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Molecular Docking of Carbohydrates to a *Mycobacterium tuberculosis* Molecule.

Sachin Soodeen[1], Nigel K. Jalsa[2], Darren Gopaul[1], Belkis Ferrer-Cosme[3]

[1] Department of Para-clinical Sciences. Faculty of Medical Sciences. The University of the West Indies. St. Augustine. 00000. Trinidad and Tobago; email: sachin.soodeen@my.uwi.edu; darren.gopaul2@my.uwi.edu

[2] Department of Chemistry. Faculty of Science and Technology. The University of the West Indies. St. Augustine. 00000. Trinidad and Tobago; email: nigel.jalsa@sta.uwi.edu

[3] "Saturnino Lora" University Hospital. Higher Institute of Medical Sciences of Santiago de Cuba. 90100. Cuba.

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ABSTRACT

This study aimed to investigate the molecular docking of carbohydrates in *Mycobacterium tuberculosis*. Mincle (macrophage-inducible C-type lectin) is predominantly expressed in macrophages, where it plays a role in the macrophage response to various microorganisms, such as mycobacteria. Docking is a computational procedure in which various software packages generate different positions at which the ligands bind to their receptors. Discovery Studio docking and the Molegro Virtual Studio software were used. ChemDraw and ChemDraw3D software were used for ligand preparation and construction, respectively. For the purposes of this research, the ligand interaction between trehalose dibenzenate (TDM) and trehalose dimycolate (TDB) and its protein receptor molecule will be investigated using a modeling technique known as molecular docking, in which Mincle plays a novel role in the induction of inflammatory signaling in response to mycobacteria. Hypothetically, TDM and TDB detected by macrophages ultimately lead to CARD9 signaling, thereby inducing pro-inflammatory cytokines and chemokines that have the potential to be used therapeutically, as *"Mycobacterium tuberculosis"* displays multidrug resistance to primary antibiotic treatment.

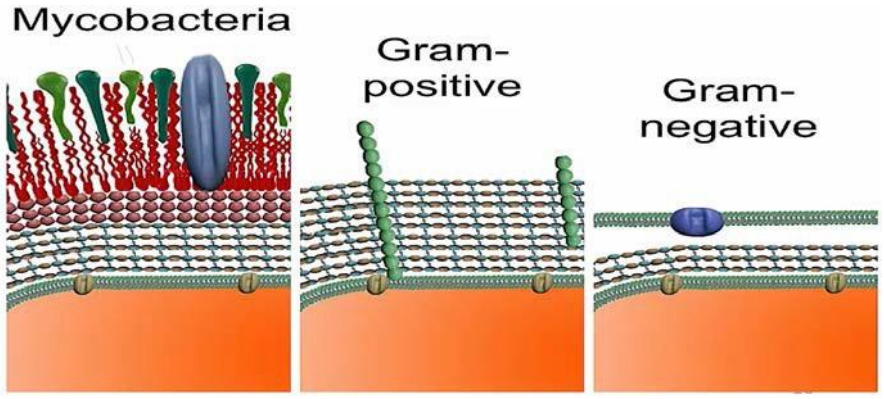
Keywords: *Mycobacterium tuberculosis*, Mincle, docking, macrophage, cytokine, trehalose dibehenate, trehalose dimycolate, Molegro Studios virtual docker, immune system.

1. INTRODUCTION

Tuberculosis is caused by *"Mycobacterium tuberculosis"* which displays multidrug resistance to primary antibiotic treatments [1]. The reasons for such resilient behavior are related to the unique physical, chemical, and biological makeup of this bacterium. It contains lipids that form a waxy impervious coat, preventing chemical interactions and even death [2].

However, Trehalose Dimycolate (TDM), a glycolipid found on the cell surface, and its derivative analog trehalose dimycolate, have been shown to elicit an immunogenic response as they are bioactive and can react with Mincle receptors on neighboring macrophages [3-5]. In this project, we visually and statistically examined how exactly these glycolipids/ligands bind to the carbohydrate domain of the c-type lectin molecule using a process called molecular docking [6-9]. Docking is a computational procedure in which various software packages generate different positions at which ligands bind to their receptors [9-11].

