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# Molecular Docking Study of Chitinase to Identify Potent Inhibitors

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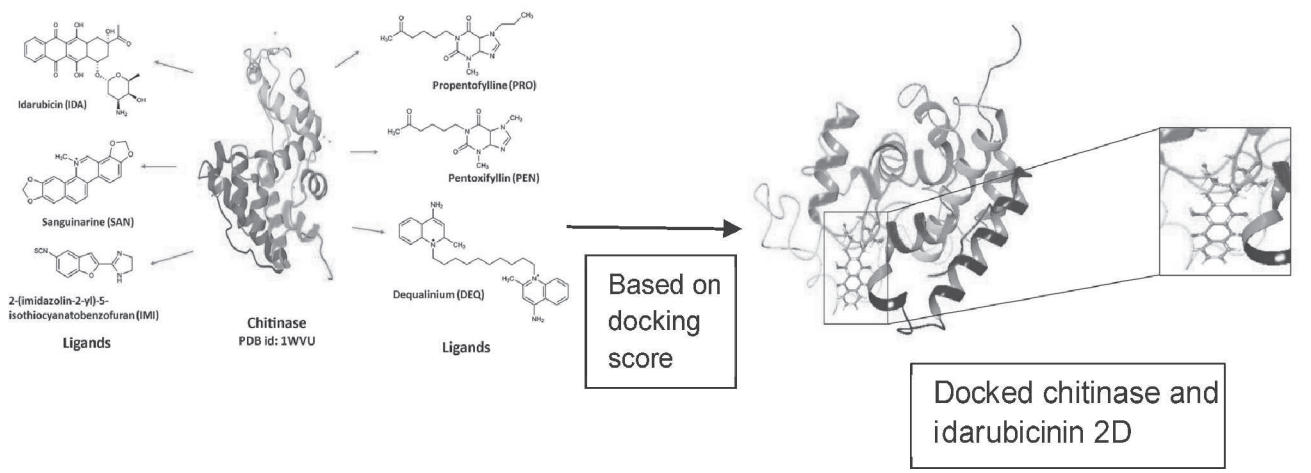
## ABSTRACT

### Abstract

Chitin is a biopolymer that consists of monomers called N-acetylglucosamine. It is found in many organisms. There are mainly two types of chitinases: endochitinase and exochitinase. Chitinase is part of the Glycoside hydrolases family. Different species of bacteria and fungi are reported to produce chitinase, and they have been extensively studied for various applications for many years. *In- silico* approaches enable the efficient screening of a large number of compounds in a short time, thus lowering costs and raising the possibility of finding promising candidates. The orientation of bound molecules can provide valuable insights into the energy profiling, strength, and stability of complexes. Our rigorous molecular docking study identified idarubicin as a potent inhibitor of bacterial chitinase, a significant breakthrough in the field. This finding, supported by our MMGBSA analysis, holds immense potential for understanding and application of chitinase inhibitors.

**Keywords :** Chitinase, molecular docking, inhibitors, MMGBSA, *in-silico* analysis.

### Graphic abstract



## INTRODUCTION

Chitin, the second most prevalent polymer on the Earth, is surpassed in abundance only by cellulose. It is composed of N-acetyl-glucosamine (GlcNAC) units, which contribute to its rigid structure. Chitin exhibits notable structural and functional similarities to cellulose. Chitinolytic bacteria possess the remarkable capability to degrade chitin in both anaerobic and aerobic settings. The exoskeletons of numerous invertebrate species contain chitin. Unlike other naturally occurring polysaccharides such as cellulose, agarose, and agar, chitin is a highly alkaline polysaccharide. Chitin demonstrates solubility in strong acids and fluoroalcohols while remaining insoluble in water and various organic solvents. Three distinct forms of chitin exist: α-chitin, β-chitin, and γ-chitin. The most common form of chitin is α-chitin (Kaya *et al*., 2015). It is notable for its inherent lack of elasticity.

The chitinase enzymes can be classified into two distinct categories: i) Endochitinase and ii) Exochitinase. Chitinase is found in various organisms, including bacteria, fungi, insects, plants, and animals. The classification of chitinases primarily involves two families, 18 and 19 of glycosyl hydrolases (GH). The families are divided into five classes (Henrissat and Bairoch, 1993). The bacterial chitinase has a molecular mass ranging from 20 to 60 kDa (Svitil and Kirchman, 1998). The breakdown of chitin is a meticulously regulated process.

