Synthesis and β-Glucuronidase Mediated Cleavage of an Alcohol Prodrug Incorporating a Double Spacer Moiety

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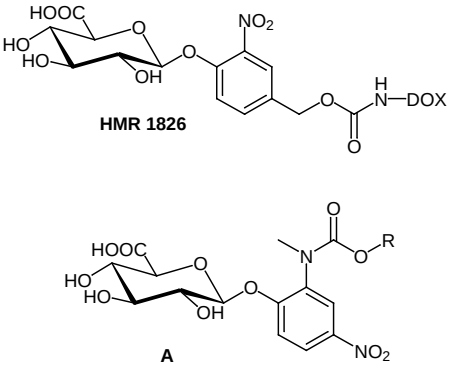
Abstract: An alcohol prodrug has been prepared by incorporation of two spacer groups between the trigger (glucuronic acid) and nitroveratryl alcohol, used as a model. Release of the latter has been observed after hydrolysis of the glycoside by β-glucuronidase. Such prodrugs may find application in ADEPT or PMT protocols in cancer chemotherapy.

Key words: prodrug, spacer, glycoside, cleavage, enzyme

A major limitation in cancer chemotherapy is the poor selectivity of cytotoxic agents for tumour cells. In order to enhance the efficiency of this therapy, the use of non-toxic prodrugs, which selectively liberate the corresponding active drugs, after enzymatic cleavage, at the tumour site in the course of ADEPT protocol have been widely studied.[1–4]

A glucuronylated prodrug of doxorubicin (DOX), HMR 1826[5] (Scheme 1), activated by a targeted fusion protein (ADEPT strategy)[6] or by β-glucuronidases present in higher concentration in necrotic areas (PMT strategy),[7] has demonstrated superior efficacy in mice and monkeys compared to standard chemotherapy.[8] This amine prodrug includes a self-immolative spacer between the drug and the enzyme substrate. The 3'-amino group of DOX is linked to the glucuronyl spacer moiety by a carbamate, which is stable in vivo. However, most known anticancer drugs do not have an amino group but one (or more) hydroxyl group (paclitaxel, etoposide…). Such a spacer can not be used to prepare alcohol prodrugs since the required plasma stability of prodrugs rules out a carbonate (instead of a carbamate) as a connecting group between the drug and the glucuronyl-spacer moiety.

Recently, phenol prodrug systems have been reported with promising results.[9–11] However, to date, only a few studies concerning glucuronide-based prodrugs of alcohols have been published.[12a,b] Monneret and co-workers designed quinine, dipyridamole and paclitaxel prodrugs (**A**) using a *N*-(-2-hydroxy-5-nitrophenyl)-*N*-methylcarbamate spacer (Scheme 1). Although in vitro preliminary tests showed the validity of this approach for the two MDR reversal agent prodrugs, the paclitaxel prodrug was poorly recognised by the enzyme due to steric hindrance.[12a] Thus, it seems worthwhile to investigate new approaches in order to prepare glucuronide prodrugs suitable to target a wide variety of alcohol-containing drugs.



Scheme 1

We report here the synthesis and preliminary studies of a model glucuronide prodrug, of type **B**, which includes two spacer groups designed for targeting hydroxy compounds (Scheme 2). In this way, the glycoside will be substantially far away from the drug in order to allow easy recognition by the enzyme. It is expected that cleavage of the first spacer should proceed as for amine prodrugs[5] to give both methylene quinone **2** (which is rapidly trapped by water to give 2-nitro-4-hydroxymethyl phenol)[5–8] and the aminocarbamate **1** which may then induce the release of a leaving group R^3OH from the aromatic carbamate by intramolecular cyclization with concomitant formation of the quinazolinone **3**.

Previous studies in our laboratory[13] have shown that *N*-methylation of the *N*-aryl carbamate ($R^1 = CH_3$, $R^2 = H$) promotes cyclisation in CH_2Cl_2 or EtOH from aminocarbamate **1**, while no cyclisation was observed with the unsubstituted compound ($R^1 = R^2 = H$).

The required *N*-methyl-2-aminobenzylamine **5** was prepared in 2 steps from anthranilonitrile (Scheme 3): *N*-methylation was carried out by the method of Bergman[14] to give **4**, which was then converted to **5**[15] by reduction with $LiAlH_4$. Compound **5** was then condensed with the known activated carbonate **6**[5] to give selectively the *mono*-carbamate **7** (DMAP, CH_3CN, r.t.). In order to introduce the model alcohol, coupling of **7** with 6-nitroveratryl chloroformate was undertaken to afford *bis*-carbamate **8**[16] (pyridine, r.t.). Finally, the glucuronic acid moiety was deprotected in two steps: first, transesterification with cat. MeONa in MeOH afforded **9**[17] after flash chromatogra-

