

Evaluation of the Inhibitory Potential of Beta Lactam Derivatives Against Bile Salt Hydrolase from *Enterococcus Faecalis* by In-Silico Approach

Neha Sharma M¹, Smiline Girija A.S² and J.Vijayashree Priyadharsini J⁴

¹Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

²Associate Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

³BRULAC-DRC, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, P.H.Road, Chennai, Tamilnadu - 600077, India

ABSTRACT

Bile Salt Hydrolase (BSH) is a vital protein in *E.faecalis* virulence and recent studies implicate that it can be an alternative target for the beta lactam derivatives to combat the menace of its complications in endodontic infections. The aim of the present study is thus undertaken to evaluate the inhibitory potential of four beta lactam derivatives against the BSH of *E.faecalis*. 3D structure of BSH was retrieved from the PDB data bank with further optimization of both the protein and ligands. In-silico inhibitory potential of the selected beta lactam derivatives against BSH was done by AutoDock 2.0 and was visualized with accelrys studio discoverer with the assessment of the molecular properties of the derivatives against BSH by molinspiration calculations for their drug likeness. Imipenem seems to be the potent inhibitory drug to target BSH with a promising binding energy of -5.54 with nine hydrogen bonds. Drug likeness parameters recorded imipenem as a promising protease and enzyme inhibitor followed by other beta lactam derivatives. The findings of the study emphasize that the beta lactam derivatives show promising interaction with bile salt hydrolase of *E.faecalis*. The findings of the study can thus have promising implications in combating the menace of drug resistance among *E.faecalis* associated with endodontic and other systemic infections with further in-vivo validation targeting the same.

KEY WORDS: BSH, E.FAECALIS, BETA-LACTAMS, AUTO-DOCK, IN-SILICO.

INTRODUCTION

Enterococcus faecalis, a gram positive diplococci, is an opportunistic pathogen commonly observed in oral

and other systemic diseases. Pertained to endodontic infections, it has been listed as one among the 25 pathogens (Anderson et al., 2016) that can lead into further complications of the root canal. However, the frequency of the dental and oral manifestations varies with individuals based on oral hygiene, age, gender, comorbidities, geographical distribution and other associated risk factors (Sun et al., 2012). They are capable of causing a variety of infections including endocarditis, sepsis, surgical wound infections, and urinary tract infections (Van Tyne et al., 2013). In recent years, the pathogen has emerged as drug resistant forms due to its drug resistant properties against the routine antimicrobials (Sedgley et al., 2005). Amidst many

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intrinsic and acquired resistance mechanisms, the vital role of bile salt hydrolase (BSH) in inducing tolerance to bile salts, has an altering effect on the action of beta lactam group of drugs.

This is due to the fact that BSH has a unique allosteric catalytic property that can de-conjugate the bile salts and is considered to be evolutionary related to penicillin V acylase (PVA), which is responsible for hydrolysing penicillin V (Chand et al., 2018). It is industrially used in the hydrolysis of penicillin V to produce 6-aminopenicillanic acid (6-APA), which is the precursor molecule for semi-synthetic β -lactam antibiotics (Chandra et al., 2005). In addition, the non-substrate ligand of PVA has a marked modulating and hydrolysing ability on BSH (Chand et al., 2016).

The β -lactam family is the most important class of antibiotics for clinical use as antimicrobial agents and constitute a major part of the global antibiotic market (Rodríguez et al., 2011). Clavulanic acid, thienamycin, cefoxitin, imipenem have shown susceptibility against *E. faecalis* characterized from various clinical specimens, with varying efficacies. Clavulanate, being considered as a potent inhibitor of β -lactamase, shows 80% susceptibility against *E. faecalis* isolated from urinary tract infections (Venkatesan et al., n.d.). Thienamycin, having a broad spectrum activity to several resistant pathogens, shows synergistic activity with other aminoglycosides against *E. faecalis* but lacks in-vivo or in-vitro activity when administered as a single drug (Eliopoulos and Moellering, 1981). Cefoxitin, being active against most bacteria that have become refractory, was not effective against *E. faecalis* (Moellering et al., 1974). Imipenem are broad-spectrum parenteral antibacterial agents that are generally reserved for use as agents of last resort in the treatment of serious nosocomial infections (Karlowsky et al., 2018). Even the extremely broad spectrum drug imipenem shows resistance among vancomycin resistant *E. faecalis* (Ono et al., 2005).

