Al-Kufa University Journal for Biology / VOL.14 / NO.2 / Year: 2022
Print ISSN: 2073-8854 Online ISSN: 2311-6544

SYNTHESIS, PHYSICAL AND BIOLOGICAL STUDY OF SOME NEW GLUCOSE BASED GLYCOLIPIDS

Nuha S. Kareem
Department of Chemistry, Faculty of Science, University of Kufa, An-Najaf, Iraq.
nuha.alahmer@uokufa.edu.iq

Abstract:

New glycolipids on glucose have been synthesized according to well-known sequencing reactions. The series of reactions started with peracetylation of glucose to produce mainly β-anomer, which is reacted directly with long chain alcohol in presence of Lewis acid as catalyst. The deacetylation of sugar head group was achieved under basic conditions. The resulting glycolipids have been modified in more three reaction steps to get the final products. The modified glycolipids have been studied under POM and DSC to confirm the phase behavior of these type of amphiphilic molecules, which is indicate that most of them form lamellar phase. These materials were also studied against two types of cancer cells to confirm their ability to interfere the bi-layer membrane of the living cell.

Keywords: Glycolipids, Glucose, Lyotropic, Liquid Crystals, Anti-cancer.

Introduction:

The term glycolipid is refer to special types of compound which containing one or more carbohydrate residues which are linked by a glycosidic bond to a hydrophobic moiety. Glycolipids were mainly classified as simple lipid derivatives such as acylglycerols, ceramides and prenols, and as glycosyl derivatives such as gangliosides and cerebrosides. Glycolipids are members of a broader class of substances identified as a glycopolymers or glycoconjugates. Glycolipid structures are highly complex, and this complexity results from the broad differences in the total carbohydrate residues, the number and bonding, the alteration of carbohydrates (e.g. phosphorylation, sulphate).[1]

Glycolipids can be synthesized under moderated conditions. They exhibit low level of toxicity and excellent biodegradability, as well as extremely compatibility with environments.[2] Furthermore, they can be obtained from natural resources like palm oil, fatty alcohols and mono and disaccharides open the possibility to get pure glycolipids. Glycolipids are significant components of the membrane which are found in nearly every living kinds extending from microbes to humans. Side by side with phospholipids and cholesterol, they form the basis of cell membrane.[3] In addition to their biological functions, glycolipids are involved in different technical applications due to their ability in self-assemblies in water-based media. Their applications include but not limited to, protein microarrays, biomolecular devices , liquid crystals, protein crystallization, and drug delivery systems. [4]

On the other hand , the cancer is a huge group of diseases characterized by uncontrolled growth and spread of the abnormal cells, and the second leading disease after cardiovascular most common causative threatening human life. The World Health Organization (WHO) has estimated 15 million deaths due to cancer worldwide by 2030. Over two hundred different types of cancers affecting major organs such as lung, brain, breast, colon, kidney, bladder and stomach have been identified thus far and more are emerging[5]

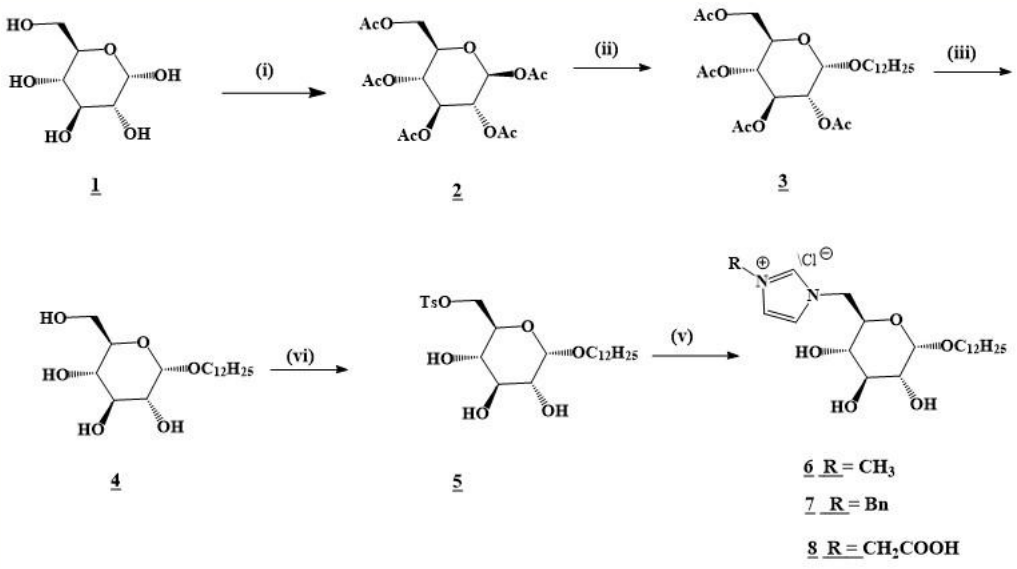
Glycolipids are not known to possess medicinal properties themselves, but they may improve drug uptake and transport properties.[6] The special complexing properties of glycolipids have led to applications as anticancer compounds and drug delivery systems and as targeting functionalities incorporated in drug derivatives and DNA-binding agents. It was shown in DNA binding studies that the positive charge of cation crown ether complexes increases the affinity of crown ether-linked compounds with the polyanionic phosphate backbone of DNA. [7]

