(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 July 2009 (30.07.2009)

PCT

(10) International Publication Number
WO 2009/092170 A1

(51) International Patent Classification:
A61K 31/7028 (2006.01) *A61K 31/7008* (2006.01)
A61K 31/70 (2006.01) *A61K 31/7016* (2006.01)
A61K 31/7004 (2006.01)

(21) International Application Number:
PCT/CA2009/000083

(22) International Filing Date: 23 January 2009 (23.01.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/023,224 24 January 2008 (24.01.2008) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

WO 2009/092170 A1

(54) **Title:** THE USE OF GLYCOSYLATED ANTITUMOR ETHER LIPIDS TO INDUCE AND/OR ENHANCE AUTOPHAGY FOR TREATMENT OF DISEASES

(57) **Abstract:** The present invention is directed to the use of glycosylated antitumor ether lipids (GAEL) of fomulas (I) - (VII) in the treatment of disorders and diseases associated with autophagy *via* the rapid induction or enhancement of autophagy in cells. Said disorders and diseases include protein aggregation diseases, neurodegenerative diseases, Parkinson's disease, Huntington's disease, spinocerebellar ataxia, dementia, ischemic/reperfusion injury, muscular diseases, and cancer..

THE USE OF GLYCOSYLATED ANTITUMOR ETHER LIPIDS TO INDUCE AND/OR ENHANCE AUTOPHAGY FOR TREATMENT OF DISEASES

PRIOR APPLICATION INFORMATION

This application claims the benefit of US Provisional Patent Application 61/023,224, filed January 24, 2008.

BACKGROUND OF THE INVENTION

Autophagy is a normal physiological process and is one of the major degradative routes in cells (*Klionsky, DJ and Emr, SD (2000), Autophagy as a regulated pathway of cellular degradation. Science 290, 1717-17201*). Autophagy degrades proteins, protein complexes, and organelles along with other cytoplasmic constituents and it supplies nutrients to cells during starvation conditions. Three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy have been identified (*Cuervo, AM (2004) Autophagy: Many paths to the same end. Mol Cell Biochem 263, 55-72*). They all ultimately shepherd molecules destined for degradation to the lysosomes. Chaperone-mediated autophagy selectively targets proteins in the cytosol that associate with the chaperone protein Hsc73 and moves them directly across the lysosomal membrane to the lumen for digestion. In microautophagy, invaginations of the lysosomal membranes results in cytoplasmic material being taken into the lysosomal lumen. The bulk of autophagy occurring in cells is macroautophagy, which is the subject of this invention. In macroautophagy, an initiating membrane called the phagophore is formed in the cytosol which grows and encompasses cytoplasmic material. The molecular mechanism and regulation of autophagy have not been elucidated but several reviews have summarized the current state of knowledge on various aspects of this process (*Levine, B and Yuan, J, 2005, Autophagy in cell death: an innocent convict? J Clin Invest 115, 2679-2688; Luo, S and Rubinsztein, DC, 2007, ATG5 and BCl2 provide novel insights into the interplay between apoptosis and autophagy, Cell Death and Diff 14, 1247-1250; Xie, Z and Klionsky, DJ, 2007, Autophagosome formation: core machinery and adaptations, Nature Cell Biology 9, 1102-1109; Pattingre, S, Espert, L, Biard-Piechaczyk, M and Codogno, P 2007, Regulation of macroautophagy by mToR and Beclin 1 complexes, Biochimie (in press)*).

A number of specific proteins produced from autophagy-related (ATG) genes are intimately involved in the various events that constitute autophagy. One of the key events appears to be the conjugation of phosphatidylethanolamine to the protein LC3 (ATG8) to form LC3-II (2). The formation of LC3-II is the accepted marker for autophagy (*Mizushima, N, 2004, Methods for monitoring autophagy. Int J Biochem Cell Biol 36, 2491-2502*). Indeed, LC3 is the only ATG protein currently known to be incorporated into the autophagasome. Consequently LC3-II is used as a definitive marker for autophagosomes. Subsequent to its formation, the autophagosome fuses with the lysosomes to form the autophagolysosome and the constituents are digested.

Autophagy is boosted in response to a variety of stimuli such as nutrient starvation, oxidative stress, and hypoxia. Pathways regulating autophagy are not well defined but currently the best known modulator of autophagy is the mTor pathway (*Rubinsztein. DC, Gestwicki, JE, Murphy, LO , Klionsky, DJ (2007) Potential therapeutic applications of autophagy. Nature Reviews Drug Discovery 6, 304-312; Pattingre, S, Espert, L, Biard-Piechaczyk, M, Codogno, P (2007), Regulation of macroautophagy by mToR and Beclin 1 complexes, Biochimie (in press)*). Activation of mTor inhibits autophagy while its inhibition promotes autophagy. Consequently, the class I phosphoinositide 3-kinase (PI3K) which positively regulates mTor activity inhibits autophagy. The class III PI3K, Vps34, positively regulates autophagy through its product phosphatidylinositol 3-phosphate (PI3-P). The role this molecule plays in autophagy is unknown but it is likely to be at the initiation stage (*Cuervo, AM (2004) Autophagy: Many paths to the same end. Mol Cell Biochem 263, 55-72*). Inhibition of the enzyme by 3-methyladenine (3-MA) inhibits autophagy (*Stroikin Y, Dalen H, Loof S and Terman A (2004) Inhibition of autophagy with 3-methyladenine results in impaired turnover of lysosomes and accumulation of lipofucsin-like material . E J Cell Biol 83, 583-5906*). Other pharmacological modulators of autophagy include rapamycin, which inhibits mTor activity and thereby activates autophagy (*Noda, T and Ohsumi, Y (1998) Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. J. Biol Chem 273, 3963-39667*); and bafilomycin, which inhibits autophagy by preventing the fusion of the autophagosome with the lysosome (*Yamamoto, A, Tagawa, Y, Yoshimori, T, Moriyama, Y, Masaki, R, Tashiro, Y (1998) Bafilomycin*

A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line H-4-II-E cells Cell Struct Funct 23, 33-42).

