **12a-m**. Reagents and conditions: (a) alkyl halide, DBU, DMF, rt - 50°C, 59-88%; (b) $NH_2NH_2{\cdot}H_2O$, CH_2Cl_2, then 2*N* HCl in dioxane, 43-96%; (c) **10a-m**, EtOH, 45-100%.

We intended to mainly synthesize a series of bis(4-hydroxy) benzophenone oxime ethers equipped with linear alkyl chains, based on the previous reports that the compounds possessing linear alkyl oxime ether chains showed anti-cancer activities.[8] As outlined in Scheme 1, a variety of compounds were conveniently synthesized from benzophenone **11** *via* a concise unified strategy. Most hydroxylamine HCls were prepared from commercially available *N*-hydroxyphthalimide (**8**), which was transformed into alkoxyphthalimides using DBU and the corresponding alkyl halides.[9] Following imide cleavage and salt formation in the presence of hydrazine monohydrate and HCl provided various alkoxyamine HCl intermediates. Other alkoxyamine salts were available from commercial sources. Concise condensations of the commercially available bis(4-hydroxy)benzophenone **11** with alkyloxyamine salts yielded the desired compounds (**12a-m**).

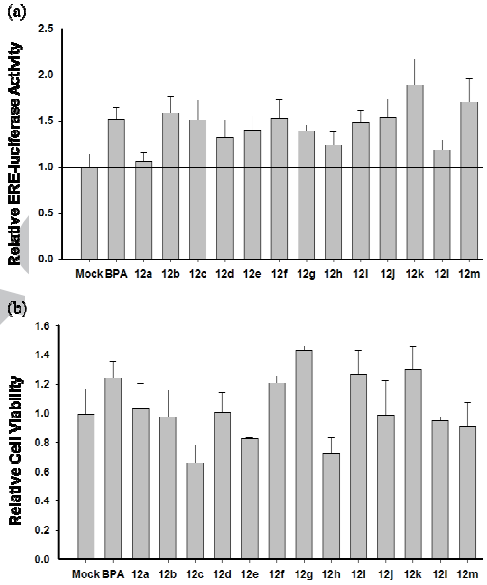
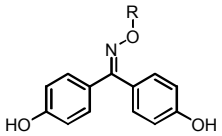


Figure 3. (a) ERE-mediated transcriptional activities of synthesized compounds at 1 μM. (b) Anti-proliferative effects of synthesized compounds on MCF-7 breast cancer cells at 10 μM.

With synthesized compounds in hands, we performed estrogen responsive element (ERE)-luciferase assay to assess the effects of the compounds on their ERE-mediated transcriptional activities and MTT assay to investigate their anti-cancer activities in breast cancer cells. Assay result of the compounds was shown in Figure 3. Most of synthesized compounds showed significant ERE-mediated transcriptional activities, similar to bisphenol A (**4, BPA**), known as one of ER agonists.[10] However, contrary to our expectation, many derivatives did not inhibit and even increase the growth of MCF-7, ER-(+)-breast cancer cells, which was in consistent with the reports that ER agonists could induce proliferation of ER-(+)-breast cancer cells.[4] Notably, a few compounds such as **12c**, **12e** and **12h** did not induce cancer cell proliferation but exhibited moderate anti-proliferative activities on MCF-7 cells. This result implied that these compounds might be novel ER agonists that have additional and complementary anti-cancer activities as we intially designed. On the basis of these results, we selected compound **12c**, **12e** and **12h** as hit compounds for further biological evaluation of their ER agonistic activities and anti-cancer activities.

Table 1. EC_{50}s of **12c**, **12e** and **12h** on ERE-mediated transcriptional activities



Compound	R	EC_{50} (μM)[a]
12c	*n*-butyl	0.075
12e	4-pentenyl	0.046
12h	*i*-pentyl	0.077
BPA (**4**)		0.290

[a]EC_{50} values of the test compounds were estimated from the sigmoidal dose-response curves using SigmaPlot 10.0 software.

