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Amino(oxo)acetate moiety: A new functional group to improve the cytotoxicity of betulin derived carbamates

Lucie Heller [a], Vincent Perl [a], Jana Wiemann [a], Ahmed Al-Harrasi [b], René Csuk [a,*]

[a] Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany
[b] University of Nizwa, Chair of Oman's Medicinal Plants and Marine Natural Products, PO Box 33, Birkat Al-Mauz, Nizwa, Oman

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ABSTRACT

While 3-*O*-acetylated betulin derivatives carrying a carbamate moiety at position C-28 are of rather low cytotoxicity for human tumor cell lines, the corresponding C-3 amino(oxo) acetates show good cytotoxicity. For example, an EC_{50} as low as 2.0 μM was found for (3β) 28-{[(hexylamino)carbonyl]oxy}lup-20 (29)-en-3-yl amino(oxo)acetate (**16**) employing the ovarian cancer cell line A2780.

Cancers are a family of diseases, and one of the hallmarks of cancer is a continuous and unregulated growth of cells leading either to a solid tumor or to diffusely spread cells. In Europe, North America and parts of Asia nearly half of people diagnosed with cancer and receiving a treatment die of cancer or because of the treatment. Cancer can be treated and even cured either by surgery, application of radiation, immunotherapy or by chemotherapy. Although it is indisputable that notable progress and major achievements in the therapy of all kinds of cancer have been made by chemotherapy, but there remains the need to search for new chemotherapeutics. Starting several decades ago, natural product derived compounds made their way into modern anticancer chemotherapy, and nowadays they play a major role in developing new cytotoxic agents. Among these natural products, many terpenoids[1–8] have shown to act as excellent starting points for finding new therapeutic drugs[2,3,5,6,8] and even as potential chemopreventive agents[1,4–7] During our search for new cytotoxic compounds derived from secondary natural products, we became interested in triterpenoic acids (cf. betulinic acid[9] (**BA**), boswellic acid,[10,11] glycyrrhetinic acid,[12] maslinic acid,[13] oleanolic acid,[14] ursolic acid,[14] tormentic acid[15] and others) and other triterpenes (cf. betulin, **BN**, Fig. 1). Interestingly enough, while there are many reports on the cytotoxicity of **BA** or derivatives,[16–22] **BN** is of reduced cytotoxicity, and the number of betulin derived cytotoxic derivatives remained limited.[23–25]

Apart from the fact that **BN** is a cheap, renewable resource usually obtained from agro-industrial waste. Thus, **BN** is easily to obtain in huge amounts from extracting birch bark, and it is an interesting scaffold for accessing derivatives displaying various biological properties. For example, in HeLa cancer cells BN, unlike BA, seems to trigger apoptosis through an intrinsic pathway by a sequential activation of caspase-9 and caspase 3/-7 and the cleavage of poly-(ADP-ribose)-polymerase.[26] Recently, **BN** derived carbamates came into the focus of scientific interest because of their increased cytotoxic activity[27–31] and an improved therapeutic index.[30,32] Hence, we decided to have a closer look onto this class of compounds, and to explore their cytotoxic properties.[32]

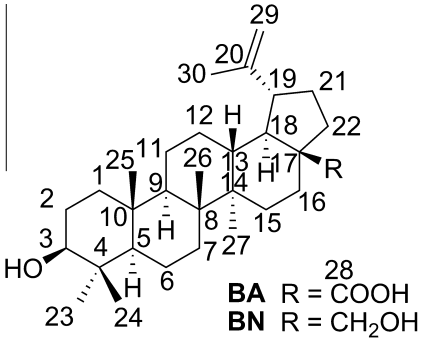

Figure 1. Structure of betulin (**BN**) and betulinic acid (**BA**).

Following well known procedures, **BN** was acetylated and diacetate **1** was obtained in excellent yields (Scheme 1). A regioselective and partial deacetylation of **1** with KOH in a mixture of THF and

* Corresponding author.

