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New design of cationic alkyl glycoglycerolipids toxic to tumor cells

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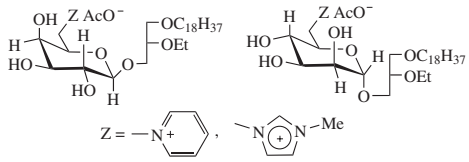
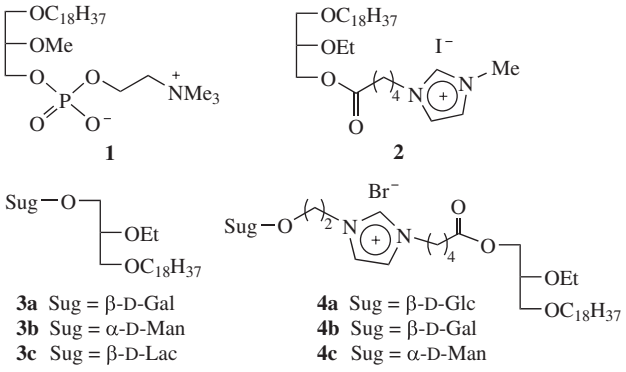
New glycosylated alkyl glycerolipids bearing a cationic group at the C^6 position of sugar moiety have been synthesized and tested as potential anticancer drugs. The compounds demonstrated cytotoxicity and selectivity against tumor cells at low micromolar concentration.



Anticancer chemotherapeutic agents typically initiate programmed cell death in an apoptosis-dependent pathway.[1,2] However, during cancer therapy the malignant cells acquire an ability to block the mechanism of self-destruction,[3,4] which results in an incomplete tumor suppression,[5] insufficient drug selectivity, mutagenic effect and the drug resistance phenomena. These disadvantages stimulated the search for chemotherapeutic agents[6,7] with an alternative mechanism of antitumor activity. In the last decade, antitumor ether lipids (AELs) and glycosylated antitumor ether lipids (GAELs) have been shown to be effective against cancer cells in an apoptosis-independent manner.[8–11] AELs and GAELs demonstrate selective toxicity to tumor cells, negligible damage to non-malignant cells and the absence of mutagenic effect.[12,13] Edelfosine **1** (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine) is a well-known prototype for AEL compounds. Despite its antitumor activity both *in vitro* and *in vivo*, edelfosine causes the rupture of red blood cells[12,14] and undergoes hydrolysis by intracellular phospholipases.[13]

To eliminate the undesired hydrolysis of lipids by phospholipases, we synthesized earlier non-phosphorus AEL-type compound **2**, in which the cationic head group was linked by a spacer to the glycerol backbone,[15,16] as well as GAEL-type compounds **3a**–**c**,[17] both types having toxicity to tumor cells comparable to that for edelfosine **1** (Table 1).[17,18] To increase the water solubility, the structure of compound **2** was further modified by additional sugar moiety linked to the cationic head group.[19] However, the resulting glycoglycerolipids **4a**–**c** did not demonstrate significant improvement in toxicity to HCT116, K562 or B16F10 tumor cell lines.[19]



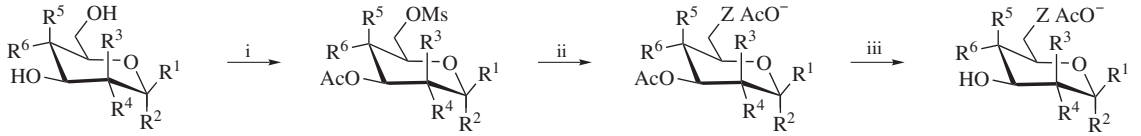
1 **2**

3a Sug = β-D-Gal
3b Sug = α-D-Man
3c Sug = β-D-Lac

4a Sug = β-D-Glc
4b Sug = β-D-Gal
4c Sug = α-D-Man

To increase antitumor activity of the compounds discussed above and to minimize their side effects, in this work we have prepared a series of new glycoglycerolipids **8a**,**b** and **9a**,**b** (Scheme 1), which can be considered as cationic modification of GAEL-type compounds **3a**,**b**[17] at the C^6 sugar position.