BSH being evolutionarily related to penicillin V hydrolase, it would be a best target to combat the clinical complication of *E. faecalis* infections and its emerging resistance against the penicillin derivatives in an alternate way. The present investigation is thus aimed to assess the potent inhibitory role of four beta lactam derivatives viz., clavulanic acid, thienamycin, cefoxitin and imipenem against BSH by in-silico docking analysis. Thus will aid in the selection of the best beta lactam derivatives targeting BSH of *Enterococcus faecalis* to treat endodontic and other systemic infections.

METHODS

Retrieval And optimisation of BSH: From the RCSB protein data bank (<http://www.rcsb.org/pdb>) the crystal structure of Bile salt hydrolase was retrieved for this. For the target optimization the hydrogen atoms were added and using kollman united atoms force field, the electronic charges were assigned to the protein using AutoDock

Tool – 1.5.6. RASMOL tool was applied to view the three dimensional structure of BSH.

Preparation and optimization of the ligand: Using ChemsSketch Software the structures of the beta lactam derivatives viz., clavulanic acid, thienamycin, cefoxitin, and imipenem were drawn and generation of their 3-D structures and 3D optimization was also done. The selected ligands were further saved in .mol file followed subsequent conversion using open babel molecular converter program and were saved in PDB format.

Molinspiration assessments: Molecular descriptors such as logP for partition coefficient, molecular weight of the compounds, the counts of hydrogen bond acceptors and donors relates to the membrane permeability and bio-availability of the compounds were assessed using molinspiration (Rizvi et al., 2013). Characterization of the absorption, distribution, metabolism and elimination (ADME) properties of the selected compounds was checked to fit into the Lipinsky's rule of five (Morris et al., 1998).

Auto-docking for drug-ligand interactions: The docking analysis to interpret the affinity between clavulanic acid, thienamycin, cefoxitin, and imipenem against BSH of *E. faecalis* was achieved by an auto-dock tool. Briefly, the target protein is embedded in a grid in pre-calculated grid maps (Autodock 4.2) (Ertl et al., 2000). AutoDock requires pre-formed grid maps, one for each atom type present in the ligand being docked. Calculation of the maps was done using the Autogrid auxiliary program by allowing the flexible molecules to move in the six spatial degrees of freedom for orientation and torsion. For the modelling of H-bonds and vanderWaals interactions, we implemented Lennard-Jones parameters 12-10 and 12-6 respectively. Evaluation of the force field was done in two steps, first by estimating the intra-molecular energetics between the transition from unbound states to the ligand-protein bound state. Secondly, evaluation of the intermolecular energetics was done by combining the ligand and protein in their bound conformation in the $\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{desolv}$ format. First three terms are for van der Waal's, hydrogen bonding, electrostatics respectively (Lipinski et al., 1997).

Visualisation of the docked molecules: The hydrogen bond interactions clavulanic acid, thienamycin, cefoxitin, and imipenem against BSH of *E. faecalis* were visualized using Discovery Studio Visualizer. The relative stabilities were evaluated using their molecular dynamics, binding affinities, energy simulations with further docking score assessments.