Bacterial chitinases have shown great potential for various commercial applications due to their ability to thrive in diverse environments, rapid reproduction, exceptional enzyme stability, and convenient genetic engineering. Family 18 exhibits a wide range of diversity in terms of evolution, encompassing chitinases from various sources such as bacteria, fungi, viruses, animals, and even certain plant chitinases. Family 19 of glycosyl hydrolases includes plant chitinases (classes I, II, and IV) and certain chitinases found in *Streptomyces* (Hart *et al*., 1995). The chitinases from families 18 and 19 of glycosyl hydrolases do not exhibit any similarity in their amino acid sequences. They possess distinct 3-D structures and molecular mechanisms, suggesting they may have originated from separate ancestors (Suzuki *et al*., 1999). The family 20 of glycosyl hydrolases encompasses the β-N-acetyl-hexosaminidases found in bacteria, *Streptomycetes,* and humans. Bacterial chitinases can be categorized into three main subfamilies, A, B, and C, by analyzing the amino acid sequence of their catalytic domains (Watanabe *et al*., 1993). The distinction between these groups lies solely in the existence of a chitin insertion domain (CID) that is exclusively present in subfamily A. The production of extracellular chitinase can be affected by various factors, including the composition of the media, such as carbon sources, nitrogen sources, and agricultural residues like wheat bran and rice bran (Bhushan and Hoondal, 1998; Dahiya *et al*., 2005).

Bacteria, fungi, and insect chitinases exhibit a wide range of diversity within family 18 of glycosyl hydrolases. These members have developed a highly efficient mechanism for breaking down chitin. They secrete a combination of chitinases to break down chitin for various purposes, such as development or defence (Kramer and Muthukrishnan, 1997). The synergistic property mentioned has been empirically demonstrated, particularly in the case of *Serratia marcescens* (Suzuki *et al*., 2002) which can produce up to four chitinolytic enzymes. The bacteria that produce GH family 18 chitinases are found in various genera such as *Serratia* (Watanabe *et al*., 1997; Purushotham and Podile, 2012), *Vibrio* (Suginta *et al*., 2016), *Bacillus* (Laribi-Habchi

*et al*., 2015), *Chromobacterium* (Lobo *et al*., 2013) and *Yersinia* (Busby *et al*., 2012).

The family 19 of glycosyl hydrolases chitinases have primarily been identified in plants, several bacteria, a limited number of viruses (as documented in the CAZy database, http://www.cazy.org), and only one fungus (Han *et al*., 2016). The production of members within this family is believed to be a defence against fungal pathogens. Classes I, II, and IV of plant chitinases are categorized under GH family 19, which includes the extensively studied chitinases from *Oryza sativa* (Kezuka *et al*., 2010), *Bryum coronatum* (Taira *et al*., 2017) and *Picea abies* (Ubhayasekera *et al*., 2009) respectively. The classes are distinguished either by the presence (class I) or absence (class II) of the chitin-binding domain (CBD). Class IV organisms are smaller due to the absence of specific additional loops in their structure (Beintema, 1994; Grover, 2012).

Given the recent advancements in computer technology and the exponential growth of data in fields such as structural, chemical, and biological research, it is clear why the utilization of *in- silico* methods such as chemoinformatics, molecular modelling, and artificial intelligence (AI) has experienced a substantial rise in the past few years. Several molecular modelling tools can aid in drug development activities, with most of them falling into the categories of ligand-based and structure-based approaches.

One way to accomplish this is by utilizing the scoring function of molecular docking. In today's world, molecular docking is commonly used to predict how small molecules (drug candidates) bind to their biomolecular target (such as protein, carbohydrate, and nucleic acid) to determine their potential binding parameters. This provides a foundation of raw data for the rational drug-designing process, enabling the development of new agents that offer improved efficacy and greater specificity. Molecular docking is essential in structural molecular biology and computer-assisted drug design. Ligand-protein docking aims to accurately predict the most likely binding mode(s) between a ligand and a protein with a known three-dimensional structure. Efficient docking methods excel at navigating complex spaces and employ a reliable scoring system to evaluate potential dockings accurately. Using docking, one can conduct virtual screening on extensive compound libraries, prioritize the outcomes, and suggest potential structural explanations for how the ligands hinder the target. This process is extremely valuable for lead optimization.

In this study, we aimed to study the comparative analysis of the different inhibitors on the enzyme derived from the Protein Data Bank (PDB) having id 1WVU. It is the chitinase C from the *Streptomyces griseus* HUT6037. Then, it was subjected to molecular docking with different inhibitors and based on the docking score, the inhibitor with the maximum binding affinity was identified. After that, a molecular mechanics generalized born surface area (MMGBA) analysis was also performed.

