Synopsis

Introduction

Glycolipids were discovered, isolated from brain tissue and named by Ernst Klenk in 1942. Glycolipids are glycosyl derivatives of lipids. They are collectively a part of larger family of substances known as glycoconjugates. Glycolipids are a structurally ubiquitous molecules found in all living organisms from prokaryotic to eukaryotic cells, from bacteria to humans. Glycolipids are essential constituents of cellular membranes and cell signalling molecules. This class of compounds has been attracting an increasing amount of attention up to today, on account of playing essential roles in diverse biological functions and bio surfactant applications due to their biodegradability. Inspite of a wide range of functions, it is surprising that these compounds are not exploited commercially. Possible reasons for this could be the lack of availability of natural resources rich in these glycolipids or commercially viable process for their isolation/purification as these lipids are found to be complex mixtures in their respective natural sources. Furthermore, to understand the complete physico-chemical and biological properties of glycolipids, pure glycolipids were required. Thus, in order to produce novel glycolipids with enhanced biological applications present study will be mainly focused on the entitling **"Design, synthesis and biological evaluation of novel glycolipid mimics"**.

Chapter 1: Introduction to Glycolipids

This Chapter gives introduction about classification of glycolipids, nomenclature, structure and different applications. Salient features of various methods available in literature for the synthesis of glycoglycerolipids, galactoceramides and phenolic, steryl and heterocyclic-based glycosides using chemical and enzymatic process are briefly reviewed. Various glycosylation methods used for the synthesis of different type of glycolipids are also briefly reviewed. The chapter also gives information on biological evaluation of various type of glycolipids isolated and/or synthesized either by partial or total synthesis.

Chapter 2: Synthesis of Dihydrosterculic acid-based Monoglucosyl Diacylglycerol and its Analogues and their Biological Evaluation

Among the available broad spectrum of lipids, glycoglycerolipids have gained significant attention in view of their diverse properties. They are major constituents present in chloroplast membranes of photosynthetic cells and in bacterial cell walls. Glycolipids are well-studied molecules exhibiting different biological activities. However, all these activities

are strictly dependent on their fatty acyl chain length and olefinic nature. Simultaneously, earlier reports suggest that the site of attachment of the sugar to the glycerol moiety to derive an anomeric configuration of the sugar seemed to play negligible roles on improving the antitumor potential.

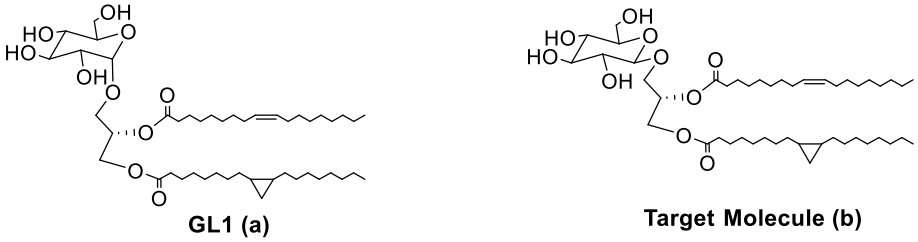
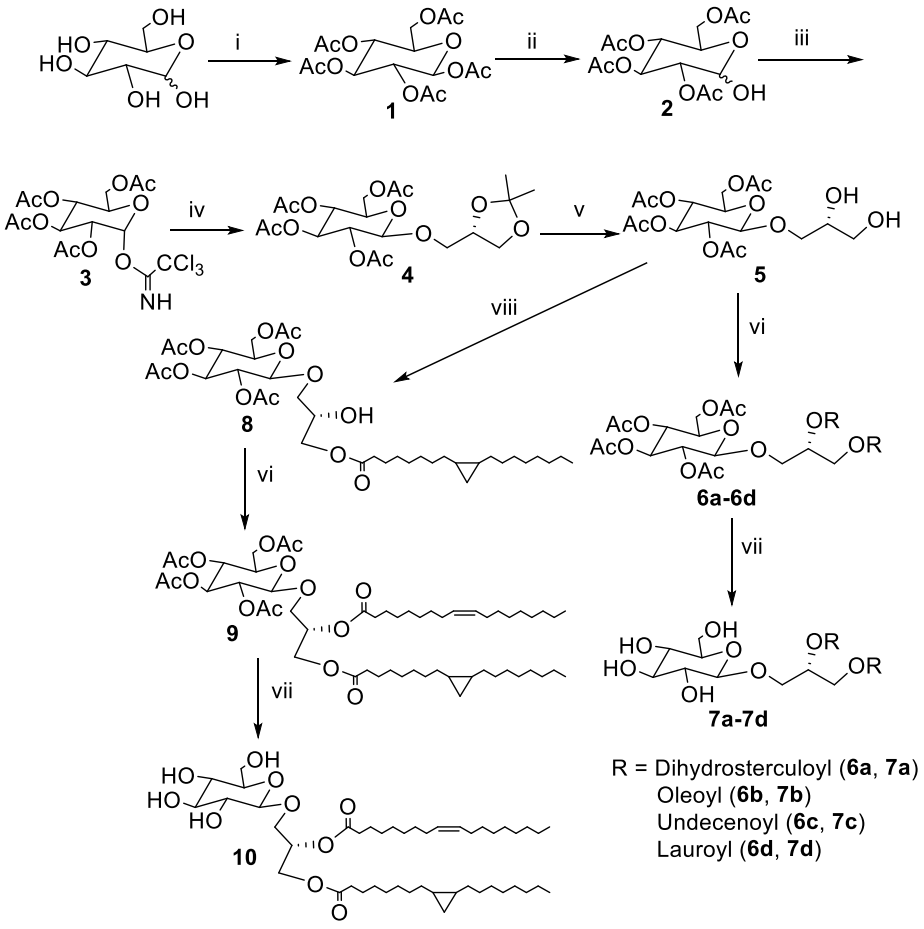
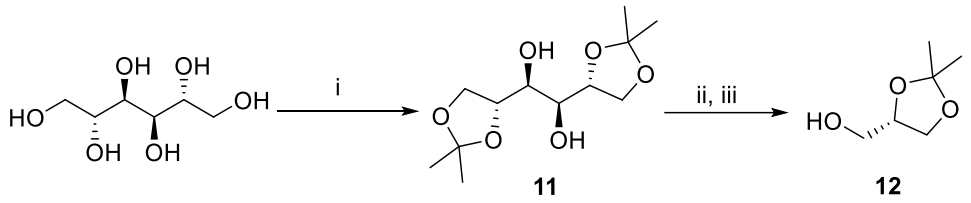


