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ADVANCED QUANTUM MECHANICAL SCREENING OF LEADS AGAINST PERIODONTITIS DRUG TARGET CYSTALYSIN FROM *TREPONEMA DENTICOLA*

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ABSTRACT : Hemolytic protein, Cystalysin from *Treponema denticola*, is a known drug target for combating Periodontitis. Cystalysin catalyzes the release of hydrogen sulphide and ammonia which is responsible for tissue hemolysis and conversion of hemoglobin to sulfhemoglobin and methemoglobin in periodontal pockets. In the present study we have performed an exhaustive screening of Natural Product Library to identify potential Leads (drug like molecules) that may inhibit the hemolytic action of this enzyme. Natural product library was screened to identify top 50 potential binders by LibDock tool followed by RMSD filtration. These were then subjected to advanced Quantum Mechanical Molecular Mechanical (QM/MM) docking on pipeline pilot. Four most promising ligands are reported along with their binding stability scores and interaction patterns. Two of the four inhibitors NP-015209 and NP-020109 appear to be the most promising leads since they specifically interact with the functionally active amino acids at the active site thereby blocking the catalytic action of the enzyme (Cystalysin). These inhibitors appear to be most promising leads for drug development against periodontitis.

Key words : Periodontitis, drug target, cystalysin, *Treponema denticola.*

INTRODUCTION

Despite advancements in oral disease science, periodontitis continues to be a worldwide health concern, affecting humans of all ages, especially children where oral infection is on the rise. Periodontitis is very common and up to 80% of the world population may suffer from some form of this disease during their lifetime (Pihlstrom *et al*, 2005; Selwitz *et al,* 2007). It is a chronic inflammatory disease affecting the supporting tissues of the teeth, leading ultimately to tooth loss. Periodontitis is initiated by accumulation of the dental plaque biofilm and also because of abnormal host response to bacterial challenge. Chronic periodontitis involves destruction of both soft and hard tissues leading to progressive loss of attachment and mobility of tooth, ultimately resulting in loss of tooth in severe case. It is now increasingly becoming clear dental caries and periodontitis are orchestrated by not a single bacterium but by a consortium of bacterial pathogen (Broadbent *et al*, 2013). Through a number of recent studies it has been recognized that periodontitis may influence the course and pathogenic mechanisms of a variety of systemic diseases and health condition such as obesity, diabetes, hypertension, cardiovascular disease and low birth weight (Hirschfeld and Kawai, 2015; Keller *et al*, 2015).

Periodontitis is often known as 'Gum Disease' and is a very common condition in which the gums and deeper periodontal structures become inflamed. This inflammation of the gums, which usually takes the form of redness, swelling and a tendency to bleed during tooth brushing, is the body's response to certain bacteria that have been allowed to accumulate on the teeth. Although part of the body's defence system, this inflammatory response can eventually cause serious damage. If left unchecked, the inflammation can spread down below the gums and along the roots of the teeth, causing destruction of the periodontal ligament and the supporting bone. This ultimately leads to the loosening and potential loss of the teeth.

Periodontitis usually develops with gingivitis, when abundant plaque and calculus beneath the gingival margin, has not been adequately treated. In periodontitis, the deep pockets can harbor anaerobic organisms that do more damage than those usually present in simple gingivitis. The organisms trigger chronic release of inflammatory mediators, including cytokines, prostaglandins and enzymes from neutrophils and monocytes. The resulting inflammation affects the periodontal ligament, gingiva,