One of the possible way to kill the cancer cells by change the balance inside the cell membrane. This way is possible by penetrate the glycolipids with the phospholipids bi-layer of cell. The glycolipids are suitable for this purpose as they resemble the phospholipids bi-layers by structure, so when they applied on cancer cell may effect the balance inside and outside the cell circumstances and as a result terminate the cancer cell life. [8]

Result and Discussion:

1. Synthesis:

The n-dodecyl β-D-glucopyaranoside **3** is synthesized by applying glycosylation reaction catalyzed by boron trifluoride from the corresponding n-dodecanol with β-D-glucose pentaacetate **2**, which was prepared from glucose **1** and acetic anhydride in the presence of sodium acetate. Unfortunately, the result compound **2** was a mixture of α- and β-anomers, which can be separated by chromatography, scheme (1).



Reagents and conditions: (i) acetic anhydride, NaOAc, (ii) n-dodecanol, $BF_3.Et_2O$, CH_2Cl_2, (iii) NaOMe, MeOH (vi) TsCl, Pyridine, (v), methyl or benzyl or carboxymethyl imidazole, MeCN ,reflux.

Scheme (3-1): Overall synthesis of 6-imidazolium derivatives on β-D-glucopyarnoside.

The peracetylated glycolipid **3** was deacetylated under basic condition applying sodium methoxide in methanol to furnish compound **4**. The selective tosylation on C-6 of glycolipid **4** can be achieved via tosylate group by treated compound **4** with tosylate chloride under basic condition applying pyridine to furnish compound **5** in good yield. The compound 5 was characterized by FTIR and NMR spectroscopy to

confirm its structure. The FTIR spectrum of **5** showed the following characteristic bands that is possible to introduce the tosylate group. The bands at 3060 cm^{-1} is can be attributed to the stretching vibration of aromatic proton. The bands at the region of (1600 – 1500) cm^{-1} which attributed to the carbon carbon double bonds of aromatic ring of tosylate group (Fig. 1).