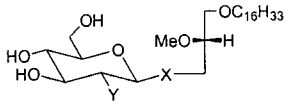
The potential to treat a number of diseases by inducing autophagy is increasingly being recognized. Dysregulation of autophagy, the mechanism via which toxic molecules including the aggregation-prone proteins found in neuronal cells are cleared from the cell, has been implicated in the development of some diseases. The beneficial and harmful effects of autophagy in various diseases have been reviewed (*Rubinsztein, DC et al. 2007, Potential therapeutic applications of autophagy, Nature Reviews, Drug Discovery 6, 304-312; Hippert, MM, O'Toole, PS and Thorburn, A, 2006, Autophagy in cancer; good, bad or both, Cancer Res 66, 9349-9351; Nixon, RA, 2006, Autophagy in neurodegenerative disease: friend, foe or turncoat? Trends Neurosci 29, 528-535*). The list includes muscle and liver diseases, neurodegenerative diseases such as Parkinson's disease, Huntington's disease, spinocerebellar ataxia type 3, and certain forms of dementia involving tau mutations. It may also be relevant in heart diseases (*Terman, A & Brunk, UT (2005) Autophagy in cardiac myocyte homeostasis aging and pathology. Cardiovasc Res 68, 355-365*). Autophagy diminishes in the aging heart (15), the reasons for which are unclear, but may play a role in cellular damage in the aging heart. In this instance the existence of drugs that boost autophagy could be of benefit in reducing the buildup of the toxic agents. The potential role of autophagy in protecting against ischemia/reperfusion injury in the heart was recently reported (*Hamacher-Brady, A, Brady, NR, Gottlieb, RA (2006) Enhancing macroautophagy protects against ischemia/reperfusion injury in cardiac myocytes J. Biol Chem 281. 29776-29787; Hamacher-Brady, A, Brady NR, Gottlieb, RA, Gustafsson, AB (2006) Autophagy as a protective response to Bnip3-mediated apoptotic signaling in the heart. Autophagy 2, 307-309.*). Enhanced autophagy may also be a strategy to kill cancer cells that are resistant to apoptosis.

Very few pharmacological agents that induce autophagy are currently known. Under conditions where autophagy is diminished or dysregulated, pharmacological intervention to boost the process could have tremendous effects on treatment. The two main pharmacological means of activating autophagy are

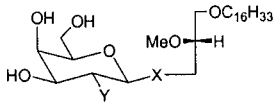
with rapamycin and lithium (*Sarkar, S, Floto, RA Berger, Z, Imaarisio, S, Cordenier, A, Pasco, M, Cook, LJ, Rubinsztein, DC (2005) Lithium induces autophagy by inhibiting inositol monophosphate J Cell Biol 170, 1101-111; Sarkar, S, Rubinsztein, DC 2006). Inositol and IP3 levels regulate autophagy Autophagy 2: 132-134*) . Lithium requires prolonged incubation for its effects, and rapamycin has a myriad of effects including immunosuppressive activities, discounting its use as a therapeutic agent to modulate autophagy. The need to develop rapid acting agents to modulate autophagy has been recognized. Recently new compounds, small-molecule enhancers of rapamycin (SMERS), have been reported that induce autophagy (*Sarkar, S , Perlstein, EO, Pineau, S, Cordenier, A et al. (2007). Small molecules enhance autophagy and reduce toxicity in Huntington disease models. Nat Chem Biol 3, 331-338*).

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a method of inducing autophagy in a cell or population of cells comprising administering an effective amount of a compound selected from the group consisting of:



I X = O, CH_2, or NH
Y = NH_2, H, or OH



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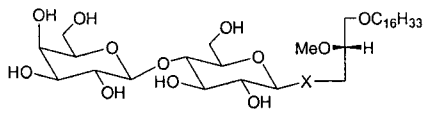

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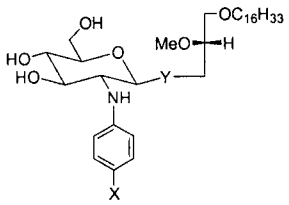
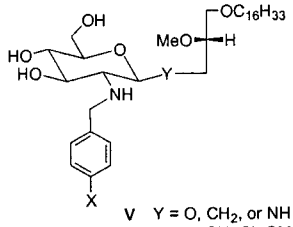


IV Y = O, CH_2, or NH
X = H, CN, Cl, OMe, or NMe_2



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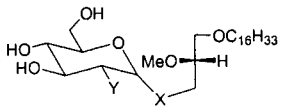
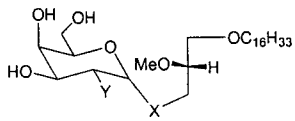


VI X = O, CH_2, or NH
Y = NH_2, H, or OH



VII X = O, CH_2, or NH
Y = NH_2, H, or OH

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Autophagolysomes in cells incubated with Gln (compound **I**, X = O, Y = NH_2). Proliferating cells were incubated with or without 7.5 μM of **Gln** for 6 h (ASK1, ATG5 Wt, ATG5 -/-,) or 24 h (BT549).

Figure 2: Generation of autophagy marker LC3-II by Gln (compound **I**, X = O, Y = NH_2) in ASK1 -/-, Caspase 3 -/-, and wild type MEF, A549 and BT549 cells. Proliferating cells were incubated with 7.5 μM Gln for 6 h (ASK1 -/-, Caspase 3 -/- WT Mef) or 24 h (A549, BT549). The cells were washed and harvested and cell lysates were prepared in the presence of protease inhibitors. Samples were run on SDS PAGE gels and subjected to Western Blot analysis with LC3 antibody.

Figure 3: Time course of generation of autophagy marker LC3-II by Gln (compound **I**, X = O, Y = NH_2) in caspase 3 -/- cells and wild type MEF. Proliferating cells were incubated with 7.5 μM Gln for various times. At the end of the incubation, the cells were washed and harvested and cell lysates were prepared in the presence of protease inhibitors. Samples were run on SDS PAGE gels and subjected to Western Blot analysis with LC3 antibody.

Figure 4: Effect of bafilomycin on Gln-induced LC3-II generation in ASK1 -/- . ATG5 WT, and ATG5 -/- cells. Proliferating cells were incubated with or without 7.5 μM or 10 μM Gln (compound **I**, X = O, Y = NH_2) for 6 h in the presence or absence of bafilomycin (200 nM). At the end of the incubation, the cells were washed and harvested and cell lysates were prepared in the presence of protease inhibitors. Samples were run on SDS PAGE gels and subjected to Western Blot analysis with LC3 antibody.

