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ORIGINAL ARTICLE

Computational study on chitinase with NPASS compounds to identify potent chitinase inhibitor

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ABSTRACT
Chitin is present in many organisms. It can be degraded by many different types of enzymes like endochitinase, exochitinase, chitin deacetylase, chitosanase, etc. The computational analysis of the aligned molecules provide valuable information on the intensity, energy distribution, and robustness of complexes. Chitinase from the protein data bank id 2DBT was downloaded and virtual screening of the Natural Product Activity and Species Source (NPASS) database with around 35,002 compounds using glide, schrodinger. Then, the top five compounds were shortlisted based on their docking score. Idarubicin, a known chitinase inhibitor was also docked with chitinase as a reference compound. Then, molecular dynamics stimulation of the chitinase with the topmost compound NPC313813 and reference compound was carried out using Meastro schrodinger. The stability of the NPC313813 was found better than reference compound. Lastly, binding free energy was calculated using Molecular mechanics with generalised Born and surface area solvation (MM/GBSA) and there too NPC 313813 scored better than reference compound.
Keywords: *chitinase, molecular docking, MD stimulation, MMGBSA, NPASS library.*

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INTRODUCTION

Chitin is the second most prevalent polymer on the earth, surpassed only by cellulose. Chitin is composed of N-acetylglucosamine (GlcNAC) units and has a rigid structure. Chitin bears significant structural and functional resemblance to cellulose. Chitinolytic bacteria have the ability to break down chitin in both anaerobic and aerobic environments. Chitin is present in the exoskeleton of many invertebrate species. Chitin is a highly alkaline polysaccharide, in contrast to the majority of naturally occurring polysaccharides (e.g., cellulose, agarose, agar), which are acidic. Chitin exhibits solubility in strong acids and fluoroalcohols but is insoluble in water and numerous organic solvents. There are three types of chitin: α-chitin, β-chitin, and γ-chitin. α-chitin is the predominant form of chitin (1). Chitin is composed of N-acetylglucosamine (GlcNAC) monomers and is characterized by its lack of elasticity. There exist two distinct categories of chitinase enzymes: i) Endochitinase and ii) Exochitinase. Chitinase is present in several bacteria, fungi, insects, plants, and animals. Chitinases are often categorized into three families: families 18, 19, and 20 of glycosyl hydrolases (GH). These families are further split into five classes [2]. Chitinases are identified based on the sources from which they have been obtained. Chitinase, can be obtained from several sources including bacteria, fungi, insects, plants, and mammals. Chitinases exhibit significant diversity and belong to a vast category of enzymes. Reported differences exist in the catalytic mechanism, molecular structure, substrate specificity [3]. Bacterial chitinase has the ability to break down chitin, which can then be used as a valuable energy source [4]. The bacterial chitinase has a molecular mass ranging from 20 to 60 kDa [5]. The breakdown of chitin is a meticulously regulated

process. Chitinases play a crucial role in various biological processes such as morphogenesis, nutrient cycling, and protection against pests and parasites having chitin [6]. They can be found in a variety of habitats. They are a key component of chitin's nutrition cycle, originating from the cells of invertebrate organisms and many other sources [7]. Chitinolytic bacteria in the rhizosphere and soil use chitin from fungus and insects as a nitrogen and carbon source [4, 8]. Chitinases produced by bacteria have nematicidal, insecticidal, and antifungal properties [9]. Therefore, chitinolytic bacteria have immense potential and serve as a perfect alternative to chemical insecticides. Various organisms synthesize a diverse range of hydrolytic enzymes that demonstrate distinct substrate specificities and other advantageous properties for various biological functions. Chitinases have various functions in different organisms. In bacteria, they are involved in nutrition and parasitism. However, in fungi, protozoa, and invertebrates, they also play a role in morphogenesis. Chitinases play a crucial role in the defence mechanisms of both plants and vertebrates. Baculoviruses are utilized in the field of biological control to manage insect pests. Additionally, these viruses are known to produce chitinases as part of their pathogenesis mechanism. The recent discovery of chitinase activity in human serum has been documented. The suggested role is a potential defence mechanism against fungal pathogens. In recent years, numerous scientists from around the world have begun investigating the numerous untapped applications of natural polymers; furthermore, natural polymers are anticipated to gain an exponentially growing market share over the next few years. In this study, we have employed various computational analysis methods like molecular docking, Molecular Mechanics Generalized Born Surface Area (MM-GBSA) and Molecular Dynamics (MD) simulation to determine which substance inhibits the chitinase the most. This can be useful in the practical approach for example, the presence of such compounds should be avoided where bacterial chitinase are being specifically being used otherwise it such compounds inhibit the bacterial chitinase activity.