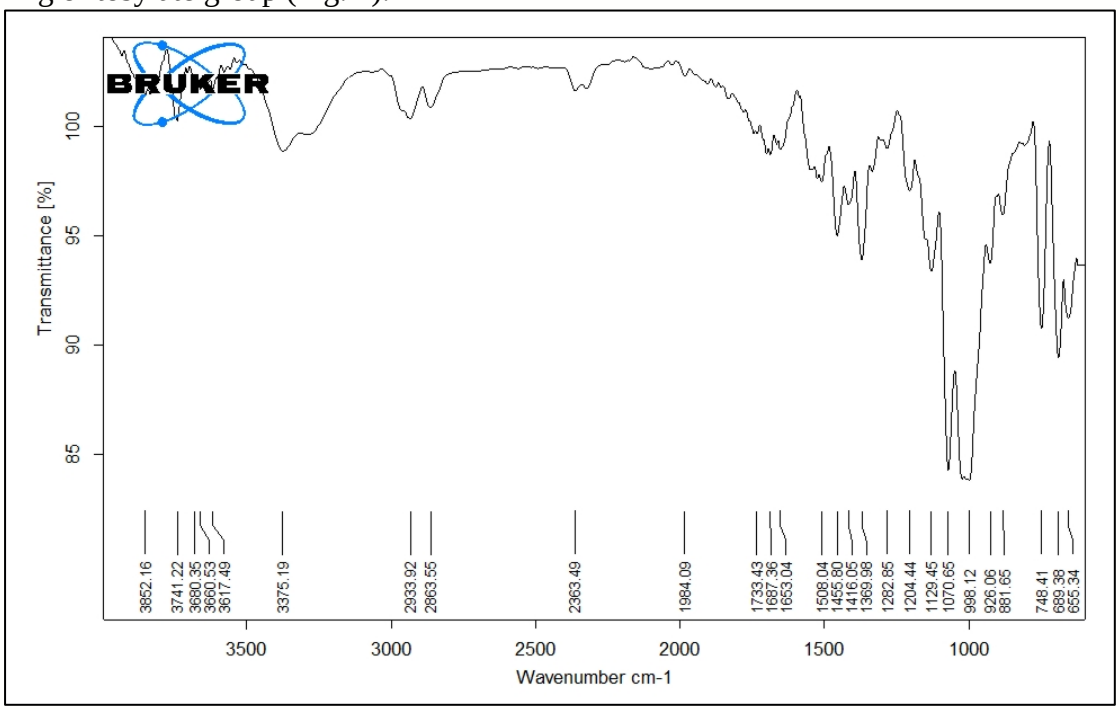


Figure 1. The FTIR spectrum of compound **5**.

In the same context, ^{1}HNMR spectrum of compound 5 showed the characteristic peaks that related to the introducing of tosylste group on carbon 6 of pyronside ring of the sugar (Fig 2). The two doublets signals at 7.6-7.0 ppm with J-coupling 8Hz is good prove that the tosylate aromatic protons are exist. In addition, the signal at 2.5 with 3 protons integration as singlet is refer to the methyl group of tosylate in the para position of the benzene residue. The other peaks is reflected the exact structure of glycolipids **5**. Moreover, the ^{13}CNMR also confirm the introducing of tosylate group through the aromatic peaks at 150-130 ppm region and the methyl carbon signal at 32 ppm. The other carbon peaks is reflected the whole structure of compound 5 exactly (Fig. 3).

