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Synthesis and Estrogen Receptor Binding Affinities of 7-Hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-ones Containing a Basic Side Chain

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Abstract—Two isoflavones containing a sulfur or oxygen hinge with an amine-bearing side chain have been designed and synthesized as potential selective estrogen receptor modulators. The target compounds exhibited low affinities for estrogen receptors (ERs), and binding affinity data indicate that oxygen hinge is more favorable than sulfur for binding. These compounds also displayed selectivity for ERα over ERβ.

Over the past few years, our group has been interested in the 4*H*-1-benzopyran-4-one ring system (Fig. 1) as a core of potential therapeutic agents for the treatment of hormone-dependent breast cancer.[1] This ring system can be found in a number of natural products termed flavonoids. Compounds in this class have demonstrated numerous biological activities such as antiviral, anti-inflamatory, antiallergic, antimutagenic and anti-carcinogenic activities.[2] In breast cancer cells, in particular, numerous flavonoids have shown interesting pharmacological activities including binding affinities for estrogen receptors (ERs),[3] antiproliferative activities,[4] and inhibitory activities against aromatase enzyme.[5]

One isoflavonoid, genistein (Fig. 1), has been extensively studied. Genistein is a weak phytoestrogen and has structural characteristics in common with the non-steroidal estrogen pharmacophore, that is, two phenolic groups separated by approximately 11–12 Å planar core.[6] It is therefore not surprising that it shows reasonable binding affinities for ERs and, more importantly, is able to discriminate between two ER subtypes (ERα and ERβ).[7] Genistein also inhibits proliferation of various breast cancer cell lines including MCF-7, T47D, MD-MBA-231, and SKBR3 by several mechanisms of action.[6,8]

Based on these findings, we became interested in identifying a new series of selective estrogen receptor modulators (SERMs) constructed on an isoflavone scaffold. SERMs are a class of nonsteroidal ER ligands with mixed estrogen agonistic/antagonistic activity and are represented by raloxifene (Fig. 2).[9,10] The amine-bearing side chain of raloxifene is reported to play a key role for SERM activity by preventing the proper positioning of helix 12 for agonistic activity.[11] For this reason, most published SERMs contain a similar amine-bearing side chain.[10]

We have initially designed several isoflavone analogues containing the basic side chain of raloxifene (Fig. 2). However, compound **A** has already been synthesized by another research group.[12] Therefore, we have focused on compounds **1** that contain a sulfur or oxygen as an isostere of the carbonyl group. We envisioned these heteroatoms could serve as a hinge to direct the basic side chain to the proper region in the binding pocket of the ER for the SERM profile.[11] Herein we wish to describe the synthesis of compounds **1** and their binding affinities for ERs as preliminary biological data.

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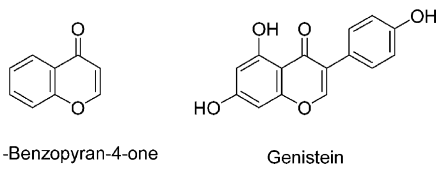


Figure 1. Structures of genistein and its core ring.

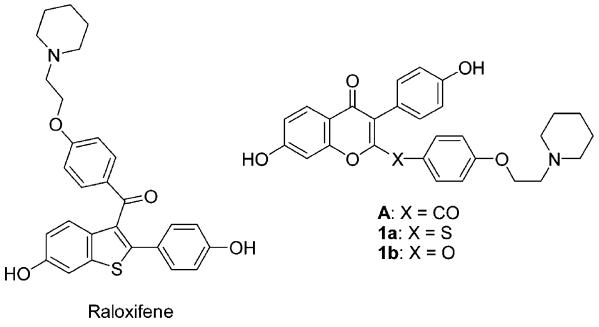
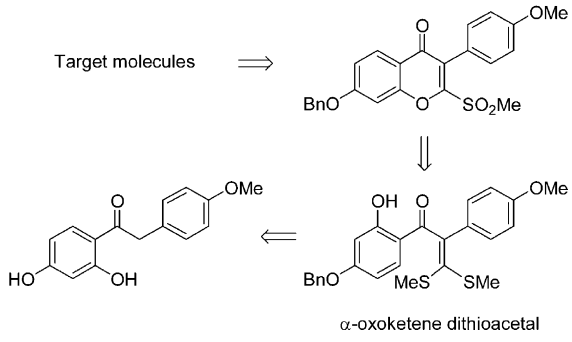


