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Synthesis and biological evaluation of the suberoylanilide hydroxamic acid (SAHA) β-glucuronide and β-galactoside for application in selective prodrug chemotherapy

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Abstract—The β-*O*-glucuronide and β-*O*-galactoside of SAHA have been prepared and evaluated as prodrugs for selective cancer chemotherapy (ADEPT, PMT). These new compounds are stable under physiological conditions and do not exhibit any antiproliferative activity compared to the parent drug after a 48-h treatment of H661 cells. The glucuronide derivative did not lead to the release of the drug in the presence of either *Escherichia coli* or bovine liver β-glucuronidase. On the other hand, under enzymatic cleavage of galactoside prodrug by the corresponding enzyme, a rapid release of SAHA was observed demonstrating that the β-*O*-galactoside of SAHA is a promising candidate for in vivo investigations.
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Although considerable effort has been invested in the discovery of selective anticancer drugs, most pharmacological approaches for the treatment of solid tumours suffer from poor selectivity. The development of more selective therapeutic agents has consequently become a major goal of current anticancer research. Within this framework, the use of non-toxic prodrugs designed to deliver the corresponding anticancer agent by specific enzymatic activation in cancerous tissues is currently being investigated.[1] In this approach, the active enzyme may be naturally present in high concentration in the vicinity of the tumour (PMT strategy)[2] or previously targeted to the tumour site using various strategies such as ADEPT,[3] GDEPT,[4] or LEAPT.[5]

Over the past 20 years, glycoside prodrugs, including β-glucuronide and β-galactoside ones, have been extensively studied and some of them have led to very promising in vivo profiles. For example, HMR 1826, a glucuronide prodrug of doxorubicin, has shown superior efficacy in the treatment of various human tumour xenografts in mice compared to standard chemotherapy, both in ADEPT[6] and PMT[2b] strategies. Very recently, Sun and co-workers have reported the evaluation of a β-galactoside prodrug of geldanamycin in combination with a humanized monoclonal antibody chemically conjugated to β-galactosidase (ADEPT strategy).[7] In this study, the authors demonstrated the high potential of β-galactoside prodrugs since the monoclonal antibody they used was found to be an ideal candidate for tumour targeting in the course of a pilot clinical trial.[8] Thus, in the light of these results, the extension of the range of prodrugs that could be activated by either β-glucuronidase and β-galactosidase seems to be warranted.

Histone deacetylase inhibitors (HDACi) have recently emerged as new potential drug targets. Several classes of small-molecule HDACi have been discovered so far, among which hydroxamic acid derivatives have received the most attention.[9–11] Suberoylanilide hydroxamic acid (SAHA) **1** is a member of the hydroxamate class of HDAC inhibitors (Scheme 1).[12–14] SAHA has

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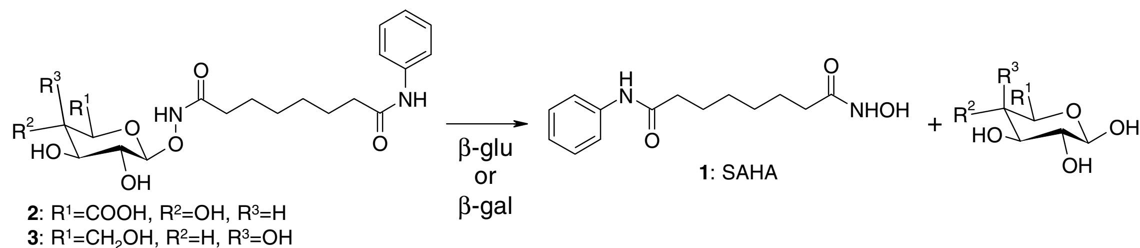


Scheme 1.