Figure 5: Effect of 3-methyladenine and wortmannin on Gln-induced LC3-II generation in ATG5 WT cells. Proliferating cells were incubated with or without 7.5 μM Gln (compound **I**, X = O, Y = NH_2) for 6 h in the presence or absence of 3-MA (10 mM) or wortmannin (0.1 μM). In experiments with 3-MA, the cells were preincubated with 3-MA for 12 h prior to the addition of 3-MA + Gln. At the end of the incubation, the cells were washed and harvested and cell lysates were prepared in the presence of protease inhibitors. Samples were run on SDS PAGE gels and subjected to Western Blot analysis with LC3 antibody.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

We developed compounds known as glycosylated antitumor ether lipids, which have with a sugar residue in place of the phosphorylcholine head group at the C-3 position of the lipid (*Guivisdalsky, PN, Bittman, R, Smith, Z, Blank, ML, Snyder, F, Howard, S, Salari, H.(1990). Synthesis and antineoplastic properties of ether-linked thioglycolipids. J Med Chem 33, 2614-2621; Lu, X, Rengan, K, Bittman, R, Arthur, G (1994). Synthesis and antineoplastic properties of ether-linked thioglycolipids. J Med Chem 33, 2614-2621; Lu, X, Rengan, K, Bittman, R, Arthur, G (1994) The α and β anomers of 1-O-hexadecyl-2-O-methyl-3-S-thioglucosyl-sn-glycerol inhibit the proliferation of epithelial cancer cell lines. Oncol Rep 1, 933-936; Erukulla, RK, Zhou, X, Samadder, P,. Arthur, G, Bittman, R (1996). Synthesis and evaluation of the antiproliferative effects of 1-O-hexadecyl-2-O-methyl-3-O-(2'-acetamido-2'-deoxy- β -D-glucopyranosyl)-sn-glycerol and 1-O-hexadecyl-2-O-methyl-3-O-(2'-amino-2'deoxy- β -D-glucopyranosyl)-sn-glycerol on epithelial cancer cell growth. J Med Chem 39, 1541-1548; Samadder, P, Byun, H-S, Bittman, R, Arthur, G, "Glycosylated antitumor ether lipids are more effective against oncogene-transformed fibroblasts than choline-containing alkyl-lysophospholipids," Anticancer Res. 18, 465-470 (1998); Yang, G, Franck, RW, Byun, H-S, Bittman, R, Samadder, P, Arthur, G (1999), "Convergent C-glycolipid synthesis via the Ramberg-Bäcklund reaction: Active antiproliferative glycolipids," Org. Lett. 1, 2149-2151; Yang, G, Franck, RW, Bittman, R, Samadder, P, Arthur, G (2001), "Synthesis and growth-inhibitory properties of glucosamine-derived glycerolipids," Org. Lett. 3, 197-2001).*

The most active compounds are lipids bearing a glucosylamine (Gln) and its *C*-glycoside analog, both of which have an amino group at position 2 of the glucose moiety. The characteristics displayed by these glycosylated ether

compounds are significantly different from the prototype antitumor ether lipid ET-18-OCH_3, which suggested a different mechanism of action.

We report in this invention that glycosylated antitumor ether lipids (GAEL) are small molecules that induce and/or enhance autophagy in cells. A major advantage of these compounds is the rapid manner with which they induce autophagy. In addition the reversible nature of their effects will be advantageous in that induction of autophagy would not necessarily be an event that culminates in cell death. The specific use of a synthetic lipid analog to enhance or induce autophagy to treat diseases or disorders such as neurodegenerative diseases like Parkinson's and Huntington's diseases is a novel concept.

The ability of GAEL to induce autophagy is clearly dependent on the presence of the sugar moiety at the C3 position of the glycerol backbone since ET-18-OCH_3, the prototype AEL with a phosphocholine group at the same position, does not induce autophagy. The molecular mechanism of autophagy is not well understood but initiation and nucleation in starvation-induced autophagy is dependent on the activity of 2 complexes, the Beclin 1 complex and the mTORC1 complex. The individual components of the Beclin 1 complex are comprised of Beclin 1, Vps34, Vps15, and UVRAG. Upon activation, the complex causes the production of phosphatidylinositol 3-phosphate (PI3P) by Vps34. PI3P then associates with WIPI-1 to initiate nucleation. The Beclin 1 complex also associates with the anti-apoptotic molecule Bcl2. The association of Bcl2 with Beclin 1 inhibits the complex whereas its dissociation relieves the inhibition. GAELs may induce autophagy through multiple mechanisms. The compounds could inhibit the association of Bcl2 with Beclin and thereby activate the complex to generate PI3P to initiate autophagy. Our observation that wortmannin and 3 methyladenine, which inhibit the production of PI3P but yet have no effect on GAEL-induce autophagy, indicates that the compounds can activate autophagy independently of Vps34. In this instance it is envisaged that GAELs bind to the PI3P-binding site on WIPI-1 and activate the molecule to initiate autophagy. Alternatively, GAEL could activate class II PI3K's, to produce PI3P, activate phosphatases to remove D4 phosphate from PI(3,4) P2, or inhibit phosphatases that remove D3 phosphate from PI3P.

mTOR is a protein kinase that has been identified as a key player in regulating the initiation of starvation-induced autophagy. Under nutrient sufficient conditions, mTOR is active, leading to the phosphorylation of ATG13, a component of the ATG1 kinase complex. Phosphorylation of ATG13 causes it to dissociate from the complex and thereby inhibits the catalytic activity, leading to the inhibition of autophagy. Under starvation conditions, mTOR is inactive and the hypophosphorylation of ATG17 allows it to associate with the ATG1 complex and results in activation of ATG1. Our studies suggest that GAEL initiates autophagy independently of mTOR inhibition. Thus GAEL may initiate autophagy through direct or mTOR-independent activation of ATG1 kinase.

