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Original article

Preparation of betulinic acid derivatives by chemical and biotransformation methods and determination of cytotoxicity against selected cancer cell lines

Leopoldo C. Baratto [a], Mariana V. Porsani [b], Ida C. Pimentel [b], Adaucto B. Pereira Netto [c], Reinhard Paschke [d], Brás H. Oliveira [a,*]

[a] Departamento de Química, Universidade Federal do Paraná, Centro Politécnico, 81531-970, CP 19081 Curitiba, PR, Brazil
[b] Departamento de Patologia Básica, Universidade Federal do Paraná, Centro Politécnico, 81531-990, CP 19031 Curitiba, PR, Brazil
[c] Departamento de Botânica, Universidade Federal do Paraná, Centro Politécnico, 81531-970 Curitiba, PR, Brazil
[d] Biozentrum, Martin-Luther University, Weinbergweg, 06120 Halle (Saale), Germany

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ABSTRACT

Several novel 2,4-dinitrophenylhydrazone betulinic acid derivatives have been prepared by chemical and biotransformation methods using fungi and carrot cells. Some compounds showed significant cytotoxicity and selectivity against some tumor cell lines. The most active, 3-[(2,4-dinitrophenyl)hydrazono]lup-(20*R*)-29-oxolupan-28-oic acid, showed IC_{50} values between 1.76 and 2.51 μM against five human cancer cell lines. The most selective, 3-hydroxy-20-[(2,4-dinitrophenyl)hydrazono]-29-norlupan-28-oic acid, was five to seven times more selective for cancer cells when compared to fibroblasts. Cell cycle analysis and apoptosis induction were studied for the most active derivatives.

1. Introduction

Betulinic acid (**1**) is a bioactive pentacyclic triterpene. It is one of the most common triterpenes found in plants together with ursolic and oleanolic acids [1]. It has been isolated from many plants such as birch tree (*Betula* spp.), *Ziziphus* spp., *Syzygium* spp., *Diospyros* spp. and *Paeonia* spp. [2], sometimes in concentrations as high as 2–3% [3,4]. Important pharmacological properties have been described for betulinic acid including antitumor, anti-HIV [5], anti-inflammatory, antibacterial, antimalarial, antitrypanosomal and analgesic [6]. Other triterpenes, e.g. boswellic acid and glycyrrhetinic acids, have also shown antitumor activity [7,8].

The activity of betulinic acid against a large spectrum of tumor cell lines has attracted considerable attention. It is active in micromolar concentrations, with high selectivity [9–13]. It is highly selective against the melanoma cells and malignant tumors, inducing apoptosis. The mechanism of action involves mitochondrial membrane permeabilization with release of factors like cytochrome *c*, Smac or AIF in a permeability transition pore-dependent manner, activating caspases and nuclear fragmentation [14,15].

The antitumor properties of betulinic acid motivated studies of structure–activity relationship. Derivatives have been prepared by chemical methods and many exhibited increased cytotoxicity [16]. Biotransformations using microorganisms or plant cells have also been used for that purpose [17,18].

In this report we describe the preparation, characterization and *in vitro* anti-cancer activity of new derivatives of **1** and also the known compounds **2** [19], **8** [20], **11** and **13** [21] for comparison. They were obtained by chemical and biotransformation methods using fungi and carrot cells. The structure–activity relationships are proposed and could contribute to the understanding of the cytotoxic profile of this class of compounds.

2. Results and discussion

2.1. Chemistry

The known betulonic acid (**2**) (Scheme 1), and compounds **8** and **11**, **13** (Schemes 3 and 4) were prepared according to literature

* Corresponding author. Universidade Federal do Paraná, Setor de Ciências Exatas, Departamento de Química, Centro Politécnico, Jardim das Américas, 81531-970 Curitiba, PR, Brazil. Tel.: +55 (41) 3361 3395.
E-mail address: bho@ufpr.br (B.H. Oliveira).

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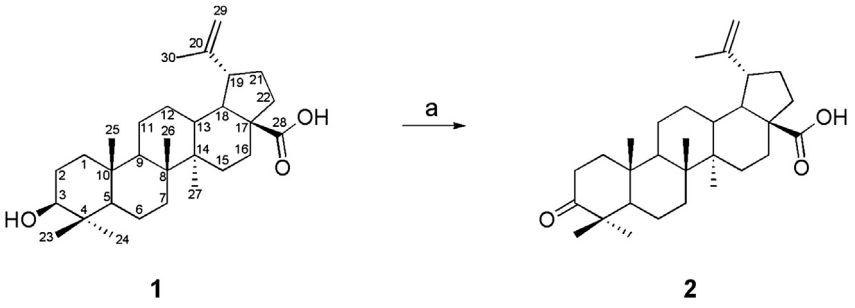


Scheme 1. a) CrO_3, H_2SO_4, 1.5 h, rt.

methods and their spectroscopic data were comparable with those previously reported [19–21].