## MATERIALS AND METHODS

### Preparation of ligands and proteins

The structure of chitinase was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/) with PDB id 1WVU. Schrodinger's protein preparation wizard was utilized to repair and polish the protein and fix the structural artefacts. This achieved great structural confidence by converting the protein structure from a raw to a refined state and appropriately preparing it for molecular docking and molecular dynamic research. Assigning the revised bond ordering, removing water molecules and other non-

specific chemical components from the crystal structure, and adding hydrogen atoms to the protein structure to rebuild the tautomeric and ionization states of amino acid residues were all part of the procedure (Schrodinger Release 2022-4). The protein preparation wizard was pre-processed by removing water molecules within 5 Å hetero groups. The chitinase structure was restricted energy minimized using the OPLS4 force field after adding missing hydrogen atoms and optimising hydrogen bonds. As in the case of metals, no conformers were generated.

**Receptor Grid Generation**

The GLIDE molecular docking method requires the presence of a single ligand that can bind to the X-ray crystal structure of the protein. This is necessary to determine the protein's active site and generate a receptor grid. The utilization of receptor grid-based molecular docking facilitates the binding of ligands in multiple potential conformations. The scaling factor and partial charge cutoff for van der Waals radius scaling are 0.25 and 1 Å, respectively. The default settings of Maestro 12.8 include other parameters such as sites, constraints, rotatable groups, and excluded volume (Friesner *et al*., 2006; Repasky *et al*., 2007).

**Molecular Docking**

The ligand and protein were prepared, and the grid on the protein's active site was defined. Subsequently, molecular docking procedures were conducted. The GLIDE employ a systematic computational simulation method to evaluate specific poses and ligand flexibility. The GLIDE systematic method is a novel, rapid, and accurate molecular docking approach. This method produces an output that is highly reliable and efficient. GScore, an empirical scoring function, is constructed by combining several parameters. The GScore is computed in units of kcal/mol and encompasses various factors such as internal energy, ligand-protein interaction energies, hydrogen bonds, hydrophobic interactions, p-p stacking interactions, and desolvation. The GLIDE module of the XP visualizer analyses the specific interactions between ligands and proteins (Friesner *et al*., 2004; Halgren *et al*., 2004).

**Calculation of binding free energy by using MM-GBSA**

Molecular mechanics generalized Born surface area (MM/GBSA) calculates the binding free energy using the single trajectory method. The MM-GBSA technique is often used to analyze the binding free energy of ligands-protein or protein-metal molecules. The Prime MMGBSA technique calculated $\Delta G_{(bind)}$ (relative binding-free energy) for each ligand molecule.

**The formula is as follows:**

$$\Delta G_{(bind)} = \Delta G_{(solv)} + \Delta E_{(MM)} + \Delta G_{(SA)}$$

Where:

$\Delta G_{(solv)}$ is the difference in GBSA solvation energy of the Chitinase-inhibitor complex and the sum of the solvation energies for unliganded chitinase and inhibitor.

$\Delta E_{(MM)}$ is a difference in the minimized energies between the Chitinase-inhibitor complex and the sum of the energies of the unliganded Chitinase and metal.

$\Delta G_{(SA)}$ is a difference in the surface area energies of the complex and the sum of the surface area energies for the unliganded Chitinase and inhibitor.

The Prime MM-GBSA software calculates the energy of optimized free ligands and receptors and the complex formed by the ligand and receptor. The ligand strain energy is calculated by placing the ligand in a solution automatically generated by the VSGB 2.0 suite. The prime energy visualizer provides a comprehensive visualization of energy.

## RESULT AND DISCUSSION

Using the lower energy score, we identified the highest-ranking compounds in the set of inhibitors. When the binding energy decreases, the binding efficiency correspondingly increases, resulting in a more potent inhibition. Details of the docking score are mentioned in table 1.

