ORIGINAL ARTICLE

A C G publications

Org. Commun. 15:1 (2022) 32-43

organic communications

Acyl glucopyranosides: synthesis, PASS predication, antifungal activities and molecular docking

Abul F.M. Sanaullah [1], Mohammed M. Matin [1*], Md. Rezaur Rahman [2] and S. M. Abdul Nayeem [1]

[1] *Bioorganic and Medicinal Chemistry Laboratory, Department of Chemistry, Faculty of Science, University of Chittagong, Chittagong, 4331, Bangladesh*

[2] *Department of Chemical Engineering and Energy Sustainability, Faculty of Engineering, Universiti Malaysia Sarawak, Jalan Datuk Mohammad Musa, Kota Samarahan, 94300, Malaysia*

(Received January 03, 2022; Revised March 12, 2022; Accepted March 13, 2022)

Abstract: Sugar esters (SEs) with fatty acyl chains showed diverse applications including antimicrobial inhibition against multidrug-resistant (MDR) microorganisms. Thus, fatty acid esters, especially 6-*O*-stearoyl glucopyranoside ester was prepared by the treatment of glucopyranoside with unimolar stearoyl chloride at low temperature. The 6-*O*-stearoyl ester thus obtained was further modified to four newer 2,3,4-*O*-acyl esters to incorporate decanoyl, lauroyl, palmitoyl, and benzoyl chains in the glucopyranoside skeleton. Prediction of activity spectrum for substances (PASS) analyses suggested that these fatty acid esters are more prone to fungal pathogens compared to bacterial pathogens. Guided by PASS analyses *in vitro* antifungal activities were screened against four fungal pathogens, which supported the PASS observation. To validate the findings molecular docking was conducted with lanosterol 14α-demethylase (CYP51), a significant fungal enzyme, which is the principal target of antifungal drugs. Corroboration of *in vitro* results with binding affinity revealed the possibility of glucopyranoside-based fatty acyl esters with stearoyl, decanoyl and lauroyl chains as highly potential compared to antifungal azole drugs.

Keywords: Antimicrobial activities; Binding affinity; Docking; Fatty acid esters; D-Glucopyranoside; Regioselectivity.

1. Introduction

In recent years, various efforts for the application of carbohydrate compounds as functionalized and chiral sources for the biological process have been reported [1,2] and bring sugar molecules as scaffolds for medicinally important compounds [3-5]. These are also related to the natural carbohydrates where nature exploits these molecules as scaffolds in building natural molecular architectures. More clearly, the cyclic structures of the available sugar units guarantee an adequate conformational rigidity, and multiple OH groups with chirality allow different positions/orientations for a wide scope for making linkages with the substrates [3,6-7]. Considering all these advantages, a variety of polyfunctional synthetic sugar derivatives are exploited as key platforms for the generation of potentially bioactive compounds [8-10]. These bioactive compounds have comparatively less N, X, etc. instead having more

* Corresponding author: E-Mail: mahbubchem@cu.ac.bd

The article was published by ACG Publications
http://www.acgpubs.org/journal/organic-communications © Jnauary-March 2022 EISSN:1307-6175
DOI: http://doi.org/10.25135/acg.oc.120.2201.2307
Available online: March 16,2022

O atoms which impose more biological relevance [11,12]. Hence, synthetic sugar derivatives like glycoconjugates, glycohybrids, sugar esters (SEs), and neoglycoconjugates are in consideration for the treatment of fatal diseases [13,14].

Among the sugars, acyl and alkyl glycoses, and their esters (SEs) participate in many crucial biological processes with specific interactions with receptors [15-17]. With the diverse biological events, SEs possess many attractive physicochemical properties like non-toxicity, biocompatibility, biodegradability, tasteless, non-allergic, and non-irritating [18,19] leading to their applications in pharmaceuticals, food additives, cosmetics, and personal care products [20,21]. Some SEs have better inhibitory activities against multidrug-resistant (MDR) pathogens [20,22-23]. For example, mannopyranose-6-*O*-lauroate was found to inhibit methicillin-resistant *S. aureus* (MRSA) [20]. SEs like Acteoside (**1**, Figure 1) with an unsaturated ester group showed neuroprotective activity [24] and are considered an important anti-Alzheimer's compound. Glucose-derived acyl sugars confer resistance to some potential insect herbivores indicating their anti-insect activity [25]. Natural and synthetic glucose-derived SEs have many advantages in biological profile as these SEs impart hydrophobicity and alter their functionality [26], and showed improved parameters in food surfactants like HLB value, emulsion stability, foam stability, emulsion stability index, etc. with antimicrobial properties [27-30]. Surface-active glucose ester namely rubiwettin RG1 (**2**) is an important biosurfactant with potential medical, pharmaceutical, and cosmeceutical applications [31].

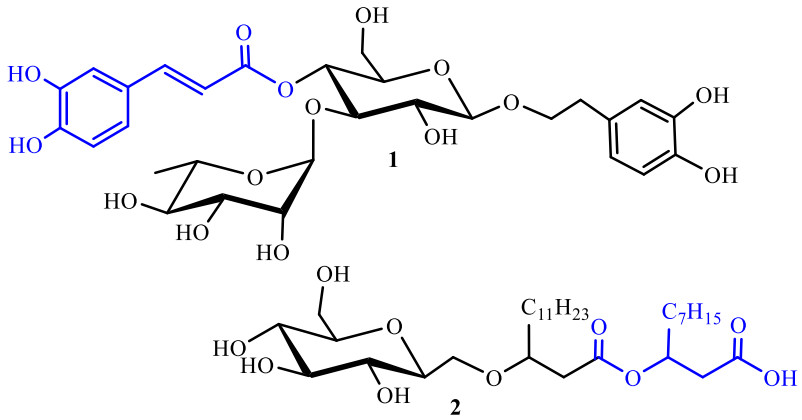


Figure 1. Carbohydrate based bioactive esters.

In the past decades, many acylation methods for sugars have been reported [32,33]. Considering the presence of many 2º OH groups of indistinguishable reactivity, different esterification methods such as direct, catalytic, enzymatic, protection-deprotection, microwave-assisted technique, etc. are used [34-41]. Of these methods, here direct method is used for monostearoylation of glucopyranoside **3**.
Fungal infections along with SARS-CoV-2 deteriorated the current pandemic situation, which indicated the urgency of new chemotherapeutics. In this perspective and our interest in SEs [42,43], several glucopyranoside-based stearates are synthesized, characterized, and subjected to *in vitro* antifungal assay. The designed biocompatible novel glucose fatty acid esters might improve the fungal inhibitory properties.

2. Experimental

Methyl α- D-glucopyranoside, acylating agents, solvents, and necessary chemicals are purchased from Merck, Germany, and were used directly (solvents were distilled before use). Evaporations were conducted under diminished pressure on a rotary evaporator (Buchi R-100, Switzerland) and temperature was kept ~40 °C. TLC (thin-layer chromatography) was performed with Kieselgel GF_{254} and column chromatography was conducted using silica gel G_{60}. Different ratio of solvents ($CHCl_3$/MeOH, *n*-hexane/EtOAc, etc) was used for TLC and CC. The functional group(s) in the products was detected by FT-IR spectrophotometer (MB 3000, ABB, Canada). Proton (400 MHz) and carbon (100 MHz) NMR spectra were scanned using a Bruker DPX-400 spectrometer (Switzerland) in deuterated chloroform

solution using TMS as an internal standard (δ scale).

