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Synthesis, Antifungal Activity, and Molecular Docking Studies of Some New Di-*O*-Isopentanoyl Glucopyranosides

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Extensive research over the past decades has shown that sugar ester (SE)-type biomolecules bring long-chain fatty acids with sugar moieties into the plant cells and play various important roles in food, surfactants, innovative green materials, and biological properties. Thus, in this study, dimolar isopentanoylation of methyl α-D-glucopyranoside (compound 4) furnished methyl-2,6-di-*O*-isopentanoyl-α-D-glucopyranoside (compound 5), indicating selectivity at C-2 and C-6 positions. The obtained compound (5) was further acylated to give 3,4-di-*O*-acyl esters (compounds 5-8) in good yields. *In vitro* antifungal activities of these compounds exhibited moderate to good zone of inhibition. To rationalize these results, molecular docking studies of compounds 4-8 were performed on lanosterol 14-α-demethylase (CYP 51). The attachment of acyl ester chain(s) to the glucopyranoside ring added more lipophilicity and affected their fungal inhibition by binding to the lanosterol 14-α-demethylase enzyme. In particular, the isopentanoyl group showed a stronger binding affinity with lauroyl groups, as in compound 8, than with the fluconazole group, indicating the higher efficiency of SEs.

Keywords: Antimicrobial, Glucose esters, HMBC, Selective acylation, Lanosterol 14-α-demethylase

INTRODUCTION

Sugar esters (SEs), also known as sugar fatty acid esters, are generally prepared by esterification between sugar/sugar alcohols and non-polar fatty acids/acyl halides [1]. Glycosidic and ester bonds with alkyl fatty acid chain showed amphiphilicity and biodegradability under aerobic and anaerobic conditions [2], indicating that SEs are environment friendly, biocompatible, non-toxic, tasteless, odorless, non-irritating, and cost-effective [3-5]. SEs-type biomolecules bring long-chain fatty acids with sugar moieties into the plant cells and play various important roles, including the gelatinization of starch, formation of emulsions, *etc*. [6]. These properties allow SEs to have broad-spectrum applications in the food, cosmetics, beverage, surfactant, chemical, and pharmaceutical sectors [7-8]. Encouragingly, many natural and synthetic SEs have been reported to have antibacterial [9], antifungal [10-11], and pesticide [12-13] properties. Recently, SEs have been used in the preparation of biocompatible materials. For instance, SEs have been found to improve the performance of high-amylose starch-based wood adhesives (HASWA) by increasing the thermal stability of starch and blocking the aggregation of latex particles [14]. In addition, several SEs have been found to resist wood decay in aspen and pine wood. Biomaterials prepared by the combination of SEs and chitosan showed superior wound healing properties *in vivo* compared to the available wound healing products (Healosol®) [15]. These wound healing properties were found to depend on the degree of substitution of the SEs

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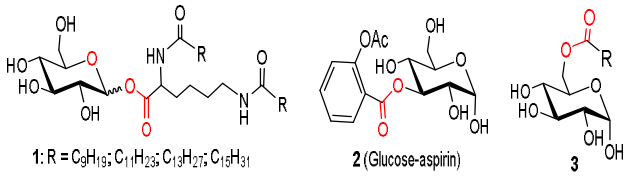


Fig. 1. Biologically active glucose esters (1-3).

[16].

Of the SEs, glucose esters have attracted enormous interest since their precursor glucose is widely available in nature [17]. These glucose-derived esters are used as synthetic intermediates and surfactants [18] and for pharmaceutical purposes [19]. For example, lysine glucose ester 1 (Fig. 1) was used as a twin-tailed anionic surfactant with continuous micellization processes [18]. Glucose-derived ester glucose-aspirin (GA, **2**) was found to be 8-9-fold more active against various cancer cell lines (SKBR3, PANC-1, and PC3) and showed better solubility in water under experimental conditions [20]. Several glucose esters (substitution at C-6 position; 3) showed significant activity against the larvae of *Aedesaegypti*, human pathogens (*K. pneumoniae*, *P. aeruginosa*, *B. subtilis, S. aureus*, *etc*.), and cancer cells (SKOV-3), leading to their application as pharmaceuticals [19]. However, the activities of SEs were found to be greatly affected by the hydrophilic-lipophilic balance (HLB), number, and position of substituents, *i.e.* acyl groups [21].

