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Glucopyranoside Dipentanoyl Esters: Synthesis, PASS Predication, Antimicrobial and *In Silico* ADMET Studies

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Abstract

Dimolar pentanoylation of methyl α-D-glucopyranoside using direct method furnished the 2,6-di-*O*-pentanoate indicating regioselectivity at C-6 and C-2 positions. To develop glucopyranoside based potential antimicrobial agents, 2,6-di-*O*-pentanoate was further converted into eight newer 3,4-di-*O*-acyl esters reasonably in good yields. Both prediction of activity spectra for substances (PASS) and *in vitro* antimicrobial activity test established them as better antifungals than antibacterials. PASS predication also indicated that these sugar esters (SEs) are more potent as anticarcinogenic agents than as antioxidant agents. Structure activity relationship along with *in silico* ADMET studies clearly indicated that combination of pentanoyl (C5) and lauroyl (C12) in the glucopyranoside framework could be a potential antifungal agent especially against *Macrophomina phaseolina*.

Keywords: Regioselective pentanoylation; 2,6-Di-*O*-pentanoyl-α-D-glucopyranoside; Sugar esters (SEs); PASS predication; Antimicrobial activities; AdmetSAR.

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1. Introduction

Sugar esters (SEs), also known as carbohydrate fatty acid (CFA) esters, are involved in many diverse biological events in organisms from all kind of life. SEs have synthetic utility as versatile intermediates in the syntheses of many natural products due to the presence of multifunctional groups and their analogues which have a broad spectrum of applications [1-4]. They are composed of hydrophilic carbohydrate moiety and one or more fatty acids as lipophilic moieties, and are generally tasteless, odorless, biodegradable, non-toxic, non-allergic and non-irritating [5]. Due to their good stabilizing and conditioning properties, SEs are increasingly used as important commodity chemicals, and have received attention in the food, cosmetics, pharmaceutical and dental care industries [6,7]. Some SEs showed biosurfactant, anticancer, insecticidal and antimicrobial activities [8-11]. For instance, glucopyranose, xylopyranose, mannose,

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fructose and galactose esters strongly inhibit the growth of various organisms like *A. flavus*, *F. graminarium*, *M. separate*, *S. mutans* and *T. cinnabarinus* [12,13].

In recent years, emergence of multiple antibiotic resistant pathogenic bacteria is a global concern, and need new chemotherapeutic agents with novel mode of action [14]. In this respect, several mannopyranose esters with different length of side chain were synthesized and evaluated their antimicrobial activities against several microorganisms [13]. It was found that the chain length containing C14 such as 6-*O*-myristoyl-D-mannopyranose was highly active against methicillin resistant *Staphylococcus aureus* ATCC 33591 (MRSA) [13]. Recently, we have also synthesized a series of octyl glucopyranoside [Fig. 1(**1**)] esters, and observed that the incorporation of alkanoyl and aromatic ester groups increased antimicrobial potentiality in a very low concentration (10 μgmL^{-1}) which may act as competitive inhibitors of lanosterol 14α-demethylase [15]. Catelani *et al*. [16] found that 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives (**2a-c**) effects hemoglobin-containing cells in treated K562cell populations and can be used for β-thalassemia treatment.

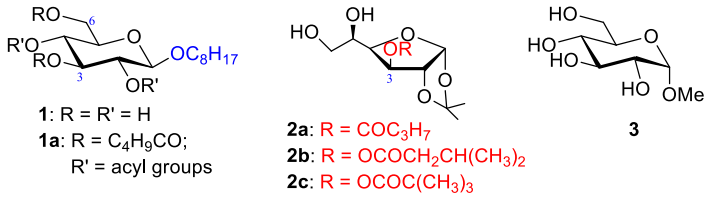


Fig. 1. Structure of SEs **1**-**3**.

Synthetic organic chemists face various challenges for selective and regioselective esterification of sugars as these molecules contain several hydroxyl groups (2º) of similar reactivity. These alcoholic groups compete during functionalization (esterification) step leading to a mono-, di-, and polyesters [17]. Different type of methods have so far been developed in the last couple of decades and employed successfully for selective esterification (acylation) such as protection-deprotection technique [18,19], organotin [20,21] and other catalyst mediated technique [22], microwave (MW) assisted method [23], enzyme catalyzed method [24] and direct method [25-27]. Some of them have many shortcomings such as increase the number of steps, tedious, expensive and generally decrease the overall yield. Thus, direct acylation technique maintaining appropriate reaction conditions is preferred for the monosaccharide-based SEs synthesis to reduce the number of steps with improved yield [15,25].

The antimicrobial property of SEs has been studied extensively, although variable results were reported for various bacterial and fungal species as well as for acyl chain lengths [28]. Hence, we considered to synthesize some novel SEs and study their antimicrobial functionality with positional and chain effects. This study is concerned with the direct regioselective dimolar pentanoylation of glucopranoside **3**, *in vitro* antimicrobial evaluation, and *in silico* pharmacokinetic study of the synthesized SEs.

2. Experimental

2.1. *Materials and methods*

All reagents and solvents used were commercially available (Merck, Germany) and were used as received, unless otherwise specified. Evaporations were carried out under reduced pressure using a Buchi rotary evaporator (R-100, Switzerland) with a bath temperature below 40 °C. Thin layer chromatography (TLC) was performed on Kieselgel GF_{254}, and the spots were detected by spraying the plates with 1 % H_2SO_4 and heating at 150–200 °C until coloration took place. FT-IR spectra were recorded on a FT-IR spectrophotometer (Shimadzu, IR Prestige-21) in $CHCl_3$ technique. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded for solutions in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard with a Bruker DPX-400 spectrometer at the Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka, Bangladesh. Column chromatography (CC) was performed with silica gel 60 GF_{254}. The solvent system employed for the TLC analyses and CC was *n*-hexane/ethyl acetate (EA) in different proportions.

