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Research Paper

Selective alkylation of genistein and daidzein

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Abstract: Regioselective 7-O- and 4-O- alkylations of isoflavones are described on example of genistein and daidzein. Derivatization of more acidic phenolic group is easily achieved via its tetra-n-butyl ammonium salt. Two modes of selective 4-O-derivatization are described, one through mono 7-O-benzyl derivative and second, one step, via double deprotonation of an isoflavone substrate.

Introduction

Genistein [**1**; 5,7,4'-trihydroxyisoflavone; 5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-chromen-4-one] is one of the best studied natural products, currently available from soybeans isolates as well as by chemical synthesis^[1], which has first attracted attention as a phytoestrogen^[2,3], later became a biochemical probe with model tyrosine kinase C inhibitor function^[4] and finally ended up as an active ingredient of great number dietary supplements and also in numerous clinical trials as a drug candidate, as well as proven pleiotropic ligand of various biomacromolecular targets of therapeutic significance^[5,6].

Thus, from commercial chemical perspective, genistein substance advanced from very expensive and scarcely available plant secondary metabolite to affordable multipurpose fine chemical with perspective of medicinal application. Consequently genistein derivatives have also become desirable but somehow difficult to obtain because of relative instability of the parent molecule under harsh chemical conditions. Daidzein [**3**; 7,4'-dihydroxyisoflavone; 7-hydroxy-3-(4-hydroxyphenyl)-4*H*-chromen-4-one] is a close structural analog of genistein, and possibly also its functional congener, which warrants investigation of its derivatization. Chemical reactivity of complex plant phenolics is seldom discussed in context of their multiple biological activities but it should be realized that even

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single phenolic functionality is capable of introducing great variety of chemical and biological transformations leading to creation of new bonds and new functions under neutral, basic or acidic conditions. Phenols are easily engaged in hydrogen atom transfer reactions, electron transfer reactions leading to free radicals, can undergo electrophilic attack or facile heterolytic fission of hydroxyl group resulting in deprotonation and formation of phenolate anion. This last reaction which is of great importance for subsequent O-acylation and O-alkylation of phenols has been studied under variety of conditions with use of different basic reagents^[7,8]. Genistein, with its three easily ionizable phenolic groups, exhibit rather complex pattern of hydrogen bonding network and proton transfer localized at the most acidic O7 – H7 center, when exposed to action of organic bases^[9,10]. Aqueous inorganic base ionization clearly shows three steps dissociation with pKa values separated by approximately two units^[11]. This distinct difference in phenolics group acidity does not translate into alkylation selectivity because nucleophilicity of the corresponding phenolates grows in the reverse order. This study attempts to answer a practical question – how to achieve a scalable, selective alkylation of genistein with reagents which allow secondary functionalization needed for preparation of more extended structures. Our first choice is exploitation of reaction conditions related to the classical Williamson synthesis^[12,13].

Materials and Methods

The principal research material for this study was high purity synthetic genistein (> 99.0% by HPLC) obtained by proprietary technical process carried out on a pilot plant scale at PRI^[14]. The process was designed on chemistry known from original literature^[14,15]. The secondary material was tetra-n-

butyl ammonium salt of genistein, which unlike earlier described “complexes” with organic bases has clearly defined deprotonation site at 7-O-^[16], promoting selectivity of various nucleophilic substitution reactions. Daidzein was purchased from LC Laboratories, Woburn, MA, USA. The remaining reagents, including solvents and sorbents were of commercial origin, certified for research use. Solvents were additionally dried before use as recommended in literature^[17]. Products of the isoflavone alkylation reactions were recognized based on silicagel TLC R_F values (in toluene / ethyl acetate= 2:1), purified by column chromatography performed on silica gel 60 (70–230 mesh, E. Merck) developed with a) methylene chloride/acetone=20/1; b) manual gradient of toluene / ethyl acetate = 15/1 to 1/ 2 v/v); c) manual gradient hexane/ethyl acetate=5/1 to 2/1, v/v) solvent systems or crystallization (methanol, ethanol), and characterized by determination of high resolution mass spectroscopy (HRMS; positive mode, Mariner Per Septive Biosystem detector using the electrospray-ionization (ESI) technique.) and assignment of the signals from ¹H and ¹³C NMR spectra (determined as DMSO-d₆ solution with TMS internal standard on 600 MHz Varian Inova 600 MHz apparatus. Melting points were determined on Koeffler apparatus and are not corrected. All evaporations were performed under diminished pressure at 50 °C. The test reactions aimed at finding suitable reaction conditions for etherification of **1** with di- or polyfunctional alkylating reagents were performed with 3-bromo-1-propanol, deliberately introducing complications resulting from the substrate self-alkylation leading to competing cyclization or oligomerization reactions. (Scheme 1). The three main concerns: 1) phenolic substrate deprotonation conditions; 2) phenolate activation conditions, and 3) choice of solvent for the alkylation reaction, were

addressed in experimental manner, by carrying out reactions applying chosen parameters. The results of reactions conducted at point modification conditions are exemplified in Table 1 presented below.

The test experiments strongly suggested that initial deprotonation of the phenolic substrate is essential for efficiency of the alkylation and pre-formation of lipophilic salt with a strong organic base offers operational advantage. While means of delivery of an activation energy seems optional, choice of solvent can be considered a critical parameter, with indication at dipolar aprotic kind, which is in perfect agreement with general theory of nucleophilic substitution reactions. Therefore following procedures have been designed for syntheses of particular derivatives of **1** and **3**:

Procedure A. (7-O-alkylation of isoflavone):

The suspension of tetra-n-butylammonium salt of genistein (**2**; 1 mmol) {or daidzein, **4**; 1 mmol} in 10 mL of dry DMF was stirred in a round bottom flask protected from moisture. The alkylating agent (1.1 mmol) was added in one portion and the reaction mixture was heated to 50° C for 24 hrs, after which time TLC analysis (toluene – ethyl acetate 2 : 1) indicated exhaustion of the substrates. Cooled reaction mixture was poured to stirred ice – water mixture (250 mL), white precipitation was collected by filtration, washed with cold water and dried. Product was crystallized from methanol or purified by flash column chromatography.

