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Research paper

Betulinic acid derived hydroxamates and betulin derived carbamates are interesting scaffolds for the synthesis of novel cytotoxic compounds

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ABSTRACT

The betulinic acid-derived hydroxamates **5**–**18**, the amides **19**–**24**, and betulin-derived bis-carbamates **25**–**28** as well as the carbamates **31**–**40** and **44**–**48** were prepared and evaluated for their antiproliferative activity in a photometric sulforhodamine B (SRB) assay against several human cancer cell lines and nonmalignant mouse fibroblasts (NIH 3T3). While for 3-*O*-acetyl hydroxamic acid **5** EC_{50} values as low as EC_{50} = 1.3 μM were found, *N*,*O*-bis-alkyl substituted hydroxamates showed lowered cytotoxicity (EC_{50} = 16–20 μM). In general, hydroxamic acid derivatives showed only reduced selectivity for tumor cells, except for allyl substituted compound **13** (EC_{50} = 5.9 μM for A2780 human ovarian carcinoma cells and EC_{50} > 30 μM for nonmalignant mouse fibroblasts). The cytotoxicity of betulinic acid derived amides **19**–**24** and of betulin derived bis-carbamates **25**–**28** was low, except for *N*-ethyl substituted **25**. Hexyl substituted **39** showed EC_{50} = 5.6 μM (518A2 cells) while for mouse fibroblasts EC_{50} > 30 was determined.

1. Introduction

Hydroxamic acids have been known since Lossen's discovery of oxalohydroxamic acid (Fig. 1) in 1869 [1,2]. Then and for many years to follow they were regarded as rather exotic compounds. Nowadays, they have been recognized [3] as a unique family of compounds that hold a wide spectrum of biological activities. They act as selective inhibitors of many enzymes, such as matrix metalloproteinases [4,5], hydrolases [6], ureases [7], lipoxygenase [8], tumor necrosis factor-α converting enzyme [9,10], carbonic anhydrase [11,12], ribonucleotide reductase [13,14] and many others. Their acidity is much weaker than that of structurally related carboxylic acids but the hydroxamic acid moiety may act as a bidentate ligand to chelate with several metal ions. It also controls multiple sites for potential hydrogen bond interactions with enzymes and receptors [15].

Nonspecific hydroxamic acid derivatives have been prepared starting inter alia from benzodiazepines [16], α-amino-suberic acids [17], *N*-alkylated amino acids [18] as well as from arylsulfonamides [19]. Although triterpenoic acids represent an important class of compounds bearing high potential as antitumor-active compounds [20,21], to our knowledge, there are only two reports describing a hydroxamic acid derived from glycyrrhetinic acid. These compounds (Fig. 1) acted as selective inhibitors of 11β-hydroxysteroid dehydrogenase 2; there were no data provided whether these compounds were cytotoxic.

During our continuing search for antitumor active compounds from natural products [20–22] we became interested in the synthesis of betulinic acid (**BA**, **1**, Fig. 1) derived hydroxamic acid and derivatives and their cytotoxicity. Previous studies suggested introducing a sulfamate [23,24] or carbamate [25,26] to the pentacyclic skeleton of triterpenes can significantly improve the cytotoxicity of these compounds. Thus, we decided to prepare several mono- and bis-carbamates derived from betulin (**2**, Fig. 1). Betulin as well as betulinic acid have been shown to be interesting scaffolds for developing analogs displaying various biological and medicinal properties especially potent anticancer effects [27–37].

2. Results and discussion

2.1. Chemistry

Our synthetic approach started from **BA** whose acetylation gave 3-*O*-acetyl-**BA** (**3**, Scheme 1) while from the Jones oxidation of **BA**

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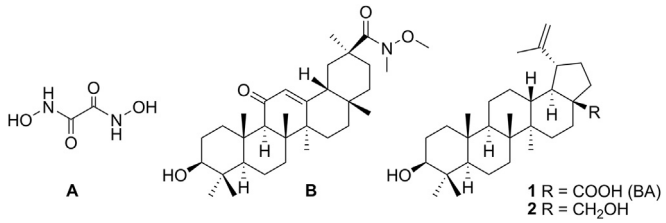


Fig. 1. Structure of Lossen's oxalohydroxamic acid (A) and a representative glycyrrhetinic acid derived hydroxamic acid (B), betulinic acid (**BA**, **1**) and betulin (**2**).

betulonic acid (**4**) was obtained [38,39]. Treatment of **3** with oxalyl chloride in DCM for 2 h at 25 °C followed by adding hydroxylammonium chloride in the presence of trimethylamine [40] provided a 68% yield of hydroxamic acid **5**. Deacetylation of **5** with potassium hydroxide in methanol gave 63% of **6** [41].