Several methods have been developed and employed for the selective and regioselective esterification of monosaccharide hydroxyls [22-23]. In the present study, methyl α-D-glucopyranoside (4)-derived di-*O*-isopentanoyl glucopyranosides (compounds 5-8) were synthesized for antifungal susceptibility tests. The *in vitro* results were further verified by the molecular docking studies of the related antifungal enzyme (*i.e.*, lanosterol 14-α-demethylase).

MATERIALS AND METHODS

General Methods

Analytical grade reagents, solvents, and chemicals (Merck, Darmstadt, Germany) were used throughout the experiments to synthesize the compounds. Evaporations were conducted in a Buchi rotary evaporator (R-100, Buchi Corporation, New Castle, DE, USA) under diminished pressure. Thin-layer chromatography (TLC) was performed on Kieselgel GF_{254} plates, which were heated at 150-200 °C by spraying with 1% methanolic sulfuric acid until coloration occurred. Purification was performed by column chromatography (CC) with silica gel G_{60}. Fourier-transform infrared spectroscopy (FT-IR, IR Prestige-21, Shimadzu, Kyoto, Japan) was performed without solvent. Deuterated chloroform ($CDCl_3$) solution was used as a solvent in the analysis of nuclear magnetic resonance (NMR, ^{1}H: 400 MHz, ^{13}C: 100 MHz) (Bruker DPX-400 spectrometer, Billerica, MA, USA) spectra. In general, chemical shifts are reported in δ unit (ppm), with tetramethylsilane (TMS) as an internal standard, and the coupling constants (*J*) are reported in Hz.

Synthesis

Methyl-2,6-di-*O*-isopentanoyl-α-D-glucopyranoside (compound 5). Isopentanoyl chloride (1.366 g, 11.329 mmol) was slowly added to a cold (-5 °C) solution of methyl α-D-glucopyranoside (4, 1.0 g, 5.15 mmol) in pyridine (12 ml) and stirred for 7 h at the same temperature, followed by 3 h at room temperature. Usual workup of the reaction mixture, concentration, and purification by column chromatography (CC) gave ester compound 5 as semi-solid in a good yield of 63% (1.175 g). R_f = 0.57 ($CHCl_3$/MeOH = 5/1); FT-IR (neat) ν_{max} (cm^{-1}): 3220-3450 (OH), 1733, 1722 (CO), 1097 (pyranose ring); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 4.90 (d, J = 3.6 Hz, 1H, H-1), 4.68 (dd, J = 10.0 and 3.6 Hz, 1H, H-2), 4.46 (dd, J = 12.2 and 4.8 Hz, 1H, H-6a), 4.29 (dd, J = 12.2 and 1.6 Hz, 1H, H-6b), 3.95 (t, J = 9.6 Hz, 1H, H-3), 3.73-3.78 (m, 1H, H-5), 3.42 (t, J = 9.6 Hz, 1H, H-4), 3.37 (s, 3H, OCH_3), 3.15-3.22 (br s, 2H, 2×OH), 2.26-2.30 [m, 4H, 2×$(CH_3)_2CHCH_2CO$], 2.08-2.16 [m, 2H, 2×$(CH_3)_2CHCH_2CO$], 0.98, 0.97 [2×s, 2×6H, 2×$(CH_3)_2CHCH_2CO$]; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 173.9, 173.0 [2×$(CH_3)_2CHCH_2CO$], 97.1 (C-1), 72.9 (C-2), 71.3 (C-3), 70.6 (C-5), 69.4 (C-4), 62.9 (C-6), 55.2 (OCH_3), 43.2(2) [2×$(CH_3)_2CHCH_2CO$], 25.8, 25.7 [2×$(CH_3)_2CHCH_2CO$], 22.4(2), 22.3, 22.2 [2×$(CH_3)_2CHCH_2CO$]. The position of signals in the NMR spectra was assigned by analyzing its 2D experiment, such as COSY, DEPT-135, HSQC, HMBC, *etc*.