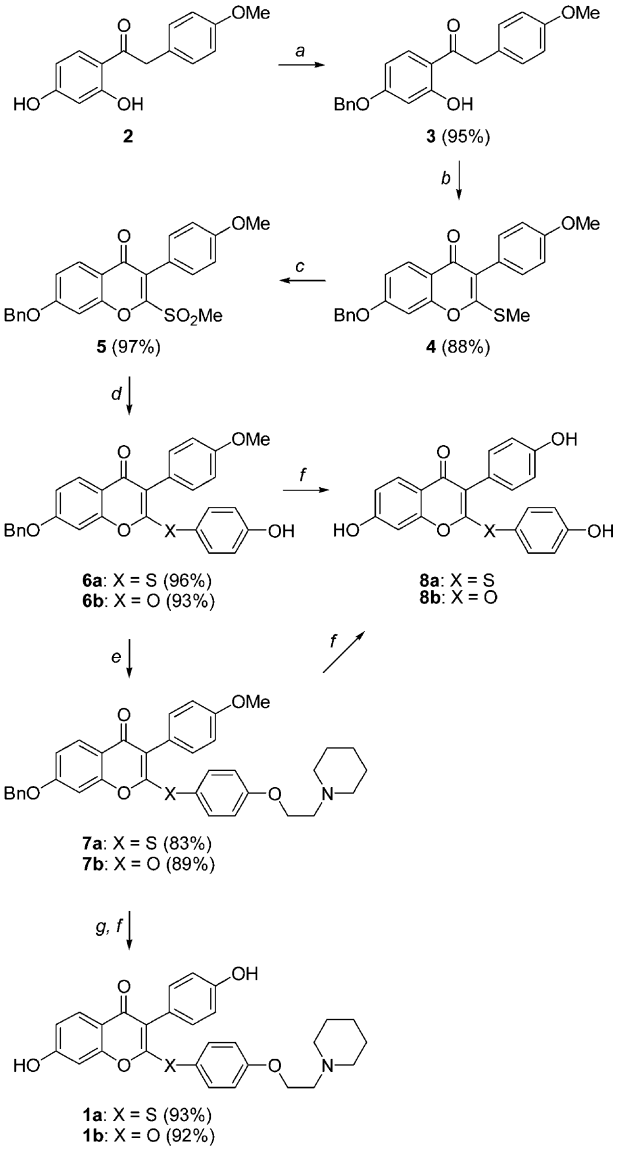
Figure 2. Structures of raloxifene and target molecules.

Many synthetic methods have been reported for the synthesis of flavonoids.[13] These previously reported methods are not ideally suited for the synthesis of our target isoflavones. Our initial goal was therefore to develop an efficient synthetic route that avoids the harsh conditions previously employed in flavonoid chemistry. One of the authors has developed a convenient method for the synthesis of 6-substituted 1,5-dialkyluracils, in which α-oxoketene dithioacetals were employed as key intermediates for the construction of pyrimidine ring.[14] We anticipated this strategy would be suitable for the synthesis of our target molecules. One of the advantages of this synthetic route is that, after construction of the ring system, the resulting methylthio substituent can be converted into the methylsulfonyl group, which serves as a good leaving group for the introduction of other substituents. Thus, we investigated the feasibility of this strategy for the synthesis of our target molecules. The retrosynthesis is outlined in Scheme 1.

