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Synthetic phenylethanoid glycoside derivatives as potent neuroprotective agents

Ying-Guo Liu [a, 1], Xiaxi Li [a, 1], De-Cai Xiong [a], Binhan Yu [a], Xiaoping Pu [a, *], Xin-Shan Ye [a, b, *]

[a] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Xue Yuan Road No. 38, Beijing 100191, China
[b] National Engineering Research Center for Carbohydrate Synthesis, Jiangxi Normal University, Nanchang 330022, Jiangxi, China

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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.

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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

* Corresponding authors. State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Xue Yuan Road No. 38, Beijing 100191, China

E-mail addresses: pxp123@bjmu.edu.cn (X. Pu), xinshan@bjmu.edu.cn (X.-S. Ye).

[1] These authors contributed equally to this work.

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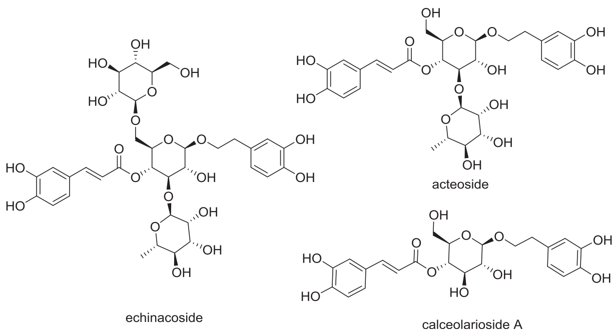


Fig. 1. Some structures of naturally occurring PGs.

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 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

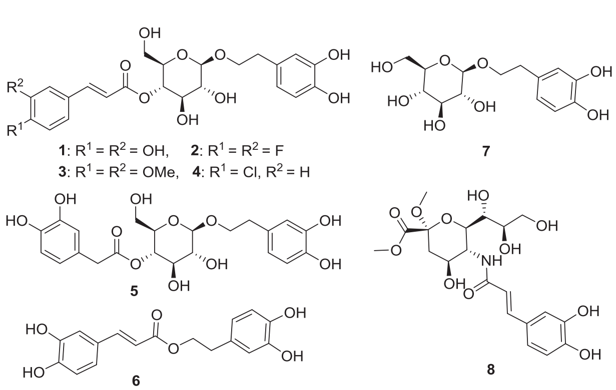
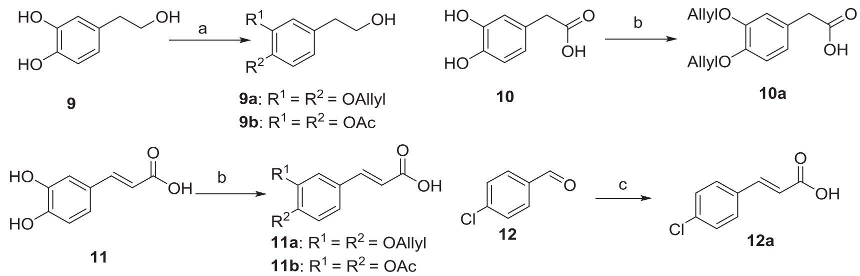
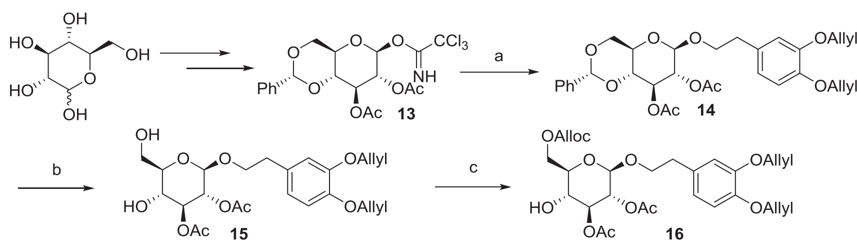


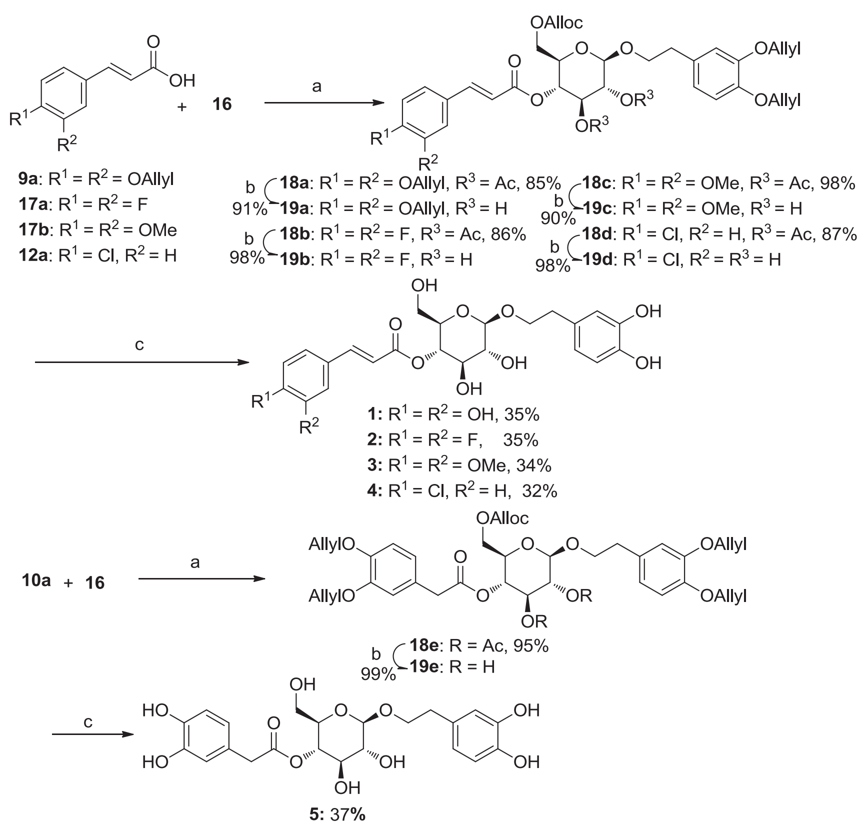
Fig. 2. The designed PGs derivatives.



Scheme 1. Synthesis of aglycone and cinnamic acid derivatives. Reagents and conditions: (a) for **9a**: allylBr, K_2CO_3, acetone, reflux overnight, 89%; for **9b**: isopropylamine, NaOH, Ac_2O, H_2O, 81%; (b) i) allylBr, K_2CO_3, acetone, reflux overnight; ii) MeOH, $NaHCO_3$ (sat.), reflux for 2 h, 88% for **10a**, 80% for **11a**; for **11b**: DMAP, pyridine, Ac_2O, 86%; (c) malonic acid, piperidine, pyridine, reflux overnight, 70%.



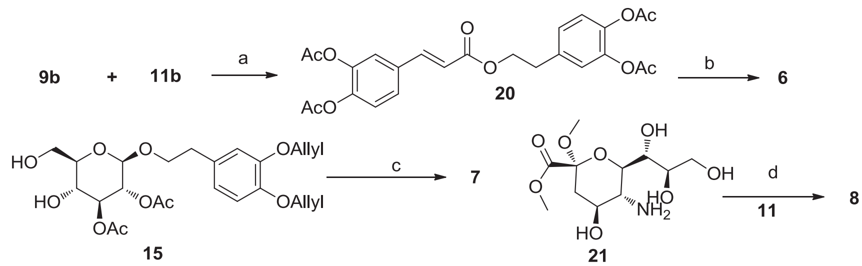
Scheme 2. Synthesis of PGs intermediates. Reagents and conditions:(a) TMSOTf, **9a**, CH2Cl2, 4 Å MS, −72 °C, 93%; (b) PPTS, MeCN, H2O, reflux, 99%; (c) AllocBt, Et3N, CH2Cl2, 92%.



Scheme 3. Synthesis of PGs derivatives **1**–**5**. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, 0 °C to r.t.; (b) AcCl, MeOH, CH_2Cl_2; (c) 10% Pd/C, MeOH, H_2O, $HClO_4$ or TsOH, reflux.

shown in Fig. 3, the cell viabilities were decreased significantly when treated with 300 μM of H_2O_2. Except for compound **3**, it was found that all other compounds **1**, **2**, **4**, **5**, **6**, **7**, **8** attenuated greatly cell death caused by H_2O_2.

To further examine the protective effects of compounds **1**, **2**, **4**, **5**, **6**, **7**, **8**, the 6-OHDA-induced damage model was chosen [33]. Neurotoxin 6-OHDA is widely used to generate PD-like models. It induces neuronal death via uncoupling mitochondrial oxidative phosphorylation resulting in energy deprivation, which is related to its ability to produce H_2O_2, hydroxyl and superoxide radicals.



Scheme 4. Synthesis of PGs analogs **6**–**8**. Reagents and conditions: (a) oxalyl chloride, CH_2Cl_2, Et_3N, 69%; (b) AcCl, CH_2Cl_2, MeOH, 82%; (c) i) 10% Pd/C, MeOH, H_2O, $HClO_4$; ii) AcCl, CH_2Cl_2, MeOH, 92% for two steps; (d) PyBOP, NMM, DMF, 39%.