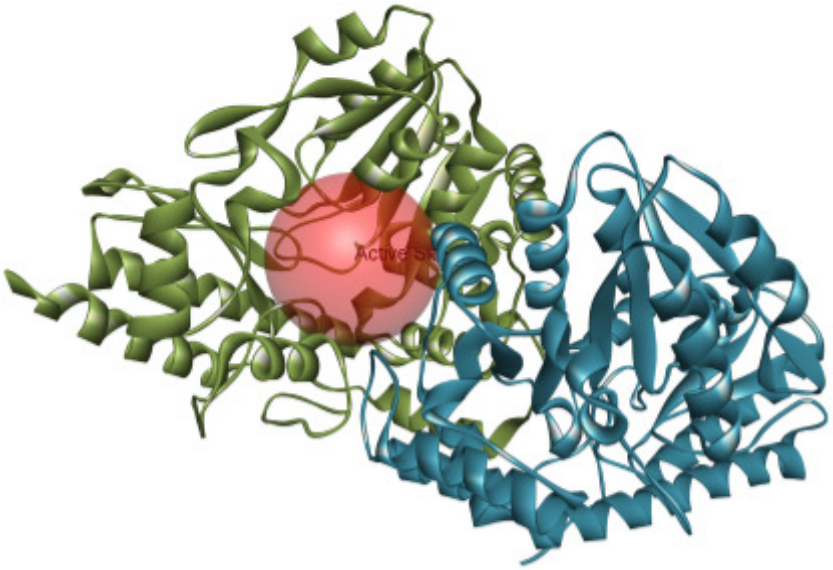

Fig. 1: Cystalysin Homodimer with active site.

cementum, and alveolar bone. The gingiva progressively loses its attachment to the teeth, bone loss begins, and periodontal pockets deepen. With progressive bone loss, teeth may loosen and gingiva recedes. Tooth migration is common in later stages and tooth loss can occur.

A large number of bacterial species, aerobe, facultative anaerobe, microareophilic and obligate anaerobes are associated with periodontitis. However, three anaerobic species, namely *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Treponema denticola* described as 'red complex' are strongly linked to periodontitis (Rocas *et al*, 2001; Holt and Ebersole, 2005). Cystalysin plays a vital role in the virulence of the anaerobic pathogen, *Treponema denticola* (Krupka *et al*, 2000; Spyrakis *et al*, 2014). Cystalysin (a hemolytic protein) dissolves erythrocytes and converts hemoglobin to sulfhemoglobin and methemoglobin (Chu *et al*, 1999). It is a $C^{\hat{a}}$-$S^{\bar{a}}$ pyridoxal 5'-phosphate based lyase which catalyses L-cysteine to release Pyruvate, ammonia and H_2S. The cytotoxicity (virulence) of Cystalysin is attributed to the chemical release of Pyruvate, ammonia and H_2S from S-containing amino acids (Krupka *et al*, 2000). Buildup of sulphides in the periodontal pockets causes tissue hemolysis and produces sulphur derivatives of hemoglobin (Spyrakis *et al*, 2014). Cystalin (a homodimer) was found to have minimum structural homology with other known hemolysins hence known drugs may prove ineffective in combating the infection caused by this pathogen (Krupka *et al*, 2000). We have performed an exhaustive search of natural product based leads against this prime virulence factor, Cystalysin (L-Cysteine Desulfhydrase, E.C. 4.4.1.1) from *Treponema denticola.*

MATERIALS AND METHODS

Target protein : Structure of Cystalysin

Crystal structure of Cystalysin is available as 1C7N in RCSB protein Data bank. This structure is shown as a homodimer with two chains (Fig. 1).

Target Protein preparation

The target protein structure was checked and corrected for any missing residues (or atoms). Conformations of the inserted side chains (if any) are optimized. Any missing loops (of size less than 12 residues) are corrected and optimized. Protein preparation was implemented using prepare protein protocol of Discovery Studio.

Active site

The active site of the holoenzyme is located near the pyridoxal phosphate pyridine nitrogen, Prime active residues include Tyr 64, Tyr123, Lys 238. Cystalysin with the active site defined in the study is shown in Fig. 1.

Ligand Library

MEGx (latest Analyticon release) plant and microbial natural product library was screened (as ligand library) to determine potential Cystalysin blockers. Present database is a collection of more than 4500 natural products. MEGx is a collection of purified Natural Products. Isolated from plants (MEGxp) and microorganisms (MEGxm). Ready to screen for fastest hit-finding in Natural Product Drug Discovery. It is time and cost-efficient way to perform Natural Product drug discovery. It is a highly diverse database of selected plants and microorganisms.

Ligand preparation

Ligand Library need to be prepared and filtered before screening. During preparations tautomers were generated, any duplicate structures were removed, standard formal charges were associated with functional groups, molecules were kekulized (if needed). Ligand preparation was implemented using Ligand prepare protocol of Discovery Studio.

Lipinski and Veber rules based filtration

Natural products in the MeGx Library were filtered on the criteria of the Lipinski rule of five which prioritize the molecules according to their bioavailability (Lipinski *et al*, 1997). Library was further screened on the basis veber rules of having atleast 10 rotatable bonds having polar surface area of no more than 1400A2 and nom more than 12 hydrogen bond donors or acceptors (Veber *et al*, 2000). These filters are implemented using Lipinski and Veber rule filter protocol of Discovery Studio.