GAEL induces autophagy in ATG5-deficient cells. The current theories of autophagy have identified ATG5 as a key molecule essential for vehicle expansion and completion. In its absence, cells do not convert LC3-I to LC3-II and there is no insertion of LC3-II into the autophagosome. The ability of GAEL to induce autophagy in cells deficient in ATG5 implies that there may be additional pathways for the expansion of the preautophagosome. Furthermore, the conversion of LC3-1 to LC3-II and insertion of the latter may not be absolutely required for Gln-induced autophagosome formation. Thus Gln-induced expansion of autophagosomes under certain conditions may involve novel pathways whose components have yet to be identified.

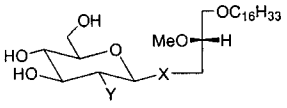
This invention deals with the methods of use of compounds having a formula selected from the group consisting of *O*-glycosylated and *C*-glycosylated antitumor ether lipids that are useful for inducing or enhancing autophagy for the treatment of disorders or diseases amenable to enhanced autophagy such as protein aggregation diseases, neurodegenerative diseases, Parkinson's disease, Huntington's disease, spinocerebellar ataxia type 3, and dementia involving tau mutations. Other uses include, but are not limited to, protection and/or amelioration of ischemic/reperfusion injury and liver and muscular diseases.

Thus, according to one aspect of the invention, there is provided a method for treating or ameliorating or treating prophylacticly a disease or disorder amenable to enhanced autophagy comprising administering to an individual in need of such treatment, for example, an individual suffering from a protein aggregation disease, a neurodegenerative disease, Parkinson's disease,

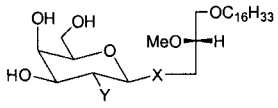
Huntington's disease, spinocerebellar ataxia type 3, dementia involving tau mutations, an ischemic/reperfusion injury or a liver or muscular disease known in the art an effective amount of one of more of the compounds described below, for example, one of compounds I-VII. As will be appreciated by one of skill in the art, an effective amount of any one of the compounds can be determined by routine experimentation if necessary and will depend of course on many factors, for example but by no means limited to age, weight, overall condition, disease progression and/or severity of symptoms of the individual.

In other embodiments, there is provided a method of preparing a pharmaceutical composition for treating or preventing or ameliorating or treating prophylacticly a disease or disorder selected from the group consiting of: protein aggregation diseases, neurodegenerative diseases, Parkinson's disease, Huntington's disease, spinocerebellar ataxia type 3, dementia involving tau mutations, ischemic/reperfusion injury and liver and muscular diseases comprising providing a compound of Formula I-VII and mixing said compound with a pharmaceutically acceptable excipient or carrier.

In one embodiment, the compounds for use in the compositions and methods provided herein have Formula I or are a compound of any one of Formulae I-VII:



I X = O, CH_2, or NH
Y = NH_2, H, or OH



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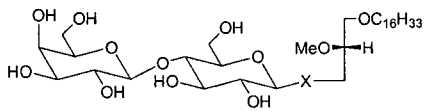
Y = NH_2, H, or OH



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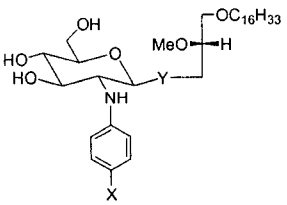
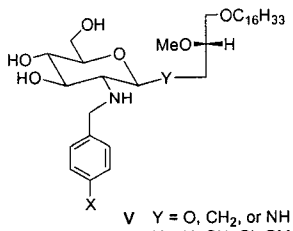


IV Y = O, CH_2, or NH
X = H, CN, Cl, OMe, or NMe_2



V Y = O, CH_2, or NH
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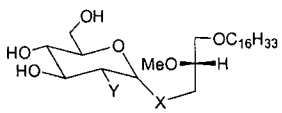
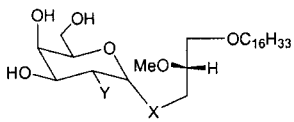


VI X = O, CH_2, or NH
Y = NH_2, H, or OH



VII X = O, CH_2, or NH
Y = NH_2, H, or OH

In some embodiments, the compound of Formula I contains a lactosyl moiety in place of the glucosyl moiety.

Results

Treatment of cells with glycosylated AELs induces autophagy in cells in a concentration- and time-dependent manner. Fig. 1 shows the presence of autophagolysosomes in **I**-treated cells (ASK1 -/-, ATG5 Wt, ATG5 -/-, BT549). The increase in levels of autophagolysosomes in **I**-treated cells is consistent with an increase in acridine orange accumulation in the cells, and an increase in lysosomal associated structures visualized by lysotracker red. The formation of autophagolysosomes is not an irreversible process, and at lower concentrations the autophagolysosmes disappear with time while at high concentrations the excessive accumulation of autophagolysomes leads to cell death.

LC3-II levels, the definitive marker for autophagosomes, increased in cells incubated with **I** (**GIn,** compound **I**, X = O, Y = NH_2). (Fig. 2). Time course studies showed the increase within 1 h of addition of **I** to the cells (Fig. 3).

Bafilomycin inhibits the fusion of autophagosomes and lysosomes to form autophagolysosomes. Its effect on cells is to cause the accumulation of autophagosomes. In the presence of bafilomycin, the LC3-II levels in ASK1 -/- cells and ATG5 wild-type mouse embryonic fibroblasts (MEFs) increased in **I** (**GIn**) treated cells relative to cells treated with **I** alone (Fig. 4).

GAELs induce autophagy by pathways that may be distinct from the known autophagy pathway involving ATG5 and LC3-II. In cells lacking ATG5, a key molecule required for LC3-II formation, **I** (**GIn**) still induced the formation of autophagolysosomes similar to the wild-type cells (Fig. 1B and 1C). We demonstrated that in ATG5 -/- cells, LC3-II was not formed in response to treatment with **I** (Fig. 4) even though autophagolysosomes were produced. In the control wild-type cells, treatment with **I** generated LC3-II. The formation of autophagolysosomes in both ATG5 and ATG5 -/- cells is inhibited by bafilomycin. 3-Methyladenine (3-MA) or wortmannin did not inhibit **I**-induced autophagolysome formation in cells with or without ATG5. Fig. 5 shows that 3MA and wortmannin did not inhibit LC3-II formation in ATG5 wild-type MEFs (Fig. 5)

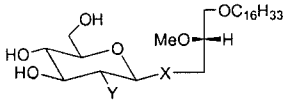
Our results indicate that GAELs induce autophagy through novel mechanisms. When ATG5 is present in cells as in the ATG5 wild type cells and ASK 1 -/- cells, **I** induced autophagosome formation via the generation of LC3-II. However, induction is formed via a 3-MA-independent pathway. In the absence of ATG5, **I** induces a novel pathway that leads to the formation of autophagolysosomes independently of LC3-II.

