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ORIGINAL RESEARCH

Synthesis and biological activities of galactose–aspirin conjugate prodrug designed for ADEPT and PMT

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Abstract Aspirin was used as the lead compound, and its structure was modified by galactosylation. Galactose-based aspirin prodrug was synthesized by using the α-D-glacto-pyranosyl bromide as glycosyl donor and aspirin as acceptor. The experimental method is simple and reproducible, has high yield and great practical value. The galactosylated aspirin prodrug was found to possess reduced cytotoxicity compared to aspirin, and selectively exhibited antiproliferative activity in the presence or in the absence of β-D-galactoside galactohydrolase with the approach of antibody-directed enzyme prodrug therapy (ADEPT) or the prodrug monotherapy (PMT).

Keywords Aspirin · Prodrug · Synthesis · Antiproliferative activity · ADEPT · PMT

Introduction

Aspirin is the non-steroidal anti-inflammatory drug as the first listing. After more than a century of clinical application, it proves that aspirin has a truly curative effect, and is the antipyretic, analgesic, and anti-inflammatory drug. After in-depth study for many years, people have found that it has the characteristics for a long-term prevention of myocardial infarction, thrombosis, stroke, and rheumatism (Jana 2008). In addition, study has shown that aspirin has anti-cancer effect (Rothwell et al. 2012). But after taking in aspirin, there are the gastric mucosal stimulation, rapid metabolism in vivo, low bioavailability, and indisposition, which greatly limit the aspirin to be more widely used in clinics (Stéphane et al. 2005).

In nature, glycosylation of natural products is very common (Huang and Mei 2014). The sugar moieties of these natural molecules can change the pharmacological activities of the parent drugs by increasing the solubility, mediation of the plasma half-life, and improving the specificity of binding. For example, enediyne antibiotics esperamycin and calicheamicin γ1, anthracycline anticancer drugs doxorubicin, digoxin, vancomycin, and other clinical therapeutic drugs (Huang et al. 2014). The chemical modification of glycosylation of molecules provides an important field for the development of new drugs. A large number of studies show that the introduction of sugar-based drugs can improve the efficacy and reduce the side effects of drugs. In addition, the use of sugar modification of existing drugs can also give them some new effects. For example, in order to make the drug targeting, we can change the drug absorption, distribution, metabolism, and excretion, and extend the half-life. At present, few researchers are participating in the work about glycosyl derivatives of aspirin. Because anticancer drugs lack selectivity, tumor chemotherapy has serious side effects. An ideal solution to the problem is the antibody-directed enzyme prodrug therapy (ADEPT) and the prodrug monotherapy (PMT) (Tietze and Schmuck 2011). Therefore, this galactose–aspirin covalent complex prodrug was prepared by chemical method in order to improve its side effects herein. In addition, the inhibitory activity of aspirin and its galactosylated conjugate prodrug to the proliferation of cancer cells was also assayed by ADEPT and PMT.

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Experimental

Synthesis of D-galactose pentaacetate 2

Seventy milliters of acetic anhydride was added, and then 5 g of D-galactose was carefully added. While stirring with a magnetic stirrer, anhydrous pyridine (70 mL) was added. This mixture was kept at room temperature while stirring for 20 h. When the reaction had proceeded, the solution was mixed with ice, which was stirred until all ice was dissolved. The filtered product was recrystallized in a solution of water/methanol ($v/v = 1/2$). D-Galactose pentaacetate **2**: yield 92%; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 6.34$ (d, $1H_\alpha$, $J_{1,2}$ 1.5 Hz, H-1α), 5.63 (d, $1H_\beta$, $J_{1,2}$ 1.5 Hz, H-1β), 5.50 (dd, 1H, $J_{3,4} < 1.0$ Hz, $J_{4,5}$ 1.3 Hz, H-4), 5.34–5.29 (m, 2H, H-2, H-3), 4.31 (dt, 1H, $J_{5,6}$ 6.5 Hz, H-5), 4.16–4.01 (m, 2H, H-6a, H-6b), 2.14, 2.10, 2.00, 1.98, 1.97, 1.96 (all s, 15H, $10 \times C(O)CH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta =$ 170.8, 170.5, 170.3, 169.3, 167.5 ($5 \times C{=}O$), 92.2 (C-1β), 89.9 (C-1α), 68.8 (C-5), 68.0 (C-3 and C-4), 66.6 (C-2), 61.5 (C-6), 21.3, 21.1, 20.9, 20.8, 20.6 ($5 \times C(O)CH_3$); ESI-MS $m/z = 413.3397$ $[M+Na]^+$.

