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Short communication

Synthesis and studies of anticancer properties of lupane-type triterpenoid derivatives containing a cisplatin fragment

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ABSTRACT

Both betulinic acid 1 and cisplatin are promising antitumor agents, which induce apoptotic cell death of cancer cells. In the present investigation a new series of betulinic acid–cisplatin conjugates were synthesized and cytotoxicity and selectivity were assessed against five different tumor cell lines. The aim was to combine two structural units, both related with apoptosis induction. The derivatives exerted a dose-dependent antiproliferative action at micromolar concentrations and the effect of these structural variations on anticancer activity was studied and discussed. Several compounds revealed significant antitumor activity, as the most active substance 3-*O*-acetylbetulinic (2-(2-aminoethyl)aminoethyl)amide ($IC_{50} = 1.30–2.24\ \mu M$). Interestingly, Betulinic acid–cisplatin conjugates were less cytotoxic than the precursors.

1. Introduction

Betulinic acid (1) and its derivatives are pluripotent compounds with numerous biological activities. Therefore they have been investigated widely over the last few years [1–5], focusing in the field of antitumor properties [6–10]. Since we have also successfully prepared highly active anticancer platinum complexes [11–13], we have developed a concept to combine both bioactive fragments into one molecule. The aim was to find out if a combination of two different apoptotic structures could lead to an increased cytotoxicity. An insufficient process of apoptosis is not only an important factor in the genesis of tumors, but also the main reason for malignant tumors getting resistant against chemo- and radiotherapies [14] (Fig. 1).

Combinational therapy is common in the field of chemotherapy [15–18]. The efficiency of this therapy depends strongly on the nature of the single components: how they can be delivered, how they are metabolized, and how and to which extent they can enter the cell. Therefore it could be advantageous when the components are covalently linked to each other. There are several examples for this approach. As a result of the combination of Wortmannin and Cetuximab in a "double drug" concept, the antiproliferative activity of both compounds could be improved [19]. Similarly the cytotoxic and phototoxic properties of a Ruthenium–Porphyrine conjugate are combined [20].

2. Results and discussion

2.1. Chemistry

The substances described in this work were prepared according to known methods which were modified appropriately (Schemes 1–3). Compound **3** – an alkyl amide (polyamine) – was prepared by reaction of 3-*O*-acetyl-betulinic acid (2) with diethylene triamine in dichloromethane (DCM) [21]. Platinum complexes **3(PtClS)**, **5($PtCl_2$)** and **6(PtClS)** were formed by having the respective ligand molecules react with dichlorobis(dimethyl sulfoxide)platinum(II) in CH_3OH [22]. The complexes **3($PtCl_2$)** and **6($PtCl_2$)** were prepared by a reaction of the appropriate DMSO platinum complexes with an aqueous LiCl solution [23]; **3($PtCl_2$)** was also obtained by reaction with $K_2[PtCl_4]$ and KCl. **6($PtCl_2$)** and **6(PtClS)** are platinum precursors used for the synthesis of **3($PtCl_2$)** and **3(PtClS)** from compound **3**, and **5($PtCl_2$)** and **5(PtClS)** from compound **5**. The diaminopropanol derivatives **4** and **5** (esters of