To further evaluate the ERE-mediated transcriptional activities of compound **12c**, **12e** and **12h**, we tried to determine EC_{50}s of **12c**, **12e** and **12h**. As summarized in Table 1, their EC_{50} values obtained from the assay were 75, 46 and 77 nM, indicating that they have significant ERE-mediated transcriptional activities, compared to BPA (**4**). With the result, we tried to confirm whether the identified compounds could activate ER mediated signal transduction.

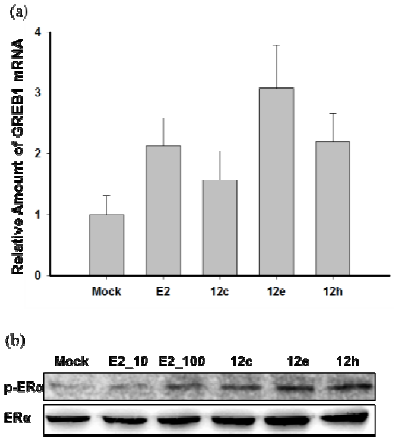


Figure 4. (a) Effects of **12c**, **12e** and **12h** on ER-mediated transcription level of GREB1 in ER-(+) MCF-7 cells at 10 μM. Data are presented as means ± SD. (b) Analysis of ER phosphorylation upon treatment with estradiol (E2, 10 nM, 100 nM), **12c** (10 μM), **12e** (10 μM) or **12h** (10 μM).

Using the quantitative polymerase chain reaction (qPCR) assay, we investigated the capabilities of compound **12c**, **12e** and **12i** to increase the cellular level of Growth Regulation by Estrogen in Breast cancer 1 (GREB1), one of the representative ER responsive gene of which expression was increased by ER activation. As depicted in Figure 4(a), treatment with 10 μM of the compounds resulted in the increase of mRNA level of GREB1 similar or more, compared to the treatment with estradiol.

To confirm ER agonistic activities of the compounds, we also examined the ERα phosphorylation, a hallmark of ER activation. As shown in Figure 4(b), treatment with **12c**, **12e** or **12h** induced significant phosphorylation of ERα, indicating its activation. Based on the all of above results, it was concluded that **12c**, **12e** and **12h** would be potent ER agonists.

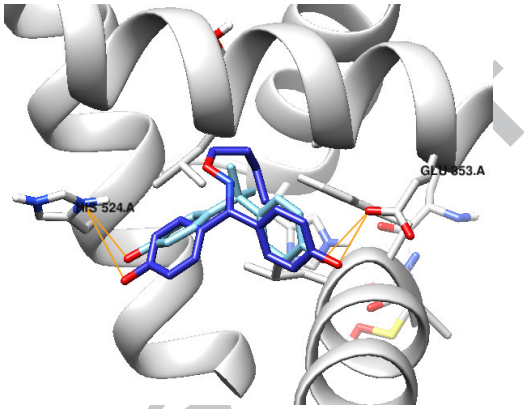


Figure 5. X-ray structure of **4** (Cyan, PDB Code: 3UU7) and molecular docking model of **12c** with ERα (Purple, PDB code: 3UU7), which were visualized using Chimera 1.10 (UCSF Chimera)[11]

To investigate the binding modes of bis(4-hydroxy) benzophenone derivatives which were identified as novel and potent ER agonists in our study, docking analysis of compound **12c** within hER LBD active site was performed using the Autodock 4.2 program (Molecular Graphic Laboratory).[12] As illustrated in Figure 5, **12c** fitted well into the active site. The estimated free energy of binding was calculated as -8.19 kcal/mol. It was plausible that two phenol moieties in our compounds interact the hER LBD through hydrogen bonding with Glu353 and His 524, respectively, whereas *n*-butyl group occupies a lipophilic pocket which was surrounded by Thr 347, Leu346, Leu 384 and Trp 383 and makes van der waals interaction with those residues. Thus, the binding mode of our compound was postulated in accordance with that of bisphenol A (**4**).