2.1. Synthesis

Methyl 6-O-stearoyl-α- D*-glucopyranoside (**4**)*: Unimolar stearoylation of methyl α- D-glucopyranoside (**3**) was conducted by stirring **3** (2.0 g, 10.31 mmol) with stearoyl chloride (4.02 g, 13.27 mmol) in arid pyridine at 0 °C for 12 h. The reaction mixture was shaken for 5 h at normal temperature. Usual workup and chromatography furnished the title compound **4** (2.46 g, 52%) as semi-solid, which withstand crystallization.

R_f = 0.28 ($CHCl_3/CH_3OH$ = 5/1); FT-IR (neat): 3225-3610 (br, OH), 1726 (CO), 1040 cm^{-1} (pyranose ring); ^1H NMR (400 MHz, $CDCl_3$): δ_H 4.82 (*J* = 4.0 Hz, 1H, d, H-1), 4.59 (dd, *J* = 12.4 and 4.0 Hz, 1H, H-6a), 4.24 (dd, *J* = 12.4 and 1.8 Hz, 1H, H-6b), 3.88-3.92 (m, 1H, H-5), 3.76 (t, *J* = 9.2 Hz, 2H, H-3 and H-4), 3.55 (dd, 1H, H-2), 3.46 (s, 3H, OCH_3), 2.40 [t, *J* = 7.6 Hz, 2H, $CH_3(CH_2)_{15}CH_2CO$], 1.71-2.11 (br s, 3H, 3×O*H*), 1.62-1.67 (m, 2H, $CH_3(CH_2)_{14}CH_2CH_2CO$), 1.22-1.37 [br m, 28H, $CH_3(CH_2)_{14}(CH_2)_2CO$], 0.90 [t, *J* = 7.2 Hz, 3H, $CH_3(CH_2)_{16}CO$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.5 ($C_{17}H_{35}CO$), 99.4 (C-1), 74.2, 72.1 (C-2/C-3), 70.2, (C-5), 69.8 (C-4), 63.3 (C-6), 55.3 (OCH_3), 34.2 [$CH_3(CH_2)_{15}CH_2CO$], 31.9 [$CH_3(CH_2)_{14}CH_2CH_2CO$], 29.7(4), 29.6(3), 29.5, 29.4, 29.3, 29.2, 29.1 [$CH_3(CH_2)_2(CH_2)_{12}(CH_2)_2CO$], 24.9 [$CH_3CH_2CH_2(CH_2)_{14}CO$], 22.7 [$CH_3CH_2(CH_2)_{15}CO$], 14.1 [$CH_3(CH_2)_{16}CO$].

*General procedure for 2,3,4-tri-O-acylation of **4***: To a stirred solution of the 2,3,4-triol **4** (0.1 g, 0.217 mmol) in pyridine (1 mL) was put on acyl halides (decanoyl/lauroyl/palmitoyl/benzoyl chloride; 3.3 molar eq) at 0 °C. After 30 min, the reaction mixture was brought into normal temperature. Stirring continued for 10 h at 25 °C followed by 1 h at 45 °C. The unreacted reagent(s) was hydrolyzed by the addition of a few drop of cooled water. The reaction mixture was extracted with dichloromethane (3×5 mL). The combined solvent extract was successively washed with dil. hydrochloric acid, saturated aq. $NaHCO_3$ solution, and brine. The chloroform layer was desiccated over $MgSO_4$, filtered, and the filtrate was evaporated off. The resulting syrup was purified by silica gel chromatography (with *n*-hexane/ethyl acetate = 16/1 as eluant) to furnish the corresponding tri-*O*-acylates **5a-d**.

Methyl 2,3,4-tri-O-decanoyl-6-O-stearoyl-α- D*-glucopyranoside (**5a**)*: Oil; yield 78%; R_f = 0.68 (*n*-hexane/ethyl acetate = 5/1); FT-IR (neat): 1755, 1750, 1738, 1735 (CO), 1088 cm^{-1} (pyranose ring); ^1H NMR (400 MHz, $CDCl_3$): δ_H 5.52 (t, *J* = 10.0 Hz, 1H, H-3), 5.09 (t, *J* = 10.0 Hz, 1H, H-4), 4.97 (d, *J* = 3.6 Hz, 1H, H-1), 4.90 (dd, *J* = 10.4 and 3.6 Hz, 1H, H-2), 4.22 (dd, *J* = 12.4 and 4.8 Hz, 1H, H-6a), 4.13 (dd, *J* = 12.4 and 2.0 Hz, 1H, H-6b), 3.97-4.02 (m, 1H, H-5), 3.42 (s, 3H, OCH_3), 2.22-2.39 [m, 8H, $CH_3(CH_2)_{15}CH_2CO$ and 3×$CH_3(CH_2)_7CH_2CO$], 1.52-1.70 [m, 16H, $CH_3(CH_2)_{13}(CH_2)_2CH_2CO$) and 3×$CH_3(CH_2)_5(CH_2)_2CH_2CO$], 1.22-1.38 [br m, 56H, $CH_3(CH_2)_{13}(CH_2)_3CO$ and 3×$CH_3(CH_2)_5(CH_2)_3CO$], 0.90 [t, *J* = 6.8 Hz, 12H, $CH_3(CH_2)_{16}CO$ and 3×$CH_3(CH_2)_8CO$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.4, 172.9, 172.6, 172.2 ($C_{17}H_{35}CO$ and 3×$C_9H_{19}CO$), 96.9 (C-1), 70.8, 69.7 (C-2/C-3), 68.4, (C-5), 67.4 (C-4), 61.9 (C-6), 55.4 (OCH_3), 34.2, 34.1(2), 34.0 [$CH_3(CH_2)_{15}CH_2CO$ and 3×$CH_3(CH_2)_7CH_2CO$], 31.9(4) [$CH_3(CH_2)_{14}CH_2CH_2CO$ and $CH_3(CH_2)_6CH_2CH_2CO$], 29.7(8), 29.6(2), 29.5, 29.4(5), 29.3(4), 29.2(2), 29.1, 29.0 [$CH_3(CH_2)_2(CH_2)_{12}(CH_2)_2CO$ and 3×$CH_3(CH_2)_2(CH_2)_4(CH_2)_2CO$], 24.9(2), 24.8(2) [$CH_3CH_2CH_2(CH_2)_{14}CO$ and 3×$CH_3CH_2CH_2(CH_2)_6CO$], 22.7, 22.6(3) [$CH_3CH_2(CH_2)_{15}CO$ and 3×$CH_3CH_2(CH_2)_7CO$], 14.1, 14.0(3) [$CH_3(CH_2)_{16}CO$ and 3×$CH_3(CH_2)_8CO$].