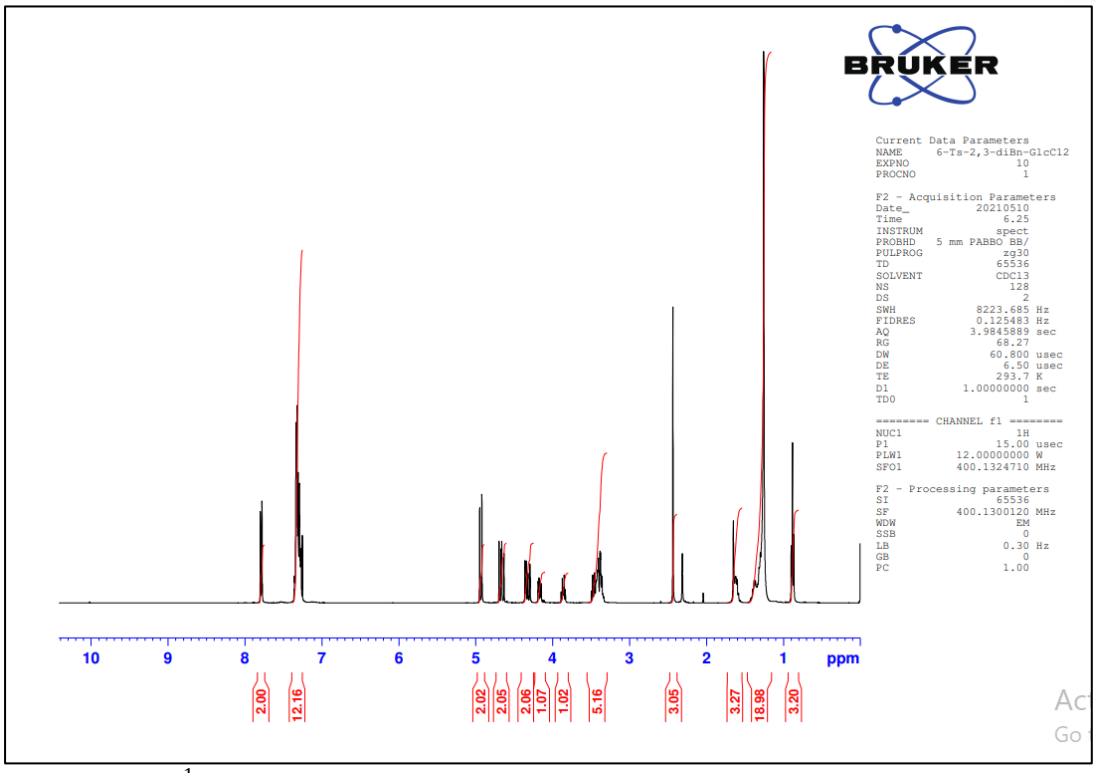


Figure 2. The ^{1}HNMR spectrum of compound **5**.

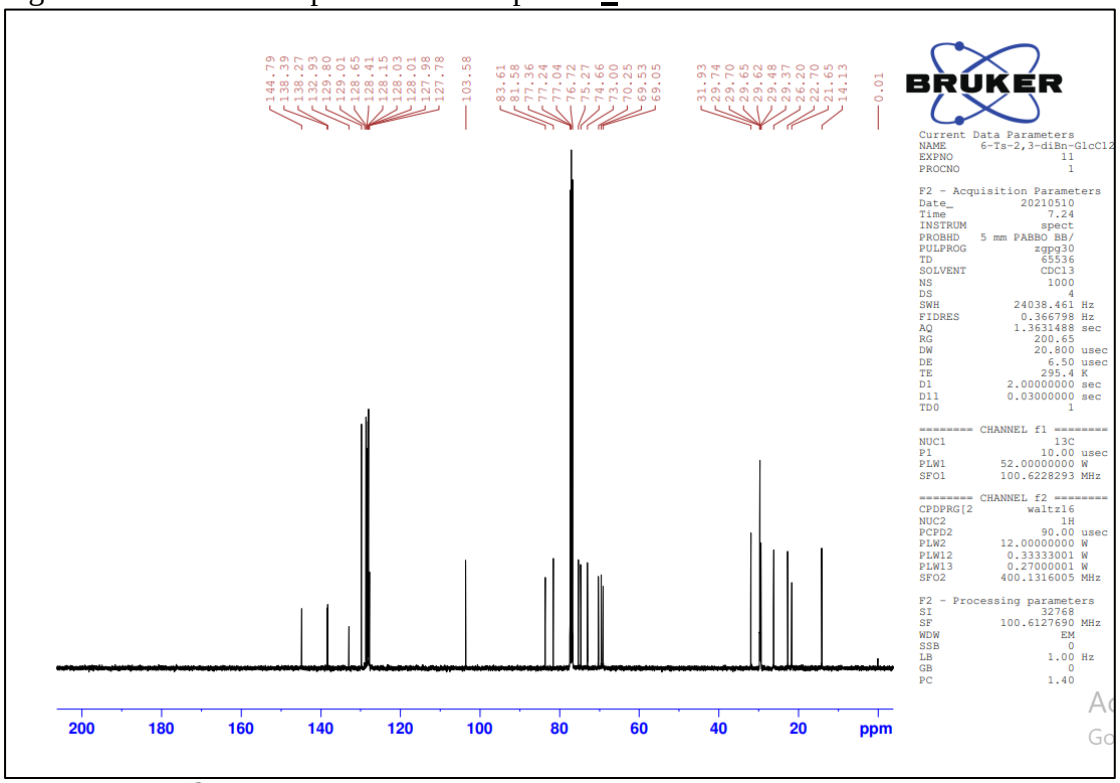


Figure 3. The ^{13}CNMR spectrum of compound **5**.

After activating carbon 6- on the pyranoside ring of glycolipids **4**, the compound is ready to inter the reaction with some alkyl imidazole derivatives to form a series of ionic liquids based on glycolipids as a new class of more polar head groups. The reaction of glycolipid **5** with methyl, benzyl and carboxymethyl imidazole in reflux

acetonitrile produce the compounds **6** , **7** and **8** in good yields. The choice of these derivatives is based on the graduation of polarity that is highest for compound **8** as it contain a carboxylic acid residue on its structures. The all compounds are confirmed by FTIR and NMR spectroscopy, that confirm the formation of these derivatives based on leaving of tosylate group on carbon 6 of the pyranoside of sugar.

