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Research paper

Selective killing of cancer cells with triterpenoic acid amides - The substantial role of an aromatic moiety alignment

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ABSTRACT

2,3-Di-*O*-acetyl-triterpenoic acid derived amides possessing a (2β, 3β) configuration in ring A and two acetyl groups were previously shown to possess high cytotoxicity for human tumor cell lines but to exhibit low cytotoxicity for non-malignant mouse fibroblasts. In this study, augustic acid (**1**) and 2-*epi*-corosolic acid (**2**) were chosen as starting points for the synthesis of analogs. While augustic acid derived 3-quinolinyl amide **9** gave low EC_{50} values in SRB assays but was cytotoxic for all lines, the isomeric 4-isoquinolinyl amide **21** was very cytotoxic for the tumor cell lines but significantly less cytotoxic for the mouse fibroblasts NIH 3T3. In addition, a triacetylated 4-isoquinolinyl derivative of asiatic acid (**28**) gave EC_{50} = 80 nM (for A2780 ovarian cancer cells). As shown by additional experiments (acridine orange/propidium iodide staining, fluorescence spectroscopy and cell cycle investigations) these compounds act mainly by apoptosis.

1. Introduction

Despite of improved technology and medical progress for the last decades, cancer remains one of the world's leading causes of death finally resulting in approximately 8 million deaths globally per year [1]. Options to treat this family of diseases include surgery, radiotherapy, chemotherapy or a combination thereof. Many of the chemotherapeutics commonly applied for the treatment of cancer are characterized altogether by a high degree of non-selective toxicity for the cells. For example, *cis*-platinum (one of the most common used chemotherapeutics accounting to annually sales of approximately 3 billion Euro) is highly active for many types of human tumors [2] (e.g. ovarian cancer as well as cancer of the head, neck, testes but also for treating small cell lung cancer), but this drug also exhibits strong nephro- and neurotoxic effects [3]. Thus, a main goal of an effective treatment of cancer pursues to kill cancer cells selectively without any (or negligible) damage to non-malignant cells. Modern therapeutic schemes to achieve these goals include, for example, prodrug targeting [4], advanced drug delivery systems [5,6] or by applying light [7,8] or heat [9,10] or by a selective drug targeting using magnetic triggering [11,12].

In the course of investigating a small library of maslinic acid derivative compounds, we discovered an interesting molecule named **EM2** (Fig. 1), and this di-*O*-acetylated benzylamide of maslinic acid exhibited rather low EC_{50} values (sulforhodamine B assays [13] (SRB), EC_{50} = 0.5 μM for human ovarian cancer cells) while being significantly less toxic for non-malignant mouse fibroblasts (NIH 3T3, EC_{50} = 33.8 μM) [14]. We considered **EM2** as an ideal basis to start additional investigations concerning the biological activity of pentacyclic triterpenoid analogs carrying an additional nitrogen containing heterocycle attached to ring E by a suitable spacer. In addition, we became also interested in possible effects of the presence/absence of acetyl groups in ring A onto cytotoxicity and/or selectivity of the compounds. Hence, a panel of analogs was synthesized, and their cytotoxicity measured using SRB assays. For selected compounds (showing low EC_{50} values for the cancer cells and high EC_{50} values for the mouse fibroblasts), cell cycle investigations, annexin V/propidium iodide staining experiments as well as visual inspections of the cells by fluorescence microscopy were carried out.

2. Results and discussion

Augustic acid (**1**, Fig. 1) and 2-epi-corosolic acid (**2**) were chosen as starting points for the synthesis of analogs. Both of these compounds carry two hydroxyl functions in ring A showing a (2β, 3β)-

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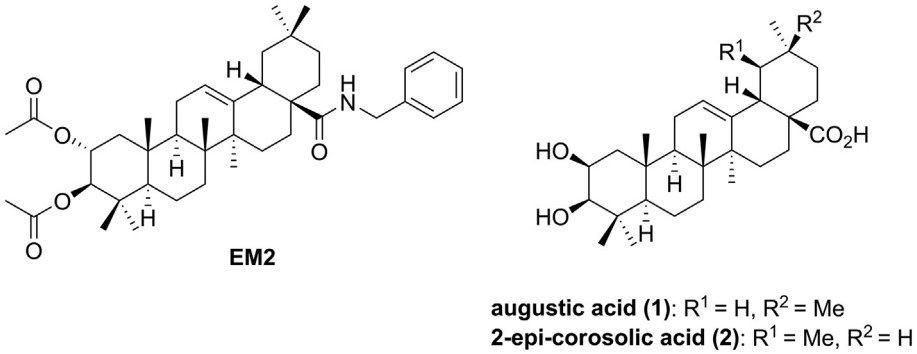


Fig. 1. Structures of **EM2**, augustic acid (**1**) and 2-epi-corosolic acid (**2**).

configuration, and they are easily accessed by synthesis [15-18]. Acetylation of **1** or **2** gave diacetates **3** and **4** (Scheme 1) whose reactions under Schotten-Baumann conditions with oxalyl chloride followed by the addition of an amine furnished amides **5**-**21** in moderate to excellent isolated yields between 38 and 90%. Amides **5**-**21** were characterized by NMR, IR, UV-vis spectroscopy and ESI-MS. Thus, in the IR spectra of these amides a strong absorption band at ν = 1506-1534 cm^{-1} was detected being characteristic for the presence of a NHCO moiety. In their UV-vis spectra the aromatic chromophore gave characteristic absorption maxima between λ = 244-279 nm for the pyridinyl substituted compounds while for the quinolinyl amides absorption maxima λ = 241-335 nm were detected. As far as their ^{1}H NMR spectra are concerned, the signal for the NH moiety was detected between δ = 10.30-10.37 ppm for the 8-quinolinyl amides while all of the other compounds this signal was found between δ = 7.77-8.10 ppm, respectively [19,20].

