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Towards cytotoxic and selective derivatives of maslinic acid

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ABSTRACT

Several novel esters and amides of maslinic acid were prepared. Their evaluation for cytotoxic activity with a panel of human cancer cell lines using a sulforhodamine B (SRB) assay revealed for some of them a noteworthy activity. The results from annexinV-FITC and caspase-assays as well as from DNA laddering experiments provided evidence for an apoptotic cell death. A diacetylated benzylamide (15) induced a G1/G0 arrest in tumor cells. It also displayed an extraordinary cytotoxicity against human ovarian cancer cells but a 300 times lower toxicity for non-malignant primary human fibroblasts.

1. Introduction

In 2009/2010 nearly three million tons of olive oil have been produced worldwide, and approximately ten million tons of olives are harvested each year.[1] Per ton of processed olives approximately 800 kg of residues, the olive pomace also known as 'alperujo' are obtained.[2] For natural product chemists this 'waste' (often used as an additive for animal feed) looks like a gold mine for the isolation of natural products. The olive pomace contains high amounts of oleuropein, tyrosol, hydroxytyrosol and the triterpenoid maslinic acid (**MA**, Fig. 1).[3] This oleane type triterpenic acid shows some promising biological properties, such as antiviral,[4–6] antioxidant[7–9] and an anticancer activity.[10–14] Improvements in activities are usually obtained by chemical modifications,[15] and thus we set out synthesizing a series of systematically modified maslinic acid derivatives trying to improve its cytotoxicity.

A SAR study using several aliphatic esters and amides at C-28 revealed that the presence of a proton donor group at position C-28 seems unfavorable for obtaining good cytotoxicity. The presence of a lipophilic residue, however, improved the anticancer activity of the compounds while still retaining their ability to trigger apoptosis. As outlined in Figure 1, bulky ester residues seem to be able to interact quite well with an up to now still unknown intracellular target/receptor. Thus, we became interested in the synthesis and biological evaluation of some substituted benzylic esters of **MA**. All new compounds were evaluated by a photometric sulforhodamin B assay (SRB) for their cytotoxic activity. For some of these compounds annexin V/propidium iodide assays, DNA-fragmentation, caspase assay as well as cell cycle investigations were performed.

2. Synthesis and evaluation of various esters of maslinic acid

Maslinic acid (**MA**) was extracted from olive pomace or from edible green or black olives. Although **MA** has been isolated from many different sources, the residue from the olive oil production seems to be an ideal source for obtaining **MA** by extraction. Unfortunately, this pomace is readily available only in countries producing olive oil.[16] It is only seasonally available, and its transport out of the producing countries is laborious (the pomace has to be kept cool during transport to avoid/slow down fermentation processes) and hence expensive. The isolation of **MA** from edible olives (to be purchased in the nearest supermarket) seems to be a feasible alternative. **MA** was transformed into the corresponding esters by its reaction with alkyl bromides in the presence of finely grounded K_2CO_3 in dry DMF (Fig. 2, Table 1). By this procedure esters **1**–**10** (and chain-extended analogs **12** and **13**) were prepared in good yields. Hydrogenation of **12** gave **13**. These esters showed in their respective IR spectra the typical signals expected for this class of

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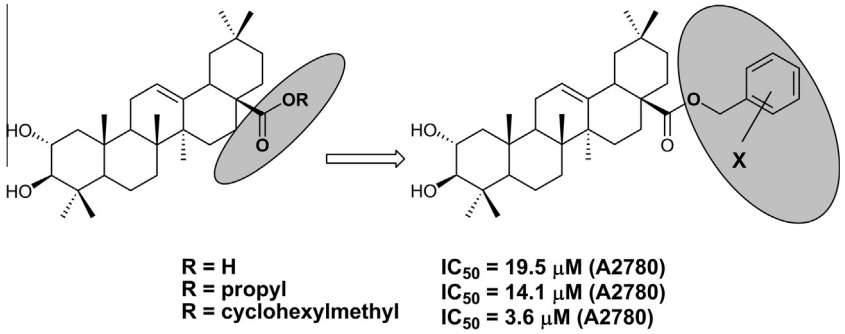

Figure 1. Structure of maslinic acid (MA) and some of its cytotoxic esters.

