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Synthesis, characterization, and estrogen receptor binding affinity of flavone-, indole-, and furan-estradiol conjugates

Naseem Ahmed, Celena Dubuc, Jacques Rousseau, Francois Bénard and Johan E. van Lier*

Department of Nuclear Medicine and Radiobiology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada J1H 5N4

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Abstract—Different flavone-, indole-, and furan-17β-estradiol conjugates, linked via alkyl spacer chains extending from the 17α-position of the estradiol moiety, were synthesized by Pd-catalyzed cross-coupling reactions. Structures were assigned based on spectroscopic data. In vitro competitive binding assays for the estrogen receptor (α-ER), using [^{3}H]estradiol (RBA = 100) as a competitor, revealed that a two-carbon alkyl linker combined with a flavone conjugate provided the highest binding affinity (RBA ~ 9), warranting further studies on their potential use as selective estrogen-receptor modulators (SERMs) for hormone-replacement therapies.

Breast cancer remains a major cause of death for women in the western world (2–10 times higher than for oriental women) with the global incidence estimated at 1.15 million in 2002.[1] The female sex hormones, estrogens, play a central role in the development of breast tumors. Many of these tumors are initially hormone-responsive and circulating estrogens have a vital role in their growth. Approximately 80% of breast cancer cases occur in post-menopausal women whose ovarian estrogen production has ceased with remaining estrogens originating in extra-glandular tissues.

Estrogen receptors (ER) are proteins present in target cells in very low concentrations (1000–10,000 molecules per cell).[2] Unlike circulatory estrogen binding proteins (with high capacity and low binding specificity) that aid in transporting steroids, the ER have low capacity but are highly specific in binding selected steroid hormones. The mechanism by which steroids and their receptors work together to effect their physiological functions is well known.[3] During the past decades bioconjugates, that is, biomolecules bearing unnatural organic structures, have found an increasing number of applications in molecular and cell biology.

Conjugated groups provide biomolecules with novel properties, such as fluorescence emission, catalytic activity, altered hydrophobicity or bio-affinity, resistance toward biodegradation or the ability to carry metal ions.[4] Many naturally occurring steroid hormones[5] and non-steroidal[6] derivatives are recognized by steroid hormone receptors (SHRs) either as agonists or antagonists depending on their interaction with the SHR. Both agonists or antagonists are used for the treatment of hormone-dependent breast cancers (HDBCs).[5,6]

Phytoestrogens are plant-derived compounds that structurally or functionally mimic mammalian estrogens. Similar to the numerous effects of estrogens on the human body, these are known to exert broad beneficial effects toward human health, especially against cancer, osteoporosis, irregular menopause syndrome, cardiovascular disease, and possibly neuro-degenerative diseases. Recently, the prenylated flavanone, 8-prenylnaringenin (8-PN) derived from hops (*Humulus lupulus* L.), has been identified as a potent estrogen showing the highest in vitro estrogenic activity among all phytoestrogens known to date.[7] Furthermore, some phytoestrogens, indoles, and furans have been shown to function as selective estrogen-receptor modulators (SERMs), eliminating

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* Corresponding author. Tel.: +1 819 564 5409; fax: +1 819 564 5442; e-mail: johan.e.vanlier@USherbrooke.ca

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unwanted side-effects often observed with synthetic estrogens used in hormone-replacement therapies.[8] However, the non-selectivity of phytoestrogens and their acute toxicity toward rapidly proliferating healthy tissues has deterred their use as antitumor agents.

