การศึกษาด้วยแบบจำลองทางคอมพิวเตอร์ของเภสัชจลนศาสตร์ ความเป็นพิษ และปฏิสัมพันธ์ระดับโมเลกุลของเบต้า 1,3/1,6 กลูแคนโอลิโกแซคคาไรด์จากเชื้อรากับตัวรับของเซลล์ภูมิคุ้มกัน

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บทคัดย่อ

เบต้า 1,3/1,6 กลูแคนเป็นโพลีแซ็กคาไรด์ในผนังเซลล์ของยีสต์ รา และเห็ด ที่มีผลต่อภูมิคุ้มกัน ปัจจุบันใช้รังสีแกมมาตัดสายให้เป็นโอลิโกแซ็กคาไรด์เพื่อเพิ่มการละลาย แต่ยังไม่ทราบเภสัชจลนศาสตร์และความเป็นพิษ และการจับกับตัวรับของเซลล์ภูมิคุ้มกัน งานวิจัยนี้ใช้แบบจำลองทางคอมพิวเตอร์ศึกษาการดูดซึม การกระจาย เมตาบอลิซึม การขับออก และการเกิดพิษ (ADMET) และปฏิสัมพันธ์ของเบต้า 1,3/1,6 กลูแคนโอลิโกแซคคาไรด์จากเชื้อรากับตัวรับแบบเดกติน-1 และ TLR2 บนนิวโทรฟิล โดยจำลองการจับกันและวิเคราะห์ผลด้วยโปรแกรม UCSF Chimera, AutoDock Vina , Proteins.plus และ PyMOL วิเคราะห์ ADMET ด้วยเว็บเซิร์ฟเวอร์ pkCSM พบว่าเบต้ากลูแคนโอลิโกแซ็กคาไรด์ทั้งขนาด 1.6 และ 0.9 กิโลดัลตัน สามารถจับกับตัวรับเดกติน-1 และ TLR2 ได้ดีกว่าสารอ้างอิง โดยการจับทั้งตัวดีกว่าจับกับตำแหน่งอ้างอิงที่ทราบอยู่เดิม เบต้ากลูแคนขนาด 1.6 กิโลดัลตันจับกับตัวรับ TLR2 ได้ดีกว่าขนาด 0.9 กิโลดัลตัน ผลการวิเคราะห์ ADMET พบว่า เบต้ากลูแคนดูดซึมได้น้อย ผ่านเข้าระบบประสาทต่ำมาก ไม่ถูกเปลี่ยนรูปโดยเอนไซม์ CYP2D6 ไม่เป็นพิษต่อตับหรือสารพันธุกรรม ผลสรุปทำนายได้ว่า เบต้ากลูแคนโอลิโกแซคคาไรด์จากเชื้อราเป็นสารที่มีความเป็นพิษต่ำ และถูกขับออกโดยไม่เปลี่ยนรูป สามารถจับกับตัวรับเดกติน-1 และ TLR2 บนนิวโทรฟิลได้ดีกว่าสารอ้างอิง และน้ำหนักโมเลกุลส่งผลต่อความสามารถในการจับ งานวิจัยนี้บ่งชี้ว่าอาจมีตำแหน่งการจับใหม่บนตัวรับทั้งสอง ซึ่งควรได้รับการศึกษาเพิ่มเติม

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In Silico Predictions of Pharmacokinetic, Toxicity, and Molecular Interactions of Fungal β-1, 3/1, 6-Glucans Oligosaccharides with Immune Cells Receptors

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Abstract

β-1, 3/1, 6-glucans are polysaccharides in fungi's cell walls (yeast, mold, and mushrooms) known to promote an immune response. Recently, gamma irradiation has been used to cut β-glucan polysaccharides into oligosaccharides with improved solubility. However, their pharmacokinetic and toxicity profiles, and molecular interactions with immune cell receptors were unknown. This study aims to use *in silico* models to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) and the interaction of fungal β-1, 3/1, 6-glucans oligosaccharides with neutrophil receptors, including Dectin-1 and TLR2. Molecular docking and analysis were performed by using UCSF Chimera, AutoDock Vina, Proteins.plus, and PyMol. *In silico* ADMET analysis was done by using the pkCSM web server. The result showed that both 1.6 and 0.9 kDa forms of 1, 3/1, 6 β-glucans oligosaccharides can bind with Dectin-1 and TLR2 better than the reference compounds. Their binding capacity with the whole molecule is better than with the known binding sites. The 1.6 kDa β-glucans bind to the macromolecules of TLR2 better than the 0.9 kDa ones. ADMET analyses suggest low absorption, very low blood–brain barrier permeability, no CYP2D6 metabolism, and no hepatotoxicity or genotoxicity. In conclusion, this study predicts that the fungal β-1, 3/1, 6-glucans oligosaccharides are low-toxic and are fecally excreted without metabolic transformation. Their binding to Dectin-1 and TLR2 receptors on neutrophils is stronger than that of reference compounds and is influenced by molecular weight. These findings suggest that they possess new binding sites to both receptors, warranting further studies.

Keywords: β-glucans, Oligosaccharides, Immune receptors, Molecular docking simulation, Pharmacokinetics and toxicity, Immunomodulation

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Introduction

Fungal β-glucans are a heterogeneous group of naturally occurring polysaccharides, primarily consisting of β-(1→3)-linked D-glucose units with varying degrees of β-(1→6)-linked side chains[1]. These complex molecules are typically found in the cell walls of fungi (yeast, mold, and mushrooms)[2]. In particular, β-1, 3/1, 6-glucans have received increasing attention due to their potent immunomodulatory, anti-inflammatory, and anticancer properties[3]. Structural variations in β-glucans—such as polymer length, branching pattern, and molecular size—have been shown to influence solubility, receptor recognition, biological activity, and safety[3]. Although it has been traditionally used in complementary medicine, recent studies have increasingly focused on the molecular mechanisms of its efficacy[4]. To improve water solubility, various modification techniques such as enzymatic hydrolysis, microfluidization and gamma irradiation have been recently employed to generate short-chain oligosaccharides from native β-glucans polymers[5]. However, the extent to which these size-reduced molecules retain or alter their immunological and toxicological functions remains insufficiently characterized.