Methyl 2,3,4-tri-O-lauroyl-6-O-stearoyl-α- D*-glucopyranoside (**5b**)*: Syrup; yield 75%; R_f = 0.74 (*n*-hexane/ethyl acetate = 5/1); FT-IR (neat): 1750(2), 1742(2) (CO), 1080 cm^{-1} (pyranose ring); ^1H NMR (400 MHz, $CDCl_3$): δ_H 5.52 (t, *J* = 10.0 Hz, 1H, H-3), 5.09 (t, *J* = 10.0 Hz, 1H, H-4), 4.97 (d, *J* = 3.6 Hz, 1H, H-1), 4.90 (dd, *J* = 10.4 and 3.6 Hz, 1H, H-2), 4.23 (dd, *J* = 12.4 and 4.8 Hz, 1H, H-6a), 4.14 (dd, *J* = 12.4 and 2.0 Hz, 1H, H-6b), 3.96-4.03 (m, 1H, H-5), 3.41 (s, 3H, OCH_3), 2.20-2.39 [m, 8H, $CH_3(CH_2)_{15}CH_2CO$ and 3×$CH_3(CH_2)_9CH_2CO$], 1.51-1.68 [m, 16H, $CH_3(CH_2)_{13}(CH_2)_2CH_2CO$) and 3×$CH_3(CH_2)_7(CH_2)_2CH_2CO$], 1.22-1.38 [br m, 68H, $CH_3(CH_2)_{13}(CH_2)_3CO$ and 3×$CH_3(CH_2)_7(CH_2)_3CO$], 0.90 [t, *J* = 6.8 Hz, 12H, $CH_3(CH_2)_{16}CO$ and 3×$CH_3(CH_2)_{10}CO$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.4, 172.9, 172.6, 172.2 ($C_{17}H_{35}CO$ and 3×$C_{11}H_{23}CO$), 96.9 (C-1), 70.8, 69.7 (C-2/C-3), 68.4, (C-5), 67.4 (C-4), 61.9 (C-6), 55.4 (OCH_3), 34.2, 34.1(2), 34.0 [$CH_3(CH_2)_{15}CH_2CO$ and 3×$CH_3(CH_2)_9CH_2CO$], 31.9(4) [$CH_3(CH_2)_{14}CH_2CH_2CO$ and 3×$CH_3(CH_2)_8CH_2CH_2CO$], 29.7(8), 29.6(5), 29.5(3), 29.4(5), 29.3(4), 29.2(2), 29.1(2), 29.0 [$CH_3(CH_2)_2(CH_2)_{12}(CH_2)_2CO$ and 3×$CH_3(CH_2)_2(CH_2)_6(CH_2)_2CO$], 24.9(2), 24.8(2) [$CH_3CH_2CH_2(CH_2)_{14}CO$ and 3×$CH_3CH_2CH_2(CH_2)_8CO$], 22.7(4) [$CH_3CH_2(CH_2)_{15}CO$ and

3×$CH_3CH_2(CH_2)_9CO$], 14.1(4) [$CH_3(CH_2)_{16}CO$ and 3×$CH_3(CH_2)_{10}CO$].

Methyl 2,3,4-tri-O-palmitoyl-6-O-stearoyl-α- D*-glucopyranoside (**5c**)*: Syrup; yield 69%; R_f = 0.53 (*n*-hexane/ethyl acetate = 5/1); FT-IR (neat): 1748, 1741(3) (CO), 1068 cm^{-1} (pyranose ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.52 (t, *J* = 10.0 Hz, 1H, H-3), 5.09 (t, *J* = 10.0 Hz, 1H, H-4), 4.97 (d, *J* = 3.6 Hz, 1H, H-1), 4.90 (dd, *J* = 10.4 and 3.6 Hz, 1H, H-2), 4.22 (dd, *J* = 12.4 and 4.8 Hz, 1H, H-6a), 4.13 (dd, *J* = 12.4 and 1.2 Hz, 1H, H-6b), 3.97-4.04 (m, 1H, H-5), 3.42 (s, 3H, OCH_3), 2.22-2.44 [m, 8H, $CH_3(CH_2)_{15}CH_2CO$ and 3×$CH_3(CH_2)_{13}CH_2CO$], 1.53-1.72 [m, 16H, $CH_3(CH_2)_{13}(CH_2)_2CH_2CO$) and 3×$CH_3(CH_2)_{11}(CH_2)_2CH_2CO$], 1.23-1.38 [br m, 92H, $CH_3(CH_2)_{13}(CH_2)_3CO$ and 3×$CH_3(CH_2)_{11}(CH_2)_3CO$], 0.90 [t, *J* = 6.8 Hz, 12H, $CH_3(CH_2)_{16}CO$ and 3×$CH_3(CH_2)_{14}CO$].

*Methyl 2,3,4-tri-O-benzoyl-6-O-stearoyl-α-D-glucopyranoside (**5d**)*: Pasty mass; yield 87%; R_f = 0.59 (*n*-hexane/ethyl acetate = 5/1); FT-IR (neat): 1748, 1732, 1728(2) (CO), 1066 cm^{-1} (pyranose ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.86-8.06 (m, 5H, Ar-*H*), 7.48-7.59 (m, 3H, Ar-*H*), 7.32-7.46 (m, 7H, Ar-*H*), 6.16 (t, *J* = 9.6 Hz, 1H, H-3), 5.61 (t, *J* = 9.6 Hz, 1H, H-4), 5.30 (dd, *J* = 10.0 and 3.6 Hz, 1H, H-2), 5.26 (d, *J* = 3.6 Hz, 1H, H-1), 5.17-5.22 (m, 1H, H-5), 4.28-4.33 (m, 2H, H-6a and H-6b), 3.50 (s, 3H, OCH_3), 2.35 [t, *J* = 7.6 Hz, 2H, $CH_3(CH_2)_{15}CH_2CO$], 1.58-1.68 (m, 2H, $CH_3(CH_2)_{14}CH_2CH_2CO$), 1.22-1.38 [br m, 28H, $CH_3(CH_2)_{14}(CH_2)_2CO$], 0.90 [t, *J* = 7.2 Hz, 3H, $CH_3(CH_2)_{16}CO$]; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.4 ($C_{17}H_{35}CO$), 165.8, 165.7, 165.2 (C_6H_5CO), 133.4, 133.3, 133.1, 129.9(2), 129.8(2), 129.7(2), 129.3, 129.1, 129.0, 128.4(4), 128.3(2) (Ar-*C*), 97.1 (C-1), 72.0, 70.4 (C-2/C-3), 69.4, (C-5), 67.6 (C-4), 62.4 (C-6), 55.6 (OCH_3), 34.0 [$CH_3(CH_2)_{15}CH_2CO$], 31.9 [$CH_3(CH_2)_{14}CH_2CH_2CO$], 29.7, 29.6(3), 29.5(2), 29.4, 29.3(2), 29.2, 29.1, 29.0 [$CH_3(CH_2)_2(CH_2)_{12}(CH_2)_2CO$], 24.8 [$CH_3CH_2CH_2(CH_2)_{14}CO$], 22.7 [$CH_3CH_2(CH_2)_{15}CO$], 14.1 [$CH_3(CH_2)_{16}CO$].

2.2. PASS Calculation

Numerous *in silico* methods for predicting biological and toxicity properties of molecules are developed. These are relying on the data-based approaches, similarity comparison, QSAR (quantitative structure−activity relationship), 3D QSAR, pharmacophore modeling, etc. [44,45]. Most of them are not freely available which limits their use in the scientific community. Thus, for the biological activity search freely available resource PASS (Prediction of Activity Spectra for Substances) software was applied (http://www.way2drug.com/passonline/) [46,47]. In this respect, all the compounds' structure was drawn with the ChemDraw 18.0, followed by conversion into their SD file format. This format was used to predict the biological spectrum like antibacterial, antifungal, anticarcinogenic, and membrane permeability inhibition properties using PASS software. The predicted results are mentioned as Pa (probability for active substance) and Pi (probability for inactive substance) on the scale of 0.000 to 1.000.