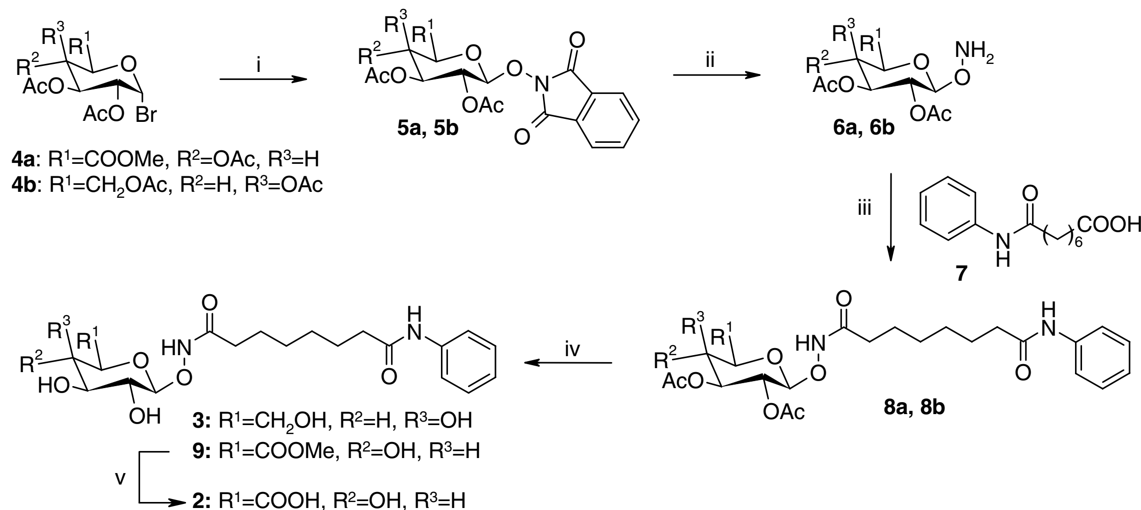
demonstrated antitumour activity in several in vivo models of cancer, including a xenograft of human prostate tumour implanted in mice,[15] a transgenic mouse model of leukaemia and a carcinogen-induced tumour model in rodents.[16] Phase I clinical trials have established that SAHA displayed antitumour activity in patients with solid tumours and haematological malignancies.[17] SAHA is currently progressing through phase III clinical trials.[18]

Although it appeared to be a promising chemotherapeutic agent, SAHA, like other hydroxamate-based inhibitors, exhibits poor pharmacodynamic properties mainly due to instability and rapid elimination (half-life ranged from 21 to 58 min).[17] As a consequence, SAHA needs daily administration to sustain its in vivo drug level. Moreover, adverse events such as anorexia, anaemia and thrombocytopenia have been reported in the course of phase I clinical trials.[17]

Thus, with the aim to enhance the therapeutic index of this promising new anticancer agent, we have undertaken the synthesis and preliminary evaluations of the two glycosyl prodrugs of SAHA **2** and **3** depicted in Scheme 1. We hypothesised that the glycosyl derivatization of the hydroxamate moiety may prevent the rapid in vivo degradation of SAHA. We also anticipated that these compounds would be less toxic than the corresponding drug since crystallographic studies demonstrated that SAHA acted by binding the zinc ion in the active site of HDAC in a bidentate fashion, through its CO and OH groups.[19–21] Furthermore, we expected that such prodrugs would be readily activated by either β-glucuronidase or β-galactosidase to release the corresponding active drug under physiological conditions.

Despite the renewal of interest for hydroxamic acids over the past decade, the development of synthetic methods leading to the corresponding *O*-glycosides has received little attention. To the best of our knowledge only one method has been reported in the literature.[22] In this study, the authors have prepared the glucuronide adduct of Trocade™, a selective inhibitor of MMP collagenase, by coupling the protected *O*-glucuronsylhydroxylamine **6a** with the corresponding carboxylic acid followed by suitable deprotection (Scheme 2). Recently, a similar strategy has been used with success by Hosokawa and co-workers for the first synthesis of Trichostatin D, the α-D-glucopyranoside of Trichostatin A.[23] Thus, in the light of these results the preparations of the requested prodrugs **2** and **3** were achieved employing the strategy depicted in Scheme 2.

The two *O*-glycosylhydroxylamines **6a** and **6b** were synthesised in two steps from the corresponding activated bromo-glycosides **4a** and **4b**. First, stereoselective gly-



Scheme 2. Reagents and conditions: (i) HONFt, TBAHS, CH_2Cl_2/Na_2CO_3 (1 M), rt, 4 h (**5a**: 60%, **5b**: 55%); (ii) N_2H_4, MeOH, rt, 5 min (**6a**: quant., **6b**: quant.); (iii) **7**, EDC, HOBt, Et_3N, CH_2Cl_2, rt, 12 h (**8a**: 73%, **8b**: 62%); (iv) MeONa/MeOH, 0 °C, 1 h then Amberlist IRC 50 (**3**: quant, **9**: 67%); (v) acetone/NaOH (1 N), −20 °C, 5 min then Amberlist IRC 50 (**2**: quant.).