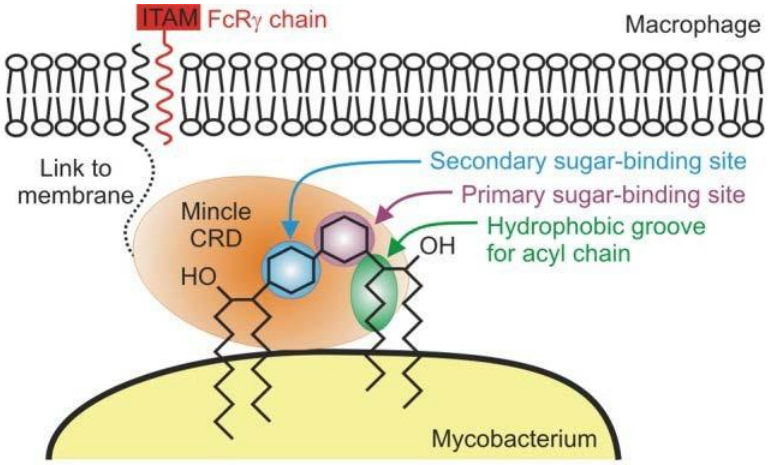
Molegro Virtual Studio and Discovery Studio Docking software were used in this study [11]. ChemDraw and ChemDraw3D were used for ligand preparation and construction because protein preparation using docking software is time consuming. Trehalose dibehenate showed the highest affinity for binding to mincle and triggered the highest release of cytokines in terms of biological activity, as its poses had the most intense docking scores for various software programs [1]. Images of these poses and interactions were captured and are presented in the results section. Trehalose dibenzenate (TDB) can be used for manufacturing drugs and as a vaccine adjuvant to trigger strong immune responses [4].



Mycobacteria (Figure 1) lack an outer membrane resembling that of Gram-positive bacteria. However, as shown above, the peptidoglycan layer is not as thick as that of gram-positive bacteria [1,2].

Mincle is a macrophage-inducible C-type lectin expressed on myeloid cell outskirts that recognizes damage-associated molecular patterns (DAMPs) and pathogen-

associated molecular patterns (PAMPs) [4]. The TDM molecule and its synthetic analog TDB act as ligands for Mincle receptors and exhibit anticancer activity by activating the spleen tyrosine kinase (Syk) and Card9- Bc110-MALT1 inflammasome pathways [12,13]. A part of the receptor binds to the C-type carbohydrate domain, which is attached to the cell surface via a transmembrane anchor [9,13-15]. After binding, a hetero-oligomer is formed with the gamma subunit of the Fc receptor, which interacts with spleen tyrosine kinase (Syk) enzymes through the immunotyrosine activation motif (ITAM), leading to the CARD9 signaling pathway cascade. This, in turn releases TH1 and TH17 molecules that induce pro-inflammatory cytokines such as IL-1, IL-7, and TNF-α. This induces inflammation in macrophages and dendritic cells [16].



The protein-ligand interaction between (TDM) and (TDB) to its protein receptor molecule for the purposes of this research will be done using a modeling technique known as molecular docking (Figure 2), which is used to predict how proteins interact with various molecules such as ligands [26]. The aftermath of such interactions can result in a complex that enhances, activates, or inhibits biological functions [9]. All docking software possess an algorithm to identify the active site, and it explores the best possible binding position of the ligands [9,11].

- Saving a lot of energy, as these trials and research are computationally based, the disadvantages of these techniques are as follows:
- The uncertainty of convergence which would require multiple independent runs
- The software is unable to differentiate between OH and NH bonds [9,17].