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.

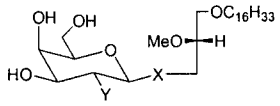
CLAIMS

1. A method of inducing autophagy in a cell or population of cells comprising administering to said cell or population of cells an effective amount of a compound selected from the group consisting of:

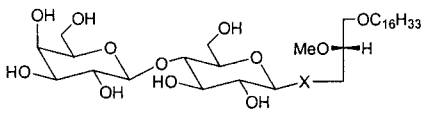
5



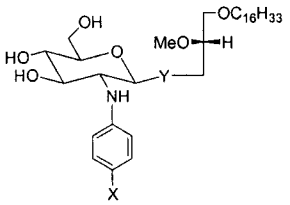
I X = O, CH_2, or NH
Y = NH_2, H, or OH



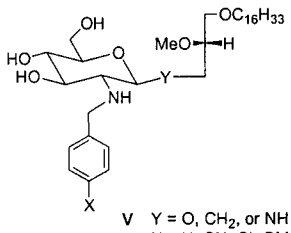
II X = O, CH_2, or NH
Y = NH_2, H, or OH



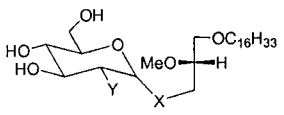
III X = O, CH_2, or NH



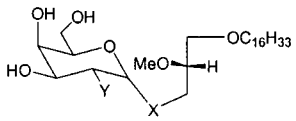
IV Y = O, CH_2, or NH
X = H, CN, Cl, OMe, or NMe_2