2.2. *Syntheses*

2.2.1. *Methyl 2,6-di-O-pentanoyl-α-D-glucopyranoside (**4**)*

A solution of methyl α-D-glucopyranoside (**3**, 5.0 g, 25.70 mmol) in pyridine (15 mL) was cooled to -5 °C whereupon pentanoyl chloride (6.58 g, 54.57 mmol) was added drop-wise to this mixture. The mixture was stirred at the same temperature for 4 h and then stirred overnight at room temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EA, 1/1), which indicated the formation of two products, the slower-moving component being the major one. A few pieces of ice were added to the flask and then extracted the product mixture with chloroform (3×10 mL). The combined organic layer was washed successively with dilute hydrochloric acid (10 %), saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution and distilled water. The chloroform layer was dried over $MgSO_4$, filtered and filtrate was concentrated under reduced pressure to leave syrup. The syrup was passed through silica gel column and eluted with chloroform/methanol (25/1). Initial elution with *n*-hexane/EA (10/1) provided a faster-moving component which could not be isolated in pure form. Further elution with *n*-hexane/EA (5/1) furnished the 2,6-di-*O*-pentanoate **4** (4.52 g, 48 %) as a clear syrup, which resisted crystallization.

R_f = 0.51 (with *n*-hexane/EA = 1/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 3350-3460 (OH), 1755, 1740 (CO); ^1H NMR (400 MHz, $CDCl_3$) δ_H ppm: 4.88 (d, J = 3.6 Hz, 1H, H-1), 4.66 (dd, J = 10.0 and 3.7 Hz, 1H, H-2), 4.47 (dd, J = 12.1 and 5.0 Hz, 1H, H-6a), 4.25 (dd, J = 12.1 and 2.0 Hz, 1H, H-6b), 3.94 (t, J = 9.3 Hz, 1H, H-3), 3.73 (t, J = 9.7 Hz, 1H, H-4), 3.43 (ddd, J = 12.8, 9.9 and 2.9 Hz, 1H, H-5), 3.34 (s, 3H, OCH_3), 2.37 [t, J = 7.5 Hz, 4H, $2\times CH_3(CH_2)_2CH_2CO$], 1.58-1.61 (m, 4H, $2\times CH_3CH_2CH_2CH_2CO$), 1.33-1.37 [m, 4H, $2\times CH_3CH_2(CH_2)_2CO$], 0.91 [t, J = 6.6 Hz, 6H, $2\times CH_3(CH_2)_3CO$].

2.2.2. *General procedure for 3,4-di-O-acylation of compound **4** by direct method*

To a solution of the **4** (0.1 g) in anhydrous pyridine (1 mL) was added corresponding acyl halide (2.2 eq.) slowly at 0 ºC followed by addition of catalytic amount of DMAP. The reaction mixture was allowed to attain room temperature and stirring was continued for 12-16 h. In some cases (for compound **8**-**9**) reaction mixture was stirred additional 1-2 h at 45°C. A small amount of cold water (0.5 mL) was added to the reaction mixture to decompose excess acyl halide and extracted with DCM (3×3 mL). The DCM layer was washed successively with 5 % hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution and brine. The DCM layer was dried and concentrated under reduced pressure. The residue thus obtained on CC (elution with *n*-hexane/EA) gave the corresponding 3,4-di-*O*-acyl esters in pure form.

*Methyl 3,4-di-O-acetyl-2,6-di-O-pentanoyl-α-D-glucopyranoside (**5**):* Oil; Yield 94 %; R_f = 0.49 (*n*-hexane/EA = 7/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 1775, 1772, 1770, 1769 (CO); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 5.46 (t, J = 9.7 Hz, 1H, H-3), 5.03 (t, J = 9.8 Hz, 1H, H-4), 4.93 (d, J = 3.6 Hz, 1H, H-1), 4.88 (dd, J = 10.1 and 3.6 Hz, 1H, H-2), 4.22 (dd, J = 12.3 and 4.8 Hz, 1H, H-6a), 4.13 (dd, J = 12.0 and 2.2 Hz, 1H, H-6b), 3.94-3.99 (m, 1H, H-5), 3.39 (s, 3H, O-C*H*$_3$), 2.29-2.36 [m, 4H, 2×$CH_3(CH_2)_2CH_2CO$], 2.01 (s, 3H, COC*H*$_3$), 1.99 (s, 3H, COC*H*$_3$), 1.55-1.61 [m, 4H, 2×$CH_3CH_2CH_2CH_2CO$] 1.19-1.34 [m, 4H, 2×$CH_3CH_2(CH_2)_2CO$], 0.88-0.91 [m, 6H, 2×$CH_3(CH_2)_3CO$].

*Methyl 2,6-di-O-pentanoyl-3,4-di-O-pivaloyl-α-D-glucopyranoside (**6**)*: Clear syrup; Yield 92 %; R_f = 0.50 (*n*-hexane/EA = 7/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 1750 (CO); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 5.50 (t, J = 9.8 Hz, 1H, H-3), 5.09 (t, J = 9.9 Hz, 1H, H-4), 4.90-4.94 (m, 2H, H-1 and H-2), 4.10-4.15 (m, 2H, H-6), 3.96-3.99 (m, 1H, H-5), 3.38 (s, 3H, O-C*H*$_3$), 2.30-2.36 [m, 4H, 2×$CH_3(CH_2)_2CH_2CO$], 1.54-1.61 (m, 4H, 2×$CH_3CH_2CH_2CH_2CO$), 1.21 [s, 9H, C(C*H*$_3$)$_3$], 1.19 [s, 9H, C(C*H*$_3$)$_3$], 1.12-1.15 [m, 2H, $CH_3CH_2(CH_2)_2CO$], 1.07-1.11 [m, 2H, $CH_3CH_2(CH_2)_2CO$], 0.88 [t, J = 6.7 Hz, 6H, 2×$CH_3(CH_2)_3CO$].

