European Journal of Medicinal Chemistry 72 (2014) 78–83

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

A "natural" approach: Synthesis and cytoxicity of monodesmosidic glycyrrhetinic acid glycosides

Stefan Schwarz [a,b], Bianka Siewert [a], Nuno M. Xavier [b], Ana R. Jesus [b], Amélia P. Rauter [b], René Csuk [a,*]

[a] Bereich Organische Chemie, Martin-Luther-Universität Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120 Halle, Saale, Germany
[b] Faculdade de Ciências, Universidade de Lisboa, Centro de Química e Bioquímica/Departamento de Química e Bioquímica, Campo Grande, Ed. C8, Piso 5, 1749-016 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form
14 November 2013
Accepted 22 November 2013
Available online 5 December 2013

Keywords:
Glycyrrhetinic acid
Glycosylation
Antitumor compounds
Apoptosis
SRB assay
Trypan blue staining

ABSTRACT

Several pentacyclic triterpenoic acids have shown noteworthy antitumor activity, among them betulinic acid as well as oleanolic acid and derivatives thereof. Glycyrrhetinic acid (**GA**) exhibits some cytotoxic activity albeit this compound is not as active as betulinic acid, but **GA** came in the focus of scientific interest since it triggers apoptosis in tumor cells. In addition, it can be extracted from the roots of liquorice in high yields. Previous studies revealed that the introduction of an extra hydrophilic moiety increases the cytotoxicity of these compounds. Thus, a series of GA glycosides was prepared utilizing hexoses as well as pentoses (in D- and L-configuration) by using glycosyl trichloroacetimidates and TMSOTf as catalyst. The compounds were screened for cytotoxic activity against seven human cancer cell lines and the not malignant murine cell line NIH 3T3using a photometric SRB assay. The compounds trigger apoptosis as shown from extra trypan blue and acridine orange/ethidium bromide staining.

1. Introduction

Glycyrrhizinic acid, the main component of the extracts from liquorice roots [1,2], is a glycosyl triterpenoic acid whose aglycon, glycyrrhetinic acid, is known for many pharmacological effects, such as anti-inflammatory [3,4], antiviral [5,6] or antitumor activities [7,8]. **GA** can be obtained very easily and in huge amounts from the extract of liquorice roots [1]. Its ability to trigger apoptosis in tumor cells [9–11] resurged scientific interest in **GA** and derivatives thereof.

This study aims to increase cytotoxicity of **GA** analogs without losing their apoptotic potential. Some improvements were achieved for other triterpenoic acids, e.g. betulinic acid [12], by the introduction of a hydrophilic sugar moiety. Triterpenoic glycosides are widely distributed in plants and marine organisms [13,14], and their biological effects are attributed to the presence of the aglycon as well as of the sugar moiety. Following this "natural model", **GA** occurs in nature as a glycoside. Hence, we attempted the synthesis of several GA glycosides differing in their anomeric configuration and exhibiting five and six-membered ring glycosyl moieties that belong to the D- or the L-series. Methyl glycyrrhetinate (**GA**–**Me**, Scheme 1) was used as a starting material, because of its enhanced antitumor activity as compared to parent **GA** [15]. All new compounds were fully characterized, and their biological activity was determined in photometric sulforhodamine B (SRB) assays. An extra acridine orange/ethidium bromide (AO/EB) staining was performed to evaluate their apoptotic potential.

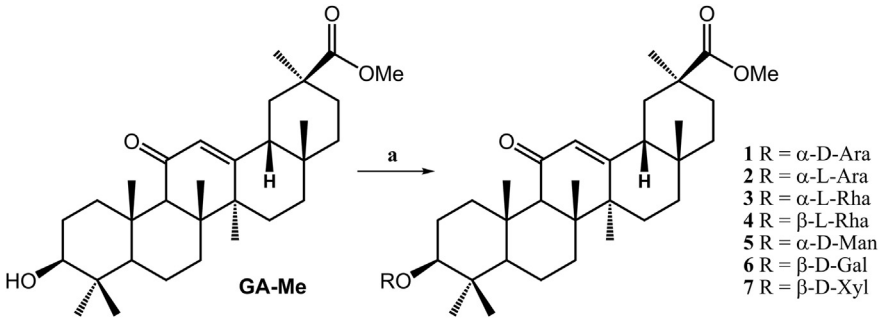
2. Results

2.1. Chemistry

For the glycoside synthesis, the activation of the anomeric center is mandatory. Ample examples show that the trichloroacetimidate group activated by the Lewis acid trifluoromethanesulfonate (TMSOTf) becomes a suitable leaving group for the nucleophilic attack of the alcohol. Hence, sugar trichloroacetimidates were prepared via a three-step protocol [16]. The synthesis of glycosides **1**–**7** proceeded nicely in dichloromethane in the presence of TMSOTf. Removal of the acetyl protecting groups was achieved using a 0.25 M sodium hydroxide solution (in water/THF/methanol, 1:2:1) to yield compounds **1**–**7**.

* Corresponding author.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.11.024



Scheme 1. Synthesis of the glycosides: a) sugar trichloro acetimidate, TMSOTf, mol sieve 4 Å, DCM, −70 °C → 25 °C, 2 h, 4–62%.