Preparation of 3,4-di-*O*-acylates 6-8 of 5: Direct acylation method was used for the preparation of compounds 6-8 from compound 5. Acylating agent(s) was added to a solution of compound 5 (0.1 g, 0.276 mmol) in dry pyridine at 0 ℃; then, the mixture was stirred. After 12 h, the reaction mixture was stirred for another 2 h at 30 ℃. The reaction was quenched with cold water, extracted with chloroform (3 × 5ml), and washed with dilute HCl, $NaHCO_3$, H_2O, and brine. The concentration of the organic layer and CC purification gave the corresponding acyl ester(s) (compounds 6-8) in good yields.

Methyl-2,6-di-*O*-isopentanoyl-3,4-di-*O*-octanoyl-α-D-glucopyranoside (6): Yield 89%; Syrup; R_f = 0.49 (n-Hex/EtOAc = 5/1); FT-IR (neat) ν_{max} (cm^{-1}): 1749, 1744, 1739, 1731 (CO), 1095 (pyranose ring); ^1H NMR (400 MHz, $CDCl_3$) δ_H: 5.53 (t, J = 9.6 Hz, 1H, H-3), 5.09 (t, J = 9.6 Hz, 1H, H-4), 4.96 (d, J = 3.4 Hz, 1H, H-1), 4.89 (dd, J = 10.0 and 3.4 Hz, 1H, H-2), 4.22-4.32 (m, 2H, H-6a and H-6b), 3.98-4.02 (m, 1H, H-5), 3.42 (s, 3H, OCH_3), 2.19-2.39 [m, 8H, 2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3(CH_2)_5CH_2CO$], 2.07-2.15 [m, 2H, 2×$(CH_3)_2CHCH_2CO$], 1.53-1.66 [m, 8H, 2×$CH_3(CH_2)_3(CH_2)_2CH_2CO$], 1.22-1.35 [br s, 12H, 2×$CH_3(CH_2)_3(CH_2)_3CO$], 0.94-0.99 [m, 2×6H, 2×$(CH_3)_2CHCH_2CO$], 0.89 [t, J = 7.2 Hz, 6H, 2×$CH_3(CH_2)_6CO$]; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 173.0, 172.7, 172.3, 172.2 [2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3(CH_2)_6CO$], 96.8 (C-1), 70.8 (C-2), 69.7 (C-3), 68.4 (C-4), 67.4 (C-5), 61.8 (C-6), 55.4 (OCH_3), 43.1(2) [2×$(CH_3)_2CHCH_2CO$], 34.1, 34.0 [2×$CH_3(CH_2)_5CH_2CO$], 31.6(2) [2×$CH_3(CH_2)_4CH_2CH_2CO$], 29.1, 29.0(2), 28.9 [2×$CH_3(CH_2)_2(CH_2)_2(CH_2)_2CO$], 25.7, 25.4 [2×$(CH_3)_2CHCH_2CO$], 24.9, 24.7 [2×$CH_3CH_2CH_2(CH_2)_4CO$], 22.6(2), 22.4(2), 22.3(2) [2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3CH_2(CH_2)_5CO$], 14.1(2) [2×$CH_3(CH_2)_6CO$].