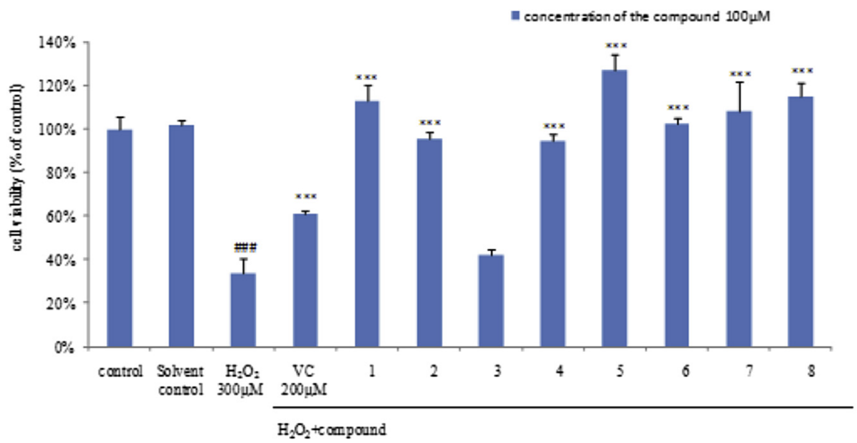


Fig. 3. Survival of PC12 cells upon exposure to H_2O_2 in the presence of Vitamin C or compounds, respectively. Cells were treated with the compound (100 μM) for 6 h followed by the treatment with H_2O_2 (300 μM) for 1 h. Values represent survival as % of PBS treated control from MTT assay. Data are presented as a mean ± SD, n = 3; compared with control, ###*P* < 0.001; compared with model, ****P* < 0.001.

Recently, it has been shown that 6-OHDA can also induce apoptosis in various cells. Therefore, the evaluation using this model was performed. The PC12 cells were incubated with PGs analogs at concentrations ranging from 6.25 μM to 100 μM for 6 h, which was followed by the incubation with 100 μM of 6-OHDA for 24 h. As shown in Fig. 4, the number of viable cells decreased significantly with 100 μM of 6-OHDA treatment. When the cells were pretreated with compounds **5**, **6**, **8**, the cell viability rates significantly increased in a concentration-dependent fashion (compound **6** displayed the effect in a concentration-dependent fashion at lower concentrations, see Fig. S5 in the supplementary data). It was observed that compounds **1**, **2**, **4**, **7** did not exhibit the protective effects against damage induced by 6-OHDA in a concentration-dependent manner, or the cell viability rates did not increase significantly. Consequently, compounds **5**, **6**, **8** were chosen for further investigations.

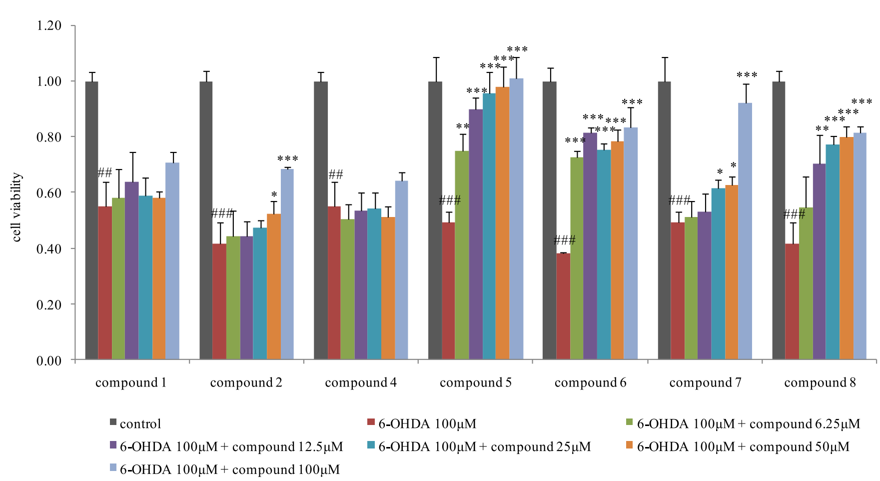


Fig. 4. Survival of PC12 cells upon exposure to 6-OHDA in the presence of selected compounds, respectively. Cells were treated with compounds (from 6.25 μM to 100 μM) for 6 h, followed by the treatment with 6-OHDA (100 μM) for 24 h. Values represent survival as % of PBS treated control from MTT assay. Data are presented as a mean ± SD, n = 3; compared with control, ##*P* < 0.01, ###*P* < 0.001; compared with 6-OHDA, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Encouraged by the above results, the protective effects of compounds **5, 6** and **8** against 6-OHDA-induced apoptosis were further investigated. The cell morphological changes were observed by staining with AnnV/PI. AnnV staining (green) represents early apoptosis and PI staining represents late apoptosis (red). Edaravone, a marketed drug for neuroprotection, was chosen as the positive control [34]. It was found that the control PC12 cells were not stained, whereas 6-OHDA-treated cells were stained red in the nucleus site with PI and green in the membrane site with AnnV (Fig. 5A), indicating that 6-OHDA induced PC12 apoptosis and necrosis. We observed the cellular membrane damage and nuclear karyorrhexis. When treated with 6-OHDA in the presence of compound **5** (6.25 μM, 25 μM, 50 μM) (Fig. 5C), compound **6** (1.56 μM, 3.125 μM, 6.25 μM) (Fig. 5D), compound **8** (6.25 μM, 12.5 μM, 25 μM) (Fig. 5E), the staining of red was not observed and the staining of green decreased in different degrees. The results indicated that all the three compounds prevented the late apoptosis of PC12 cells. More importantly, PC12 cells were controlled in the early stage of apoptosis when pretreated with compounds **5, 6,** and **8**, and with the increase of concentrations of the compounds, the apoptosis of PC12 cells significantly decreased even vanished. For example, when PC12 cells were treated with compound **6** (3.125 μM), the apoptosis was not observed (no stained green). By contrast, when the cells were treated with Edaravone at a high concentration (200 μM), the apoptosis was still detected (stained green) (Fig. 5B).

Finally, the effect of compounds **5**, **6** and **8** on the intracellular ROS level of PC12 cells was measured using the ROS test kit. DCFH-DA can passively enter the cell and react with ROS to produce a fluorescent compound dichlorofluorescein (DCF). As shown in Fig. 6 and Fig. S6, when PC12 cells were exposed to 100 μM of 6-OHDA for 24 h, the intracellular ROS increased obviously. Preconditioning with compounds **5**, **6** and **8** significantly reduced the 6-OHDA induced ROS level in a dose-dependent manner. Edaravone was again used for the positive control. The experiments demonstrated that our synthetic compounds **5**, **6** and **8** showed better ROS-lowering activities than Edaravone.

2.3. Preliminary structure–activity relationships

We began the first screening by assessing the effect of synthetic PGs analogs against H_2O_2-induced cell death. Surprisingly, all the compounds can protect the cell from the free radical damage at the same level except the di-methylated one (compound **3**). Additionally, the protective effects of the compounds on the 6-OHDA-induced damage model were further evaluated. The cell survival