MATERIAL AND METHODS

Protein preparation of 2 DBT and Ligand preparation of NPASS compounds

Fetching the three-dimensional X-ray crystal structure of chitinase with PDB ID: 2DBT from Protein Data Bank at https://www.rcsb.org/ in .pdb format was done in high resolution. The co-crystallized ligand and water molecules were removed because they did not contribute significantly to ligand binding— as an effort to empty the binding pocket of any potential water molecules that might have distorted the pose search calculations. The addition of hydrogen atoms to the structure and optimization of missing side chain atoms while aiming to assign bond orders was facilitated using Protein Preparation Wizard tool available in Maestro version 12.5. Moreover, in the Protein Preparation Wizard tool of Maestro 12.5, PROPKA program was used to assign protonation states for residues which is further used to predict protein pKa at pH = 7.0. After pre-processing the protein structure, optimization and minimization of the structure were done by using OPLS3 force field (optimised Kanhesia for Liquid Simulations) with default constraints through protein preparation wizard in Maestro 12.5. Afterwards, we conducted virtual screening by extracting the 3D conformations of 35,002 natural compound structures from the Natural Product Activity and Species Source Database (NPASS) in .sdf file format. The known chitinase inhibitor idarubicin was taken as the reference molecule (ligand) for docking and simulation analysis. It was obtained from the pubchem database (https://pubchem.ncbi.nlm.nih.gov/) also in ".sdf" file format [10]. Then we used Schrödinger suite Maestro with LigPrep module to prepare ligands from both NPASS compounds and idarubicin. LigPrep created a large number of ligand molecules by generating various combinations with enantiomers and tautomers of all compounds in NPASS library along with idarubicin. Moreover, Epik module was employed to adjust ligand protonation states at pH 7.4 while refining them. In ligand preparation with LigPrep module the applied force field was constrained to OPLS3 force field. Optimisation of ligands were done through the LigPrep module by determining ring confirmation, partial atomic charges correction, ionization state at pH 7.0 along with the promoters, tautomers and stereoisomers (32 per ligand) which was then followed by subsequent maestro format export for suitable docking calculations.

Receptor grid generation and Structure based virtual screening

The ligand, which was co-crystallized and situated at the active site of the target protein chitinase (PDB ID: 2DBT), was chosen as a point of interest for grid generation. At the central coordinates of the active site, a grid box sized X: 76.85, Y: 18.98, Z: 11.68 was established for subsequent docking of ligands into chitinase (PDB ID: 2DBT)'s catalytic pocket. The Schrödinger suite Maestro glide module was used in conjunction with the Virtual Screening Workflow (VSW) method which consisted of three docking protocols: High Throughput Virtual Screening (HTVS), Standard Precision (SP), and Extra Precision (XP). In transferring around 10% of total NPASS compounds from HTVS to SP, the aim was to identify false

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positives since even though HTVS provides valuable scoring states it is important to validate these through decent scoring states. After the SP docking approach, another set comprising around 10% of resultant SP docked NPASS compounds were subjected to XP docking protocol— as per the XP protocol these are expected to be best scoring states.