Methyl-3,4-di-*O*-decanoyl-2,6-di-*O*-isopentanoyl-α-D-glucopyranoside (7): Yield 87%; Syrup; R_f = 0.52 (n-Hex/EtOAc = 5/1); FT-IR (neat) ν_{max} (cm^{-1}): 1748, 1741, 1739, 1733 (CO), 1092 (pyranose ring); ^1H NMR (400 MHz, $CDCl_3$) δ_H: 5.54 (t, J = 9.8 Hz, 1H, H-3), 5.07 (t, J = 9.8 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), 4.24-4.33 (m, 2H, H-6a and H-6b), 3.96-4.00 (m, 1H, H-5), 3.43 (s, 3H, OCH_3), 2.18-2.37 [m, 8H, 2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3(CH_2)_7CH_2CO$], 2.08-2.14 [m, 2H, 2×$(CH_3)_2CHCH_2CO$], 1.55-1.67 [m, 8H, 2×$CH_3(CH_2)_5(CH_2)_2CH_2CO$], 1.21-1.38 [br s, 20H, 2×$CH_3(CH_2)_5(CH_2)_3CO$], 0.94-0.98 [m, 2×6H, 2×$(CH_3)_2CHCH_2CO$], 0.88 [t, J = 7.0 Hz, 6H, 2×$CH_3(CH_2)_8CO$]; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 173.2, 172.8, 172.3, 172.0 [2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3(CH_2)_8CO$], 97.0 (C-1), 71.2 (C-2), 70.0 (C-3), 68.4 (C-4), 67.7 (C-5), 61.7 (C-6), 55.5 (OCH_3), 43.2(2) [2×$(CH_3)_2CHCH_2CO$], 34.2, 34.0 [2×$CH_3(CH_2)_7CH_2CO$], 31.4 (2) [2×$CH_3(CH_2)_6CH_2CH_2CO$], 29.2, 29.1(2), 28.6 [2×$CH_3(CH_2)_4(CH_2)_2(CH_2)_2CO$], 25.8, 25.6 [2×$(CH_3)_2CHCH_2CO$], 25.0, 24.8 [2×$CH_3(CH_2)_3CH_2(CH_2)_4CO$], 22.8(2), 22.6(3), 22.4(2), 22.3(3) [2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3(CH_2)_3(CH_2)_5CO$], 14.0(2) [2×$CH_3(CH_2)_8CO$].

Methyl-2,6-di-*O*-isopentanoyl-3,4-di-*O*-lauroyl-α-D-glucopyranoside (8): Yield 81%; Thick liquid; R_f = 0.56 (n-Hex/EtOAc = 5/1); FT-IR (neat) ν_{max} (cm^{-1}): 1751, 1744, 1736, 1731 (CO), 1095 (pyranose ring); ^1H NMR (400 MHz, $CDCl_3$) δ_H: 5.56 (t, J = 9.6 Hz, 1H, H-3), 5.05 (t, J = 9.6 Hz, 1H, H-4), 4.95 (d, J = 3.6 Hz, 1H, H-1), 4.86 (dd, J = 10.0 and 3.6 Hz, 1H, H-2), 4.22-4.34 (m, 2H, H-6a and H-6b), 3.99-4.04 (m, 1H, H-5), 3.41 (s, 3H, OCH_3), 2.16-2.38 [m, 8H, 2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3(CH_2)_9CH_2CO$], 2.09-2.14 [m, 2H, 2×$(CH_3)_2CHCH_2CO$], 1.59-1.70 [m, 8H, 2×$CH_3(CH_2)_7(CH_2)_2CH_2CO$], 1.18-1.38 [br s, 28H, 2×$CH_3(CH_2)_7(CH_2)_3CO$], 0.93-0.99 [m, 2×6H, 2×$(CH_3)_2CHCH_2CO$], 0.89 [t, J = 7.2 Hz, 6H, 2×$CH_3(CH_2)_{10}CO$]; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 173.5, 172.9, 172.2, 172.0 [2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3(CH_2)_{10}CO$], 97.2 (C-1), 71.0 (C-2), 70.4 (C-3), 68.2 (C-4), 67.9 (C-5), 61.4 (C-6), 55.5 (OCH_3), 43.2, 43.0 [2×$(CH_3)_2CHCH_2CO$], 34.6, 34.2 [2×$CH_3(CH_2)_9CH_2CO$], 31.6, 31.1 [2×$CH_3(CH_2)_8CH_2CH_2CO$], 29.8, 29.5, 29.0, 28.8 [2×$CH_3(CH_2)_6(CH_2)_2(CH_2)_2CO$], 25.5, 25.2 [2×$(CH_3)_2CHCH_2CO$], 25.0, 24.7 [2×$CH_3(CH_2)_5CH_2(CH_2)_4CO$], 23.1, 23.0(2), 22.8(2), 22.6(3), 22.4(2), 22.3(3), 22.0 [2×$(CH_3)_2CHCH_2CO$ and 2×$CH_3(CH_2)_5(CH_2)_5CO$], 13.9(2) [2×$CH_3(CH_2)_8CO$].