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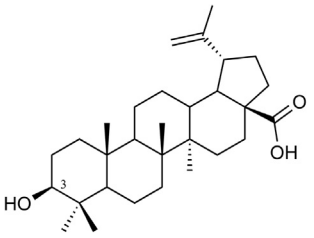
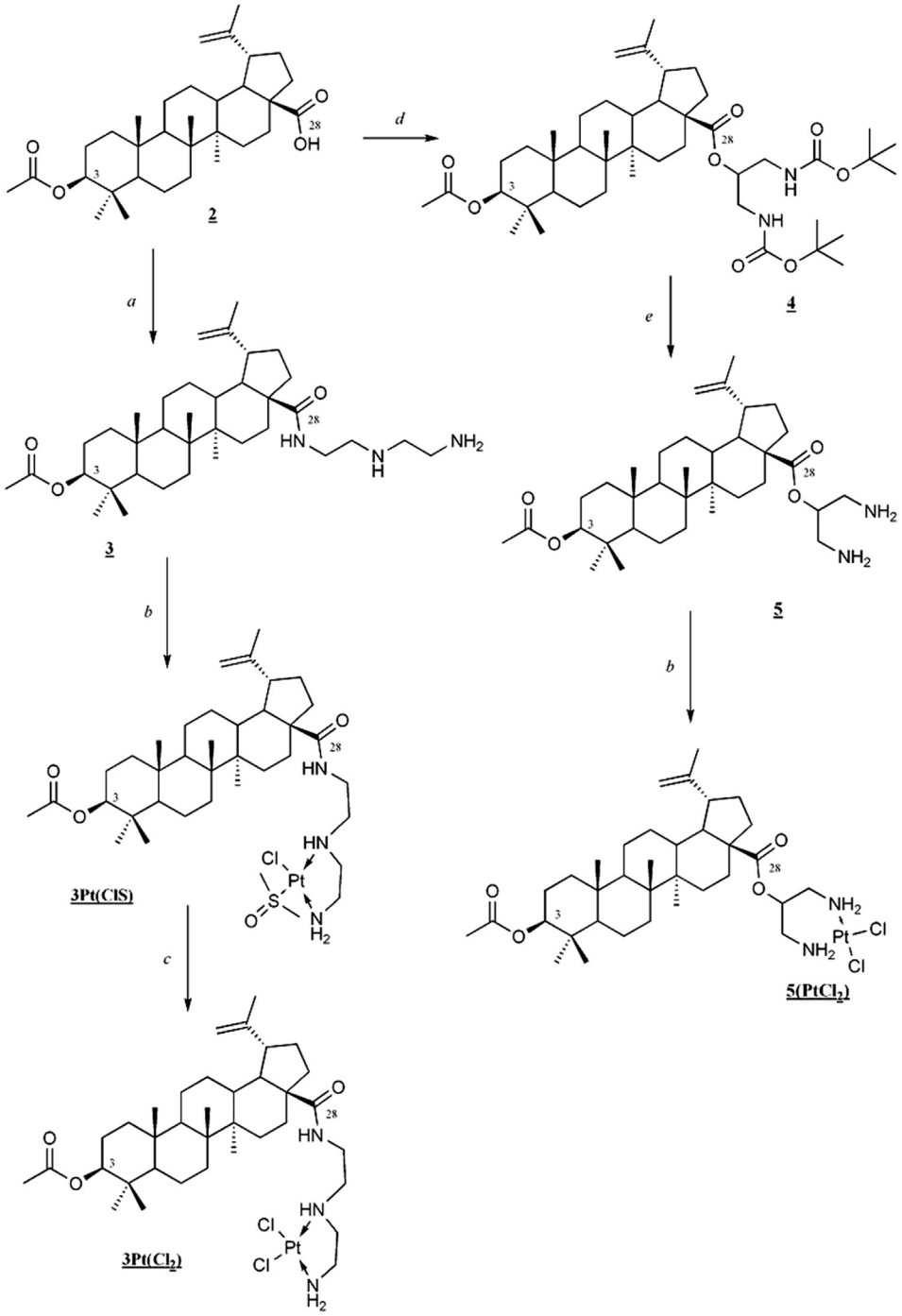


Fig. 1. Betulinic acid **1**.

compound **2**) and **6b** have been prepared according to literature [24], as well as dichlorobis(dimethyl sulfoxide)platinum(II) [25].

2.2. Cytotoxicity

In the present study *in vitro* cytotoxic activity of betulinic acid **1** and its derivatives containing cisplatin similar ligands were studied on five different cancer cell lines: 518A2 (melanoma), A2780 (ovarian), A549 (lung carcinoma), MCF-7 (breast) and 8505C (anaplastic thyroid) as well as on one non-tumorous cell line (WWO70327) by SRB colorimetric assay method [26]. The



Scheme 1. Synthesis of 3-*O*-acetyl-betulinic acid derivatives. (a) Oxalyl chloride, diethylene triamine/DCM, 16 h rt, 6 h reflux; (b) dichlorobis(dimethyl sulfoxide)platinum(II)/CH_3OH, 2 h, rt; (c) $LiCl/H_2O$, 2 h, 80 °C; (d) oxalyl chloride, **6b**/trimethylamine/DCM, 20 h rt, 30 min reflux; and (e) TFA/DCM, 1.5 h rt.