Bioorganic Chemistry 72 (2017) 42–50

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Protective effects of hepatocyte-specific glycyrrhetic derivatives against carbon tetrachloride-induced liver damage in mice

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ARTICLE INFO

Article history:
Received 5 January 2017
Revised 10 February 2017
Accepted 17 March 2017
Available online 20 March 2017

Keywords:
Glycyrrhetic acid
Anethol trithione
Hepatoprotective
Hepatic injury

ABSTRACT

Glycyrrhetic acid (GA), the main hydrolysate of glycyrrhizic acid extracted from the roots of the Chinese herb Glycyrrhiza glabra, was reported to be accumulated in hepatocytes due to the extensive distribution of GA receptors in liver. A series of hepatocyte-specific derivatives on the basis of anetholtrithione and glycyrrhizic were designed and synthesized. The potential beneficial effect was evaluated in carbon tetrachloride (CCl_4)-induced liver injury model. In addition, the hepatoprotective activity of these derivatives was assessed by measuring levels of serum marker enzymes, including serum glutamate oxaloacetate transaminase (GOT), serum glutamate pyruvate transaminase (GPT), alkaline phosphatase (AKP), lactate dehydrogenase (LDH) and the ratio of GSH to GSSG. Gratifyingly, compounds **5a–c** (100 mg/kg, p.o.) markedly prevented CCl_4-induced elevation of levels of serum GPT, GOT. A comparative histopathological study of liver exhibited almost a normal liver lobular architecture and cell structure of the livers, as compared to CCl_4-treated group. These findings were confirmed with the histopathological observations, where hepatocyte-specific glycyrrhetic acid derivatives **5a–c** were capable of reversing the toxic effects of CCl_4 on hepatocytes.

1. Introduction

Hepatitis is one of the major causes of mortality worldwide, which is induced by various hepatotoxic substances. It can lead to liver fibrosis, cirrhosis and increase incidence of hepatocellular carcinoma. Current medical treatments for hepatitis are often limited in efficacy and safety [1,2]. Therefore efforts are being made to develop pharmacologically effective hepatoprotective agents, especially from natural products due to their few side effects [3].

Licorice (*Glycyrrhiza species*) is one of the most commonly used herb in traditional Chinese medicine. Its major bioactive ingredients of the roots, glycyrrhizin and glycyrrhetic acid (GA, Fig. 1), are considered to have the hepatoprotective effects [4,5]. Glycyrrhizin inhibits Hepatitis C Virus dose dependently by the inhibition of HCV core gene expression or function both at mRNA and protein levels. In the LPS/D-galactosamine-induced liver injury, glycyrrhizin prevents inflammatory responses and suppresses the effect of IL-18 of increasing ALT levels [6–12]. Glycyrrhizin was also reported to exert its hepatoprotective effect by preventing deranged microcirculatory flow and leukocyte-endothelium interaction in ischemia-reperfusion (I/R)-induced liver injury [13]. GA, the aglycone of glycyrrhizin also has been revealed the hepatoprotective potency. After oral administration of glycyrrhizin, it can be converted to GA through the intestinal bacterial hydrolysis [14]. Another study discovered that GA can bind specifically to a particulate fraction in liver [15]. Recently, several GA derivatives have shown to possess hepatoprotective effects [6,16–19]. Mikhailova et al. synthesized triterpene 1,2-trans-glycosides and 18,19-dehydro-GA, which exhibited hepatoprotective activity on the alcohol-induced hepatitis and decreased the level of TNF-α protein. Maitraie et al. synthesized 18β-GA derivatives by Bayer-Villiger oxidation and evaluated the inhibition of inflammation [20].

Anethol trithione (AT, Fig. 1) is considered to protect the liver via increasing the activity of phase II enzymatic detoxification systems [21]. It is widely used in clinic to treat hepatobiliary dysfunctions with its high potency and low toxicity [22]. In order to combine both the therapeutic benefits of GA and anethol trithione, we coupled GA with demethyl anethol trithione to afford a series of pro-drugs. Vicker et al. reported that the therapeutic activities were improved when a 2 or 3-atom linker was introduced into C-30 position of GA [13]. Therefore, compounds **5a–c** were designed and synthesized. We expected the high specific binding

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http://dx.doi.org/10.1016/j.bioorg.2017.03.009
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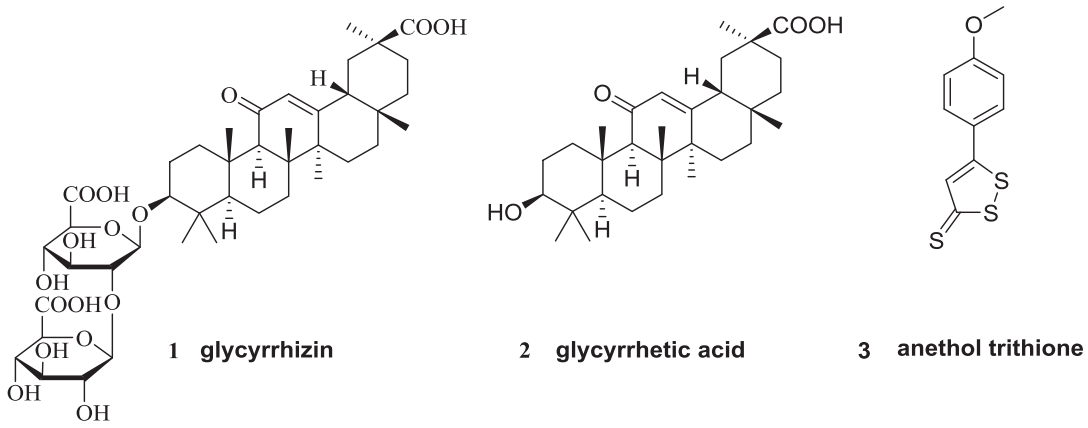