cosidations were carried out in the presence of *N*-hydroxyphthalimide (HONFt) and tetrabutylammonium hydrogenosulfate (TBAHS) to afford **5a**[22] and **5b** exclusively as the β-anomers in 60% and 55% yields, respectively. Second, treatment of **5a** and **5b** with hydrazine in methanol gave *O*-glycosylhydroxylamines **6a**[22] and **6b**, both in quantitative yields. Condensations of **6a** and **6b** with the well-known carboxylic acid **7**[24] were next undertaken by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt) and triethylamine to yield the corresponding protected β-glycosides of SAHA (**8a**: 73%, **8b**: 62%). The β-galactoside **8b** was deprotected in the presence of 10 equiv of MeONa in MeOH at 0 °C to give prodrug **3** in quantitative yield.[25] Deprotection of the glucuronide **8a** was achieved using a two-step procedure. Thus, the acetyl groups were first cleaved by treatment with 1 equivalent of MeONa in MeOH and the methyl ester was subsequently saponified at −20 °C (acetone/NaOH (1 N) 5 equiv) to afford prodrug **2** (67% over 2 steps).[25]

Stability and enzymatic hydrolysis of prodrugs **2** and **3** were carried out in 0.02 M phosphate buffer (pH 7) at 37 °C and monitored by HPLC.[26] As expected, no decomposition of these two compounds was detected after 72 h under these conditions.

Enzymatic hydrolysis of **2** and **3** was conducted in the presence of an excess of the corresponding enzyme.[26] First, prodrug **2** (100 μg mL^{-1}) was incubated with *Escherichia coli* β-glucuronidase (100 U mL^{-1}). Aliquots were taken at 7 and 30 min. The release of SAHA was not observed under these conditions. Thus, further experiments were undertaken using higher concentrations of the enzyme. However, in these cases **2** was found to be perfectly stable even at a β-glucuronidase concentration of 700 U mL^{-1}. Further enzymatic assays were then carried out with prodrug **2** (100 μg mL^{-1}) in the presence of various concentrations of bovine liver β-glucuronidase (from 200 to 1000 U mL^{-1}). In the course of these experiments, **2** was again found to be stable and release of SAHA was not detected.

Prodrug **3** (430 μg mL^{-1}) was incubated for 7 min in the presence of *E. coli* β-galactosidase (40 U mL^{-1}) and the mixture was then analysed by HPLC. Total disappearance of **3** was observed together with the release of SAHA (Fig. 1).

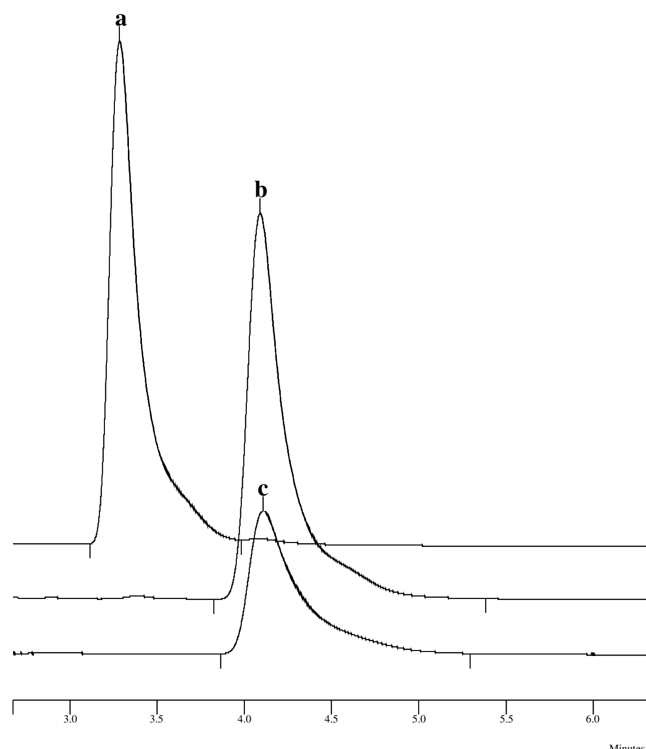


Figure 1. HPLC analysis (for HPLC conditions, see Ref. 26). (a) prodrug **3**; (b) prodrug **3** + *E. coli* β-galactosidase; (c) SAHA.