Synthesis of α-D-galactopyranosyl bromide 4

At 0 °C, a solution of hydrogen bromide in acetic acid (33%, 50 mL) was added dropwise to 1,2,3,4,6-penta-*O*-acetyl-(α,β)-D-galactopyranose **2** (5 g, 12.8 mmol). After stirring for 2 h at room temperature, CH_2Cl_2 (150 mL) was added to the reaction. The solution was carefully washed with ice-cold water, and the organic layer was dried over $MgSO_4$. The solvent was evaporated under vacuum to obtain galactosyl bromide **3** in 97% yield. Compound **3** was hydrolyzed with dilute hydrobromic acid aqueous solution (1 mol/L, 20 mL) at room temperature for 2 h to obtain α-D-galactopyranosyl bromide **4** in 90% yield. α-D-Galactopyranosyl bromide **4**: ^{1}H NMR (D_2O, 300 MHz): $\delta = 6.79$ (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.42 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 1.2 Hz, H-4), 5.21 (dd, 1H, $J_{2,3}$ 10.4 Hz, H-3), 5.00 (dd, 1H, H-2), 4.33 (m, 1H, H-5), 4.15 (dd, 1H, $J_{6a,6b}$ 11.6 Hz, $J_{5,6}$ 6.0 Hz, H-6a), 3.99 (dd, 1H, $J_{5,6b}$ 6.8 Hz, H-6b); ^{13}C NMR (D_2O, 75 MHz): $\delta = 86.8$ (C-1), 70.4 (C-5), 66.3, 66.0 (C-3/C-4), 64.9 (C-2), 59.9 (C-6).

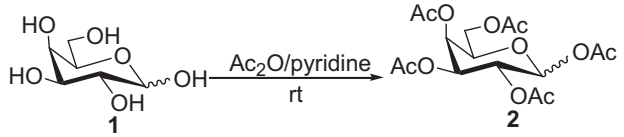

Fig. 1 Synthesis of galactose pentaacetate **2**

Synthesis of galactose–aspirin conjugate 5

The anhydrous dimethyl sulfoxide (DMSO) (30 mL) was added to three-necked flask, and oil bath was heated to 40 °C. Under violent agitation, α-D-galactopyranosyl bromide **4** (5 mmol), aspirin (5.3 mmol), and triethylamine (5.3 mmol) were sequentially added to the reaction flask. The reaction went on for 5 h at 40 °C. After the reaction was completed, a slurry was obtained under the reduced pressure. The slurry was dissolved with absolute alcohol, and cooled with ice water. The obtained product was recrystallized with anhydrous ethanol to offer galactose–aspirin conjugate **5** in 80% yield. Galactose–aspirin conjugate **5** ^{1}H NMR (D_2O, 300 MHz): $\delta = 7.09$–8.03 (4m, 4H, ArH), 5.89–5.86 (d, 1H, $J = 7.73$ Hz, H_β-1), 5.34–5.27 (m, 2H, H-2, H-3), 5.24–5.16 (q, 1H, H-4), 4.35–4.29 (q, 1H, H-6), 4.13–4.12 (d, 1H, H-6′), 4.08–3.88 (q, 1H, H-5), 3.84 (s, 3H, $-OCOCH_3$); IR (KBr): ν (cm^{-1}) = 2939 (C–H), 1755 (C = O), 1605, 1486, 1450 (Ph), 1223 (C–O), 1073 (C–O–C), 918 (β-galactosidic bond), 752 (two adjacent substituents on benzene ring).

Cell culture

The lung adenocarcinoma cell line A549 was cultured in RPMI-1640 nutrient fluid, and then was cultured with 5% CO_2 at 37 °C in incubator.

MTT colorimetric assay

The A549 cells in logarithmic growth phase were seeded in 96-well plates for 5×103 cells/hole. The culture medium was replaced after 24 h, the experimental group (with different concentrations of the sample), blank withered group (not inoculated cells), and control group (with only the same amount of solvent) were designed. Each group had four holes. MTT (20 μL) was added into each hole after 48 h. After the cultivation which lasted for 4 h in the absence or in the presence of β-D-galactoside galactohydrolase, it was centrifuged at 1000 r/min for 5 min, and the supernatant was

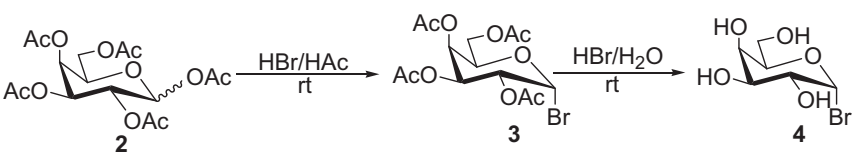

Fig. 2 Synthesis of α-D-galactopyranosyl bromide **4**

Fig. 3 Synthesis of galactose–aspirin conjugate **5**

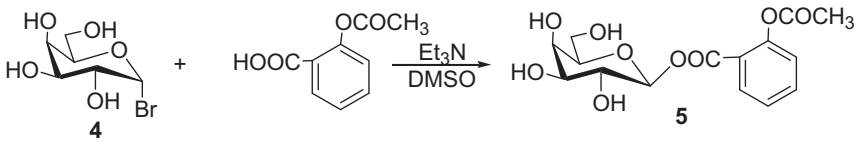

Table 1 The antiproliferative activity of aspirin and galactose–aspirin conjugate prodrug ($n = 5$)