5-hydroxy-7-(2-propargyloxy)-3-(4-hydroxyphenyl)chromen-4H-one (5)

¹H NMR : 3.68(t, 1H, J=2.4 Hz, CH), 4.96(d, 2H, J=2.5 Hz OCH₂), 6.47(d, 1H, J=2.3 Hz,

H-8), 6.72(d, 1H, J=2.3 Hz, H-6), 6.83(AA'XX', 2H, J=8.8 Hz, H-3', H-5'), 7.40(AA'XX', 2H, J=8.6 Hz, H-2', H-6'), 8.42(s, 1H, H-2), 9.60(s, 1H, 4'-OH), 12.96(s, 1H, 5-OH).
¹³C NMR: 56.27(OCH₂), 78.30(CCH), 79.03(CH), 93.33(C-8), 98.58(C-6), 105.77(C-4), 115.60(C-3', C-5'), 120.96(C-1'), 122.55(C-3), 130.12(C-2', C-6'), 154.47(C-2), 157.24(C-4'), 157.48(C-8), 161.96(C-5), 162.96(C-7), 180.43(C-4)

5-hydroxy-7-benzyloxy -3-(4-hydroxyphenyl)chromen-4H-one (6)

¹H NMR : 5.24 (s; 2H; PhCH₂-), 6.50, 6.75 (2 x d; 1H; J=2.3 Hz; H-6, H-8), 6.83 (AA'XX'; 2H; J=8.4 Hz; C-3', C-5'), 7.34-7.48 (m; 7H; Ph, C-2', C-6'), 8.40 (s; 1H; H-2), 9.60 (s; 1H; 4'-OH), 12.95 (s; 1H; 5-OH)
¹³C NMR: 70.48 (PhCH₂-), 93.74 (C-8), 99.14 (C-6), 105.99 (C-4a), 115.52 (C-3', C-5'), 121.48 (C-1'), 122.97 (C-3), 128.35, 128.59 128.97 (C-2'', C-3'', C-4'', C-5'', C-6''), 130.60 (C-2', C-6'), 136.52 (C-1''), 154.86 (C-2), 157.88 (C-8a), 157.92 (C-4'), 162.21 (C-5), 164.65 (C-7), 180.86 (C-4).

5-hydroxy-7-(2-hydroxyetoxy)-3-(4-hydroxyphenyl)chromen-4H-one (7)

¹H NMR : 3.70 – 3.78 (m; 2H; -CH₂O-), 4.11 (t; 2H; J=9.6 Hz; -CH₂O-), 4.97 (br s; 1H; -CH₂OH), 6.40, 6.65 (2 x d; 2H; J=2.2 Hz; H-6, H-8), 6.83 (AA'XX'; 2H; J=8.7 Hz; H-3', H-5'), 7.39 (AA'XX'; 2H; J=8.7 Hz; H-2', H-6'), 8.40 (s; 1H; H-2), 9.63 (br s; 1H; 4'-OH), 12.96 (s; 1H; 5-OH).
¹³C NMR : 59.97, 71.20 (-OCH₂-), 93.52 (C-8), 99.08 (C-6), 106.06 (C-4a), 115.77 (C-3', C-5'), 121.75 (C-1'), 123.18 (C-3), 130.85 (C-2', C-6'), 155.04 (C-2), 158.17 (C-4', C-8a), 162.42 (C-5), 165.38 (C-7), 181.07 (C-4).

5-hydroxy-7-(3-hydroxypropyloxy)-3-(4-hydroxyphenyl)chromen-4H-one (8)

^1H NMR : 1.84 – 1.92 (m; 2H; $-\text{CH}_2-$), 3.54 – 3.59 (m; 2H; $-\text{CH}_2\text{O}-$), 4.16 (dd; 2H; $J=6.2$ Hz, $J=6.4$ Hz; $-\text{CH}_2\text{OH}$), 4.62 (t; 1H; $J=6.4$ Hz; $-\text{CH}_2\text{OH}$), 6.39, 6.65 (2 x d; 2 H; $J=2.2$ Hz; H-6, H-8), 6.83 (AA'XX'; 2H; $J=8.6$ Hz; H-3', H-5'), 7.39 (AA'XX'; 2H; $J=8.6$ Hz; H-2', H-6'), 8.41 (s; 1H; H-2), 9.64 (br s; 1H; 4'-OH), 12.96 (s; 1H; 5-OH).

^{13}C NMR : 31.78 ($-\text{CH}_2-$), 65.60, 57.60 (2 x $-\text{OCH}_2-$), 92.76 (C-8), 98.35 (C-6), 105.33 (C-4a), 115.07 (C-3', C-5'), 121.06 (C-1'), 122.47 (C-3), 130.17 (C-2', C-6'), 154.38 (C-2), 157.48, 157.51 (C-4', C-8a), 161.74 (C-5), 164.66 (C-7), 180.37 (C-4).