2.3. Antifungal Activity Evaluation

For *in vitro* antifungal susceptibility testing, the food poisoning technique was employed [48]. Necessary test tube cultures of the tested fungal pathogens were collected from the Microbiology Laboratory, University of Chittagong, Bangladesh. Sabouraud medium (agar and broth, PDA) was employed for the culture of fungi. The results are measured after 3~5 days of incubation as linear mycelial growth of the fungus. In general, the percentage susceptibility of radial mycelial growth of the fungal organisms was computed using the formula: $I = \left\{\frac{(C-T)}{C}\right\} \times 100$

where, I = percentage of inhibition, C = diameter of the fungal colony in control (DMF), T = diameter of the fungal colony in treatment. To validate and compare antifungal efficacy, standard antifungal antibiotic nystatin (100 µg/mL medium) was tested under similar conditions.

2.4. Molecular Docking

Most of the antifungal drugs (azoles, polyenes, etc.) are targeted to inhibit the lanosterol C-14 α-demethylase (a cytochrome P450 [CYP450] enzyme). Thus, related protein 3LD6 was selected for

molecular docking.

2.4.1. Ligand Preparation

Structures of the compounds are drawn in ChemDraw with appropriate geometry. Each of them is optimized by DFT (RB3LYP, 6-31G, *d,p*) [49]. The optimized structure of each of the compounds is then saved as a PDB file format and used as ligands.

2.4.2. Protein Preparation

The selected protein structure of human lanosterol 14alpha-demethylase (CYP51, PDB ID: 3LD6) was downloaded as a three-dimensional (3D, Resolution: 2.80 Å) crystal structure from RSCB Protein Data Bank (PDB) [50]. The 3LD6 PDB file is opened in Discovery Studio followed by removal of H_2O and ketoconazole chain (present as docked with 3LD6 in PDB), and saving as a PDB file. Its energy is then minimized in software called Swisspdb.

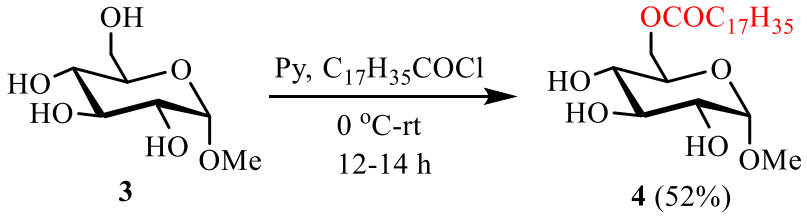
2.4.3. Molecular Docking

To know the binding affinity of glucopyranosides with 3LD6 protein, their bonding interaction, bonding mode molecular docking was performed. Initially, both the ligands and protein are loaded in PyMOL software (PyMOL V2.3; https://pymol.org/2/) [51]. Protein (3LD6) is marked as a macromolecule. Ligands (compounds) are made energy minimized and converted to the corresponding pdbqt file formats. Both the protein and ligand are forwarded to AutodockVina wizard with maximum grid box size to cover to wrap the protein's substrate-binding region. For example, the grid center points for **5a** were set in X = 38.7752 Å, Y = -1.2252 Å, Z = -3.8245 Å, and the dimension in X = 61.8917883587 Å, Y = 61.1866195297 Å, Z = 70.6893203354 Å. Docking result files are saved and opened in the BIOVIA Discovery Studio Visualizer 2017 for necessary 2D and 3D interaction analyses.

3. Results and Discussion

3.1. Selective Stearoylation: Formation of 6-O-stearoyl-glucopyranoside

Selective stearoylation of methyl α- D-glucopyranoside (**3**) was conducted by stirring **3** with unimolar stearoyl chloride at 0 °C for 12 h, which gave a semi-solid in 52% (Scheme 1). The appearance of a carbonyl stretching at 1726 cm^{-1} in its FT-IR spectrum informed the attachment of the stearoyl group in the molecule. The existence of additional thirty-five aliphatic protons in its ^{1}H NMR and related carbon signals in its ^{13}C NMR spectrum confirmed the attachment of one stearoyl group in the molecule. The substantial downfield shift of H-6 protons (at δ 4.59 and 4.24 ppm) was indicative of bonding of the acyl group at the C-6 position of the glucopyranoside structure. Thus, this product was designated as methyl 6-*O*-stearoyl-α- D-glucopyranoside (**4**).



3 **4** (52%)

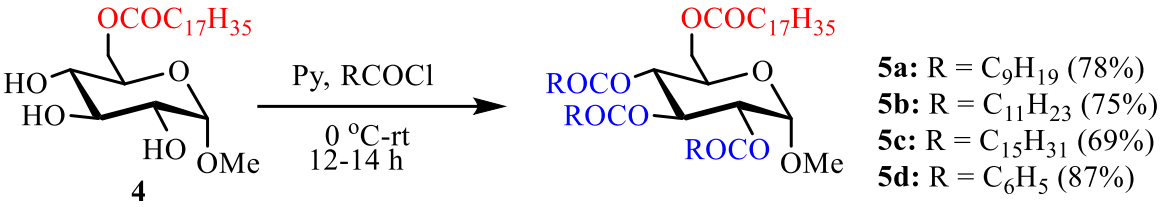
Scheme 1. Selective stearoylation of glucopyranoside **3**

The formation of **4** in lower yield (52%) was anticipated for the appearance of the inseparable complex mixture during stearoylation reaction at 0 °C. However, 6-*O*-stearate **4** indicated the better reactivity of the primary OH group compared to secondary OH groups as present in the glucopyranoside **3**. The similar compound **4** was also prepared by Xia and Hui [52]. In their procedure, treatment of **3** with S-stearoyl-*p*-nitrothiophenol in pyridine for 12 h followed by treatment with acetic acid gave **4** in lower

yield (59.8%). The reaction conditions are harsher than that we reported here.

*3.2. Derivatization of Stearate **4***

Glucose esters with different chain length maintaining HLB are known to have different applications [27,24,53,54]. Thus, having stearate **4** in hand, 2,3,4-tri-*O*-acylation was conducted by the direct method in pyridine (Scheme 2). Initial treatment of **4** with decanoyl chloride furnished an oil (Scheme 2). Its FT-IR spectrum indicated the absence of OH bands indicating complete decanoylation. In its ^{1}H NMR spectrum, fifty-seven additional aliphatic protons resonated compared to the stearate **4**, which clearly informed the attachment of three decanoyl groups in this molecule. The fact was again confirmed by analyzing its ^{13}C NMR spectrum where four carbonyl carbons appeared at δ 173.4, 172.9, 172.6, and 172.2. A careful observation indicated that H-2 (δ 4.90), H-3 (δ 5.52), and H-4 (δ 5.09) shifted considerably downfield than its precursor **4**, thereby confirming the attachment of decanoyl groups at C-2, C-3 and C-4 positions, respectively. Thus, the structure of the oil is named methyl 2,3,4-tri-*O*-decanoyl-6-*O*-stearoyl-α-D-glucopyranoside (**5a**).



Scheme 2. 2,3,4-Tri-*O*-acyl esters of stearate **4**

Similarly, the separate treatment of stearate **4** with lauroyl chloride, palmitoyl chloride, and benzoyl chloride furnished the corresponding acylate **5b**, **5c**, and **5d**, respectively (Scheme 2). All of these pure compounds were characterized by spectroscopic methods.

