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Original article

Synthesis of genistein coupled with sugar derivatives and their inhibitory effect on nitric oxide production in macrophages

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ABSTRACT

The isoflavone genistein **1** and some derivatives modulate IL-12, TNF-α and NO production by macrophages and lung cancer cell lines, and improve the clinical signs of experimental autoimmune encephalomyelitis (EAE). Seven genistein derivatives connected at C-6 position of a sugar, such as D-glucose and D-galactose, were synthesized. The ability to modulate macrophage response was evaluated, showing variable inhibition capacity of NO and TNF-α production in J774.A1 and RAW 264.7. Five of the seven compounds were non-cytotoxic; compound **8** was more effective to inhibit NO and TNF-α production, without affecting cell viability.

1. Introduction

Innate immune response is an essential first line of defense against aggressors, and acts in the polarization of the adaptive response [1]. Macrophages are one of the components of innate immunity, and play an important role by releasing inflammatory mediators, such as nitric oxide (NO) and tumor-necrosis factor-alpha (TNF-α) [2,3].

Nitric oxide is a reactive molecule involved in several biological activities. Inducible nitric oxide synthase (iNOS), an enzyme formed in response to pathological conditions, controls NO production. The upregulation of iNOS is implicated in inflammatory processes [4]. Macrophages can be activated by addition of interferon-γ plus lipopolysaccharide, which promotes the release of large amounts of NO induced by iNOS [2,3].

TNF-α and its receptors (TNF-R1 and TNF-R2) are involved in autoimmune and inflammatory diseases characterized by excessive TNF-α production [5]. The blocking of the biochemical effects of TNF-α by soluble pre-ligand assembly domain (PLAD) of TNF receptors has been proved to inhibit arthritis in animal models [6].

Genistein **1** (4′,5,7-trihydroxyisoflavone) (Fig. 1) is a natural isoflavone that modulates IL-12, TNF-α and NO production by macrophages and lung cancer cell lines through the inhibition of the signaling pathway of nuclear factor kappa B (NF-κB) [7]. Moreover, genistein and its derivative 4′-*O*-tetradecanoyl-genistein **2** (Fig. 1) have been shown to reduce the clinical signs of experimental autoimmune encephalomyelitis (EAE), a murine autoimmune disease used in the study of multiple sclerosis (MS) [8,9].

Isoflavones are found in a number of plants in a glycosylated form [10]. Genistin **3** (Fig. 1), the glycoside form of genistein **1** [11], is metabolized to its aglycone form in the low gut by intestinal glycosidases [12,13]. However, it has been shown that genistin is partially absorbed without previous hydrolysis in the rat small intestine [14]. Furthermore, genistein bioavailability is greater when it is administered in the glycosylated form, in comparison with the aglycone form, due to the protection provided by the glycoside group and the lower solubility of aglycones [15]. Moreover, the transportation of the flavonoid quercetin 4′-β-glycoside by sodium-dependent glucose transporter was demonstrated in Caco-2 and G63 cells [16].

In order to obtain compounds with physicochemical properties similar to those exhibited by glycosides of genistein, this work

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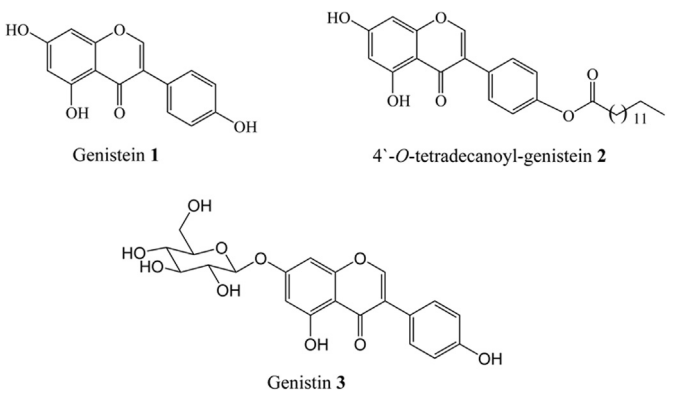


Fig. 1. Structure of **1**, **2** and **3**.

describes the synthesis of genistein derivatives attached to the C-6 position of D-galactose and D-glucose derivatives, and evaluates their effect on the production of NO and TNF-α by J774A.1 and RAW 264.7 cells, activated by LPS plus IFN-γ.

2. Results and discussion

2.1. Chemistry

The C7-OH group of genistein **1** exhibits a 100-fold increase in acidity, compared to the C4′-OH group. However, the C4′-phenolate or C4′-OH groups are better nucleophiles than the C7-phenolate or C7-OH groups, respectively. The C5-OH group is the less nucleophilic one of genistein. Because of this difference in reactivity, it is easy to make modifications at C4′-OH or/and C7-OH positions [17,18].