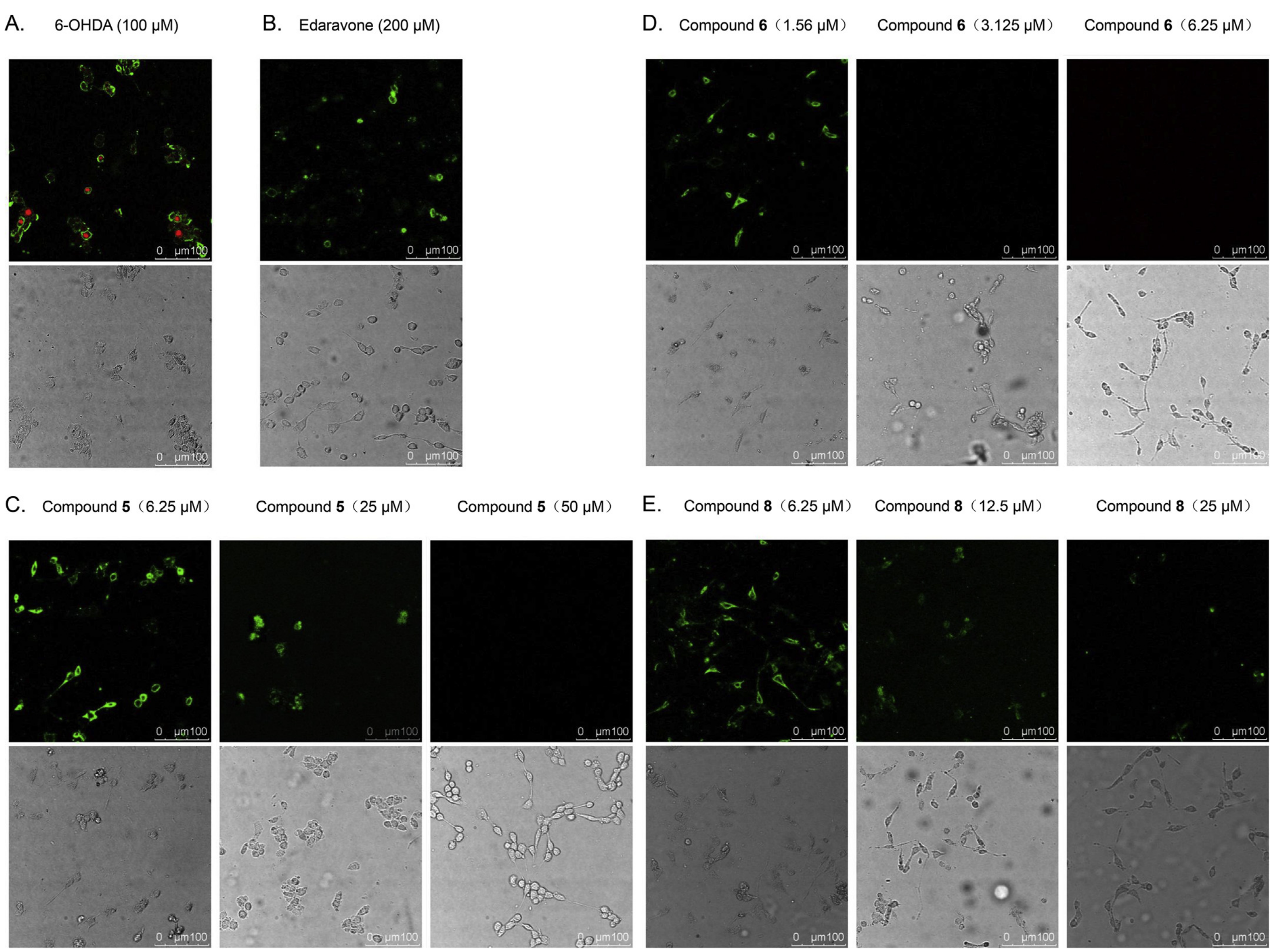


Fig. 5. The neuroprotective effect of compounds **5**, **6**, and **8** by staining Annexin V/PI assay. PC12 cells were pretreated with compounds **5**, **6**, **8** and Edaravone for 6 h, respectively, followed by the treatment with 6-OHDA (100 μM) for 24 h, then the cells were staining with Annexin V/PI and imaged under a fluorescence microscope. The figures are presented on the merged images of Annexin V and PI, Scale bar, 100 μm.

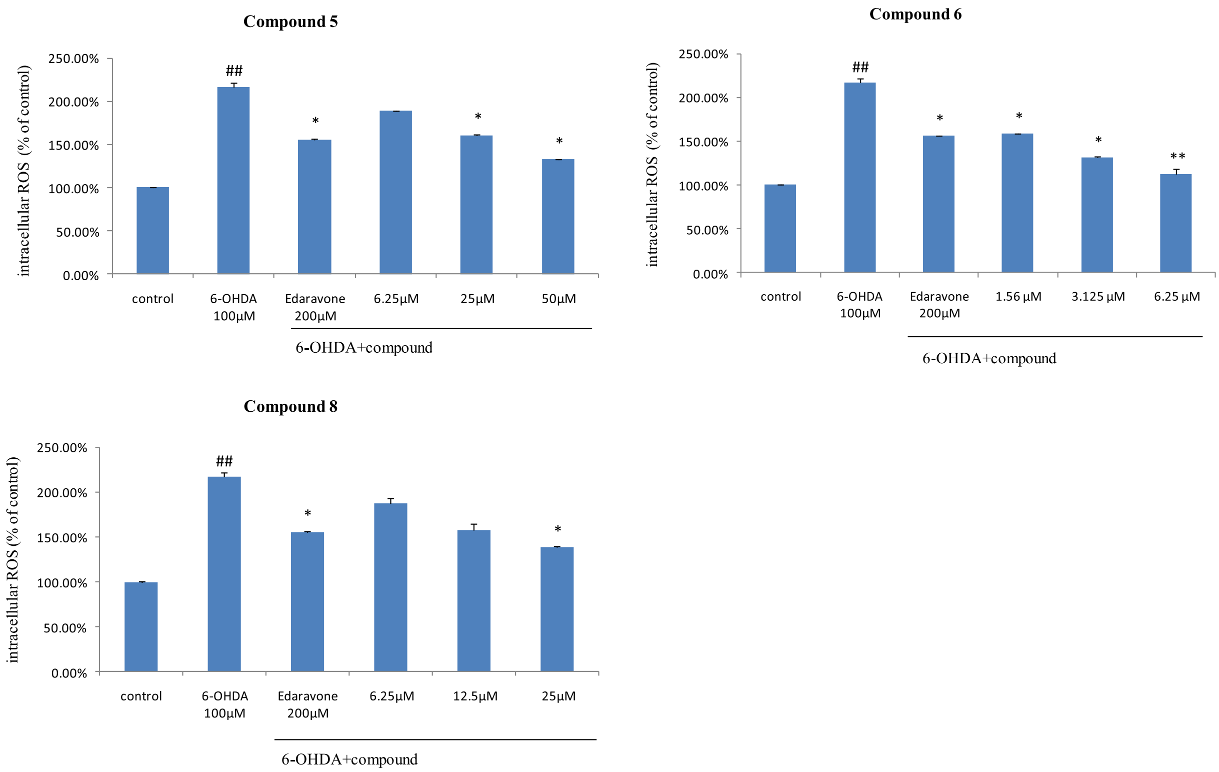


Fig. 6. Intracellular ROS level of PC12 cells upon exposure to 6-OHDA in the presence of compounds **5**, **6**, **8**, respectively. PC12 cells were exposed to 100 μM of 6-OHDA for 24 h, which was preconditioned with compounds **5**, **6**, **8** at different concentrations for 6 h. Edaravone was employed as the positive control. The fluorescence intensity of cells cultured in normal medium was designated as 100%. Data are presented as a mean ± SD, n = 3; compared with control, ##$P < 0.01$; compared with model, *$P < 0.05$, **$P < 0.01$.

data demonstrated that, the derivatives (**2**, **4**, **7**) exhibited similar protective effects to the natural compound (compound **1**), however, compound **5** displayed the superior effect to compound **1**. The inherent metabolite of PGs (compound **7**) did not show improved neuroptotective effect below 100 μM. These results might indicate that the catechol moiety may not monopolize the action but probably contribute partially to neuroprotection. On the other hand, the glucose moiety seems not to be necessary for the neuroprotection since compound **6** exhibited better neuroprotective activities. This finding also agrees with the recent biological activity studies on caffeic acid phenethyl ester analogs [35,36]. Moreover, as expected, compound **8**, a sialic acid derivative, displayed better neuroprotective effect at the concentration of 25 μM than the positive drug (Edaravone at the concentration of 200 μM). The introduction of sialic acid moiety in place of glucose moiety may serve as a new starting point to modify the PGs.

2.4. The celluar target and BBB permeability

This class of compounds have broad pharmacological spectrum related to neuroprotection such as free radical scavenging activity, inhibition of glutamate excytotoxicity, repression of the Parkin protein decomposition and the α-synuclein aggregation, preventing the cell programmed death via inhibition of the bioactivities of caspase-3 and caspase-8 in rat nerve cells [37]. No single identified cellular target or further research have been reported at present. However, with highly bioactive PGs in hand, we have the chance to identify the target in our future work.

PGs' penetration through BBB is suspected because of their low liposolubility. Nevertheless, it was verified that PGs can reach rat brains although they have low oral bioavailability [38–40].

3. Conclusion

Our previous studies demonstrated that the naturally-occurring PGs such as acteoside could protect the dopaminergic neurons SH-SY5Y cells from rotenone-induced apoptosis [41], echinacoside displayed neuroprotective effects in the mouse MPTP model of Parkinson's disease [6], and phenylethanoid glycosides from *Cistanches salsa* inhibited apoptosis induced by 1-methyl-4-phenylpyridinium ion in neurons [7]. However, as the concentration of the compounds increased, some toxicity appeared. So we want to search for new PGs analogs with better activity and less toxicity. Hence, based on the structures of natural PGs, several new PGs derivatives were designed and synthesized. It was found that the pretreatment of PC12 cells with our synthetic compounds **1**, **2**, **4**, **5**, **6**, **7**, **8** can prevent the H_2O_2-induced cell death. Furthermore, by using the 6-OHDA-induced damage model in PC12 cells, it was demonstrated that compounds **5**, **6**, **8** can significantly increase the cell viability rates in a concentration-dependent fashion. And these compounds did not show obvious cytotoxicity (Fig. S4). To further investigate the mechanism underlying their neuroprotective effects, the 6-OHDA-induced apoptosis in PC12 cells was detected by using an AnnV/PI staining assay. Previous reports proved that the exposure of PC12 cells to a variety of stimuli, including acute deprivation of serum and neurotoxins such as 6-OHDA, resulted in apoptosis accompanied by activation of caspase-3-like proteases. Our results showed that compounds **5**, **6**, **8** can prevent 6-OHDA-induced apoptotic and necrotic cells in a dose-dependent manner. And their protective effects are better than that of the positive drug (Edaravone). The effects of compounds **5**, **6**, **8** on the intracellular ROS level of PC12 cells were also examined. It was found that these compounds are able to significantly reduce the ROS increase resulting from 6-OHDA treatment. And increasing the dosage of compounds **5**, **6**, **8** can cause more reduction of ROS level. Again, the ROS-lowering effects are better than Edaravone. Thus, the synthetic compounds **5**, **6**, **8** may hold the potential as promising neuroprotective agents and new lead compounds for the treatment of neurodegenerative diseases. The disclosed results will benefit the discovery of more potent and less toxic PGs analogs with neuroprotection activities as well as the understanding of their structure–activity relationships.