*Methyl 3,4-di-O-hexanoyl-2,6-di-O-pentanoyl-α-D-glucopyranoside (**7**)*: Thick syrup; Yield 87 %; R_f = 0.61 (*n*-hexane/EA = 7/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 1758, 1752, 1748, 1740 (CO); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 5.49 (t, J = 9.8 Hz, 1H, H-3), 5.06 (t, J = 9.7 Hz, 1H, H-4), 4.93 (d, J = 3.8 Hz, 1H, H-1), 4.88 (dd, J = 10.0 and 3.8 Hz, 1H, H-2), 4.17 (dd, J = 12.2 and 4.6 Hz, 1H, H-6a), 4.12 (dd, J = 12.2 and 2.2 Hz, 1H, H-6b), 3.92-3.98 (m, 1H, H-5), 3.38 (s, 3H, O-C*H*$_3$), 2.17-2.36 [m, 8H, 2×$CH_3(CH_2)_2CH_2CO$ and 2×$CH_3(CH_2)_3CH_2CO$], 1.48-1.64 [br 8H, m, 2×$CH_3CH_2CH_2CH_2CO$ and 2×$CH_3(CH_2)_2CH_2CH_2CO$], 1.17-1.39 [br m, 12H, 2×$CH_3CH_2(CH_2)_2CO$ and 2×$CH_3(CH_2)_2(CH_2)_2CO$], 0.80-0.94 [br m, 12H, 2×$CH_3(CH_2)_3CO$ and 2×$CH_3(CH_2)_4CO$].

*Methyl 3,4-di-O-lauroyl-2,6-di-O-pentanoyl-α-D-glucopyranoside (**8**)*: Syrup; Yield 81 %; R_f = 0.53 (*n*-hexane/EA = 8/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 1758, 1744, 1730, 1725 (CO); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 5.48 (t, J = 9.9 Hz, 1H, H-3), 5.05 (t, J = 9.7 Hz, 1H, H-4), 4.92 (d, J = 3.7 Hz, 1H, H-1), 4.87 (dd, J = 10.1 and 3.7 Hz, 1H, H-2), 4.17 (dd, J = 12.1 and 4.6 Hz, 1H, H-6a), 4.11 (dd, J = 12.1 and 2.2 Hz, 1H, H-6b), 3.93-3.98

(m, 1H, H-5), 3.37 (s, 3H, O-C*H*$_3$), 2.20-2.37 [m, 8H, 2×$CH_3(CH_2)_2CH_2CO$ and 2×$CH_3(CH_2)_9CH_2CO$], 1.49-1.64 [br m, 8H, 2×$CH_3CH_2CH_2CH_2CO$ and 2×$CH_3(CH_2)_8CH_2CH_2CO$], 1.15-1.38 [br m, 36H, 2×$CH_3CH_2(CH_2)_2CO$ and 2×$CH_3(CH_2)_8(CH_2)_2CO$], 0.78-0.92 [br m, 12H, 2×$CH_3(CH_2)_3CO$ and 2×$CH_3(CH_2)_{10}CO$].

Methyl 3,4-di-O-myristoyl-2,6-di-O-pentanoyl-α-D-glucopyranoside ***(9)***: Semi-solid: Yield 82 %; R_f = 0.57 (*n*-hexane/EA = 8/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 1754, 1748, 1740, 1738 (CO); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 5.49 (t, J = 9.8 Hz, 1H, H-3), 5.09 (t, J = 9.7 Hz, 1H, H-4), 4.93 (d, J = 3.6 Hz, 1H, H-1), 4.88 (dd, J = 10.0 and 3.6 Hz, 1H, H-2), 4.09-4.22 (m, 2H, H-6), 3.90-3.94 (m, 1H, H-5), 3.37 (s, 3H, O-C*H*$_3$), 2.22-2.39 [br m, 8H, 2×$CH_3(CH_2)_2CH_2CO$ and 2×$CH_3(CH_2)_{11}CH_2CO$], 2.06-2.10 (m, 4H, 2×$CH_3CH_2CH_2CH_2CO$), 2.00-2.06 [m, 4H, 2×$CH_3(CH_2)_{10}CH_2CH_2CO$], 1.62-1.71 (m, 8H, 2×$CH_3(CH_2)_8(CH_2)_2(CH_2)_2CO$], 1.16-1.38 [br m, 36H, 2×$CH_3CH_2(CH_2)_2CO$ and 2×$CH_3(CH_2)_8(CH_2)_4CO$], 0.80-0.93 [br m, 12H, 2×$CH_3(CH_2)_3CO$ and 2×$CH_3(CH_2)_{12}CO$].

Methyl 3,4-di-O-methansulfonyl-2,6-di-O-pentanoyl-α-D-glucopyranoside ***(10)***: Syrup; Yield 90 %; R_f = 0.51 (*n*-hexane/EA = 7/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 1750, 1741 (CO), 1370, 1365 (SO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 5.11 (t, J = 9.8 Hz, 1H, H-3), 4.90-4.96 (m, 2H, H-4 and H-1), 4.74 (dd, J = 10.0 and 3.7 Hz, 1H, H-2), 4.30-4.39 (m, 2H, H-6), 3.95-3.99 (m, 1H, H-5), 3.38 (s, 3H, O-C*H*$_3$), 3.15 (s, 3H, SO_2CH_3), 3.11 (s, 3H, SO_2CH_3), 2.37-2.41 [m, 4H, 2×$CH_3(CH_2)_2CH_2CO$], 1.57-1.63 (m, 4H, 2×$CH_3CH_2CH_2CH_2CO$) 1.28-1.36 [m, 4H, 2×$CH_3CH_2(CH_2)_2CO$], 0.89-0.92 [m, 6H, 2×$CH_3(CH_2)_3CO$].