2. Physical study: phase determination:

2.1 Lyotropic phase behavior of compound 8:

OPM pictures for lyotropic behavior of the compound at ambient temperature showed a clear birefringent line at the ended of the sample, which usually characteristic of the lamellar phase (Lα) upon reaching water-rich side. Moreover, beside the producing of the lamellar phase, the compound was form a cubic phase at side when water-poor as shown in (Fig. 4). The modified glycolipid with imidazolium ionic liquids, which somewhat increases the head-group size with increasing of the hydrophilicity, can promote the formation of the non-lamellar phase.[9] However, The results are noticeably proves the effect of water making a hydrogen bond network with the monosaccharides head-groups and the imiadazolium residue [10].

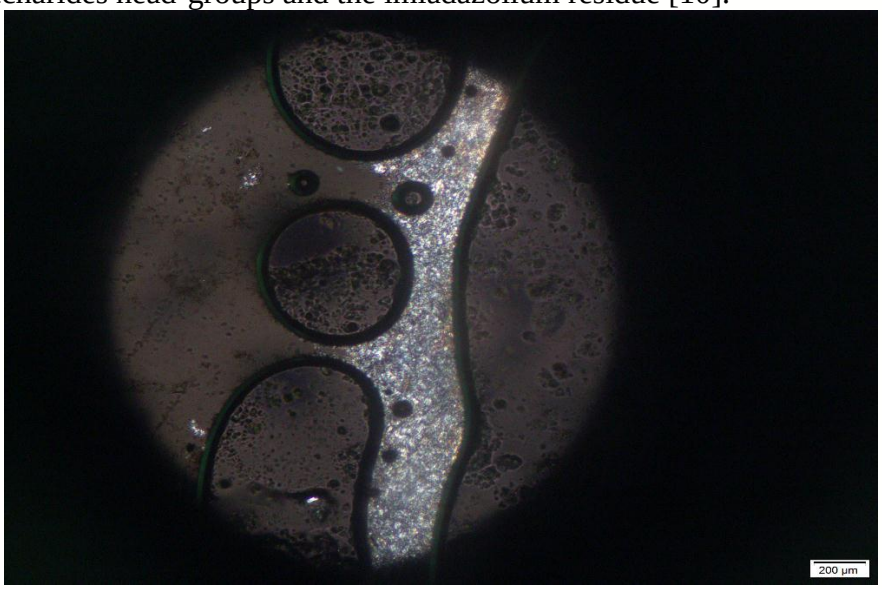


Figure 5: Texture of compound **8** water penetrate at zoom (X3)

2.2 Differential Scanning Calorimeter (DSC) of compound 8: -

The transition temperature of phase was getting calorimetrically during the DSC second heating round of the sample to remove the thermal history and minimize the kinetic effect. Figure (6) shows that the compound exhibit recrystallization in the reheating process which is followed by a melting transition at 52°C (ΔH= 14.32 J/g) upon further heating A similar observation has been reported by monoalkylated glucosides [11]. The OPM texture confirms this assignment. The liquid crystal phase transition [12].

