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Research paper

Synthesis and antiproliferative properties of new hydrophilic esters of triterpenic acids

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ABSTRACT

To improve the properties of cytotoxic triterpenoid acids **1**–**5**, a large set of hydrophilic esters was synthesized. We choose betulinic acid (**1**), dihydrobetulinic acid (**2**), 21-oxoacid **3** along with highly active des-E lupane acids **4** and **5** as a model set of compounds for esterification of which the properties needed to be improved. As ester moieties were used - methoxyethanol and 2-(2-methoxyethoxy)ethanol and glycolic unit (type **a**-**d**), pyrrolidinoethanol, piperidinoethanol and morpholinoethanol (type **f**-**h**), and monosaccharide groups (type **i**-**l**). As a result, 56 triterpenic esters (49 new compounds) were obtained and their cytotoxicity on four cancer cell lines and normal human fibroblasts was tested. All new compounds were fully soluble at all tested concentrations, which used to be a problem of the parent compounds **1** and **2**. 16 compounds had IC_{50} < 10 μM on at least one cancer cell line, 12 compounds had cytotoxicity of <10 μM against at least three of four tested cancer cell lines. The highest activity was found for compound **3c** (1.8 μM on MCF7, 2.8 μM on HeLa, and 1.6 μM on G-361 cells) which also had no toxicity on non-cancerous BJ fibroblasts at the highest tested concentration (50 μM). High selective cytotoxicity was also found in compounds **1k**, **2k**, **3c**, and **3i** that are ideal candidates for drug development. Therefore, more studies to identify the mechanism of action were performed in case of **1k**, **3c**, and **3g** such as effects on cell cycle and apoptosis. It was found that compounds **3c** and **3g** can induce apoptosis via caspase-3 activation and modulation of protein Bcl-2 in G-361 cells. In conclusion, compounds **1k**, **3c**, and **3g** show high and selective cytotoxicity, therefore they are significantly better candidates for anti-cancer drug development than the parent acids **1**–**5**.

1. Introduction

Triterpenes are natural compounds with many biological activities [1], most importantly they are cytotoxic [2], antibacterial [3], antifungal [4], antimalarial [5], antileishmanial [6], and some of them inhibit [7] HIV proliferation. In our research group, we have been investigating cytotoxic semisynthetic derivatives of betulin and betulinic acid and found dozens of new compounds with IC_{50} in low micromolar ranges. The most important are heterocyclic triterpenes [8–10], derivatives with modified A-ring [11], and compounds with highly degraded skeleton - des-E lupane derivatives [12]. Throughout all of these subgroups, most active compounds are often carboxylic acids [13]. The presence of a polar carboxyl group on a highly lipophilic triterpene, however, causes problems with solubility and bioavailability. In order to improve those properties of the promising compounds, polar esters were prepared from triterpenoid acids **1**–**5**. Ester groups were chosen to increase the solubility in water based media while retaining or improving

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the cytotoxic activity.

Triterpenoid acids **1**–**5** (derivatives of lupane and des-E-lupane) were chosen because of their high and selective cytotoxic activity on multiple cancer cell lines. All of them having low solubility in organic solvents which causes difficulties with their isolation and purification and additionally, having also low solubility in water based media which keeps causing problems during the *in vivo* tests [8,11]. In general, transforming lupane acids to common alkyl-esters always improved their chemical properties and solubility in organic solvents; however, the cytotoxic activity dropped significantly [14,15]. To avoid this, Pom (pivaloyloxymethyl) and Acm (acetoxymethyl) ester moiety was used and it was found that the Pom esters are mostly inactive while Acm esters had IC_{50} similar to the parent acids [12,14,16]. The solubility of Acm esters in water, however, is still low. There are several literature precedents describing the synthesis of various polar functional derivatives from triterpenoid acids in order to obtain active compounds with favorable solubility in water based media. Some advance in solubility was achieved with esters of terpenic acids with amino-alcohols, ammonium salts, or hydroxylamines [17–21] amides of terpenic acids with aminoacids [7,22–28]; sugar esters [29]. Also hemiesters prepared at the C-3 hydroxy group or C-2 amino group were moderately successful [30,31], however, they required γ–cyclodextrine formulation which then showed low bioavailability in *in vivo* tests [21]. A recent review summarizes more information about triterpenic prodrugs [32,32a]. From this reason, a new type of polar group was sought that would improve polarity of terpenes and retain their high cytotoxicity.

In this work, we selected a set of hydrophilic ester moieties that would be easy to prepare, isolate and purify and which would improve the solubility of triterpenoid lupane or des-E-lupane acids in water based media while retaining or improving the IC_{50}. Betulinic acid (**1**) and dihydrobetulinic acid (**2**) were chosen because they are well known, accessible and widely studied compounds and they may serve as standards in this study. Compounds **3**, **4** and **5** were found earlier in our research group [12,16]. The cytotoxic activity of acids **1**–**3** and **5** on many cancer cell lines (IC_{50}) is in low micromolar range. Structure of starting compounds is in Fig. 1. Due to instability of β-oxoacid **5**, in some cases we had to alkylate an inactive but stable precursor - hydroxyacid **4** from which the derivatives of β-oxoacid **5** are easily available by oxidation. (Scheme 1).