The bioactivity of β-glucans is closely tied to their interaction with pattern recognition receptors (PRRs) on immune cells[6]. Among these, Dectin-1—a type II transmembrane C-type lectin receptor—plays a pivotal role by specifically recognizing β-1, 3/1, 6-glucans structures[7]. Upon ligand binding, Dectin-1 activates a cascade involving spleen tyrosine kinase (Syk) and the caspase-recruitment domain 9 (CARD9) signaling adaptor, which in turn stimulates the release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6)[8]. These signaling events promote phagocytosis, neutrophil recruitment, and activation of adaptive immune pathways including antigen presentation and T-helper 1 (Th1)-mediated responses. Additionally, Toll-like receptor 2 (TLR2) interacts synergistically with Dectin-1 to amplify cytokine production through the recognition of pathogen-associated molecular patterns (PAMPs) [9]. While this cooperative signaling enhances host defense, overstimulation of these pathways may induce systemic inflammatory responses. Therefore, a mechanistic evaluation of β-glucan-mediated immune activation is required. Structurally, fungal β-glucan polysaccharides can exist as long-chain polymers with more than ten glucose units and branched side chains, forming intricate three-dimensional molecular frameworks[10]. These complex structures are believed to influence binding avidity and receptor clustering[9]. In contrast, oligo-β-glucan—comprising 2 to 10 glucose residues—are more soluble but may differ in

receptor affinity and immunological potency[11]. The influence of molecular weight and size reduction on immune receptor interactions, especially for Dectin-1 and TLR2, remains a critical area of investigation[12], particularly in the context of neutrophil function and cytokine regulation. Despite mounting evidence of immune stimulation, there is limited understanding of whether short-chain oligosaccharides derived from fungal β-glucan can effectively engage immune receptors in a manner comparable to their macromolecular counterparts. Furthermore, the pharmacokinetics and safety profile represented by ADMET of oligo- β-1, 3/1, 6-glucans were unclear.

For early-phase screening, *in silico* modeling is a perfect approach to investigate ligand–receptor interactions with the advantage of minimal ethical concerns[13]. Computational methods such as molecular docking enable the prediction of binding conformations, receptor selectivity, interaction energies, and key contact residues at an atomic-level resolution. These tools can model potential orientation and flexibility of β-glucan structures when interacting with immune receptors such as Dectin-1 and TLR2. Furthermore, *in silico* ADMET (absorption, distribution, metabolism, excretion, and toxicity) prediction offers early insights into pharmacokinetic profiles and safety risks[14]—including hepatotoxicity, mutagenicity, and metabolic enzyme interactions such as cytochrome P450 (CYP2D6)[14]. Such predictive models can highlight whether β-glucan are likely to be bioavailable, cross biological barriers, or pose toxicity risks[15]. The *in silico* model reduces the need for extensive laboratory research by providing preliminary valuable information in further designing cost-effective *in vitro* and *in vivo* studies[13].

Previous studies have evaluated the immunomodulatory effects of native fungal β-glucan polysaccharides and compared their receptor-binding capacity across different molecular weights using structural docking models[16]. However, the molecular interaction of fungal β-1, 3/1, 6-glucans oligosaccharides with immune cell receptors, such as Dectin-1 and TLR2, and the influence of molecular weight variation on the binding capacity have never been explored.

This present study aims to use *in silico* ADMET to predict the pharmacokinetic and safety profiles and employ molecular docking to simulate the molecular interactions between fungal β-1, 3/1, 6-glucans oligosaccharides of varying sizes and neutrophil receptors (Dectin-1 and TLR2).

Materials and Methods

The list of software used in *in silico* molecular docking and ADMET analysis is shown in Table 1.

Table 1. Software tools and their purposes used in the *in silico* molecular modeling and ADMET analysis.

Software	Version	Purpose
UCSF Chimera	1.19	Protein/ligand preparation
AutoDock Vina	1.1.2	Docking
PyMOL	2.x	3D visualization
Proteins.plus	(Web/API/CLI)	Molecular interaction 2D
pkCSM	Web server	*In silico* ADMET prediction

Molecular docking

- Preparation of protein and ligand

The 3-dimensional (3D) crystal structures of the Dectin-1 protein (PDB ID: 2CL8, https://www.rcsb.org/structure/2CL8[17] [accessed on March 2025]) and TLR2 (PDB ID: 3A79, https://www.rcsb.org/structure/3A79[18] [accessed on March 2025]) were obtained from the RCSB Protein Data Bank. These proteins have been used in previous studies to predict binding interactions using molecular docking analysis[19]. Prior to docking, all protein structures were prepared by removing nonstandard molecules, adding hydrogen atoms, and assigning Gasteiger charges using UCSF Chimera software. Then, protein structures were saved in PBD format for molecular docking studies. For ligand preparation, fungal β-1, 3/1, 6-glucans oligosaccharides with molecular weights of 0.9 kDa and 1.6 kDa were manually constructed using the ChemDraw program, based on the structure of schizophyllan derived from fungus *Schizophyllum commune*, which is characterized by a linear β-1, 3-glucan backbone with β-1, 6-linked monoglucose side chains occurring approximately at every third residue along the main chain (1:3 branching)[20, 21]. Then structures were converted into 3D structures using Open Babel graphical user interface (GUI) program. All 3D ligand structures were prepared by removing solvents, adding hydrogen atoms, and determining the charge using UCSF Chimera software and then saved in PBD format for molecular docking studies[22].

This present study aimed to investigate the binding interactions between β-1, 3/1, 6-glucans oligosaccharides and immune cell receptors, with a focus on the effect of molecular weight on binding affinity. The oligosaccharides comprised 2

to 10 glucose units, with molecular weights of 0.9 kDa and 1.6 kDa consistent to structures containing 5 and 9 glucose units, respectively. However, a limitation of this study is that β-glucans with molecular weights exceeding 1.6 kDa could not be evaluated using the molecular docking program due to computational constraints. Therefore, the 0.9 kDa and 1.6 kDa oligosaccharides were selected for investigation in this study, as they represented distinct molecular weights within the permissible range of the computational modeling tools employed.

- Method validation

The docking procedure was validated by removing the original ligand from the crystal protein structures and re-docking it into the protein's binding site. The root-mean-square deviation (RMSD) of the re-docked co-crystallized compound was subsequently calculated. RMSD values below two angstroms were considered to be indicative of a reliable docking solution[23]. In the present study, the re-docking results of Dectin-1and TLR2 showed an RMSD value of 0.0 Å.