2. MATERIALS AND METHODS

The protein data bank ID code for the carbohydrate domain of C-type lectin mincle was obtained [3]. After downloading the protein receptor, the file was saved in the exact format (. pdb) using a docking software. Several images of trehalose dibenzoate (TDB) and trehalose dimycolate (TDM) were obtained using Google Scholar and Google, respectively. Using ChemDraw Professional [10], the shapes of the TDB and TDM ligands acquired online

using the same software ensured that no errors were found in the structure. Subsequently, the structure is copied.

The copied structure was then pasted in CHEM3D to obtain the 3-Dimensional structure of the ligand. Following the 3-Dimensional conversion the MM2 function was selected, where the 3D shape was altered to minimize steric interaction and energy, showing the most feasible configuration of the ligand molecule.

Using the Molegro virtual docker [11], the (4KZV) carbohydrate region domain of the protein receptor Mincle was imported into the software. All water molecules and external hydrogen bonds were removed for a concise visualization of the binding site. One ligand (GLC-GLC) was already present in the receptor and was removed, allowing only two ligands to be present: TDB and TDM. The TDB molecule was imported as the ligand. The preparation option was selected for small molecules as the ligand was prepared for docking. The authors selected the preparation and detected all cavities. A docking and docking wizard was selected, which allowed it to choose the ligand to dock, allowing for five possible poses, and running the docking simulation. It saved the energy values and rearranged them in order of the most negative docking score. The moldock energy values are tabulated for each pose. This procedure was repeated for the TDM.

Procedure for docking using discovery studios

After obtaining the configured files of the ligand from CHEMDRAW in a “cdxml” file format, using sourceforge.net to download open babel in which was used to convert the file format into a pdb format to be used in discovery studios docker, the 4KZV protein file and TDB files were imported separately. A TDB file was prepared for the ligand and was used as the input ligand. The protein was prepared and all water molecules were removed. Using the libdock function, docking was started with the prepared molecules, five out of the 11 poses were made visible for viewing. The energy values were calculated, and libdock values were obtained. The docking view was then converted into a two-dimensional image to view the interactions with the ligand [9]. Subsequently, the values and images were recorded.

3. RESULTS

3.1.1 Docking of mincle and TDM

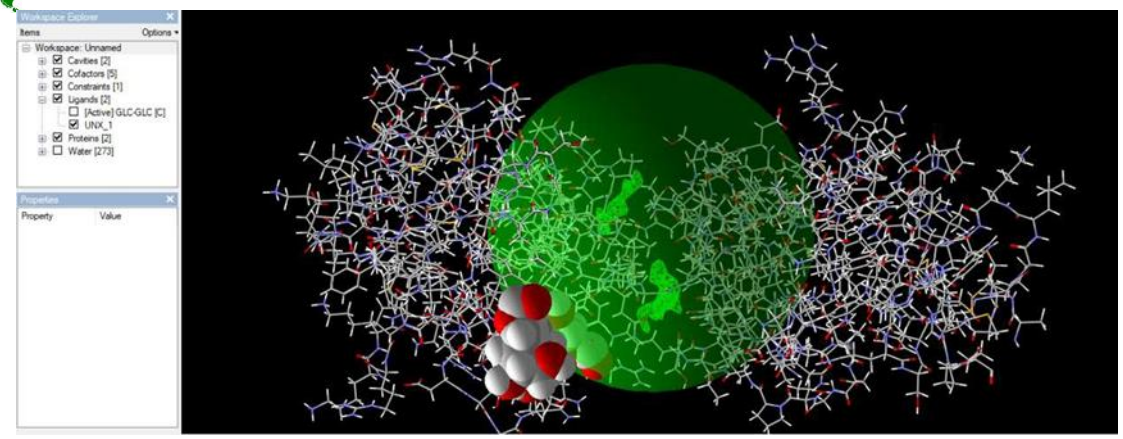
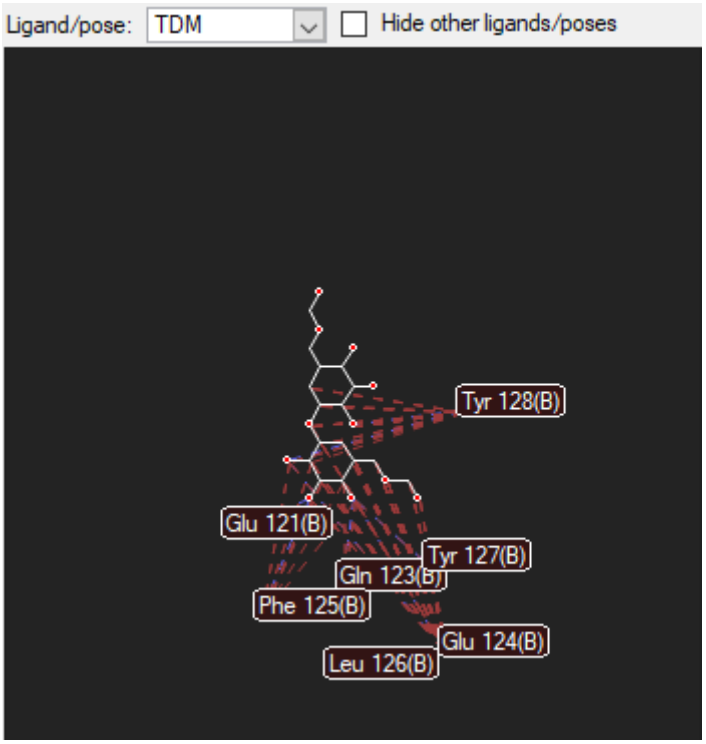


