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Original article

Synthesis, antimicrobial and cytotoxic activities, and structure–activity relationships of gypsogenin derivatives against human cancer cells

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ABSTRACT

A series of gypsogenin (**1**) derivatives (**1a**–**i**) was synthesized in good yields, and the derivatives' structures were established using UV, IR, ^{1}H NMR, ^{13}C NMR, and LCMS spectroscopic data.

Among the tested compounds, **1a**, **1b**, **1d**, **1e**, and gypsogenin (**1**) showed antimicrobial activities against *Bacillus subtilis* and *Bacillus thrungiensis*, with inhibition zones of 10–14 mm. In addition, compounds **1b**, **1d**, and **1e** showed antimicrobial activities against *Bacillus cereus*, with inhibition zones of 9–14 mm. Using six human cancer cell lines *in vitro*, the cytotoxic activities of all tested compounds were determined by calculating the IC_{50} values. Doxorubicin and paclitaxel were used as controls. Among the tested compounds, **1a**, **1c**, and **1d** had inhibitory effects with IC_{50} values of 3.9 μM (HL-60 cells), 5.15 μM (MCF-7 cells), and 5.978 μM (HL-60), respectively. To determine the type of cell death, Hoechst 33258 (HO) and propidium iodide (PI) double staining was used. Especially, gypsogenin (**1**) and compound **1a** triggered the apoptotic mechanism at a concentration of 20 μM. Thus, gypsogenin (**1**) and compounds **1a**, **1c**, and **1d** possess varying degrees of biological activities and can be considered as potential antitumor agents.

1. Introduction

Saponins, which are detected in a number of plant families, are glycosides with a polycyclic aglycone and a sugar moiety. Sugars can attach to aglycone, which is also called sapogenin, at one or two different positions; thus, they are named monodesmosidic and bidesmosidic saponins, respectively [1–3]. These secondary metabolites have hydrophilic and hydrophobic sides; hence, they have surface activity, and saponin-containing plants are used as soap.

Saponins have various structure-dependent biological activities such as glucosidase inhibiting [4], antiviral [5,6], anti-inflammatory [7], spermicidal [8], hypocholesterolemic [9], antitumor [10,11], anticarcinogenic [12], and antioxidant activities [13]. Moreover, saponins have been evaluated against cancer cells for anticancer activity [14,15].

Triterpene saponins can be found in many plant species [16–19], and several recent studies have reported on saponins produced from *Gypsophila* species [20–22].

Some saponins from *Gypsophila* have shown a variety of biological activities including anticarcinogenic [23], immunostimulatory [24], and cytotoxic activities [25].

Gypsophila accumulate gypsogenin aglycone with sugar chains, which has been attributed to various biological properties. For example, these compounds have exhibited inhibitory activity [26] and have shown significant growth inhibition in *in vitro* cultures [27]. In addition, some of them have shown very high activity against different human cancer cell lines [28,29].

Thus, there is strong evidence that gypsogenin has anticancer activity.

Gypsogenin aglycone is found at high concentrations in *Gypsophila* [30]; therefore, it can be obtained with ease [31]. In this study, nine new gypsogenin derivatives (**1a**–**i**) were synthesized from gypsogenin aglycone (**1**). In addition, they were evaluated for their antibacterial and antifungal activities as well as cytotoxic activities against six different human cancer cell cultures.

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2. Results and discussion

2.1. Chemistry

Nine gypsogenin derivatives (**1a**–**i**) were synthesized by a series of reactions as outlined in Scheme 1.

The starting material, gypsogenin (**1**), was obtained from the commercially available *Gypsophila arrostii* root extract, and its isolation has been explained in our previous work [32].

All compounds were obtained in good yields, as shown in Table 1.