- Molecular docking analysis

In this procedure, UCSF Chimera 1.19 software was used with all default parameters for conducting the molecular docking analysis[24]. The grid box values for the binding site of Dectin-1 were set in the center at -41.1813 × -0.547616 × -4.7604 in the x, y, and z dimensions, respectively, with sizes of grid box was set at 14.9697 × 11.6809 × 13.3471. These values represented the size and coordinates of the grid box defined to encompass the binding site, corresponding to the region where ligand binding was anticipated before running the molecular docking program. For the binding site of TLR2, a center of the grid box was set at 19.5337 × -41.6442 × 44.7529, with a size of the grid box set at 37.1996 × 22.1954 × 34.8597. Additionally, this study examined alternative binding sites on the whole macromolecule of protein to investigate whether β-glucans could interact with other sites beyond the binding site that previously had an original ligand attached to it. The reference binding sites were identified based on the crystallographic structures of Dectin-1 and TLR2, obtained from the RCSB Protein Data Bank under PDB IDs 2CL8 and 3A79, respectively. The grid box values for the whole macromolecule of Dectin-1 were set in the center at -41.6767 × -0.675602 × -13.0797, with the sizes of the grid box set at 47.8504 × 67.1839 × 60.5906. For whole macromolecules of TLR2, a center grid box was set at 59.4294 × -33.2135 × 30.5163, with a size of the grid box set at 93.7094 × 92.3559 × 101.093. Finally, the conformation with the

lowest binding free energy scores, indicating the most favorable and stable interaction with Dectin-1 and TLR2, was selected to investigate the interaction between protein and ligand using AutoDock Vina 1.1.2 software[25].

In silico 2D and 3D modeling of binding activities

These selected docking results were subsequently used for structural molecular analyses and molecular images. The 2D interaction diagrams were generated using the ProteinsPlus web server[26] (https:// proteins.plus/ [accessed on May 2025]). Finally, the PyMOL Molecular Graphics System was then used to visualize high-quality of 3D molecular structures[27].

ADMET prediction

An *in silico* ADMET analysis was performed using SMILES (Simplified Molecular Input Line Entry System) format, which was generated using the ChemDraw software. SMILES format was submitted to the pkCSM pharmacokinetics web server (https://biosig.lab.uq.edu.au/pkcsm/ [accessed on April 2025]) to predict the potential biological activities and evaluate the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles of the molecules[28].

Results

Molecular docking with Dectin-1 receptors

To understand the interactions between Dectin-1 and fungal β-1,3/1,6-glucans oligosaccharides of different molecular weights, a molecular docking approach was used to examine the Dectin-1-β-glucans interactions, both at the binding site for reference ligands and the whole macromolecule of the Dectin-1. The results were evaluated based on the lowest binding free energy scores to identify potential interactions between Dectin-1 and the oligo-β-1, 3/1, 6-glucans. As shown in Table 2, both 0.9 and 1.6 kDa β-glucans demonstrated more favorable binding energies (-9.1 and -9.3 kcal/ mol, respectively) with the macromolecule of Dectin-1, compared to the original ligand (−8.0 kcal/mol). In contrast, their binding energy at the binding site is slightly less than that of the reference ligand. Since this study docks at the binding site of the reference ligand, the findings suggest that the binding capacity of the oligo-β-1, 3/1, 6-glucans with the whole molecule of Dectin-1 is better than at the commonly known binding sites. The finding suggests that oligo-β-1, 3/1, 6-glucans may also bind to other sites besides the widely known binding site of the reference ligand. Future studies are warranted to identify the new binding sites of Dectin-1.

Table 2. Molecular docking results of β-glucans with binding site and whole macromolecule of Dectin-1

	Interactions at binding sites			Interactions at macromolecules		
Ligand	**Binding energy (kcal/mol)**	**Amino acid interaction**		**Binding energy (kcal/mol)**	**Amino acid interaction**	
		Hydrogen bond	**Hydrophobic bond**		**Hydrogen bond**	**Hydrophobic bond**
BGC (Glucose) Reference ligand: Laminaritriose (2CL8)	−8.0	Glu243B	His153B	−8.0	Arg145A Ser148A Glu243B	His153B
0.9 kDa β-glucan	−7.3	Arg145A Glu243B Ser148A Leu154B Ala152B	His153A His153B	−9.1	Arg145A Asp158B Asp195A Glu194A Glu194B Glu243B Lys156A Ser148B	
1.6 kDa β-glucan	−7.9	Arg145A Lys156B Gln149A Glu194A Glu194B Ser148B	Glu243B	−9.3	Ser129A Ser148A Ser148B Glu194B Asp158A Leu154A	His153A

Regarding the different molecular weights, Table 2 suggests that the binding of 1.6 kDa oligo-β-1, 3/1, 6-glucans with either the binding sites or macromolecule of Dectin-1 is slightly stronger than that of 0.9 kDa. The docking results of Dectin-1 binding site with β-glucans showed that the 0.9 kDa β-glucans had a binding energy at −7.3 kcal/mol, forming hydrogen bonds with Arg145A, Glu243B, Ser148A, Leu154B, and Ala152B, as well as hydrophobic bonds with His153A and His153B. In contrast, the 1.6 kDa β-glucans exhibited a slightly stronger binding energy, with a binding energy of −7.9 kcal/mol. This interaction involved hydrogen bonds with Arg145A, Lys156B, Gln149A, Glu194A, Glu194B, and

Ser148B, along with a hydrophobic interaction with Glu243B. The docking results across the entire Dectin-1 macromolecule with β-glucans revealed that the 1.6 kDa β-glucans exhibited the strongest binding energy at −9.3 kcal/mol. It formed hydrogen bonds with Ser129A, Ser148A, Ser148B, Glu194B, Asp158A, and Leu154A, in additional to a hydrophobic interaction at His153A. Comparatively, the 0.9 kDa β-glucans showed a slightly weaker binding energy at −9.1 kcal/mol, forming hydrogen bonds with Arg145A, Asp158B, Asp195A, Glu194A, Glu194B, Glu243B, Lys156A, and Ser148B. The 2D and 3D interaction structures of these complexes are illustrated in Figures 1 and 2, respectively. Such findings suggest that the 1.6 kDa β-glucans bind to Dectin-1 receptors slightly better than the 0.9 kDa ones.