4. Materials and methods

4.1. Chemistry

All reagents and solvents were dried prior to use according to the standard methods. Commercial reagents were used without further purification, unless otherwise stated. All reactions were performed with a glass stopper or rubber septa under a positive pressure of argon. The reactions were monitored by TLC on silica gel 60 F-254 precoated on aluminum plates. Detection was done by both UV light and charring with acidic ceric ammonium molybdate solution. Column chromatography was performed on Silica Gel 200–300 mesh. ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC and HMBC spectra were recorded on INOVA-500 or Bruker AVANCE III-400 spectrometer. Low-resolution mass spectra were recorded on PE SCLEX QSTAR or Autospec-Ultimate TOF mass spectrometer through electron spray ionization (ESI). High-resolution mass spectra were recorded with APEX IV FT-MS mass spectrometer in ESI mode. Elemental analyses were performed on Vario EL III instrument. Optical rotations were measured at 25 °C and 589 nm in Hanon P850 polarimeter.

4.1.1. Preparation of 3,4-bis(allyloxy)phenylethanol (*9a*)

A mixture of **9** (0.83 g, 5.4 mmol, 1.0 equiv), allyl bromide (2.59 g, 21.6 mmol, 4.0 equiv), and K_2CO_3 (3.73 g, 27 mmol, 5.0 equiv) in acetone was stirred under reflux for 12 h. The solids were filtered out and the solvent was evaporated. The residue was purified by column chromatography on silica gel (eluant: EtOAc/petroleum ether, 3:1) to produce **9a** (1.12 g, 89% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (d, J = 8.0 Hz, 1H), 6.78–6.73 (m, 2H), 6.12–6.02 (m, 2H), 5.44–5.36 (m, 2H), 5.28–5.24 (m, 2H), 4.62–4.56 (m, 4H), 3.81 (t, J = 6.5 Hz, 2H), 2.78 (t, J = 6.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.6, 147.2, 133.6, 133.5, 131.5, 121.4, 117.5, 117.4, 115.3, 114.6, 70.1, 70.0, 63.6, 38.6; MS (*m/z*): calcd for $C_{14}H_{18}O_3$: 234; found: 234 $[M]^+$; Anal. Calcd for $C_{14}H_{18}O_3$ (%): C, 71.77; H, 7.74; found C, 71.72; H, 7.84.

4.1.2. Preparation of 3,4-dihydroxyphenylacetic acid diacetate (*9b*)

To a solution of NaOH (0.5 g) in H_2O (1.5 mL) were added isopropylamine (30 mL), 3, 4-dihydroxyphenylethanol (1.0 g, 6.49 mmol, 1 equiv) and Ac_2O (1.6 mL). The mixture was stirred for 40 min at room temperature and then diluted with EtOAc (150 mL), washed with ice water, neutralized with HOAc at 0 °C, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:3) to give **9b** (1.13 g, 81%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (s, 2H), 7.05 (s, 1H), 3.82 (t, J = 6.3 Hz, 2H), 2.83 (t, J = 6.3 Hz, 2H), 2.28 (s, 6H), 1.76 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.42, 168.36, 141.9, 140.5, 137.7, 127.2, 123.8, 123.3, 63.2, 38.5, 20.6; ESI-HRMS $[M+Na]^+$ calcd for $C_{12}H_{14}NaO_5$ 261.0733, found 261.0730. The spectroscopic data coincide with the previous report [42].

4.1.3. General procedure for the preparation of 3,4-bis(allyloxy) phenylacetic acid (*10a*) and 3,4-bis(allyloxy)cinnamic acid (*11a*)

A mixture of acid (1.0 equiv), allyl bromide (2.0 equiv per phenolic OH), and K_2CO_3 (5.0 equiv) in acetone was stirred under reflux for 12 h. The solids were filtered out and acetone was evaporated. To this residue was added methanol/$NaHCO_3$ (sat.) (1:1). The mixture was heated under reflux for 2 h. After it was cooled down, the pH value of the mixture was adjusted to 6 with HCl (aq. 1 M). The precipitation was collected and further purified by recrystallization from EtOAc/petroleum ether to give the product.

Compound **10a** was prepared from compound **10** as white solids (88% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89–6.86 (m, 2H), 6.83 (dd, J = 1.9 Hz, 8.2 Hz, 1H), 6.15–6.05 (m, 2H), 5.47–5.41 (m, 2H), 5.31–5.29 (m, 2H), 4.63–4.62 (m, 4H), 3.60 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 148.6, 148.0, 133.5, 133.4, 126.2, 122.0, 117.6, 117.5, 115.5, 114.4, 70.1, 70.0, 40.5; MS (*m/z*): calcd for $C_{14}H_{16}O_4$: 248, found: 248 $[M]^+$. Anal. Calcd for $C_{14}H_{16}O_4$ (%) C, 67.73; H, 6.50; found C, 67.51; H, 6.75.

Compound **11a** was prepared from compound **11** as white solids (80% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 15.9 Hz, 1H), 7.12–7.10 (m, 2H), 6.88 (d, J = 8.1 Hz, 1H), 6.28 (d, J = 15.9 Hz, 1H), 6.08 (m, 2H), 5.47–5.41 (m, 2H), 5.33–5.29 (m, 2H), 4.66–4.64 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 151.1, 148.6, 146.9, 133.0, 132.8, 127.2, 123.1, 118.0, 117.9, 114.9, 113.4, 112.9, 70.0, 69.7; ESI-HRMS $[M+H]^+$ calcd for $C_{15}H_{17}O_4$ 261.1121, found 261.1121.

4.1.4. Preparation of 3,4-dihydroxycinnamic acid diacetate (*11b*)

To a solution of **11** (1.0 g, 5.56 mmol, 1.0 equiv) in pyridine (50 mL) was added Ac_2O (2 mL). The reaction mixture was stirred for 3 h and concentrated under *vacuum*. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:1) to give **11b** (1.26 g, 86% yield) as white solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 15.9 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.32 (s, 1H), 7.18 (d, J = 9.2 Hz, 1H), 6.32 (d, J = 15.9 Hz, 1H), 2.24 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 168.0, 145.0, 143.8, 142.5, 132.9, 126.7, 124.0, 123.0, 118.4, 20.64, 20.59; ESI-HRMS $[M+K]^+$ calcd for $C_{13}H_{12}KO_6$ 303.0842, found 303.0839.

4.1.5. Preparation of 3,4-bis(allyloxy)phenylethyl 2,3-di-O-acetyl-4,6-di-O-benzylidene-β-D-glucopyranoside (*14*)

To a mixture of **13** (0.50 g, 10.1 mmol, 1.0 equiv), **9a** (0.25 g, 10.6 mmol, 1.05 equiv) and 4 Å molecular sieves (2.0 g) in anhydrous CH_2Cl_2 (60 mL) was added TMSOTf (20 μL) at −72 °C under Ar. The reaction mixture was stirred for 2 h, and then was neutralized with Et_3N. The mixture was filtered over a pad of silica gel and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:3) to give **14** (0.53 g, 93% yield) as a colorless oil. $[\alpha]_D^{25}$ − 17.1 (*c* 0.0203, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.41 (m, 2H), 7.35–7.34 (m, 3H), 6.81 (d, J = 8.1 Hz, 1H), 6.74 (d, J = 1.6 Hz, 1H), 6.71 (dd, J = 1.6 Hz, 8.1 Hz, 1H), 6.13–6.01 (m, 2H), 5.48 (s, 1H), 5.44–5.36 (m, 2H), 5.31–5.23 (m, 3H), 4.99 (t, J = 9.1 Hz, 7.9 Hz, 1H), 4.60–4.56 (m, 5H), 4.35 (dd, J = 4.9 Hz, 10.5 Hz, 1H), 4.07 (dt, J = 6.5 Hz, 9.4 Hz, 1H), 3.77 (t, J = 10.3 Hz, 1H), 3.71–3.62 (m, 2H), 3.51 (dt, J = 4.8 Hz, 9.8 Hz, 1H), 2.80 (t, J = 6.4 Hz, 2H), 2.03 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 169.5, 148.4, 147.1, 145.6, 136.8, 133.6, 133.5, 131.0, 129.3, 128.2, 126.1, 124.7, 121.3, 117.43, 117.37, 115.3, 114.4, 101.5, 101.3, 78.3, 72.1, 71.7, 70.9, 70.1, 69.9, 68.5, 66.3, 35.5, 20.7, 20.5; ESI-HRMS $[M+H]^+$ calcd for $C_{31}H_{37}O_{10}$ 569.2381, found 569.2381.

4.1.6. Preparation of 3,4-bis(allyloxy)phenylethyl 2,3-di-O-acetyl-β-D-glucopyranoside (*15*)

To a stirred solution of **14** (1.00 g, 1.76 mmol, 1.0 equiv) in CH_3CN/H_2O (100 mL, 9:1) was added pyridinium *p*-toluenesulfonate (PPTS) (840 mg, 3.35 mmol, 2.0 equiv). The mixture was heated under reflux for 12 h, then diluted with $NaHCO_3$ (sat. 50 mL), and extracted with EtOAc (150 mL × 3). The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:1), affording **15** (770 mg, 99% yield) as a colorless oil. $[\alpha]_D^{25}$ − 12.8 (*c* 0.027, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (d, *J* = 8.1 Hz, 1H), 6.73 (d, *J* = 1.8 Hz, 1H), 6.70 (d, *J* = 1.8 Hz, 8.1 Hz, 1H), 6.13–6.01 (m, 2H), 5.44–5.36 (m, 2H), 5.28–5.23 (m, 2H), 4.99 (dd, *J* = 9.5 Hz, 9.2 Hz, 1H), 4.91 (dd, *J* = 7.8 Hz, 9.7 Hz, 1H), 4.61–4.56 (m, 4H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.06 (dt, *J* = 6.5 Hz, 9.5 Hz, 1H), 3.92 (dd, *J* = 3.4 Hz, 11.9 Hz, 1H), 3.82 (dd, *J* = 4.9 Hz, 12.0 Hz, 1H), 3.75 (t, *J* = 9.2 Hz, 1H), 3.64 (dt, *J* = 7.3 Hz, 9.4 Hz, 1H), 3.43–3.39 (m, 1H), 2.79 (t, *J* = 6.8 Hz, 2H), 2.08 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 169.5, 148.5, 147.2, 133.7, 133.6, 131.5, 121.4, 117.5, 117.4, 115.5, 114.5, 100.8, 76.2, 75.5, 71.1, 70.8, 70.2, 70.0, 69.6, 62.2, 35.6, 20.8, 20.6; ESI-HRMS $[M+Na]^+$ calcd for $C_{24}H_{32}NaO_{10}$ 503.1888, found 503.1890.