As shown in Scheme 2, our synthesis starts with deoxybenzoin **2**, which can be prepared by the published procedure from resorcinol and 4-methoxyphenylacetic acid.[15] Since we wished to eventually apply this method to a solid-phase synthesis, we decided to use benzyl group as a surrogate for hydroxymethyl polystyrene resin. The 4-hydroxyl group of **2** was therefore selectively protected with benzyl group under Mitsunobu reaction conditions to give monobenzyl ether **3**. To construct the 4*H*-1-benzopyran-4-one scaffold, we previously developed a convenient one-pot synthesis using a phase transfer catalyst.[1c] Since this method is not readily amenable to solid-phase synthesis, we modified the reaction conditions in which sodium hydride was utilized as a base. The resulting 2-(methylthio)isoflavone **4** was oxidized by *m*CPBA to **5**, whose methylsulfonyl group was then successfully displaced with sodium salt of 4-mercaptophenol or hydroquinone to afford 2-(4-hydroxythiophenoxy)-isoflavone **6a** and 2-(4-hydroxyphenoxy)isoflavone **6b**, respectively. The hydroxyl group of each compound **6** was alkylated with 1-(2-chloroethyl)piperidine to give compounds **7**. Initial attempts to generate the target molecules **1a** and **1b** using typical reaction conditions for demethylation,



Scheme 1. Retrosynthesis.



Scheme 2. Synthesis. Reagents and conditions: (a) BnOH, Ph_3P, DIAD, THF, 0 °C, 0.5 h; (b) NaH, CS_2, DMF, 0 °C, 5 min; MeI, 0 °C→rt, 1 h; (c) *m*CPBA, CH_2Cl_2, reflux, 1 h; (d) 4-mercaptophenol (for **6a**) or hydroquinone (for **6b**), NaH, DMF, 0 °C, 1 h; (e) 1-(2-chloroethyl)piperidine monohydrochloride, Cs_2CO_3, DMF, rt, overnight; (f) BBr_3, CH_2Cl_2, rt, overnight; (g) HCl, EtOAc.

such as boron tribromide in dichloromethane, were unsuccessful; the competitive cleavage of piperidinyl ethoxy group led to compounds **8** as major products, which can be obtained from compounds **6** using the same reaction conditions. This partial cleavage of the basic side chain has been previously noted by Katzenellenbogen and co-workers with their pyrazole derivatives.[10b] They have successfully overcome this problem using the milder $AlCl_3$-EtSH reagent. However, the application of this reagent to our compounds which contain a Michael acceptor was considered to be inappropriate because of the intrinsic nucleophilicity of ethanethiol.[16] The basic amine was considered to be responsible for the susceptibility of the side chain, presumably by coordinating with the Lewis acid and thereby allowing access of the Lewis acid to the susceptible ethoxy group. Based on this explanation, we were able to avoid the undesired cleavage of the side chain by using hydrochloride salts of compounds **7** as substrates. The positively charged ammonium species might prevent the access of Lewis acid to the ethoxy group in the side chain. Thus the desired targets **1a** and **1b** were synthesized from deoxybenzoin **2** in seven steps in excellent overall yields (60 and 62%, respectively).[17]

Since SERMs display their activities through the estrogen receptors, we evaluated binding affinities of compounds **1** for human ERα and ERβ. We also included the triphenolic compounds **8** in the assay. The binding affinities were determined by fluorescence polarization using a modified protocol of the previously reported procedure.[18] All the compounds showed no significant binding to ERβ below 5 μM (data not shown). Binding results for ERα are listed in Table 1 as relative binding affinity.

As previously mentioned, no significant binding to ERβ was observed for both **1** and **8** while they showed binding affinities for ERα. This is opposite to our expectation because genistein is known to bind to ERβ with higher affinity than to the other subtype. Albeit with a preference for ERα, binding affinities of **1** for the subtype are still low. However, oxygen linkage seems to be a more favorable hinge than sulfur based on the binding affinity of **1b**, which is almost four times greater than that of **1a**. It has been reported that, in raloxifene analogues, oxygen linker provides an enhanced affinity for ER relative to carbonyl group of raloxifene.[19] It is interesting to note that their structurally related compound **A** has been reported to bind well to estrogen receptors with K_i value of 3.9 ± 0.3 nM.[12] While further investigation is needed, the result indicated that both sulfur and oxygen atoms in this series may significantly alter the orientation of the basic side chain, which may not be well tolerated by the receptors. To our surprise, both compound **8a** and **8b**, which lack the bulky side chain, bind to ERα with even lower affinities. This is interesting because most triphenolic compounds tend to bind reasonably well to the estrogen receptors.[20] It is plausible that, in this series of compounds, the basic side chain may provide an additional interaction with ER in the binding pocket, which can compensate for its bulkiness.