Methyl 3,4-di-O-(4-chlorobenzoyl)-2,6-di-O-pentanoyl-α-D-glucopyranoside ***(11)***: Pasty mass; Yield 84 %; R_f = 0.52 (*n*-hexane/EA = 6/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 1758, 1750, 1745, 1730 (CO); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 7.78-7.84 (m, 4H, Ar-*H*), 7.29-7.35 (m, 4H, Ar-*H*), 5.88 (t, J = 9.9 Hz, 1H, H-3), 5.43 (t, J = 9.8 Hz, 1H, H-4), 5.14 (dd, J = 10.2 and 3.7 Hz, 1H, H-2), 5.01 (d, J = 3.7 Hz, 1H, H-1), 4.18-4.24 (m, 3H, H-6 and H-5), 3.46 (s, 3H, O-C*H*$_3$), 2.30 [t, J = 7.6 Hz, 2H, $CH_3(CH_2)_2CH_2CO$], 2.23 [t, J = 7.6 Hz, 2H, $CH_3(CH_2)_2CH_2CO$], 1.54-1.58 (m, 2H, $CH_3CH_2CH_2CH_2CO$), 1.38-1.42 [m, 2H, $CH_3CH_2CH_2CH_2CO$], 1.22-1.26 [m, 2H, $CH_3CH_2(CH_2)_2CO$], 1.10-1.15 [m, 2H, $CH_3CH_2(CH_2)_2CO$], 0.88 [t, J = 6.6 Hz, 3H, $CH_3(CH_2)_3CO$], 0.69 [t, J = 6.6 Hz, 3H, $CH_3(CH_2)_3CO$].

Methyl 3,4-di-O-(2,6-dichlorobenzoyl)-2,6-di-O-pentanoyl-α-D-glucopyranoside ***(12)***: Thick syrup; Yield 80 %; R_f = 0.48 (*n*-hexane/EA = 6/1); FT-IR ($CHCl_3$) υ_{max} (cm^{-1}): 1752, 1748, 1740, 1733 (CO); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ppm: 7.79-7.86 (m, 3H, Ar-*H*), 7.25-7.32 (m, 3H, Ar-*H*), 5.86 (t, J = 9.8 Hz, 1H, H-3), 5.44 (t, J = 9.9 Hz, 1H, H-4), 5.14 (dd, J = 10.1 and 3.6 Hz, 1H, H-2), 5.02 (d, J = 3.6 Hz, 1H, H-1), 4.17-4.22 (m, 3H, H-6 and H-5), 3.44 (s, 3H, O-C*H*$_3$), 2.37 [t, J = 7.5 Hz, 4H, 2×$CH_3(CH_2)_2CH_2CO$], 1.31-1.35 (m, 4H, 2×$CH_3CH_2CH_2CH_2CO$), 1.22-1.26 [m, 4H, 2×$CH_3CH_2(CH_2)_2CO$], 0.90 [6H, t, J = 6.8 Hz, 2×$CH_3(CH_2)_3CO$].

2.3. *Evaluation of in vitro antimicrobial activities*

Four Gram-positive and five Gram-negative human pathogenic bacteria, and four human fungal pathogens were collected from the Biochemistry Laboratory, Department of Biochemistry and Molecular Biology, University of Chittagong, Bangladesh (Table 1). Antibacterial activities were screened *in vitro* disc diffusion method [29-31] and antifungal efficacy was evaluated *in vitro* food poisoning technique [32-35]. Proper control was maintained with DMF (dimethylformamide) without chemicals. Each experiment was carried out three times. All the results were compared with the standard antibacterial antibiotic Ampicillin (FISONS Bangladesh Ltd.) standard antifungal antibiotic Nystatin (Beximco Pharmaceuticals Ltd., Bangladesh) under identical conditions.

Table 1. Name of the microorganisms.

Strain	Reference	Source
Bacteria		
Bacillus cereus, G-(+)ve	BTCC 19	Clinical strains
Bacillus megaterium, G-(+)ve	BTCC18	Clinical strains
Bacillus subtilis, G-(+)ve	ATCC 6633	Gastrointestinal tract
Staphylococcus aureus, G-(+)ve	ATCC 6538	Clinical strains
Escherichia coli, G-(-)ve	ATCC 25922	Clinical strains
Shigella dysenteriae, G-(-)ve	AE 14369	Stool
Salmonella paratyphi, G-(-)ve	ATCC 9150	Clinical strains
Salmonella typhi, G-(-)ve	AE 14612	Natural sugars
Vibrio cholera, G-(-)ve	ICDDR, B	Water
Fungi		
Aspergillus flavus	Human pathogenic	-
Aspergillus ochraceus	Human pathogenic	-
Macrophomina phaseolina	Human pathogenic	-
Fusarium equiseti	Human pathogenic	-

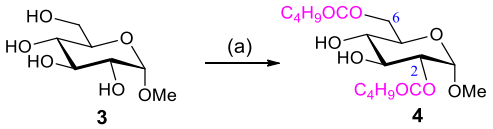
2.4. *ADMET analysis*

Absorption, distribution, metabolism, excretion and toxicity (ADMET) analyses were conducted by using computational approaches. At first all the structures of glucopyranoside esters were drawn in Chem Draw 18.0 to collect InChI Key, isomeric SMILES and SD file format. ADMET of all the SEs were predicted by using AdmetSAR [32,36] and SwissADME [35,37] free web tools. SMILES (simplified molecular-input line-entry system) strings were used throughout the process.