5-hydroxy-7-(5-acetoxypentyloxy)-3-(4-hydroxyphenyl)chromen-4H-one (9)

^1H NMR : 1.43 – 1.77 (m; 6H; 3 x $-\text{CH}_2-$), 2.00 (s; 3H; AcO), 4.02 (t; 2H; $J=6.4$ Hz; $-\text{CH}_2\text{O}-$), 4.09 (t; 2H; $J=6.4$ Hz; $-\text{CH}_2\text{O}-$), 6.39, 6.64 (2 x d; 2H; $J=2.2$ Hz; H-6, H-8), 6.82 (AA'XX'; 2H; $J=8.7$ Hz; H-3', H-5'), 7.38 (AA'XX'; 2H; $J=8.7$ Hz; H-2', H-6'), 8.40 (s; 1H; H-2), 12. (s; 1H; 5-OH).

^{13}C NMR : 20.71 (AcO), 21.95 ($-\text{CH}_2-$), 27.79, 27.98 (2 x $-\text{CH}_2\text{CH}_2\text{O}-$), 66.67, 68.31 (2 x $-\text{CH}_2\text{O}-$), 92.77 (C-8), 98.32 (C-6), 105.35 (C-4a), 115.17 (C-3', C-5'), 120.64 (C-1'), 122.55 (C-3), 130.10 (C-2', C-6'), 154.28 (C-2), 157.50 (C-8a), 157.99 (C-4'), 161.76 (C-5), 164.58 (C-7), 170.40 (C=O), 180.40 (C-4).

5-hydroxy-7-methoxy-3-(4-hydroxyphenyl)chromen-4H-one (10)

^1H NMR : 3.87 (s; 3H; $-\text{OCH}_3$), 6.41, 6.65 (2 x d; 2H; $J=2.1$ Hz; H-6, H-8), 6.83 (AA'XX'; 2H; $J=8.8$ Hz; H-3', H-5'), 7.40 (AA'XX'; 2H; $J=8.8$ Hz; H-2', H-6'), 8.40 (s; 1H; H-2), 9.60 (s; 1H; 4'-OH), 12.96 (s; 1H; 5-OH).

^{13}C NMR : 56.54 ($-\text{OCH}_3$), 92.86 (C-8), 98.48 (C-6), 105.87 (C-4a), 115.52 (C-3', C-5'), 121.49 (C-1'), 122.96 (C-3), 130.59 (C-2', C-6'), 154.83 (C-2), 157.92 (C-4'), 157.95 (C-8a), 162.20 (C-5), 165.68 (C-7), 180.87 (C-4).

7-(2-propargyloxy)-3-(4-hydroxyphenyl)chromen-4H-one (11).

^1H NMR (600 MHz, DMSO d_6): 3.68(t, 1H, $J=2.4$ Hz, CH), 5.00(d, 2H, $J=2.0$ Hz OCH_2), 6.83(AA'XX', 2H, $J=8.7$, H-3', H-5'), 7.12(dd, 1H, $J=8.9$ $J=2.4$ Hz, H-6d), 7.22(d, 1H, $J=2.4$ Hz, H-8d), 7.41(AA'XX', 2H, $J=8.7$ Hz, H-2'd, H-6'd), 8.06(d, 1H, $J=8.9$ Hz, H-5d), 8.38(s, 1H, H-2d), 9.58(s, 1H, 4'-OH).

^{13}C NMR (150 MHz, DMSO d_6): 56.22(OCH_2), 78.26(CCH), 78.94(CH), 101.73(C-8), 114.91(C-3', C-5', C-6), 118.04(C-4), 122.24(C-1'), 123.69(C-3), 126.97(C-5), 129.98(C-2', C-6'), 153.11(C-2), 157.01(C-4'), 157.19(C-8), 161.32(C-7), 174.61(C-4)

Procedure B. (4-O-alkylation of isoflavone):

Genistein (500 mg, 1.85 mmol) was suspended in dry DMF (15 mL) in a round bottom flask equipped with magnetic stirring bar and protected from moisture, potassium tert-butanolate (685 mg, 6.10 mmol) was added and the reaction mixture was left stirring for 2 hours at ambient temperature. Then, alkylating agent (1.85 mmol) was added and stirring was continued until TLC analysis (toluene – ethyl acetate 2:1) indicated disappearance of **1** (approx. 16 hours). The reaction mixture was poured to ice-water mixture (200 mL) and products were extracted with methylene dichloride (3 x 50 mL). Organic layer was dried with anhydrous magnesium sulfate and solvent was evaporated on rotary evaporator under

reduced pressure. An oily residue was chromatographed on silicagel column using manual gradient (system b.)

5,7-dihydroxy-3-(4-(2-benzoiloxoethoxy)phenyl)chromen-4H-one (12)

^1H NMR : 4.39 (t; 2H; $J=4.3$ Hz; $-\text{CH}_2\text{O}-$), 4.63 (t; 2H; $J=4.3$ Hz; $-\text{CH}_2\text{O}-$), 6.24, 6.40 (2 x d; 2H; $J=2.1$ Hz; H-6, H-8), 7.07 (AA'XX'; 2H; $J=8.8$ Hz; H-3', H-5'), 7.50 – 7.56 (m; 4H; H-2', H-6', H-3'', H-5''), 7.67 (ddd; 1H; $J=7.5$ Hz, $J=1.3$ Hz; H-4''), 7.97 (m; 2H; H-2'', H-6''), 8.38 (s; 1H; H-2), 10.92 (br s; 1H; 7-OH), 12.93 (s; 1H; 5-OH).