Table 1. Binding affinities for ERα of compounds **1** and **8**

Compd	EC_{50}, μM	Log EC_{50} (±SE)[a]	RBA[b]
4-OHT[c]	0.0157	−7.81 (±0.12)	26.0
1a	0.77	−6.11 (±0.07)	0.51
1b	0.20	−6.70 (±0.11)	1.95
8a	1.86	−5.73 (±0.55)	0.21
8b	1.74	−5.76 (±0.13)	0.22

[a]Log EC_{50} values were calculated by a nonlinear regression analysis (GraphPad Prizm). Each dose–response curve contained eleven concentrations, each in quadruplicate.
[b]Relative binding affinity (RBA) values, where estradiol = 100.
[c]4-Hydroxytamoxifen. Published RBA value for 4-OHT is 26 using this fluorescence polarization assay.[18]

In summary, we have designed new isoflavone derivatives as potential SERMs and have developed an efficient synthetic route for the preparation of these molecules. Although these isoflavones display common structural features that are found in many of published SERMs, the ER binding data indicated they are poor ligands for estrogen receptors. Further work is currently underway to understand the consequences of this study. Our medicinal chemistry efforts are now focused on manipulating functional groups around the isoflavone core to identify new lead compounds for the treatment of hormone-dependent breast cancer.

Acknowledgements

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17. Spectral and physical data. **1a**: mp 188–190 °C (dec); IR (KBr) 1593, 1493, 1444, 1371, 1276, 1248 cm^{-1}; ^1H NMR (DMSO-d_6, 250 MHz) δ 7.79 (d, $J=8.7$ Hz, 1H), 7.47 (d, $J=8.7$ Hz, 2H), 7.10 (d, $J=8.5$ Hz, 2H), 7.00 (d, $J=8.7$ Hz, 2H), 6.83–6.78 (m, 3H), 6.32 (d, $J=2.0$ Hz, 1H), 4.07 (d, $J=5.8$ Hz, 2H), 2.63 (t, $J=5.8$ Hz, 2H), 2.42–2.25 (m, 4H), 1.53–1.25 (m, 6H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 173.94, 163.12, 162.13, 160.65, 158.29, 158.08, 137.39, 132.78, 128.13, 123.34, 122.10, 118.47, 116.42, 116.07, 115.85, 102.13, 66.71, 58.13, 55.27, 26.41, 24.76; HRMS calcd for $C_{28}H_{28}NO_5S$ $(M + H)^+$ 490.1688, found 490.1669. **1b**: mp 188–190 °C (dec); IR (KBr) 1610, 1558, 1500, 1385, 1270, 1243, 1194 cm^{-1}; ^1H NMR (DMSO-d_6, 250 MHz) δ 7.86 (d, $J=8.7$ Hz, 1H), 7.17 (d, $J=8.4$ Hz, 2H), 7.11 (d, $J=9.0$ Hz, 2H), 6.93–6.859 (m, 3H), 6.72 (d, $J=8.4$ Hz, 2H), 6.56 (d, $J=1.7$ Hz, 1H), 4.01 (bt, 2H), 2.60 (bt, 2H), 2.46–2.2n8 (m, 4H), 1.55–1.28 (m, 6H); ^{13}C NMR (DMSO-d_6, 62.9 MHz) δ 177.33, 163.38, 160.52, 157.43, 156.54, 155.07, 147.64, 132.50, 128.11, 121.60, 120.61, 116.39, 115.84, 115.44, 107.70, 102.60, 66.86, 58.22, 55.26, 26.41, 24.77; HRMS calcd for $C_{28}H_{28}NO_6$ $(M + H)^+$ 474.1916, found 474.1900.
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