Figure 1. Structures of GL1 and Target Molecule

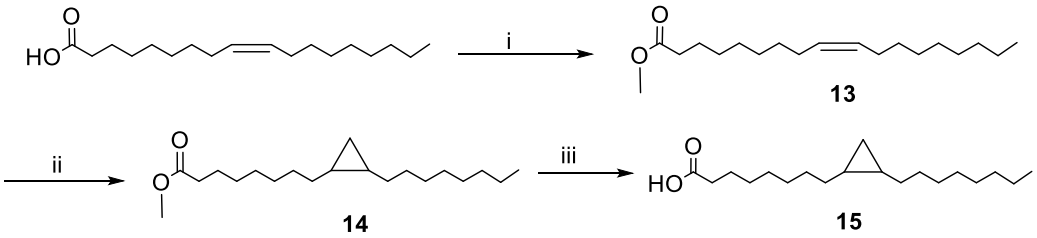


Scheme 1. Reagents and conditions: i) Ac_2O, NaOAc, reflux 4 h, 92%; ii) Hydrazine Acetate, DMF, 50 ^oC, 2 h, 92%; iii) CCl_3CN, DBU, DCM, rt, 3 h, 77 %; iv) 12, TMSOTf, DCM, 4 A^o MS., 0 ^oC to rt, overnight, 82 %; v) $Zn(NO_3)_2.6H_2O$, MeCN, 50 ^oC, 6 h, 75%; vi) EDC-HCl, DMAP, DCM, Fatty acid, 0 ^oC to rt, overnight, 88-92%; vii) $N_2H_4.H_2O$, aq EtOH (85 %), 44 ^oC, 6 h, 75- 78 %; viii) EDC-HCl, DMAP, DCM, Fatty acid (1 eq.), 0 ^oC, 6h, 98%.

Recently, Sauvageau and coworkers reported the isolation and characterization of dihydrosterculic acid-based glycolipids from *Lactobacillus plantarum* (GL1) with α-configuration (Figure 1a). Considering the above facts, in the present study, we herein report the synthesis of dihydrosterculic acid-based glycolipids with β-configuration (Figure 1b) using trichloroacetimidate method (Scheme 1 to Scheme 3) with good yields for the first time. The β-configuration of the GL1 molecule was unambiguously assigned by NMR studies using 2D-ROESY (NOE) and *J*-coupling analysis. Dihydrosterculic acid was synthesized using Furukawa's reagent and the selective esterification of dihydrosterculic acid at C-3 position of glycerol was achieved with EDC-HCl at 0 °C. All the products were characterized by FT-IR, NMR and MS analysis.



Scheme 2. Reagents and conditions **:** i) Anhydrous $ZnCl_2$, Acetone, K_2CO_3, 2.5 h, rt, 90% ; ii) $NaIO_4$, $NaHCO_3$, DCM, rt, 45 min; iii) EtOH, $NaBH_4$, NH_4Cl, 2 h, rt, 85%.



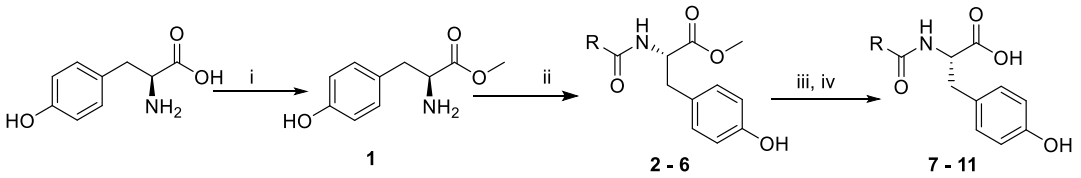
Scheme 3. Reagents and conditions **:** i) MeOH-H_2SO_4, rt, 3 h, 98%; ii) $(C_2H_5)_2Zn$, CH_2I_2, DCM, **-** 5 ^oC to rt, overnight, 100%; iii) LiOH, THF:H_2O (7 : 3), overnight, rt, 99 %.

In vitro cytotoxicity of the GL1 molecule and its fatty acid analogues were evaluated against DU145, A549, SKOV3 and MCF7 cell lines. Among all the synthesized molecules, the GL1 molecule and compound **7d** showed moderate activity, while the compound **7b** showed promising activity against all the tested cell lines with IC_{50} values of 20.1, 18.2, 19.1 and 17.6 µM, respectively. In addition, all tested compounds showed poor cytotoxicity against normal HUVEC cells. The MCF7 cells when treated with compound **7b** showed lower bromodeoxyuridine incorporation levels as compared to untreated cells, suggesting that the compound **7b** was highly effective and inhibited the cell proliferation. In addition, the

compounds showed significant increase in caspases 3 and 9 levels by inducing apoptosis in MCF 7 cells.