*3.3. PASS Analysis of Compounds **3-5***

Prediction of activity spectra for substances (PASS) program is used for the analyses of a plethora of biological spectrum of a new compound(s) with safety and efficacy [46]. Online-based this program is available (http://www.pharmaexpert.ru/ PASSonline/index.php). With the help of PASS, any researcher can choose the synthesized new molecule for the selection of the most desirable/suitable biological activities. In short, PASS helps for selecting the prospective compounds for high throughput screening, and hence, saves time and cost. PASS results of the glucopyranoside compounds in the form of Pa (probability for active compound) and Pi (probability for inactive compound) are presented in Table 1.

Table 1. PASS-based biological calculations of glucose eaters

Drug	Biological activity analysis							
	Antibacterial		Antifungal		Anti-carcinogenic		MPI	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
3	0.514	0.013	0.628	0.016	0.731	0.008	0.917	0.003
4	0.528	0.014	0.669	0.012	0.769	0.006	0.954	0.002
5a	0.551	0.012	0.673	0.011	0.614	0.012	0.929	0.003
5b	0.551	0.012	0.673	0.011	0.614	0.012	0.929	0.003
5c	0.551	0.012	0.673	0.011	0.614	0.012	0.929	0.003
5d	0.505	0.016	0.642	0.014	0.632	0.011	0.911	0.003
NYS	0.967	0.000	0.986	0.000	0.416	0.028	0.959	0.000
FCZ	--	--	0.726	0.008	--	--	--	--
ICZ	--	--	0.846	0.003	--	--	--	--

NYS = nystatin; FCZ = fluconazole; ICZ = itraconazole; MPI = Membrane permeability inhibitor; Pa>0.7 indicates higher probability of activity experimentally.

Analyzed Pa for antibacterial 0.50<Pa<0.55 and Pa for antifungal 0.62<Pa<0.67 (Table 1) clearly indicated the higher antifungal nature of the glucopyranoside compounds. Although addition of one stearoyl group increases anti-carcinogenic properties (as in **4**, Pa = 0.769) in the glucopyranoside **3** (Pa =

0.731), further incorporation of acyl group(s) decrease anti-carcinogenic potentiality (**5a-d**, Pa = 0.61-0.63). However, all the glucopyranoside esters are found excellent membrane permeability inhibitors (Pa>0.91) and are comparable to the standard drug nystatin. The rate of passive diffusion of molecules through the membrane is termed membrane permeability. During stress or ischemia, intracellular Na^{+} and Ca^{++} can pass rapidly through the mitochondrial membrane and overloaded in its inner part resulting in cell death. In this regard, membrane permeability inhibitors (e.g. cyclosporine A) are used [55,56]. The higher Pa values of glucose esters indicate their significance in this field.

3.4. Antifungal Efficacy of Glucose Fatty Acid Esters

Azole drugs like fluconazole and itraconazole are found comparatively safer than amphotericin B in several opportunistic and endemic fungal infections [57]. However, these drugs face inactivity due to the emerging drug-resistant fungi [58].

Table 2. Antifungal efficacy of the glucose esters

Compoun	Fungal mycelial growth inhibition in % (100 µg dw/ mL PDA)			
d	***A. niger***	***F. equiseti***	***M. phaseolina***	***P. ascomycetous***
3	**--**	**--**	**--**	**--**
4	**16.32±3.05**	**28.72±1.15**	**23.52±1.00**	**77.89±3.78***
5a	**27.65±3.46**	**38.89±2..00**	**73.86±1.52***	**78.39±0.58***
5b	**28.36±1.15**	20.00±2.00	**49.01±1.00**	**65.32±2.64***
5c	**53.19±1.00**	**26.86±1.53**	**71.24±0.57***	79.39±0.57*
5d	34.75±1.15	**17.58±2.08**	78.43±1.00*	**75.38±4.16***
Nystatin	**71.63±2.45***	**55.48±2.97***	**76.77±1.13***	**79.99±1.53***

* = good inhibition; nystatin = standard antibiotic, dw = dry weight, for rigorous validation the results are compared with nystatin.

To overcome such a threat, alternative antifungals with enhanced efficacy and fungal-specific adjuvants are essential. Many sugar esters are reported to possess antifungal potentiality [59]. Thus, the synthesized glucopyranoside stearates **4-5a,b,c,d** are tested *in vitro* against four fungi (*Aspergillus niger*, *Fusarium equiseti*, *Macrophomina phaseolina*, and *Penicillium ascomycetous*), and susceptibility testing results are summarized in Table 2 and, it clearly shows the effectiveness of glucose stearates against the four tested fungi. With the increase of lipophilic nature (**5a-d**) the antifungal susceptibility gradually increased. The glucopyranoside esters are especially found more susceptible against *M. phaseolina* and *P. ascomycetous* compared to *A. niger* and *F. equiseti*. Such excellent results may help these esters to establish themselves as promising antifungal agents instead of azoles**.**

3.5. Molecular Docking with Lanosterol 14α-Demethylase (CYP51)

In recent years, molecular docking appeared as a significant tool for drug discovery compared with traditional experimental high-throughput screening (HTS). In this approach, behavior/interactions of the compounds with target proteins can be studied and understand the comprehensive insight of the biochemical behavior in the 3D model [60,61]. As the glucopyranoside esters of the present study showed better antifungal susceptibility (Section 3.4), the fact is further verified by molecular docking. In most cases, antifungal drugs are designed to inhibit lanosterol 14α-demethylase (CYP51) as it plays a crucial step in the conversion of lanosterol to ergosterol in the fungal cell membrane [62]. So, lanosterol 14α-demethylase related protein 3LD6 was selected for molecular docking of the synthesized compounds and the obtained results are mentioned in Table 3. Table 3 shows that the binding affinity of compounds **5a** (-7.4 kcal/mol) and **5b** (-7.7 kcal/mol) is higher than the other glucopyranosides (**3**, **4**, **5c,d**) and comparable to the standard drug fluconazole (-7.5 kcal/mol). However, the binding affinities are lower than the ketoconazole (-10.2 kcal/mol). A careful observation indicated that the attachment of the lipophilic stearoyl group at the C-6 position of **3** (as in **4**) increases its binding affinity (-6.7 kcal/mol). Further addition of decanoyl groups (10C) (as in **5a**, -7.4 kcal/mol) and lauroyl groups (12C) (as in **5b**, -7.7 kcal/mol) increase its binding affinity to an excellent level. However, further elongation of chain length to the palmitoyl group (16C) decreases its binding affinity (as in **5c**, -4.9 kcal/mol). Even the addition of the benzoyl group at C-2, C-3, and C-4 positions (**5d**) didn't improve considerable binding affinity (-5.8

kcal/mol). Thus, 6-*O*-stearate with 2,3,4-tri-*O*-acylates having chain lengths 10C (**5a**) and 12C (**5a**) are found to show better antifungal susceptibility. Both the compounds showed various bonding and nonbonding interactions with 3LD6 protein (Figure 2). Like fluconazole, compound **5a** and **5b** formed conventional H-bond with THR135 of chain A. These results are also supported by the *in vitro* antifungal test results (Section 3.4).

Table 3. Molecular docking of glucose esters with 3LD6.