The protected carbohydrates **4** and **5** were first prepared for the subsequent condensation with genistein **1**. Iodide **4** was obtained (58% yield) through the acetalization of the hydroxyl groups at C1, C2, C3 and C4 positions of D-galactose using dry acetone, zinc chloride and catalytic amounts of sulfuric acid followed by an iodination reaction at C-6 [19] (Scheme 1). Iodide **5** was obtained (62% yield) through the iodination at C-6 of methyl α-D-glucopyranoside followed by acetylation of the hydroxyl groups [20] (Scheme 1).

The treatment of genistein **1** with potassium carbonate followed by the addition of 3 equivalents of iodide **4** afforded to obtain the mono-ether **6** and di-ether **7** (30 and 9% yield, respectively; Scheme 2). The structure of compound **6** was confirmed by nuclear Overhauser effect enhancement spectroscopy (NOEDIFF)-NMR experiments: the presence of specific NOE signals between H-6″ and H-6 and H-8 of **6** proves that the ether was formed at the *O*-7 position of the genistein. Hydrolysis of the isopropylidene groups of compound **6** resulted in the formation of compound **8** (70% yield, Scheme 2).

The reaction of genistein with potassium carbonate and 3 equivalents of iodide **5** led to the formation of the ethers **9** and **10** (55 and 20% yield, respectively). Compounds **9** and **10** were deacetylated with sodium methoxide in MeOH to afford genistein derivatives **11** and **12** (95 and 87% yield, respectively; Scheme 3).

2.2. Biological evaluation

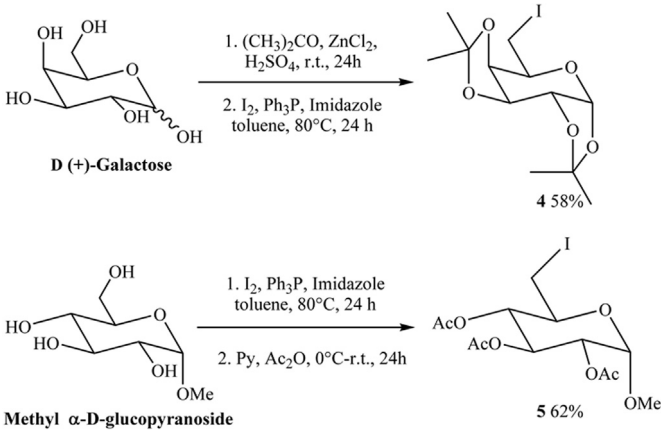
2.2.1. Cytotoxic activity

The inhibitory activity of genistein in the release of inflammatory mediators by macrophages has been previously described [2,21]. In the present work, seven genistein derivatives were synthesized and their ability to inhibit the production capacity of NO and TNF-α production in J774.A1 and RAW 264.7 macrophages was evaluated.

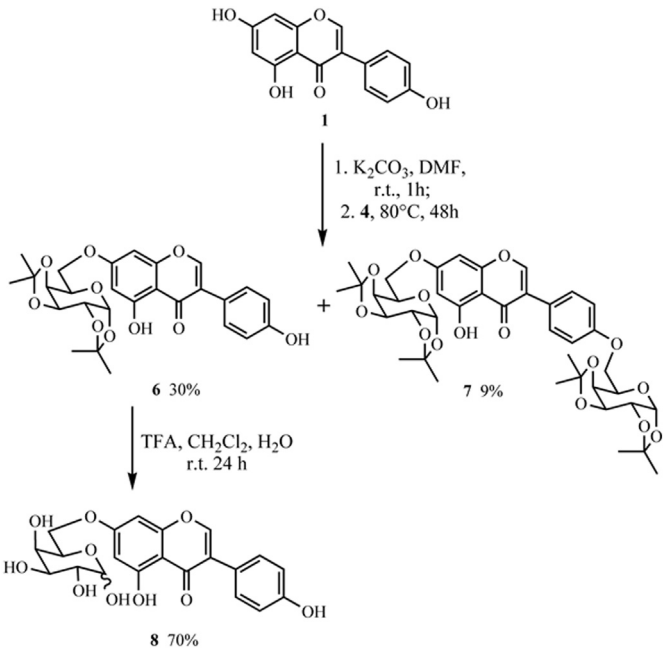
The cytotoxic activity of the synthesized compounds was evaluated by the determination of cell viability (Tables 1 and 2). None of these compounds was cytotoxic against RAW 264.7 macrophages. Monoether **11** was toxic against J774A.1 cells at all concentrations tested, and the protected monoether **6** was cytotoxic at 26 μM.

2.2.2. Inhibition of NO production

Nitric oxide has been implicated in several inflammatory diseases as septic shock, rheumatoid arthritis, and platelet aggregation. Inhibition of its production provides a useful therapy for inflammatory disorders [4,22]. The reduction of NO production after treatment of the cells with glycosylated flavonoids has been



Scheme 1. Synthesis of iodide **4** and **5**.



Scheme 2. Synthesis of **6**, **7** and **8**.

described: it has been shown that quercetin 7-*O*-β-D-