3. Results and Discussion

3.1. *Synthesis of methyl 2,6-di-O-pentanoyl-α-D-glucopyranoside (4)*

In an attempt to investigate the dimolar regioselectivity of glucopyranoside **3**, we employed pentanoyl chloride and its direct reaction with **3** in anhydrous pyridine for 12 h gave a faster-moving syrupy mass in 48 % (Scheme 1).

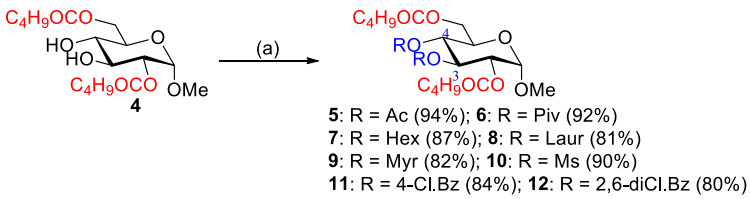


Scheme 1. Reagents and conditions: (*a*) Py, C_4H_9COCl, 0 °C-rt, 12 h, 48 %.

FT-IR spectrum of this syrup showed the presence of two carbonyl stretching bands at 1755 and 1740 cm^{-1} and a hydroxyl stretching band at 3350-3460 cm^{-1} indicating the attachment of partial pentanoyl group(s) in the molecule. In its ^{1}H NMR spectrum, a four-proton triplet at δ 2.37, two four-proton multiplets at δ 1.58-1.61 and 1.33-1.37, and a six-proton triplet at δ 0.91 totaling eighteen protons were indicative of the attachment of two pentanoyloxy groups. In addition, the downfield shift of H-2 (δ 4.66) and H-6 protons (δ 4.25 and 4.47) as compared to the precursor compound **3** [38] confirmed the attachment of the pentanoyloxy group at position C-2 and C-6. The H-1 of this compound resonated at δ 4.88 as doublet with small coupling constant (*J* = 3.6 Hz). Such coupling constant requires axial-equatorial vicinal protons. Since in D-glucose H-2 is axially oriented, H-1 must be in equatorial position resulting OMe in axial position i.e. α in the compound. The rest of the ^{1}H NMR spectrum was in complete accord with the structure assigned as methyl 2,6-di-*O*-pentanoyl-α-D-glucopyranoside (**4**). It should be noted that, this direct method gave methyl 2,6-di-*O*-pentanoate **4** in somewhat lower yield (48 %) probably because of the formation of inseparable other products. However, formation of **4** clearly indicated that the hydroxyl group at C2 position had higher reactivity (selectivity) among the secondary hydroxyl groups of glucopyranoside **3**.

3.2. *Synthesis of methyl 3,4-di-O-acyl-2,6-di-O-pentanoyl-α-D-glucopyranosides 5-12*

Having 2,6-di-*O*-pentanoate **4** in hand we prepared its eight 3,4-di-*O*-acyl esters with various acyl groups of different chain length(s) (Scheme 2) to get newer glucopyranoside based SEs of biological importance. Thus, reaction of diol **4** with excess acetic anhydride in dry pyridine provided an oil (94 %, Scheme 2). Its FT-IR spectrum showed peaks at 1775, 1772, 1770 and 1769 cm^{-1} (CO) and no band for hydroxyl stretching. The ^{1}H NMR spectrum of this compound showed two three-proton singlets at δ 2.01 and 1.99 corresponding to two acetyl methyl groups, and hence indicated the attachment of two acetyloxy groups in the molecule. Proton H-3 and H-4 were found to appear downfield positions at δ 5.46 (as t, *J* = 9.7 Hz) and 5.03 (as t, *J* = 9.8 Hz), respectively as compared to its precursor compound diol **4** (H-3 at δ 3.94 and H-4 at δ 3.73) which were affirmative of the attachment of two aceyloxy groups at C-3 and C-4 positions. On the basis of FT-IR and ^{1}H NMR spectra the structure was accorded as methyl 3,4-di-*O*-acetyl-2,6-di-*O*-pentanoyl-α-D-glucopyranoside (**5**). The formation of 3,4-di-*O*-acetate **5** also confirmed the structure of 2,6-di-*O*-pentanoate **4**.



Scheme 2. Reagents and conditions: (*a*) Py, $Ac_2O/(CH_3)_3CCOCl/C_5H_{11}COCl/C_{11}H_{23}COCl/C_{13}H_{27}COCl$/MsCl/4-Cl.BzCl/2,6-di-Cl.BzCl, DMAP, 0 °C-rt, 11-18 h.

Similarly, separate treatment of diol **4** with dimolar pivaloyl chloride, hexanoyl chloride, lauroyl chloride, myristoyl chloride, mesyl chloride, 4-chlorobenzoyl chloride and 2,6-dichlorobenzoyl chloride formed corresponding 3,4-di-*O*-acyl esters **6-12**, respectively in good yields (Scheme 2). Attachment of new acyl groups at C-3 and C-4 position was confirmed from the considerable downfield shifts of H-3 and H-4 protons in their ^{1}H NMR spectra (Table 2).

Table 2. ^{1}H NMR shift values of protons (δ ppm, *J* in Hz).