Chapter 3: Synthesis and Cytotoxic Evaluation of Novel Glycosylated *N*-Fattyacyl-*L*-Tyrosine Derivatives

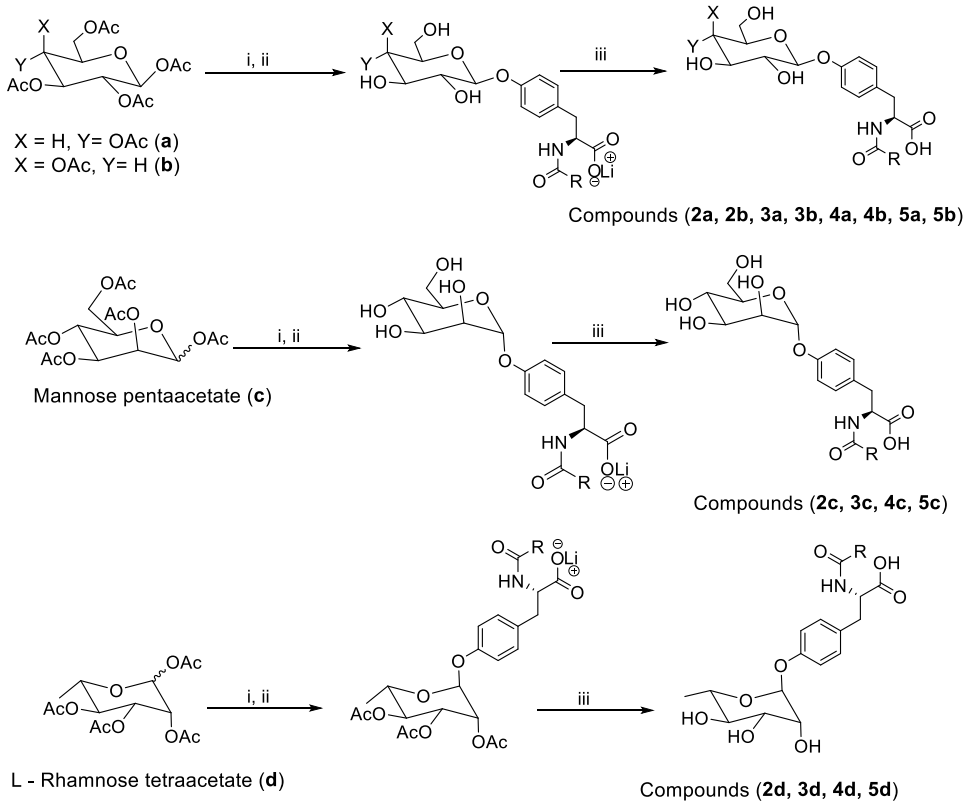
In general, glycosides are biologically active compounds and the glycosidic residue dictates their biological activity. In some cases, the glycosidic residue or overall molecular structure improves the pharmacokinetic parameters. Especially, the combination of carbohydrate moiety to aglycone plays a functional role in the bioactivity. On the other hand, many reports are available on biologically active lipoamino acids and fatty amino acid conjugates. These endogenous substances exhibit multiple biological activities such as analgesic, anti inflammatory and inhibition of cell proliferation. Based on the above facts, it was necessary to place the amino acid acylated with fatty acids and glycosylated with carbohydrates into a separate class of lipid derivatives which are worthy to investigate.



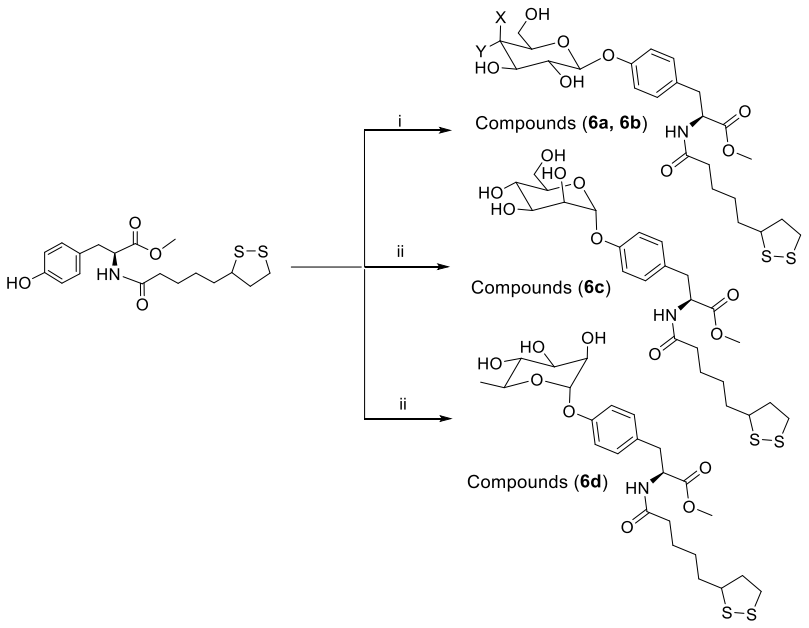
Scheme 1. Reagents and conditions: i) H_2SO_4-MeOH, 4 h, reflux, 70% ; ii) Fatty acid, EDC, HOBT, DCM, rt, 12 h, 80 - 89%; iii) LiOH, THF, rt ,16 h; iv) 2N HCl, 96 - 98%.