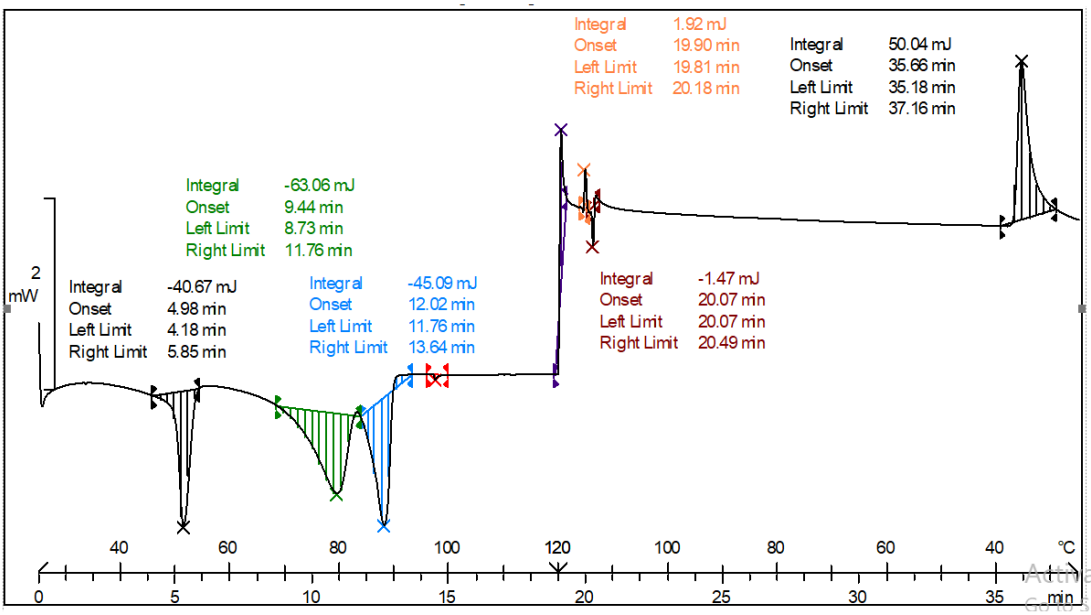


Figure 6: DSC of compound ***8***.

3. Biological applications

The glycolipids have been used for broad therapeutic applications due to their diverse biological activities i.e., antimicrobial antiviral, anti-inflammatory, analgesic, anticancer, antifungal and anticonvulsant [13]. In this regard, these privileged scaffolds have drawn considerable attention in the field of medicinal chemistry [14].

In this context, the glycolipids **6**, **7** and **8** derivatives are subjected to test them against two types of carcinoma cell lines. These are human lung epithelial carcinoma cells (A549 cells) and breast cancer cell (MCF-7 cell lines) beside the normal cell line (MCF-12A) which is used as control. The compound **8** is tested on the MCF-7, MCF-12A and A549 cell lines which are grown as monolayers in Dulbecco's modified Eagle's Medium (DMEM) with 10% fetal calf serum (FCS) (Sigma) and 1% penicillin/streptomycin in a humidified 5% CO_2. [15] However. The results are showed that IC_{50} values for **8** on A549 cell line was (3.856 µM), and the percentage of survival A549 cells was (5-47 %), as demonstrated in Fig (7). The results also shows that IC_{50} values of **8** on MCF-7 cell line was (11. 4µM), and the percentage of survival MCF-7 cells was (7-52. Interestingly, IC_{50} value shows with non-tumorigenic cells (MCF-12A) about (11.44 µM). It seems that the effect of compound **8** can inhibits the carcinoma cells not non-carcinoma cells.

