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Design, synthesis and antitumor properties of glycosylated antitumor ether lipid (GAEL)- chlorambucil-hybrids

Temilolu Idowu[a], Pranati Samadder[b], Gilbert Arthur[b,*], Frank Schweizer[a,**]

[a] Department of Chemistry, University of Manitoba, 144 Dysart Rd, Winnipeg MB, R3T 2N2, Canada
[b] Department of Biochemistry and Medical Genetics, University of Manitoba, 745 Bannatyne Avenue, Winnipeg MB, R3E 0J9, Canada

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ABSTRACT

Glycosylated antitumor ether lipids (GAELs) kill cancer cells and cancer stem cells via a novel, apoptosis-independent mechanism. In contrast, chlorambucil, a drug in clinical use for the treatment of chronic lymphocytic leukemia reacts with nucleophiles within the major groove of DNA, leading to apoptosis. We hypothesized that hybrid molecules that combine apoptosis-dependent and apoptosis-independent mode of actions in a single molecule may lead to enhanced antitumor activity. Here, we describe the antitumor activities of chlorambucil-linked glucosamine-derived glycerolipid hybrids and investigate the role of the chlorambucil moiety and the effect of cationic charge on the hybrid molecule. Three hybrids and two control GAELs were synthesized and their activities against breast (JIMT1, MDA-MB-231, BT474), pancreas (MiaPaCa2) and prostate (DU145, PC3) cancer cell lines were determined using MTS assay. Hybrid **3** displayed the most potent activity on DU145 at CC_{50} of 6.0 μM while hybrid **4** displayed the best activity on JIMT1 at 7.5 μM. Hybrid **5** exhibited no activity at the highest concentration tested (CC_{50} >20 μM), underscoring the significance of the cationic charge at *C*-2 position as previously reported. Although chlorambucil **(2)** itself showed very little activity against all the six cell lines (CC_{50} >150 μM), GAELs **6** and **7** which lack the chlorambucil moiety were consistently less active than **3** and **4**, suggesting that the chlorambucil moiety contributes to the overall activity. The hybrids were however not as active as the parent GAEL **1** against MiaPaCa2 whereas **6** restored activity comparable to **1**.

1. Introduction

Cancer is a class of disease in which a group of cells display uncontrolled growth, invasion and metastasis. The magnitude of threat posed by this simple but yet sophisticated disease is quite alarming and it remains a leading cause of death worldwide. The number of new cases, which currently stands at about 14 million, is expected to rise by 70% over the next two decades (WHO, 2014). More frightening is the rate and manner with which cancer cells develop resistance to clinically used chemotherapeutics (Gottesman, 2002), thereby presenting a daunting task of effectively managing this disease. The major classes of anticancer drugs (antimetabolites, anthracyclines, taxanes and alkylating agents), which are mostly pro-apoptotic, act by disrupting cellular DNA, preventing DNA synthesis and/or targeting microtubules (MacFarlane, 2009). Cancer cells often have increased expression of multidrug resistant (MDR) proteins, P-glycoproteins of the ATP-binding cassette transporter which actively extrude drugs from the cells (Gottesman et al., 2002; Longley and Johnston, 2005). Furthermore, some cells may harbor mutations in the apoptotic pathways that enable them to escape spontaneous or therapy-induced apoptosis thereby developing resistance to the very pathways activated by chemotherapeutic agents (Hanahan and Weinberg, 2011; Wong and Goodin, 2009; Soengas et al., 2001; Deming et al., 2004). It therefore becomes imperative to develop anticancer agents whose mechanistic pathways do not rely solely on apoptosis. Professor Robert Bittman's research in the area of antitumor ether lipids, AELs (Fig. 1), eventually led to the discovery of anticancer compounds that kill cells by an apoptosis-independent mechanism. Novel analogs of these compounds are the subject of the research presented herein.

AELs represent a group of small molecular weight compounds with antiproliferative and cytotoxic activities against a wide range of cancer cell lines. The prototypic compounds, the alkyllysophospholipids (ALPs), are structurally similar to naturally occurring lysophosphatidylcholine with the replacement of the ester bond at the *C*-1 position of the glycerol backbone with an ether bond and a

* Corresponding author. Fax: +1 204 789 3900.
** Corresponding author. Fax: +1 204 474 7608.
E-mail addresses: gilbert.arthur@umanitoba.ca (G. Arthur), schweize@cc.umanitoba.ca (F. Schweizer).

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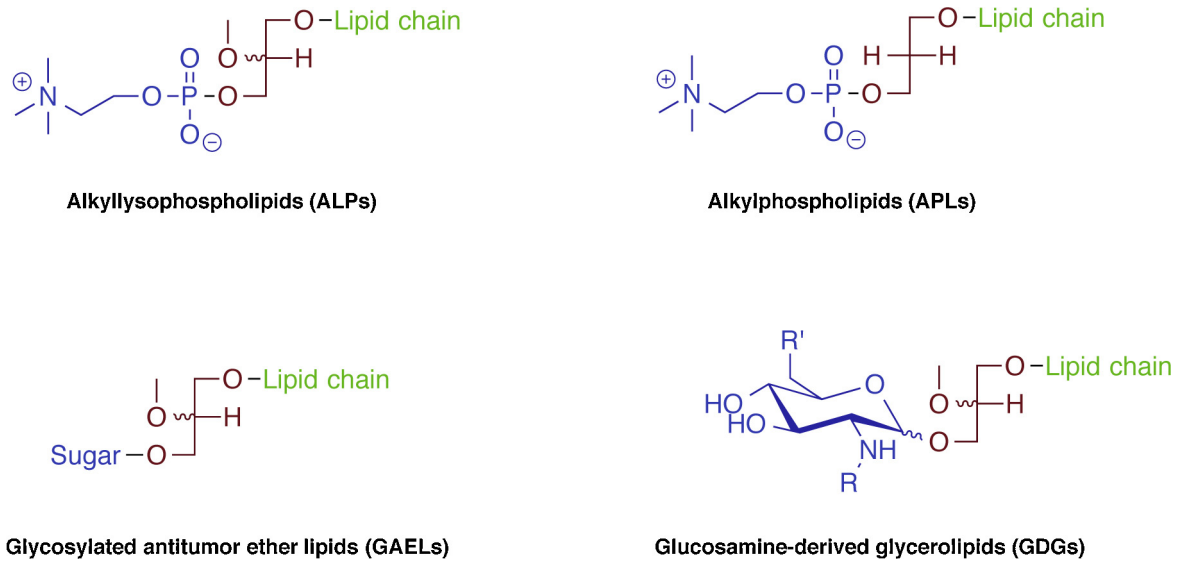