V Y = O, CH_2, or NH
X = H, CN, Cl, OMe, or NMe_2



VI X = O, CH_2, or NH
Y = NH_2, H, or OH



VII X = O, CH_2, or NH
Y = NH_2, H, or OH

ASK1 -/-

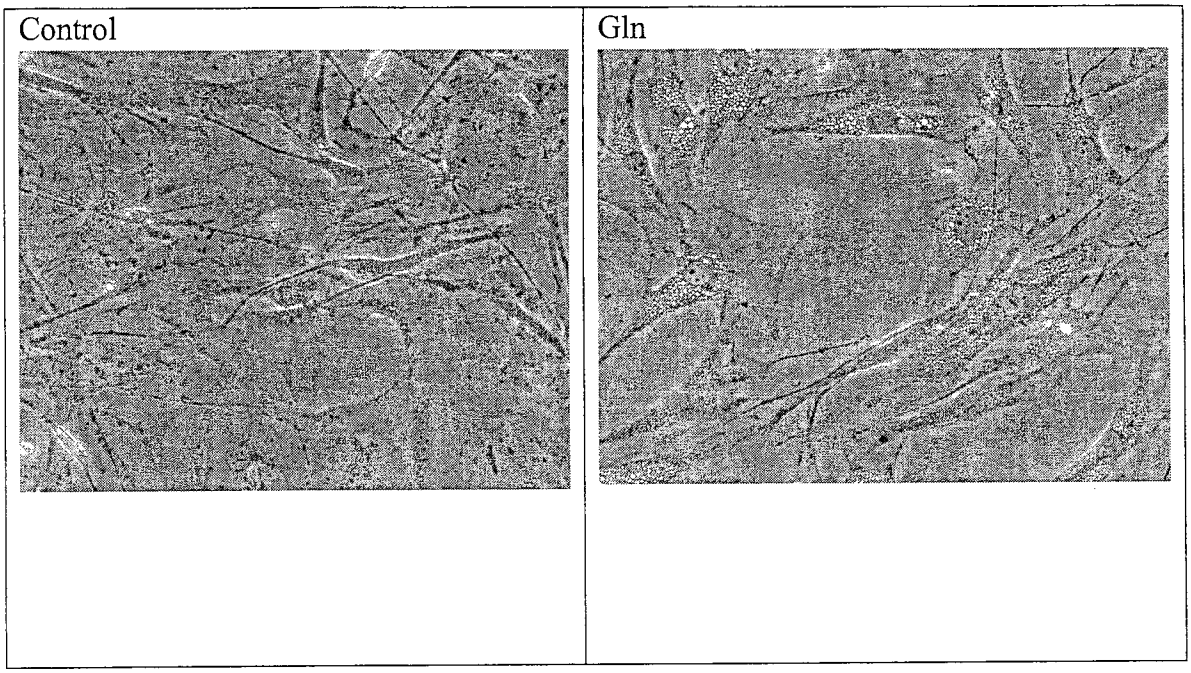


Figure 1A

ATG5 WT

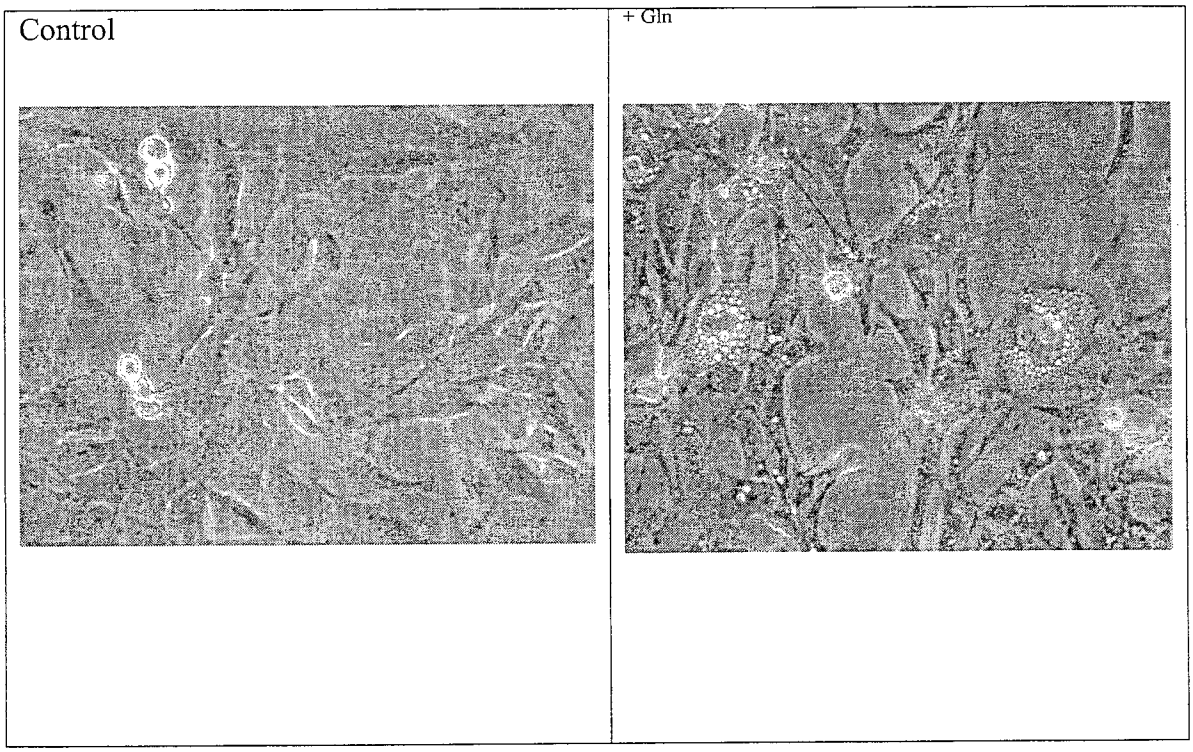


Figure 1B

ATG5 -/-

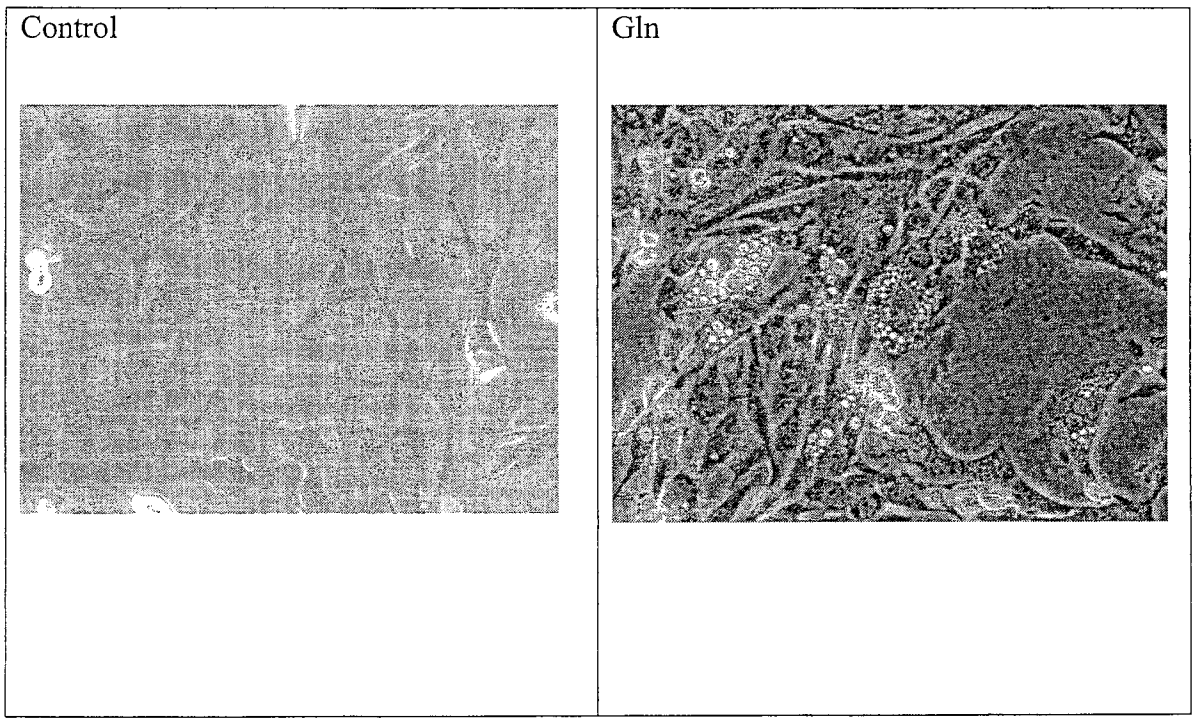


Figure 1C

BT549

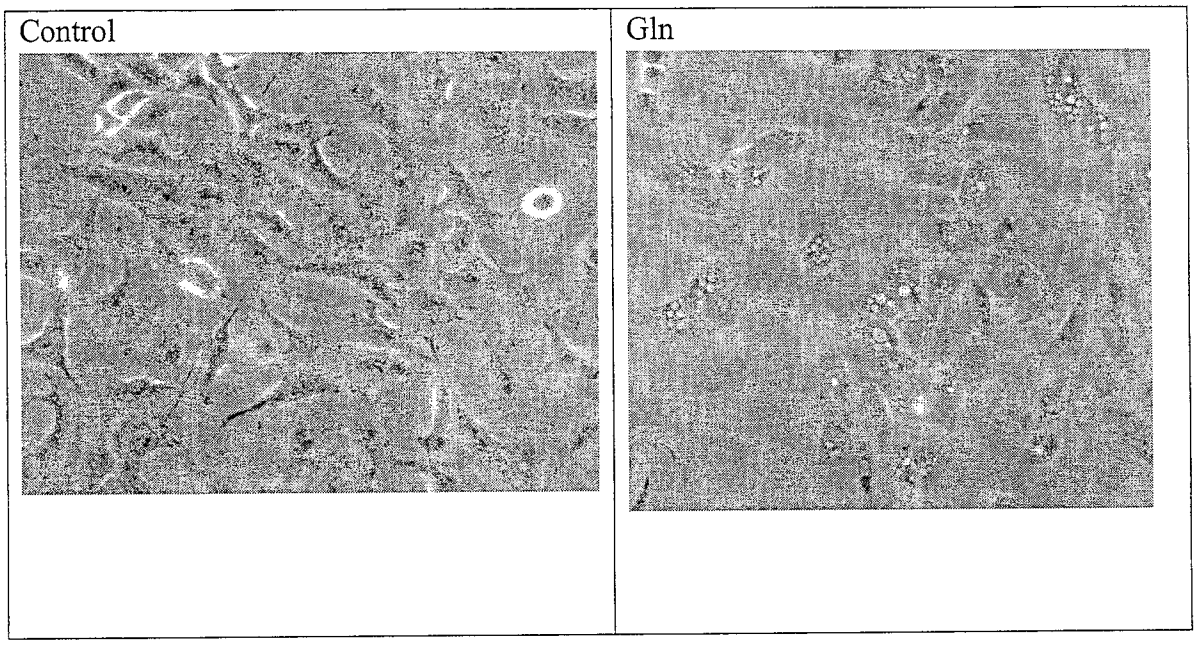