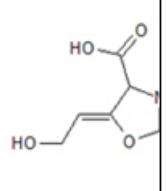

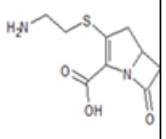
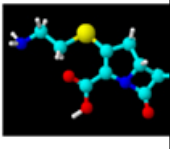
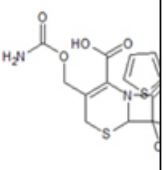

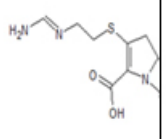
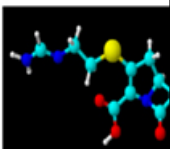
RESULTS AND DISCUSSION

Structure retrieval of the BSH protein from *E. faecalis*: The Crystal structure of a trimeric variant of the Bile Salt Hydrolase from *E. faecalis* (strain T2) is downloaded from PDB database and its structure is documented as 4WL3-A Chain. Optimization was successfully achieved

by the removal of the water molecules with merging of hydrogen atoms to the receptor molecule. RASMOL tool showed 3D structure of BSH with pink colour indicating the alpha-helix, yellow arrow indicating the beta sheets and white colour indicating the turns.

Beta lactam derivatives structure optimizations (the ligands): Optimization of the ligand was successful using ACD Chems sketch and the compatible format was retrieved using Open Babel molecular converter tool. The retrieved 2D and 3D structures of the ligands and its SMILES format are shown in table 1.

Drug likeness by molinspiration estimation: The bioactivity scores prediction of clavulanic acid, thienamycin, cefoxitin, and imipenem against BSH of *E. faecalis* based on the calculation of the GPCR ligand, ion channel modulation, kinase inhibitor, nuclear receptor ligand, protease inhibition and enzyme inhibition towards drug likeness is scored and tabulated in Table 2. Imipenem scores to be the best drug candidate followed by apigenin and cirsimaritin in targeting the bile salt hydrolase.

Table 1. 2D and 3D structures and SMILES format of the selected beta lactam derivatives under study			
Compounds	2D	3D	SMILES
Clavulanic acid			<chem>C1C2N(C1=O)C(C(=CCO)O2)C(=O)O</chem>
Thienamycin			<chem>CC(C1C2CC(=C(N2C1=O)C(=O)O)SCCN)O</chem>
Cefoxitin			<chem>COC1(C2N(C1=O)C(=C(CS2)COC(=O)N)C(=O)O)NC(=O)CC3=CC=CS3</chem>
Imipenem			<chem>CC(C1C2CC(=C(N2C1=O)C(=O)O)SCCN=CN)O</chem>

Docking analysis of the beta lactam derivatives against BSH of *E. faecalis*: Based on the lowest energy and minimal solvent accessibility, selection of the best conformers was made using LGA of the docked structure. Accelrys discovery studio visualization of the hydrogen bond interactions in the stick model between the Clavulanic acid, Thienamycin, Cefoxitin, and Imipenem against BSH of *E. faecalis* is given in Figure 1,2,3& 4. The number of hydrogen bonds formed, torsional energy and the docking scores between the drug and ligands are given in Table 3.

The present study involved an in-silico approach to target bile salt hydrolase of *E. faecalis*, to achieve a preliminary understanding of the drug interactions using computational tools prior experimental approaches. In-sights into such an approach reveals the potent

virulent based interactions which will further enhance the application of effective therapeutics in endodontic treatments (J et al., 2018). Structure based computational analysis in drug designing with the 3D knowledge in the prediction of the receptor bound to the ligands with a given or congeneric molecule optimization by molecular docking has spurred interest in recent decades in the field of dentistry. The conformational flexibility of virulent protein playing a vital role in endodontic infections is best achieved by docking analysis which is otherwise a perplexing problem and quite challenging.

In the present study, the beta lactam derivatives viz., clavulanic acid, thienamycin, cefoxitin and imipenem docked against the bile salt hydrolase of *E. faecalis* resulted in a promising receptor – ligand complex. Computational docking analysis predicts the exact

orientation of the protein-ligand docked structures based on the scores achieved by pose and strength (Wang et al., 2003). In addition, the molecular surface structures and interaction of BSH of *E.faecalis* with the beta lactam drugs were analysed based on several properties like interactive charges, and other chemical bonds like Vander Waal's forces which is further determined by

hydrogen bonding (Jiang and Kim, 1991). In this context, accelrys studio visualised and docked structures of BSH of *E.faecalis* yielded excellent docking scores with promising hydrogen bond interactions and the affinity distance scores for the beta lactam derivatives and BSH interactions.