Fig. 1. Generic structures of subclasses of antitumor ether lipids (AELs).

short chain ether moiety at the *C*-2 position to prevent acylation (Fig. 1). Work by Bittman and associates led to the development of several ALP analogs including compounds with phosphonocholine bonds at the *C*-3 position to minimize phospholipase *C* hydrolysis of the compound, thereby creating more metabolic-resistant and long-lived analogs (Salari et al., 1992; Bittman et al., 1993, 1994, 1997; Samadder et al., 2004). The ALP class of AELs were shown to kill cells by inducing apoptosis (Smets et al., 1999; Gajate et al., 1998; Jackson et al., 1998; Mollinedo et al., 2004). AEL analogs with a monosaccharide replacing the phosphocholine found in ALPs, the glycosylated AELs (GAELs), were developed along with the ALPs, but they showed weak antiproliferative and cytotoxic activity relative to the ALPs (Fig. 1). Professor Bittman's initial foray into the GAELs field involved the synthesis of thio-analogs to enhance lipophilicity, and synthesis of alpha- and beta-anomeric compounds to study the effect of the anomeric position on activity (Guivisdalsky et al., 1990). The activities of the resulting compounds were however not significantly different from the existing compounds (Guivisdalsky et al., 1990). In 1996, Professor Bittman and associates synthesized a GAEL analog with an amino group at the *C*-2 position of glucose, 1-*O*-hexadecyl-2-*O*-methyl-3-*O*-(2′-amino-2′-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (glucosamine-derived glycerolipid (GDG) **1**, Fig. 2). Assessment of the cytotoxic effects of **1** against human epithelial cell lines revealed that the compound's activity was comparable to the activity of the other AEL classes (Erukulla et al., 1996). This simple modification of

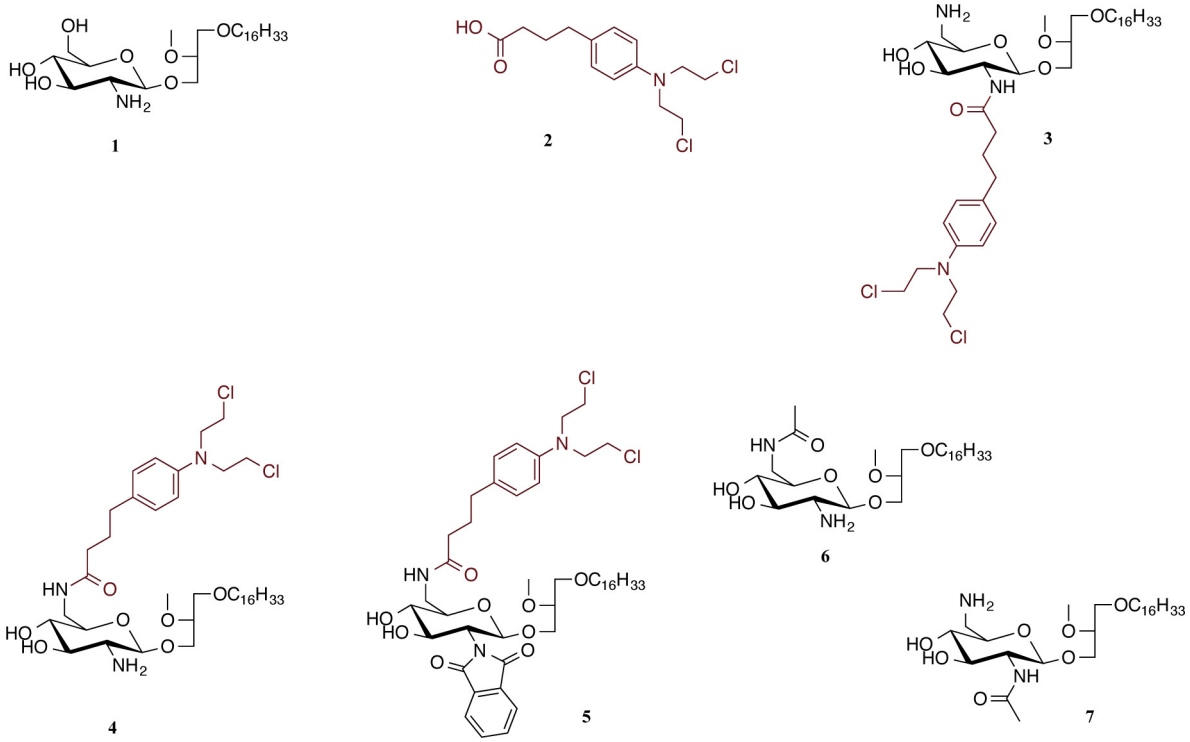

Fig. 2. Structures of GAEL-chlorambucil hybrids and corresponding reference compounds used in this study.