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Synthetic phenylethanoid glycoside derivatives as potent neuroprotective agents

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ABSTRACT

Several phenylethanoid glycoside derivatives were designed and synthesized. Most of the synthetic compounds showed significant neuroprotective effects, including antioxidative and anti-apoptotic properties. Specifically, target compounds displayed potent effects against various toxicities such as H_2O_2 and 6-hydroxydopamine (6-OHDA) in PC12 cells. Among the synthetic derivatives, three compounds (**5**, **6**, **8**) exhibited much superior activities to the marketed drug Edaravone. The compounds were able to prevent the 6-OHDA-induced damage in PC12 cells in a dose-dependent manner. The anti-apoptotic effects could be observed via cell morphological changes. Moreover, the compounds significantly reduced the intracellular ROS increase resulting from 6-OHDA treatment. The preliminary structure–activity relationships were also explored. Compounds **5**, **6**, **8** may hold the potential as promising neuroprotective agents and new lead compounds for the treatment of neurodegenerative diseases or cerebral ischemia.

1. Introduction

The pathological process, the damage or death of nerve cells, caused by oxidative stress or free radical damage, is shared in many neurological diseases such as neurodegenerative disorder [1] and ischemic stroke [2]. Neurodegenerative diseases are characterized by the progressive loss of structure and function of neurons, including death of neurons [3]. In this way, many degenerative diseases including Parkinson's (PD), Alzheimer's (AD), and Huntington's (HD) diseases occur. Another common disease resulting in nerve injury is cerebral ischemia. Ischemia leads to cerebral hypoxia and thus to the death of brain tissue, via the multiple, progressive, independently-lethal processes including oxidative stress and free radical damage [4]. These processes have not been well understood presently, so that the diseases stemming from them have, as yet, no cures. Currently, the drugs used in medication are not radical, but palliative. Most of them were developed based on the new use of the existing drugs to relieve symptom of neural lesion. One of strategies for the treatment of these diseases is to search for antioxidants or radical scavengers.

Over the past few years, our group and other research groups identified a class of new chemical entities with neuroprotective activities, namely, phenylethanoid glycosides (PGs) such as acteoside [5], echinacoside [6,7], and calceolarioside A [8,9]. PGs are widely distributed in the dicotyledons which have similar skeletons (Fig. 1) [10]. This class of compounds exhibit broad pharmacological spectrum related to neurodegenerative disorders and ischemic stroke, especially exhibit antioxidant activity and radical scavenging activity [11]. These properties make them promising in finding new drug candidates.

Cistanche is a genus of Orobanchaceae family, in which the prominent components are PGs. PGs have been on the market as traditional Chinese medicine for the neuroprotective effect and it is expected that new drugs will be developed from them in all likelihood, several dilemmas still lie ahead of us. PGs are widely distributed but sparsely contained in plant kingdom (about 0.02%–0.4%). And they are difficult to be extracted and purified in the presence of iridoids and other glycosides [12]. Furthermore, the synthesis of this class of compounds is not easy due to their inherent structures. In addition, PGs' low lipophilicity may disfavor its druggability. Currently, the synthesis of PGs derivatives and their structure–activity relationships, especially the neuroprotective

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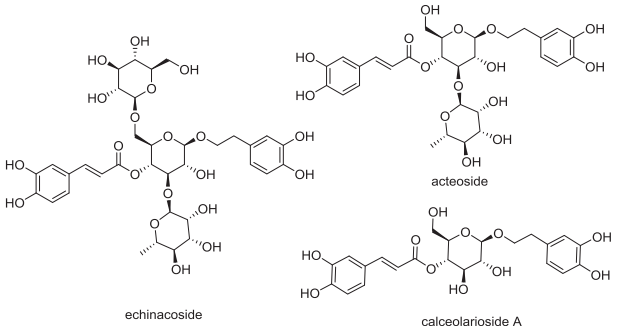

Fig. 1. Some structures of naturally occurring PGs.

effect, are less studied. Therefore, it will be of great significance that new PGs derivatives with improved activities are discovered to be potentially used in the treatment of neural lesion.

Herein we report the design and synthesis of a series of PGs derivatives as well as their neuroprotective activities. Previous studies suggested that the antioxidant properties of many polyphenols are related to the neuroprotection effect, but there is no clear consensus on the precise biologically active form (the glycosylated/esterified form and the type of sugar moiety present) [13]. PGs are naturally occurring products consisting of three moieties: the acid, sugar, and hydroxytyrosol. The role of each moiety in antioxidation and free radical scavenging has not been recognized yet. Therefore, calceolarioside A (compound **1**) was chosen as the basic target molecule (Fig. 2). To explore the substituent effect, the fluoro-, chloro-, and methoxyl-substituted analogs (compounds **2**–**4**) were designed. To reveal the function of the double bond, one analog (compound **5**) without the double bond was designed. To elucidate the contribution of the sugar moiety or ester moiety to the biological activities, we split the structure of compound **1**, removing the sugar moiety to give compound **6**, and removing the ester moiety to give compound **7**. In addition, since sialic acid plays an important role in protecting nerve cells such as its free radical scavenging ability [14–16] and the binding to myelin associated glycoprotein [17,18], based on the structure of **1**, we replaced the glucose moiety by the sialic acid moiety, compound **8** was thus designed.