5,7-dihydroxy-3-(4-(3-benzoiloxo-propyloxy)phenyl)chromen-4H-one (13)

^1H NMR : 2.21 (tt; 2H; $J=6.2$ Hz; $-\text{CH}_2-$), 4.19 (t; 2H; $J=6.2$ Hz; $-\text{CH}_2\text{O}-$), 4.46 (t; 2H; $J=6.2$ Hz; $-\text{CH}_2\text{O}-$), 6.23, 6.40 (2 x d; 2H; $J=2.0$ Hz; H-6, H-8), 7.02 (AA'XX'; 2H; $J=8.9$ Hz; H-3', H-5'), 7.48 – 7.56 (m; 4H; H-2', H-6', H-3'', H-5''), 7.67 (ddd; 1H; $J=7.5$ Hz, $J=1.3$ Hz; H-4''), 7.99 (m; 2H; H-2'', H-6''), 8.37 (s; 1H; H-2), 10.90 (br s; 1H; 7-OH), 12.93 (s; 1H; 5-OH).

5,7-dihydroxy-3-(4-(5-acetoxypentyloxy)phenyl)chromen-4H-one (14)

^1H NMR (DMSO- d_6): 1.41 – 1.50 (m; 2H; $-\text{CH}_2-$), 1.63 (tt; 2H; $J=7.0$ Hz; $-\text{CH}_2-$), 1.71 (tt; 2H; $J=7.0$ Hz, $J=6.7$ Hz; $-\text{CH}_2-$), 2.00 (s; 3H; AcO), 4.00 (2 x t; 4H; $J=7.0$ Hz; 2 x $-\text{CH}_2\text{O}-$), 6.23, 6.39 (2 x d; 2H; $J=2.1$ Hz; H-6, H-8), 6.98 (AA'XX'; 2H; $J=9.0$ Hz; H-3', H-5'), 7.47 (AA'XX'; 2H; $J=9.0$ Hz; H-2', H-6'), 8.35 (s; 1H; H-2), 10.90 (br s; 1H; 7-OH), 12.93 (s; 1H; 5-OH).

^{13}C NMR : 20.66 (AcO), 22.02 ($-\text{CH}_2-$), 27.82, 28.22 ($-\text{CH}_2\text{CH}_2\text{O}-$), 63.67, 67.30 ($-\text{CH}_2\text{O}-$), 93.65 (C8), 98.97 (C-6), 104.42 (C-4a), 114.17 (C-3', C-5'), 121.92 (C-1'),

122.77 (C-3), 130.08 (C-2', C-6'), 154.17 (C-2), 157.50 (C-4'), 157.54 (C-8a), 161.96 (C-5), 164.29 (C-7), 170.34 (C=O), 180.06 (C-4).

Procedure C. (4-O-alkylation of 7-O-benzyl- or 7-O-methyl genistein):

The substrate – 7-O-benzyl genistein (**6**) (943 mg, 2.61 mmol) was dissolved in dry DMF (30 mL) in a round bottom flask protected from moisture.. Anhydrous potassium carbonate (360 mg, 2.61 mmol) was added, followed by DBU (38.98 μL , 0.261 mmol) and alkylating reagent (2.61 mmol) and the reaction mixture was stirred at 50° until TLC analysis (toluene – ethyl acetate 2:1) indicated disappearance of **6** (approx. 24 hours). The reaction mixture was poured to ice-water mixture (200 mL) and products were extracted with methylene dichloride (3 x 50 mL). Organic layer was dried with anhydrous magnesium sulfate and solvent was evaporated on rotary evaporator under reduced pressure. An oily residue was chromatographed on silicagel column using manual gradient system b.

5-hydroxy-7-benzyloxy-3-(4-(5-acetoxypentyloxy)phenyl)chromen-4H-one (15)

^1H NMR : 1.44-1.50 (m; 2H; $-\text{CH}_2-$), 1.62-1.67 (m; 2H; $-\text{CH}_2-$), 1.73-1.77 (m; 2H; $-\text{CH}_2-$), 2.00 (s; 3H; AcO), 4.01 (t; 2H; $J=6.5$ Hz; $-\text{CH}_2\text{O}-$), 4.02 (t; 2H; $J=6.6$ Hz; $-\text{CH}_2\text{O}-$), 5.25 (s; 2H PhCH_2-), 6.51, 6.76 (2 x d; 2H; $J=2.3$ Hz; H-6, H-8), 7.00 (AA'XX'; 2H; $J=8.8$ Hz; H-3', H-5'), 7.34-7.38 (m; 1H; Ph), 7.40-7.43, (m; 2H; Ph), 7.46-7.49 (m; 2H; Ph), 7.50 (AA'XX'; 2H; $J=8.8$ Hz; H-2', H-6'), 8.45 (s; 1H; H-2), 12.92 (s; 1H; 5-OH)

^{13}C NMR : 21.50 (AcO), 22.51 ($-\text{CH}_2-$), 28.31 ($-\text{CH}_2\text{CH}_2\text{O}-$), 28.70 ($-\text{CH}_2\text{CH}_2\text{O}-$), 64.16 ($-\text{CH}_2\text{O}-$), 67.80 ($-\text{CH}_2\text{O}-$), 70.49

(PhCH₂-) 93.81 (C-8), 99.20 (C-6), 106.00 (C-4a), 114.71 (C-3', C-5'), 122.66 (C-1'), 123.09 (C-3), 128.36, 128.60, 128.98 (C-2'', C-3'', C-4'', C-5'', C-6''), 130.60 (C-2', C-6'), 141.00 (C-1'), 155.18 (C-2), 157.89 (C-4', C-8a), 162.20 (C-5), 164.71 (C-7), 170.86 (C=O), 180.77 (C-4).