Molecular dynamics (MD) simulation

Molecular dynamics simulations examined the structural stability of receptor-ligand complexes formed by molecular docking. The molecular dynamics simulations of the docked posture chitinase and NPASS compound, also chitinase and reference target were performed using the Desmond package [11, 12]. The docked posture file was utilized as the initial structure for 100 nanoseconds simulations in maestro format wit. Initially, the system builder was employed to enclose the complete receptor-ligand complex within an orthorhombic simulation box. Periodic box conditions were configured to dictate the form and magnitude of the repeating unit, which was buffered at 10Å intervals. The system was then solvated utilizing an SPC (single point charge) water model [13] coupled with an Optimized Potentials for Liquid Simulations (OPLS) all-atom force field 2005 [14]. In order to make the system electrically neutral, a sufficient amount of counter NA^+/Cl^- ions were introduced into the solvated system at random intervals to equalize the system charge. The system was first reduced using the steepest descent gradient approach, which may go up to 1000 iterations. After the equilibration, the unrestrained production phase was conducted using an NPT ensemble in which the number of atoms, pressure, and temperature remained constant for 100 nanoseconds at 300 K temperature, as monitored by a Nosé-Hoover thermostat (relaxation time = 1 ps), and 1.013 bar pressure, as monitored by an isotropic Martyna–Tobias–Klein barostat (relaxation time = 2 ps). We utilized the smooth particle mesh Ewald (PME) method with the RESPA integrator to calculate long-range electrostatic interactions. For short-range interactions, we employed a cutoff of 9.0 Å. The simulation trajectory in (.cms) format was analyzed using the simulation interaction diagram functionality. This analysis allowed for the creation of detailed reports on protein-ligand interactions at different time scales, system evaluative metrics as well as dynamic properties specific to the protein and ligand.

Binding free energy calculations using MM-GBSA

The MM-GBSA methodology was used to investigate the binding free energies of chitinase protein (receptor) and ligands such as the NPC313813 complex and chitinase and idarubicin. Calculation involved the use of OPLS 2005 force field, VSGB solvent model, and rotamer search methods to determine the binding free energy via prime module's Python script thermal mmgbsa.py. Equations were employed for the determination of binding free energy upon ligand-receptor binding with NPC313813 and idarubicin

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand}) \quad (1)$$

where ΔG_{bind} = binding free energy,

$G_{complex}$ = free energy of the complex,

$G_{protein}$ = free energy of the target protein, and

G_{ligand} = free energy of the ligand.

RESULT AND DISCUSSION

Structure based virtual screening

Virtual screening identified a chemical from the NPASS library that has the ability to inhibit the chitinase protein. The dataset of 35,002 compounds derived from NPASS. Then, all the compounds of NPASS were filtered by using the high throughput virtual screening module. By employing the lower energy score, the top-ranking compounds in the dataset were determined. As the binding energy decreases, the binding efficiency increases, leading to stronger inhibition. The glide scores of 5 compounds that exhibit high binding affinity are mentioned in the table 1 below along with the chemical compound named Idarubicin as control.

Table 1: The variants filtered after the high through output virtual screening with their docking score.

Variants	Docking score
	(kcal/mol)
NPC313813	-14.551
NPC89105	-13.971
NPC477081	-13.865
NPC116229	-13.849
NPC477613	-13.676
Idarubicin (Reference compound)	-7.566