2. Results and discussion

2.1. Chemistry

A new synthetic route to prepare the PGs derivatives was developed. The syntheses of phenylethanol and acid building

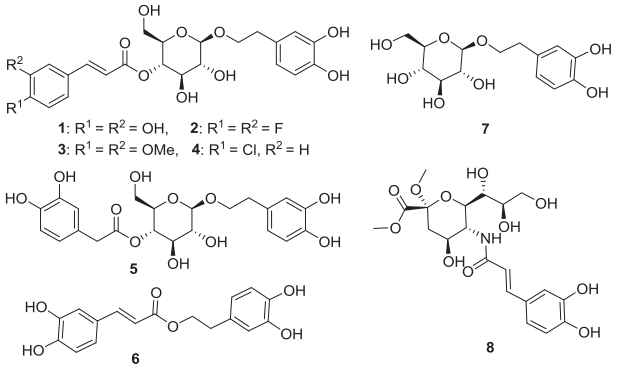

Fig. 2. The designed PGs derivatives.

blocks were shown in Scheme 1. The allyl group was employed to block the phenolic OH groups in aglycone and cinnamic acid derivatives (compounds **9**–**11**). Compounds **9** and **11** were also treated with acetic anhydride to yield the corresponding acetyl-protected products **9b** and **11b**, respectively. Compound **12a** was prepared by the condensation of malonic acid and *p*-Cl-benzaldehyde **12** using piperidine in pyridine according to the reported procedure [19].

Next, we began to synthesize the PGs intermediates. The glycosyl donor **13** was prepared from glucose by the reported protocol [20–22]. The glycosylation reaction of **13** and **9a** in the presence of TMSOTf as catalyst successfully afforded the β-linked glucoside **14** in 93% isolated yield. Removal of the benzylidene group in **14** by using pyridinium *para*-toluenesulfonate (PPTS) [23] provided diol **15** in 99% isolated yield. The primary OH group in **15** was selectively protected with allyl 1H-benzo[d][1,2,3]triazol-1-yl carbonate (AllocBt) to obtain the key intermediate **16** in 92% isolated yield (Scheme 2).

With the intermediate **16** in hands, the synthesis of designed target PGs derivatives **1**–**5** was carried out. As shown in Scheme 3, the condensation of compound **16** with acids (**9a**, **17a**–**b**, **12a**) in the presence of DCC and DMAP afforded compounds **18a**–**d** very smoothly. The acetyl functionality in **18a**–**d** was removed selectively using AcCl in MeOH/CH_2Cl_2 to obtain the corresponding alcohols **19a**–**d** in high yields [24]. Finally, compounds **19a**–**d** were treated by 10% Pd/C in MeOH/H_2O with a catalytic amount of TsOH or $HClO_4$ to remove the allyl and alloc groups simultaneously [25], yielding the target compounds **1**–**4**. In the similar way, compound **5** was prepared. As the acyl migration could occur in the preparation of PGs [26–28], the position of acyl groups in target molecules was unambiguously identified by their NMR (COSY, HSQC, HMBC, etc.) analyses.

Attention was next turned to the preparation of target molecules **6**–**8**. The condensation of alcohol **9b** and acid **11b** provided compound **20** smoothly. Subsequently, the acetyl groups were selectively removed by the treatment with acetyl chloride in methanol/CH_2Cl_2, affording **6** in high yield (Scheme 4). The target compound **7** was successfully prepared from intermediate **15** by the sequential removal of allyl and acetyl groups. Moreover, the target molecule **8** was prepared by the coupling reaction of compound **11** with the sialic acid derivative **21** [29] in the presence of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and *N*-methylmorpholine (NMM). All compounds were characterized by their ^{1}H NMR, ^{13}C NMR, and HRMS analyses.

2.2. Biological evaluation

With all the target compounds in hand, their neuroprotective properties were assessed in the hope of identification of more effective PGs analogs which prevent the damage or death of nerve cells. It is known that reactive species are closely related to neurodegeneration. Antioxidants can react with reactive species to invalidate them and they can be used as potential therapeutics. In order to evaluate the antioxidant properties of target compounds in neural cells, compounds **1**–**8** were first tested by the H_2O_2 model on PC12 cells [30]. H_2O_2 can generate exogenous free radicals, which are highly reactive species that lead to lipid, protein, and DNA damage and high concentration of H_2O_2 can help us find active compounds in preliminary screenings. PC12 cells are usually used as a screening model for studying neurodegenerative diseases [31,32]. To that end, PC12 cells were pretreated with 100 μM of PGs analogs for 6 h, and then treated with 300 μM of H_2O_2 for 1 h. The protection effect against H_2O_2 was determined by the cell viability through MTT assay. Vitamin C was used as the positive control. As