The antiproliferative activities of prodrugs **2** and **3** were evaluated after a 48-h treatment of non-small cell lung cancer H661 cells and compared to SAHA (IC_{50} = 1 μM). In the course of these experiments, both compounds **2** and **3** did not present any detectable antiproliferative activities when tested from 1 to 25 μM.

The two β-glycoside prodrugs of SAHA **2** and **3** were stable under physiological conditions and exhibited a reduced cytotoxicity compared to the parent drug against H661 cells. The prodrug **2** did not lead to the release of SAHA in the presence of a large excess of either *E. coli* or bovine liver β-glucuronidase. This result was surprising taking into account that β-glucuronidase is known to lack structural specificity for the aglycone moiety and the results obtained by Mitchell et al. who have shown that the Trocade™ glucuronide is a substrate for this enzyme.[22] Nevertheless, our result clearly demonstrated that such a glucuronide is not useful as a prodrug for β-glucuronidase-mediated PMT or ADEPT strategies. On the other hand, the β-galactoside **3** was totally and rapidly converted to SAHA in the presence of an excess of the corresponding enzyme (which is always the case in the course of an ADEPT protocol).

In summary, we have prepared the non-toxic β-glucuronide **2** and β-galactoside **3** of SAHA. The compound **2** was found to be inappropriate for both ADEPT and PMT strategies. However, it should be mentioned that this derivative may be of biological interest since SAHA-glucuronide was recently found to be one of the drug metabolites.[27] Under enzymatic cleavage of the galactosyl moiety by *E. coli* β-galactosidase, a rapid and efficient release of the drug was observed. Thus, as these data are compatible for an ADEPT strategy, the β-galactoside prodrug of SAHA **3** seems to be a good candidate for further in vivo investigations.

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25. Compound **2**: ^{1}H NMR (D_2O) δ (ppm): 1.25–1.35 (m, 4H), 1.50–1.70 (m, 4H), 2.16 (t, 2H, $J = 7.1$ Hz), 2.38 (t, 2H, $J = 7.3$ Hz), 3.40–3.60 (m, 3H), 3.70 (d, 1H, $J = 9.3$ Hz), 4.65 (d, 1H, $J = 7.8$ Hz), 7.23 (dd, 1H, $J = 8.7$ and 3.8 Hz), 7.35–7.40 (m, 4H); ^{13}C NMR (D_2O) δ (ppm): 25.0, 25.5, 28.0, 28.1, 32.7, 36.7, 71.6, 71.8, 75.7, 75.9, 105.0, 122.6, 126.0, 129.6, 137.1, 173.7, 175.9, 176.4. Compound **3**: ^{1}H NMR (CD_3OD) δ (ppm): 1.30–1.40 (m, 4H), 1.60–1.70 (m, 4H), 2.12 (m, 2H), 2.33 (t, 2H, $J = 7.3$ Hz), 3.40–3.80 (m, 6H), 4.42 (d, 1H, $J = 7.8$ Hz), 7.03 (t, 1H, $J = 8.4$ Hz), 7.25 (t, 2H, $J = 8.4$ Hz), 7.51 (d, 2H, $J = 8.4$ Hz); ^{13}C NMR (CD_3OD) δ (ppm): 25.2, 25.6, 28.6, 28.7, 32.4, 36.7, 61.4, 68.9, 69.3, 73.4, 76.1, 107.0, 120.1, 124.0, 128.6, 138.7, 172.2, 173.5.
26. HPLC conditions: Analytical HPLC was carried out using a Waters™ HPLC system with UV detection at 254 nm. The separation was performed on a reversed phase column chromatography (Discovery® RP amide 16, 150 × 4.6 mm, 5 μm) using isocratic conditions (1 mL/min), eluent CH_3CN/H_2O/TFA 3:7:0.028. Retention times for compounds **2**, **3** and SAHA, respectively, were 3.40, 3.31 and 4.15 min. *E. coli* β-glucuronidase, bovine liver β-glucuronidase and β-galactosidase were purchased from Sigma–Aldrich (reference Sigma: G2513-1KU, G0501-100KU and G7896-2KU, respectively).
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