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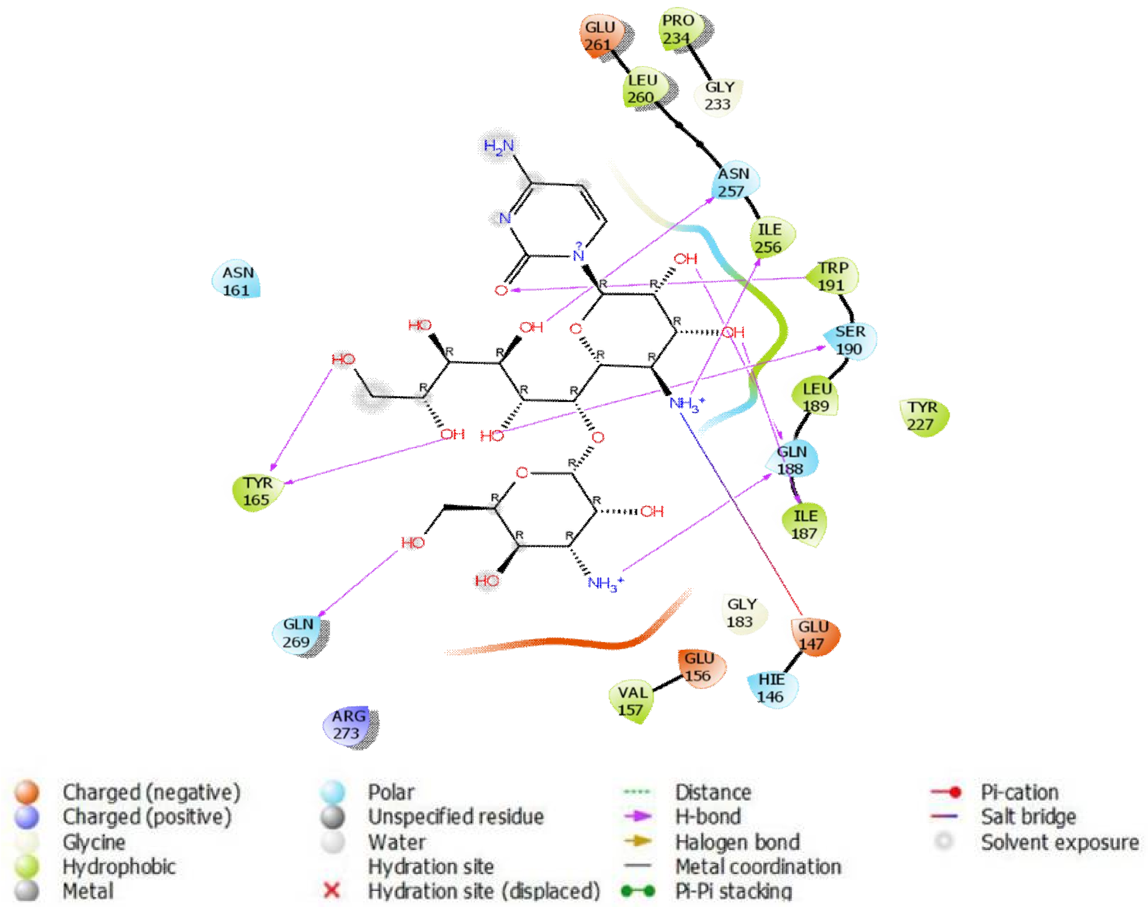

Figure 1: 2D image of chitinase docked with NPC 313813

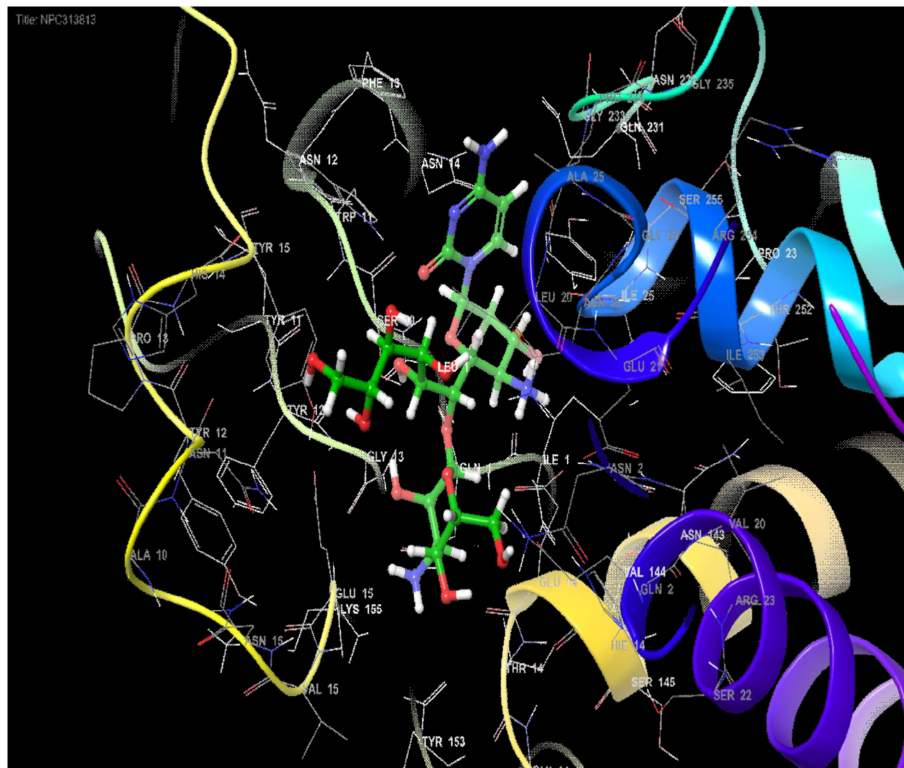

Figure 2: 3D image of chitinase docked with NPC 313813

The hydrophobic regions surrounding the active site of chitinase like Val 157, Try 165, Ile 187, Leu 189, Trp 191, Tyr 227, Pro 234, Ile 256, Leu 260 whereas, Hie 146, Asn 161, Gln 188, Ser 190, Asn 257, Gln 269 are polar amino acids. There is positively charged amino acid Arg 273 and negatively charged amino acids including Glu 147, Glu 156, Glu 261.