Virtual screening of Natural product Library

Virtual Screening was done by LibDock on Discovery Studio platform via Pipeline Pilot (Diller *et al*, 2001). Docking and scoring software is used widely to enhance the drug design and development processes in the pharmaceutical industry (Coupez *et al*, 2006). Major use of these programs is in virtual screening exercises to increase the number of hits versus the number of compounds tested in high throughput biological assays. A number of different metrics are used to measure the success of these tools (Rao *et al*, 2007). Most commonly used measures are the compatibility of docked poses with the corresponding poses in the X-ray structures, retrieval of active compounds over inactive ones, and the correlation between experimental binding constants and computed scores. A number of validation studies of the various docking and scoring functions have been reported in the literature (Ferrara *et al*, 2004). LibDock is based on the algorithm developed by Diller and Merz and is one of the few commercially available docking programs that uses protein binding site features to guide docking. The LibDock methodology was originally developed to handle the rapid docking of combinatorial libraries of compounds with the goal of prioritizing the selection of libraries rather than rank ordering the compounds themselves. The algorithm has four functional aspects: conformation generation of the ligands, creating a binding site image (hot spot identification), matching the binding site image and the ligand, and a final optimization stage and scoring. The binding site image consists of lists of polar and apolar hot spots. These are generated by laying a grid in the binding site volume and then scoring an apolar and polar probe at each grid point. The apolar and polar hot spots are clustered to generate their respective hot spot maps, which are then used to match the ligand apolar and polar features to the binding site. The optimization step includes pruning the list of matches for steric clashes, ranking using an atom pairwise score, and clustering. In the final stage of refinement hydrogen bonding and steric potentials are used with the BFGS optimization algorithm (Diller *et al*, 2003).

Quantum Mechanical Molecular Mechanical (QM/MM)

Exhaustive second stage docking study was performed using Quantum Mechanical Molecular Mechanical (QM/MM) docking on DS using Pipeline Pilot (Fig. 3). Quantum mechanics (QM) precisely reproduces experimental conditions. There are several advantages of QM methods over empirical molecular mechanics (MM) methods, *i.e.* it is better suited for the treatment of metal ions in protein systems and efficiently accounts for charge delocalization/polarization. QM/MM have following direct advantages above traditional molecular docking (1) facilitating the atomic elucidation of weakly resolved electron density (2) effect of molecule conformation and probing the nature of the interactions within the protein active site and (3) studying the impact of subtle substituent changes on the binding conformation or in the assessment of alternate scaffolds. The implemented pipeline is shown in Fig. 3 (Gleeson and Gleeson, 2009; Halgren and Damm, 2001; Raha *et al*, 2007).

RESULTS AND DISCUSSION

Cystalysin is known to exist as a homodimer (Fig. 1). The active site was defined from the PDB active site records (Fig. 1). MeGx Ligand Library was prepared. After preparation, it contained 3101 unique molecules. After Lipinski and Veber rule-based filtration the library contains 2679 unique molecules (Appendix I). This Lipinski filtered library was subjected to undergo virtual screening by LibDock (on Biovia Pipeline Pilot) against Cystalysin (1C7O). The LibDock runtime workflow followed by RMSD (root-mean-square deviation) pose filtration and CHARMm (Chemistry at HARvard Macromolecular Mechanics, Brooks *et al*, 2009) *in situ* Minimization is shown in Fig. 2.

LibDock was able to generate 13930 successfully docked poses from 2672 (7 conformers were rejected) unique Lipinski & Veber filtered Natural molecules. The RMSD pose filter calculates the RMSD to the top pose (highest Sort Property) and removes any lower scoring poses having less than the specified RMSD Cut-off. This