Figure 3 shows a feasible pose in which trehalose dimycolate (TDM) binds to the mincle receptor. Among the five poses found, this pose has the lowest energy value, indicating minimal energy, as shown in nature.

3.1.2 The nearby amino acids in which the TDM binds to.



Similar to pattern recognition receptors, such as NOD-like receptors (NLRs), Toll-like receptors (TLRs), or RIG-I-like receptors (RLRs), a C-type lectin receptor can bind to a series of ligands and mincle, as shown in Figure 4.

3.2.1. Docking of mincle and TDB.

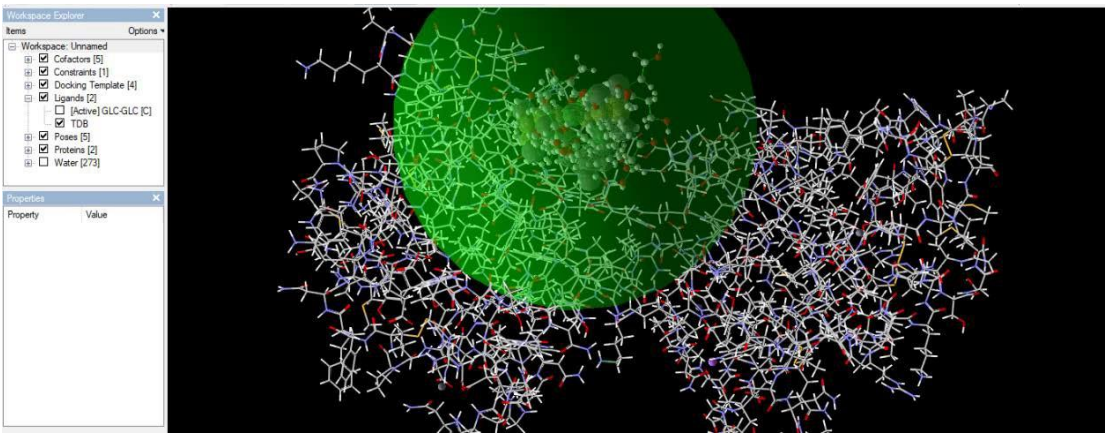


Figure 5. shows a feasible pose in which the TDB binds to the mincle receptor. Among the five poses found, this pose has the lowest energy value, indicating minimal energy, as shown in nature.