5-hydroxy-7-benzyloxy-3-(4-(3-hydroxypropyloxy)phenyl)chromen-4H-one (16)

¹H NMR (DMSO-d₆): 1.88 (tt; 2H; J=6.6 Hz; -CH₂-), 3.57 (dt; 2H; J=5.4 Hz; -CH₂O-), 4.08 (t; 2H; J=6.6 Hz; -CH₂O-), 4.57 (t; 1H; J=5.4 Hz; -CH₂OH), 5.24 (s; 2H; PhCH₂-), 6.50, 6.75 (2 x d; 2H; J=2.1 Hz; H-6, H-8), 7.00 (AA'XX'; 2H; J=9.0 Hz; H-3', H-5'), 7.35-7.48 (m; 5H; Ph), 7.50 (AA'XX'; 2H; J=9.0 Hz; H-2', H-6'), 8.44 (s; 1H; H-2), 12.92 (s; 1H; 5-OH)

¹³C NMR (DMSO-d₆): 32.54 (-CH₂-), 57.74 (-CH₂O-), 65.11 (-CH₂O-), 70.49 (PhCH₂-), 93.79 (C-8), 99.18 (C-6), 105.98 (C-4a), 114.69 (C-3', C-5'), 122.66 (C-1'), 123.06 (C-3), 128.35, 128.59, 128.97 (C-2'', C-3'', C-4'', C-5'', C-6''), 130.58 (C-2', C-6'), 136.50 (C-1'), 155.13 (C-2), 157.88 (C-4'), 159.10 (C-8a), 162.20 (C-5), 164.69 (C-7), 180.76 (C-4).

5-hydroxy-7-metoksy-3-(4-(5-acetoksypentyloxy)phenyl)chromen-4H-one (17)

¹H NMR (DMSO-d₆): 1.12-1.50 (m; 2H; -CH₂-), 1.58-1.67 (m; 2H; -CH₂-), 1.69-1.78 (m; 2H; -CH₂-), 1.99 (s; 3H; AcO), 3.85 (s; 3H; -OCH₃), 3.99 (t; 2H; J=6.3 Hz; -CH₂O-), 4.01 (t; 2H; J=6.3 Hz; -CH₂O-), 6.40, 6.66 (2 x d; 2H; J=2.2 Hz; H-6, H-8), 6.98 (AA'XX'; 2H; J=8.8 Hz; H-3', H-5'), 7.49 (AA'XX'; 2H; J=8.8 Hz; H-2', H-6'), 8.44 (s; 1H; H-2), 12.92 (s; 1H; 5-OH)

¹³C NMR : 20.73 (AcO), 22.07, 27.87, 28.27 (3 x -CH₂-), 56.13 (-OCH₃), 63.73 (-CH₂O-),

67.35 (-CH₂O-), 92.48 (C-8), 98.10 (C-6), 105.42 (C-4a), 11.25 (C-3', C-5'), 122.19 (C-1'), 122.65 (C-3), 130.16 (C-2', C-6'), 154.70 (C-2), 157.53 (C-4'), 158.60 (C-8a), 161.75 (C-5), 165.29 (C-7), 170.44 (C=O), 180.34 (C-4)

Procedure D. The acylated product **9** (5 mM) and magnesium shavings in 50 mL of dry methanol or acylated product **12 -17** and sodium methanolate 1M 10 mL were stirred at temperature 40⁰ C for 24 h. After the completion of reaction, the mixture was neutralized with an Amberlyst 15 followed by filtration. The filtrate was distilled to form white solid. Recrystallization of the solid from 100 mL hot methanol gave product.

5-hydroxy-7-(5-hydroxypentyloxy)-3-(4-hydroxyphenyl)chromen-4H-one (23)

¹H NMR : 1.42 – 1.50 (m; 4H; 2 x -CH₂-), 1.71 – 1.76 (m; 2H; -CH₂-), 3.39 – 3.45 (m; 2H; -CH₂O-), 4.08 (t; 2H; J=6.6 Hz; -CH₂O-), 4.41 (t; 1H; J=5.1 Hz; -CH₂OH), 6.39, 6.64 (2 x d; 2H; J=2.2 Hz; H-6, H-8), 6.83 (AA'XX'; 2H; J=8.7 Hz; H-3', H-5'), 7.39 (AA'XX'; 2H; J=8.7 Hz; H-2', H-6'), 8.40 (s; 1H; H-2), 9.62 (s; 1H; 4'-OH), 12.95 (s; 1H; 5-OH).

¹³C NMR : 22.67, 28.92, 32.77 (3 x -CH₂-), 61.20, 69.18 (2 x -OCH₂-), 93.44 (C-8), 98.97 (C-6), 105.96 (C-4a), 115.71 (C-3', C-5'), 121.71 (C-1'), 123.11 (C-3), 130.79 (C-2', C-6'), 154.99 (C-2), 158.11 (C-8a), 158.14 (C-4'), 162.39 (C-5), 165.31 (C-7), 181.02 (C-4).