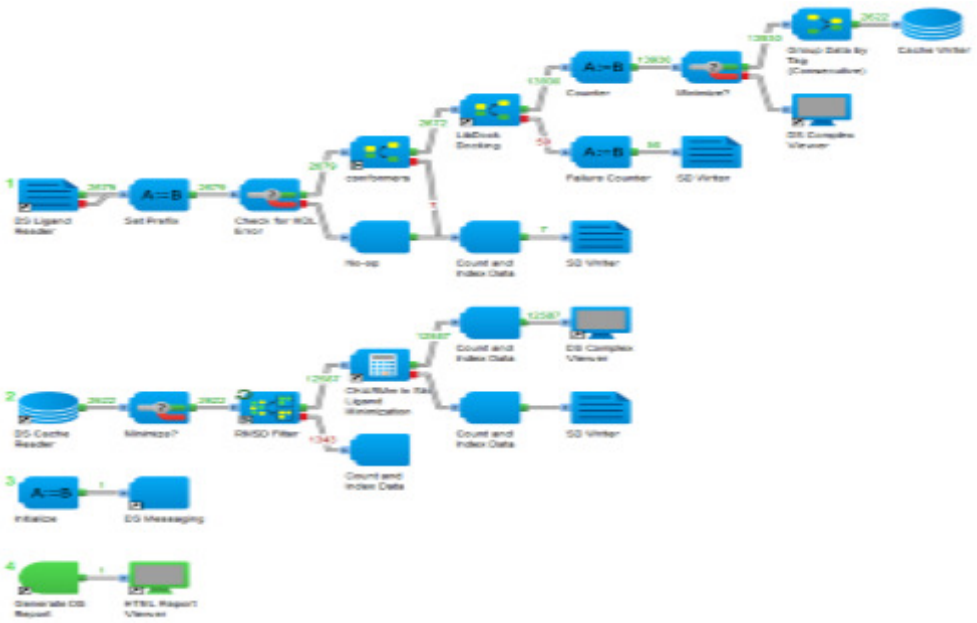

Fig. 2 : LibDock and RMSD workflow.

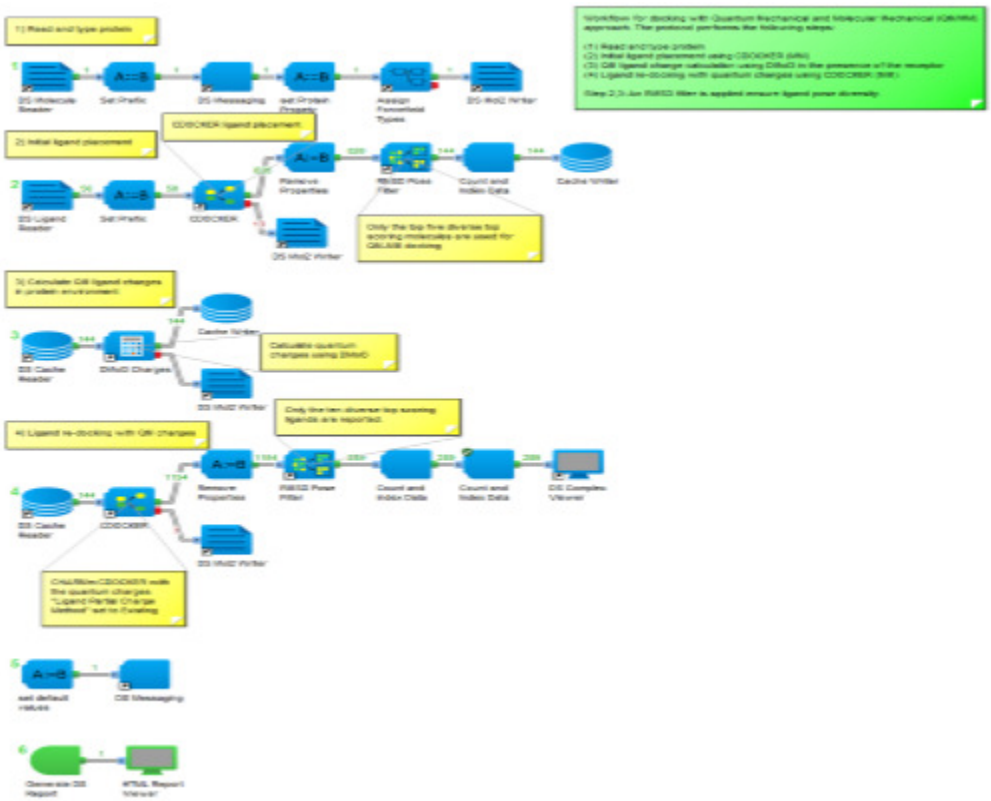

Fig. 3 : QM/MM workflow.

procedure is repeated for the next best scoring pose and so on. This reduces the number of similar poses while keeping the highest scoring poses. In our case 1343 poses were filtered. While retaining 12587 poses. Top 50 unique LibDock scorers (Appendix II) were further subjected to exhaustive QM/MM docking, the implemented pipeline is shown in Fig. 3.