3.2.2 The nearby amino acids which TDM binds to.

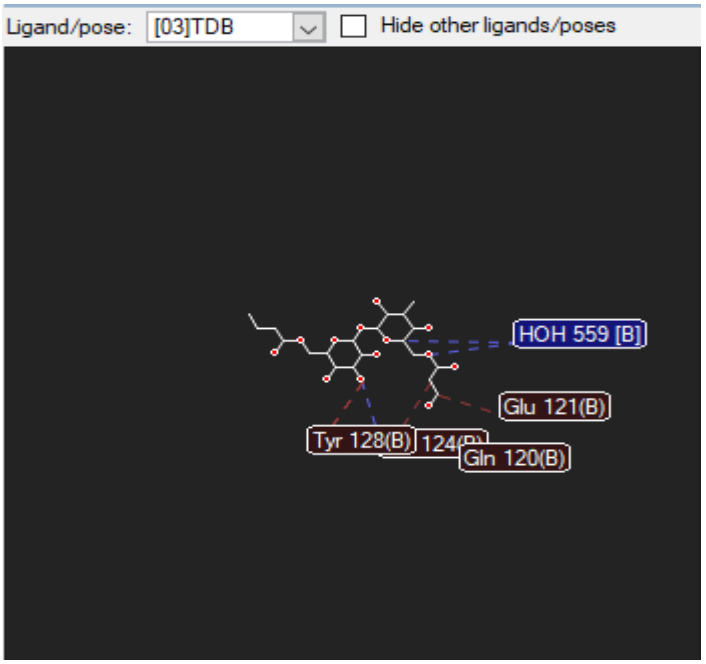


Figure 6. illustrates the van der Waals and hydrogen bond-to-bond interactions between TDB and (as shown above) amino acids. These amino acids are found on the mincle receptor, which is the orientation in which they interact with the TDB ligand.

Table 1. Showing energy values using the molegro studios virtual docker for TDB.

POSE	LIGAND	MolDock SCORE	Rerank	Docking

			Score	score
4	TDB	-40.4109	-31.3595	-402.02
3	TDB	-36.6981	2.50891	-410.682
2	TDB	-24.9085	-9.17407	-414.356
5	TDB	-24.6662	-30.2681	-401.288
1	TDB	-16.5354	61.5971	-442.523

Table 1 lists the results of the calculations performed using Molegro Studio virtual docker. This software provides a forcefield-generated energy value in the form of a "moledock score, where the more negative the value is, the more likely it is to exist in nature. Therefore, from the results, pose number 4 with a value of -40.4109 has the highest chance of existing biologically, whereas pose number 1 with a value of -16.5354 is the least likely to exist in nature, as it requires too much energy to do so.

Table 2. Showing energy values using the molegro studios virtual docker for TDM.

POSE	LIGAND	MolDock SCORE	Rerank Score	Docking score
3	TDM	-42.1683	-38.2711	-261.883
4	TDM	-34.7130	-16.1923	-247.830
1	TDM	-30.0261	-4.84853	-268.438
2	TDM	-27.6750	-17.0551	-265.637
5	TDM	-2.75511	-28.6257	-244.610

Table 2 shows the results of the calculations performed using Molegro Studio virtual docker. This software provides a forcefield-generated energy value in the form of a "moledock score, where the more negative the value is, the more likely it is to exist in nature. Therefore, from the results, pose number 3 with a value of -42.1683 holds the highest chance of existing biologically, whereas pose number 5 with a value of -2.75511 is the least likely pose to exist in nature, as it requires too much energy to do so.

Table 3. Showing docking scores using Discovery studios docker.

POSE No.	LIGAND	LibDOCK SCORE
6	TDB	89.9593
5	TDB	96.6506
4	TDB	99.7128
3	TDB	116.655
1	TDB	124.043

For this particular part of the research experiment, discovery studios docker is used and its docking score or energy value for each individual pose is converted to a "libdock score" where a higher reading would signify a greater energy requirement for natural existence (Table 3). Therefore, we can say that pose 6 values of 89.9593 would require the least amount of energy to exist in such a pose. However, the pose number 1 value at 124.043 would require the highest amount of energy to exist in nature, and is therefore almost enviable.

