European Journal of Medicinal Chemistry 127 (2017) 1–9

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Rhodamine B conjugates of triterpenoic acids are cytotoxic mitocans even at nanomolar concentrations

Sven Sommerwerk, Lucie Heller, Christoph Kerzig, Annemarie E. Kramell, René Csuk*

Martin-Luther University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany

ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form
19 December 2016
Accepted 20 December 2016
Available online 23 December 2016

Keywords:
Triterpenes
Maslinic acid
Mitocan
Cytotoxicity
Rhodamine B
Betulinic acid
Oleanolic acid
Ursolic acid
Platanic acid
Glycyrrhetinic acid

ABSTRACT

Triterpenoic acids **1**–**6** exhibited very low or no cytotoxicity at all, but their corresponding 2,3-di-*O*-acetyl-piperazinyl amides **13**–**18** showed low EC_{50} values for several human tumor cell lines. Their cytotoxicity, however, was also high for the non-malignant mouse fibroblasts NIH 3T3. A significant improvement was achieved by preparing the rhodamine B derivatives **19**–**24**. While rhodamine B is not cytotoxic (up to a concentration of 30μM – cut-off of the assay), the triterpenoid piperazine-spacered rhodamine B derivatives were cytotoxic in nano-molar concentration. Compound **24** (a diacetylated maslinic acid derivative) was most toxic for several human tumor cell lines but less toxic for mouse fibroblasts NIH 3T3. Staining and double-staining experiments revealed **24** to act as a mitocan.

© 2016 Elsevier Masson SAS. All rights reserved.

1. Introduction

As early as 1924 Nobel laureate O. H. Warburg linked impaired mitochondrial functions to the growth of tumor cells [1–4]. This hypothesis was neglected for many years but experienced a renaissance during the last decade, and mitochondria have been identified as an emerging target for developing anti-cancer agents. All compounds targeting mitochondria as final target are regarded as mitocans [5]. Mitocans have a large potential to be developed into selective and efficient anti-cancer drugs [6–11]. This is corroborated by recent findings that cells of a tumor might differ due to mutations within the same tumor [12–14], and mutations have been observed for the same type of tumor in different patients [15,16]. Mitocans can be divided into eight different categories [17], one of them being lipophilic cations acting onto the inner mitochondrial membrane [18]. Previous studies showed an increased mitochondrial membrane potential for malignant cells compared to nonmalignant cells [19,20]. Thus, an increased potential difference led to an increased accumulation of cationic compounds, and as a result, an increased cytotoxic activity can be expected for these compounds as well as a higher selectivity for tumor cells. In 1980 Johnson etal. [21] showed rhodamine-123 to accumulate in mitochondria of src-transformed cells, and Lampidis etal. [22] proved these compounds to exhibit selectivity for cancer cells. In addition, a fluorinated rhodamine-docetaxel derivative targeted mitochondria and showed good cytotoxicity [23]. Several derivatives of triterpenoic acids (for example, ursolic, oleanolic, betulinic, maslinic acid) [24–33] have been shown to exhibit high cytotoxicity for human tumor cell lines, and the presence of an extra amine function increased cytotoxicity [24,25]. As a consequence of these observations we planned to synthesize triterpenoic acid derivatives holding an extra amine substituent (in the form of a piperazine amide) that is covalently bonded to rhodamine B as the lipophilic component.

2. Results and discussion

2.1. Chemistry

Ursolic (**1**, Fig.1), oleanolic (**2**), glycyrrhetinic (**3**) and platanic

* Corresponding author.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

http://dx.doi.org/10.1016/j.ejmech.2016.12.040
0223-5234/© 2016 Elsevier Masson SAS. All rights reserved.

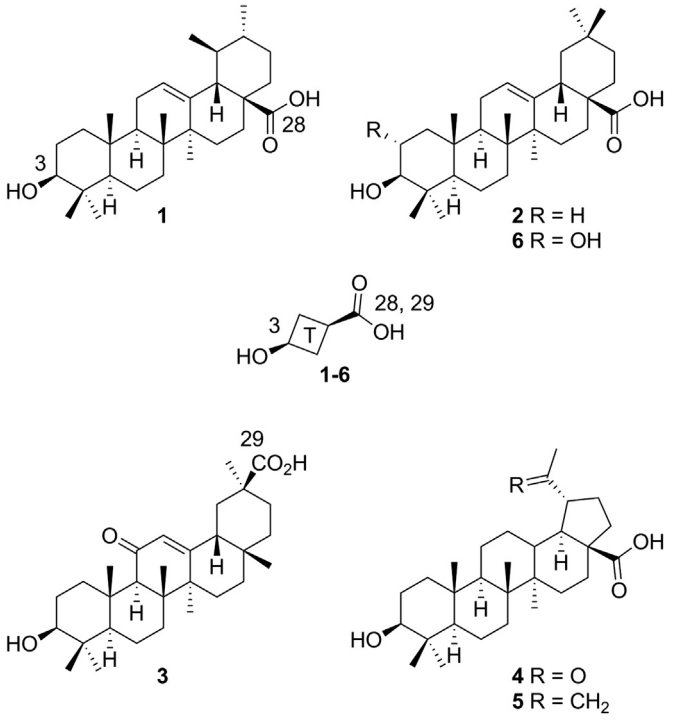


Fig. 1. Structures of triterpenoic acids **1**–**6** and a generalized representation.

acid (**4**) were bought from different suppliers, betulinic acid (**5**) was prepared by oxidation of betulin [34,35], and maslinic acid (**6**) was synthesized as previously described [36,37].