While the reaction of **1** with oxalyl chloride/hydroxylammonium chloride gave target compound **5** nicely, the reaction of **1** with propylphosphonic anhydride (T3P) or with 1,1′-carbonyldiimidazole failed to give high yields under a broad variety of different conditions [42–44].

Hydroxamic acid **5** is characterized in its ^{13}C NMR spectrum by a signal at $\delta = 175.1$ ppm being assigned to the **C**ONHOH moiety (C-28) of **5**. For comparison, C-28 was found in **3** at $\delta = 182.5$ ppm. This shifting of the resonance signal of carbonyl carbon C-28 to higher fields is typical for hydroxamic acids [45,46]. In the IR spectrum the C–O stretch vibration was detected at $\nu = 1643$ cm^{-1}.

Reaction of **3** with oxalyl chloride followed by reaction with *N*,*O*-dimethylhydroxylammonium chloride, or *N*-methylhydroxylammonium chloride or *O*-methylhydroxylammonium chloride or *O*-allylhydroxylammonium chloride in the presence of triethylamine furnished products **7**, **9**, **11** and **13**; their deacetylation yielded compounds **8**, **10**, **12** or **14**, respectively.

Under similar conditions betulonic acid (**4**) gave substituted hydroxamic acids **15**–**18**. Yields dropped slightly for these reactions because of the accompanying formation of C-3-oximes.

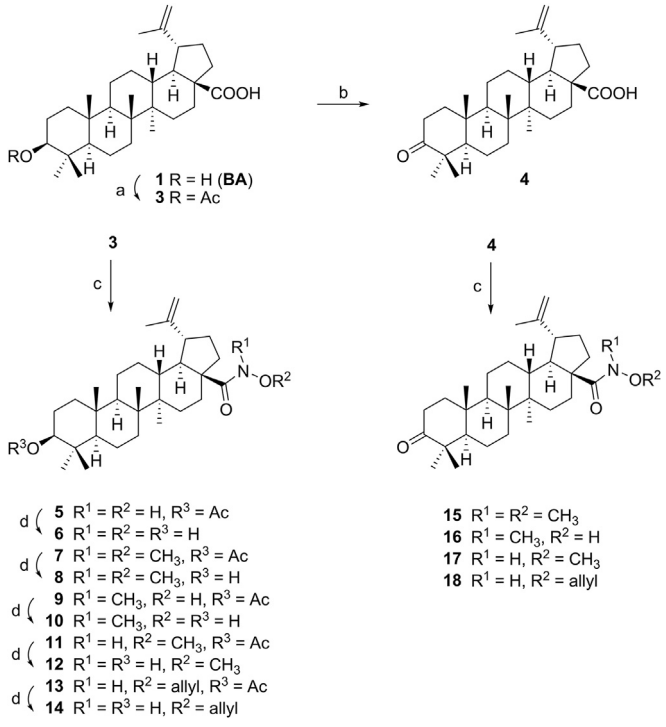
For comparison, we prepared several betulinic acid derived amides as well as betulin derived carbamates. Reaction of 3-*O*-acetyl-betulinic acid (**3**) with oxalyl chloride (Scheme 2) followed by a reaction with dry ammonia in DCM furnished amide **19** in 95% yield. Deacetylation of **19** with potassium hydroxide in methanol gave amide **20** [47,48]. In a similar way, from the reaction of **3** with oxalyl chloride and benzylamine, benzylamide **21** was obtained whose deacetylation yielded **22**; the Jones oxidation of **21** gave 3-oxo compound **23**. Following the procedure given for the synthesis of **12**, from betulonic acid (**4**) with dry ammonia in DCM 3-oxo-amide **24** [49–52] was obtained. Compound **19** is characterized in its ^{1}H NMR spectrum by the presence of a signal at $\delta = 5.55$ ppm that was assigned to the CON**H$_2$** moiety. In addition, the signal for H-19 was shifted to lower fields (compared to parent compound **3** showing $\Delta\delta = 0.08$ ppm). In the ^{13}C NMR spectrum the **C**ONH$_2$ moiety was detected at $\delta = 179.3$ ppm.