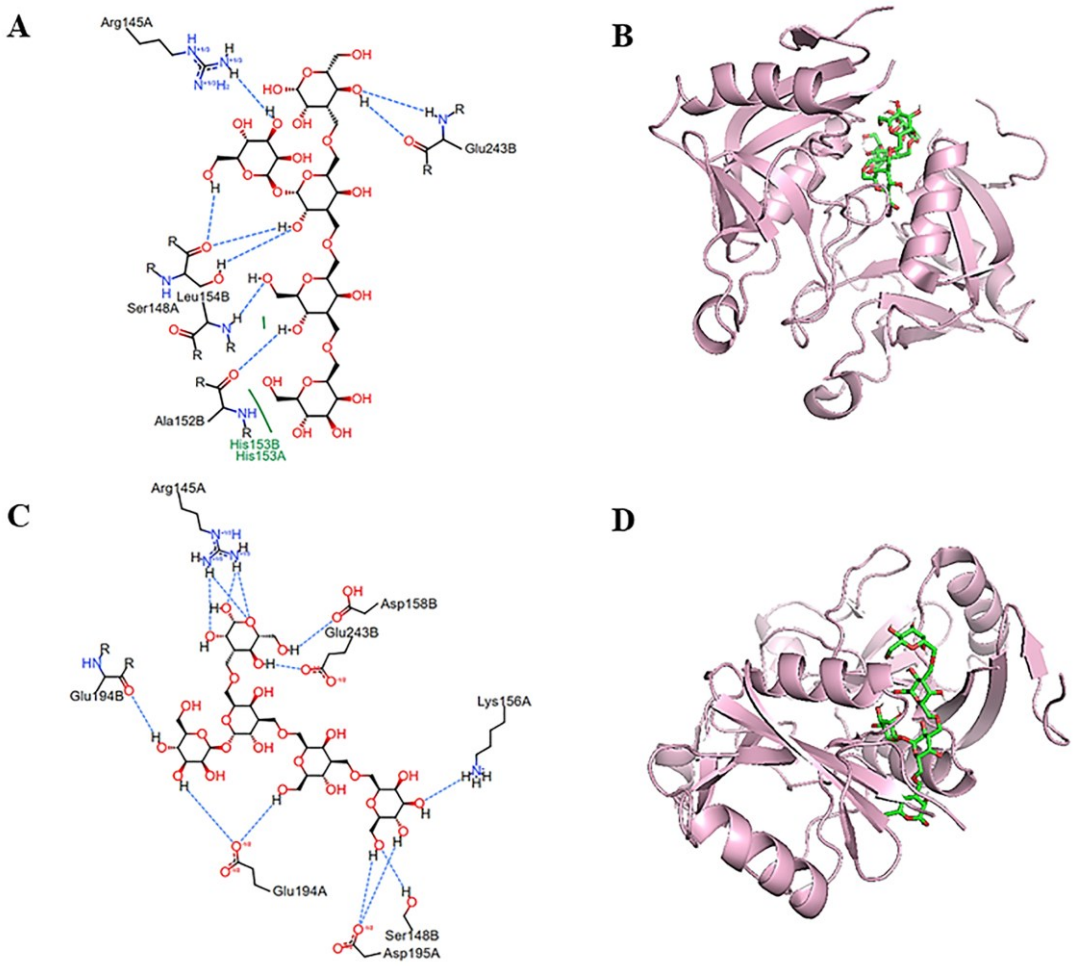

Figure 1. Molecular interaction of 0.9 kDa β-glucans with Dectin-1. (A) Schematics of amino acid interactions of β-glucans and Dectin-1 binding site, (B) 3D visualization of β-glucans and Dectin-1 binding site complex, (C) Schematics of amino acid interactions of β-glucans and Dectin-1 macromolecule, (D) 3D visualization of β-glucans and Dectin-1 macromolecule complex.

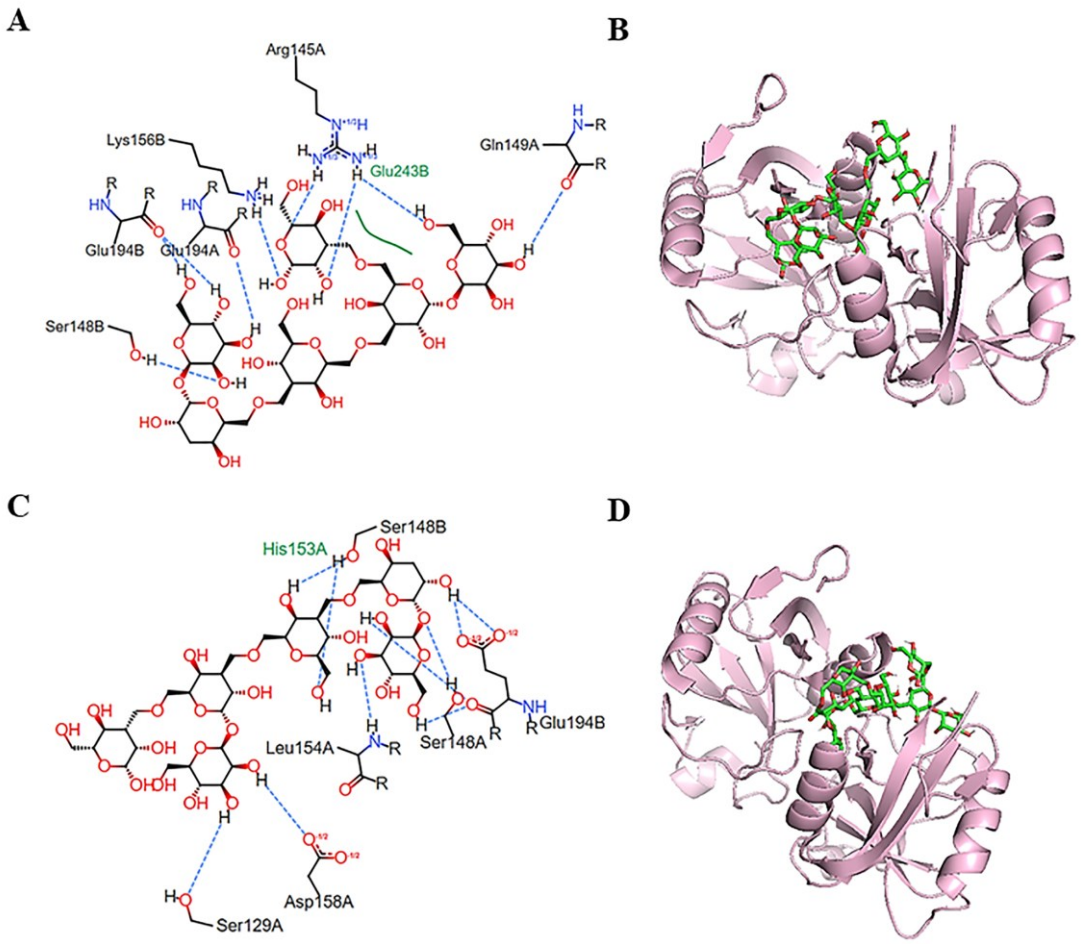

Figure 2. Molecular interaction of 1.6 kDa β-glucans with Dectin-1. (A) Schematics of amino acid interactions of β-glucans and Dectin-1 binding site, (B) 3D visualization of β-glucans and Dectin-1 binding site complex, (C) Schematics of amino acid interactions of β-glucans and Dectin-1 macromolecule, (D) 3D visualization of β-glucans and Dectin-1 macromolecule complex.