Hence, in the present study, a series of different fatty acids-based (short, medium and long unsaturated chains) glycosylated *N*-fattyacyl-*L*-tyrosines and *N*-lipoyl-*L*-tyrosine methyl esters were synthesized and evaluated for their cytotoxic and antimicrobial activities. The aglycone moiety was synthesized from *L*-tyrosine methyl ester. This methyl-*L*-tyrosinate (**1**) was further derivatized to afford *N*-fattyacyl-*L*-tyrosine methyl esters (**2-6**) with different fatty acids using EDC-HCl and HOBt coupling reagents (Scheme 1). The glycosylation of aglycone moiety with different carbohydrates was performed using Lewis acid, $BF_3.Et_2O$ (Scheme 2 and 3). The glucose and galactose *N*-fattyacyl-*L*-tyrosines were formed in β–configuration and the mannose and rhamnose *N*-fattyacyl-*L*-tyrosines were formed in α–

configuration due to NGP participation of C2-acetate group of the sugar during the glycosylation.



Scheme 2. Reagents and conditions : i) Compounds **2 - 5,** $BF_3.Et_2O$, DCM, rt, over night ; ii) LiOH, THF, rt ,16 h; iii) 2N HCl, 65-77 %



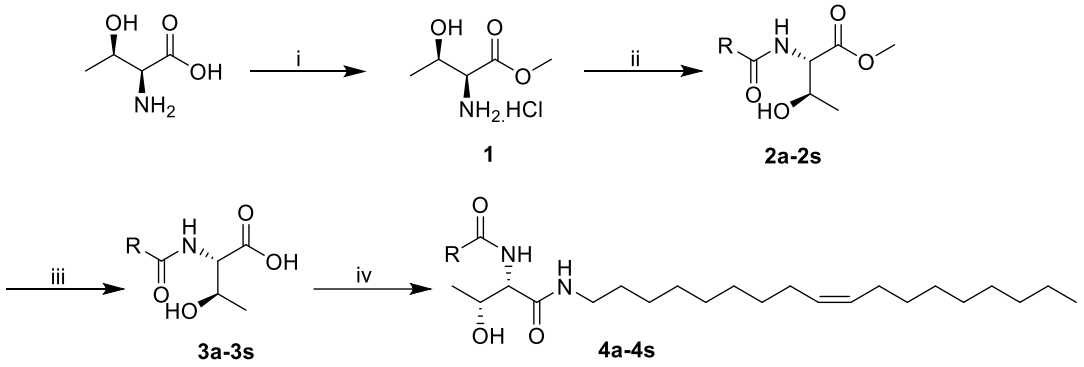
Scheme 3. Reagents and conditons: i) A) a or b, $BF_3.Et_2O$, DCM, rt, over night ; B) NaOMe, MeOH, 70- 75% ii) A) c or d, $BF_3.Et_2O$, DCM, rt, over night ; B) NaOMe, MeOH, 70 - 75%.

All the synthesized compounds were tested against a panel of four cancer cell lines, namely, A549, PC3, MDA-MB-231 and HepG2. Among all the tested compounds, glycosylated *N*-fattyacyl-*L*-tyrosines showed moderate activity against all the cell lines irrespective of carbohydrate and lipid moiety and the IC_{50} values were in the range of 15.6-45.6 µM. However, the oleic acid analogues (**5a, 5d**) exhibited minimum IC_{50} values of 15.6 and 17.6 µM, respectively, against MDA-MB-231 cell line. In case of *N*-lipoyl-*L*-tyrosine methyl esters, **6b-6d** showed promising activity against all the tested cell lines and the IC_{50} values ranged between 9.4-13.8 µM. The compound **6d** exhibited significant cytotoxicity with IC_{50} values 10.5, 9.4, 10.9 and 12.1 µM against A549, PC3, MDA-MB-231 and HepG2 cell lines respectively. It was observed that among all the synthesized compounds, *N*-fattyacyl-*L*-tyrosine methyl esters (**2-6**) showed a poor antimicrobial activity against both bacterial and fungal strains. However, *N*-fattyacyl-*L*-tyrosines (**7-11**) exhibited promising antimicrobial activity against both bacterial and fungal strains.

Chapter 4: Design, Synthesis and Cytotoxic Evaluation of Threonine-based Galacto-ceramide with Aromatic Groups and Various Fattyacyl Chains

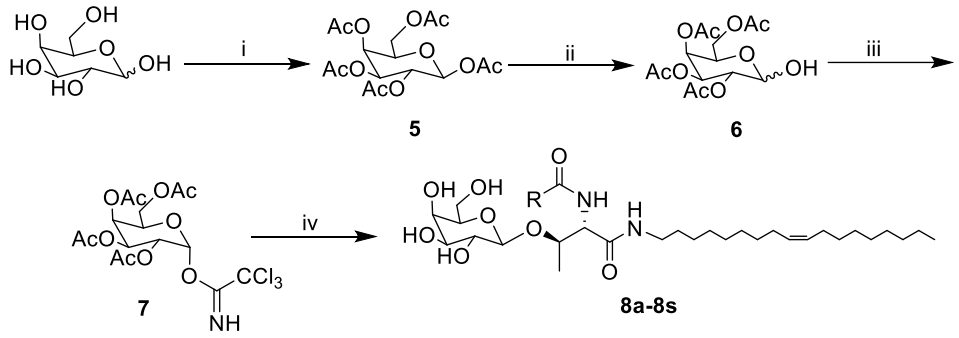
Galactoceramide and its derivatives have been identified as potential anti-tumour compounds. From the last two decades, α-GalCer and its synthetic analogues were gained much attention in the scientific community due to their high potential antitumor activity in the view of versatile utility as invariant natural killer T (iNKT) cell activators and adjuvants in many diseases like malaria, HIV, tuberculosis, tumour immunotherapy. Moreover, the Phase I clinical trials proved that α-GalCer was ineffective in the treatment of solid tumors due to less selectivity toward either Th1 or Th2 cytokines (hormonal messengers) responses. Therefore, new α-GalCer analogues will require increasing toward either Th1 or Th2 cytokines responses. In addition, the SAR study reported in literature on α-GalCer showed that modification on the sphingosine OH groups leads to only change in the binding effect but not destroy the total activity of α-GalCer. Hence, the modification of fatty acyl group on amide moiety or phytosphingosine group allowed for more selective Th2 response.