This involves initial ligand placement using CDOCKER: it is a program for calculating the mechanical docking modes of small molecules in protein binding sites using a genetic algorithm methodology with the advantage that it allows for both unconstrained ligand flexibility and partial flexibility of the binding pocket (Wu *et al*, 2003). Docking annealing parameters for van der Walls and hydrogen bonding were set to 5.0 and 2.5 respectively. Following parameters were used for genetic

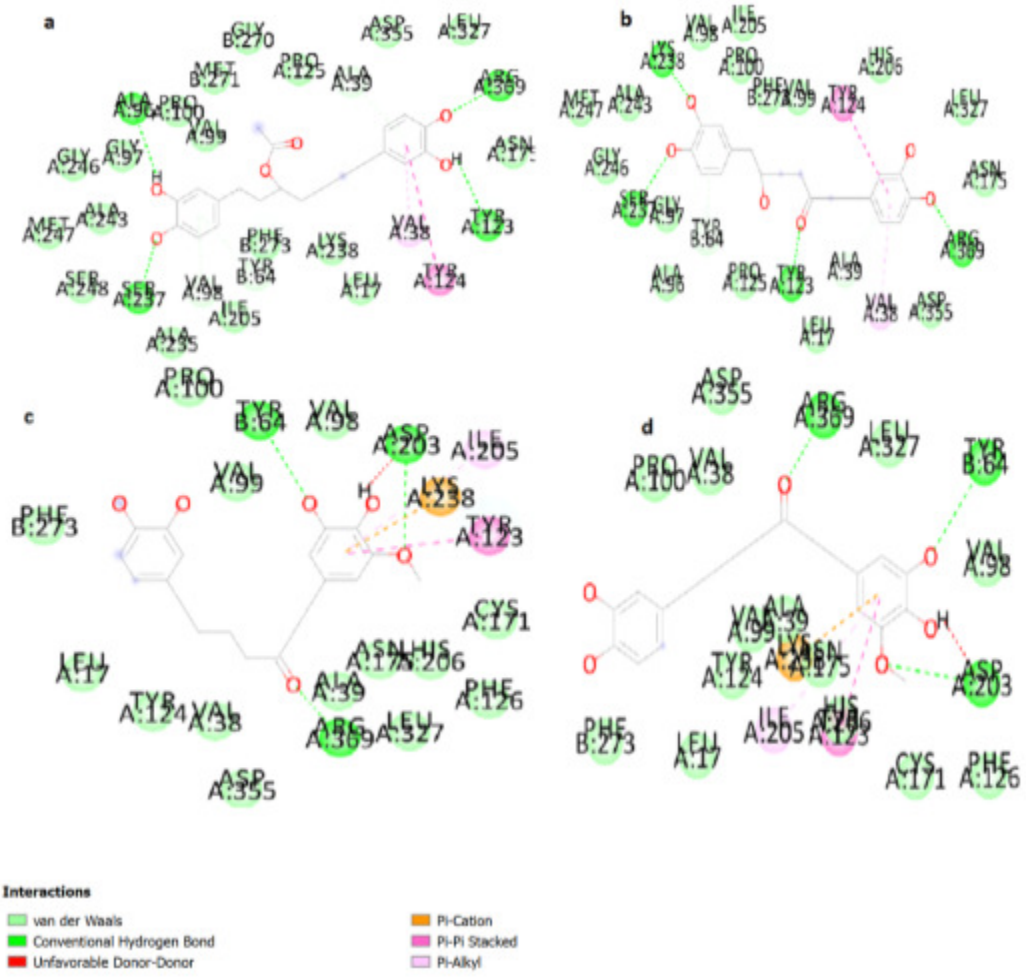

Fig. 4 : 2D images of interaction of top 4 Ligands with Cystalysin. a) NP-003624; b) NP-015209; c) NP-020109; d) NP-003872.

Table 1 : Top five ligands with binding scores.

Ligand	CDOCKER_ ENERGY	LibDock Score	Hydrogen Bonds
NP-003624	-66.7065	153.011	4
NP-015209	-61.343	144.095	4
NP-020109	-59.3338	152.643	3
NP-003872	-54.0796	142.468	3

algorithm population size 100, selection pressure 1.2, number of operations 1,00,000, number of islands 5, niche size 2, migrate 10, mutate 100 and cross -over 100. Thirteen Ligands were rejected in the initial molecular docking. DMol3 calculates the ligand quantum charges followed by CHARMm based re docking with QM charges. Finally, top four redocking scorers (Ligands) are reported as top binders. Table 1 reports the type of interactions and CDOCKER and LibDock scores.