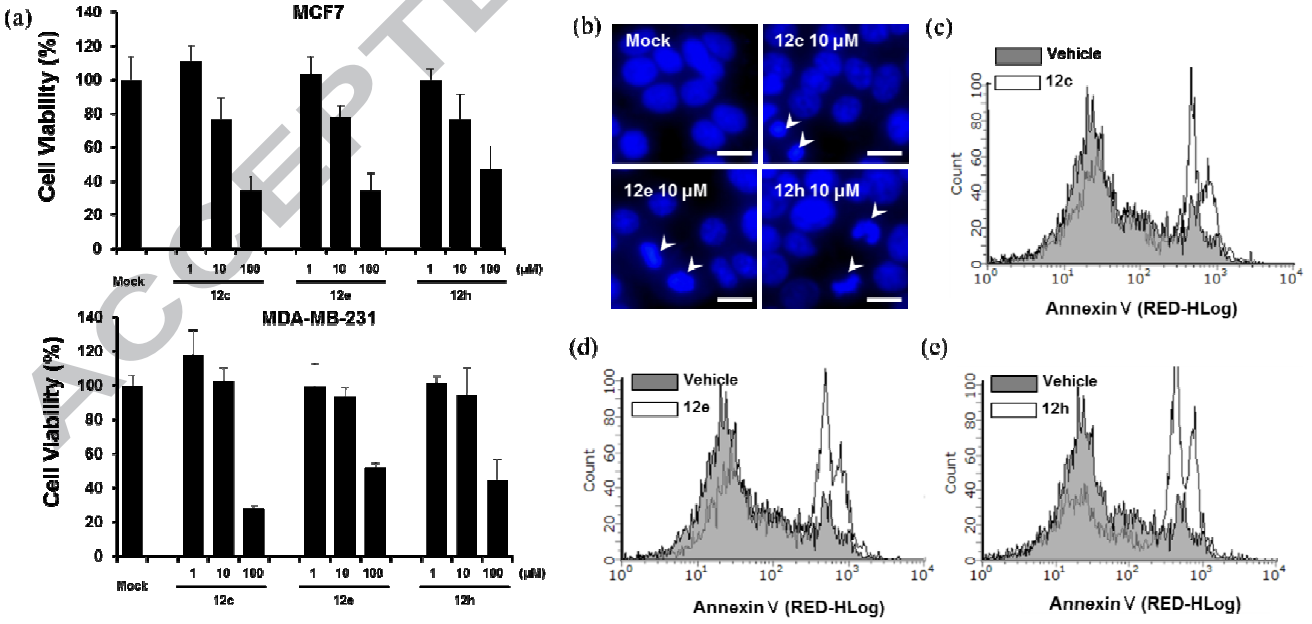


Figure 6. Anti-proliferative activities of **12c**, **12e** and **12h** against breast cancer cells. (a) Dose-dependent anti-proliferative activities of **12c**, **12e** and **12h** against MCF-7 and MDA-MB-231 breast cancer cell lines as determined by MTT assays. (b) DAPI staining of MCF-7 cells treated with **12c**, **12e** and **12h** at 10 μM. Scale bar: 10 μm. Arrows indicate condensed nuclei. (c)-(e) Analysis of apoptosis of MCF-7 cells treated with **12c** (c), **12e** (d) and **12h** (e) at 10 μM by Annexin V staining.

With the result that **12c**, **12e** and **12h** showed potent ER agonistic activities, we turned our attention to further study of their additional and complementary anti-cancer activities, neutralizing ER-mediated cell proliferation in cancer cells. Through the MTT assay results (Figure 3), **12c**, **12e** and **12h** were found to have anti-cancer activities in MCF-7, breast cancer cells. To investigate whether their anti-cancer activities are associated with ER dependent mechanism, we performed MTT assay with increasing concentrations at ER-(+) or ER-(-) cancer cells. These compounds inhibited the growth of MCF7, ER-(+) breast cancer cells in dose dependent manners. Notably, the compounds also showed dose dependent anti-proliferative activities toward MDA-MB231 cells, ER-(-) breast cancer cell lines, indicating that anti-cancer activities of these compounds might not be resulted from their ER-mediated signaling but from ER-independent signaling (Figure 6(a)). Next, we have examined whether anti-cancer activities of these compounds were caused by the induction of apoptosis. As shown in Figure 6(b), DAPI staining of MCF-7 cells treated with 10 μM of the compounds showed the morphological changes including membrane blebbing, or condensed nuclei, which have been known as typical features of apoptosis in cancerous cells. To further evaluate the apoptotic cell death by the compounds, additional analysis of MCF7 cells treated with **12c**, **12e** and **12h** was performed. As shown in Figure 6(c)-(e), the cell death of cancer cell, caused by the compounds, was associated with apoptosis as determined by AnnexinV staining, followed by flow cytometry analysis. Moreover, increment of subG1 population in **12c**, **12e** and **12h** treated cells were observed in cell cycle analysis by PI staining. All of these results indicated that the compounds have anti-proliferative activities by inducing ER-independent apoptosis of cancer cells (Figure 7).[13]