Table 1. 2D and 3D structures and SMILES format of the selected beta lactam derivatives under study

Compounds	M. wt	Mol formula	H Bond Donor	H Bond Acceptor	Log P	TPSA	Rotatable bonds	Vol.	N atoms	Compounds
Clavulanic acid	199.162	C ₈ H ₉ NO ₅	2	6	-2.42	2	0	87.07	161.4	14
Thienamycin	272.319	C ₁₁ H ₁₆ N ₂ O ₄ S	4	6	-1.32	5	0	103.86	232.02	18
Cefoxitin	427.446	C ₁₆ H ₁₇ N ₃ O ₇ S ₂	4	10	0.02	8	0	148.27	337.31	28
Imipenem	299.345	C ₁₂ H ₁₇ N ₃ O ₄ S	4	7	-0.86	6	0	116.222	255.28	20

Drug likeness calculation of beta lactam derivatives against BSH of *E.faecalis*
Table 2. Molinspiration calculations of beta lactam derivatives

Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Clavulanic acid	-0.34	-0.02	-0.98	-0.43	0.70	0.80
Thienamycin	0.09	-0.19	-0.60	-0.38	1.00	0.89
Cefoxitin	-0.33	-0.49	-0.82	-0.64	0.70	0.01
Imipenem	0.29	-0.22	-0.58	-0.24	1.15	0.98

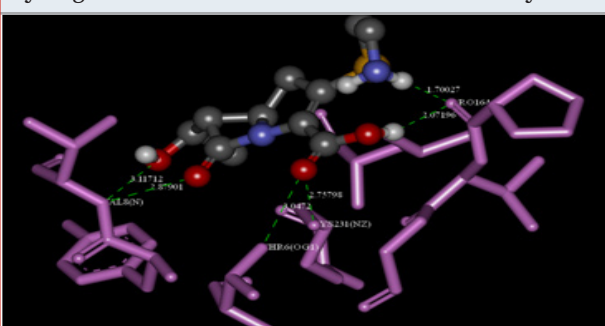
Table 3. Overall docking results of ligands with BSH of *E.faecalis*

BSH docking with compounds	Number of hydrogen bonds	Binding energy	Ligand efficiency	Intermolecular energy desolv Energy	vdW + H Bond +	Electrostatic energy	Torsional energy Unbound	Total internal
Clavulanic acid	6	-5.4	-0.39	-6.59	-6.59	0.01	1.19	-1.29
Thienamycin	6	-4.24	-0.24	-6.63	-5.54	-1.09	2.39	-3.73
Cefoxitin	5	-4.63	-0.17	-7.32	-7.22	-0.1	2.68	-3.22
Imipenem	9	-5.54	-0.28	-8.23	-7.36	-0.86	2.68	-1.51

Figure 1: Accelrys discovery studio visualisation of the hydrogen interactions between BSH and clavulanic acid



Figure 2: Accelrys discovery studio visualisation of the hydrogen interactions between BSH and thienamycin



The number of hydrogen bonds between the donors and the acceptors determines the best conformers and the binding energy values predicts the bioactivity value for a ligand to the corresponding receptor suggesting the inhibitory effect of the drugs to target *E. faecalis* based endodontic infections mediated by BSH. In this view, imipenem scores to be the best inhibitory drug of choice against BSH with nine hydrogen bonds followed by clavulanate and thienamycin with six bonds and finally by cefoxitin with five hydrogen bonds. The promising inhibitory activity of imipenem has already been documented in an earlier study (Beadle and Shoichet, 2002) and in the present investigation, we suggest BSH of *E. faecalis* can be best targeted by imipenem.