Figure 1D

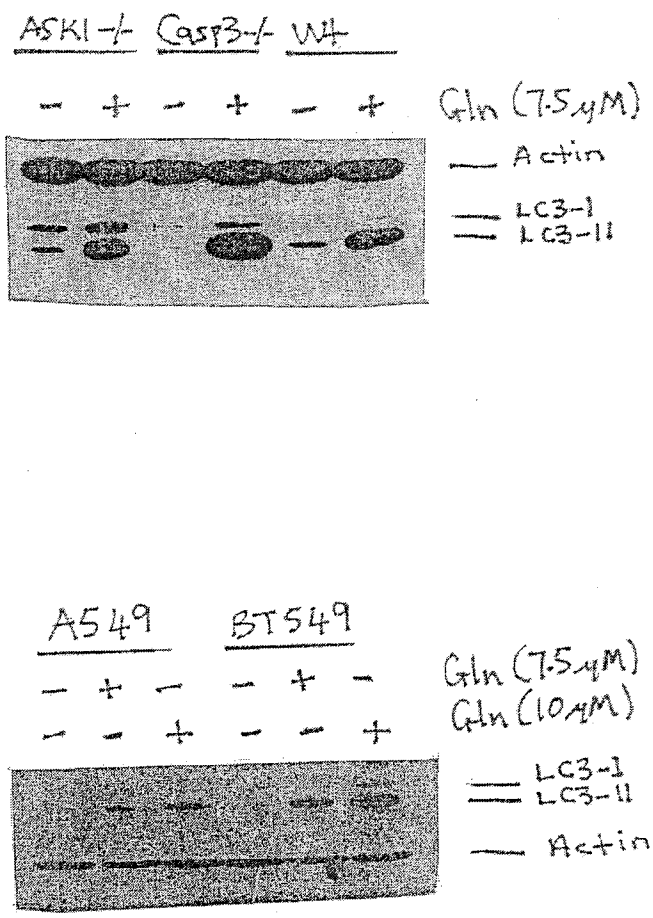

ASK1-/-
Casp3-/-
Wt
- + - + - +
Gln (7.5μM)
Actin
LC3-I
LC3-II
A549
BT549
- + - - + -
- - + - - +
Gln (7.5μM)
Gln (10μM)
LC3-I
LC3-II
Actin

Figure 2

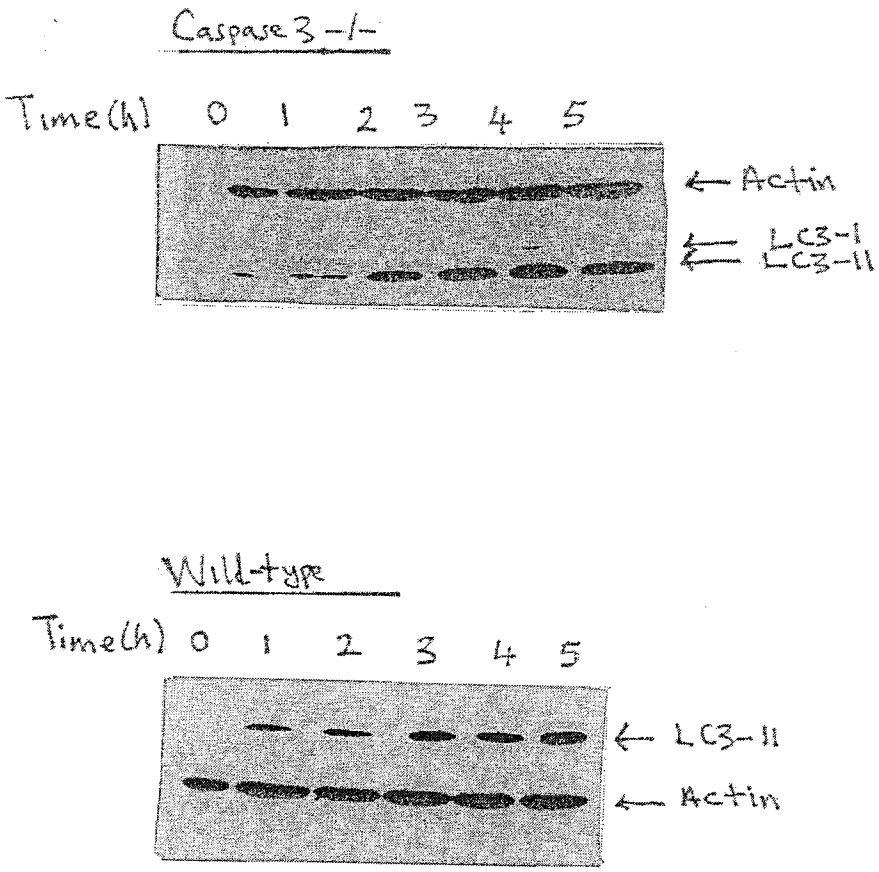

Caspase 3 -/-
Time(h) 0 1 2 3 4 5
Actin
LC3-I
LC3-II
Wild-type
Time(h) 0 1 2 3 4 5
LC3-II
Actin