Previous studies suggested the introduction of a sulfamate [23] or carbamate [25] to the skeleton of a pentacyclic triterpenoid can significantly improve its cytotoxicity. Thus, we decided to prepare several bis-carbamates. The synthesis of these bis-carbamates **25**–**29** started from betulin (**2**, Scheme 3). While the reaction of betulin with ethyl isocyanate in refluxing chloroform for 48 h gave only low yields, the microwave assisted reaction of **2** with ethyl isocyanate in dry THF worked nicely, and 3,28-bis-*N*-ethyl-carbamate **25** was obtained in 81% isolated yield. Similarly bis-carbamates **26**–**28** were prepared.

For the synthesis of 3-*O*-acetyl-28-*N*-alkyl-carbamates **31**–**35**, 3-*O*-acetyl-betulin (**30**) was used as a starting material. Compound **30** is easily accessible from betulin; thus, diacetylation of betulin gave diacetate **29** whose selective deacetylation with KOH in MeOH/THF at 0 °C yielded 57% of monoacetate **30**.

The microwave assisted reaction of **30** with alkyl isocyanates or phenyl isocyanate allowed a quick and reliable synthesis of 3-*O*-acetylated 28-*N*-substituted carbamates **31**–**35**. From their deacetylation with potassium hydroxide in MeOH compounds **36**–**40** were obtained.

The synthesis of 3-oxo-28-*N*-alkyl-carbamates **44**–**48** (Scheme 4) started from 3-oxo-betulin **43**, and the microwave assisted reaction of **43** with alkyl isocyanates in THF gave target compounds **44**–**48** in good yields. The starting material for these reactions, **43**, was obtained from a deacetylation reaction of **42** (KOH in MeOH) in 90% isolated yield. Compound **42** was easily prepared by Jones oxidation of **41** (72% yield); the latter was made by a selective acetylation of betulin in 60% isolated yield.



Scheme 1. Synthesis of betulinic acid derived hydroxamic acids **5**–**18**: a) Ac_2O, NEt_3, pyridine, DMAP, 12 h, 25 °C, 75%; b) Jones oxidation (4 h, 25 °C), 81%; c) oxalyl chloride, DCM, 2 h, 25 °C, then NHR^{1}OR^2, NEt_3, DCM, 2–12 h, 25 °C: **5** (from $NH_2OH \cdot HCl$, 68%), **7** (from HNMeOMe·HCl, 77%), **9** (from HNMeOH·HCl, 66%), **11** (from $NH_2OMe \cdot HCl$, 63%), **13** (from $NH_2OAll \cdot HCl$, 90%), **15** (from HNMeOMe·HCl, 52%), **16** (from HNMeOH·HCl, 81%), **17** (from $NH_2OMe \cdot HCl$, 45%), **18** (from $NH_2OAll \cdot HCl$, 68%); d) KOH in MeOH, 25 °C: **6** (4 d, 63%), **8** (5 d, 89%), **10** (5 d, 60%), **12** (5 d, 95%), **14** (7 d, 94%).

2.2. Biology

The betulinic acid-derived hydroxamates **5**–**18**, the amides **19**–**24**, and betulin-derived bis-carbamates **25**–**28** as well as the carbamates **31**–**40** and **44**–**48** were evaluated for their antiproliferative activity in a photometric sulforhodamine B (SRB) assay [53–56] against several human cancer cell lines and nonmalignant mouse fibroblasts (NIH 3T3). For comparison, betulinic acid (**1**), betulin (**2**), betulonic acid (**4**) and acetates **3**, **29** and **30** were included into this screening (Table 1).

Betulin (**2**) and its diacetate **29** displayed no ($EC_{50} > 30$ μM; cut-off of the assay) cytotoxicity; low activity was found for the betulin-monoacetate **30**. Betulinic acid (**1**) is well-known for its