Molecular docking with TLR2 receptors

Table 3 shows that both 0.9 and 1.6 kDa β-glucans bind to both binding sites (binding energy of -6.4 and -6.5 kcal/ mol, respectively) and macromolecules (-7.3 and -8.3 kcal/ mol, respectively) of TLR2 better than those of the reference ligand (binding energy of -4.3 and -4.8 kcal/mol, respectively). Similar to those of Dectin-1, the binding of oligo-β-glucans to the whole molecule of TLR2 is stronger than with the known binding site (-7.3 vs -6.4 kcal/mol for 0.9 kDa; -8.3 vs -6.5 kcal/mol for 1.6 kDa). The finding suggests that oligo-β-1, 3/1, 6-glucans may also bind with other sites besides the commonly known binding site of the reference ligand. Future studies are warranted to identify the new binding sites of TLR2.

Regarding the different molecular weights, Table 3 suggests that the binding of 1.6 kDa oligo-β-1,3/1,6-glucans with the

macromolecule of TLR2 is stronger than that of 0.9 kDa (-8.3 vs -7.3 kcal/ mol). In contrast, the molecular interaction of 0.9 kDa and 1.6 kDa oligo-β-1, 3/1, 6-glucans at the binding sites of TLR2 is not much different (-6.4 vs -6.5 kcal/ mol). The docking results of the TLR2 binding site showed that the 0.9 kDa β-glucans had a binding energy at −6.4 kcal/mol, forming hydrogen bonds with residues Arg291B, Ile292B, Asp293B, Arg294B, Asn314B, Phe317B, Leu318B, Phe319B, Ser320B, Lys321B, and Tyr325B. It also formed hydrophobic interactions with Phe319B, Lys321B, and Leu318B. The 1.6 kDa β-glucans exhibited a binding energy at −6.5 kcal/mol, establishing hydrogen bonds with Val316B, Phe317B, Ser320B, Lys321B, and Glu322B, along with hydrophobic interaction at Lys321B. The docking results of TLR2 macromolecule with β-glucans revealed that the 1.6 kDa β-glucan exhibited the strongest binding energy at −8.3 kcal/mol. This interaction formed hydrogen bonds with Pro205B, Ser226B, Asn227B, Thr255B, Gln257B, His258B, and Tyr284B, as well as hydrophobic interactions with His258B, His311B, Asn285B, Tyr284B, and His200B. In contrast, the 0.9 kDa β-glucan showed a slightly weaker binding energy at −7.3 kcal/mol, forming hydrogen bonds with Asn110B, Ser226B, Asn227B, His258B, and Asn285B, as well as hydrophobic interactions with Pro205B, Val202B, and Phe130B. The 2D and 3D interaction structures are shown in Figures 3 and 4, respectively. Such findings suggest that the 1.6 kDa β-glucans bind to the whole macromolecule of TLR2 receptors better than the 0.9 kDa ones.

Taken together, the findings of this study predict that β-1, 3/1, 6-glucans oligosaccharides can bind to the macromolecule of both Dectin-1 and TLR2 better than those of reference ligands. Since their binding capacity with the whole molecule of Dectin-1 and TLR2 is better than with the docked binding sites, oligo-β-1, 3/1, 6-glucans likely can bind with other sites besides the commonly known binding site of the reference ligand. The binding between 1.6 kDa oligo-β-1, 3/1, 6-glucans and the macromolecule of TLR2 is apparently stronger than that of the 0.9 kDa one, while such a difference for Dectin-1 is consistent, albeit to a lesser extent. Since Dectin-1 and TLR2 are major receptors of neutrophils, the findings of this study imply potential application of fungal β-1, 3/1, 6-glucans oligosaccharides in the modulation of innate immune response against fungal infection

Table 3. Molecular docking results of β-glucans with binding site and whole macromolecule of TLR2

	Interactions at binding sites			Interactions at macromolecules		
Ligand	**Binding energy (kcal/mol)**	**Amino acid interaction**		**Binding energy (kcal/mol)**	**Amino acid interaction**	
		Hydrogen bond	**Hydrophobic bond**		**Hydrogen bond**	**Hydrophobic bond**
Reference ligand: Pam2CSK4 (3A79)	–4.3	Ser320B Lys321B Glu322B Ile344B	Lys321B Ile344B His345B	–4.8	His85B Arg87B Asn110B Asp132B Asp177B Ser180B Asn227B	His85B Pro205B
0.9 kDa β-glucan	–6.4	Arg291B Ile292B Asp293B Arg294B Asn314B Phe317B Leu318B Phe319B Ser320B Lys321B Tyr325B	Phe319B Lys321B Leu318B	–7.3	Asn110B Ser226B Asn227B His258B Asn285B	Pro205B Val202B Phe130B
1.6 kDa β-glucan	–6.5	Val316B Phe317B Ser320B Lys321B Glu322B	Lys321B	–8.3	Pro205B Ser226B Asn227B Thr255B Gln257B His258B Tyr284B	His258B His311B Asn285B Tyr284B His200B