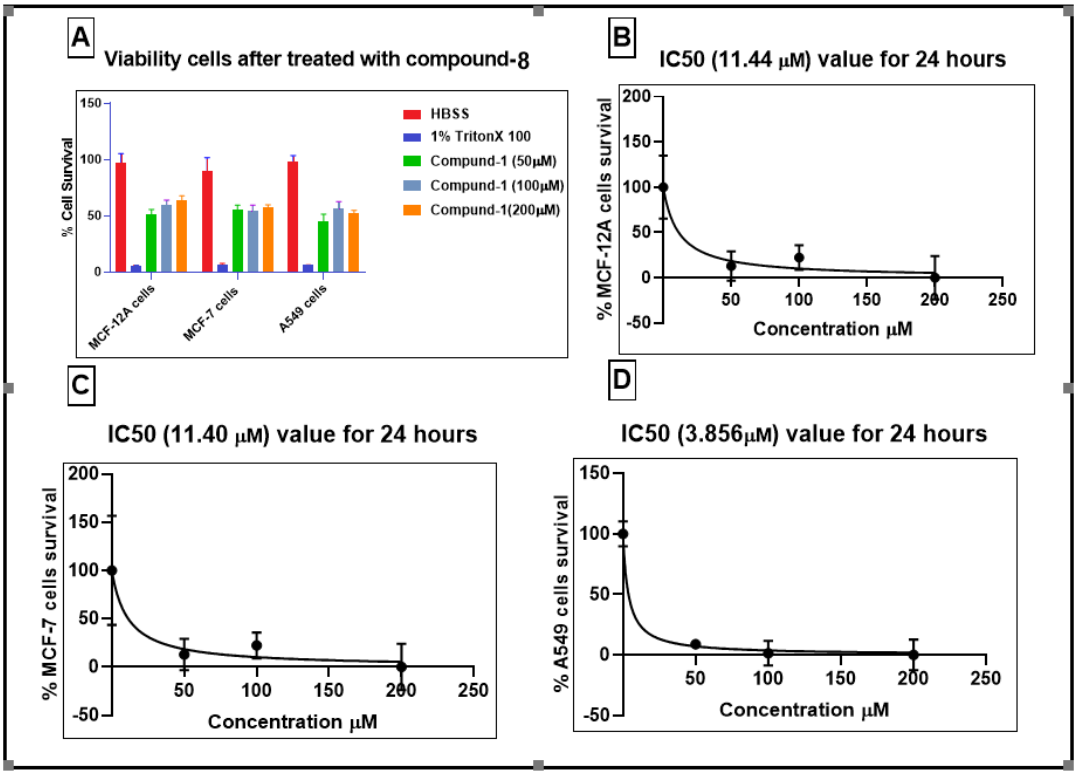


Figure (7) IC50 measurements of the non and tumorigenic cells treated with compound **8**. Data are represented as the mean ± SD (n=2).

Experimental:

1. Synthesis of glycolipids:

The glycolipid **4** have been synthesized according to the sequence that published in reference 21 with some modifications.[16]

2. Synthesis of n-dodecyl 6-tosyl-β-D-glucopyarnoside 5.

In round bottom flask contain (10 mmol, 3.48 g) of compound 4 in dry pyridine (50 mL) that cool down to (0^{o}C) and a solution of (12 mmol, 2.1 g) of tosylate in pyridine has been added dropwise to the mixture with continues stirring. After complete the addition the temperature is rising to room temperature gradually and the stirring is continue for further 24 hours. The solvent was evaporated in vacuum and the residue was washed extensively with dilute HCl solution and water. The residue was purified by column chromatography applying gradient of ethyl acetate: hexane eluent to furnish compound 5 as yellow syrup (3.8 g, 72 %).

3. General procedure for synthesis of ionic liquid glycolipids (6-8)

Compound 5 (1 mmol, 0.51 g) was dissolved in acetonitrile in round bottom flask and then (1 mmol) of imidazole derivatives and the mixture was reflux for 12 hours. The solution of KCl (2 N) was added and the solution left to stirred for 24 hours. The precipitate of potassium tosylate was removed by filtration. The resulting solution was subject to rotary evaporater to remove the solvent left over the products.

3.1 Compound 6.

Compound 5 (1 mmol, 0.51 g) and methyl imidazole (1mmol, 0.08 g) was dissolved in acetonitrile according to the general procedure to give compound 6 as colourless syrup (0. 6 g, 94 %).

3.2 Compound 7.

Compound 5 (1 mmol, 0.51 g) and methyl imidazole (1mmol, 0.158 g) was dissolved in acetonitrile according to the general procedure to give compound 6 as yellow wax (0. 6.5 g, 96 %).

3.3 Compound 8.

Compound 5 (1 mmol, 0.51 g) and methyl imidazole (1mmol, 0.126 g) was dissolved in acetonitrile according to the general procedure to give compound 6 as white crystal (0. 6.2 g, 95 %).

Conclusion:

In conclusion, new glycolipids derivatives have been synthesized successfully based on ionic liquid residues. The resulting glycolipids have been studied under POM and DSC to confirm the phase behavior of these type of amphiphilic molecules, which is indicate that most of them form lamellar phase. Also the demonstration of highly potent in vivo anticancer activities have been monitor. An unexpected but exciting finding was the existence of unusually wide in vivo therapeutic windows, which may contribute to the remarkable in vivo efficacy of these new agents.

References:

[1] K. Garidel, Patrick Kaconis, Yani Heinbockel, Lena Wulf, Matthias Gerber, Sven Munk, Ariane Vill, Volkmar and Brandenburg, "Self-Organisation, Thermotropic and Lyotropic Properties of Glycolipids Related to their Biological Implications," *Open Biochem. J.*, vol. 9, pp. 49–72, Aug. 2015, doi: 10.2174/1874091X01509010049.

[2] O. K. Zahid, N Idayu Ji, Lei Khyasudeen, M Faisal Friedrich, Alexandra Hashim, Rauzah Marder, Todd B and Abou-Zied, "Evidence of Increased Hydrophobicity and Dynamics inside the Tail Region of Glycolipid Self-Assemblies Using 2-n-Alkyl-Pyrene Derivatives to Probe Different Locations," *Langmuir*, vol. 35, no. 29, pp. 9584–9592, Jul. 2019, doi: 10.1021/acs.langmuir.9b01767.