**Table 1 : Docking score of the different inhibitors**

| | Docking score (kcal/mol) | XP GScore (kcal/mol) | Glide emodel (kcal/mol) | Glide energy (kcal/mol) |
|---|---|---|---|---|
| Idarubicin | -7.906 | -7.906 | -71.653 | -49.247 |
| Propentofylline | -4.22 | -4.220 | -40.126 | -32.002 |
| Pentoxifylline | -4.071 | -4.071 | -40.203 | -31.946 |
| Dequalinium | -3.868 | -3.868 | -54.478 | -43.967 |
| IMI | -3.332 | -3.332 | -38.264 | -29.47 |
| Sangunarine | -2.672 | -2.672 | -34.332 | -27.367 |

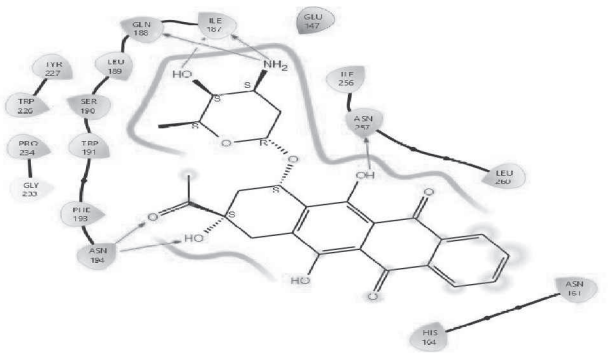


**Figure 1 : 2D image of the Idarubicin docked with the chitinase.**

The hydrophobic areas around the active site of chitinase are made up of amino acid residues such as ILE187, LEU 189, TRP 191, PHE 193, TRP 226, TYR 227, PRO 234, ILE 256, and LEU 260, whereas ASN 161, HIS 164, GLN 188, SER 190, and ASN 194 are polar amino acids. There is one negatively charged amino acid, GLU 147.

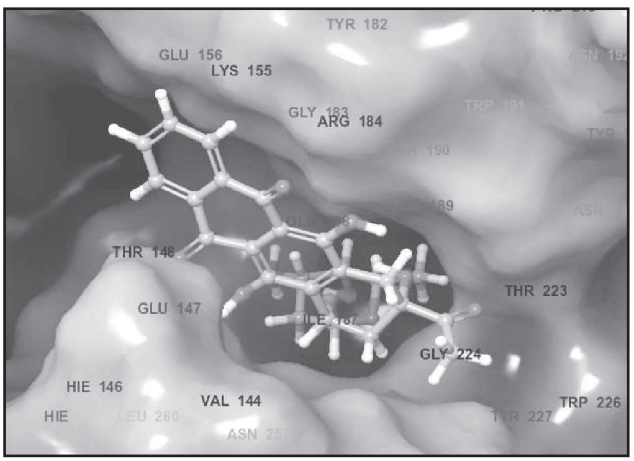



Figure 2: 3D image of the Idarubicin docked with the chitinase.

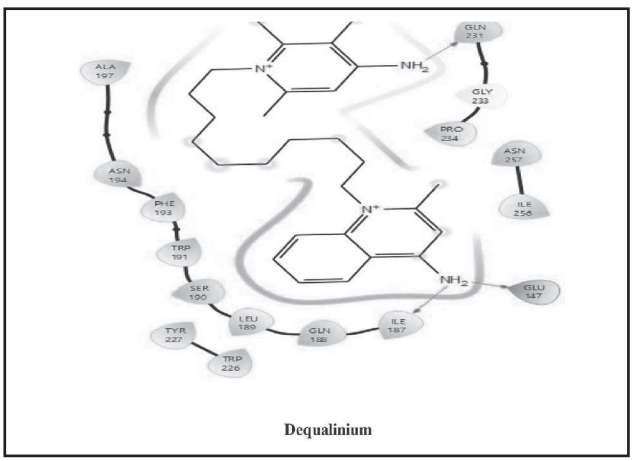



Figure 3: 2D image of different compounds docked with the chitinase.

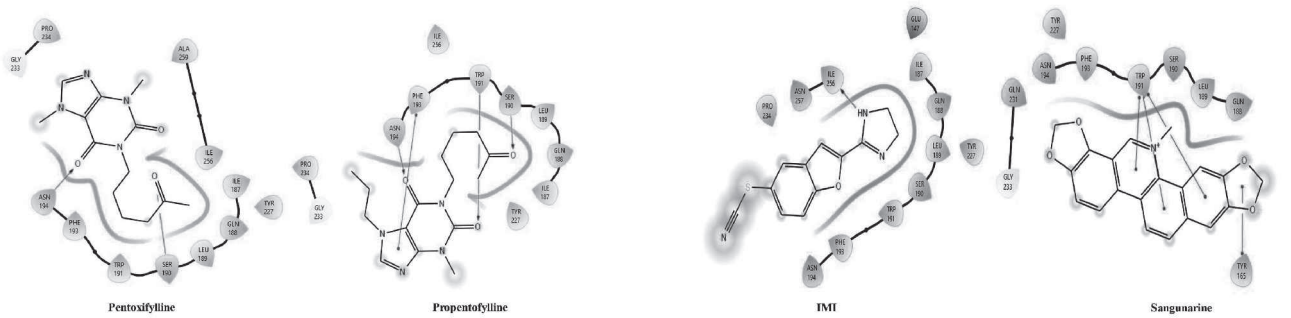



Figure 3: 2D image of different compounds docked with the chitinase.

