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Synthesis of daidzein derivatives for targeted drug delivery

Alena I. Sidorova, Vasiliy S. Koval,* Vitalia V. Kulikova, Svetlana V. Revtovich, Elena A. Morozova, Andrey A. Stomakhin, Natalia V. Anufrieva and Pavel N. Solyev*

V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 1405; e-mail: tokojami@yandex.ru; solyev@gmail.com

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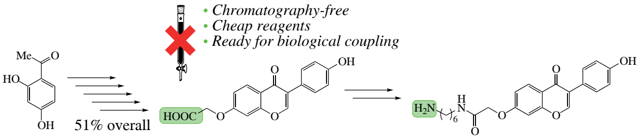
A new protocol was developed for the synthesis of daidzein derivatives bearing 7-positioned linkers with carboxy and amino functions. The key step of the scheme is the Suzuki cross-coupling, the optimized procedures provide high yields and do not require chromatographic purification. This approach is advantageous for further linker elongation necessary for anchoring and drug-coupling; the compounds obtained are regarded as tumor-directing carriers.



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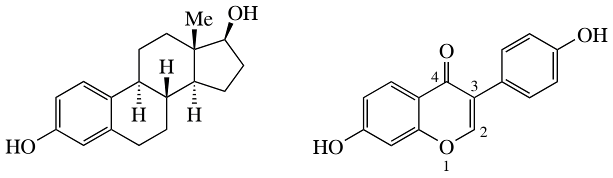
Isoflavones are nonsteroidal diphenolic compounds isostructural with 17β-estradiol, an endogenous gonadal steroid.[1] About 70% of breast cancers show increased expression of estrogen receptor α (ERα).[2] Due to the similarity of the chemical structure of isoflavones to that of 17β-estradiol, this class of compounds competitively binds to ERα, which affects intracellular signaling mechanisms and can be used in the treatment of estrogen-dependent tumors, as well as in targeted drug delivery to these cancer cells (Figure 1).[2] In addition to competitive inhibition of the overexpressed estrogen receptors, there are other mechanisms of flavone and isoflavone interactions.[3] By binding to the ERα, they can induce transcription of estrogen-dependent genes leading to an increased activity of antioxidant enzymes such as catalase and glutathione peroxidase.[4,5] The compounds can lead to non-genomic effects by binding to membrane estrogen receptors coupled to G proteins (GPER-1/GPR30).[6,7] Furthermore, isoflavones can also affect the bioavailability of sex hormones by inactivating globulin that binds these hormones or by stimulating its synthesis.[8]

Previously, we explored the ability of isoflavones to deliver drugs directly to the cancer cell in the enzyme therapy for ERα-positive tumors.[2,9–11] Such therapy requires profound amounts of daidzein-based drug-carriers, and their synthesis is desirable with reduced solvent waste. Isoflavones with amino- and carboxy-functionalized linkers capable of coupling with active molecules by ionic or covalent binding are in demand for prodrug design.[12,13] Isoflavone-containing linkers in the carrier's structure can also provide benefits after their chemical or enzymatic *in vivo* degradation into the parent compounds. Daidzein, itself being active in antitumor treatment, often synergistically enhances effects of the main drug in cancer cells thus disrupting additional biochemical targets.[14]



17β-Estradiol

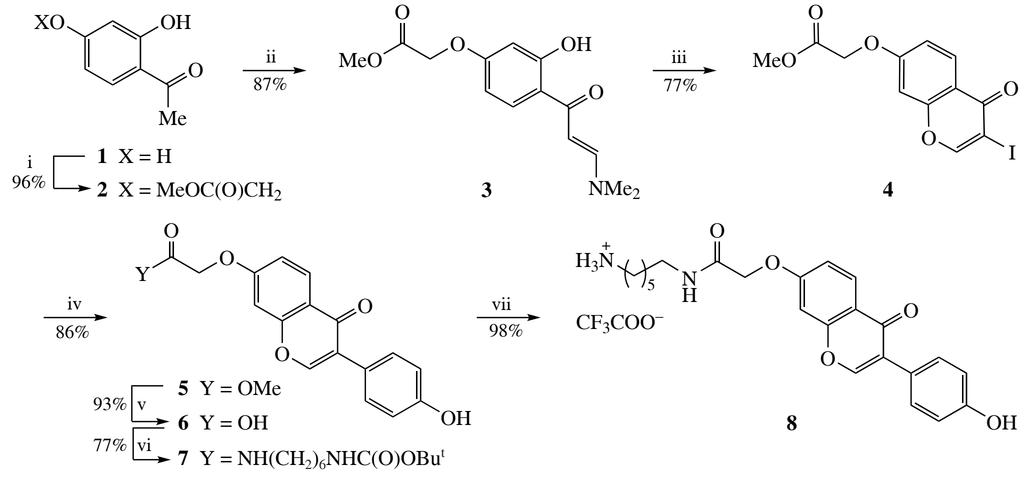
Daidzein

Figure 1 Structural similarity of 17β-estradiol and daidzein.

In this article, we propose an optimized protocol for the synthesis of daidzein-based anchors with the most commonly used in biology carboxy and amino groups. The late-stage linker coupling was repurposed to the first step for double functioning as a protective group, to omit unnecessary protection/deprotection steps for employment as a prospective drug carrier. However, this small change allowed us to maintain all the other steps intact and, moreover, to eliminate chromatographic purification of the isoflavone derivatives.