4.1.7. Preparation of 3,4-bis(allyloxy)phenylethyl 2,3-di-O-acetyl-6-O-alloc-β-D-glucopyranoside (16)

To a solution of **15** (100 mg, 0.208 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (10 mL) was added allyl 1H-benzo[d][1,2,3]triazol-1-yl carbonate (AllocBt) (54 mg, 0.250 mmol, 1.2 equiv) and Et_3N (140 μL, 1 mmol). The mixture was stirred at room temperature for 8 h and then neutralized with AcOH and evaporated. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:2) to furnish **16** (114 mg, 92% yield) as a colorless oil. $[\alpha]_D^{25}$ − 27.1 (*c* 0.008, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (d, *J* = 8.1 Hz, 1H), 6.73 (d, *J* = 1.8 Hz, 1H), 6.70 (d, *J* = 1.8 Hz, 8.1 Hz, 1H), 6.13–6.01 (m, 2H), 5.97–5.87 (m, 1H), 5.45–5.34 (m, 3H), 5.29–5.23 (m, 3H), 4.99 (d, *J* = 8.7 Hz, 1H), 4.92 (d, *J* = 7.8 Hz, 9.6 Hz, 1H), 4.64–4.62 (m, 2H), 4.60–4.55 (m, 4H), 4.47–4.44 (m, 3H), 4.06 (dt, *J* = 6.5 Hz, 9.5 Hz, 1H), 3.68–3.58 (m, 2H), 3.56–3.52 (m, 1H), 2.90 (d, *J* = 3.1 Hz, 1H), 2.79 (d, *J* = 6.9 Hz, 2H), 2.08 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 169.4, 155.3, 148.4, 147.1, 133.7, 133.6, 131.6, 131.3, 121.4, 119.2, 117.5, 117.4, 115.4, 114.5, 100.7, 75.8, 74.0, 71.0, 70.7, 70.2, 70.0, 69.1, 68.9, 66.3, 35.5, 20.8, 20.6; ESI-HRMS $[M+Na]^+$ calcd for $C_{28}H_{36}NaO_{12}$ 587.2103, found 587.2104.

4.1.8. General procedure for the preparation of 18a–e

To a stirred and cooled (0 °C) solution of **16** (1.0 equiv) and corresponding acid (1.5 equiv) in CH_2Cl_2 was added DCC (1.5 equiv) and DMAP (1.5 equiv), and the mixture was stirred for 1 h at 0 °C. The mixture was allowed to be gradually warmed to room temperature, and stirred overnight. The solvent was removed and the residue was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:4 to 1:3) to give the coupling product.

3,4-Bis(allyloxy)phenylethyl 2,3-di-*O*-acetyl-4-*O*-((trans)-3,4-bis(allyloxy)cinnamoyl)-6-*O*-alloc-β-D-glucopyranoside (compound **18a**) was obtained from the condensation of compounds **16** and **9a** as a colorless oil (85% yield): $[\alpha]_D^{25}$ − 9.8 (*c* 0.008, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 15.9 Hz, 1H), 7.08–7.05 (m, 2H), 6.87 (d, *J* = 8.1 Hz, 1H), 6.80 (d, *J* = 8.1 Hz, 1H), 6.75 (d, J = 1.8 Hz, 1H), 6.71 (dd, *J* = 1.8 Hz, 8.1 Hz, 1H), 6.18 (d, *J* = 15.9 Hz, 1H), 6.13–6.01 (m, 4H), 5.94–5.84 (m, 1H), 5.46–5.21 (m, 11H), 5.15 (t, *J* = 9.6 Hz, 1H), 5.01 (dd, *J* = 8.0 Hz, 9.6 Hz, 1H), 4.65–4.56 (m, 10H), 4.52 (d, *J* = 7.9 Hz, 1H), 4.22–4.26 (m, 2H), 4.08 (dt, *J* = 6.4 Hz, 9.4 Hz, 1H), 3.81–3.77 (m, 1H), 3.65 (dt, *J* = 6.4 Hz, 9.4 Hz, 1H), 2.80 (t, *J* = 6.8 Hz, 2H), 1.96 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.3, 165.6, 154.6, 151.1, 148.7, 148.5, 147.2, 146.6, 133.7, 133.6, 133.0, 132.8, 131.6, 131.4, 127.1, 123.2, 121.4, 119.0, 118.0, 117.9, 117.4, 117.3, 115.5, 114.6, 113.9, 113.5, 112.9, 100.7, 72.6, 72.1, 71.3, 70.7, 70.2, 70.03, 70.00, 69.7, 68.7, 66.2, 35.6, 20.6, 20.5; ESI-HRMS $[M+Na]^+$ calcd for $C_{43}H_{50}NaO_{15}$ 829.3042, found 829.3052.

3,4-Bis(allyloxy)phenylethyl 2,3-di-*O*-acetyl-4-*O*-((trans)-3,4-difluorocinnamoyl)-6-*O*-alloc-β-D-glucopyranoside (compound **18b**) was obtained from the condensation of compounds **16** and **17a** (commercially available) as a colorless oil (86% yield): $[\alpha]_D^{25}$ − 16.5 (*c* 0.018, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 15.9 Hz, 1H), 7.36–7.31 (m, 1H), 7.25–7.15 (m, 2H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 1.9 Hz, 1H), 6.71 (dd, *J* = 1.9 Hz, 8.3 Hz, 1H), 6.27 (d, *J* = 15.9 Hz, 1H), 6.13–6.01 (m, 2H), 5.94–5.84 (m, 1H), 5.45–5.22 (m, 7H), 5.15 (t, *J* = 9.6 Hz, 1H), 5.02 (dd, *J* = 8.0 Hz, 9.6 Hz, 1H), 4.64–4.55 (m, 6H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.31–4.24 (m, 2H), 4.09 (dt, *J* = 6.4 Hz, 9.5 Hz, 1H), 3.82–3.77 (m, 1H), 3.65 (dt, *J* = 6.4 Hz, 9.5 Hz, 1H), 2.81 (t, *J* = 6.8 Hz, 2H), 1.97 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.2, 164.8, 154.6, 148.5, 147.1, 144.2, 133.7, 133.6, 131.6, 131.3, 125.1, 121.4, 119.0, 118.0, 117.8, 117.5, 117.4, 116.7, 116.5, 115.4, 114.6, 100.7, 72.5, 71.9, 71.2, 70.8, 70.2, 70.0, 69.1, 68.7, 66.0, 35.5, 20.55, 20.48; ESI-HRMS $[M+Na]^+$ calcd for $C_{37}H_{40}NaO_{13}F_2$ 753.2329, found 753.2337.

3,4-Bis(allyloxy)phenylethyl 2,3-di-*O*-acetyl-4-*O*-((trans)-3,4-dimethoxycinnamoyl)-6-*O*-alloc-β-D-glucopyranoside (compound **18c**) was obtained from the condensation of compounds **16** and **17b** (commercially available) as a colorless oil (98% yield): $[\alpha]_D^{25}$ − 9.3 (*c* 0.013, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 15.9 Hz, 1H), 7.11–7.03 (m, 2H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.74–6.70 (m, 2H), 6.22 (d, *J* = 15.9 Hz, 1H), 6.13–6.02 (m, 2H), 5.94–5.85 (m, 1H), 5.45–5.21 (m, 7H), 5.15 (t, *J* = 9.6 Hz, 1H), 5.02 (dd, *J* = 8.1 Hz, 9.5 Hz, 1H), 4.60–4.52 (m, 7H), 4.28–4.27 (m, 2H), 4.09 (dt, *J* = 6.3 Hz, 9.4 Hz, 1H), 3.91 (s, 6H), 3.82–3.77 (m, 1H), 3.65 (dt, *J* = 7.4 Hz, 9.4 Hz, 1H), 2.81 (t, *J* = 6.8 Hz, 2H), 1.97 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.2, 165.6, 154.6, 151.6, 149.3, 148.5, 147.1, 146.7, 133.7, 133.6, 131.6, 131.3, 126.9, 123.2, 121.4, 119.0, 117.4, 117.3, 115.4, 114.6, 113.8, 111.0, 109.7, 100.7, 72.7, 72.6, 72.1, 71.3, 70.7, 70.2, 69.9, 68.7, 66.2, 56.0, 55.9, 35.5, 20.6 20.5; ESI-HRMS $[M+Na]^+$ calcd for $C_{39}H_{46}NaO_{15}$ 777.2729, found 777.2764.