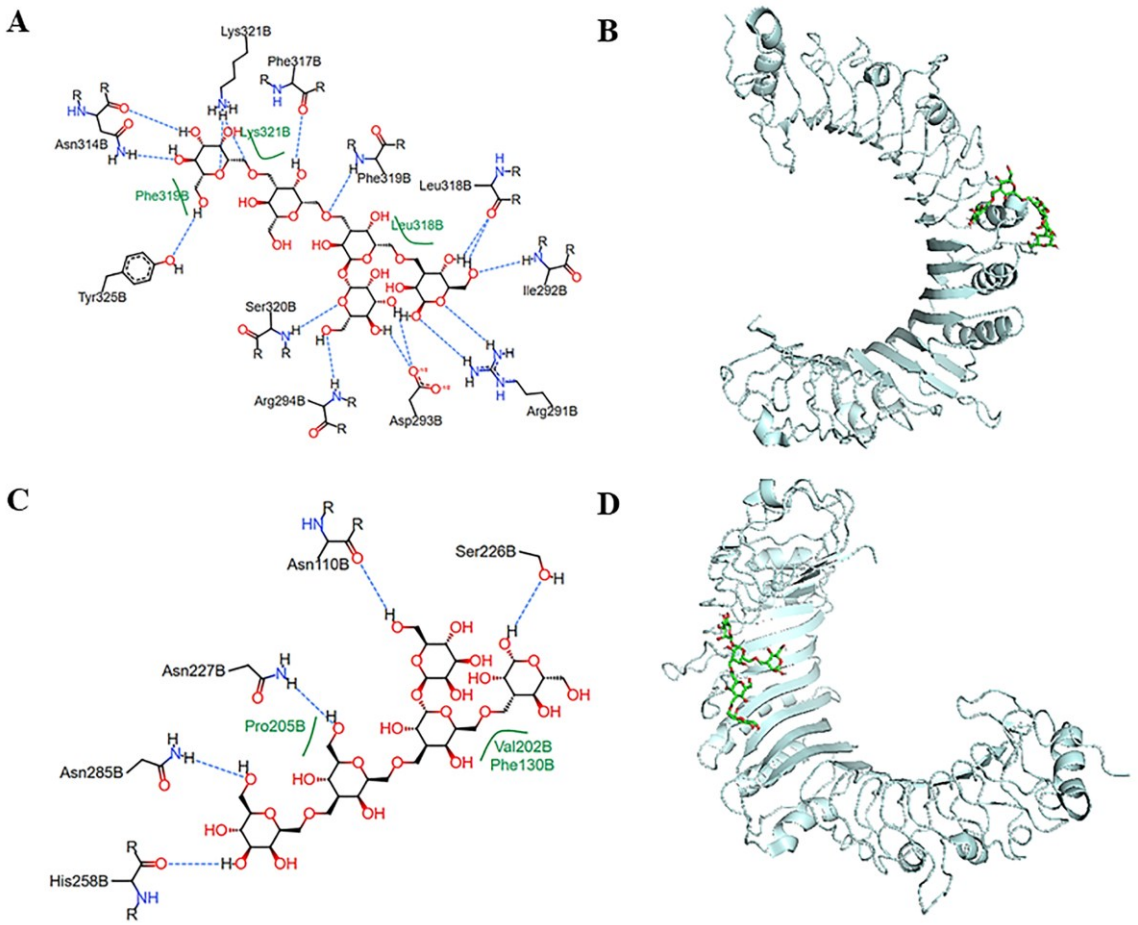

Figure 3. Molecular interaction of 0.9 kDa β-glucans with TLR2. (A) Schematics of amino acid interactions of β-glucans and TLR2 binding site, (B) 3D visualization of β-glucans and TLR2 binding site complex, (C) Schematics of amino acid interactions of β-glucans and TLR2 macromolecule, (D) 3D visualization of β-glucans and TLR2 macromolecule complex.

ADMET analysis

ADMET profiling revealed that both 0.9 and 1.6 kDa β-glucans exhibit low oral bioavailability, as demonstrated by poor predicted intestinal absorption and limited permeability across Caco-2 cell monolayers. The absence of gastrointestinal (GI) absorption predictions suggests that these compounds are likely to remain confined to the gastrointestinal lumen, reducing the likelihood of systemic exposure. In addition, the predicted blood-brain barrier (BBB) permeability was extremely low, with logBB values below -3, indicating minimal central nervous system (CNS) penetration and, consequently, a low risk of neurotoxicity. Furthermore, metabolic predictions further indicated that neither β-glucans is absent of cytochrome P450 2D6 (CYP2D6), suggesting a low potential for hepatic metabolism-related toxicity and minimal risk of drug-drug interactions involving this enzyme. Furthermore, the *in silico* models predict that the oligosaccharides have no liver

toxicity and genotoxicity (as determined by virtual Ames tests), as shown in Table 4. Overall, the ADMET profile supports the safety of fungal β-glucans, particularly for localized mucosal applications, where systemic absorption is not required for efficacy.

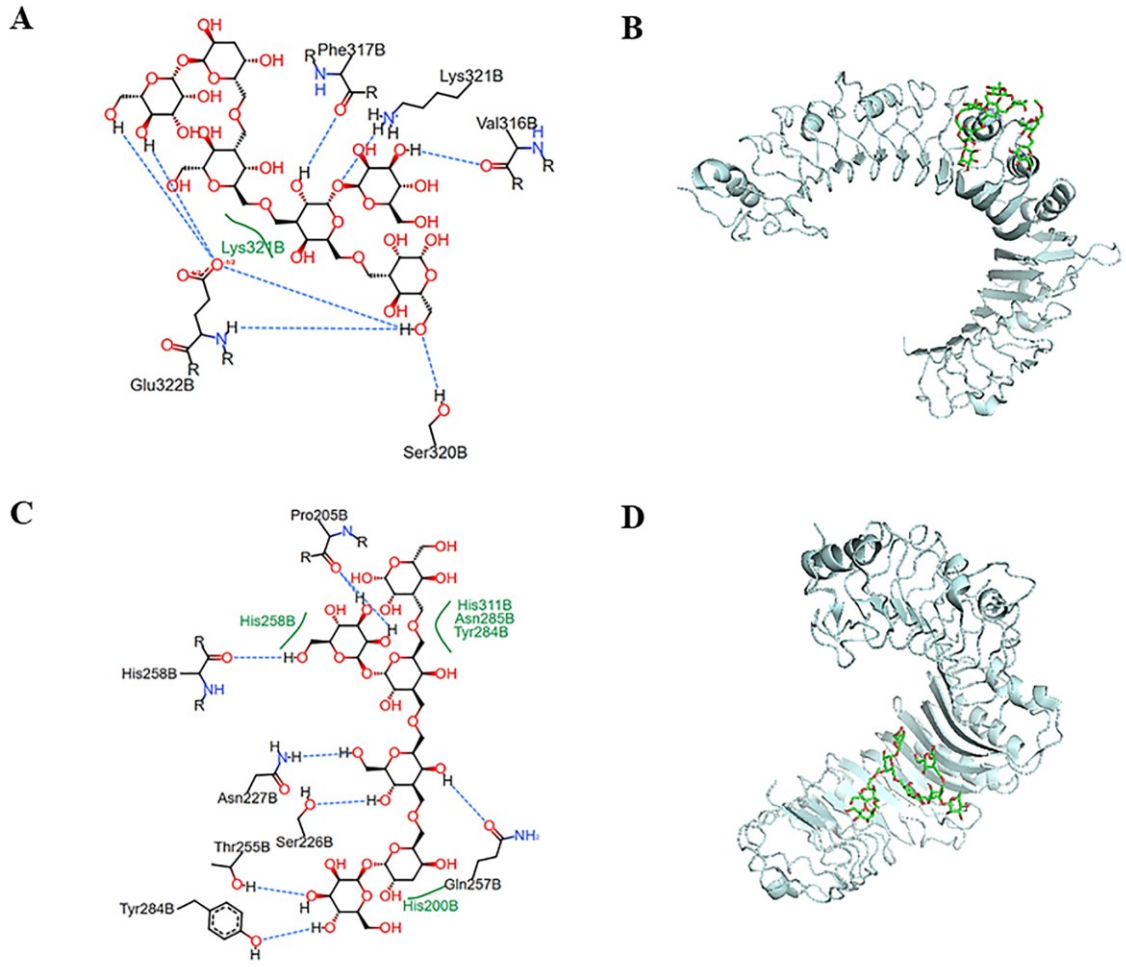

Figure 4. Molecular interaction of 1.6 kDa β-glucans with TLR2. (A) Schematics of amino acid interactions of β-glucans and TLR2 binding site, (B) 3D visualization of β-glucans and TLR2 binding site complex, (C) Schematics of amino acid interactions of β-glucans and TLR2 macromolecule, (D) 3D visualization of β-glucans and TLR2 macromolecule complex.