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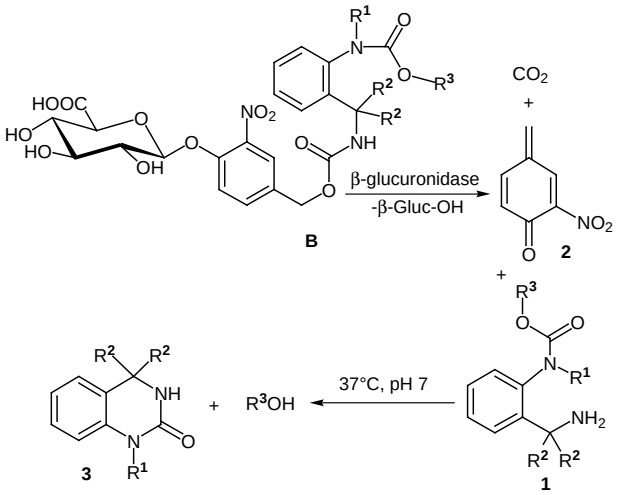

Scheme 2

phy, then saponification using $Ba(OH)_2 \cdot 8H_2O$ in MeOH gave the target prodrug **10** without further purification.

Stability and enzymatic hydrolysis of **10** were carried out at 37 °C in a 0.02 M phosphate buffer (pH = 7.2) and monitored by HPLC.[18] As expected, no decomposition of the prodrug was detected after 24 hours under these conditions. After addition of *E. Coli* β-glucuronidase (β-glucuronidase: 750 U mL^{-1}; **10**: 65 μg mL^{-1}), rapid disappearance of the prodrug was observed (t < 2 min) together with formation of two compounds. The first one was identified as 4-hydroxy-3-nitrobenzyl alcohol resulting from nucleophilic addition of water to the intermediate methylene quinone **2**.[19] The second one (**1**, $R^1 = CH_3$, $R^2 = H$, R^3 = 6-nitroveratryl alcohol) slowly disappeared, with a half-life of 18 hours, to give quinazolinone **3** ($R^1 = CH_3$, $R^2 = H$) and 6-nitroveratryl alcohol.

In conclusion, a new model for glucuronylated prodrugs including a double spacer has been proposed. Such a prodrug was easily prepared in few steps and was found to be stable under physiological conditions and easily recognised by β-glucuronidase. Clean decomposition of both spacers was observed leading to the release of the alcohol moiety. However, in contrast with cyclisation rates measured in organic / aqueous media,[14] the release of the alcohol is slower under aqueous conditions. Further studies are underway to increase the rate of drug liberation.

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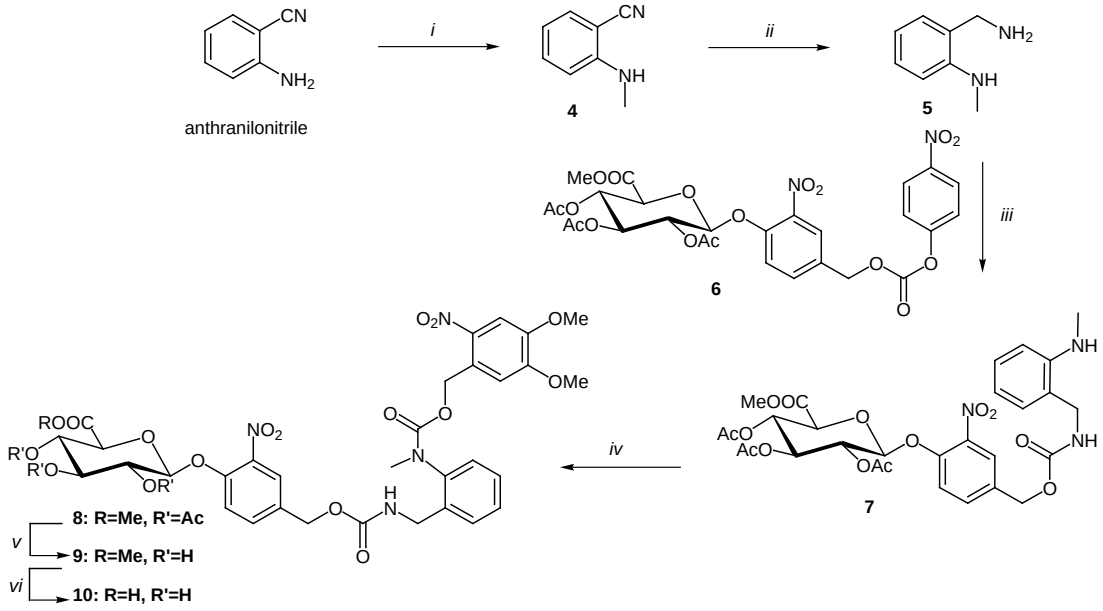