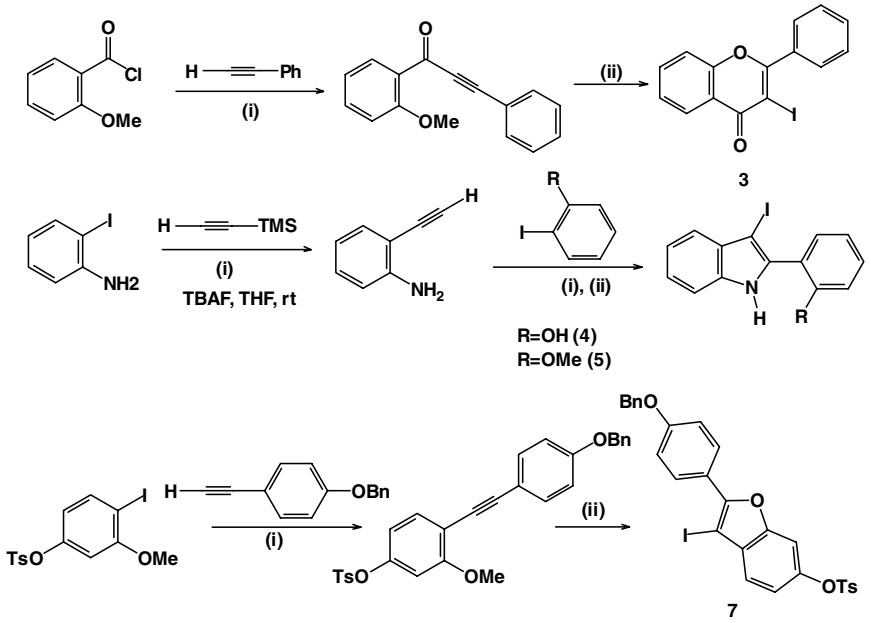
One approach to overcome this non-selectivity is to couple the phytoestrogen to a carrier with strong affinity for a particular tissue from which the tumor is derived.[9a] This might enable the selective delivery of the cytotoxic agent to the cells or tissues bearing a high concentration of binding sites for the carrier.[9b,c] To target breast cancer, estrogens are of particular interest since the majority of breast cancers are ER-positive and markedly concentrate estrogens within the cell nucleus.[9d] Several attempts have been made to utilize this approach by conjugating estradiol with alkylating moieties such as nitrogen mustards,[10a] nitrosoureas,[10b] aziridines,[10c] dichloroplatinum.[10d] Such conjugates exhibit antitumor activity and also possess sufficient binding to the ER, allowing selective accumulation of the conjugates in ER-rich cells. Several conjugates of 17β-estradiol (E2) linked directly or indirectly through an alkyne-type rigid spacer at the 17α-position have been advanced as synthetic ligands for therapeutic applications targeting the ER.[11]

In search for agents that would be useful for the management of estrogen-dependent pathologies, we previously synthesized steroid-nucleoside conjugates and tested them for their ER-binding affinity and cytotoxicity toward ER-positive animal tumors.[12] Here we report a similar approach to improve the binding affinity of phytoestrogens for the ER by conjugating a series of flavone, indole, and furan derivatives with estradiol attached to the 17α-position via alkyne chains of varying lengths.

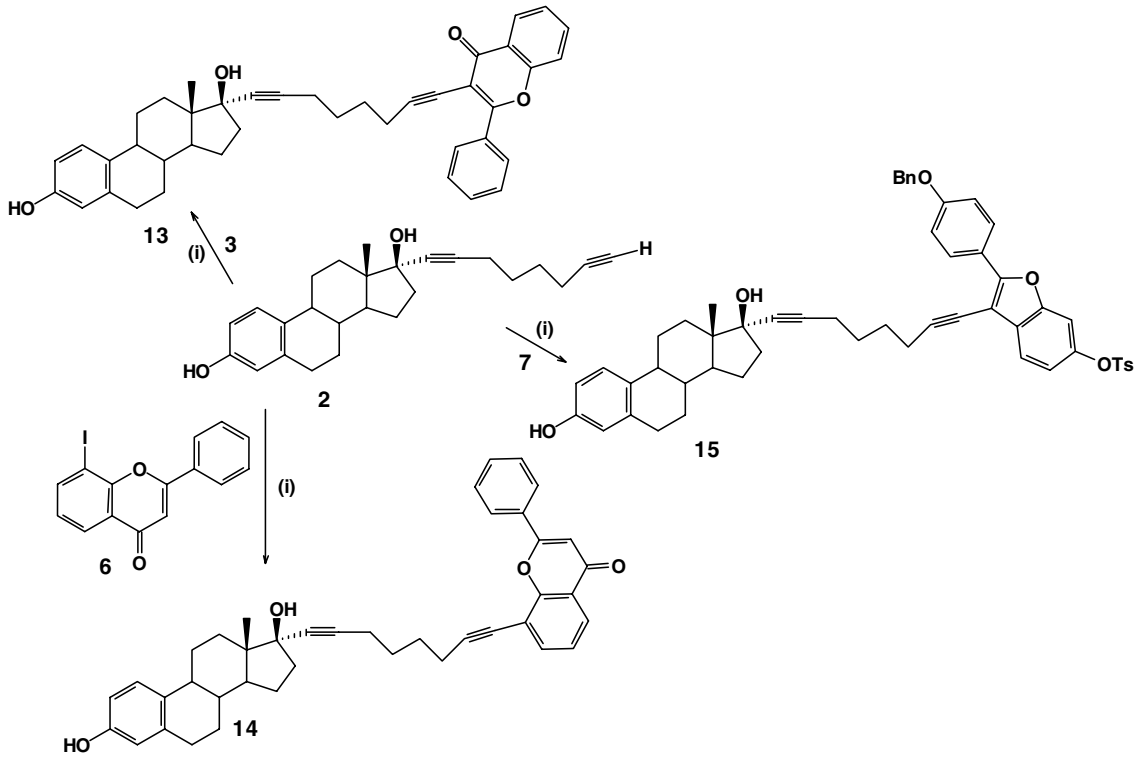
The alkynone intermediates were prepared via Pd-catalyzed Sonogashira cross-coupling reactions and subsequent iodine or monochloroiodine-induced cyclization of the alkynone to give the iodoflavone (**3**) and iodofuran (**7**) in high yields (Scheme 1).[13] However, using a similar approach to prepare the iodoindoles (**4** and **5**) gave the latter in low yields (10–20%) likely due to the presence of the free hydroxyl and amino groups on the substrate. In the case of iodoflavone **6**, iodination was accomplished by heating equimolar amounts of flavone (commercially available), I_2, and $Cu(OAc)_2$ at 60 °C in acetic acid for 2–3 h. All products were characterized on the basis of their spectroscopic data and comparison with literature values.[13] Based on the high electrophilic nature of the C-8 of the A-ring, the iodine in compound **6** is assigned to C-8 position, although this is difficult to confirm by ^{1}H NMR due to the clustering of the aromatic protons.