CDOCKER score is reported as the negative value (*i.e.*, -CDOCKER_ENERGY), where a higher value indicates a more favorable binding. This enables the energy to be used like a score. This score includes internal ligand strain energy and receptor-ligand interaction energy (Wu *et al*, 2003) and is used to sort the poses of each input ligand. Interaction of the top four ligands with cystalysin is shown in Figs. 4a-d and 5a-d.

It can be observed that all the top four binders interact with the active site amino acids (Tyr 64, Tyr123, Lys 238). NP-003624 establishes four hydrogen bonds with the active site and have the least CDOCKER_Energy however NP-015209 establishes specific hydrogen bonds with functionally critical amino acids (Tyr123, Lys 238). It also establishes hydrogen bonds with ser237 and Arg369. NP-020109 undergoes hydrogen bonding with functionally active Tyr64 (B chain) along with Arg369 and Asp203. NP-020109 also undergoes Pi-cation interaction and Pi-Pi stacked interaction with functionally critical Lys238 and Tyr123, respectively. NP-003872 have similar interaction pattern and hydrogen bonding as NP-020109 however it also includes non-favorable Donor-Donor interaction which reduces the stability of the complex. It is important to note that functionally active Tyr64 belongs to the B chain (Cystalysin exists as

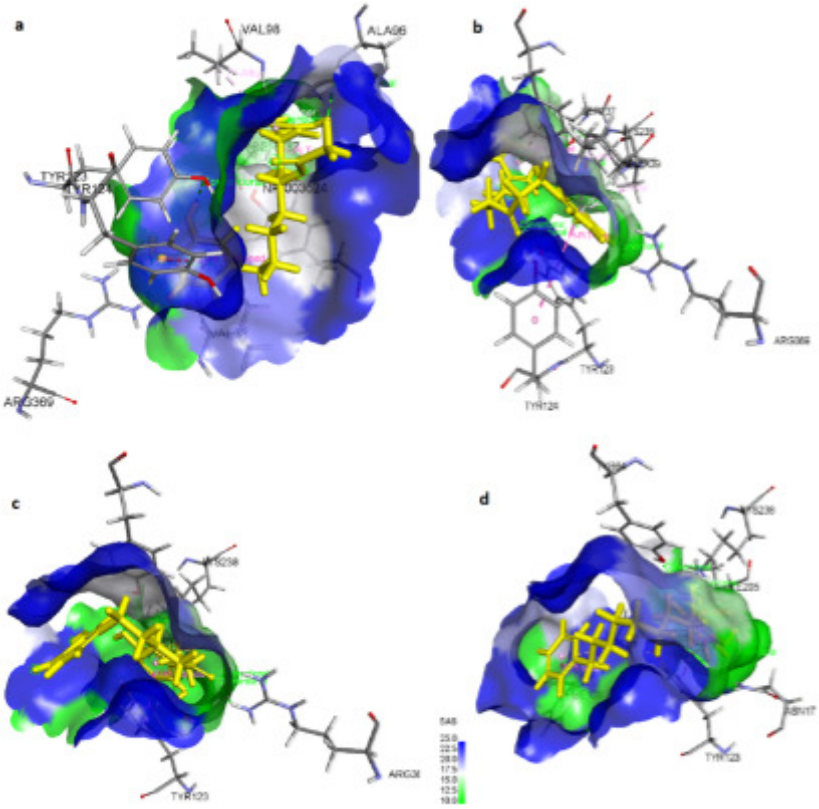

Fig. 5 : 3D images of Interaction of top 4 Ligands with Cystalysin with solvent accessibility. a) NP-003624; b) NP-015209; c) NP-020109; d) NP-003872.

homodimer) since this active site is located in the cleft near the intersection of the two monomers. Solvent accessibility of the active site is represented in Fig. 5. It can be observed the four top ligand fit in the high solvent accessible active site.

NP-015209, NP-020109 appear to be the most promising candidates for blocking the catalytic action of Cystalysin. Both NP-015209, NP-020109 have high LibDock Score, very low CDOCKER_Energy and also they specifically interact strongly with the amino acids (Tyr 64, Tyr123, Lys 238) playing the central role in catalytic mechanism of Cystalysin.

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