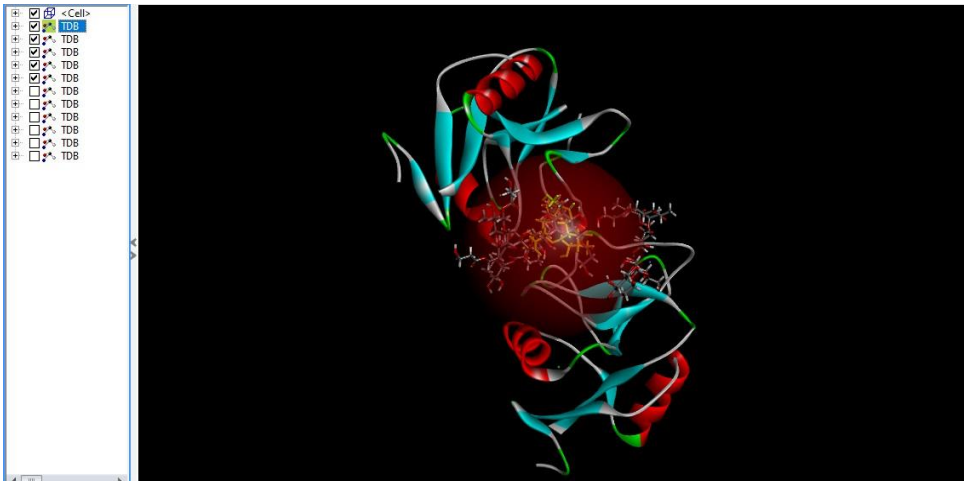
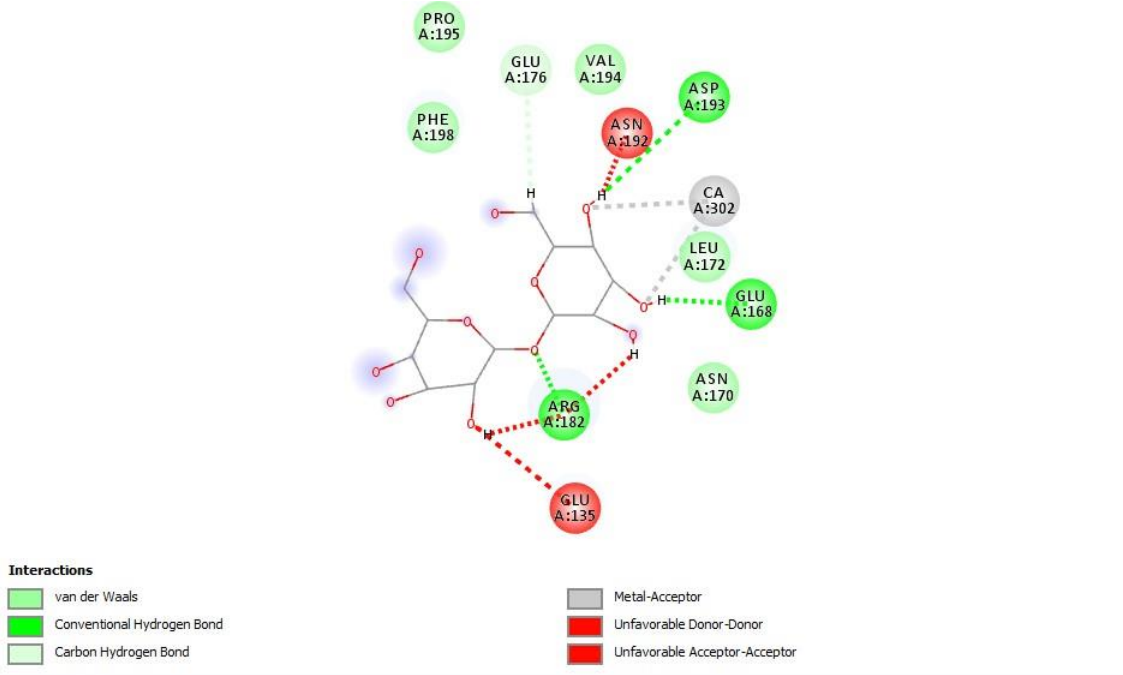


Figure 7. Diagram above illustrates all 5 poses are connected.