Based on the above findings, in the present study, threonine-based β-galactoceramide and its derivatives were prepared by modifying the fatty acyl group on amide moiety with different unusual, branched, saturated and unsaturated fattyacyl moieties and aromatic acids employing trichloroacetimidate methodology.



R = Cinnamoyl (**2a, 3a, 4a**), 4-Nitro cinnamoyl (**2b, 3b, 4b**), Indole-3-acetanoyl (**2c, 3c, 4c**), Indole-3-propanoyl (**2d, 3d, 4d**), Indazole-3-cabonyl (**2e, 3e, 4e**), Indole-2-cabonyl (**2f, 3f, 4f**), Phenyl acetonyl (**2g, 3g,4g**), 4-Methoxy phenylacetonyl (**2h, 3h, 4h**), 4-Methoxy phenyl butanoyl (**2i, 3i, 4i**), Hexanoyl (**2j, 3j, 4j**), Octanoyl (**2k, 3k, 4k**), 2-Propylpentanoyl (**2l, 3l, 4l**), Undecenoyl (**2m, 3m, 4m**), Undecanoyl (**2n, 3n, 4n**), Dodecanoyl (**2o, 3o, 4o**), Tetradecanoyl (**2p, 3p, 4p**), Hexadecanoyl (**2q, 3q, 4q**), Octadecanoyl (**2r, 3r, 4r**), Oleoyl (**2s, 3s, 4s**)

Scheme 1. Reagents and conditions: i) $SOCl_2$, MeOH, rt, 3 h, 98%; ii) Acid, EDC, HOBT, DCM, rt, Overnight, 78 - 84%; iii) $LiOH.H_2O$, THF, H_2O, rt, 16 h, 88 - 95%; iv) Oleyl Amine, EDC, HOBT, DCM, rt, Overnight, 75 - 80%.



R = Cinnamoyl (**8a**), 4-Nitro cinnamoyl (**8b**), Indole-3-acetanoyl (**8c**), Indole-3-propanoyl (**8d**), Indazole-3-cabonyl (**8e**), Indole-2-cabonyl (**8f**), Phenyl acetonyl (**8g**), 4-Methoxy phenylacetonyl (**8h**), 4-Methoxy phenyl butanoyl (**8i**), Hexanoyl (**8j**), Octanoyl (**8k**), 2-Propylpentanoyl (**8l**), Undecenoyl (**8m**), Undecanoyl (**8n**), Dodecanoyl (**8o**), Tetradecanoyl (**8p**), Hexadecanoyl (**8q**), Octadecanoyl (**8r**), Oleoyl (**8s**)

Scheme 2. Reagents and conditions: i) NaOAc, Ac_2O, reflux, 4 h, 92%; ii) Benzyl amine, THF, rt, 20 h, 84% ; iii) CCl_3CN, DBU, DCM, rt, 2.5 h, 77%; iv) **4(a-s),** TMSOTf, DCM, rt, 12 h, NaOMe, MeOH, Amberlite-IR-120, rt, 1 h, 65 - 74%.

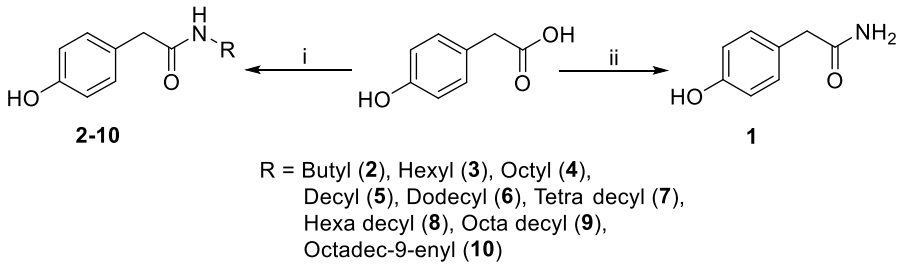
The threonine-based ceramide part was synthesized (Scheme 1) in multi-step process using different reagents. This ceramide part was glycosylated with galactose using trichloroacetimidate (Scheme 2) as donor. Further, all the synthesized compounds were evaluated for *in vitro* cytotoxicity against three cancer cell lines and one normal cell line. All the compounds exhibited moderate to good activity against all the tested cancer cell lines

with IC_{50} values in the range 14.08 to 64.05 µM. In aromatic derivatives, compound **8i** exhibited promising activity against MCF7, A549 and HeLa cancer cell lines with IC_{50} values 14.08, 14.78 and 16.70 µM, respectively. In fatty acid derivatives, the compound **8m** and compound **8n** exhibited a promising activity against HeLa and MCF7 cancer cell lines with IC_{50} values 16.34 and 18.05 µM, respectively. Based on structure activity relationship, aromatic acid derivatives exhibited better activity compared to fatty acid derivatives. As well, influence of some of the key factors such as spacer chain length between aromatic residue and amide functional group, methoxy substituents on aryl group, terminal unsaturation of fatty acid and branching chain effect on the cytotoxicity was described in this study.