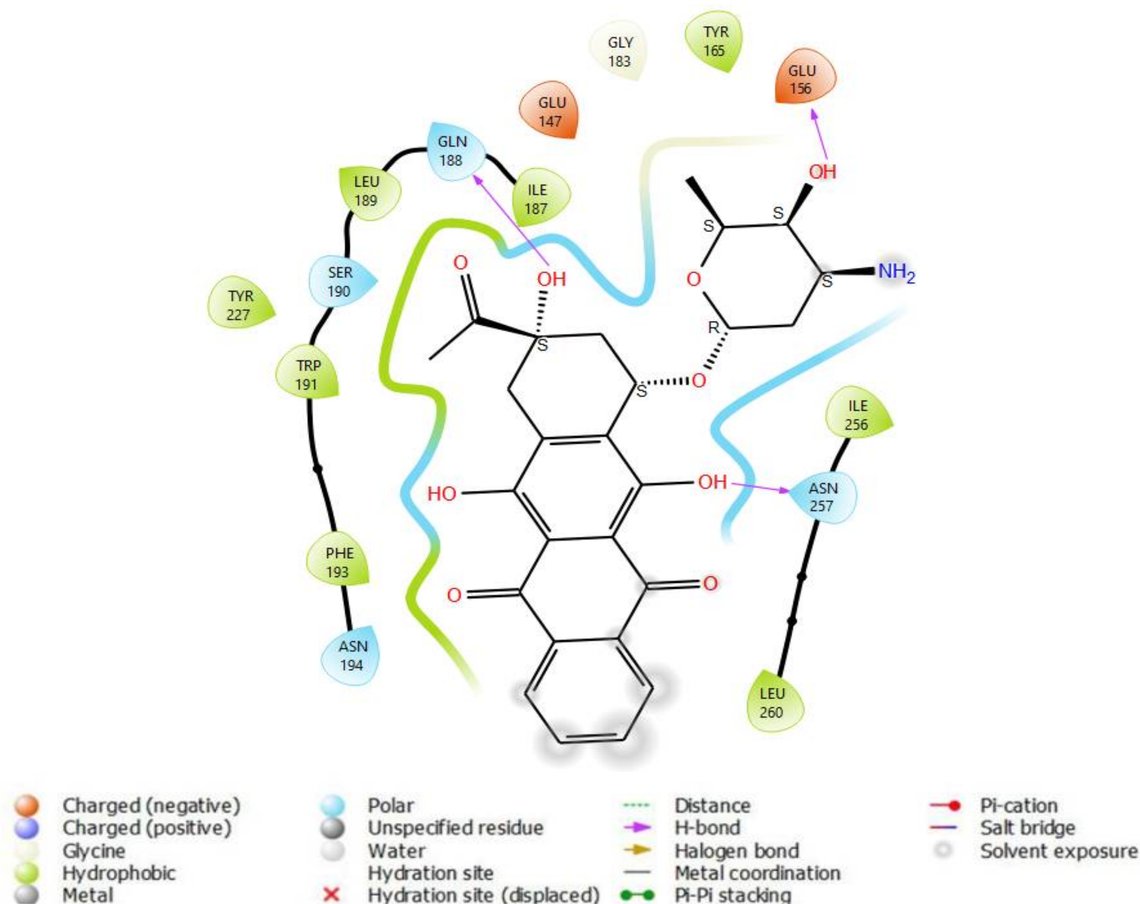

Figure 3: 2D image of chitinase docked with Idarubicin

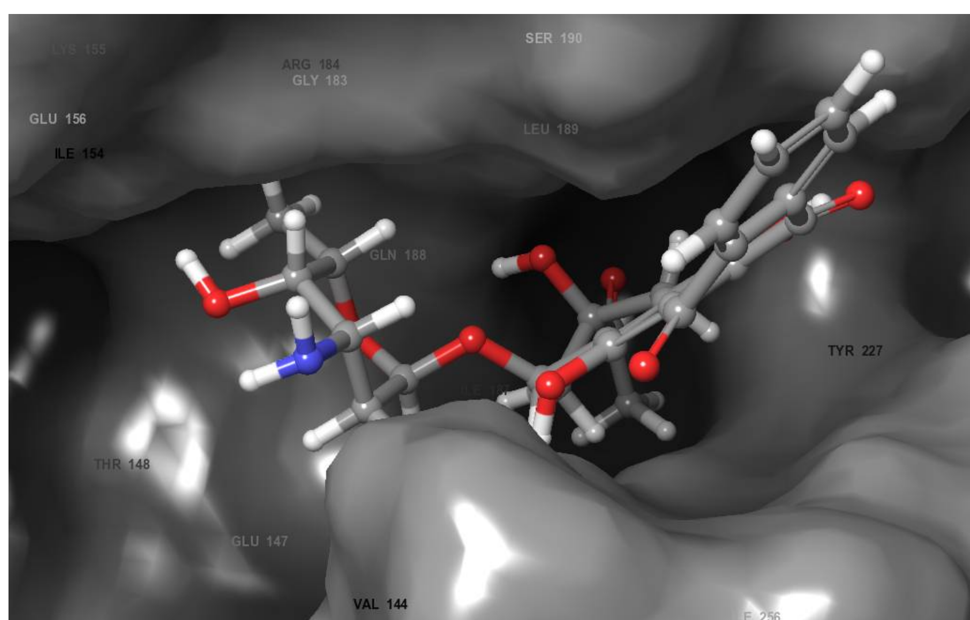

Figure 4: 3D image of chitinase docked with Idarubicin

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Hydrophobic regions like Ile 187, Leu 189, Trp 191, Phe 193,Tyr 227, Ile 256, Leu 260 are found to surround the active site of chitinase, whereas Ser 190, Asn 194, Gln 188, Asn 257 are polar amino acids. Negatively charged amino acids are Glu 147 and Glu 156. Glycine (Gly 183) is also found.

Molecular Dynamic Simulation

Simulation results clearly show the stability of NPC313813 and chitinase near 5 ns at 1.2 Å, with minor fluctuations. Ligand marginally drifted away from the protein two times first between 10 to 30 ns and approximately from 35 to 60 ns but later attached with the protein and stayed bonded till the end of the simulation. The RMSD value of NPC313813 and chitinase as found to be within an acceptable range (1–3 Å).