SEs	H-2	H-3	H-4	H-6
4	4.66 (dd, *J* 10.0 & 3.7)	3.94 (t, *J* 9.3)	3.73 (t, *J* 9.7)	4.47 (dd, *J* 12.1 & 5.0 6a), 4.25 (dd, *J* 12.1 & 2.0 6b)
5	4.88 (dd, *J* 10.1 & 3.6)	5.46 (t, *J* 9.7)	5.03 (t, *J* 9.8)	4.22 (dd, *J* 12.3 & 4.8 6a), 4.13 (dd, *J* 12.0 & 2.2 6b)
6	4.90-4.94 (m)	5.50 (t, *J* 9.8)	5.09 (t, *J* 9.9)	4.10-4.15 (m)
7	4.88 (dd, *J* 10.0 & 3.8)	5.49 (t, *J* 9.8)	5.06 (t, *J* 9.7)	4.17 (dd, *J* 12.2 & 4.6 6a), 4.12 (dd, *J* 12.2 & 2.2 6b)
8	4.87 (dd, *J* 10.1 & 3.7)	5.48 (t, *J* 9.9)	5.05 (t, *J* 9.7)	4.17 (dd, *J* 12.1 & 4.6 6a), 4.11 (dd, *J* 12.1 & 2.2 6b)
9	4.88 (dd, *J* 10.0 & 3.6)	5.49 (t, *J* 9.8)	5.09 (t, *J* 9.7)	4.09-4.22 (m)
10	4.74 (dd, *J* 10.0 & 3.7)	5.11 (t, *J* 9.8)	4.90-4.96 (m)	4.30-4.39 (m)
11	5.14 (dd, *J* 10.2 & 3.7)	5.88 (t, *J* 9.9)	5.43 (t, *J* 9.8)	4.18-4.24 (m)
12	5.14 (dd, *J* 10.1 & 3.6)	5.86 (t, *J* 9.8)	5.44 (t, *J* 9.9)	4.17-4.22 (m)

3.3. *Computational biological activities: Prediction of activity spectra for substances (PASS)*

Initially drawn SMILES (simplified molecular-input line-entry system) of the compounds were used to find biological activities from PASS software (www.pharmaexpert.ru/ PASS online/index.php) [10,15]. PASS results, as shown in Table 3, indicated that the synthesized SEs had potential antimicrobial activities. Especially these SEs could be more active against fungal pathogens ($0.54 < Pa < 0.69$) as compared to bacterial organisms ($0.46 < Pa < 0.57$). The PASS study also revealed that these SEs have various potential

biological activities such as anticarcinogenic (0.46 < Pa < 0.72) and antioxidant (0.31 < Pa < 0.48) properties which need further studies to validate these predictions.

Table 3. Predicted biological activities of **4-12** using PASS software.

Drug	Biological Activity							
	Antibacterial		Antifungal		Anticarcinogenic		Antioxidant	
	Pa	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**
3	0.541	0.013	0.628	0.016	0.731	0.008	0.667	0.004
4	0.568	0.011	0.695	0.010	0.723	0.008	0.481	0.007
5	0.574	0.010	0.692	0.010	0.672	0.010	0.457	0.008
6	0.550	0.012	0.684	0.011	0.575	0.014	0.423	0.010
7	0.551	0.012	0.673	0.011	0.614	0.012	0.463	0.008
8	0.551	0.012	0.673	0.011	0.614	0.012	0.463	0.008
9	0.551	0.012	0.673	0.011	0.614	0.012	0.463	0.008
10	0.463	0.020	0.549	0.024	0.456	0.024	0.310	0.021
11	0.507	0.016	0.685	0.010	0.559	0.015	0.367	0.015
12	0.479	0.018	0.668	0.012	0.509	0.019	0.347	0.017

Pa = Probability 'to be active'; Pi = Probability 'to be inactive'

3.4. *In vitro antimicrobial evaluation of SEs 4-12*

3.4.1. *Effects of glucopyranoside esters **4-12** against bacteria*

In the present study, we used four Gram-positive and five Gram-negative organisms using disc diffusion method [39,40]. Gram-positive organisms were *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis* and *Staphylococcus aureus*. Five Gram-negative organisms were *Escherichia coli*, *Shigella dysenteriae*, *Salmonella paratyphi*, *Salmonella typhi* and *Vibrio cholera*. The effects are shown in Table 4 as diameter of inhibition zone (mm, Fig. 2).

Table 4. *In vitro* effects of SEs **4-12** against bacteria.

Drug	Diameter of zone of inhibition in mm (100 µg dw /disc)								
	B. cereus	*B. megaterium*	*B. subtilis*	*S. aureus*	*E. coli*	*S. dysenteriae*	*S. paratyphi*	*S. typhi*	*V. cholera*
3	NI	NI	NI	NI	NI	NI	NI	NI	NI
4	NI	7.9±.18	NI	NI	11.0±.18	NI	8.5±.34	NI	7.5±.50
5	NI	NI	NI	NI	7.5±.19	NI	NI	NI	6.3±.18
6	NI	NI	NI	NI	NI	NI	6.5±.28	NI	7.5±.5
7	NI	NI	NI	NI	NI	NI	6.5±.28	NI	6.2±.20
8	*11.4±.22	*12.0±.29	*11.5±.43	*13.0±.43	*12.4±.31	*12.9±.34	*11.6±.42	9.5±.33	9.5±.50
9	NI	NI	NI	NI	NI	NI	NI	NI	NI
10	NI	6.0±.33	NI	NI	NI	NI	NI	NI	NI
11	10.6±.28	11.3±.24	9.5±.20	NI	NI	NI	NI	NI	9.5±.42
12	NI	*10.2±.39	9.2±.20	NI	NI	*10.6±.34	NI	NI	7.5±.50
Amp**	*19.2±.54	*18.1±.24	*16.0±.48	*22.6±.86	10.0±.68	*21.9±.61	*18.0±.34	*19.5±.58	*32.2±.71

Data are presented as Mean±SD; * = Good inhibition; ** = Reference antibiotic Ampicillin (Amp; 25 µg/disc); dw = Dry weight; NI = No inhibition; NI was observed for control (DMF); Values are represented for the triplicate of all the experiments.