We previously showed that in the case of steroid-nucleoside conjugates increasing the length of the carbon spacer chain at the 17α-position enhances ER-binding affinities.[12a] Therefore, we synthesized 1,7-octadiynyl-17α-estradiol (**2**) following the above procedure and subsequent conjugation with **3**, **6**, and **7** using the Pd-catalyzed reaction to yield the desired products **13**–**15** in good yield (Scheme 2). All conjugated products were characterized on the basis of their spectroscopic data (^{1}H NMR, EIMS, and HR MS).[14]

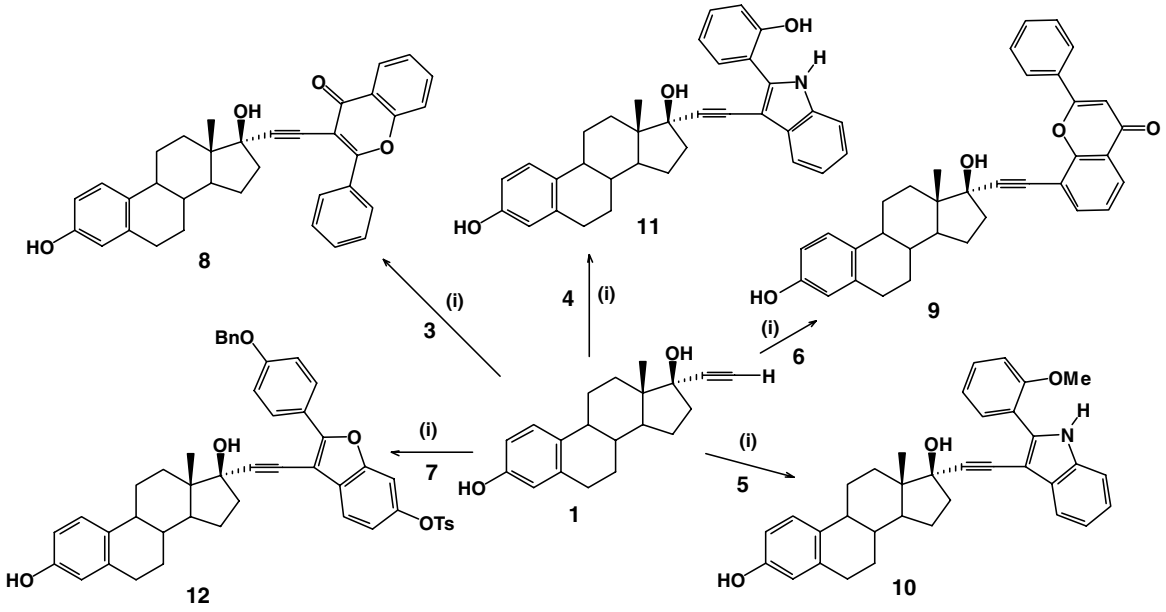
The syntheses of E2 conjugates **8**–**12** were accomplished by employing a palladium-catalyzed Sonogashira cross-coupling reaction using equimolar amounts of 17α-ethynylestradiol (**1**) and products **3**, **4**, **5**, **6** or **7** (Scheme 3) in the presence of CuI (0.04 mol) and $Pd[PPh_3]_4$ (0.02 mol) as catalysts and triethylamine base refluxed at 70 °C for 3 h under an argon atmosphere. After cool-



Scheme 1. Reagents and conditions: (i) $PdCl_2(PPh_3)_2$ (2 mol%), CuI (1 mol%), Et_3N, THF, rt, 16 h; (ii) I_2 or ICl, CH_2Cl_2, rt, 10–15 min.