3,4-Bis(allyloxy)phenylethyl 2,3-di-*O*-acetyl-4-*O*-((trans)-*p*-chlorocinnamoyl)-6-*O*-alloc-β-D-glucopyranoside (compound **18d**) was obtained from the condensation of compounds **16** and **12a** [19] as a colorless oil (87% yield): $[\alpha]_D^{25}$ − 22.6 (*c* 0.019, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 15.9 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.74 (d, *J* = 1.9 Hz, 1H), 6.71 (d, *J* = 1.9 Hz, 8.2 Hz, 1H), 6.33 (d, *J* = 15.9 Hz, 1H), 6.12–6.02 (m, 2H), 5.94–5.84 (m, 1H), 5.45–5.36 (m, 2H), 5.34–5.21 (m, 5H), 5.15 (t, *J* = 9.6 Hz, 1H), 5.01 (dd, *J* = 8.0 Hz, 9.6 Hz, 1H), 4.62–4.55 (m, 6H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.27–4.25 (m, 2H), 4.08 (dt, *J* = 6.4 Hz, 9.5 Hz, 1H), 3.80 (m, 1H), 3.65 (ddd, *J* = 7.3 Hz, 7.5 Hz, 9.5 Hz, 1H), 2.80 (d, *J* = 6.8 Hz, 2H), 1.96 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.3, 165.1, 154.6, 147.1, 145.2, 136.8, 133.7, 133.6, 132.4, 131.5, 131.3, 129.5, 129.2, 121.4, 119.0, 117.5, 117.45, 117.4, 116.8, 115.3, 114.5, 100.7, 72.5, 71.9, 71.2, 70.8, 70.2, 69.9, 69.0, 68.8, 66.1, 35.5, 20.6, 20.5; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{37}H_{45}NO_{13}Cl$ 746.2574, found 746.2564.

3,4-Bis(allyloxy)phenylethyl 2,3-di-*O*-acetyl-4-*O*-((trans)-3,4-bis(allyloxy)phenylacetyl)-6-*O*-alloc-β-D-glucopyranosid (compound **18e**) was obtained from the condensation of compounds **16** and **10a** as a colorless oil (95% yield): $[\alpha]_D^{25}$ − 10.3 (*c* 0.016, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85–6.68 (m, 6H), 6.12–6.00 (m, 4H), 5.95–5.86 (m, 1H), 5.46–5.32 (m, 5H), 5.29–5.22 (m, 5H), 5.18 (t, *J* = 9.5 Hz, 1H), 5.00 (dd, *J* = 9.6 Hz, 9.8 Hz, 1H), 4.93 (dd, *J* = 9.0 Hz, 9.7 Hz, 1H), 4.62–4.55 (m, 10H), 4.47 (d, *J* = 8.0 Hz, 1H), 4.21–4.10 (m, 2H), 4.05 (dt, *J* = 6.5 Hz, 9.5 Hz, 1H), 3.71–3.66 (m, 1H), 3.62 (dt, *J* = 9.5 Hz, 6.5 Hz, 1H), 3.48 (dd, *J* = 14.6 Hz, 2.6 Hz, 2H), 2.78 (t, *J* = 6.8 Hz, 2H), 1.88 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.0, 169.1, 154.5, 148.6, 148.4, 147.9, 147.1, 133.7, 133.6, 133.4, 133.3, 131.5, 131.3, 126.0, 121.8, 119.0, 117.5, 117.46, 117.37, 117.3, 115.4, 115.2, 114.6, 114.4, 100.6, 72.3, 71.7, 71.2, 70.7, 70.1, 70.0, 69.93, 69.87, 68.9, 68.7, 65.8, 40.5, 35.5, 20.4, 20.3; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{42}H_{54}NO_{15}$ 812.3488, found 812.3465.

4.1.9. General procedure for the preparation of 19a–e

To a solution of the *O*-acetyl-protected compound in MeOH/

CH_2Cl_2 (1:1) was added AcCl. The reaction mixture was stirred for 12 h. After the starting material was consumed by TLC detection, the mixture was neutralized with $NaHCO_3$. The solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:2; or CH_2Cl_2/MeOH, 10:1 to 5:1) to yield the product.

3,4-Bis(allyloxy)phenylethyl 4-*O*-((trans)-3,4-bis(allyloxy)cinnamoyl)-6-*O*-alloc-*β*-D-glucopyranoside (compound **19a**) was obtained from compound **18a** as white solids (91% yield): $[\alpha]_D^{25}-3.2$ (*c* 0.018, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67–7.63 (d, *J* = 15.9 Hz, 1H), 7.07–7.06 (m, 2H), 6.87–6.73 (m, 4H), 6.26 (d, *J* = 15.9 Hz, 1H), 6.12–6.01 (m, 4H), 5.91–5.83 (m, 1H), 5.44–5.20 (m, 10H), 4.96 (t, *J* = 9.6 Hz, 1H), 4.64–4.56 (m, 10H), 4.33–4.27 (m, 3H), 4.10 (dt, *J* = 6.9 Hz, 9.3 Hz, 1H), 3.76–3.69 (m, 3H), 3.49 (dd, *J* = 8.3 Hz, 8.7 Hz, 1H), 2.94 (br, 1H), 2.87 (t, *J* = 7.5 Hz, 2H), 2.60 (br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.8, 154.7, 151.0, 148.51, 148.48, 147.1, 146.6, 133.6, 133.5, 133.0, 132.8, 131.3, 131.2, 127.1, 123.1, 121.3, 119.1, 118.0, 117.9, 117.5, 117.4, 115.1, 114.4, 114.2, 113.3, 112.7, 102.4, 74.6, 74.0, 72.1, 70.9, 70.1, 70.0, 69.9, 69.7, 68.7, 66.5, 35.6; ESI-HRMS $[M+H]^+$ calcd for $C_{39}H_{47}O_{13}$ 723.3011, found 723.2986.

3,4-Bis(allyloxy)phenylethyl 4-*O*-((trans)-3,4-difluorocinnamoyl)-6-*O*-alloc-*β*-D-glucopyranoside (compound **19b**) was obtained from compound **18b** as white solids (98% yield): $[\alpha]_D^{25}-4.8$ (*c* 0.002, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 15.9 Hz, 1H), 7.37–7.32 (m, 1H), 7.28–7.24 (m, 1H), 7.24–7.15 (m, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.78–6.73 (m, 2H), 6.36 (d, *J* = 15.9 Hz, 1H), 6.13–6.02 (m, 2H), 5.93–5.83 (m, 1H), 5.44–5.21 (m, 6H), 4.99 (t, *J* = 9.7 Hz, 1H), 4.60–4.57 (m, 6H), 4.32 (d, *J* = 7.8 Hz, 1H), 4.28–4.26 (m, 2H), 4.12 (dt, *J* = 7.1 Hz, 9.6 Hz, 1H), 3.77–3.68 (m, 3H), 3.48 (dt, *J* = 2.2 Hz, 8.5 Hz, 1H), 2.87 (t, *J* = 7.6 Hz, 2H), 2.76 (d, *J* = 3.5 Hz, 1H), 2.49 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 154.7, 148.6, 147.3, 144.2, 133.6, 133.58, 131.4, 131.3, 125.0, 121.4, 119.0, 118.0, 117.95, 117.94, 117.8, 117.5, 117.4, 116.6, 116.4, 115.3, 114.6, 102.5, 74.5, 74.1, 72.1, 71.2, 71.0, 70.2, 70.0, 68.7, 66.4, 35.6; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{33}H_{40}NO_{11}F_2$ 664.2564, found 664.2565.

3,4-Bis(allyloxy)phenylethyl 4-*O*-((trans)-3,4-dimethoxycinnamoyl)-6-*O*-alloc-*β*-D-glucopyranoside (compound **19c**) was obtained from compound **18c** as white solids (90% yield): $[\alpha]_D^{25}-48.0$ (*c* 0.0007, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 15.9 Hz, 1H), 7.11–7.05 (m, 2H), 6.87–6.73 (m, 4H), 6.31 (d, *J* = 15.9 Hz, 1H), 6.13–6.02 (m, 2H), 5.93–5.83 (m, 1H), 5.44–5.21 (m, 6H), 4.97 (t, *J* = 9.6 Hz, 1H), 4.60–4.57 (m, 5H), 4.33 (d, *J* = 7.8 Hz, 1H), 4.29 (m, 2H), 4.11 (dt, *J* = 7.0 Hz, 9.4 Hz, 1H), 3.92–3.88 (m, 6H), 3.80–3.69 (m, 4H), 3.50 (t, *J* = 9.2 Hz, 1H), 2.97 (d, *J* = 3.0 Hz, 1H), 2.87 (t, *J* = 7.6 Hz, 2H), 2.60 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 154.7, 151.5, 149.3, 148.6, 147.2, 146.7, 133.63, 133.57, 131.4, 131.3, 127.0, 123.1, 121.4, 119.0, 117.5, 117.4, 115.3, 114.5, 114.2, 111.1, 109.8, 102.5, 74.7, 74.1, 72.1, 71.02, 70.96, 70.1, 70.0, 66.5, 56.0, 55.9, 35.6; ESI-HRMS $[M+H]^+$ calcd for $C_{35}H_{43}O_{13}$ 671.2698, found 671.2698.