Compound	Antiproliferative activity (1.25–20 mmol/L) (%)
Aspirin	3.8–63.9
Galactose–aspirin conjugate (ADEPT)	6.3–90.2
Galactose–aspirin conjugate (PMT)	4.5–71.8

Table 2 The cytotoxicity of aspirin and galactose–aspirin conjugate prodrug ($n = 5$)

Compound	IC_{50} (mmol/L)
Aspirin	$11.30 \pm 0.16^{*}$
Galactose–aspirin conjugate	$1256.83 \pm 0.10^{*}$

$^{*}p < 0.05$

sucked out. DMSO (100 μL) was added into each hole, and gently shaked for 10 min. The absorbance (*A* value) in each hole was determined at 570 nm with the microplate reader. The inhibition rate of cell proliferation was calculated: the inhibition rate = (1−the average value of all *A* values for experimental group/the average value of all *A* values for control group) × 100%.

Results and discussion

In order to prepare α-D-galactose pentaacetate, the catalysts mainly used had proton acids, Lewis acids, solid acids, and enzymes. The traditional homogeneous catalysts have small phase diffusion resistance, are easy to control, and can be conveniently operated in the catalytic process, but the selectivity of reactions and the yields of product need to be further improved. Moreover, the homogeneous catalysts are not easy to reuse. In the heterogeneous catalysts, solid acid catalysts have high selectivity and yields, but the preparation process of catalysts is very complex. So, it was very important to develop the rapid, simple, and effective catalysts with high catalytic activity and selectivity, improve the yield of a single configuration, and reduce the production costs (Huang et al. 2016). Herein, using pyridine as catalyst, galactose pentaacetate **2** was synthesized by esterification of galactose **1** and acetic anhydride (Fig. 1). The molar ratio of galactose and acetic anhydride was 1:26, the dosage of catalyst was 13 times of the mass of galactose, reaction time was 20 h, the reaction temperature was room temperature, the esterification yield of galactose and acetic anhydride was up to 92%. The molar ratio of α-galactose pentaacetate and β-galactose pentaacetate was 3.2:1.

1,2,3,4,6-Penta-*O*-acetyl-(α,β)-D-galactopyranose **2** reacted with a solution of hydrogen bromide in acetic acid (33 wt%). After stirring for 2 h at room temperature, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **3** was obtained in the yield of 97%. Compound **3** was hydrolyzed with dilute hydrobromic acid aqueous solution at room temperature to obtain α-D-galactopyranosyl bromide **4** in 90% yield (Fig. 2).

Aspirin was neutralized with triethylamine to obtain the corresponding salt. Compound **4** reacted with the salt of aspirin in anhydrous DMSO at 40 °C for 5 h, and the target compound, namely galactose–aspirin conjugate **5**, was obtained in 80% yield (Fig. 3).

After the human lung adenocarcinoma cell line A549 was treated with aspirin at a concentration of 1.25–20 mmol/L for 48 h, the inhibition rate of cancer cell proliferation was 3.8–63.9%, which was in a dose-dependent manner. However, the inhibition rate when treated with galactose–aspirin conjugate prodrug was 6.3–90.2% under the same conditions by ADEPT in the presence of β-D-galactoside galactohydrolase. Moreover, ADEPT was better than PMT. The related results are shown in Table 1. Therefore, it was proved that the galactosylation of aspirin could enhance its inhibitory activity against the proliferation of cancer cells by ADEPT and PMT.

The IC_{50} of prodrug **5** and free aspirin were 1256.83 and 11.30 mmol/L, respectively. In the presence of β-D-galactoside galactohydrolase, the cytotoxicity of prodrug **5** was restored to that of the free aspirin. The ratio of cytotoxicity between the prodrug and the free drug was 111 (Table 2). This value is compatible with ADEPT or PMT strategy where a relatively non-cytotoxic prodrug releases a cytotoxic compound.

Conclusion

The target prodrug **5** was synthesized by four steps, namely, acetylation, bromination, deacetylation, and coupling. The method is easy to operate with high yield. In addition, the galactosylation of aspirin could enhance its inhibitory activity against the proliferation of cancer cells by ADEPT

or PMT in the presence or in the absence of β-D-galactoside galactohydrolase.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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