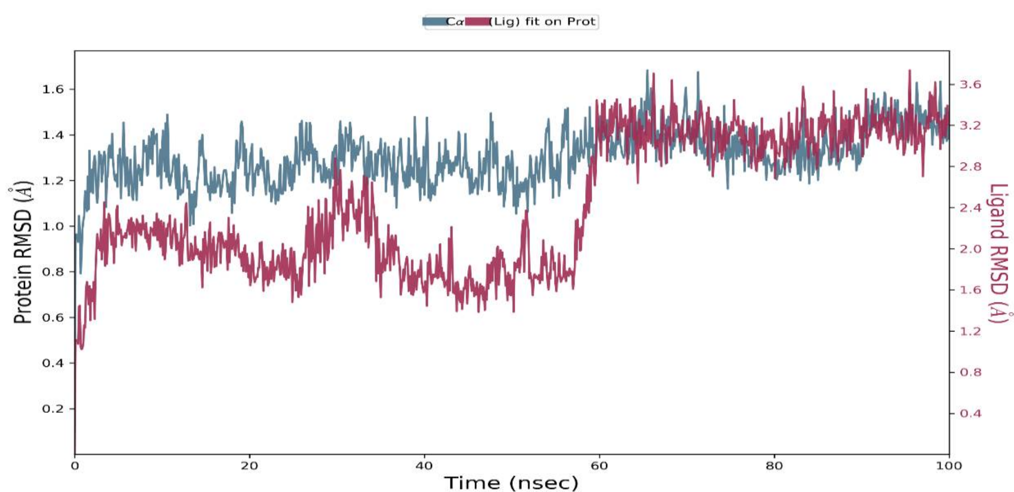

Figure 5: RMSD of NPC313813

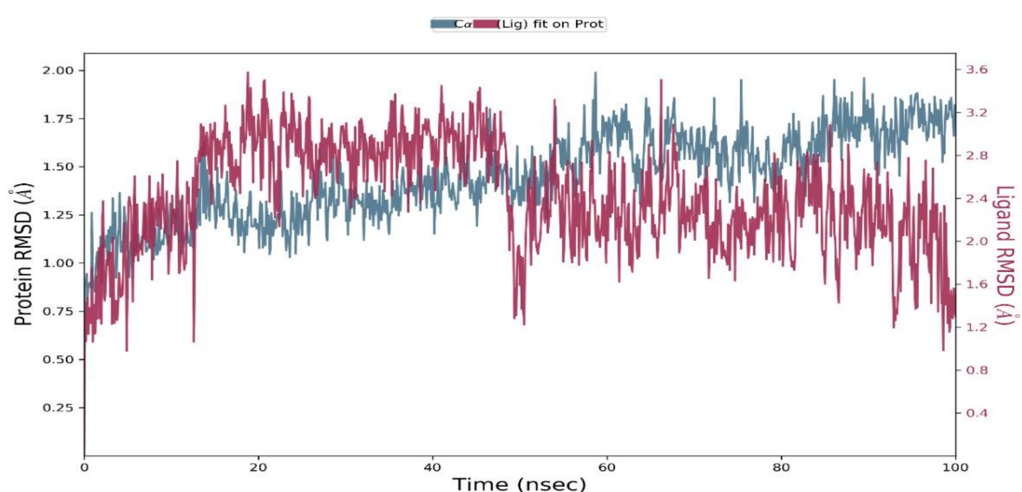

Figure 6: RMSD of Idarubicin

Simulation results indicate the stability of idarubicin and chitinase attached together nearly upto 18 ns, then, ligand marginally drifted away from the protein between 18 to 45 ns and remained stable binding from 50 to 80 ns however, slightly fluctuated away from 85 to 100 ns till the end of the simulation. The RMSD value of Idarubicin and chitinase as found to be within an acceptable range (1–3 Å).

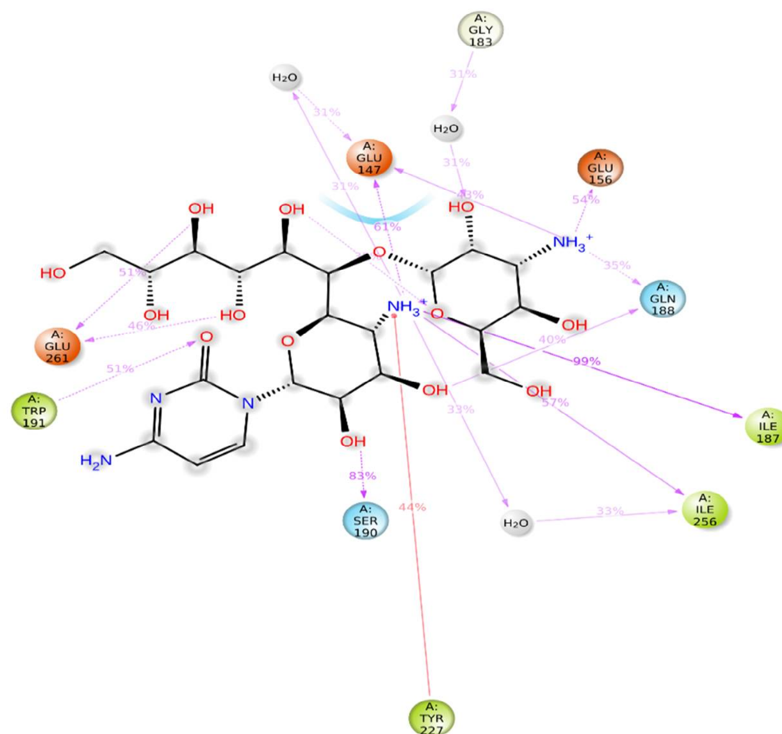

Figure 7: Plot of ligand interaction diagram displaying total time (in %) a particular amino acid of the protein over the course of simulation (ligand is NPC313813 and protein is chitinase)

Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown. The Hydrogen bond interaction of Gln 188, Ser 190, Tyr 165, Asn 257, Trp191, Ile 256, Gln 269 as retained with less 10% of the complete simulation time.