The diagram above (Figure 7) shows the five most feasible poses in which TDB, the derivative analog of TDM, binds to mincle because of the low force field energy shown above in a reddish hemisphere, which is the area in which most hydrogen bond interactions



occur.

Figure 8. The most feasible ligand poses interacting with molecules to bind.

The diagram above (Figure 5) shows the five most feasible poses in which TDB, the derivative analog of TDM, binds to Mincle because of the low force field energy shown above in the reddish hemisphere, which is the area in which most hydrogen bond interactions occur. All five poses presented in the previous diagram illustrate that this pose is only feasible or energy-viable because of amino acid interactions and hydrogen bonding with the TDB molecule. The scale underneath shows the different types of bonding, not just hydrogen bonding but also van der Waals, conventional hydrogen bonds, carbon hydrogen bonds, metal acceptors, unfavorable donor donors, and unfavorable acceptors.

4. DISCUSSION

In this study, molecular docking of tuberculosis proteins was conducted using Molegro virtual docker and Discovery Studio docking. Docking is a computational study used to determine the position or manner in which TDB and TDM bind to the carbohydrate domain of mincle. These values explain why POSE 1 of TDB has a higher affinity for binding to Mincle and releasing cytokines than TDM in both docking and biological activity studies [6,9]. For this research, I chose to draw my ligands using ChemDraw and optimized them using ChemDraw3D, despite it being a longer and tedious route. However, this has enabled me to view and examine my molecular interactions. The use of discovery studios for docking is a lengthy and time-consuming process for preparing protein receptors. Obtaining docking scores and biological activity data from online sources is difficult, because this is a specific and targeted study. Therefore, results showing the release of inflammatory cytokines directly related to the binding and activation of the Mincle receptor were used [18].

The Docking score used for the Molegro Virtual Studios Docker was the value generated for the binding energies, which were the determinants of the two factors.

1. Force field based
2. Empirical scoring functions [9,19].

A lower or higher negative docking score indicates less energy in the pose [11,16]. The other criterion was used to predict the affinity. This score includes hydrogen bonding and electrostatic terms, which the scoring function does not, and a more positive value indicates better outcome. Furthermore, there are other criteria and yet another way to generate accurate results: Molegro's re-ranking score, which combines both and, produces a value that signifies the best possible docking pose. According to the data in Tables 1 and 2, pose 1 had the highest values of 69.5971 and -4.84853 respectively as their docking diagrams and interactions are shown [11,20].

For Discovery Studios, the docking and calculations in this study were performed with LibDock, which combines the values for hydrogen bonding, van der Waals forces of attraction, pi interactions, and other values to generate a score that indicates a high

probability of ligand-receptor binding as the LibDock score increases [21]. As shown in Table 3, Pose 1 had the highest score of 124.03 as its docking diagram is highlighted in yellow, followed by a diagram showing its interactions. [9].

In biological assays, TDM and TDB are detected by macrophages in the body, which ultimately leads to CARD9 signaling, thereby producing pro-inflammatory cytokines and chemokines. Therefore, by determining the highest concentrations of cytokine production, one can determine which ligand and concentration result in the highest activation and can be used therapeutically [1,9,21].

Mincle was first described as the target of the macrophage transcription factor, NF-IL6. Mincle mRNA in response to innate immune responses and inflammatory stimuli, including IL-6 and LPS in murine macrophages, may serve as a receptor for various fungi, bacteria, other microorganisms, and molecules and signals *via* FcRγ chain association, resulting in NF-κB activation [22].