Fig. 1. The structures of glycyrrhizin (1), glycyrrhetic acid (2) and anethol trithione (3).

of GA to liver would improve the hepatoprotective potency of anethol trithione. In addition, two glycosylated derivatives were synthesized to investigate the effect of glycosylation to hepatoprotective activity.

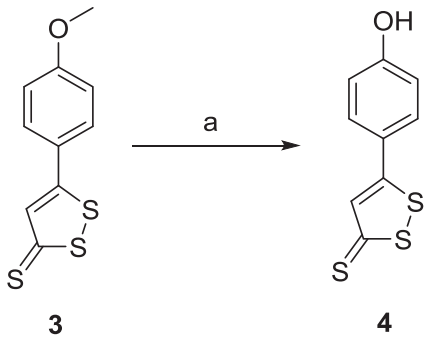
2. Results and discussion

2.1. Chemistry

In total, five compounds (**5a–c**, **11**, **15**) were designed and synthesized based on chemical and physical properties and structural features of pentacyclic triterpenoid. The synthetic route to demethyl anethol trithione is shown in Scheme 1, and the synthetic routes to GA derivatives are outlined in Schemes 2–4. The details of the synthetic procedures and structural characterizations (IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy) are described in synthetic procedures of target products section. Purification by column chromatography was carried out over silica gel (200–300 mesh) and checked for purity using HPLC before being tested in biological evaluation (purity was >97%).

As shown in Scheme 1, anethol trithione (**3**) was heated to 220 °C with pyridine hydrochloride to afford demethyl anethol trithione (**4**).

The target compounds **5a–c** was synthesized starting from commercial material glycyrrhetic acid (**2**) shown in Scheme 2. The starting material compound **2** was condensed with demethyl anethol trithione (**4**) to give the target compound **5a**. In addition, glycyrrhetic acid (**2**) was condensed with $Br(CH_2)_nBr$ to get the key intermediates **6a** and **6b**. Then intermediates **6a** and **6b** were treated with demethyl anethol trithione (**4**) to give target compounds **5b** and **5c**, respectively.



Scheme 1. Reagents and conditions: (a) pyridine hydrochloride, 220 °C.

The glycyrrhizin derivative **11** was prepared by starting material compound **7**. Compound **7** was acetylated with acetic anhydride to give intermediate **8**. Then compound **8** was further treated with deacetylation selectively using ammonium carbonate to prepare the intermediate **9** [23]. The key intermediate **10** was obtained by condensation reaction using intermediate **9** and trichloroacetonitrile. The target compound **11** was prepared by condensing key intermediate **10** with compound **5a** as shown in Scheme 3.

As shown in Scheme 4, glycyrrhizin (**1**) was treated with acetyl chloride to give the intermediate **12**. Then intermediate **13** was prepared by acetylation reaction using compound **12**. Intermediate **13** was condensed with anethol trithione (**3**) in the presence of DCC, HOBT and triethylamine to afford the key intermediate **14.** The desired compound **15** was obtained by deacetylation reaction using the key intermediate **14**.

2.2. Biological evaluation

2.2.1. Biochemical assays

In the assessments of AST and ALT, all five derivatives decreased the AST and ALT levels in serum compared with the CCl_4 model group. Their effects were equivalent to anethol trithione and significantly higher than glycyrrhizin and GA. Especially, compound **15** was the most active and its effect was higher than anethol trithione (Table 1). This result indicated that glycosylated derivatives could increase effect of glycosylation to hepatoprotective activity. The varied number of glycosylation exhibited different hepatoprotective activities. In the assessment of LDH and ALP, all derivatives decreased the LDH and ALP levels compared with the CCl_4 model group. However, their effects were no significant difference with anethol trithione (Tables 1 and 2). In the assessments of GSH, GSSG and T-GSH/GSSG ratio, all derivatives increased the T-GSH/GSSG ratio in serum compared with the CCl_4 model group, among which compound **5c** was most active and its effect was higher than anethol trithione (Table 2). This result indicated that the different length of linker introduced into C-30 position of GA exhibited varied hepatoprotective effects. Especially, 3-atom linker of compound **5c** increased the hepatoprotective activities markedly.

2.2.2. Histopathological studies

The cellular architecture of the liver tissue of different group of mice as studied by the histopathological analysis is presented in Fig. 1. The liver tissues of normal group (Fig. 2A and B) showed normal architecture of hepatic cells and hepatic lobule. No congestion and eurysma was observed in central veins and sinus hepaticus. In CCl_4-treated group (Fig. 2C and D), the liver showed distorted