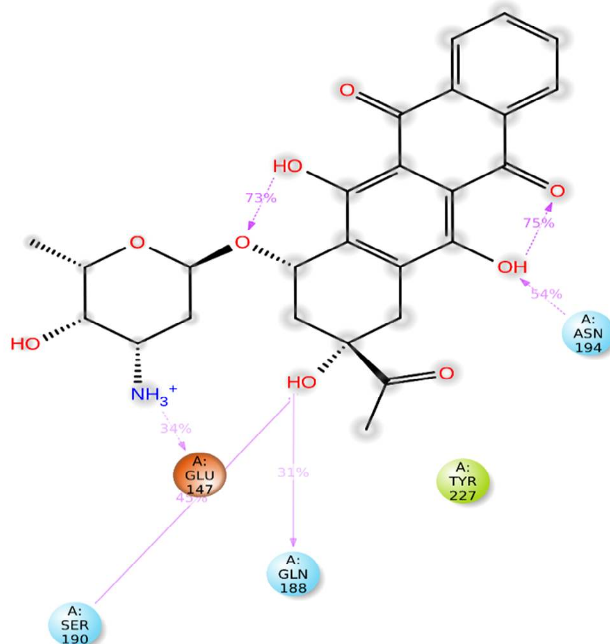

Figure 8: Plot of ligand interaction diagram displaying total time (in %) a particular amino acid of the protein over the course of simulation (ligand is Idarubicin and protein is chitinase)

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Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown. The Hydrogen bond interaction of Gln 188, Ser 190, Tyr 165, Asn 257, Trp191, Ile 256, Gln 269 as retained with less 10% of the complete simulation time.

Table 2: MMGBSA energy calculations

MM/GBSA energy terms	NPC313813 (kcal/mol)	Idarubicin (Control) (kcal/mol)
ΔG_Bind[a]	-65.9023	-64.8494
ΔG_Bind_Coulomb[b]	-126.135	-50.7813
ΔG_Bind_Covalent[c]	4.0929	2.5697
ΔG_Bind_Hbond[d]	-7.7645	-2.5489
ΔG_Bind_Lipo[e]	-10.7687	-18.0600
ΔG_Bind_Solv GB[f]	117.2789	53.1954
ΔG_Bind_vdW[g]	-42.2504	-47.2104

[a]Free energy of binding
[b]Energy term of Coulomb
[c]Covalent binding energy
[d]Hydrogen-bonding correction
[e]Lipophilic energy
[f]Generalized Born electrostatic solvation energy
[g]Van der Waals energy

The MM-GBSA method is frequently employed to evaluate the binding free energy between ligands and chitinase protein molecules [11, 15]. The calculation was performed on the binding free energy of the chitinase with NPC313813 (based on docking score) is -65.9023 (kcal/mol) and reference compound idarubicin is -64.8494 (kcal/mol). The non-bonded interactions for example ΔG_Bind_Coulomb, ΔG_Bind_Covalent, ΔG_Bind_Hbond, ΔG_Bind_Lipo, ΔG_Bind_Solv GB control the binding free energy (ΔG_Bind). It can be considered that in protein-ligand interaction, non-bonded interactions can significantly influence the average binding energy however, ΔG_Bind_Covalent and ΔG_Bind_Solv GB have demonstrated adverse energy contributions and hence been antagonistic to binding. Therefore, the MMGBSA calculations provided strong validation for the molecular docking-derived binding energy estimates.

CONCLUSIONS

Chitinase is the one the key enzyme in chitin degradation. In this study, we have designed computational based protein ligand workflow to identify the potent chitinase inhibitors from the NPASS library. We first carried out the molecular docking, molecular dynamics simulation, MM-GBSA analysis. The structure based virtual screening of the 35,002 compounds of the NPASS was carried out. Then from the best docking score top five hits (NPC313813, NPC89105, NPC477081, NPC116229, NPC477613) were identified. For the stability study of the compounds molecular dynamics stimulation was also performed where NPC 313813 has better stability compare to idarubicin (a reference compound). Further, free binding energy calculated using Molecular mechanics with generalised Born and surface area solvation (MM/GBSA) where score of NPC 313813 was better than idarubicin, a reference compound.

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