Acetylation of the triterpenoic acids gave the acetates **7**–**12** (Scheme 1). Reaction of **7**–**12** with oxalyl chloride followed by a reaction with piperazine furnished amides **13**–**18**. Reaction of rhodamine B with **13**–**18** gave violet-colored compounds **19**–**24** showing strong fluorescence.

The main plot of Fig.2 compares the absorption spectra of **24** and rhodamine B (Fig.2). In both spectra, the shapes as well as the positions of all absorption bands above λ=250nm are quite similar. The characteristic visible absorption band of the rhodamine B chromophore in **24**, however, was red-shifted by about 15nm.

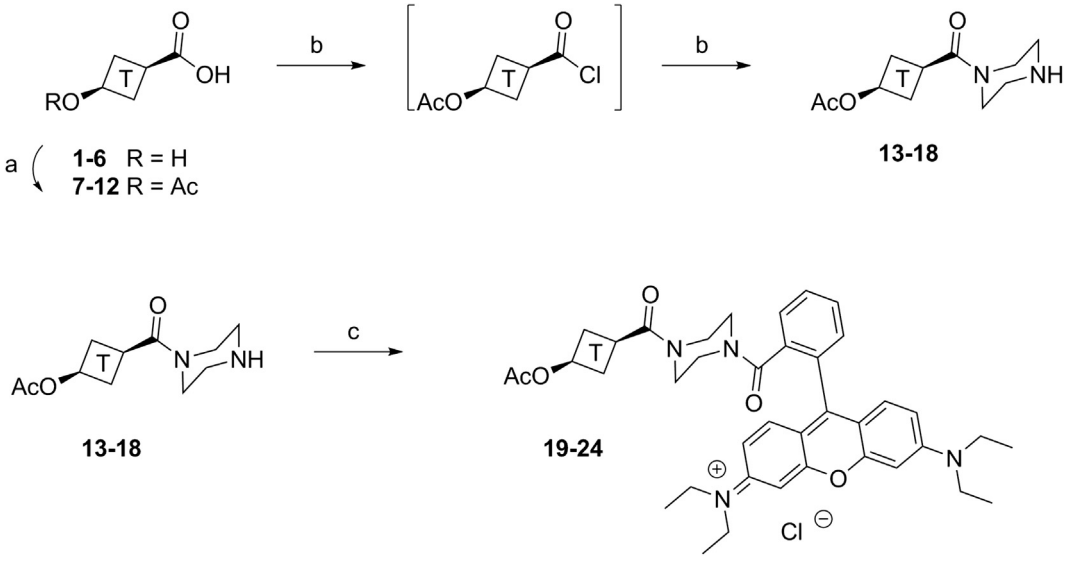
The fluorescence emission spectrum of **24** exhibited a maximum at λ=587nm, as shown in the inset of Fig.2, and it is independent of the excitation wavelength in the range from λ=250–500nm. Moreover, excitation spectra of **24** were recorded with three detection wavelengths (λ=600, 610 and 620nm). The observed wavelength-independence of both excitation and emission spectra clearly indicates that compound **24** does not contain any fluorescent impurities. These promising results prompted us to perform a determination of the fluorescence quantum yield of **24**. A methanolic solution of rhodamine B was chosen as reference system, and a fluorescence quantum yield of 0.82 has been determined (the fluorescence spectrum of pure rhodamine B is also shown in Fig.2). The quantum yield determination was carried out using excitation wavelengths λ=355 and 500nm, respectively. For these measurements very similar quantum yields were obtained with an average value of 0.57±3%. All these results provided unambiguous additional evidence (besides NMR and MS) that the fluorescence active rhodamine B moiety is present in **24**.

2.2. Biology

The cytotoxicity of the compounds was determined using the SRB assay. The results from these assays employing different human tumor cell lines have been compiled in Table1 using parent maslinic acid as a reference compound. As a result, compound **24** is approximately 1000-times more cytotoxic than parent maslinic acid, and the selectivity F_{Si} (defined as EC_{50} A2780 tumor cell line compared to EC_{50} nonmalignant mouse fibroblasts NIH 3T3) was increased by factor 50.

No cytotoxicity was measured for rhodamine B (EC_{50}>30μM; cut-off of the assay). Hence, to the best of our knowledge, compound **24** is the most toxic triterpenoic acid derivative so far; its cytotoxicity is comparable to that of commercial and well established cytotoxic therapeutics, such as doxorubicin or paclitaxel.

At the cellular level, compound **24** probably acts as a mitocan. To support these assumptions extra investigations were carried out.



Scheme 1. Synthesis of compounds **7**–**24**: a) Ac_2O, NEt_3, DMF (cat.), DCM, 25 °C, 1 day: yields: **7** (83%), **8** (85%), **9** (81%), **10** (87%), **11** (81%), **12** (78%); b) oxalyl chloride, NEt_3, DMF (cat.), DCM, 25 °C, 5 h, then piperazine, DCM, NEt_3, DMAP, 0° → 25 °C, 30 min: yields: **13** (80%), **14** (85%), **15** (67%), **16** (85%), **17** (79%), **18** (86%); c) rhodamine B, NHS, DCC, DMF, 25 °C, 3 days: yields: **19** (58%), **20** (76%), **21** (64%), **22** (70%), **23** (66%), **24** (70%).

Download English Version:

https://daneshyari.com/en/article/5158543

Download Persian Version:

https://daneshyari.com/article/5158543

Daneshyari.com