Table 4. The pharmacokinetic properties of β-glucans

Pharmacokinetic properties	0.9 kDa β-glucan	1.6 kDa β-glucan
Absorption		
Water solubility (log mol/L)	-2.804	-2.892
Caco2 permeability (log Papp in 10−6 cm/s)	-1.515	-2.579
Intestinal absorption (human) (% absorbed)	0	0
Distribution		
BBB permeability (log BB)	-3.788	-6.489
Metabolism		
CYP2D6 substrate	No	No
CYP2D6 inhibitor	No	No
Excretion		
Total clearance (log ml/min/kg)	1.994	2.156
Toxicity		
AMES toxicity	No	No
Hepatotoxicity	No	No

Note. BBB = blood–brain barrier; BB = brain: blood drug concentration ratio.

Discussion

This study provides novel insights into the molecular interactions and pharmacokinetic profiles of fungal β-1, 3/1, 6-glucans oligosaccharides, which remain underexplored compared to their well-characterized polysaccharide counterparts. This is the first *in silico* study to show that both 0.9 kDa and 1.6 kDa oligo-β-glucans exhibit stronger binding affinities to the whole macromolecular structures of Dectin-1 and TLR2 than to the known reference ligand binding sites. Notably, the superior binding of the 1.6 kDa oligosaccharide, particularly to TLR2, underscores the importance of molecular weight in modulating receptor affinity. This is consistent with the docking results, which showed that several amino acid residues involved in β-glucans binding at the reference ligand site were also engaged in the whole macromolecule of Dectin-1. The additional interactions suggest that both larger 0.9 kDa and 1.6 kDa β-glucans extend beyond the conventional pocket to contact nearby surface grooves on the receptor. This expanded interface likely contributes to their higher binding affinity

and structural stability compared with the smaller native ligand, 0.5 kDa laminaritriose.

In molecular docking studies, a reference (native) ligand is the molecule co-crystallized with the target protein at its active site, providing an accurate representation of the binding mode and serving as a benchmark for evaluating the binding behavior of new compounds[29]. In this study, laminaritriose and Pam2CSK4 were used as reference ligands for Dectin-1 and TLR2, respectively. Laminaritriose binds within the carbohydrate-recognition domain (CRD) of Dectin-1, a Ca^{2+}-independent region responsible for β-glucan recognition. Key amino acid residues involved include Glu243B and His153B, which contribute to receptor stabilization and activation of the Syk–CARD9 signaling pathway, leading to cytokine production and phagocytosis[17]. Pam2CSK4 interacts with a hydrophobic pocket at the TLR2 interface, engaging residues such as Ser320B, Lys321B, Glu322B, Ile344B, and His345B to trigger MyD88-dependent NF-κB signaling and induce inflammatory cytokine production[18]. Our docking results revealed that β-glucan oligosaccharides bind strongly to both Dectin-1 and TLR2 and also interact with additional or extended regions beyond the canonical ligand-binding sites. These multivalent hydrogen bonds and hydrophobic interactions likely contribute to stronger and more stable ligand–receptor complexes. Such extended binding may enhance receptor clustering, amplify downstream signaling, and broaden immune responses[30].

The higher docking scores of oligo-β-1, 3/1, 6-glucans to macromolecules of both Dectin-1 and TLR2 suggest their superior structural complementarity with these innate immune receptors, compared to the reference ligands[31]. The co-engagement of Dectin-1 and TLR2 has been shown to amplify innate immune activation and shape the adaptive immune response, particularly by promoting the differentiation and function of T-helper (Th) 1 and Th17 cells, which are critical for the clearance of fungal infections such as *Candida albicans* and *Aspergillus fumigatus*[32]. Th17 cells play a pivotal role in host defense against fungal infections, by stimulating the production of pro-inflammatory cytokines including TNF-α, IL-6, and IL-1β, and by enhancing the recruitment and activation of neutrophils, which are critical for the effective elimination of pathogens[33]. Therefore, our *in silico* findings suggest that oligo-β-glucans may serve as potent immunostimulants that bridge innate recognition and adaptive immunity. These results indicate the potential of fungal β-glucan oligosaccharides in enhancing host

defense mechanisms against opportunistic fungal pathogens. Future studies may explore the molecular interactions between Th17 and TLR2 through docking simulations to further elucidate the mechanisms underlying Th17-mediated antifungal immunity.

Our study revealed that β-glucans exhibited stronger binding affinities when docked across the entire macromolecule when compared to the binding sites of both Dectin-1 and TLR2, especially with 1.6 kDa β-glucans. Since the binding of β-glucans to both receptors are stronger than those of the reference ligands and the docked binding sites are known sites for reference ligands, the findings of this study imply that the fungal β-1, 3/1, 6-glucans oligosaccharides may have better conformational fit with the receptors or have additional binding sites beyond the known binding sites of reference ligands. In this present study, distinct grid box parameters were defined for two docking scenarios: one targeting the original ligand-binding site and the other encompassing the entire macromolecule. Therefore, docking to the entire macromolecule enables β-glucans to explore a broader conformational landscape, including solvent-accessible grooves and surface clefts that are not considered in the limited binding site model. This increases the probability of identifying alternative favorable binding poses with higher binding energies, particularly for flexible and high molecular weight β-glucans[34]. Based on molecular docking results, the additional interactions between β-glucan oligosaccharides and Dectin-1 were located adjacent to the CRD rather than in a completely separate region. Several amino acid residues were positioned close to, but slightly beyond, the CRD, suggesting that β-glucan oligosaccharides interact within a partially overlapping region of the receptor surface. This indicates that the functional integrity of Dectin-1 in β-glucan recognition and activation of the Syk–CARD9 signaling pathway is preserved. However, the engagement of additional residues likely enhances binding stability, promotes receptor clustering, and prolongs signal persistence, potentially leading to a stronger or more sustained immunomodulatory response. This is consistent with previous reports on pattern-recognition receptors, suggesting that such strengthened binding, rather than altering the receptor's intrinsic function or generating new activities, likely optimizes its established role in immune recognition[35]. These findings advance the understanding of Dectin-1's binding plasticity and have implications for modulating innate immune responses through rational ligand design.