The GAEL-type compounds **8a**,**b** and **9a**,**b** were synthesized starting from the neutral glycoglycerolipids **3a**,**b**.[17] The introduction of cationic group at the C^6 atom of sugar moiety involved

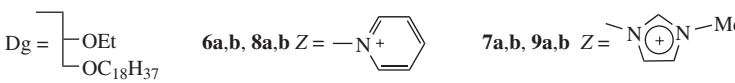


3a R^1 = ODg, R^4 = R^5 = OH, R^2 = R^3 = R^6 = H
3b R^2 = ODg, R^3 = R^6 = OH, R^1 = R^4 = R^5 = H

5a R^1 = ODg, R^4 = R^5 = OAc, R^2 = R^3 = R^6 = H
5b R^2 = ODg, R^3 = R^6 = OAc, R^1 = R^4 = R^5 = H

6a, **7a** R^1 = ODg, R^4 = R^5 = OAc, R^2 = R^3 = R^6 = H
6b, **7b** R^2 = ODg, R^3 = R^6 = OAc, R^1 = R^4 = R^5 = H

8a, **9a** R^1 = ODg, R^4 = R^5 = OH, R^2 = R^3 = R^6 = H
8b, **9b** R^2 = ODg, R^3 = R^6 = OH, R^1 = R^4 = R^5 = H



Scheme 1 *Reagents and conditions*: i, MsCl, pyridine, –15 °C, 2 days, then Ac_2O, 20 °C, 1 day; ii, pyridine, 115 °C, 1–3 days; or *N*-methylimidazole, MeCOEt, 80 °C, 2–3 days; then chromatography (silica gel, $CHCl_3$–MeOH–1% aq. AcOH, 40:10:1 v/v); iii, MeONa, MeOH, CH_2Cl_2, 24 °C, 1 h; then chromatography (silica gel, $CHCl_3$–MeOH–1% aq. AcOH, 20:10:1 v/v).

selective mesylation of starting compounds followed by peracetylation (see Scheme 1), quaternization of pyridine or *N*-methylimidazole with these acetylated mesylates and finally complete deacetylation.

Selective mesylation of glycoglycerolipids **3a**,**b** at the primary OH group of the carbohydrate moiety was performed with an equimolar amount of MsCl in pyridine at –15 °C.[20] Then *in situ* peracetylation with an excess of acetic anhydride at 20 °C was performed. After the chromatographic purification on silica gel the yields of protected glycosyl diglycerides **5a** and **5b** were 46 and 93%, respectively. The structures of new compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry. In ^{1}H NMR spectra of compounds **5a**,**b** the signals from acetyl groups with chemical shifts 2.0–2.2 ppm and signals from mesyl groups (two singlets for the two diastereoisomers due to the stereocenter in glycerol moiety) with the chemical shift of ~3.0 ppm were detected (see Online Supplementary Materials). The mass spectrometric data of compounds **5a**,**b** agreed well with calculated mass values for the corresponding molecular ions.

Compounds **6a**,**b** and **7a**,**b** were obtained by quaternization of pyridine with no additional solvent or *N*-methylimidazole in methyl ethyl ketone, respectively, by mesylates **5a**,**b** under reflux. After column chromatography on silica gel, onium glycoglycerolipids **6a**,**b** and **7a**,**b** were isolated in 34–96% yields. The low yield of compounds **6a** and **7a** could be explained by steric hindrance at C^4 and C^6 atoms of the galactose ring.

As compared with the ^{1}H NMR spectra of the starting glycoglycerolipids **5a**,**b**, the spectra of compounds **6a**,**b** demonstrate additional signals in the weak field at 8.0–9.2 ppm, corresponding to the pyridine moiety. Analogously, the spectra of compounds **7a**,**b** exhibit signals at 7.3–7.6 ppm due to *N*-methylimidazole ring and a singlet at 3.9–4.0 ppm from *N*-methyl group (see Online Supplementary Materials).

The final compounds **8a**,**b** and **9a**,**b** were obtained by deacetylation of the quaternization products **6a**,**b** and **7a**,**b**, respectively, using sodium methylate in methanol–dichloromethane. Glycoglycerolipids **8a**,**b** and **9a**,**b** were isolated by column chromatography in 54–85% yields.