Initial experiments resulted in rather poor yields (compared to those ranging from 41% to 50% reported in the literature for betulinic acid [16]). However, changes in the ratio donor/**GA**–**Me**, amount of TMSOTf and variation of the reaction temperature, as shown in Table 1 for the synthesis of compound **6**, led to a significant yield improvement, allowing glycosylation yields ranging from 48% to 62 % using a **GA**–**Me**/glycosyl donor (2/1) ratio and applying one equivalent of TMSOTf at −70 °C.

The configuration of the anomeric center was assigned from their ^{1}H and ^{13}C NMR spectra, in particular considering the chemical shifts of the anomeric carbon and proton and the anomeric proton coupling constant, which were also compared to those reported in the literature for other glycosides with the same glycon configuration. Thus, for compound **1** a chemical shift of δ = 99.4 ppm was found, and for **2** a chemical shift δ = 104.8 ppm was determined. This parallels previous findings of Mitzutani et al. [18] and Evtushenko et al. [19] for other α-D- and α-L- arabinoglycosides. The chemical shift of the anomeric carbon of the β-D-xylopyranoside **7** is also in agreement with data given in the literature for this residue [17,20,23]. The coupling constant of the anomeric proton exhibited by the three pentopyranosides ranges from 5.4 Hz to 6.3 Hz and suggests that a preferred conformation is adopted where most of the substituents tend to be close to the equatorial position as it occurs in conformation $^{1}C_4$ for compound **1** and $^{4}C_1$ for compounds **2** and 7. Compound **3** is the α-L-rhamnoside (δ = 102.2 ppm, is in excellent agreement with previous findings of Gorin et al. [17] and Breitmaier et al. [20]), whereas compound **4** is the β-L-configurated anomer (δ = 97.2 ppm) [21]. For the mannoside **5** the α-D-configuration was assigned from the presented chemical shift for the signal of C1 (δ = 98.2 ppm), in accordance with the values previously published by Khan et al. [22], and from the H-1 chemical shift (δ = 5.40 ppm) as well as the expected anomeric proton coupling constant J = 1.5 Hz. A β-D-configuration was assigned to compound **6** as a result of the presented NMR chemical shifts and coupling constant that are expected for this stereochemistry [17,20,24].

2.2. Biology

The cytotoxic activity of the new derivatives was evaluated using SRB assays, and the IC_{50} values for different human tumor cell lines as well as for mouse fibroblasts (NiH 3T3) are reported in Table 2. For comparison purposes the cytotoxicity data for the aglycon **GA**–**Me** [15] is also presented (Fig. 1).

The arabinosides **1** and **2** did not show any remarkable activity and the *xylo*-configurated glycoside 7 was inactive in these assays (cut-off 30 μM). For the pair of rhamnosyl substituted compounds **3** and **4** cytotoxicity was found for several human tumor cell lines, and IC_{50} values <30 μM were obtained. All of these IC_{50} values, however, ranged between 20 and 30 μM, thus being comparable to those found for **GA**–**Me**.

The mannosyl derivative **5**, however, was the most active compound of this series showing IC_{50} values as low as 9.48 μM on breast carcinoma MC7 cells, thus being twice as active as the parent **GA**–**Me**. In addition, the IC_{50} value for mouse fibroblasts NiH 3T3 was >30 μM. This pronounced selectivity toward MCF7 cancer cells distinguishes this compound from the aglycon **GA**–**Me**. Compound **6** containing a galactose moiety exhibited similar cytotoxicity as **GA**–**Me**.

Apoptosis is a naturally occurring process directing cells to programmed cell death. As a consequence of this process, cells that are detrimental to an organism are disposed off in an orderly manner. This prevents the development of an inflammatory response being often associated with necrotic cell death. Since SRB tests *a priori* do not allow any conclusion for an apoptotic cell death, selected compounds were chosen for further studies using a dye exclusion test (AO/EB). In these assays (applying MCF7 and A549 tumor cells) the presence of green fluorescent cells with sections of varying intensity were observed. Although this test does not permit quantifying the percentage of apoptosis as compared to necrosis, it revealed that the compounds trigger apoptosis.

In conclusion, we were able to synthesize new monodesmosidic triterpenoid glycosides starting from **GA**–**Me**. These derivatizations did not lead to an increase in cytotoxicity with the exception of compound **5**, that was twice as active as the parent **GA**–**Me**. This parallels previous studies on the cytotoxicity of glycosidic triterpenes. For example, Cmoch et al. [25] observed an increase of the cytotoxicity of lupeol after introducing an extra α-D-mannosyl moiety at position C-3 while Gauthier et al. [12,26] showed decreasing and increasing cytotoxicity of several derivatives with no correlation between the monosaccharide structure and the cytotoxicity. Similar results were found by Thibeault et al. [27] for betulin and betulinic acid glycosides. Extra AO/EB tests indicated that our glycosides are able to trigger apoptosis. Tosum up, the α-D-mannoside **5** showed an improved cytotoxicity against a variety of different human tumor cells lines, and was shown to trigger apoptosis in A549 lung carcinoma cells. Together with its higher

Table 1
Conditions for the glycosylation of **GA**–**Me** with galactosyl trichloroacetimidate and TMSOTf as activator.

Entry	Glycosyl donor	Methyl glycyrrhetinate	TMSOTf	Temperature	Yield
1	1 eq.	1/3 eq.	100 μL	25 °C	4%
2	1 eq.	1/3 eq.	1 eq.	−70 °C	5%
3	1 eq.	2/3 eq.	100 μL	25 °C	10%
4	1 eq.	1 eq.	1 eq.	−50 °C	21%
5	1 eq.	2 eq.	1 eq.	−70 °C	62%