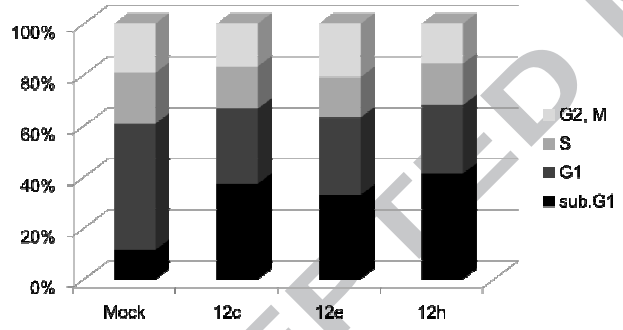


Figure 7. Cell cycle analysis of MCF-7 cells treated with **12c**, **12e** and **12h** at 10 μM by PI staining

In summary, we identified a series of bis(4-hydroxy)benzophenone oxime ether derivatives as ER agonists that have additional and complementary anti-proliferative activities *via* ER-independent mechanism in cancer cells, based on rational design, convenient synthetic approaches and biological evaluation. In particular, the compounds such as **12c**, **12e** and **12h** showed significant estrogenic activities but inhibit the growth of cancer cells through ER-independent mechanism, which is distinguished from existing ER agonists such as estradiol and tamoxifen, which have been known to induce the proliferation of cancer cells. Further work for the development of therapeutically useful novel ER modulators based on our current study is in progress.