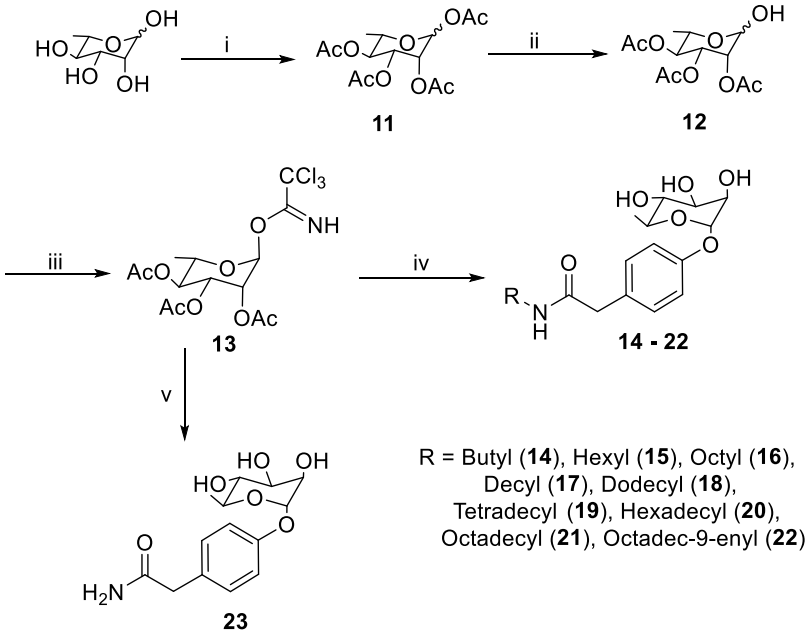
Chapter 5: Synthesis and Biological Evaluation of Phenolic, Thio and Steryl Glycosides

Section A: Synthesis and Biological Evaluation of Marumoside A and its Lipid Derivatives

Moringa oleifera (horseradish tree) is a fast growing, multipurpose tree native to tropical and subtropical areas, for human, animal feeding and medicinal uses. The green pods, flowers and leaves are used as vegetables in many countries. In folklore medicine, the various parts of the plant have long been recognized for ailments like pain and inflammation. From the different parts of *Moringa oleifera* plant, several types of bioactive compounds have been isolated. Recently, Marumoside A and other compounds were isolated from the leaves of *Moringa oleifera* of Thai origin. In Marumoside A, 4-hydroxyphenylethanamide was glycosylated at anomeric hydroxyl group of L-rhamnose in α-configuration. Further, 4-hydroxyphenylethanamide is structurally related to bioactive compounds like homovanillic acid amides (Capsaicin) and hydroxyphenylacetamides. These compounds have profound anti inflammatory, antinociceptive, analgesic and antiirritant activities.



Scheme 1. Reagents and conditions: i) Fatty amine, HOBT, EDC. HCl, DCM, rt, overnight, 70%; ii) $(COCl)_2$, Aq.NH_3 sol., DCM, rt, 5 h, 80-90%.



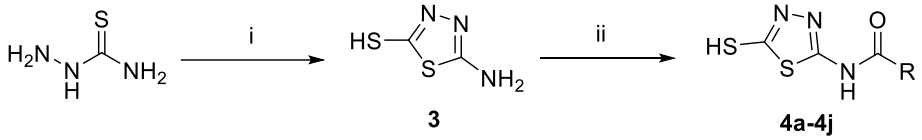
Scheme 2. Reagents and conditions: i) Ac_2O, Pyridine, 12 h, rt, 98%; ii) Hydrazine acetate, DMF, 50 ^oC, 2 h, 92%; iii) CCl_3CN, DBU, DCM, rt, 2 h, 80%; iv) Step 1: **2-10** , TMSOTf, DCM, 4 A^o molecular sieves, 0 ^oC to rt, overnight, Step 2: NaOMe, MeOH, rt, 45 min, 65 - 74%; v) Step 1: **1**, DCM, 4 A^o molecular sieves, 0 ^oC to rt, overnight, Step 2: NaOMe, MeOH, rt, 45 min, 62%.

Considering the above facts, in the present study, Marumoside A was synthesized for the first time using trichloroacetimidate donor as a key step. The aglycone 4-hydroxy phenylacetamide was prepared from 4-hydroxyphenyl acetic acid using oxallyl chloride and aqueous ammonia. The lipid derivatives of Marumoside A were synthesized (Scheme 1 and 2) using different fatty amines. The antiinflammatory activity of the Marumoside A and its lipid derivatives was evaluated for the inhibition of TNF-α and IL-1β secretion levels. Among all the synthesized molecules, the oleyl amine lipid (unsaturated) derivative showed significant inhibition of TNF-α and IL-1β secretion with IC_{50} value of 16.7 µM and 23.4 µM, respectively when compared to Marumoside A and its saturated lipid derivatives.