There are many approaches to the synthesis of isoflavones described in literature.[15] The most common routes to obtain isoflavones are the Houben–Hoesch reaction of deoxybenzoins or the Friedel–Crafts acylation,[16] the Suzuki[17] or the Stille cross-coupling,[18] or the chalcone route.[19] The main criteria for choosing a synthesis route were the availability of starting reagents, feasibility of gaining high yields and the absence of side reactions. Among the abovementioned ways which often necessitate the use of complex initial reagents or feature the formation of by-products, the ways employing the Suzuki cross-coupling look most promising (for the graphical survey of the literature precedents, see Online Supplementary Materials, Scheme S1). For the Suzuki cross-coupling, chromenone bearing 3-positioned iodine substituent is required; an access to it was described by Biegasiewicz[20,21] starting from commercially available 2,4-dihydroxyacetophenone.

Herein, we report an efficient synthesis of new daidzein derivatives containing 7-positioned substituents with carboxy and amino groupings. All synthetic steps occurred with high yields and the products were isolated without chromatographic purification. This pathway has been accomplished through the utilization of an enamine addition, a ring closure and iodination, and the Suzuki coupling to afford our desired products (Scheme 1). The starting compound was 2,4-dihydroxyacetophenone (resacetophenone) **1**. Its treatment with methyl bromoacetate not only ensures the introduction of a carboxy



Scheme 1 *Reagents and conditions*: i, K_2CO_3 (1.5 equiv.), $BrCH_2COOMe$ (1.05 equiv.), DMF, 6 h; ii, $Me_2NCH(OMe)_2$ (1 equiv.), DMF, 24 h; iii, I_2 (2.35 equiv.), MeOH, 24 h; iv, 4-$HOC_6H_4B(OH)_2$ (1.5 equiv.), PEG 3350, $Pd(OAc)_2$ (0.1 equiv.), Na_2CO_3 (2.5 equiv.), 3 h; v, 50% AcOH, 8 h; vi, $BocNH(CH_2)_6NH_2$ (1.1 equiv.), HBTU (1.1 equiv.), DIPEA (1.1 equiv.), DMF, 45 min; vii, CF_3CO_2H, 30 min.

group into the future linker but also protects the 4-OH group from unwanted methylation at the next step. At the next step, enamine **3** is obtained on treatment of compound **2** strictly with 1 molar equivalent of DMF dimethyl acetal (to minimize the formation of difficultly separable by-products). To purify product **3**, crystallization was carried out from EtOAc–hexane. It was also found that purification of compound **3** could be by-passed, because at the next stage iodochromone **4** crystallized during its formation whereas the closure of the chromene ring in methanol in the presence of iodine occurred selectively. To improve the yield of iodo derivative **4**, we used minimal amounts of the solvent, and upon completion of the reaction, the mixture was cooled down to –20 °C. The formed precipitate was washed with a small amount of methanol cooled to –20 °C, and the residue was treated with an aqueous solution of sodium sulfite. It was also found that treatment with sodium thiosulfate solutions should be avoided since it caused the reduction of iodine at the chromene ring.

The typical Suzuki cross-coupling of iodide **4** with 4-$HOC_6H_4B(OH)_2$ afforded the desired isoflavonoid **5**. We have noticed that in the course of washing of the crude material with water solutions, the target compound **5** still remained mixed with the catalyst. As the latter is insoluble in EtOAc, multiple hot extractions can separate **5**; however, it appeared more productive to use the Soxhlet extractor, which allowed us to increase the yield. Hydrolysis of the ester group in compound **5** was initially attempted under alkaline conditions, but this promoted polymerization of the product into resin. It was decided to use 50% AcOH, as described by Cao,[22] but at the same time to select its optimal volume for the reaction completion and crystallization of the product from the mixture.

The amine derivative of daidzein was then prepared in two steps from carboxy derivative **6** by the condensation with mono-Boc-protected hexane-1,6-diamine (synthesized according to the Kohen method[23]). The acid chloride method did not give satisfactory yields, and after several attempts we settled on using HBTU as a condensing agent. At that stage, purification of compound **7** from by-products occurred successfully through the acid–base extraction sequence. First, the remnants of the Boc-protected amine were passed into the aqueous phase in the hydrochloride form when washed with diluted HCl, while the second extraction eliminated the unreacted acid **6** in the form of sodium salt when washed with a $NaHCO_3$ solution. Final elimination of Boc protection afforded the target product **8** when carbamate **7** was treated with CF_3CO_2H, proceeding quantitatively and smoothly without any by-products formation.

All structures **2–8** were confirmed by ^1H, ^{13}C NMR spectroscopy and characterized by high-performance liquid chromatography–high-resolution mass spectrometry data.

To sum, the preparation of isoflavone-containing linkers, namely, daidzein-O^7-acetic acid **6** and daidzein-O^7-(6-aminohexyl)acetamide **8** from 2,4-dihydroxyacetophenone **1** without chromatographic purification has been developed with overall yields of 51 and 39%, respectively. These daidzein derivatives are suitable for implementation in the targeted delivery of therapeutical agents to ERα-positive tumors.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7722.

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