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Research paper

11-Keto-boswellic acid derived amides and monodesmosidic saponins induce apoptosis in breast and cervical cancers cells

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ABSTRACT

Beta-boswellic acids are considered the main bioactive components of frankincense. Their potential to act as cytotoxic agents, as well as that of their derivatives remained unexploited so far. In this study we were able to prepare derivatives of 11-keto-β-boswellic acid (KBA) that showed lower IC50 values as determined by a sulphorhodamine B (SRB) assay using several different human tumour cell lines. Monodesmosidic saponins of KBA are as cytotoxic as 3-acetyl-KBA. The presence of a free hydroxyl group at position C-3 seems to lower cytotoxicity while the presence of an amide function at C-24 improves cytotoxicity. The most active compound of this series gave IC_{50} values as low as 4.5 μM. Cell death proceeded mainly via apoptosis.

1. Introduction

The importance of the development of plant medicines as a key to global health is evident: over one billion people lack access to health care systems. In this context, the study of traditional medicines has been a neglected aspect of global health care for many years [1]. One of these traditional remedies is olibanum. Olibanum (frankincense) is a resin obtained from trees of the genus *Boswellia* in the family *Burseraceae* and has been used in traditional remedies for decades [2]. This precious material has been traded on the Arabian peninsula for more than 5000 years, and it has been used in the traditional medicines of India, Africa, China but also in Western countries [3]. Frankincense has been used to treat fevers and dysentery [4,5], but also – among other applications [6–10] - as an antiseptic [4,5], antihelmintic [6] and as an antitumor agent [11–14].

The resin contains different types of secondary metabolites, such as di-, sesqui- and triterpenes but also essential oils [2,15]. Among all of these, triterpenoids [16] are the most abundant, and many studies have shown among other effects the cytotoxic and antitumor properties of triterpenoids from frankincense.

Frankincense has been used and examined from various perspectives for millennia. The chemical investigations of the resin started as early as 1788 by Baer [17], and in 1898, β-boswellic acid (**1**, Fig. 1) was first isolated [18,19]. Boswellic acids and derivatives are among the major bioactive compounds of frankincense. Compared to other triterpenoic acids, such as betulinic, ursolic, oleanolic, glycyrrhetinic or maslinic acid, β-boswellic acids received less attention probably due their limited availability and high costs of the resin. Several years ago, J. Jauch et al. [20,21] improved older extraction and purification techniques [22,23] significantly; this allowed a more convenient isolation of 3-acetyl-11-keto-β-boswellic acid (**AKBA**, **2**), together with 11-keto-β-boswellic acid (**KBA**, **3**) and 3-acetyl-β-boswellic acid (**ABA**, **4**).

Recently boswellic acids and derivatives came into the focus of scientific interest due to their antitumor activity, and several compounds have been synthesized showing promising activity [10,24,25]. One major drawback of most of these compounds, however, is their poor solubility in aqueous systems. This limits their solubilization and the formulation for biological tests as well as for medicinal applications [26,27]. Therefore, we set out to synthesize a few derivatives either by transformation of the carboxylic group C-24 into amino acid/amino alcohol derived amides

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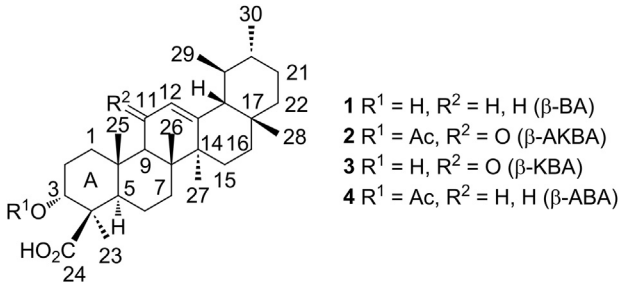

Fig. 1. Structure and ring numbering for β-boswellic acid (**1**, β-**BA**), AKBA (**2**), KBA (**3**) and ABA (**4**).

or by converting **KBA** into monodesmosidic saponins (using the hydroxyl group at C-3), and to test these derivatives for their cytotoxic activity employing several human tumor cell lines. We previously reported [28–32] a higher cytotoxicity for amino acid/amino alcohol derived amides of glycyrrhetinic acid compared to the parent compound, and a betulinic acid derived saponin [33] is regarded [34] as a very promising compound for advanced biological testing.

Triterpenoid glycosides, saponins, are known for their increased hydrosolubility [27,35]compared to their parent aglyca, and they are widely spread throughout the plant kingdom [36]. While there are many saponins derived from triterpenoids such as betulinic, glycyrrhetinic and other triterpenoic acids, to the best of our knowledge, there have been no reports for boswellic acid glycosides.

2. Results and discussion

2.1. Chemistry

Frankincense was purchased from different local suppliers; extraction of the finely grounded resin followed by oxidation and acetylation [20] allowed the convenient isolation of **AKBA** on a larger preparative scale. Due to incomplete oxidation, 3-acetyl-β-boswellic acid (**ABA**, **4**) was obtained as a side product.