Section B: Synthesis and Biological Evaluation of 5-Fattyacylamido-1, 3, 4-Thiadiazole-2-Thioglycosides

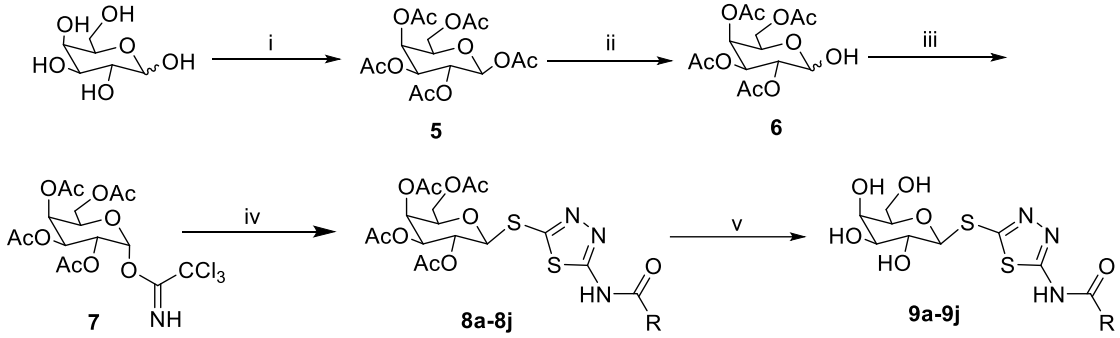
Recently thioglycosides have gained much attention because of their broad spectrum of properties such as antitumour, antidiabetic, biological inhibitors, inducers, *in vitro* inhibitory effects on the replication of a number of DNA viruses. Many of the thioglycoside derivatives are having common glycosylthio moiety attached to carbon linked to nitrogen either in an open chain or cyclic form (heterocyclic ring). These glycosylthio moiety attached to the

heterocyclic ring is known as glycosylthio heterocycles. In these glycosylthio heterocycles, glycone and aglycone (different heterocyclic rings) were modified for extensive biological activities.1, 3, 4-Thiadiazoles and its derivatives having a wide range of biological applications in different fields such as agriculture, petroleum and medicine. Many drugs are available in the market with thiadiazole nucleus such as sulfamethazole, methazolamide, acetazolamide etc.



R = - $(CH_2)_4$-CH_3 (**a**), - $(CH_2)_6$-CH_3 (**b**), - $(CH_2)_8$-CH_3 (**c**), - $(CH_2)_8$-CH=CH_2 (**d**), - $(CH_2)_{10}$-CH_3 (**e**), - $(CH_2)_{12}$-CH_3 (**f**), - $(CH_2)_{14}$-CH_3 (**g**), - $(CH_2)_{16}$-CH_3 (**h**), - $(CH_2)_7$-CH=CH-$(CH_2)_7$-CH_3 (**i**), - $(CH_2)_{11}$-CH=CH-$(CH_2)_7$-CH_3 (**j**).

Scheme 1. Reagents and conditions: i) EtOH, CS_2, reflux, 4 h, 84%; ii) Acid chloride, Et_3N, DCM, rt, 6 h, 80-88%.



Scheme 2. Reagents and conditions: i) Ac_2O, NaOAc, reflux, 4 h, 92%; ii) Hydrazine Acetate, DMF, 50 ^oC, 2 h, 92%; iii) CCl_3CN, DBU, DCM, rt, 3h, 77%; iv) **4a-4j**, TMSOTf, DCM, 4 A^o MS., 0 ^oC to rt, overnight, 72-80%; v) NaOMe, MeOH, Amberlight IR-120, rt, 3 h, 95-97%.

Based on the above facts, in the present study, the synthesis of 1, 3, 4-thiadiazole-based thioglycosides were accomplished in good yields. In Scheme 1, 5-Amino-1, 3, 4-thiadiazole-2-thiol was synthesized and this was followed by synthesis of a series of 5- fattyacylamido-1, 3, 4-thiadiazole-2-thiols with fatty acid chlorides. In Scheme 2, the glycosylation of 5-fattyacylamido-1, 3, 4-thiadiazole-2-thiols were achieved with trichloroacetimidate methodology. All the compounds were in β–configuration. This was supported by ^{1}H-NMR analysis, the signals of H-1′ proton was observed as a doublet at δ = 4.97 ppm, J = 10.07 Hz. Anti microbial and anti cancer activities of 1, 3, 4-thiadiazole-based thioglycosides were