Compounds containing the 2,4-dinitrophenylhydrazone (2,4-DNPH) moiety, e.g. 4,4′-dihydroxybenzophenone-2,4-dinitrophe nylhydrazone, have been shown to be active against cutaneous cancer metastasis [22,23]. Thus, we decided to prepare some 2,4-DNPH derivatives of betulinic acid.

The ^{13}C NMR spectra of compounds **3**–**10** and **12** (Schemes 2–4), containing a 2,4-DNPH group, showed the characteristic signals of C3 (~167.0 ppm) and C20 (161–162.5 ppm). The ^{1}H NMR spectra also showed the signal of –NH group at 11.00 ppm.

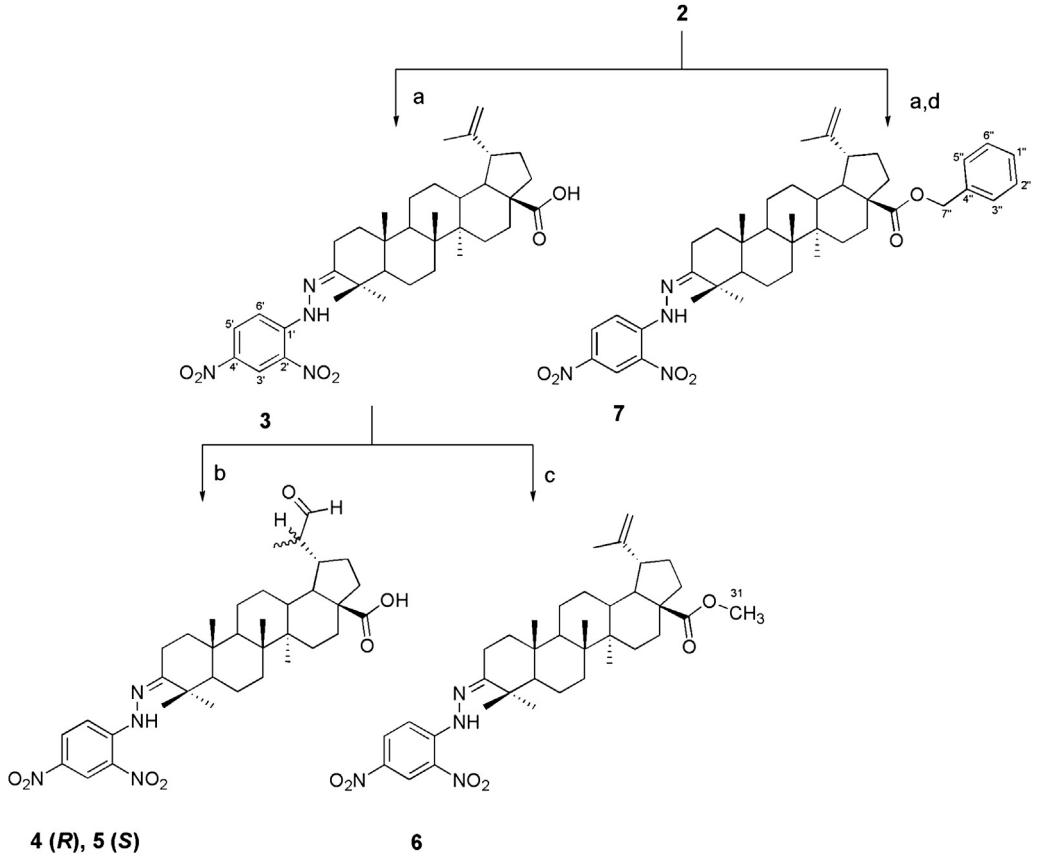
An attempt was made to prepare the epoxide of **3** which could not be isolated because it quickly rearranged into **4** and **5**. Their ^{1}H and ^{13}C NMR spectra showed the characteristic signals of aldehyde groups 9.87, 207.2 and 9.66, 204.8 ppm, respectively. This 1,2 hydrogen-shift rearrangement of terminal epoxides has been explored for the preparation of chiral aldehydes [24].

The biotransformation experiments produced compounds **2**, **14** and **15** (Scheme 5). They have been also obtained by biotransformations using *Bacillus megaterium* [25] and *Nocardia* sp. [26,27].

2.2. Cytotoxicity

The *in vitro* cytotoxic activity was evaluated against 518A2 (melanoma), 8505c (anaplastic thyroid tumor), A2780 (ovarian cancer), A549 (lung cancer) and MCF-7 (breast cancer) cell lines, by SRB colorimetric assay. The compounds were initially dissolved in DMSO and then with RPMI-1640 medium. The DMSO concentration was kept below 0.5% which was non-toxic to the cells. The compounds showed antitumor activity in a dose–response manner and the IC_{50} values are listed on Table 1.

Our results show that most derivatives have superior cytotoxic activity compared to the parent compound, **1** (IC_{50} = 8.75–14.8 μM).



Scheme 2. a) 2,4-DNPH, H_2SO_4, 15 h, rt; b) *m*-CPBA, 5 h, 0 °C; c) diazomethane, rt; d) benzyl chloride, K_2CO_3, 2 h, reflux.