The glucopyranoside derived SEs were found to possess weak to moderate activity against both Gram-positive and Gram-negative organisms. Only 3,4-di-*O*-lauroate **8** with twelve-carbon ester chain (C12) showed moderate inhibition against both types of organisms.

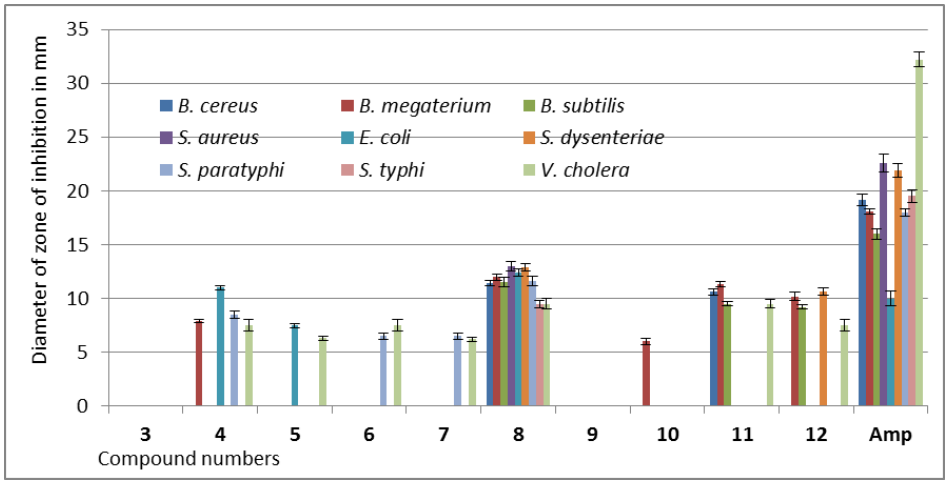


Fig. 2. Activities against bacterial pathogens.

3.4.2. *Effects of SEs **4-12** against fungal pathogens*

The effect of the SEs **4-12** against four pathogenic fungi viz. *Aspergillus flavus*, *Aspergillus ochraceus*, *Fusarium equiseti* and *Macrophomina phaseolina* are presented in Table 5 and Fig. 3.

Table 5. *In vitro* inhibition against fungal pathogens by **4-12**.

Drug	% Inhibition of fungal mycelial growth (100 µg dw / mL PDA)			
	A. flavus	*A. ochraceus*	*F. equiseti*	*M. phaseolina*
3	NI	NI	NI	NI
4	NI	NI	28.0±.33	27.0±.33
5	NI	NI	24.5±.33	NI
6	16.6±.18	NI	NI	NI
7	NI	NI	30.1±.29	*65.9±.91
8	21.5±.50	27.9±.29	NI	*74.0±.84
9	NI	NI	NI	19.2±.58
10	NI	23.6±.74	24.0±.48	40.5±.33
11	15.5±.66	18.0±.34	42.3±.48	*50.5±.33
12	NI	NI	26.8±.29	NI
Nys**	26.5±.33	22.9±.37	44.7±.74	*71.8±.84

Data are presented as Mean ± SD; * = Good inhibition; ** = Reference antibiotic nystatin (Nys; 12.5 µg/mL PDA); dw = Dry weight; NI = No inhibition; NI was observed for control (DMF); Values are represented for the triplicate of all the experiments.

The results are presented as the percentage inhibitions of mycelial growth [39,40], which indicated that these SEs were more prone against fungal pathogens compared to bacterial organisms, and was found to be in consistent with our previous observations for **1a** [15]. Among the fungi *M. phaseolina* was found the most sensitive by these SEs. In addition, pentanoyl chain (C5) along with lauroyl chain (compound **8**, C12, *74.0±0.84 %) showed highest inhibition against this fungus followed by nystatin (*71.8±0.84 %), hexanoyl chain (compound **7**, C6, *65.9±0.91 %) and 4-chlorobenzoyl group (compound **11**, *50.5±0.33 %).

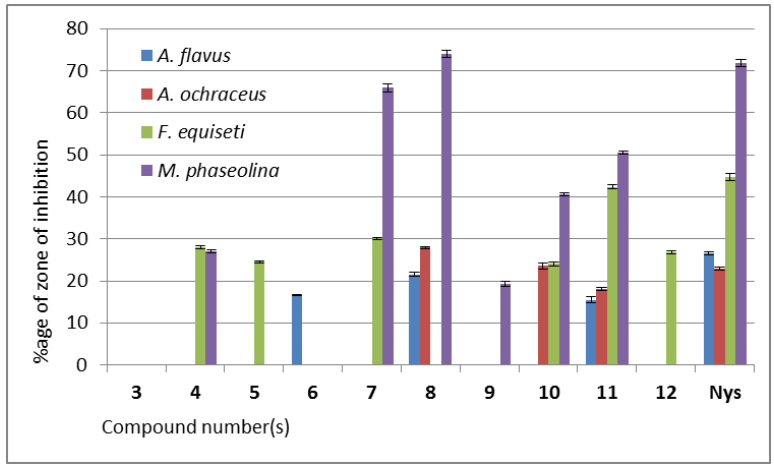


Fig. 3. Activities against fungal pathogens.