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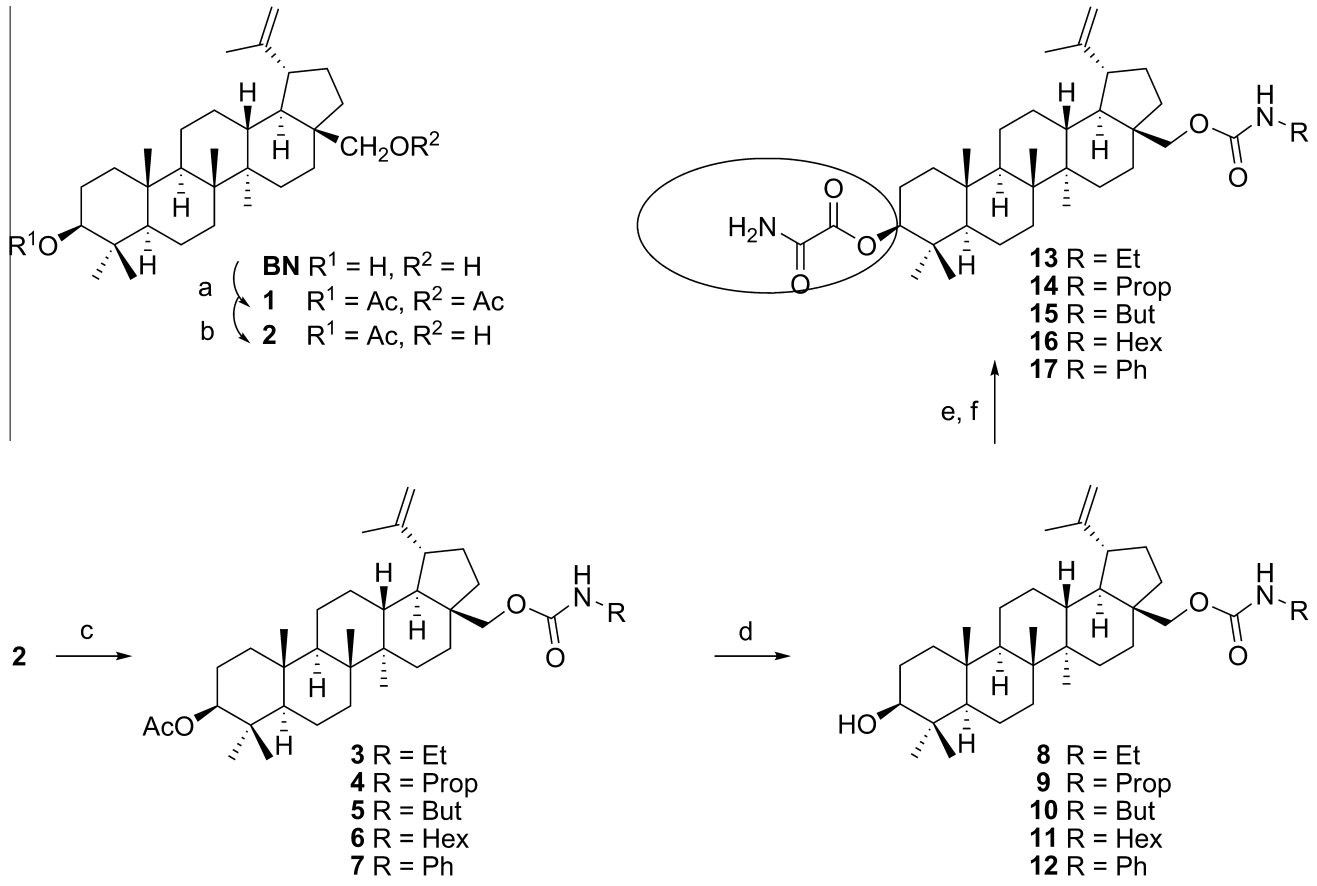

Scheme 1. Synthesis of target compounds **13**–**17**: (a) Ac_2O, NEt_3, DMAP, DCM, 12 h, 25 °C, 90%; (b) KOH, MeOH, THF, 0 °C, 30 min, 57%; (c) microwave-assisted, 7 h, 120 °C, THF, RNCO, **3** (81%), **4** (83%), **5** (81%), **6** (73%), **7** (87%); (d) KOH, MeOH, THF, 25 °C, **8** (2 d, 95%), **9** (1 d, 93%), **10** (2 d, 94%), **11** (1 d, 88%), **12** (2 d, 89%); (e) $(COCl_2)_2$, DCM, 25 °C, 1 h; (f) NH_3, DCM, 25 °C, 12 h, **13** (72%), **14** (63%), **15** (78%), **16** (76%), **17** (60%).

methanol advanced smoothly at 0 °C and provided monoacetate **2** in 57% yield. Microwave assisted reaction of **2** with alkyl isocyanates or phenyl isocyanate gave 3-*O*-acetylated carbamates **3**–**7**. They were deacetylated with KOH in methanol[32] to yield carbamates **8**–**12**.

Previous screening of compounds **3**–**7** in photometric sulforhodamine B (SRB)[33] assays showed these compounds of low cytotoxicity (EC_{50} >30 μmol = cut-off of the assay)—these compounds were of the same low cytotoxicity as parent **BN** (Table 1). Interestingly enough, on deacetylation of **3**–**7**, for compounds **8**–**12** cytotoxicity was restored, and EC_{50} values in the same magnitude as observed for **BA** were measured.