3,4-Bis(allyloxy)phenylethyl 4-*O*-((trans)-*p*-chlorocinnamoyl)-6-*O*-alloc-*β*-D-glucopyranoside (compound **19d**) was obtained from compound **18d** as white solids (98% yield): $[\alpha]_D^{25}-12.5$ (*c* 0.004, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 16.0 Hz, 1H), 7.45 (m, 2H), 7.37 (m, 2H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.78 (d, *J* = 1.6 Hz, 1H), 6.74 (dd, *J* = 1.6 Hz, 8.0 Hz, 1H), 6.41 (d, *J* = 16.0 Hz, 1H), 6.13–6.02 (m, 2H), 5.93–5.83 (m, 1H), 5.45–5.37 (m, 2H), 5.35–5.20 (m, 4H), 4.99 (t, *J* = 9.6 Hz, 1H), 4.61–4.56 (m, 6H), 4.32 (d, *J* = 7.7 Hz, 1H), 4.28–4.27 (m, 2H), 4.12 (dt, *J* = 7.2 Hz, 9.6 Hz, 1H), 3.77–3.68 (m, 3H), 3.51–3.46 (m, 1H), 2.87 (t, *J* = 7.8 Hz, 2H), 2.74 (d, *J* = 3.3 Hz, 1H), 2.43 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 154.7, 148.6, 147.3, 145.2, 136.7, 133.7, 133.6, 132.5, 131.4, 131.3, 129.3, 121.4, 119.0, 117.5, 117.4, 117.3, 115.3, 114.5, 102.5, 74.5, 74.1, 72.1, 71.1, 71.0, 70.2, 70.0, 68.7, 66.4, 35.6; ESI-HRMS $[M+H]^+$ calcd for $C_{33}H_{38}O_{11}Cl$ 645.2097, found 645.2071.

3,4-Bis(allyloxy)phenylethyl 4-*O*-((trans)-3,4-bis(allyloxy)phenylacetyl)-6-*O*-alloc-*β*-D-glucopyranoside (compound **19e**) was obtained from compound **18e** as white solids (99% yield): $[\alpha]_D^{25}-7.6$ (*c* 0.003, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87–6.71 (m, 6H), 6.12–6.01 (m, 4H), 5.94–5.84 (m, 1H), 5.45–5.35 (m, 5H), 5.30–5.23 (m, 5H), 4.84 (t, *J* = 9.6 Hz, 1H), 4.57 (br, 10H), 4.25 (d, *J* = 7.7 Hz, 1H), 4.17 (dd, *J* = 5.9 Hz, 11.8 Hz, 1H), 4.10–4.04 (m, 2H), 3.71–3.57 (m, 5H), 3.41 (dd, *J* = 8.1 Hz, 8.8 Hz, 1H), 2.84 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 154.6, 148.6, 148.5, 147.8, 147.2, 133.6, 133.5, 133.44, 133.36, 131.2, 126.2, 121.3, 119.1, 117.6, 117.54, 117.49, 115.2, 115.1, 114.4, 114.3, 102.4, 74.4, 74.0, 71.9, 71.2, 71.0, 70.1, 71.0, 70.1, 70.0, 69.9, 68.7, 66.1, 40.7, 35.6; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{38}H_{50}NO_{13}$ 728.3277, found 728.3290.

4.1.10. Preparation of 3,4-bis(allyloxy)phenylethyl (trans)-3,4-bis(allyloxy)cinnamate (**20**)

To a solution of **11b** (100 mg, 0.379 mmol, 1 equiv) in CH_2Cl_2 (2 mL) was added oxalyl chloride (0.11 mL). The mixture was stirred under Ar at room temperature for 6 h and then evaporated under *vacuum*. To the residue was added **9b** (90.2 mg, 0.379 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL), Et_3N (0.5 mL). The reaction mixture was stirred for another 12 h. The solvent was removed and the residue was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:3) to give **20** (126 mg, 69% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 16.0 Hz, 1H), 7.42–7.36 (m, 2H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.14–7.01 (m, 3H), 6.36 (d, *J* = 16.0 Hz, 1H), 4.42 (t, *J* = 6.7 Hz, 2H), 3.00 (t, *J* = 6.7 Hz, 2H), 2.30 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 168.2, 168.0, 167.9, 166.4, 143.5, 143.1, 142.4, 142.0, 140.7, 136.7, 133.3, 127.0, 126.4, 123.9, 123.4, 122.8, 119.1, 64.5, 34.5, 20.6, 20.5; ESI-HRMS $[M+Na]^+$ calcd for $C_{25}H_{24}NaO_{10}$ 507.1272, found 507.1262.

4.1.11. General procedure for the preparation of **1, 2, 3, 4, 5**

To a stirred solution of the *O*-allyl-protected compound in MeOH/H_2O (20:1) were added 10% Pd/C and TsOH (or $HClO_4$). The reaction mixture was heated under reflux for 12–24 h. The solids were filtered off and the solvent was removed under *vacuum*. The residue was purified by preparative thin layer chromatography (CH_2Cl_2/MeOH, 10:1 to 5:1) to afford the final product.

3,4-Dihydroxyphenylethyl 4-*O*-((trans)-3,4-dihydroxycinnamoyl)-*β*-D-glucopyranoside (compound **1**) was obtained from compound **19a** as a light yellow oil (35% yield): $[\alpha]_D^{25}-25.7$ (*c* 0.026, CD_3OD); ^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (d, *J* = 15.8 Hz, 1H), 7.04 (d, *J* = 1.3 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.70 (m, 2H), 6.58 (m, 1H), 6.28 (d, *J* = 15.8 Hz, 1H), 4.35 (d, *J* = 7.8 Hz, 1H), 4.08 (m, 2H), 3.74 (m, 1H), 3.65 (m, 2H), 3.55 (m, 2H), 3.35–3.25 (m, 1H), 2.81 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 168.6, 149.7, 147.6, 146.8, 146.1, 144.6, 131.5, 127.7, 123.1, 121.3, 117.1, 116.5, 116.3, 115.2, 114.7, 104.4, 76.1, 75.8, 75.2, 72.5, 72.2, 62.5, 36.6; ESI-HRMS $[M+Na]^+$ calcd for $C_{23}H_{26}NaO_{11}$ 501.1367, found 501.1369.

3,4-Dihydroxyphenylethyl 4-*O*-((trans)-3,4-difluorocinnamoyl)-*β*-D-glucopyranoside (compound **2**) was obtained from compound **19b** as a light yellow oil (35% yield): $[\alpha]_D^{25}-10.6$ (*c* 0.012, CD_3OD); ^{1}H NMR (400 MHz, CD_3OD) δ 7.68 (d, *J* = 16.0 Hz, 1H), 7.66–7.58 (m, 1H), 7.46–7.43 (m, 1H), 7.34–7.27 (m, 1H), 6.70–6.66 (m, 2H), 6.58–6.54 (m, 2H), 4.88 (t, *J* = 9.4 Hz, 1H), 4.36 (d, *J* = 7.8 Hz, 1H), 4.08–4.02 (m, 1H), 3.75–3.70 (m, 1H), 3.64 (m, 2H), 3.58–3.49 (m, 2H), 3.35–3.28 (m, 1H), 2.80 (dt, *J* = 2.3 Hz, 7.5 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.5, 146.1, 144.7, 144.4, 133.4, 131.5, 126.6, 126.5, 121.3, 120.1, 119.0, 118.8, 117.7, 117.5, 117.1, 116.3, 104.4, 76.0, 75.8, 75.2, 72.9, 72.2, 62.4, 36.6; ESI-HRMS $[M+Na]^+$ calcd for $C_{23}H_{24}NaO_9F_2$ 505.1281, found 505.1286.

3,4-Dihydroxyphenylethyl 4-*O*-((trans)-3,4-dimethoxycinn amoyl)-β-D-glucopyranoside (compound **3**) was obtained from compound **19c** as a light yellow oil (34% yield): $[\alpha]_D^{25}$ − 5.4 (*c* 0.010, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.67 (d, *J* = 15.9 Hz, 1H), 7.22 (d, *J* = 1.9 Hz, 1H), 7.17 (dd, *J* = 1.8 Hz, 8.4 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.68–6.65 (m, 2H), 6.55 (dd, *J* = 2.1 Hz, 8.1 Hz, 1H), 6.44 (d, *J* = 15.9 Hz, 1H), 4.86 (t, *J* = 9.4 Hz, 1H), 4.36 (d, *J* = 7.8 Hz, 1H), 4.07–4.01 (m, 1H), 3.85 (m, 6H), 3.74–3.68 (m, 1H), 3.64–3.58 (m, 2H), 3.56–3.48 (m, 2H), 3.32–3.28 (m, 1H), 2.79 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 168.4, 153.0, 150.8, 147.0, 146.1, 144.7, 131.5, 128.8, 124.1, 121.3, 117.1, 116.3, 116.2, 112.7, 111.7, 104.4, 76.1, 75.9, 75.3, 72.6, 72.2, 62.5, 56.5, 36.6; ESI-HRMS $[M+H]^+$ calcd for $C_{25}H_{31}O_{11}$ 507.1861, found 507.1867.