Scheme 3 *Reagents and conditions: i*, dimethyl oxalate (1.5 equiv), *t*-BuOK (1.25 equiv), DMF, reflux, 16 h, (85%); *ii*, $LiAlH_4$ (12 equiv), Et_2O, r.t., 1.5 h, (95%); *iii*, DMAP (1 equiv), CH_3CN, **6** (0.8 equiv), r.t., 1.5 h, (67%); *iv*, pyridine, 6-nitroveratryl chloroformate (2 equiv), CH_2Cl_2, r.t., 3 h, (93%); *v*, MeOH/MeONa (1 equiv), 0 °C, 3 h, (60%); *vi*, $Ba(OH)_2 \cdot 8H_2O$ (1.2 equiv), MeOH, r.t., 3.5 h, (quant.).

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(16) Preparation of **8**: 6-Nitroveratryl chloroformate (85 mg, 0.30 mmol) and pyridine (0.075 mL, 0.9 mmol) were added to a stirred solution of **7** (100 mg, 0.15 mmol) in CH_2Cl_2 (0.65 mL) at r.t. After 3 h the mixture was worked up with a saturated solution of $NaHCO_3$ and the aqueous layer was washed with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered, concentrated to dryness and the remaining yellow solid was purified by flash chromatography (petroleum ether–EtOAc 1:1) to give **8** (116 mg, 0.13 mmol, 87%). ^{1}H NMR (300 MHz, CD_3COCD_3), δ: 2.04, 2.05, 2.07 (3 × s, 9 H, CH_3COO), 3.26 (s, 3 H, CH_3N), 3.50 (s, 3 H, OCH_3), 3.70 (s, 3 H, $COOCH_3$), 3.87 (s, 6 H, OCH_3), 4.37 (d, 2 H, *J* = 6 Hz, CH_2N), 4.65 (d, 1 H, *J* = 9.5 Hz, H-5), 5.00 (s, 2 H, CH_2O), 5.18–5.69 (m, 6 H, H-1,2,3,4, CH_2O), 6.48 (s, 1 H, H_{arom}), 7.02 (s, 1 H, NH), 7.30–7.84 (m, 8 H, H_{arom}) ppm; ^{13}C NMR (75 MHz, CD_3COCD_3), δ: 37.9 (CH_3N), 41.3 (CH_2N), 53.0 ($COOCH_3$), 56.6 (OCH_3), 65.0–65.1 (CH_2O), 69.9, 71.1, 72.1, 72.8 (C-2,3,4,5), 100.0 (C-1), 121.2–149.6 (C_{arom}), 155.0–156.1 (C_{arom}), 167.7 (COOMe), 169.5, 170.0, 170.2 (CH_3CO) ppm; mp 100 °C.
(17) Data for **9**: $[\alpha_D]$ = +6,4 (c 0.95, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3COCD_3), δ: 3.51 (s, 3 H, CH_3N), 3.59–3.62 (m, 3 H, H-2,3,4), 3.71 (s, 3 H, $COOCH_3$), 3.87 (s, 6 H, OCH_3), 4.20 (d, 1 H, *J* = 9.5 Hz, H-5), 4.37 (d, 2 H, *J* = 6 Hz, CH_2N), 5.00 (s, 2 H, CH_2O), 5.33 (s, 2 H, CH_2O), 5.51 (d, 1 H, *J* = 16 Hz, H-1), 7.00 (broad s, 1 H, HNH), 7.31–7.83 (m, 9 H, H_{arom}) ppm; ^{13}C NMR (75 MHz, CD_3COCD_3), δ: 37.9 (CH_3N), 41.3 (CH_2N), 52.5 ($COOCH_3$), 56.5, 56.6 (OCH_3), 65.0, 65.1 (CH_2O), 72.3, 74.1, 76.4, 76.9 (C-2,3,4,5), 101.9 (C-1), 118.1–150.0 (C_{arom}), 155.2, 157.1 ($C_{carbamate}$), 169.5 (COOMe) ppm; mp 90 °C. The highly polar compound **10** does not lead to well resolved NMR spectra (this is observed for all glucuronylated prodrugs).
(18) HPLC conditions : C-16 reverse phase column : Discovery RP amide, 5 m (15 cm × 4.6 mm) and pre-column Discovery, 5 μm, UV detection (λ 254 nm), mobile phase (acetonitrile–0.065 M acetate ammonium solution, 30:70, 1 mL min^{-1}). Retention time: **10**: 7.8 min; 4-hydroxy-3-nitrobenzyl alcohol: 3.6 min; **3** (R^1 = CH_3, R^2 = H): 4.1 min; 6-nitroveratryl alcohol: 5.2 min; **1** (R^1 = CH_3, R^2 = H, R^3 = 6-nitroveratryl alcohol): 6.3 min.
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