Acknowledgments

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References and notes

1. (a) Green, S.; Walter, P.; Kumar, V.; Krust, A.; Bornert, J. M.; Argos, P.; Chambon, P. *Nature* **1986**, *320*, 134; (b) Kuiper, G. G.; Enmark, E.; Pelto-Huikko, M.; Nilsson, S.; Gustafsson, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, 93, 5925.
2. Dahlman-Wright, K.; Cavailles, V.; Fuqua, S. A.; Jordan, V. C.; Katzenellenbogen, J. A.; Korach, K. S.; Maggi, A.; Muramatsu, M.; Parker, M. G.; Gustafsson, J. A. *Pharmacol. Rev.* **2006**, 58, 773.
3. Couse, J. F.; Lindzey, J.; Grandien, K.; Gustafsson, J. A.; Korach, K. S. *Endocrinology* **1997**, 138, 4613.
4. (a) Rossouw, J. E.; Anderson, G. L.; Prentice, R. L.; LaCroix, A. Z.; Kooperberg, C.; Stefanick, M. L.; Jackson, R. D.; Beresford, S. A.; Howard, B. V.; Johnson, K. C.; Kotchen, J. M.; Ockene, J.; *JAMA.* **2002**, 288, 321. (b) Chlebowski, R. T.; Hendrix, S. L.; Langer, R. D.; Stefanick, M. L.; Gass, M.; Lane, D.; Rodabough, R. J.; Gilligan, M. A.; Cyr, M. G.; Thomson, C. A.; Khandekar, J.; Petrovitch, H.; McTiernan, A. *JAMA* **2003**, 289, 3243. (c) Kelly, J. P.; Kaufman, D. W.; Rosenberg, L.; Kelley, K.; Cooper, S. G.; Mitchell, A. A. *Pharmacoepidemiol. Drug Saf.* **2005**, 14, 837.
5. (a) Shang, Y.; Brown, M. *Science* **2002**, 295, 2465. (b) Black, L. J.; Sato, M.; Rowley, E.; Magee, D.; Bekele, A.; Williams, D.; Cullinan, G.; Bendele, R.; Kauffman, R.; Bensch, W. *J. Clin. Invest.* **1994**, 93, 63. (c) Jordan, V. C. *J. Med. Chem.* **2003**, 46, 883. (d) Jordan, V. C. *J. Med. Chem.* **2003**, 46, 1081. (e) Maximov, P. Y.; Lee, T. M.; Jordan, V. C. *Curr. Clin. Pharmacol.* **2013**, 8, 135.
6. (a) Fisher, B.; Costantino, J. P.; Redmond, C. K.; Fisher, E. R.; Wickerham, D. L.; Cronin, W. M. *J. Natl. Cancer Inst.* **1994**, 86, 527. (b) Mourits, M. J.; De Vries, E. G.; Willemse, P. H.; Ten Hoor, K. A.; Hollema, H.; Van der Zee, A. G. *Obstet. Gynecol.* **2001**, 97, 855. (c) Cohen, I. *Gynecol. Oncol.* **2004**, 94, 256.
7. Jordan, V. C. *Cancer Cell* **2004**, 5, 207.
8. (a) Tseng, C.-H.; Chen, Y.-L.; Lu, P.-J.; Yang, C.-N.; Tzeng, C.-C. *Bioorg. Med. Chem.* **2008**, 16, 3153. (b) Tseng, C.-H.; Chen, Y.-L.; Yang, S.-H.; Peng, S.-I.; Cheng, C.-M.; Han, C.-H.; Lin, S.-R.; Tzeng, C.-C. *Bioorg. Med. Chem.* **2010**, 18, 5172. (c) Chakravarti, B.; Akhtar, T.; Rai, B.; Yadav, M.; Akhtar Siddiqui, J.; Dhar Dwivedi, S. K.; Thakur, R.; Singh, A. K.; Singh, A. K.; Kumar, H.; Khan, K.; Pal, S.; Rath, S. K.; Lal, J.; Konwar, R.; Trivedi, A. K.; Datta, D.; Mishra, D. P.; Godbole, M. M.; Sanyal, S.; Chattopadhyay, N.; Kumar, A. *J. Med. Chem.* **2014**, 57, 8010.
9. Kim, J. N.; Kim, K. M. Ryu, E. K. *Synth. Commun.* **1992**, 22, 1427.
10. Matsushima, A.; Liu, X.; Okada, H.; Shimohigashi, M.; Shimohigashi, Y. *Environ. Health Perspect.* **2010**, 118, 1267.
11. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.;Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, 25, 1605.
12. Morris, G. M.; Huey, R.; Lindstrom, A.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. *J. Comput. Chem.* **2009**, 30, 2785.
13. Compound **12h** also showed the anti-cancer effects on endometrial cancer cells *via* ER-independent mechanism. Kim, H. I.; Kim, T.; Kim, J. E.; Lee, J.; Heo, J.; Lee, N. R.; Kim, N. J.; Inn, K. S. *Int. J. Oncol.* **2015**, 47, 280.

Supplementary Material

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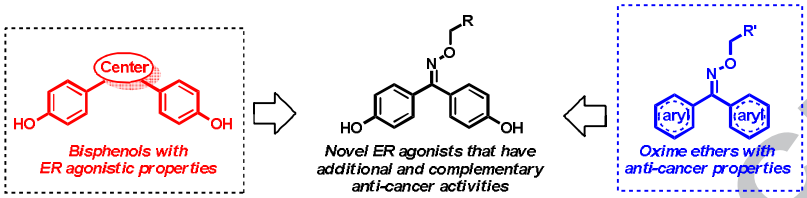

In this study, we identified a series of bis(4-hydroxy)benzophenone oxime ether derivatives as **novel ER agonists that have additional and complementary anti-proliferative activities via ER-independent mechanism** in cancer cells, based on rational design, convenient synthetic approaches and biological evaluation. In particular, the compounds such as **12c**, **12e** and **12h** showed significant estrogenic activities but inhibit the growth of cancer cells through ER-independent mechanism, which is **distinguished from existing ER agonists such as estradiol and tamoxifen, which have been known to induce the proliferation of cancer cells**.