Antifungal Screening

Antifungal susceptibility testing (AFST) is a tool to determine the fungal inhibitory property of compounds and develop novel antifungal drugs. Of the available methods, including broth dilution, disk diffusion, gradient diffusion, azole agar method, *etc*., to determine antifungal susceptibility, poisoned food technique has been found to be suitable for *in vitro* tests [24-25]. In the present study, this established method was used to evaluate antifungal activities of the synthesized esters (compounds 5-8) against two fungi, namely, *Alternaria alternata* (Fr.) Keissler and *Macrophomina phaseolina* (Tassi) Goid. Antifungal screening was performed using a literature procedure [26]. The tests were conducted three times, and the antifungal indices were calculated after 48 h. Also, the results were compared with the available standard drug called fluconazole (100 µg ml^{-1} medium; brand name Omastin, Beximco Pharmaceuticals Ltd., Bangladesh). The antifungal activity was measured as the reduction of mycelial growth in the poisoned plate compared to the control plate and expressed in the form of the mycelial growth inhibition percentage using the Bruce-Vincent formula [26]:

$$\%inhibition = \frac{mean\,diameter\,fungal\,colony(control) - mean\,diameter\,fungal\,colony(compd.)}{mean\,diameter\,fungal\,colony(control)} \times 100$$

Molecular Docking

The basic geometry of glucopyranose was obtained from PubChem. Then, the structures of compounds 4-8 were drawn using Chem3D 18.0 software, optimized with B3LYP functional, and visualized using the GaussView program. Finally, the optimized geometries were saved as PDB files and used as ligands during molecular docking analysis.

Lanosterol 14-alpha demethylase (LDM)-related crystal structure (PDB id: 5V5Z; organism: *Candida albicans*) was obtained from the RCSB Protein Data Bank and opened in PyMOL V2.3 (https://pymol.org/2/). Water molecules and unnecessary ligands were removed from the PDB files to prepare the proteins.

Once the proteins and ligands (compounds 4-8) were prepared, molecular docking was performed using AutoDock Vina (PyRx software) using a method described in the literature [27-28]. Maximum dimensions of the grid-box were used during the docking. Finally, Discovery Studio Visualizer 17 was used for the analyses and visualization of docked complexes.

Calculation of Physicochemical Properties

The DFT optimized structures of compounds 4-8 were used for the calculation of various physicochemical properties. In particular, the electronic energy (EE), enthalpy (ΔH), Gibbs free energy (GFE), dipole moment (DM), HOMO and LUMO energy gap, hardness, and softness of the compounds were calculated. In this regard, the HOMO-LUMO gap (Δε), hardness (η), and softness (S) were calculated using the following equations [5, 11]:

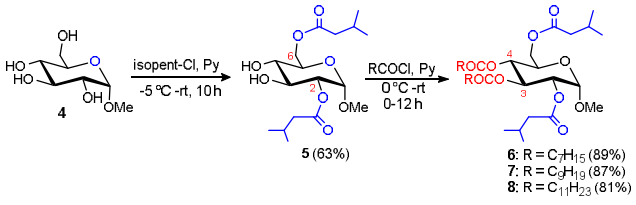
$$\Delta\varepsilon = [\varepsilon LUMO - \varepsilon HOMO]; \quad \eta = \frac{\varepsilon LUMO - \varepsilon HOMO}{2}; \quad S = \frac{1}{\eta}$$

RESULTS AND DISCUSSION

Synthesis of Glucose-based Isopentanoyl Esters

Sugar-based biomaterials are of great significance [29, 30]. To get selective isopentanoyl esters of glucopyranoside, glucopyranoside 4 was subjected to direct dimolar isopentanoylation at a low temperature (-5 °C) for 7-10 h, and a thick syrup in 63% yield was obtained (Scheme 1).

The FT-IR spectrum showed both OH (3220-3450 cm^{-1}) and CO (1733 and 1722 cm^{-1}) stretching bands, indicating partial isopentenylation of the molecule. In the ^{1}H and ^{13}C NMR spectra, the appearance of additional 18 protons and two carbonyl carbons (δ 173.9 and 173.0) indicated the attachment of two isopentanoyl groups. The downfield shift of H-2 (δ 4.68) and H-6 (δ 4.46 and 4.29) protons compared to those of compound 4 indicated the successful attachment of isopentanoyl groups at C-2 and C-6 positions. This



Scheme 1. The synthesis of glucopyranoside-derived acyl esters 5-8

was finally confirmed by the results of the 2D HMBC experiment, in which carbonyl carbons showed multiple bond correlations with H-2 and H-6 protons (Fig. 2). Thus, the syrup was identified as methyl-2,6-di-*O*-isopentanoyl-α-D-glucopyranoside (compound 5).

The formation of 2,6-di-*O*-isopentanoate (compound 5) in a moderate yield was probably due to the formation of an inseparable mixture of mono- and poly-substituted by-products (glucose esters). However, the formation of compound 5 clearly indicated the higher reactivity of C-2 OH among the three secondary hydroxyl groups under direct acylation conditions.

The treatment of compound 5 with dimolar octanoyl chloride gave a homogeneous syrup in a good yield (89%; Scheme 1). The absence of OH stretching bands in the FT-IR spectrum indicated the complete acylation of the syrup. The ^{13}C NMR spectrum showed the presence of four characteristic carbonyl carbons (δ173.0, 172.7, 172.3, and 172.2) and two methyl carbons (δ 14.1). The ^{1}H NMR spectrum showed the presence of 30 extra protons, corresponding to two octanoyl groups in addition to the protons of compound 5. More information was inferred from a comparison of the downfield shift of H-3 (δ 5.53) and H-4 (δ 5.09) protons with that of compound 5 (H-3 at δ 3.95 and H-4 at δ 3.42), which confirmed the attachment of octanoyl groups at C-3 and C-4 positions. Based on all the spectral data, the compound was identified as methyl-2,6-di-*O*-isopentanoyl-3,4-di-*O*-octanoyl-α-D-glucopyranoside (compound 6).

Similarly, dimolar decanoylation and lauroylation of compound 5 furnished decanoate 7 and laureate 8, respectively, in good yields (Scheme 1), which were characterized by spectroscopic methods.

Antifungal Potentiality

Due to the multi-drug resistance of common antifungal agents, searching for and developing new antifungal agents to combat antifungal resistance is of significant importance [24]. As SEs have antimicrobial properties [19-20,31], attention was paid to the antifungal bioassays of the synthesized glucopyranoside esters (compounds 5-8) against two fungal pathogens (*i.e.*, *A. alternata* and *M. phaseolina*). The results are presented in Table 1.