5-hydroxy-7-benzyloxy-3-(4-(5-hydroxypentyloxy)phenyl)chromen-4H-one (18)

¹H NMR : 1.42-1.51 (m; 4H; -CH₂-), 1.71-1.75 (m; 2H; -CH₂-), 3.42 (dt; 2H; J=4.9Hz, J=5.9 Hz; -CH₂O-), 4.00 (t; 2H; J=6.4 Hz; -

CH₂O-), 4.39 (t; 1H; J=4.8 Hz; CH₂OH), 5.24 (s; 2H; PhCH₂-), 6.50, 6.76 (2 x m; 2H; H-6, H-8), 7.00 (AA'XX'; 2H; J=8.4 Hz; H-3', H-5'), 7.35-7.48 (m; 5H; Ar), 7.50 (AA'XX'; 2H; J=8.4 Hz; H-2', H-6'), 8.44 (d; 1H; J=1.2 Hz; H-2), 12.91 (s; 1H; 5-OH)
¹³C NMR : 22.57 (-CH₂-); 28.99 (-CH₂CH₂O-); 32.65 (-CH₂CH₂O-); 61.06 (-CH₂O-); 67.99 (-CH₂O); 70.49 (PhCH₂-); 93.80 (C-8); 99.19 (C-6); 105.98 (C-4a); 114.69 (C-3', C-5'); 122.67 (C-1'); 123.04 (C-3); 128.35, 128.59, 128.98 (C-2'', C-3'', C-4'', C-5'', C-6''); 130.58 (C-2', C-6'); 136.51 (C-1''); 155.15 (C-2); 157.88 (C-4'); 159.10 (C-8a); 162.21 (C-5); 164.70 (C-7); 180.77 (C-4).

5-hydroxy-7-methoxy-3-(4-(5-hydroxypentyloxy)phenyl)chromen-4H-one (19)

¹H NMR : 1.42 – 1.50 (m; 4H; 2 x -CH₂-), 1.71 – 1.76 (m; 2H; -CH₂-), 3.87 (s; 3H; -OCH₃), 3.42 (t; 2H; J=6.2 Hz; -CH₂O-), 4.00 (t; 2H; J=6.4 Hz; -CH₂O-), (2 x d; 2H; J=2.1 Hz; H-6, H-8), 6.99 (AA'XX'; 2H; C-3', C-5'), 7.50 (AA'XX'; 2H; C-3', C-5'), 8.45 (s; 1H; H-2), 12.93 (s; 1H; 5-OH)
¹³C NMR : 22.58 (-CH₂-); 29.00 (-CH₂CH₂O-); 32.65 (-CH₂CH₂O-); 56.56 (-OCH₃); 61.06 (-CH₂O-); 67.99 (-CH₂O); 92.92 (C-8); 98.53 (C-6); 105.87 (C-4a); 114.68 (C-3', C-5'); 122.65 (C-1'); 123.05 (C-3); 130.59 (C-2', C-6'); 155.12 (C-2); 157.96 (C-4'); 159.10 (C-8a); 162.19 (C-5); 165.72 (C-7); 180.78 (C-4).

5,7-dihydroxy-3-(4-(2-hydroxyethyloxy)phenyl)chromen-4H-one (20)

¹H NMR : 3.73 (m; 2H; -CH₂O-), 4.01 (t; 2H; J=4.9 Hz; -CH₂O-), 4.90 (br s; 1H; CH₂OH), 6.22, 6.63 (2 x d; 2H; J=2.1 Hz; H-6, H-8), 6.99 (AA'XX'; 2H; J=8.9 Hz; H-3', H-5'), 7.48 (AA'XX'; 2H; J=8.9 Hz; H-2', H-6'),

8.34 (s; 1H; H-2), 10.90 (br s; 1H; 7-OH), 12.92 (s; 1H; 5-OH).

¹³C NMR (DMSO-d₆): 59.57, 69.58 (-CH₂O-), 93.71 (C-8), 99.02 (C-6), 104.46 (C-4a), 114.24 (C-3', C-5'), 121.96 (C-1'), 122.87 (C-3), 130.14 (C-2', C-6'), 154.22 (C-2), 157.58 (C-8a), 158.61 (C-4'), 162.00 (C-5), 164.33 (C-7), 180.10 (C-4)

Procedure E. Hydrogenolysis.

7-O-benzyl genistein 4-O-substituted derivatives (**17,19**) (1.5 mmol) was suspended in cyclohexene (1 mL) and catalyst (Pd/C, 10%, 2g) was added and reaction mixture was stirred 50⁰ until TLC analysis (toluene – ethyl acetate 2:1) indicated disappearance of benzyl ethers (approx. 4 hours. Crude products were purified by column chromatography (solvent system c).

5,7-dihydroxy-3-(4-(3-hydroxypropyloxy)phenyl)chromen-4H-one (21)

¹H NMR (DMSO-d₆): 1.87 (tt; 2H; J=6.1 Hz, J=6.3 Hz; -CH₂-), 3.55 (m; 2H; -CH₂O-), 4.05 (t; 2H; J=6.3 Hz; -CH₂O-), 4.56 (br s; 1H; -CH₂OH), 6.22, 6.38 (2 x d; 2H; J=1.9 Hz; H-6, H-8), 6.98 (AA'XX'; 2H; J=8.7 Hz; H-3', H-5'), 7.47 (AA'XX'; 2H; J=8.7 Hz; H-2', H-6'), 8.35 (s; 1H; H-2), 10.85 (br s; 1H; 7-OH), 12.92 (s; 1H; 5-OH).

5,7-dihydroxy-3-(4-(5-hydroxypentyloxy)phenyl)chromen-4H-one (22)

¹H NMR (DMSO-d₆): 1.35 – 1.55 (m; 4H; 2 x -CH₂-), 1.60 – 1.80 (m; 2H; -CH₂-), 3.20 – 3.45 (m; 2H; -CH₂O-), 3.98 (t; 2H; J=6.4 Hz; -CH₂O-), 4.38 (m; 1H; -CH₂OH), 6.22, 6.38 (2 x d; 2H; J=2.0 Hz; H-6, H-8), 6.98 (AA'XX'; 2H; J=8.8 Hz; H-3', H-5'), 7.47 (AA'XX'; 2H; J=8.8 Hz; H-2', H-6'), 8.36 (s; 1H; H-2), 10.90 (br s; 1H; 7-OH), 12.92 (s; 1H; 5-OH).