The cytotoxicity of newly synthesized compounds **8a**,**b** and **9a**,**b** and the reference lipids **1**–**3a**,**b** was evaluated using cancer cell lines of different origin, namely human chronic myelogenous leukemia K562, human promyelocytic leukemia HL60, mouse melanoma B16 and human colon carcinoma HCT116 (see Table 1). The effect of galactose-containing compound **8a** with a pyridinium head group on HL60, B16 and HCT116 cells was higher than the effect of non-cationic analogue **3a** and higher or comparable with that for reference compounds **1** and **2**. Mannose-containing compound **8b** with a pyridinium head group appeared to be less active than compound **3b** against K562 cells, more active than **3b** against HL60 and HCT116 cells, and had comparable activity for B16 cells. Lipid **8b** was less active than edelfosine **1** against B16 and HCT116 cells but had similar activity to edelfosine for HL60 cells and was more active against K562 cells. The cytotoxic effect of compounds **9a**,**b** bearing *N*-methylimidazole head group was in general lower than that of compounds **8a**,**b** with pyridinium head group. The selectivity indices in relation to human postnatal fibroblasts ($SI = IC_{50}^{\text{non-cancer cells}}/IC_{50}^{\text{cancer cells}}$)[21] for compound **8a** were 11.8 and 2.8 for HCT116 and HL60 cell lines, respectively.

Table 1 Cytotoxicity of non-phosphorus glycerolipids *vs.* edelfosine against K562, HL60, B16 and HCT116 cell lines.

Compound	Sugar moiety	IC_{50}[a]/μmol dm^{-3}			
		K562	HL60	B16	HCT116
Edelfosine **1**	–	>50	3.2±0.2	6.0±0.7	0.8±0.1
2[b]	–	16.5±0.5	3.8±0.6	14.1±0.5	10.8±0.3
3a	β-D-Gal	2.5±0.9[b]	11.3±0.3[c]	13.5±0.8[b]	15.9±0.2[c]
8a	β-D-Gal	5.0±0.4	2.1±0.5	8.1±0.7	0.5±0.2
9a	β-D-Gal	11.9±0.3	4.0±0.6	12.8±0.2	4.0±0.5
3b[b]	α-D-Man	3.0±0.9	16.0±0.7	12.0±0.7	15.0±0.4
8b	α-D-Man	11.0±0.4	3.1±0.4	11.2±0.7	3.0±0.6
9b	α-D-Man	10.1±0.6	40.2±0.6	37.3±0.6	13.3±0.6

[a] Concentration that caused a decrease of cell viability by 50%. Values are mean ± SD of three independent measurements. [b] Known data for compounds **2**[18] and **3a**,**b**.[17] [c] Unpublished data for compound **3a**.

Table 2 Hemolytic effect of compounds **8a**, **9a** and edelfosine **1**.

Compound	Concentration/μmol dm^{-3}	Hemolysis[a] (%)
8a	5	0.6
	10	1.3
	20	1.7
9a	5	0.5
	10	1.4
	20	1.8
Edelfosine **1**	2	6
	4	14
	8	19

[a] Hemolysis caused by resuspension of erythrocytes in pure water was taken as 100%.

Finally, the ability of cationic glycoglycerolipids **8a** and **9a** to damage human red blood cells *in vitro* was tested (Table 2). Compounds **8a** and **9a** had negligible effect on the integrity of erythrocytes, similar to the reference compound **3a** (2.9% hemolysis),[17] whereas edelfosine **1** was found to have hemolytic effect.

In summary, the new galactose-containing cationic alkyl glycoglycerolipid **8a** bearing a pyridinium head group may be proposed as a promising candidate for the cancer therapy due to its high cytotoxicity and selectivity to several cancer cell lines along with negligible hemolytic effect.

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Online Supplementary Materials

Supplementary data associated with this article (general synthetic procedures, characterization of compounds and details for cytotoxicity and hemolytic activity assays) can be found in the online version at doi: 10.1016/j.mencom.2019.03.016.

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