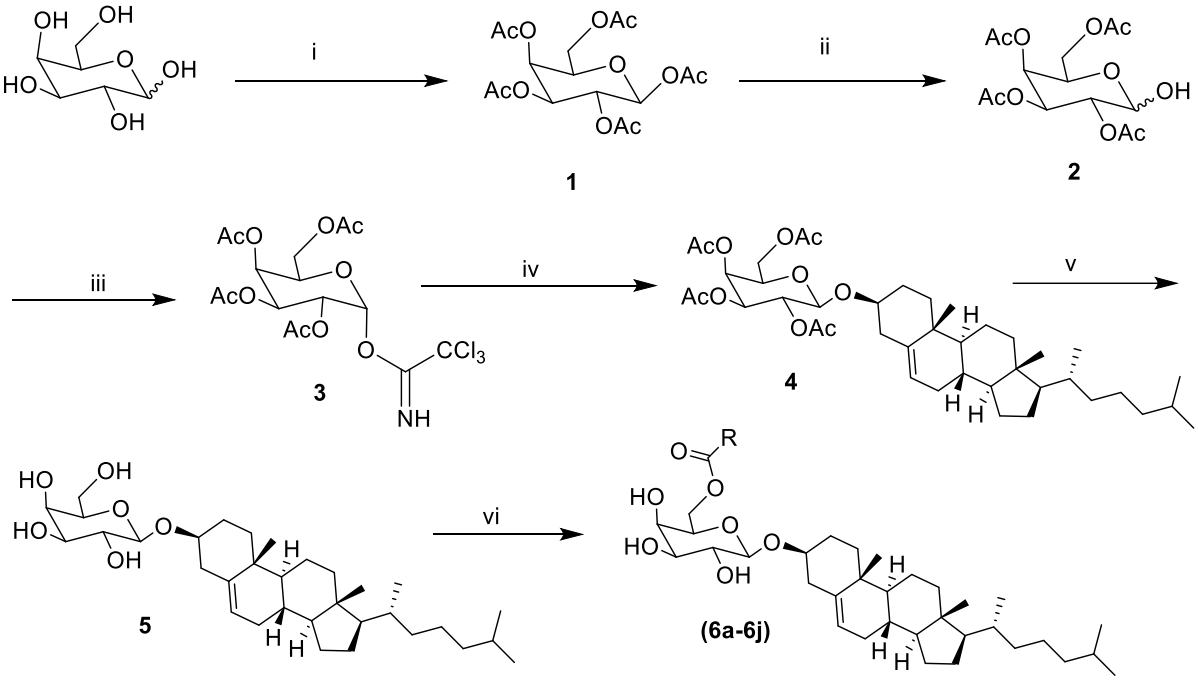
evaluated against three Gram-positive and three Gram-negative bacterial strains and four cancer and one normal cell line. Among the entire tested compounds lauric acid and myristic acid derivatives showed good antimicrobial activity against Gram-negative bacterial strain *Klebsiella pneumonia* with MIC values 12.5, 25 µg/mL and moderate activity exhibited against Gram-positive bacterial strain *Bacillus subtilis* with MIC values 25 and 50 µg/mL, respectively. In case of cytotoxicity, the free hydroxyl lauric (**9e**) and oleic acid derivative (**9i**) exhibited promising activity against cervical cancer cell line with IC_{50} values 8.7, 8.8 µM, respectively. Further, the free hydroxyl compounds **9a, 9c-9j** did not show any toxicity towards normal CHO-K1 cell line and acylated compounds **8a-8j** exhibited toxicity against CHO-K1 cell line.

Section C: Synthesis and Cytotoxic Evaluation of Cholesteryl 6-*O*-acyl-β-D-Galactopyranosides

In recent years, the isolation and/or synthesis of steryl glycosides have been motivated because of their remarkable biological functions, including regulation of host defences against pathogens, lipid metabolism and imunomodulatory properties. These steryl glycosides are widely found in various plants especially vascular plants, fungi, some animals and a few bacteria. Two major glycolipids were isolated from *Borrelia burgdorferi*, in 2001 they were initially identified as galactosyl diacyl glycerols. Later, these glycolipids were corrected as cholesteryl 6-*O*-acyl-β-D-galactopyranoside (BbGL1) and 1, 2-di-*O*-acyl-3-*O*-α-D-galactopyranosyl-*sn*-glycerol (BbGL2) with major fatty acid composition oleate and palmitate. Among these compounds, BbGL1 is a steryl glycoside, it is antigenic lipid component of *Borrelia burgdorferi,* and the research is in progress on these BbGL1 to develop potential vaccines against Lyme disease. Moreover, recent evidence reveals that cholesterol may directly mask glycosphingolipids and it protects the carbohydrate from toxin-derived proteins.

Based on the above findings, in the present study, Cholesteryl 6-*O*-acyl-β-D-galactopyranosides were synthesized using trichloroacetimidate methodology (Scheme 1) with unusual, short, medium, long and unsaturated fatty acids. The anomeric acetyl group of galactose penta acetate was chemo selectively removed by hydrazine acetate and this was followed by preparation of trichloroacetimidate using trichloroacetonitrile in the presence of DBU. Glycosylation of cholesterol was achieved using trichloroacetimidate donor and

TMSOTf as promoter. Acetate groups were selectively deprotected with Zemplen deacylation and subsequently, Cholesteryl 6-β-D-galactopyranoside was selectively esterified at C-6 position with EDC. HCl. All the compounds were evaluated for cytotoxicity against four cancer and one normal cell lines, namely human ovarian cancer (SKOV3), cervical cancer (HeLa), breast cancer (MDAMB-231), human prostate cancer (DU145) cell lines and normal Chinese hamster ovary cancer (CHO-K1) cell line. Among all the tested compounds long chain saturated palmitic and stearic acid derivatives (**6g, 6h**) exhibited significant cytotoxicity against cervical cancer cell line with IC_{50} values 17.3 and 20.3 µM respectively. All the tested compounds did not show any toxicity towards normal CHO-K1 cells.



R = Hexanoyl (**6a**), Octanoyl (**6b**), Decanoyl (**6c**), undecenoyl (**6d**), Dodecanoyl (**6e**), Tetradecanoyl (**6f**), Hexadecanoyl (**6g**), Octadecnoyl (**6h**), Oleoyl (**6i**), Erucioyl (**6j**)

Scheme 1. Reagents & Conditions: i) NaOAc, Ac_2O, reflux, 4 h, 92%; ii) Benzyl amine, THF, rt, 20 h, 84%; iii) CCl_3CN, DBU, DCM, rt, 2.5 h, 77%; iv) Cholesterol, TMSOTf, DCM, rt, 1.5 h, Et_3N, 80%; v) NaOMe, MeOH, DCM, rt, 30 min, 72%; vi) Fatty acid, EDC.HCl, DMAP, DCM, Pyridine, 0 ^oC to rt, 2 days, 45-52%.