The reaction of secondary alcohols with oxalyl chloride is known to yield alkyl chloro(oxo) acetates,[34–38] but their reactions with ammonia or amines leading to alkyl amino(oxo)acetates have only scarcely been applied.[39–44] As an alternative, decomposing halonitro-acetic acid derivatives has been suggested.[45]

Table 1
Cytotoxicity of selected compounds, betulin (**BN**) and betulinic acid (**BA**, standard) (EC_{50} values in μM from SRB assays after 96 h of treatment; the values are averaged from three independent experiments performed each in triplicate; confidence interval CI = 95 %; cut-off of the assay 30 μM)

EC_{50}	518A2	A2780	HT29	MCF-7	A549	HeLa
BA	11.9 ± 1.7	11.0 ± 1.9	14.4 ± 1.5	14.8 ± 1.9	14.9 ± 1.6	8.8 ± 0.7
BN	>30	>30	>30	>30	>30	>30
1	>30	20.3 ± 2.4	25.7 ± 2.9	>30	21.5 ± 3.0	>30
2	>30	28.5 ± 1.9	>30	20.2 ± 2.0	>30	>30
3	10.2 ± 1.3	11.1 ± 1.4	>30	20.4 ± 2.6	14.1 ± 1.2	17.0 ± 1.6
4–7	>30	>30	>30	>30	>30	>30
8	12.5 ± 3.1	4.6 ± 1.0	>30	12.1 ± 2.1	12.0 ± 2.6	>30
9	15.9 ± 2.3	10.6 ± 1.1	16.3 ± 2.0	15.5 ± 3.0	14.2 ± 1.7	9.4 ± 0.6
10	8.0 ± 1.1	7.0 ± 0.9	7.5 ± 1.3	12.5 ± 1.4	8.6 ± 1.4	10.1 ± 1.7
11	5.6 ± 0.7	7.6 ± 1.3	>30	>30	12.7 ± 2.6	7.4 ± 1.9
12	23.9 ± 3.0	7.2 ± 0.7	16.3 ± 1.8	24.6 ± 2.1	>30	11.5 ± 1.5
13	11.4 ± 1.1	10.0 ± 0.9	15.3 ± 2.0	11.2 ± 0.9	13.7 ± 1.1	14.1 ± 2.4
14	9.9 ± 0.9	7.0 ± 0.9	12.8 ± 2.7	12.5 ± 2.5	10.5 ± 1.7	12.8 ± 1.9
15	7.9 ± 1.2	6.4 ± 1.0	10.1 ± 1.2	10.0 ± 1.5	9.1 ± 1.8	11.2 ± 1.8
16	6.5 ± 1.1	2.0 ± 0.4	7.3 ± 1.6	5.6 ± 1.1	7.4 ± 1.3	10.1 ± 1.5
17	15.3 ± 1.0	17.3 ± 0.7	21.4 ± 0.6	17.6 ± 0.6	20.4 ± 0.8	10.5 ± 0.7

Human cancer cell lines: 518A2 (melanoma), A2780 (ovarian carcinoma), HT29 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma), A549 (lung adenocarcinoma), and HeLa (epitheloid cervix carcinoma).

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As a result, the influence of the presence of an amino(oxo)-acetate onto the biological activity of a molecule has never been examined before. Thus, compounds **8**–**12** were allowed to react with oxalyl chloride followed by reacting the intermediary (and not isolated) chloro(oxo)-acetates with ammonia, and products **13**–**17** were obtained in good to moderate yields. Screening of these compounds in photometric SRB assays gave surprising results: While acetates **3**–**7** were of rather low cytotoxicity for a variety of different human tumor cell lines, the amino(oxo) acetates **13**–**17** showed good cytotoxicity. For example, an EC_{50} as low as 2.0 μM was found for compound **16** employing the ovarian cancer cell line A2780.

In conclusion: there are numerous reports describing the positive influence of esters, amides, carbamates, hydroxamates and other derivatives onto the biological/cytotoxic activity of triterpenes. The deduction of general rules how to improve cytotoxicity while gaining an high therapeutic index remains difficult inasmuch as small structural modifications may alter the mode of action in a most dramatic way: for example, chloroacetylation of **BN** seems to improve cytotoxicity[46] while acetylation of **BN** gave less active derivatives[46,47] and introducing a long chain alkyl ester at HO-C (28) in **BN** caused a loss of cytotoxicity.[46] Carbamates exhibited enhanced selectivity toward different tumor cell lines[28,31,48] but the effect of introducing an amino(oxo)acetate has never been studied before. Calculations of log*P* values suggest, that the amino(oxo)acetates **13**–**17** possess log*P* values similar to analogs with an unprotected hydroxyl group (**8**–**12**), while for acetates **3**–**7** significantly higher log*P* values were calculated. This 'new' functional variation might have some positive and significant influence onto the bioactivity of potential drugs thus warranting future investigations.

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Supplementary data

Supplementary data (experimental procedures and full analytical data of all compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.055.

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