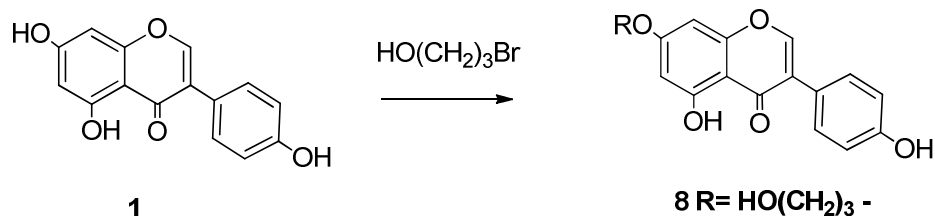
Conclusions

Genistein has been investigated as a molecular probe for several decades and although ample knowledge was accumulated on its biological activity towards prospective drug targets, its physicochemical and pharmacokinetic properties are considered sub-optimal for direct medical application. However, its constant presence on lists of drug leads and object of clinical trials, poses continuous challenge to rational drug design based on target oriented small molecular weight ligands. Although chemo- and bio-informatics based structure activity relationship has not yet advanced to a point which allows indicating feasible molecular modification for such pleiotropic ligand rendering its efficacy, existing strategies like pro-drug design and reverse pharmacology, strongly suggests that studies along that line are warranted. Since isoflavones are rather difficult objects for chemical transformations^[18], development of reliable methods of their derivatization is timely. We have developed simple experimental protocols, which can secure availability of selectively protected isoflavone derivatives with ether linkages, which can serve as temporary protecting groups as well as permanent

linker for installing structural elements of typical pro-drugs or pharmacophore conjugates. Usefulness of such constructs for preclinical investigations has been already demonstrated in our cooperative studies^[19,20]. 7-O-alkyl derivatives of genistein and daidzein can be obtained by treatment of isoflavone tetra-n-butyl ammonium salt with near stoichiometric amount of an alkylating agent. 4-O-alkylations can also be performed selectively but require either temporary protection of phenolic 7-OH or double deprotonation of the isoflavone substrate with a strong base, as previously suggested by Wähälä for selective esterifications^[21]. Benzylation can be treated as temporary protection since its reductive removal is no threat to integrity of the benzochromen-4-one heterocycle.

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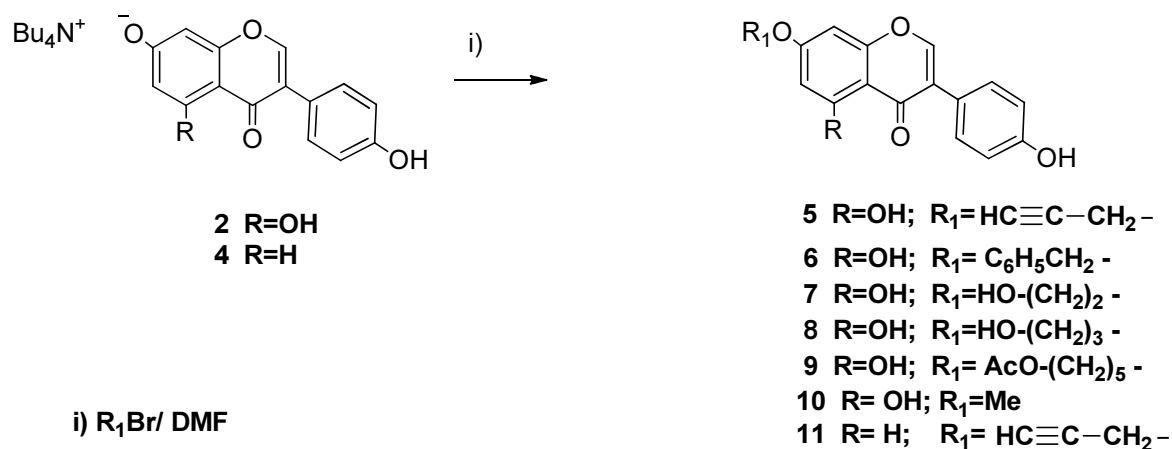


Scheme 1. Regioselective hydroxyalkylation of genistein (**1**)

Table 1. Test alkylations of genistein (**1**) to 7-O-hydroxypropyl-ether (**8**)

Solvent	Base (mol eq.)	Reagent (RX) (mol eq.)	Temp. (°C)	Yield (%)
acetone	K ₂ CO ₃ (1 eq)	1.5 eq	40	n. d.
THF	K ₂ CO ₃ (5 eq)	5 eq	20 ^a	n. d.
toluene	t-AmONa (0.5 eq)	5 eq	50 ^a	n. d.
DMF	t-AmONa (1 eq)	1 eq	r. t.	53
DMF	K ₂ CO ₃ (5 eq)	1 eq	r. t. ^b	40
DMF	DBU (1 eq.)	1.2 eq.	r. t.	47
DMF	t-AmONa (1 eq)	1 eq	r. t. ^b	n. d.
DMF	Bu ₄ NOH	1 eq.	50	91

a) Microwave irradiation, b) Sonification; n.d. – not detectable; r.t. room temperature (20°C +/- 2°)