Scheme 2. Reagents and conditions: (i) $Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), Et_3N, THF, 70 °C, 3 h.



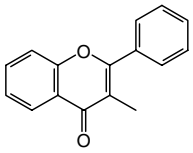
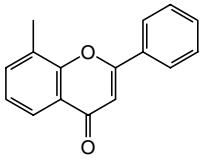
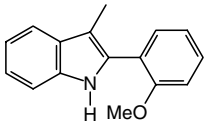
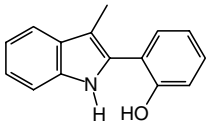
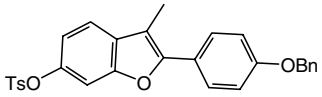
Scheme 3. Reagents and conditions: (i) $Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), Et_3N, THF, 70 °C, 3 h.

ing, the solvent was evaporated and the residue was purified by flash chromatography using 20–40% EtOAc/hexane to give the corresponding desired products **8**–**12**.

Relative binding affinity (RBA). Relative binding affinities (RBA) were measured by a competitive assay against [^{3}H]estradiol (concentration: 0.6 pM/well) using purified ERα (Panvera, Madison, WI) and FlashPlate technology.[15] The RBA values, taking estradiol as 100, are summarized in Table 1.

In addition to the nature of the phytoestrogen and length of the spacer chain, steric effects and changes in the overall molecular conformation of the estradiol conjugates also modulate the binding affinity for the ER (Table 1). Among the different heterocyclic moieties the flavone conjugate **8**, featuring a C-2 tether, exhibits

Table 1. Relative binding affinities (RBA) and K_i-values for ERα of flavone-, indole- and furan-estradiol conjugates

Heterocycle moiety	E2 conjugate	RBA[a] (C-2 tether)	RBA[a] (C-8 tether)	K_i (nM)
—	Estradiol	100	100	12
	**8**	9.17		37
	13	—	0.01	63,052
	**9**	0.92		100
	14	—	0.24	382
	**10**	8.71	—	35
	**11**	4.05	—	75
	**12**	0.80		332
	15	—	0.01	17,879

[a] Relative binding affinities (RBA) of the estradiol conjugates for the ERα were determined by a competitive [^{3}H]estradiol binding assay using Flash Plate technology. Conjugates (0.1–10 μM) were incubated at 25 °C for 1–2 h to establish EC_{50} values from which the RBA were calculated, taking estradiol as unity.

the highest binding affinity (RBA = 9.17), followed by the indole conjugate **11** (RBA = 4.05). Increasing the length of the spacer consistently diminishes the binding affinity (i.e., **8** vs **13**, **9** vs **14**, and **12** vs **15**). This contrasts our earlier findings with a series of nucleoside-17β-estradiol conjugates, where an increase in the length of the spacer augmented the binding affinities.[12a] Blocking the hydrogen bonding capacity of the indole moiety (e.g., OMe in **10** vs an OH in **11**) increases the binding affinity twofold suggesting that the latter improves interaction with the receptor. Varying the position of attachment in the estradiol–flavone conjugates (i.e., **8** vs **9** and **13** vs **14**) also strongly affects RBA values likely reflecting steric effects and changes in the molecular conformation. Overall our data indicate that the lengths of the spacer chain combined with steric effects are important factors affecting the ER binding capacity for these estradiol–phytoestrogen conjugates.

In conclusion, we have explored the synthesis and receptor binding properties of a new series of estradiol–phytoestrogen conjugates as a core scaffold for developing novel ER ligands with potential therapeutic applications. Some of these conjugates retain good binding affinity for the ER and the entailing SAR merit further examination of this class of conjugates to develop improved ER ligands for steroid hormone therapy.

Acknowledgments

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14. *Spectroscopic data*: Compound EI/HRMS *m/z*: **8/9**, 516.2301 (Calcd for $C_{35}H_{32}O_4$), $(M)^+$ 516.2201; **10**, 517.2617 (Calcd for $C_{35}H_{35}NO_3$), $(M+H)^+$ 518.2568; **11**, 503.2460 (Calcd for $C_{34}H_{33}NO_3$), $(M+H)^+$ 504.2259; **12**, 764.2808 (Calcd for $C_{48}H_{44}SO_7$), $(M)^+$ 764.2811; **13/14**, 596.2927 (Calcd for $C_{41}H_{40}O_4$), $(M+2H)^+$ 598.2898; **15**, 844.3434 (Calcd for $C_{55}H_{54}SO_7$), $(M)^+$ 844.3416.
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