TDM activates macrophages to synthesize inflammatory cytokines. It has been observed that cytokine production is fully inhibited in Mincle-deficient macrophages. This was not the case in our study because we did not look for it. In vivo, TDM administration strongly increased serum levels of inflammatory cytokines. It is one of the features of lung inflammation, including granuloma formation, which is the most prominent anatomopathological sign of tuberculosis. Similarly, no TDM-induced lung granulomas have been observed in mincle-deficient animal models [23].

Mincle is a pattern recognition receptor (PRR). It detects infections caused by mycobacteria via cell wall TDM recognition. It was shown that splenic cDCs, which express Mincle, assisted in the innate immune reconstitution of attenuated splenic mycobacteria in a Mincle-knockout mouse model after intravenous administration of BCG. This constitutes a critical mechanism that allows for BCG infection control in mice [24].

Dectin-3 stimulation by TDM can lead to the expression of Mincle on antigen-presenting cells as DCs and macrophages, which may stimulate an inflammatory response to detect mycobacterial infections in the host. However, Dectin-3 involving mechanisms as treated here are not yet well understood. It has been shown that TDM-induced Mincle expression depends on Dectin-3-mediated NF-κB [25].

Mincle activation leads to neutrophil infiltration, and chemokine and cytokine production. Mincle can also stimulate immunity by acting as an adjuvant receptor [14,27]. Furthermore, TDM binds to Mincle and activates T-lymphocyte immunity via the Syk/Card9 pathway. TDB, a synthetic form of TDM, may be used in CNS therapy to improve neuroinflammation in microglia [28].

Schick et al. (2023) experimentally demonstrated the downregulation of Mincle expression in macrophages and monocytes by IL-4, possibly leading to Th17 cell immune responses in the presence of helminth infections [29].

Macrophages are antigen-presenting cells that can be classified as M1(classically activated/inflammatory) or M2 (alternatively activated/regenerative) [32,33]. Six M1 polarization markers were identified using mRNA expression analysis. These include IL-12p35, CCR7, CXCL10, CCL5, CXCL11, and IDO1. Likewise, five markers of M2 polarization were identified using the same technique, including CCL14, TGF-β, CCL22, SR-B1, and PPARγ [33-35]. These two fenotypes have immunological and inflammatory implications because M1 is present in inflammatory diseases and is induced by IFN-γ and LPS, and M2 is present in infectious diseases such as viral and bacterial infections and is induced by IL-4 and IL-10 [36-40].

Mincles are maintained by the M1 macrophage phenotype [41]. Mincle expression in bone marrow-derived macrophages was markedly induced by cancer cells *in vivo* (melanoma and lung cancer) and *in vitro,* in M2-like tumor-associated macrophages (TAM) Mincle was also expressed. It promoted the M1 phenotype when unexpected, suggesting a novel discovery: the Mincle/Syk/NF-κB signaling pathway by which TAM killed their targets via a TLR4-independent mechanism [42]. In summary, molecular docking of Mincle to Mycobacterium tuberculosis components was performed. The different software used to dock molecules in this study suggested that hypothetical activation of the cell wall and a pathway for the activation of genes could occur, as shown in Figure 6. Furthermore, these genes activate the transduction of cytokines and other products secreted by macrophages, which hypothetically regulate immune system functions and protect against *Mycobacterium tuberculosis* infection. It is known which of the different products released by antigen-presenting cells can be used to design therapeutic molecules for infection control. The answer to this question could be therapeutic for the preparation of humanized monoclonal antibodies designed with the molecular properties of Mincles.

5. Conclusions

In retrospect, it was possible to perform molecular docking of carbohydrates with *the M. tuberculosis* components. The data show how Mincle bridges the surfaces of antigen-presenting cells (macrophages and DCs) and mycobacteria and suggest the likelihood of availing these interactions in the fields of chemical medicine in designing adjuvants that mimic the ability of bacteria to stimulate immune responses to immunization.

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