Ester types (**a**-**l**) were selected and prepared from triterpenoid acids **1**–**5**. Ester types are ethylene glycol monomethyl ether (glym, **a**) and diethylene glycol monomethyl ether (diglym, **b**), glycolates (**c** and **d**), bromoethylesters (**e**) were used as precursors for heterocyclic esters (**f**-**h**), and finally we prepared glucosyl and galactosyl esters (**i**-**l**).

2. Results and discussion

2.1. Chemistry

Series of esters **1a**-**1e**, **2a**-**2e**, **3a**-**3e**, and **4e** were prepared by reaction of triterpenic acids with appropriate bromoderivatives in the presence of DBU (Scheme 1). Due to instability of β-ketoacid **5** under basic conditions, esters **5a** – **5c** were prepared using silver carbonate in DMF instead of DBU, however, we did not succeed with the synthesis of the **5e** analog as we always obtained products of decarboxylation. Carboxymethyl esters **1d**, **2d**, **3d**, **5d** were prepared by hydrogenolysis of benzyloxycarbonyl esters **1c**, **2c**, **3c** and **5c** in an autoclave (0.4–0.5 MPa) on Pd/C. Scheme 1.

Heterocycle containing esters of the type **f**-**h** were prepared from corresponding 2′-bromoethylesters **1e** – **4e** by their reaction with each heterocyclic amine. Since the compound **5e** was unavailable by the direct alkylation of **5** using 1,2-dibromoethane and silver carbonate, we could not use it for the preparation of the desired esters **5f**-**5h**. An alternative route, the reaction of acid **5** with morpholinoethanol in the presence of EEDQ afforded compound **5h**, however the isolated yield was 4% and we did not succeed with any optimization of this procedure. We used **5h** for all necessary biological tests, however, for another derivatives, a longer but more efficient reaction pathway was designed. First, compound **4** was alkylated to give **4e**, which was treated with appropriate heterocyclic amines to give esters **4f** and **4g**. Following oxidation of **4f** and **4g** by sodium dichromate afforded desired derivatives **5f** and **5g**. (Scheme 1). In the cases, when desired esters **5a-5d** could be prepared directly from acid **5**, corresponding esters of acid **4** were not synthesized. Esters **4e-4g, 4i** and **4k** were prepared only due to impossibility to synthesize esters **5e - 5g, 5i**, and **5k** directly from acid **5**.

Monosaccharide esters of triterpenic acids were another important set of target molecules in this study. Presence of a monosaccharide moiety in a triterpenoid molecule should make the compound more soluble in aqueous media. Monosaccharide esters were prepared by modified literature procedure [29,33] using reaction of triterpenic acids and corresponding tetra-*O*-acetyl-α-D-glucopyranosyl bromide or tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of potassium carbonate. Because all acids **1**–**5** were not sufficiently soluble in the original reaction media, it was necessary to find the optimum mixture of solvents and base for each acid. Acetylated sugar esters **1i, 1k, 2i, 2k** were synthesized in a mixture of acetonitrile and acetone. In case of acid **3**, better results were obtained with DBU as a base in a mixture of DCM and acetonitrile (Scheme 1). Potassium carbonate was used for synthesis of esters derived from acid **4**, but the best reaction solvent was a mixture of DMF and acetonitrile. The deacetylated glucopyranosyl **1j** – **3j** and galactopyranosyl **1l** – **3l** esters were prepared from **i** and **k** type of esters by the Zemplén deprotection method using sodium methanolate in methanol or mixture THF/MeOH (Scheme 1). Unfortunately, analogous procedure for the deprotection of acetylated compounds **4i** and **4k** resulted in decomposition. The same result was obtained in all attempts for oxidation of **4i** and **4k** to analogous derivatives of acid **5**. From this reasons, all attempts to get monosaccharide esters of acid **5** were unsuccessful.

Oxidation of morpholinoethyl betulinate (**1h**) by SeO_2 gave 30-oxoanalogue **6** and analogous oxidation of compound **3a** gave diketone **7** (Scheme 2). Both derivatives **6** and **7** were prepared

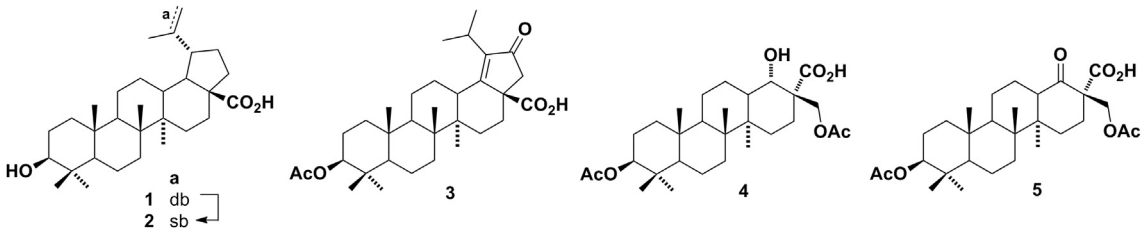

Fig. 1. Starting material - cytotoxic triterpenoid acids **1**–**5**.