Compound	3LD6 (kcal/mol)	Conventional Hydrogen Bond	Hydrophobic interactions	
			Alkyl	Pi-Alkyl
3	-4.8	HIS489; UNK0; ILE488	-	-
4	-6.7	HIS489; MET487	-	PHE139; PHE234
5a	-7.4	THR135; TYR145	ILE377; MET487; ILE488; LEU159 MET304; CYS449; ILE450	HIS314; PHE234; PHE23; PHE152
5b	-7.7	THR135; LYS156; CYS449; TYR145	ILE377; CYS449; PRO376; MET381; MET378; ILE379	HIS236; TRP239; PHE442; HIS489
5c	-4.9	ALA228	LEU204; VAL101; LEU240; LEU241	TYR182
5d	-5.8	ARG253; ARG253; ARG253	PRO137; ARG133	LYS141
Fluconazole	-7.5	THR135; MET487; ILE379	-	TYR131; PHE234
Ketoconazole	-10.2	HIS447	ALA144; MET487; LYS156	PHE152; ALA144; ALA311

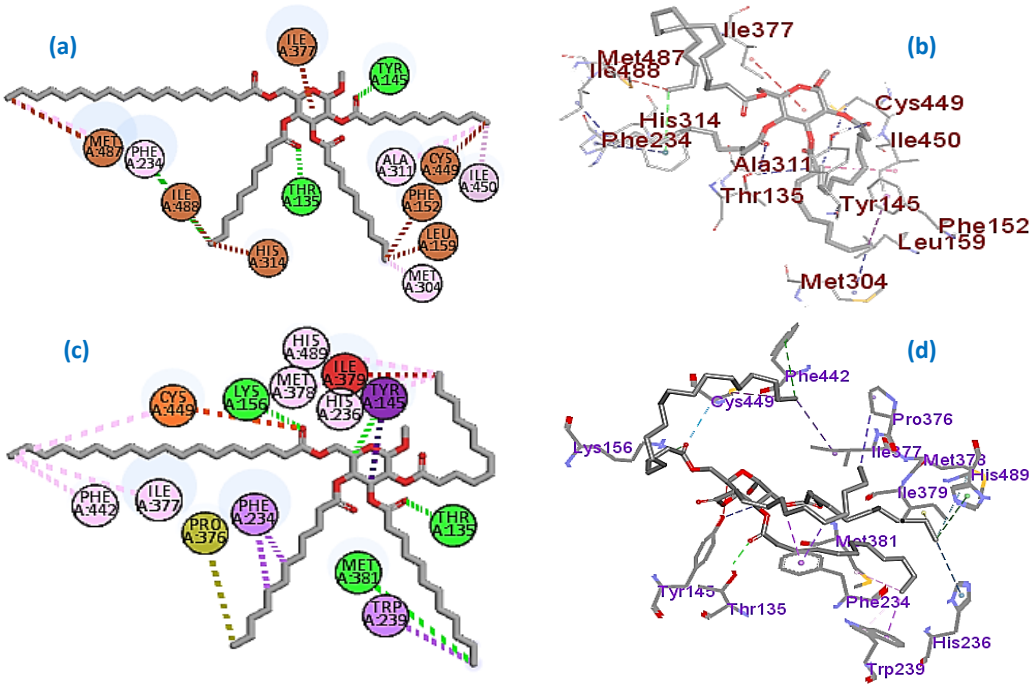


Figure 2. Docking interactions with 3LD6- (a) 2D of **5a**, (b) 3D of **5a**, (c) 2D of **5b**, and (d) 3D of **5b**

4. Conclusion

New antifungal drugs featuring promising therapeutic profiles are essential to mitigate antifungal tolerance. In this respect, several glucopyranoside stearate esters are synthesized and antifungal susceptibility is tested *in vitro*. Fully esterified glucopyranosides **5a-d** with maximum lipophilic character showed better antifungal susceptibility. The fact was further supported by molecular docking with lanosterol 14α-demethylase (CYP51; PDB id: 3LD6) which indicated a good binding affinity for **5a** (-7.4 kcal/mol) and **5b** (-7.7 kcal/mol). Corroborating all the *in vitro* and *in silico* results in the current study may help to design biodegradable and non-azole-type antifungal drugs with broader and better efficacy.

Acknowledgements

The authors would like to thank Ministry of Science and Technology, Bangladesh for financial support (Physical Science, sl. no. 624, 2021-2022) in completing this work.

ORCID ID
Abul F.M. Sanaullah: 0000-0002-4653-2059
Mohammed M. Matin: 0000-0003-4965-2280
Md. Rezaur Rahman : 0000-0002-7151-0687
S.M. Abdul Nayeem: 0000-0002-3167-0651

References

[1] Tiwari, V.K. Development of diverse range of biologically relevant carbohydrate-containing molecules: Twenty years of our journey. *The Chem. Rec.* **2021**, *21*, 1-21
[2] Campo, V.L.; Carvalho, I. In: *Click Chemistry in Glycoscience* (New Developments and Strategies), **2013**, 325–357.
[3] Velter, I.; La Ferla, B.; Nicotra, F. Carbohydrate-based molecular scaffolding. *J. Carbohydr. Chem.* **2006**, *25(2-3)*, 97-138.
[4] Matin, M.M.; Sharma, T.; Sabharwal, S.G.; Dhavale, D.D. Synthesis and evaluation of glycosidase inhibitory activity of 5-hydroxy substituted isofagomine analogues. *Org. Biomol. Chem.* **2005**, *3*(*9*), 1702–1707.
[5] Dhavale, D.D.; Matin, M.M.; Sharma, T.; Sabharwal, S.G. Synthesis and evaluation of glycosidase inhibitory activity of octahydro-2H-pyrido[1,2-a]pyrimidine and octahydro-imidazo[1,2-a]pyridine bicyclic diazasugars. *Bioorg. Med. Chem.* **2004**, *12*(*15*), 4039–4044.
[6] Cipolla, L.; Peri, F.; La Ferla, B.; Redaelli, C.; Nicotra, F. Glycidic scaffolds for the production of bioactive compounds. *Curr. Org. Synth.* **2005**, *2*, 153–173.
[7] Gruner, S.A.W.; Locardi, E.; Lohof, E.; Kessler, H. Carbohydrate-based mimetics in drug design: sugar amino acids and carbohydrate scaffolds. *Chem. Rev.* **2002**, *102*, 491–514.
[8] Le, G.T.; Abbenante, G.; Becker, B.; Grathwohl, M.; Halliday, J.; Tometzki, G.; Zuegg, J.; Meutermans, W. Molecular diversity through sugar scaffolds. *Drug Discov. Today* **2003**, *8*(*15*), 701-709.
[9] Bajad, N.G.; Swetha, R.; Gutti, G.; Singh, M.; Kumar, A.; Singh, S.K. A systematic review of carbohydrate-based bioactive molecules for Alzheimer's disease. *Future Med. Chem.* **2021**, *13*(*19*), 1695-1711.
[10] Muhammad, D.; Matin, M.M.; Miah, S.M.R.; Devi, P. Synthesis, antimicrobial, and DFT studies of some benzyl 4-O-acyl-α-L-rhamnopyranosides. *Orbital: Electron. J. Chem.* **2021**, *13*(*3*), 250-258.
[11] Dodd, R.B.; Drickamer, K. Lectin-like proteins in model organisms: implications for evolution of carbohydrate-binding activity. *Glycobiology* **2001**, *11*, 71R–79R
[12] Slovin, S.F.; Keding, S.J.; Ragupathi, G. Carbohydrate vaccines as immunotherapy for cancer. *Immunol. Cell Biol.* **2005**, *83*, 418–428.
[13] Kilcoyne, M; Joshi, L. Carbohydrates in therapeutics. Cardiovasc. *Hematol. Agents Med. Chem.* **2007**, *5*(*3*), 186-97.
[14] Devi, P.; Matin, M.M.; Bhuiyan, M.M.H.; Hossain, M.E. Synthesis, and spectral characterization of 6-O-octanoyl-1,2-O-isopropylidene-α-D-glucofuranose derivatives. *J. Turkish Chem. Soc. Sect. A: Chem.* **2021**, *8*(*4*), 1003-1024.
[15] Pöhnlein, M.; Slomka, C.; Kukharenko, O.; Gärtner, T.; Wiemann, L.O.; Sieber, V.; Syldatk, C.; Rudolf H. Enzymatic synthesis of amino sugar fatty acid esters. *Eur. J. Lipid Sci. Technol.* **2014**, *116*, 423–428.
[16] Grzywacz, D.; Liberek, B.; Myszka, H. Synthesis, modification and biological activity of diosgenyl β-d-glycosaminosides: An overview. *Molecules* **2020**, *25*, e5433.