[3] B. G. Trewyn, J. A. Nieweg, Y. Zhao, and V. S. Lin, "Biocompatible mesoporous silica nanoparticles with different morphologies for animal cell membrane penetration," vol. 137, pp. 23–29, 2008, doi: 10.1016/j.cej.2007.09.045.

[4] R. W. Ruddon, *Cancer biology*. Oxford University Press, 2007.

[5] C. D. Mathers and D. Loncar, "Projections of global mortality and burden of disease from 2002 to 2030," *PLoS Med.*, vol. 3, no. 11, p. e442, 2006.

[6] M. V Douglass, F. Cléon, and M. S. Trent, "Cardiolipin aids in lipopolysaccharide transport to the gram-negative outer membrane.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 118, no. 15, Apr. 2021, doi: 10.1073/pnas.2018329118.

[7] A. Arenaza-Corona *et al.*, "Further Approaches in the Design of Antitumor Agents with Response to Cell Resistance: Looking toward Aza Crown Ether-

dtc Complexes," *Inorg. Chem.*, vol. 59, no. 20, pp. 15120–15134, 2020, doi: 10.1021/acs.inorgchem.0c02068.

[8] M. Pinteala, M. J. M. Abadie, and R. D. Rusu, "Smart Supra-and Macro-Molecular Tools for Biomedical Applications," *Materials (Basel).*, vol. 13, no. 15, p. 3343, 2020.

[9] B. Donnio, J. M. Seddon, and R. Deschenaux, "A Ferrocene-Containing Carbohydrate Surfactant: Thermotropic and Lyotropic Phase Behavior," *Organometallics*, vol. 19, no. 16, pp. 3077–3081, Aug. 2000, doi: 10.1021/om0001568.

[10] R. F. Moore, Jackson E Mccoy, Thomas Marlow, Joshua B Pottage, Matthew J Mudie, Stephen T Pearson, Graeme R Wilkinson, Brendan and Tabor, "Rich liquid crystal phase behavior of novel alkyl-tri (ethylene glycol) -glucoside carbohydrate surfactants," *J. Colloid Interface Sci.*, vol. 22, no. 540, pp. 410–419, 2018, doi: 10.1016/j.jcis.2018.12.092.

[11] R. Patrick, Melonney Zahid, N Idayu Kriechbaum, Manfred and Hashim, "Guerbet glycolipids from mannose : liquid crystals properties Guerbet glycolipids from mannose : liquid crystals properties," *Liq. Cryst.*, vol. 45, no. 13, pp. 1–17, 2018, doi: 10.1080/02678292.2018.1492036.

[12] R. Hashim, S. M. Mirzadeh, T. Heidelberg, H. Minamikawa, T. Yoshiaki, and A. Sugimura, "A reevaluation of the epimeric and anomeric relationship of glucosides and galactosides in thermotropic liquid crystal self-assemblies," *Carbohydr. Res.*, vol. 346, no. 18, pp. 2948–2956, 2011, doi: 10.1016/j.carres.2011.10.032.

[13] D. Jacková, M. Brunderová, M. Fábian, M. Martinková, J. Gonda, and M. B. Pilátová, "Synthesis and anticancer profile of novel sphingoid base-like compounds with a quaternary stereocentre," *Carbohydr. Res.*, vol. 487, no. September 2019, p. 107862, 2020, doi: 10.1016/j.carres.2019.107862.

[14] J. L. Arias, "Novel strategies to improve the anticancer action of 5-fluorouracil by using drug delivery systems," *Molecules*, vol. 13, no. 10, pp. 2340–2369, 2008, doi: 10.3390/molecules13102340.

[15] M. Marjanovic, M. Kralj, F. Supek, L. Frkanec, I. Piantanida, and L. Tus, "Antitumor Potential of Crown Ethers : Structure - Activity Relationships , Cell Cycle Disturbances , and Cell Death Studies of a Series of Ionophores," pp. 1007–1018, 2007.

[16] K. J. Sabah and R. Hashim, "Novel mixed-heteroatom macrocycles via templating: A new protocol," *Tetrahedron Lett.*, vol. 54, no. 12, pp. 1534–1537, 2013.