MMGBSA calculations tend to support the estimation of molecular docking-derived binding energy. The MMGBSA dG Bind values in kcal/mol of different inhibitors are Idarubicin -42.16, Propentofylline -42.92, Pentoxifylline -36.39, Dequalinium -62.66, IMI -25.03, Sangunarine -45.41. Details are mentioned in table 2.

**Table 2 : Calculation of binding free energy using MM-GBSA of different inhibitors.**

| | Docking score (kcal/mol) | Prime Energy (kcal/mol) | MMGBSA dG Bind (kcal/mol) |
|---|---|---|---|
| Idarubicin | -7.906 | -8703.0 | -42.16 |
| Propentofylline | -4.220 | -8794.3 | -42.92 |
| Pentoxifylline | -4.071 | -8799.1 | -36.39 |
| Dequalinium | -3.868 | -8719.3 | -62.66 |
| IMI | -3.332 | -8667.9 | -25.03 |
| Sangunarine | -2.672 | -8646.4 | -45.41 |

A quantifiable binding pose or conformation of the ligand confined to the protein is predicted by the molecular interaction between the protein and the ligand-based on electrostatic interaction and its shape. The estimated sum of all observed interactions is the docking score of the ligand within the protein's binding pocket. The docking score is quantified in negative energy units (Kcal/mol), with a higher negative total energy indicating a more robust interaction between ligand and protein. The docking technique utilizes computational methods to determine the optimal binding conformation of substances within the protein's binding pocket. It also analyses the interaction between ligand and residues located at the enzyme's active site. Using extra precision (XP) ensures a highly accurate docking result. The inhibition of the enzyme relies on forming various types of bonds between amino acid residues and the ligand at the active site.

The initial aim of docking analysis is to determine the optimal conformation of a drug/compound of interest during interactions with the receptor, in order to minimize the binding free energy (Ferreira *et al*., 2015). Biomolecular docking algorithms often compute score functions to assess the binding energies of interactions between drugs and receptors (Tripathi and Bankaitis,

2017). The resulting binding energy (ΔG bind) is determined by combining many energies including torsional free vibrations, Hydrogen bonds, total internal, van der Waals dispersion, core repulsion, unbound system's desolation, electrostatic interactions, etc. The dissociation constant (Kd) is a measure of the binding energy represented by Gibbs' free energy (ΔG) (Hall *et al*., 2020). Drug-receptor binding prediction is influenced by several mechanisms including intermolecular interactions, solitude, and entropic effects. By increasing the estimate of the physio-chemical parameters, the precision of the scoring function is correspondingly enhanced (Agrawal *et al*., 2019).

**MMGBSA**

The binding strength of biological macromolecule and compound of interest is governed by the free energy of binding, denoted as ΔGbind. Conventionally, design campaigns are conducted through experimentation, which is a laborious and expensive approach. Hence, computational techniques have been devised to decrease the time and expenses involved in designing novel pharmaceuticals. The selection of the approach relies on the specific phase of the campaign in which it will be used, therefore including a standard balance between precision and effectiveness. Consequently, they fall between the accuracy and computing complexity of empirical scoring and rigorous alchemical perturbation approaches. These techniques have been implemented in several systems with different degrees of success. The integration of molecular mechanics energies with the Poisson-Boltzmann or generalized Born and surface area continuum solution (MM/PBSA and MM/GBSA) techniques is widely used for determining the free energy associated with the interaction between biological macromolecules and small ligands. Their effective use relies in the replication and rationalization of experimental results, as well as the enhancement of virtual screening and docking outcomes.

## CONCLUSION

Here, we carried out the molecular docking of the bacterial chitinase (chitinase C from *Streptomyces griseus* HUT6037) with different inhibitors, such as Idarubicin, Propentofylline, Pentoxifylline, Dequalinium, IMI, and Sangunarine. Among all of them, Idarubicin has the highest binding affinity. The MMGBSA analysis also supports the same.

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**Conflict of Interest**

The authors declare no competing interests.

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