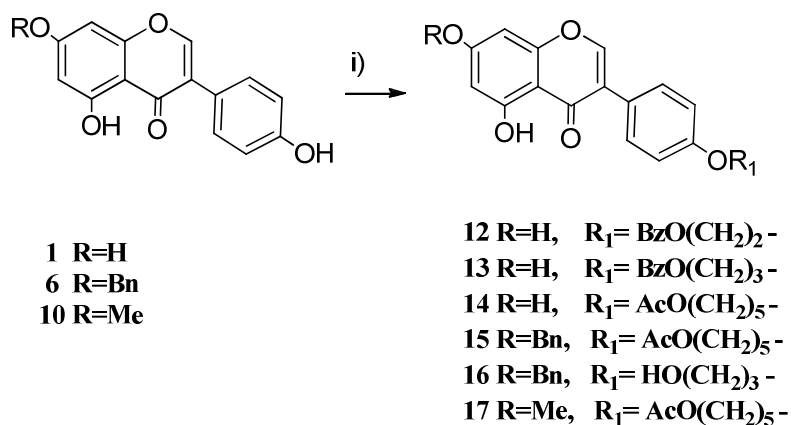


Scheme 2. Regioselective 7-O-alkylation of genistein (**2**) and daidzein (**4**) tetrabutylammonium salt.

Table 2. Synthesis of 7-O-derivatives of genistein (**1**) and daidzein (**3**).

Compound	R ¹	Time (h)	Yield (%)	m.p.(⁰ C)	HRMS (M+Na)
5	HC≡C—CH ₂ —	1.5	69 ^a	195-198	331.0582
6	C ₆ H ₅ CH ₂ —	20	63 ^b	222-225	383.0895
7	HO—(CH ₂) ₂ —	24	88 ^b	219-221	337.0666
8	HO—(CH ₂) ₃ —	24	89 ^b	201-203	351.0833
9	AcO—(CH ₂) ₅ —	24	85 ^b	118-124	421.2011
10	CH ₃ —	1	70 ^b	238-242	307.0582
11	HC≡C—CH ₂ —	1	72 ^a	218-221	315.0633

a) column chromatography (system a), b) crystallization, methanol



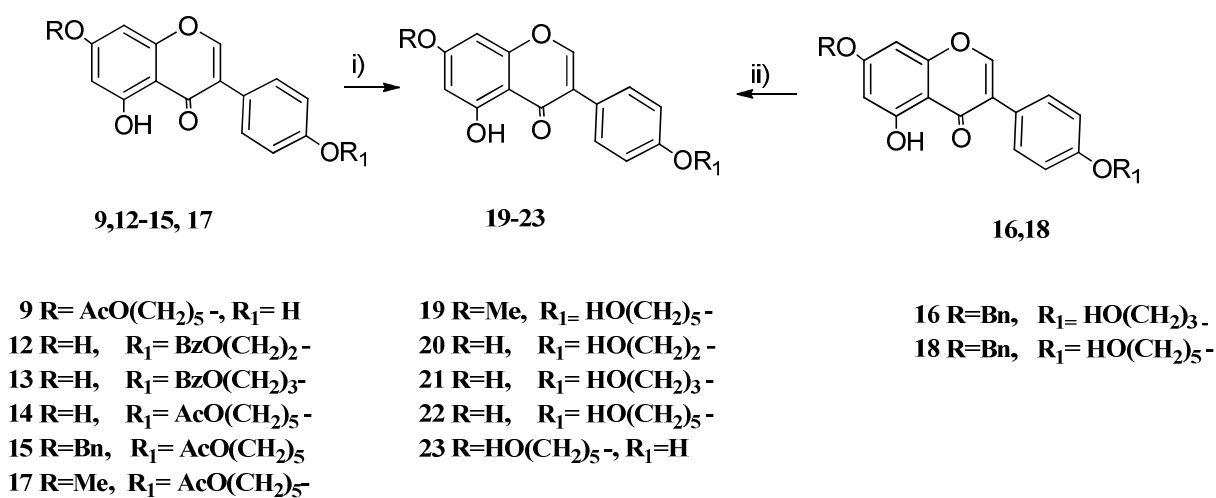
i) Procedure B: R₁Br, Bu^tOK/ DMF ; Procedure C: R₁Br, DBU, K₂CO₃/ DMF;

Scheme 3. Regioselective 4-O-alkylation of genistein (**1**), and 7-O-alkyl genistein (**6**, **10**)

Table 3. The synthesis of 4-O-derivatives of genistein (**1**)

Substrate	Product	Procedure	Yield (%)	m.p.(⁰ C) ^c	HRMS [M+Na]
1	12	B	40 ^a	168-171 (decomposition)	441.1201
1	13	B	39 ^b	156-159 (decomposition)	455.1346
1	14	B	48 ^a	116-118 (decomposition)	421.2182
1	21	B	9	206-208 (decomposition)	
1	22	B	22	200-202 (decomposition)	
6	15	C	52	168-172	511.1763
6	16	C	40 ^b	185-188	441.1328
10	17	C	36	84-87	435.1662

Column chromatography: a (system b); b (system c); c) crystallization, methyl alcohol



i) MeONa, MeOH or Mg(OMe)₂, MeOH; ii) Cyclohexene, Pd/C

Scheme 4. Deprotection of acyl and benzyl derivatives

Table 4. The synthesis of hydroxyalkyl 4-O- derivatives of genistein (**1**)

Substrate	Product	Procedure	Yield (%)	m.p.(⁰ C)	HRMS [M+Na]
9	23	D, Mg(OMe) ₂	88	154-157 ^a	379.1163
12	20	D, NaOMe	79	decomposition	337.0643
13	21	D, NaOMe	89	decomposition	351.0655
14	22	D, NaOMe	88	decomposition	379.0949
15	18	D, NaOMe	84	168-172 ^a	469.1560
17	19	D, NaOMe	88	118-122 ^a	393.1340
16	21	E	89	decomposition	351.0655
18	22	E	88	decomposition	379.0949

a) crystallisation, methyl alcohol

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