The compounds were subjected to SRB assays [13], the results of which are summarized in Table 1.

Compounds **5**-**8** carrying a pyridine substituent showed an improved cytotoxicity as compared to acetylated acids **3** and **4**. Thus, compound **5** gave an EC_{50} = 0.9 μM for the human carcinoma cell line A2780 but selectivity between the human tumor cell lines and non-malignant mouse fibroblasts NIH 3T3 remained low for these compounds. A different performance, however, was observed during the biological evaluation of quinolinyl substituted compounds - depending on their substitution pattern. While the cytotoxicity of compounds **9**-**12** and **15**-**18** was similar to that of the pyridinyl substituted amides, 8-quinolinyl derivatives (**19** and **20**) showed selective cytotoxicity towards different human tumor cell lines. However, their overall cytotoxicity was low, and their solubility in water was quite poor. As a consequence, we discarded these compounds from additional investigations. 5-Quinolinyl substituted compounds **13** and **14**, however, performed better because they gave rather low EC_{50} values, they were cytotoxic to the human tumor cell lines but of significantly lowered cytotoxicity for the non-malignant mouse fibroblasts. From these findings we presumed a potentially existing connection between cytotoxicity and the orientation of the aromatic system, and - as a consequence - we synthesized 4-isoquinolinyl compound **21**. Compounds **9** (a 3-quinolinyl derivative) and **21** are formally distinct from each other by a re-orientation of the aromatic substituent (Fig. 2).

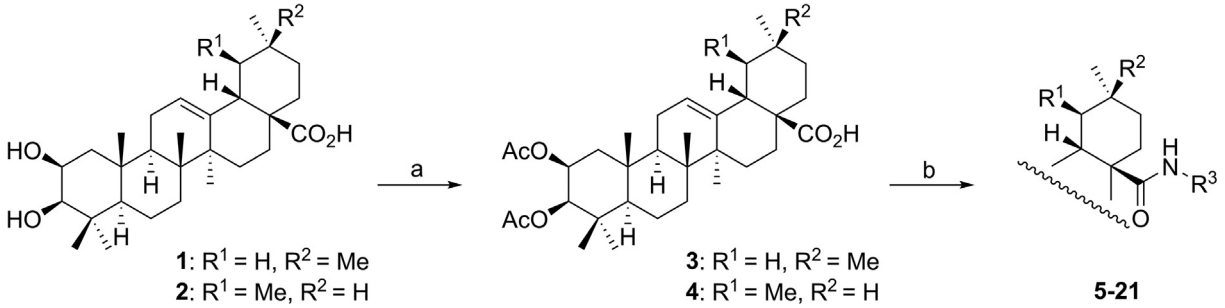
While this re-orientation had only a slight but significant effect in gaining high cytotoxicity, its impact on the selectivity for tumor cells was dramatically enhanced, and selectivity factors greater/equal 50 were observed.

To get a first impression of the impact of the presence of the hydroxyl groups of ring A, some derivatives of ursolic and asiatic acid were synthesized (Scheme 2).

The cytotoxic activities of acetylated amides of ursolic acid [21-26] has already been investigated by several groups in detail while for asiatic acid mainly amides without additional acetyl groups in ring A have been investigated so far. [27, 28] Screening of our analogs for their cytotoxic activity showed (cf. Table 2) gave for compound **26** (an ursolic acid derived 5-quinolinyl derivative) an almost complete loss of selectivity between cancer cells and nonmalignant mouse fibroblasts. For analog **27** (derived from asiatic acid), however, selectivity was maintained, and an increased cytotoxicity was gained.

Within the panel of compounds investigated so far, two factors appear particularly critical for obtaining cytotoxic as well as tumor-selective compounds, viz. the presence of an isoquinolinyl moiety at position 28 as well as a minimum of three *O*-acetyl groups attached to ring A. Combining these factors led to the synthesis of compound **28**, an 4-isoquinolinyl derivative of asiatic acid whose EC_{50} values are depicted in Fig. 3.

Hence, we managed the synthesis of a triterpenoid derivative showing both low EC_{50} values (for example, EC_{50} = 80 nM for



Scheme 1. Synthesis of compounds **3**-**21**: (a) Ac_2O, TEA, DMAP, DCM, 25 °C, 20 h, 65-79%; (b) oxalyl choride, TEA, DMF, DCM, 25 °C, 2 h; then: R^3-NH_2, TEA, DMAP, DCM, 25 °C, 2 d, 38-90%.