[17] Wolfrom, M.L.; Bhat, H.M. Trichloroacetyl and trifluoroacetyl as N-blocking groups in nucleoside synthesis with 2-amino sugars. *J. Org. Chem.* **1967**, *32*, 1821–1823
[18] Matin, M.M.; Bhuiyan, M.M.H.; Kabir, E.; Sanaullah, A.F.M.; Rahman, M.A.; Hossain, M.E.; Uzzaman, M. Synthesis, characterization, ADMET, PASS predication, and antimicrobial study of 6-O-lauroyl mannopyranosides. *J. Mol. Struct.* **2019**, *1195*, 189-197. https://doi.org/10.1016/j.molstruc.2019.05.102
[19] Matin, M.M.; Bhattacharjee, S.C.; Chakraborty, P.; Alam, M.S. Synthesis, PASS predication, in vitro antimicrobial evaluation and pharmacokinetic study of novel n-octyl glucopyranoside esters. *Carbohydr. Res.* **2019**, *485*, 107812.
[20] AlFindee, M.N.; Zhang, Q.; Subedi, Y.P.; Shrestha, J.P.; Kawasaki, Y.; Grilley, M.; Taemoto, J.Y.; Chang, C.W.T. One-step synthesis of carbohydrate esters as antibacterial and antifungal agents. *Bioorg. Med. Chem.* **2018**, *26*, 765-774.
[21] Perinelli, D.R.; Lucarini, S.; Fagioli, L.; Campana, R.; Vllasaliu, D.; Duranti, A.; Casettari, L. Lactose oleate as new biocompatible surfactant for pharmaceutical applications. *Eur. J. Pharm. Biopharm.* **2018**, *124*, 55–62.
[22] Matin, M.M.; Iqbal, M.Z. Methyl 4-O-(2-chlorobenzoyl)-α-L-rhamnopyranosides: Synthesis, characterization, and thermodynamic studies. *Orbital: Electron. J. Chem.* **2021**, *13*(*1*), 19-27.
[23] Matin, M.M. Synthesis and antimicrobial study of some methyl 4-O-palmitoyl-α-L-rhamnopyranoside derivatives. *Orbital: Electron. J. Chem.* **2014**, *6*(*1*), 20-28.
[24] Peng, X.M.; Gao, L.; Huo, S.X.; Liu, X.M.; Yan, M. The mechanism of memory enhancement of acteoside (verbascoside) in the senescent mouse model induced by a combination of D-gal and $AlCl_3$. *Phytother. Res.* **2015**, *29*(*8*), 1137–1144.
[25] Hare, J.D. Biological activity of acyl glucose esters from *Datura wrightii* glandular trichomes against three native insect herbivores. *J. Chem. Ecol.* **2005**, *31*(*7*), 1475-1491.
[26] Yang, Y.; Liu, M.; Zhang, W.; Cao, Y.; Li, C.; Wang, W. Identification and characterization of two new 1-O-acyl-glucose-ester forming glucosyltransferases from *Erigeron breviscapus*. *J. Agric. Food Chem.* **2019**, *67*, 10, 2848–2855.
[27] El-Baz, H.A.; Elazzazy, A.M.; Saleh, T.S.; Dourou, M.; Mahyoub, J.A.; Baeshen, M.N.; Madian, H.R.; Aggelis, G. Enzymatic synthesis of glucose fatty acid esters using SCOs as acyl group-donors and their biological activities. *Appl. Sci.* **2021**, *11*, 2700.
[28] Marathe, S.J.; Dedhia, N.; Singhal, R.S. Esterification of sugars and polyphenols with fatty acids: Techniques, bioactivities, and applications. *Curr. Opin. Food Sci.* **2013**, *40*, 427-452.
[29] Matin, M.M.; Bhuiyan, M.H.; Hossain, M.M.; Roshid, M.H.O. Comparative antibacterial activities of some monosaccharide and disaccharide benzoates. *Orbital: Electron. J. Chem.* **2015**, *7*(*2*), 160-167.
[30] Rahman, M.A.; Matin, M.M.; Kumer, A.; Chakma, U.; Rahman, M.R. Modified D-glucofuranoses as new black fungus protease inhibitors: Computational screening, docking, dynamics, and QSAR study. *Phys. Chem. Res.* **2022**, *10*(*2*), 189-203.
[31] Clements, T.; Ndlovu, T.; Khan, S.; Khan, W. Biosurfactants produced by *Serratia species*: Classification, biosynthesis, production and application. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 589-602.
[32] Lawandi, J.; Rocheleau, S.; Moitessier, N. Regioselective acylation, alkylation, silylation and glycosylation of monosaccharides. *Tetrahedron* **2016**, *72*, 6283-6319.
[33] Ferrier, R.J.; Middleton, S. The conversion of carbohydrate derivatives into functionalized cyclohexanes and cyclopentanes. *Chem. Rev.* **1993**, *93*(*8*), 2779–2831.
[34] Hanee, U.; Rahman, M.R.; Matin, M.M. Synthesis, PASS, in silico ADMET, and thermodynamic studies of some galactopyranoside esters. *Phys. Chem. Res*. **2021**, *9*(*4*), 591-603.
[35] Islam, F.; Rahman, M.R.; Matin, M.M. The effects of protecting and acyl groups on the conformation of benzyl α-L-rhamnopyranosides: An in silico study. *Turk. Comp. Theo. Chem.* **2021**, *5*(*1*), 39-50.
[36] Ko, Y.C.; Tsai, C.F.; Wang, C.C.; Dhurandhare, V.M.; Hu, P.L.; Su, T.Y.; Lico, L.S.; Zulueta, M.M.L.; Hung, S.C. Microwave-assisted one-pot synthesis of 1,6-anhydrosugars and orthogonally protected thioglycosides. *J. Am. Chem. Soc.* **2014**, *136*, 14425–14431
[37] Ali, M.; Karim, M.H.; Matin, M.M. Efficient synthetic technique, PASS predication, and ADMET studies of acylated n-octyl glucopyranosides. *J. Appl. Sci. Process Eng.* **2021**, *8*(*1*), 648-659.
[38] Matin, M.M.; Islam, N.; Siddika, A.; Bhattacharjee, S.C. Regioselective synthesis of some rhamnopyranoside esters for PASS predication, and ADMET studies. *J. Turk. Chem. Soc. Sect. A: Chem.* **2021**, *8*(*1*), 363-374.
[39] Zhang, X.; Wei, W.; Cao, X.; Feng, F. Characterization of enzymatically prepared sugar medium-chain fatty acid monoesters. *J. Sci. Food Agri.* **2014**, *95*(*8*), 1631-1637.