Table 1 shows that with the addition of acyl group(s),

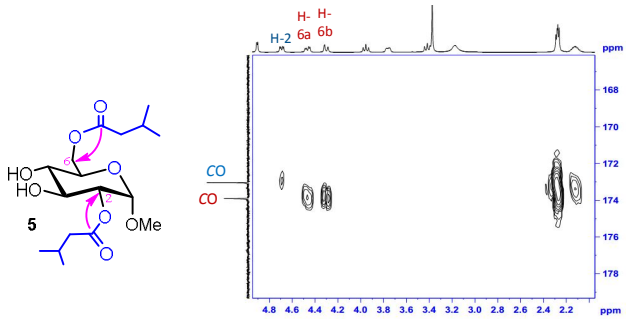


Fig. 2. The 2D HMBC correlation of 2,6-diester (compound 5).

Table 1. The Percentage (%) Inhibition of Organisms by the Glucopyranoside Esters

Compound	Percentage (%) of inhibition of mycelial growth (100 µg dw ml^{-1} PDA)	
	A. alternata	*M. phaseolina*
4	NI	NI
5	49.6 ± 0.33	44.6 ± 0.91
6	58.3 ± 0.74	*66.2 ± 0.62
7	55.2 ± 0.28	53.8 ± 0.54
8	*62.8 ± 0.48	*70.5 ± 0.24
Fluconazole	*65.2 ± 0.54	*69.4 ± 0.64

Note: Good inhibition; dw = dry weight; PDA = potato dextrose agar; NI = no inhibition; standard deviation is shown with ±.

the antifungal efficacy of glucose esters increased. The octanoyl compound (6) showed better inhibition (*66.2%) against *M. phaseolina*. The lauroyl compound (8) exhibited higher efficacy against *A. alternata* (*62.8%) and *M. phaseolina* (*70.5%). Thus, octanoyl/lauroyl groups, together with isopentanoyl group(s) in glucopyranoside skeleton, were found to be promising inhibitors against the tested fungal pathogens (Table 1). The results are consistent with other monosaccharide esters such as mannofuranose esters [32].

Molecular Docking

Most antifungal drugs (*e.g.*, azole drugs) target the cytochrome P450 enzyme lanosterol 14 α-demethylase

(LDM), where one of the nitrogen atoms in the azole ring coordinates with the LDM heme iron and several hydrophobic interactions [33]. Such binding affinity enables antifungal molecules to deactivate LDM to convert precursor lanosterolin to the ergosterol, which is essential for yeast and fungal cell membrane. Thus, in the present study, the LDM crystal structure (PDB id: 5V5Z; *Candida albicans* SC5314) [34] was selected for docking with the synthesized glucose esters (compounds 5-8), as shown in Table 2. For comparison and validation, glucopyranoside 4 and fluconazole were also docked against 5V5Z.

Table 2 indicates that the addition of isopentanoyl and other acyl groups increased the binding affinity of compounds 5-8 (-6.1 to -8.2 kcal mol^{-1}) with 5V5Z more than it did that of the non-ester glucopyranoside-4 (-5.4 kcal mol^{-1}) with 5V5Z. The highest binding affinity was found for compound 8 (-8.2 kcal mol^{-1}), followed by compound 5 (-7.6 kcal mol^{-1}). The binding affinity of these two compounds (*i.e.*, 8 and 5) was higher than that of the standard drug fluconazole (-7.3 kcal mol^{-1}). These two esters formed several hydrogen and hydrophobic interactions with the enzyme (5V5Z), as shown in Fig. 3. Interestingly, hydrophobic interactions involving TYR-118 (pi-pi stacked), as in the 5-5V5Z complex, and TYR-132 (Pi-Pi T-shaped), as in the 8-5V5Z complex, were similar to that of the fluconazole-5V5Z complex. Overall, compounds 5 and 8 showed stronger non-bonded interactions than did fluconazole (Table 2).