Figure 3

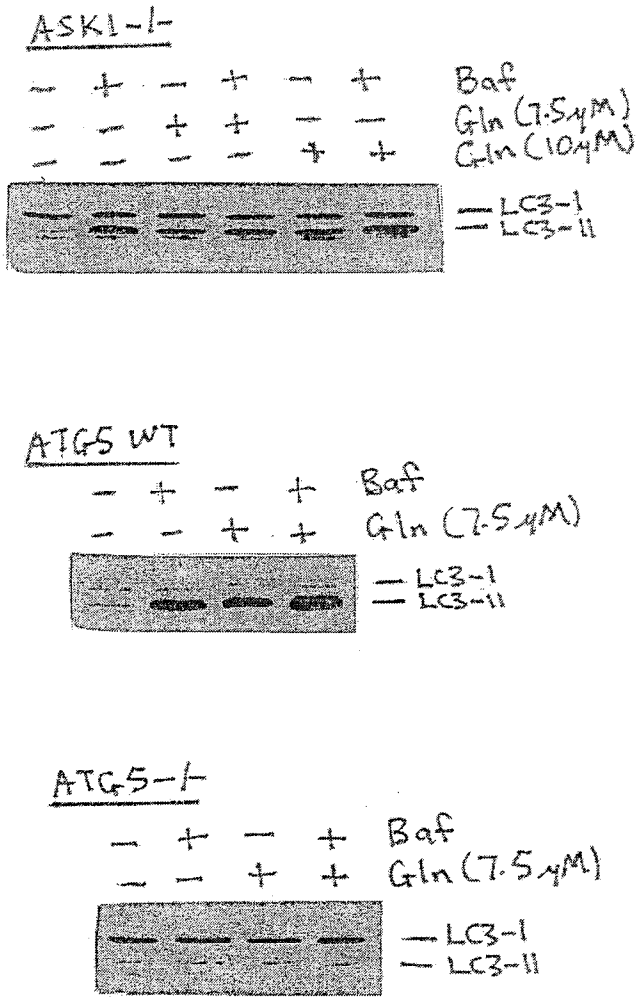

ASK1-/-
Baf
Gln (7.5μM)
Gln (10μM)
LC3-I
LC3-II
ATG5 WT
Baf
Gln (7.5μM)
LC3-I
LC3-II
ATG5-/-
Baf
Gln (7.5μM)
LC3-I
LC3-II

Figure 4

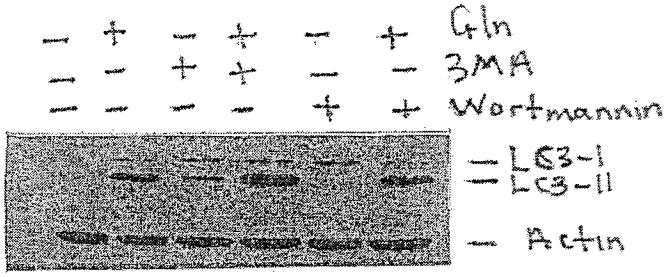


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/000083

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 31/7028*** (2006.01), ***A61K 31/70*** (2006.01), ***A61K 31/7004*** (2006.01), ***A61K 31/7008*** (2006.01), ***A61K 31/7016*** (2006.01).
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/7028 (2006.01), ***A61K 31/70*** (2006.01), ***A61K 31/7004*** (2006.01), ***A61K 31/7008*** (2006.01), ***A61K 31/7016*** (2006.01).

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
C07H*

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, Delphion, Scopus, Pubmed: autophag* ; GAEL / ether +lipid +glyco* / glucos* / gln; hexadecyl*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
X	Anticancer Res., 18(1A), pp. 465 - 470. SAMADDER *et al.* [JAN/FEB-1998] *Glycosylated antitumor ether lipids are more effective against oncogene-transformed fibroblasts than alkyllysophospholipids.* [ISSN:0250-7005] **D1**: See p 467, right column; Figure 6; and p 469, left column.	1
X	J. Med. Chem., 39 (17), pp. 3241 - 3247. MARINO-ALBERNAS *et al.* [16-AUG-1996] *Synthesis and growth inhibitory properties of glycosides of 1-O-hexadecyl-2-O-methyl-sn-glycerol, analogs of the antitumor ether lipid ET-18-OCH3 (edelfosine).* [ISSN:0022-2623] **D2**: See compounds 2 - 6; Table 1; and page 3244, left column.	1

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 2009 (15-04-2009)	4 June 2009 (04-06-2009)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	C. Bourque 819- 934-3596

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/000083

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 1

because they relate to subject matter not required to be searched by this Authority, namely :

Claim 1 is directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the therapeutic effect of the compounds defined in claim 1.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/000083

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. Med. Chem., 39 (7), pp. 1545 - 1548. ERUKULLA *et al.* [29-MAR-1996] *Synthesis and evaluation of the antiproliferative effects of 1-O-hexadecyl-2-O-methyl-3-O-(2'-acetamido-2'-deoxy-beta-D-glucopyranosyl)-sn-glycerol and 1-O-hexadecyl-2-O-methyl-3-0-(2'-amino-2'-deoxy-beta-D-glucopyranosyl)-sn-glycerol on epithelial cancer cell growth.* [ISSN:0022-2623] **D3**: See compound 5; and paragraph bridging pp 1547-1548.	1
X	WO97/011707 A1 BITTMAN *et al.* [03-APR-1997] *Modified Ether Gyceroglycolipids* **D4**: See page 10, lines 26 - 34; and example18.	1
X	WO02/060911 A2 BITTMAN *et al.* [08-AUG-2002] *C-Glucosyl Ether Lipids* **D5**: See page 2, lines 10 - 21;page 4, line 20 - 27; Examples 2 & 3; and Table 1.	1

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2009/000083

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO 9711707A1	03-04-1997	AU 705056B2	13-05-1999
		AU 7200596A	17-04-1997
		CA 2233168A1	03-04-1997
		EP 0852500A1	15-07-1998
		EP 0852500A4	14-11-2007
		JP 11514992T	21-12-1999
		US 6153736A	28-11-2000
		US 6573246B1	03-06-2003
WO 02060911A2	08-08-2002	CA 2431589A1	08-08-2002
		EP 1341800A2	10-09-2003
		JP 2004518692T	24-06-2004
		US 6613748B2	02-09-2003
		US 2002128214A1	12-09-2002
		WO 02060911A3	03-01-2003