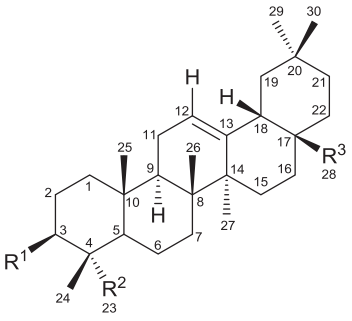
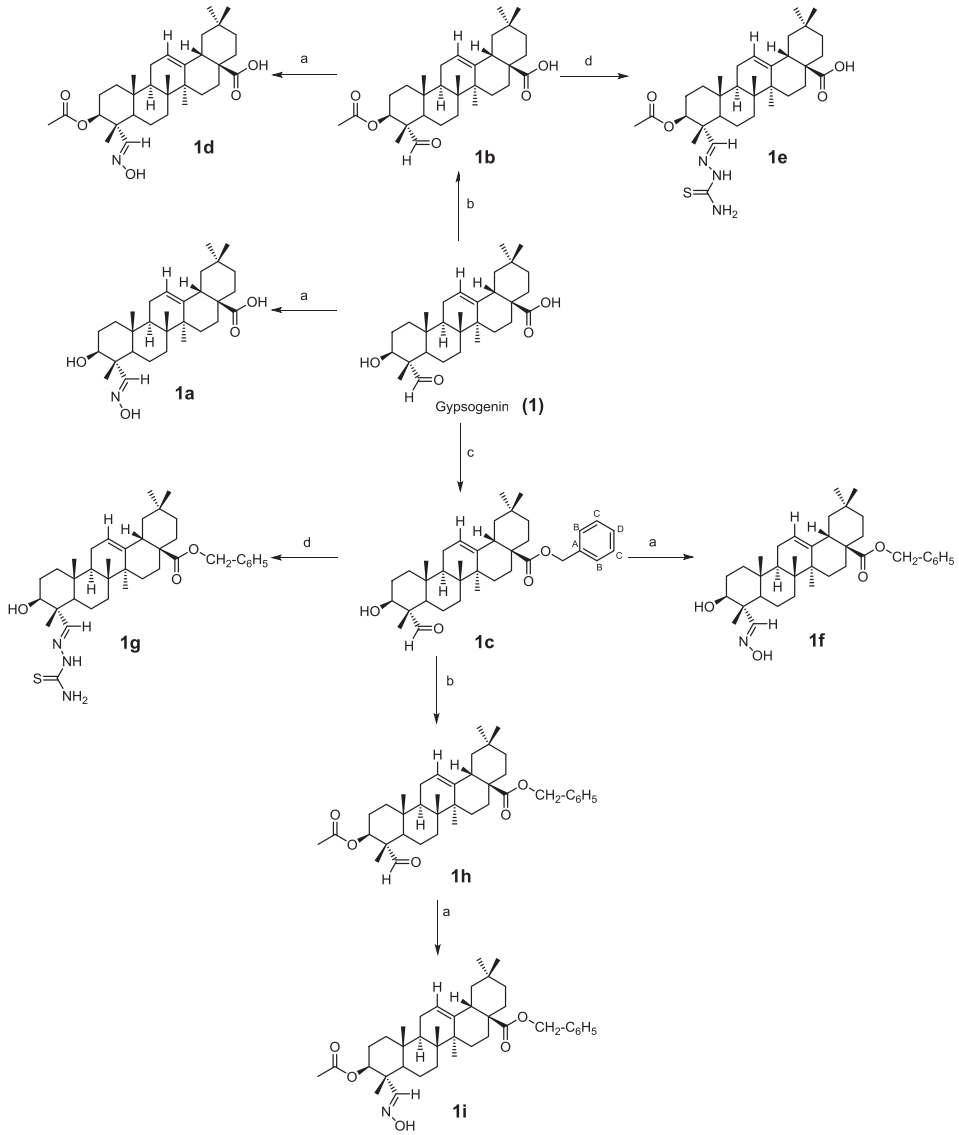


Table 1
Structures and yields of gypsogenin (**1**) and its derivatives (compounds **1a**–**i**).

Compound	R^1	R^2	R^3	Yield (%)
Gypsogenin (**1**)	−OH	−COH	−COOH	–
1a	−OH	−CH=NOH	−COOH	70.2
1b	$-OCOCH_3$	−CHO	−COOH	97.8
1c	−OH	−CHO	$-COOCH_2C_6H_5$	54.6
1d	$-OCOCH_3$	−CH=NOH	−COOH	97.2
1e	$-OCOCH_3$	$-CH=NNHCSNH_2$	−COOH	45.8
1f	−OH	−CH=NOH	$-COOCH_2C_6H_5$	88.3
1g	−OH	$-CH=NNHCSNH_2$	$-COOCH_2C_6H_5$	56.9
1h	$-OCOCH_3$	−CHO	$-COOCH_2C_6H_5$	97.7
1i	$-OCOCH_3$	−CH=NOH	$-COOCH_2C_6H_5$	91.2

Gypsogenin (**1**) was treated with hydroxylamine hydrochloride and sodium acetate in 3:1 acetonitrile: water at room temperature to provide compound **1a**. The intermediate compounds **1b** and **1c** were synthesized by substitution reactions involving acetylation at C-3 and benzylation at C-28, respectively. Usually, this reaction is preferred to protect the carboxyl group of aglycone [33–35]. These intermediates were then reacted with thiosemicarbazide in 1:1 methanol: water to yield compounds **1e** and **1g**, respectively.



Scheme 1. Reagents and conditions: (a) hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$), sodium acetate, 3:1 acetonitrile: water, rt; (b) acetic anhydride, pyridine, rt; (c) benzyl bromide, triethylamine, reflux; (d) thiosemicarbazide, 1:1 MeOH: water, reflux.