Physicochemical Properties

The conformation of acyl chains in the glucopyranoside ring influences the structural and thermodynamic properties of a product. Several physicochemical, including thermodynamic and orbital, properties of glucopyranoside esters are shown in Table 3. As can be seen in Table 2, the negative electronic energy of compounds gradually increased from compound 4 to compound 8 as the number and chain length of acyl groups increased. Thus, the electrons were more tightly bound with the nucleus in the ester compounds than in the non-ester compound (4).

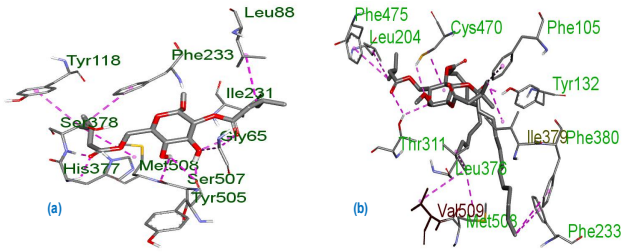


Fig. 3. 3D binding pose of compounds (a) 5 and (b) 8 with 5V5Z.

Again, the magnitude of dipole moments of esters (compounds 5-8) was higher than that of the non-ester compound (4) (Table 3), indicating the greater polar nature of the bonds in compounds 5-8. It should be noted that dipole moment is a measure of the polarity/polarization of a net neutral system.

The non-ester compound 4 possessed the highest HOMO-LUMO gap (~8.3 eV), and esters (compounds 5-8) had a lower and almost similar HOMO-LUMO gap (~6.5 eV). Hence, esters (5-8) had lower hardness and higher softness than the glucopyranoside 4. The higher softness of esters (compounds 5-8) may have contributed to their higher binding or reactivity with proteins and enzymes.

Table 2. The Binding Energy and Name of Interactions between 5V5Z and the Glucopyranoside Esters

Drug	Binding affinity (kcal mol^{-1})	No. of H bonds	No. of hydrophobic bonds	No. of van der Waals bonds	Total bonds
4	-5.4	3	0	Absent	3
5	-7.6	7	5	Absent	12
6	-6.1	4	6	Absent	10
7	-7.5	2	9	Absent	11
8	-8.2	4	10	Absent	14
FCZ	-7.3	3	3	Absent	6

Note: Standard docking score value was ≤-6.00 kcal mol^{-1}; FCZ = fluconazole.

Table 3. Physicochemical Properties of the Glucopyranoside Esters

Compound	MF	MW	EE (Hartree)	Enthalpy (Hartree)	GFE (Hartree)	DM (Debye)	Gap ($\Delta\varepsilon$)	Hardness (η)	Softness (S)
4	$C_7H_{14}O_6$	194.18	-722.468	-722.229	-722.284	1.7464	8.324	4.162	0.240
5	$C_{17}H_{30}O_8$	362.42	-1260.754	-1260.251	-1260.340	4.5236	6.416	3.208	0.312
6	$C_{33}H_{58}O_{10}$	614.82	-2033.618	-2032.669	-2032.817	3.1483	6.714	3.357	0.298
7	$C_{37}H_{66}O_{10}$	670.93	-2190.023	-2188.954	-2189.115	3.0685	6.702	3.351	0.298
8	$C_{41}H_{74}O_{10}$	727.03	-2346.429	-2345.239	-2345.413	3.1256	6.711	3.356	0.298

CONCLUSIONS

One-step dimolar isopentanoylation of methyl α-D-glucopyranoside (compound 4) easily furnished di-*O*-isopentanoate (compound 5) with selectivity at C-2 and C-6 positions. The resultant compound was then converted into three 3,4-di-*O*-acyl esters (compounds 6-8) in good yields and with higher fatty acyl halides. The sugar esters 5-8 with hydrophobic alkyl chains (4-11 carbons) showed antifungal potentiality against the tested organisms. The *in vitro* antifungal susceptibility results can be attributed to the bonding and non-bonding interactions between the above-mentioned compounds (6-8) and the fungal essential enzyme sterol 14-α-demethylase. These results can be helpful for the design and development of non-azole-type next-generation antifungal agents.

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