3,4-Dihydroxyphenylethyl 4-*O*-((trans)-*p*-chlorocinnamoyl)-β-D-glucopyranoside (compound **4**) was obtained from compound **19d** as a yellow oil (32% yield): $[\alpha]_D^{25}$ − 13.7 (*c* 0.010, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.69 (d, *J* = 16.0 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 6.69–6.65 (m, 2H), 6.58–654 (m, 2H), 4.87 (t, *J* = 9.4 Hz, 1H), 4.36 (d, *J* = 7.8 Hz, 1H), 4.08–4.01 (m, 1H), 3.74–3.68 (m, 1H), 3.66–3.61 (m, 2H), 3.56–3.49 (m, 2H), 3.33–3.28 (m, 1H), 2.79 (ddd, *J* = 2.3 Hz, 2.5 Hz, 7.7 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.7, 146.1, 145.3, 144.7, 137.4, 134.5, 131.5, 130.8, 130.2, 121.3, 119.4, 117.1, 116.3, 104.4, 76.0, 75.8, 75.2, 72.8, 72.2, 62.4, 36.6; ESI-HRMS $[M+NH_4]^+$ calcd for $C_{23}H_{29}ClNO_9$ 498.1525, found 498.1525.

3,4-Dihydroxyphenylethy 4-*O*-((trans)-3,4-dihydroxyphenylacetyl)-β-D-glucopyranoside (compound **5**) was obtained from compound **19e** as a yellow oil (37% yield): $[\alpha]_D^{25}$ − 6.4 (*c* 0.004, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 6.76–6.81 (m, 5H), 6.56–6.62 (m, 1H), 4.30 (d, *J* = 7.6 Hz, 1H), 4.14–3.98 (m, 2H), 3.89–3.79 (m, 2H), 3.65–3.76 (m, 3H), 3.27–3.38 (m, 1H), 3.15–3.22 (m, 2H), 2.84 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.9, 146.3, 146.1, 145.5, 144.7, 131.6, 126.9, 131.7, 121.3, 117.4, 117.1, 116.4, 116.3, 104.4, 77.9, 75.4, 75.0, 72.1, 71.7, 65.0, 41.4, 36.6; ESI-HRMS $[M+Na]^+$ calcd for $C_{22}H_{26}NaO_{11}$ 489.1367, found 489.1376.

4.1.12. Preparation of 3,4-dihydroxyphenylethyl (trans)-3,4-dihydroxycinnamate (6)

Following the general procedure for the synthesis of compounds **19a–e**, compound **6** was obtained from compound **20** as a colorless oil (82% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (d, *J* = 15.8 Hz, 1H), 7.04 (s, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.71 (m, 2H), 6.58 (d, *J* = 8.0 Hz, 1H), 6.24 (d, *J* = 15.8 Hz, 1H), 4.30 (t, *J* = 6.9 Hz, 2H), 2.84 (t, *J* = 6.9 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 169.3, 149.6, 146.9, 146.3, 144.9, 146.8, 130.8, 127.7, 122.9, 121.2, 117.1, 116.5, 116.4, 115.2, 115.1, 66.5, 35.6; ESI-HRMS $[M+Na]^+$ calcd for $C_{17}H_{16}O_6Na$ 339.0842, found 339.0839. The spectroscopic data coincide with the previous report [43].

4.1.13. Preparation of 3,4-dihydroxyphenylethyl β-D-glucopyranoside (7)

To a solution of **15** (150 mg, 0.315 mmol, 1 equiv) in MeOH/H_2O (20:1, 8 mL) were added 10% Pd/C and $HClO_4$ (cat.). The reaction mixture was heated under reflux for 12 h. The solids were filtered off and the solvent was removed under *vacuum*. The residue was deacetylated using the general procedure for the synthesis of **19a–e**, affording **7** as white solids (92% yield). $[\alpha]_D^{25}$ − 0.59 (*c* 0.025, CD_3OD); ^{1}H NMR (400 MHz, CD_3OD) δ 6.68–6.65 (m, 2H), 6.55 (d, *J* = 8.1 Hz, 1H), 4.28 (d, *J* = 7.3 Hz, 1H), 4.01 (dd, *J* = 8.6 Hz, 8.3 Hz, 1H), 3.83 (m, 2H), 3.75–3.70 (m, 1H), 3.70–3.60 (m, 3H), 3.17 (t, *J* = 8.2 Hz, 1H), 2.77 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 146.0, 144.6, 131.6, 121.3, 117.1, 116.3, 104,3, 78.1, 77.9, 75.1, 72.0, 71.6, 62.7, 36.6; ESI-HRMS $[M+H]^+$ calcd for $C_{14}H_{21}O_8$ 317.1231, found 317.1229. The spectroscopic data coincide with the previous report [44].

4.1.14. Preparation of 2-O-methyl-β-D-N-(trans-dihydroxycinnamoyl)neuraminic acid methyl ester (8)

To a solution of **21** (100.0 mg, 0.34 mmol, 1.0 equiv) and **11** (91.6 mg, 0.51 mmol, 1.5 equiv) in DMF (50 mL) were added PyBOP (265.0 mg, 0.51 mmol, 1.5 equiv) and NMM (103.0 mg, 1.02 mmol, 3.0 equiv). The mixture was stirred for 12 h and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/EtOH, 10:1) to give **8** (60.0 mg, 39% yield) as a light yellow oil. $[\alpha]_D^{25}$ − 11.3 (*c* 0.006, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.44 (d, *J* = 15.6 Hz, 1H), 7.03 (d, *J* = 1.7 Hz, 1H), 6.91 (dd, *J* = 2.1 Hz, 8.2 Hz, 1H), 6.77 (d, *J* = 8.2 Hz, 1H), 6.43 (d, *J* = 15.6 Hz, 1H), 4.09 (td, *J* = 4.8 Hz, 10.7 Hz, 1H), 3.99 (t, *J* = 10.2 Hz, 1H), 3.81–3.91 (m, 3H), 3.75–3.80 (m, 3H), 3.68 (dd, *J* = 5.0 Hz, 11.1 Hz, 1H), 3.55 (d, *J* = 8.0 Hz, 1H), 3.29 (s, 3H), 2.38 (dd, *J* = 5.0 Hz, 12.9 Hz, 1H), 1.69 (dd, *J* = 1.6 Hz, 12.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.1, 170.6, 148.9, 146.8, 143.0, 128.2, 122.3, 118.0, 116.5, 115.0, 100.5, 72.6, 71.4, 70.1, 67.7, 65.2, 53.8, 53.3, 51.7, 41.6; ESI-HRMS $[M-H]^-$ calcd for $C_{20}H_{26}NO_{11}$ 456.1506, found 456.1511.

4.2. Biology

4.2.1. Cell culture and treatments

Rat pheochromocytoma (PC12) cells were purchased from Shanghai Cell Culture Center, China, grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma, Aldrich), supplemented with 15% fetal bovine serum and 1% antibiotic mixture comprising penicillin–streptomycin (100 U/mL of penicillin and 100 μg/mL of streptomycin), in a humidified atmosphere at 37 °C with 5% CO_2. Cells were sub-cultured every 3 days and in culture for 2 weeks before being used for experiments.

4.2.2. Cell viability assay

PC12 cells were incubated in 96-well microplates (1 × 10^5 cell/well in 100 μL) for 24 h. Cell viability was measured using the MTT assay [45,46]. Briefly, PC12 cells were pretreated with the synthetic phenylethanoid glycoside analogs (dissolved in dimethyl sulphoxide (DMSO) and then diluted in phosphate buffered saline (PBS)) for 6 h, and then treated with 300 μM of H_2O_2 for 1 h at 37 °C, or 100 μM of 6-hydroxydopamine (6-OHDA) for 24 h, respectively. The controls were exposed to the same solvent. After treatment with 5 mg/mL of MTT for 4 h and DMSO for 10 min, the absorbance was read at 570 nm on a microplate reader. Cell viability was expressed as a percent of the control culture value.

4.2.3. Measurement of apoptosis

Apoptotic levels were measured using an Annexin V-FITC Apoptosis Detection Kit (KeyGen Biotech). PC12 cells were plated on coverslips and pre-treated with the synthetic phenylethanoid glycoside analogs for 6 h, and then treated with 100 μM of 6-OHDA for 24 h at 37 °C in 24-well plates (1 × 10^5 cell/well in 500 μL). The medium was removed and the cells were washed with PBS twice. Annexin V and propidium iodide were diluted in Binding Buffer (1:100) and mixed (1:1), and added to the cells (100 μL/well). To assess viability, cellular morphology was observed under a fluorescence microscope (LEICA TCS SP2, Germany). Excitation wavelengths and emission wavelengths were 488 nm, 515–545 nm and 488 nm, 575–635 nm. Three separate groups of samples were analyzed respectively, and the number of apoptotic and necrotic cells in five representative fields were counted per experiment [47].

4.2.4. Measurement of intracellular ROS

Intracellular ROS levels were measured by using the DCFH-DA fluorescent probe [48], as described previously [45]. One day after seeding, PC12 cells were pre-treated with compounds for 6 h, and then treated with 100 μM of 6-OHDA for 24 h at 37 °C in 96-well plates (1×10^5 cell/well in 100 μL). The medium was removed and the cells were incubated for 30 min with DCFH-DA (1:1000 in DMEM) at 5% CO_2 and 37 °C. Then the cells were washed with PBS three times. The fluorescence of the cells (488/525 nm, Spectrofluor, TECAN) was measured in five representative fields per well.

4.2.5. Statistical analysis

All experiments were conducted in triplicate. Data were expressed as the means ± S.D. Statistical comparisons were performed by ANOVA. $P < 0.05$ was considered significant.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.03.038.

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