3.5. *In silico ADME/T analysis*

Pharmacokinetic profiles of a drug molecule are constituted by absorption, distribution, metabolism, excretion, and toxicity (ADMET). ADMET is very essential in evaluating drugs pharmacodynamic activities. In the present study ADMET properties, as derived from admet SAR server [36], revealed that (Table 6) all the CFA esters, except **3** and **4**, showed positive result for blood brain barrier (BBB) criteria, predicting that they can easily pass through the BBB. They are noncarcinogenic and relatively harmless for oral administration bearing III category (safe) acute oral toxicity. All compounds (except **3** and **4**) are P-glycoprotein inhibitor where, P-glycoprotein inhibition can interrupt the absorption, permeability and retention of the chemical species. However, these SEs showed weak inhibitory properties for human ether-a-go-go-related gene (hERG) which might lead to long QT syndrome.

Table 6. AdmetSAR calculation of glucopyranoside derived SEs.

Drug	Blood brain barrier	Human intestinal absorption	P-glyco-protein inhibitor	hERG inhibition	Carcinogen	Acute oral toxicity	RAT LD_{50} (mol/kg)
3	-0.6148	-0.8373	NI 0.9393	WI 0.9535	NC 0.9654	III	1.1350
4	-0.6226	-0.5948	NI 0.5563	WI 0.9446	NC 0.9550	III	2.2438
5	0.9074	0.9233	I 0.7251	WI 0.9110	NC 0.9045	III	2.0661
6	0.8947	0.9264	I 0.6991	WI 0.9689	NC 0.8693	III	2.0084
7	0.9271	0.8974	I 0.8144	WI 0.9146	NC 0.9027	III	1.9138
8	0.9271	0.8974	I 0.8144	WI 0.9146	NC 0.9027	III	1.9138
9	0.9271	0.8974	I 0.8144	WI 0.9146	NC 0.9027	III	1.9138
10	0.9330	0.9689	NI 0.7602	WI 0.7365	NC 0.5600	III	2.4933
11	0.9160	0.9635	I 0.8169	WI 0.8651	NC 0.8745	III	2.6145
12	0.9199	0.9536	I 0.7908	WI 0.8979	NC 0.8844	III	2.6764

hERG = Human ether-a-go-go-related gene, RAT = Rat acute toxicity, NI = Non-inhibitor, I = Inhibitor, WI = Weak inhibitor, NC = Non-carcinogenic

Additional drug likeliness test was studied from SwissADME calculation (Table 7). SwissADME calculation [37] indicated that all the SEs have good hydrogen bonds donor and acceptor (except dimesylate **10**).

Table 7. Calculation of drug likeliness using SwissADME program.

Drug	HB accep-tors	HB don-ors	TPSA ($Å^2$)	Inhibitor(s)					PAINS alerts
				CYP 1A2	CYP 2C19	CYP 2C9	CYP 2D6	CYP 3A4	
3	6	4	99.38	No	No	No	No	No	0
4	8	2	111.52	No	No	No	No	No	0
5	10	0	123.66	No	No	No	Yes	No	0
6	10	0	123.66	No	Yes	No	Yes	Yes	0
7	10	0	123.66	No	Yes	No	No	No	0
8	10	0	123.66	No	No	No	No	No	0
9	10	0	123.66	No	No	No	No	No	0
10	12	0	174.56	No	No	No	No	No	0
11	10	0	123.66	No	Yes	Yes	Yes	Yes	0
12	10	0	123.66	No	No	Yes	Yes	No	0

*HB = Hydrogen bond, TPSA = Topological polar surface area, PAINS = Pan-assay interference compounds.

Topological polar surface area (TPSA) data showed the good polarity of the compounds, where the TPSA value should be less than 140 $Å^2$, more the value more the polarity. The CYP enzymes, particularly isoforms 1A2, 2C9, 2C19, 2D6, and 3A4, are responsible for about 90 % oxidative metabolic reactions. Inhibition of CYP enzymes will lead to inductive or inhibitory failure of drug metabolism. Pan-assay interference compounds (PAINS) are chemical compounds that often give false positive results in high-throughput screens with numerous biological targets. Here PAINS revealed no violation with these glucopyranoside esters (Table 7).

2.6. *Structure activity relationship (SAR)*

It is necessary to understand the structure activity relationship to know the mechanisms of antimicrobial action for the design and improved antimicrobial agents. Thus, we attempted to derive structure activity relationship (SAR) of the SEs on the basis of our results. It was evident from Tables 4 and 5 (Figs. 2 and 3) that incorporation of different acyl groups especially pentanoyl group at C-2 and C-6 positions and lauroyl group at C-3 and C-4 positions as in **8**, increased the antimicrobial potentiality of methyl α-D-glucopyranoside (**3**). Incorporation of pentanoyl, lauroyl and other acyl group(s) gradually increased lipophilicity of the CFA esters **4**-**12**, which is an important parameter with respect to bioactivity such as toxicity or alteration of membrane integrity, because it is directly related to membrane permeation [26,41-44]. More importantly it was observed that, these glucopyranoside based esters **4**-**12** were more prone against fungal pathogens especially *M. phaseolina* than that of bacterial organisms.

4. Conclusion

Dimolar pentanoylation of methyl α-D-glucopyranoside (**3**) and a series of its 3,4-di-*O*-acyl esters were successfully synthesized in view of their *in vitro* antimicrobial studies. PASS predication and *in silico* pharmacokinetic calculations were found in favour of drug likeliness of these SEs. Combination of pentanoyl (C5) and lauroyl (C12) groups in glucopyranoside framework, as in CFA ester **8**, showed excellent antifungal potentiality against *M. phaseolina*, and hence, could be a future lead compound for the development of antifungal antibiotics.

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