Reaction of **2** with oxalyl chloride and ethyl glycinate (Scheme 1) afforded **5** in 88% yield, while the reaction of **2** with oxalyl chloride and methyl 3-aminopropionate furnished **6** in 64%. Under similar conditions dimethylglutamate yielded **7**. This compound is characterized in its ^{13}C NMR spectrum by the presence of two additional signals for carbonyl groups at $\delta = 173.4$ and 172.4 ppm for the dimethylester; the carbonyl group of the α,β-unsaturated system at C-11 was detected at $\delta = 190.0$ ppm. Under similar conditions, the amino alcohol derivatives **8** and **9** were obtained from **AKBA** and **KBA** respectively in good yields.

KBA (**3**) served also as a precursor for the synthesis of the monodesmosidic saponins. Thus, **KBA** was transformed (Scheme 2) into the corresponding benzylester **10** [25,37]. Glycosylation of **10** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloracetimidate or 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloracetimidate [38,39] in the presence of $BF_3 \cdot Et_2O$ [40] at −25 °C gave a 52% yield of **11** and 48% of **12**, respectively. Lower yields, however, were achieved with trimethylsilyl trifluormethanesulfonate [40] as a catalyst under various conditions; this parallels our previous findings for the synthesis of glycyrrhetinic acid derived saponins [41]. Similar yields were obtained when Königs-Knorr conditions were applied for the synthesis of these saponins.

The anomeric configuration of the glycosides was deduced from their ^1H NMR spectra. For *gluco*-configured **11** a coupling constant $J_{H-1',H-2'} = 8.0$ Hz was detected being typical for a β-D-configuration of the anomeric center. For the galactosyl derivative **12** the signal for the anomeric proton H−1′ was detected as a dublet at $\delta = 4.46$ ppm ($J_{H-1',H-2'} = 8.0$ Hz) – hence further supports the presence of a β-configuration at the anomeric center.

Debenzylation of **11** with ammonium formate in methanol in the presence of Pd/C [42–44] gave a 79% yield of **13**; similarly, from **12** debenzylated **14** was obtained. Both compounds were deacetylated under Zemplén conditions to afford **15** and **16** in almost quantitative yield.

2.2. Biology

The in vitro cytotoxic activity of triterpenes **5**–**9** and of the saponins **15** and **16** was evaluated against a panel of different human tumor cell lines using a photometric sulforhodamine B assay (SRB) [45]. The results of these assays are summarized in Table 1. **AKBA** and **KBA** were used as positive controls in these assays. Compounds with IC_{50} values >100 μM were considered inactive. As a result, lowest cytotoxicity was established for **KBA**. Acetylation at position C-3 (→ **AKBA**) improved cytotoxicity significantly; depending on the human tumor cell line ratios $IC_{50,KBA}/IC_{50,AKBA} = 2.06$ (MCF-7, human breast adenocarcinoma) to 3.97 (A431, human epithelial cervical carcinoma) were determined. Loss of the protecting group at C-3 led to a lowered cytotoxicity – thus **9** always showed higher IC_{50} values than acetylated **8**. Transformation of the carboxyl group C-24 of **AKBA** into an amino acid derived amide did not significantly alter the cytotoxicity of the compounds. These derivatives showed the same IC_{50} values as **AKBA**. Glycosidation at position C-3 led to compounds **15** and **16**: while *gluco*-configured **R52** showed slightly improved IC_{50} values (as compared to **AKBA**), slightly increased IC_{50} values were obtained for *galacto*-configured **16**. The best result in this series of compounds was obtained for hydroxypropyl substituted **8**. For this compound in MCF-7 human breast cancer cells an IC_{50} value was as low as 4.5 μM.

Several triterpenoids induce apoptosis, and to evaluate the anticancer activity of our most active compound, compound **8** was tested for an induction of apoptosis using a DNA-laddering assay. During apoptosis, endonucleases cleave DNA into smaller fragments that were stained and finally detected by gel electrophoresis as "ladders". Thus, floating A2780 cancer cells (obtained after treatment with IC_{90}-concentrations of **8** for 24 h) were analyzed by DNA gel electrophoresis, and the typical DNA ladders were found (Fig. 2). In addition, a trypan blue dye exclusion test was performed to distinguish between cells having died by apoptosis or by necrosis. As depicted in Fig. 2, the presence of an intact cell membrane in A2780 human ovarian cancer cells in most of the cells (having been treated with an IC_{90} concentration of **8** for 24 h) confirmed that this compound is able to trigger apoptosis.

3. Conclusion

Here we prepared several amide derivatives and monodesmosidic saponins from **KBA**. All synthesized compounds showed increased cytotoxicity towards a broad panel of human tumor cell lines. The presence of a free hydroxyl group at position C-3 seems to lower cytotoxicity while the presence of an amide function at C-24 improves cytotoxicity. Monodesmosidic saponins of **KBA** are as cytotoxic as **AKBA**. The most cytotoxic compound of this series gave IC_{50} values as low as 4.5 μM and the cell death proceeded mainly by apoptosis as demonstrated by DNA laddering experiments and a trypan blue dye exclusion test.