The molecular docking results demonstrated that molecular weight significantly influenced the binding affinity and interaction patterns of β-glucans with both Dectin-1 and TLR2. The 1.6 kDa β-glucans consistently showed stronger binding energies than the 0.9 kDa molecule, especially for the macromolecule of TLR2. The 1.6 kDa β-glucan is characterized by a higher branching frequency and a longer chain than the 0.9 kDa β-glucans, which may lead to better access to receptors. This data suggests that larger β-glucans may provide more extensive surface interaction or multivalent binding, consistent with previous reports that high molecular weight of β-glucans enhances receptor clustering and immune activation[11, 16]. Additionally, the interaction between β-glucans and protein receptor residues is influenced by structural features such as molecular weight and degree of branching, which affect their binding affinity and subsequent biological activity[10, 26]. This enhanced binding affinity across the whole macromolecule can be attributed to several factors. Some studies have reported that high-molecular-weight β-glucans can adopt flexible, extended-chain conformations that facilitate simultaneous interactions with multiple domains or residues on the receptor surface macromolecule. This multivalent interaction supports greater binding stability and has been shown to promote receptor clustering and immune activation[36, 37].

Regarding the influence of molecular weight on receptor binding, a previous study by Methacanon et al. reported that β-glucan oligosaccharides with a molecular weight of 5 kDa had higher IL-8-stimulating activity than the polysaccharides with higher molecular weights[38]. Nevertheless, our present study indicates that, when comparing oligosaccharides, the 1.6 kDa β-glucans consistently exhibit stronger binding affinities than the 0.9 kDa β-glucans, suggesting a greater potential to stimulate immune responses. Taken together, we may conclude that oligosaccharides likely have better activity than polysaccharides. However, oligosaccharides with longer chains bind to the receptor more effectively than those with shorter chains. β-glucans with a molecular weight range of 1.6–5 kDa may possess enhanced immunomodulatory properties. This may also explain why β-glucan oligosaccharides are more effective than their native reference ligands. Both native ligands of Dectin-1 (laminaritriose), and TLR2 (Pam2CSK4) have lower molecular weights (0.5 and 1.3 kDa, respectively) than the range associated with optimal immunomodulatory activity[17,18]. Therefore, β-glucan oligosaccharides of moderate molecular weight may confer a structural advantage, enabling stronger,

more stable receptor interactions and thereby enhancing immunomodulatory effects.

Lipinski's rule of five serves as a small-molecule filter to predict oral bioavailability, indicating that compounds with a molecular weight ≤ 500 Da, hydrogen bond acceptors ≤ 10, hydrogen bond donors ≤ 5, and MlogP ≤ 4.15 are considered drug-like properties[39]. This rule was not applied in the present study because β-1, 3/1, 6-glucan oligosaccharides have higher molecular weights and are intended to act as biological response modifiers rather than conventional small-molecule drugs. Moreover, β-1, 3/1, 6-β-glucans exert immunomodulatory effects locally in the gastrointestinal tract through receptor-mediated mechanisms involving Dectin-1 and TLR2, unlike compounds designed for systemic absorption. Therefore, *in silico* ADMET modeling was employed as a more appropriate approach to evaluate their pharmacokinetic and safety profiles.

Regardless of molecular weight, the fungal oligo-β-1, 3/1, 6-glucans are likely confined to the gastrointestinal lumens after oral consumption and excreted without transformation. This is because the human body lacks enzymes to digest β-1, 3/1, 6-glucans into absorbable sugars[40]. Therefore, it travels through the digestive tract largely unchanged. To access the immune cells with Dectin-1 and TLR receptors, previous studies suggest that β-glucans can be internalized through microfold cells (M cells) in Peyer's patches[41], a part of gut-associated lymphoid tissue (GALT) in the small intestine that plays a key role in the immune system's surveillance of the digestive tract[42]. M cells are specialized epithelial cells that transport antigens and microorganisms from the intestinal lumen to immune cells below[43]. Via M cells, β-glucans could meet with immune cells and interact with the immune receptors[41]. Thus, β-glucans likely act as luminal immunomodulators through localized receptor interactions rather than systemic distribution.

The ADMET profile supports the safety of fungal β-glucans. No neuro-toxicity, hepatotoxicity, or genotoxicity effects are found in our *in silico* model. Consistently, a recent study demonstrated that daily supplementation with up to 2,000 mg of β-1, 3/1, 6-glucans oligosaccharide isolated from the fungus *Ophiocordyceps dipterigena* BCC 2073 for two weeks is safe in healthy volunteers[44]. Only mild and reversible gastrointestinal-related symp-toms, such as diarrhea and constipation, were reported. The findings are consistent with our *in silico* ADMET result showing that β-1, 3/1, 6-glucans only confined to the gastrointestinal tract. Further studies are warranted to verify the computational

predictions and fully assess the clinical pharmacological properties of β-1, 3/1, 6-glucan oligosaccharides.

This research has several strengths. First, these *in silico* studies docked the fungal oligo-β-1, 3/1, 6-glucans to both macromolecules and the known binding sites of the receptors. This approach allows us to discover that the receptors may have new binding sites. Second, docking both Dectin-1 and TLR2 receptors in the same study enables us to observe the common trend for the role of molecular weight on binding capacity. Third, the ADMET analysis encompasses all aspects, including both genotoxicity and liver toxicity, providing a comprehensive picture of the safety profile. Nevertheless, this work has some limitations. First, β-glucans with molecular weights exceeding 1.6 kDa could not be evaluated using the molecular docking program due to computational constraints. Some evidence suggests that the accuracy of molecular docking decreases with the use of extensive and flexible ligands[45]. This is consistent with existing data indicating that molecular docking techniques enable the prediction of interactions between small molecules and target proteins at the atomic level[46,47]. Second, in this study, we manually drew the structure of fungal oligo-β-1, 3/1, 6-glucans based on descriptions in previous literature. Then, the 3D structure used in docking is artificially intelligence-generated. Therefore, the accuracy of the structure may not be to the same extent as those formed by the structure crystallography. Nevertheless, the insight from this work is still helpful as preliminary information for further *in vitro* and *in vivo* studies.

Conclusions

This study predicts that fungal β-1,3/1,6-glucans oligosaccharides can bind strongly to Dectin-1 and TLR2 receptors on neutrophils, compared to reference compounds, and that their molecular weights may affect the binding capacity. The oligo-β-1, 3/1, 6-glucans likely remain confined within the gastrointestinal lumen, are excreted without metabolic transformation, and pose a low risk of toxicity. These findings indicate the potential of β-glucans oligosaccharides as effective immunomodulatory agents with an appreciable safety profile.

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