[40] Matin, M.M.; Chakraborty, P. Synthesis, spectral and DFT characterization, PASS predication, antimicrobial, and ADMET studies of some novel mannopyranoside esters. *J. Appl. Sci. Process Eng.* **2020**, *7*(*2*), 572-586.
[41] Matin, M.M.; Ibrahim, M. Synthesis of some methyl 4-O-octanoyl-α-L-rhamnopyranoside derivatives. *J. Appl. Sci. Res.* **2010**, *6*(*10*), 1527-1532.
[42] Matin, M.M.; Chakraborty, P.; Alam, M.S.; Islam, M.M.; Hanee, U. Novel mannopyranoside esters as sterol 14α-demethylase inhibitors: Synthesis, PASS predication, molecular docking, and pharmacokinetic studies. *Carbohydr. Res.* **2020**, *496*, 108130.
[43] Matin, M.M.; Hasan, M.S.; Uzzaman, M.; Bhuiyan, M.M.H.; Kibria, S.M.; Hossain, M.E.; Roshid, M.H.O. Synthesis, spectroscopic characterization, molecular docking, and ADMET studies of mannopyranoside esters as antimicrobial agents. *J. Mol. Struct.* **2020**, *1222*, 128821.
[44] Kumer, A.; Chakma, U.; Matin, M.M.; Akash, S.; Chando, A.; Howlader, D. The computational screening of inhibitor for black fungus and white fungus by D-glucofuranose derivatives using in silico and SAR study. *Org. Commun.* **2021**, *14*(*4*), 305-322
[45] Matin, M.M.; Nath, A.R.; Saad, O.; Bhuiyan, M.M.H.; Kadir, F.A.; Hamid, S.B.A.; Alhadi, A.A.; Ali, M.E.; Yehye, W.A. Synthesis, PASS-predication and in vitro antimicrobial activity of benzyl 4-O-benzoyl-α-L-rhamnopyranoside derivatives. *Int. J. Mol. Sci.* **2016**, *17*(*9*), 1412.
[46] Filimonov, D.A.; Lagunin, A.A.; Gloriozova, T.A.; Rudik, A.V.; Druzhilovskii, D.S.; Pogodin, P.V.; Poroikov, V.V. Prediction of the biological activity spectra of organic compounds using the PASS online web resource. *Chem. Hetero. Comp.* **2014**, *50*, 444–457.
[47] van de Waterbeemd, H.; Gifford, E. ADMET in silico modelling: towards prediction paradise? *Nature Rev. Drug Disc.* **2003**, *2*, 192−204.
[48] Kabir, A.K.M.S.; Matin, M.M.; Sanaullah, A.F.M.; Sattar, M.A.; Rahman, M.S. Antimicrobial activities of some lyxoside derivatives. *Bangladesh J. Microbiol.* **2001**, *18*(*1*), 89-95.
[49] Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G.A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H.P.; Izmaylov, A.F.; Bloino, J.; Zheng, G.; Sonnenberg, J.L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr, J.A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J.J.; Brothers, E.; Kudin, K.N.; Staroverov, V.N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S.S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J.M.; Klene, M.; Knox, J.E.; Cross, J.B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R.E.; Yazyev, O.; Austin, A.J.; Cammi, R.; Pomelli, C.; Ochterski, J.W.; Martin, R.L.; Morokuma, K.; Zakrzewski, V.G.; Voth, G.A.; Salvador, P.; Dannenberg, J.J.; Dapprich, S.; Daniels, A.D.; Farkas, O.; Foresman, J.B.; Ortiz, J.V.; Cioslowski, J.; Fox, D.J. Gaussian 09W, Revision D.01, Gaussian, Inc, Wallingford CT, **2013**.
[50] Strushkevich, N.; Tempel, W.; MacKenzie, F.; Arrowsmith, C.H.; Edwards, A.M.; Bountra, C.; Weigelt, J.; Park, H.; Consortium, S.G. Crystal structure of human lanosterol 14alpha-demethylase (CYP51) in complex with ketoconazole. *J. Mol. Biol.* **2010**, *397*, 1067-1078.
[51] DeLano, W.L. The PyMOL user's manual. **2002**. http://www.pymol.org.
[52] Xia, J.; Hui, Y. A New Highly regioselective reaction of unprotected sugars for chemical synthesis of 6-acyl-D-glycopyranosides. *Syn. Commun*. **1995**, 25(15), 2235-2251.
[53] Cheng, Y.-G.; Tan, J.-Y.; Li, J.-L.; Wang, S.-H.; Liu, K.-L.; Wang, J.-M.; Ying-Li Wang, Y.-L. Chemical constituents from the aerial part of *Polygala tenuifolia*. *Nat. Prod. Res.* **2020**, *34*(*20*), 2900-2906.
[54] Komvongsa, J.; Mahong, B.; Phasai, K.; Hua, Y.; Jeon, J.-S.; Cairns, J.R.K. Identification of fatty acid glucose esters as Os9BGlu31 transglucosidase substrates in rice flag leaves. *J. Agric. Food Chem.* **2015**, 63, 9764–9769.
[55] Kroemer, G.; Galluzzi, L.; Brenner, C. Mitochondrial membrane permeabilization in cell death. *Physiological Rev.* **2007**, *87*, 99–163.
[56] Martel, C.; Huynh, L.H.; Garnier, A.; Ventura-Clapier, R.; Brenner, C. Inhibition of the Mitochondrial Permeability Transition for Cytoprotection: Direct versus Indirect Mechanisms. *Biochem. Res. Int.* **2012**, e213403.
[57] Matin, M.M.; Matin, P.; Rahman, M.R.; Hadda, T.B.; Almalki, F.A.; Mahmud, S.; Ghoneim, M.M.; Alruwaily, M.; Alshehri, S. Triazoles and their derivatives: Chemistry, synthesis, and therapeutic applications. *Front. Mol. Biosci.* **2022**, accepted.

[58] Arastehfar, A.; Gabaldón, T.; Garcia-Rubio, R.; Jenks, J.D.; Hoenigl, M.; Salzer, H.J.F.; Ilkit, M.; Lass-Flörl, C.; Perlin, D.S. Drug-resistant fungi: An emerging challenge threatening our limited antifungal armamentarium. *Antibiotics* **2020**, *9*(*12*), *877*. doi: 10.3390/antibiotics9120877.
[59] Kabir, A.K.M.S.; Matin, M.M.; Mridha, M.A.U.; Shahed, S.M. Antifungal activities of some methyl 6-O-trityl-α-D-mannopyranosides. *Chittagong Univ. J. Sci.* **1998**, *22*(*1*), 41-46.
[60] Wang, G.; Zhu, W. Molecular docking for drug discovery and development: a widely used approach but far from perfect. *Future Med Chem.* **2016**, *8*, 0143.
[61] Yuriev, E.; Agostino, M.; Ramsland, P.A. Challenges and advances in computational docking: 2009 in review. *J. Mol. Recognit.* **2011**, *24*(*2*), 149–164.
[62] Odds, F.C.; Brown, A.J.P.; Gow, N.A.R. Antifungal agents: mechanisms of action. *Trends Microbiol.* **2003**, *11*, 272-279