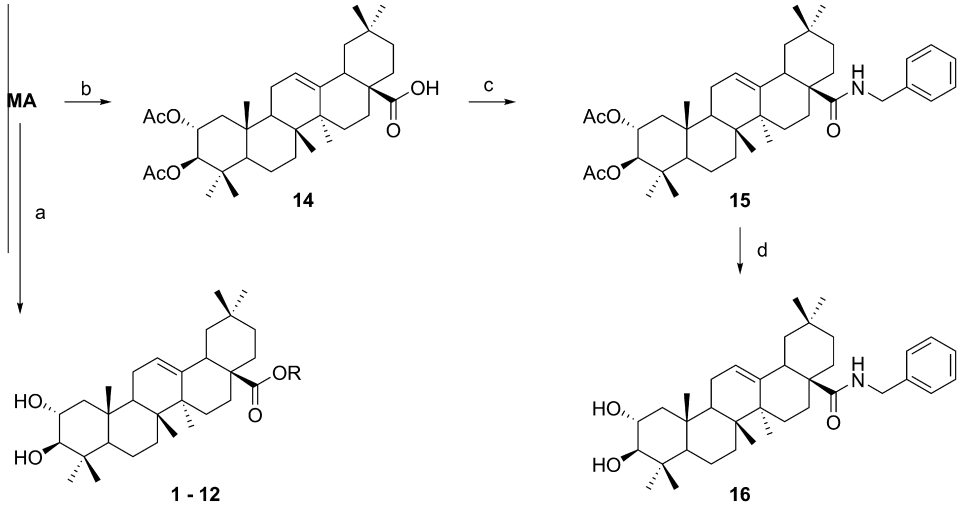

Figure 2. Synthesis of compounds 1–16: (a) K_2CO_3, R-Br, DMF, 25 °C, 24 h; (b) Ac_2O, NEt_3, DCM, 25 °C, 24 h, 88%; (c) $SOCl_2$, NEt_3, DCM/THF (1:1), then $BnNH_2$, DCM, 25 °C, 24 h, 82%; (d) MeOH/KOH, 25 °C, 24 h, 66%.

compounds, and in the ^{1}H NMR spectra the signals of the aromatic protons were detected between δ = 7.20 and 7.50 ppm. For example, for compound **5**, the signals of the aromatic protons were found at δ = 7.47 and 7.21 ppm, each as a doublet exhibiting a coupling constant of J = 8.3 Hz. In addition, compound **3** is characterized in its ^{19}F NMR spectrum by the presence of a signal at δ = −114.18 ppm showing a $J_{H,F}$ = 8.6 Hz to the adjacent and a $^4J_{H,F}$ = 5.4 Hz to the protons in m*eta*-position. Diacetyled **MA** (**14**) was obtained by acetylation of **MA**. The benzyl amide **16** was prepared from **14** using a Schotten-Baumann route followed by a deprotection of intermediate **15** (Fig. 2).

Compounds **1**–**13**, **16** and **MA** (as a reference) were submitted to SRB assays.[17] The IC_{50} values were calculated from dose response curves applying a non-linear regression using the two parametric Hills-slope equation. The results of these assays are compiled in Table 1.

The results from the SRB assay for **MA** and compounds **1**–**13** and **16** using the human ovarian carcinoma cell line A2780 are presented in more detail in Figure 3. Almost all of the compounds showed an increased cytotoxic activity as compared to parent compound **MA**.

The cytotoxicity of compounds **2**–**13** and **16** is similar to that of the cyclohexylmethylester (**1**, Table 1 and Fig. 2). For A2780 cells (Fig. 3) the activity of the *para* substituted compounds increases with the atomic radius of the halogen substituent. In addition, the *para*-bromobenzyl derivative **5** shows a quite promising selectivity in cytotoxicity discriminating between the cancer cell lines and non-malignant mouse fibroblasts NiH 3T3 with a ratio $IC_{50\ [NiH3\ 3T3]}/IC_{50\ [A2780]}$ = SI = ca. 3. The amide **16** and the methoxy substituted benzyl ester **7** exhibit SI values of similar magnitude. Except for a substitution in *para*-position, other substitution patterns seem to lower cytotoxicity. Also the presence of an *ortho* substituent led to a significant lower selectivity between malignant and non-malignant cells, and chain-elongation (as exemplified in compounds **12** and **13**) resulted in a loss in selectivity.

To sum up, these modifications yield some small increase in cytotoxicity and a low but significant improvement for the selectivity of the compounds.

Next, modifications of ring A (concerning the number of hydroxyl groups) and of ring E (α-amyrin or β-amyrin skeleton) came in our focus of our investigations. Whereas **MA** holds two hydroxyl groups, oleanolic acid (**OA**, Fig. 4) and ursolic acid (**UA**) carries only one. As previously shown for glycyrrhetinic acid (**GA**), a 20-fold increase in cytotoxicity can be gained by converting **GA** into its corresponding benzyl ester.[18] Thus, the structure–activity relationships of benzyl oleanoates (**17**–**20**) and of substituted benzyl esters (**24**–**27**) of ursolic acid (**UA**) were investigated in more detail. Compounds **17**–**20** and **24**–**27** were obtained by esterification of **OA** and **UA**, respectively. Acetylation of **OA** or **UA** (Fig. 4) gave acetates **21** and **28**; reaction of these compounds with thionyl chloride/triethylamine and benzylamine furnished amides **22** and **29**. Their deacetylation (methanolic KOH) gave amides **23** and **30**,