Figure 3: Accelrys discovery studio visualisation of the hydrogen interactions between BSH and cefoxitin

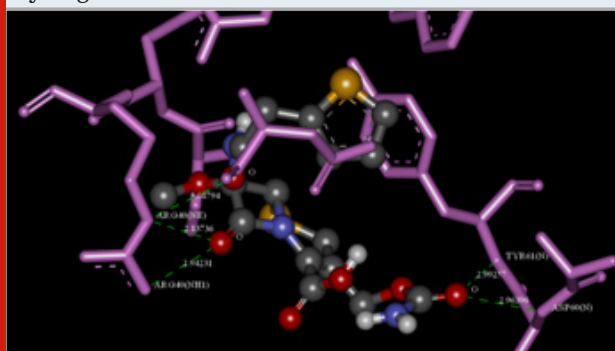
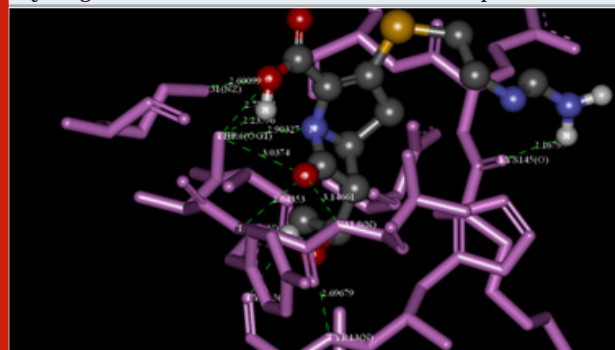


Figure 4: Accelrys discovery studio visualisation of the hydrogen interactions between BSH and imipenem



We used auto dock 4.2 to evaluate the interactions of *E. faecalis* BSH, which is considered as a suite of automated docking tools with a software for modelling flexible small molecule binding to 3D structures of receptor proteins. Using genetic algorithms for the conformational search a rapid grid based method of energy evaluation of *E. faecalis* BSH with the drugs was achieved in the study in analysing the docking simulations. In this study, the Lamarckian Genetic Algorithm (LGA) was used to explore the binding conformational landscape of the beta lactam derivatives chosen for inhibiting the BSH. The docking scores on bile salt hydrolase indicated that there is a direct relationship between the energy of the binding affinity, referring to the lowest docking scores and the stability. In accordance with this, the binding scores between the beta lactam derivatives and BSH in

the present study showed potent binding affinity to the binding sites with -5.4, -4.24, -4.63 and -5.54 Kcal/mol for clavulanic acid, thienamycin, cefoxitin and imipenem respectively with more potent and stable binding towards imipenem with the active sites of BSH. In addition, the intermolecular energy, van der waals energy and torsional energy were also at a higher end for imipenem when compared to the other three beta lactam derivatives and the beta lactam inhibitor clavulanate in interacting with the BSH of *E. faecalis*.

In the present study, molinspirational results were very promising with the n-violations of the beta lactam derivatives against BSH as zero satisfying the Lipinsky's rule of five. The application of molinspiration calculations of beta lactam derivatives with bile salt hydrolase suggests its importance in the practical approach to calculate the molecular properties and predicted the bioactivity scores to assess the best inhibitory agent for BSH. In molinspiration analysis, topological polar surface area (TPSA) of a molecule is considered as a useful descriptor to characterize the drug absorption and bioavailability and the values of TPSA and OH-NH interactions indicate that the selected ligands viz, the beta lactam derivatives to possess a smooth and efficient binding to the target proteins. It is a known fact that the drug molecules with TPSA values of >140Å or higher possess a low-absorption (miLogP) substantiating its role for its oral bioavailability for the drugs (Davies et al., 2007), (Priyadharsini et al., 2018). In this context, except cefoxitin all the other ligands score high absorption with clavulanate with high membrane penetration with a TPSA score of 87.07.

In the present study, we also predicted the bioactivity scores of clavulanic acid, thienamycin, cefoxitin, and imipenem towards drug likeness by calculating the activity scores of various inhibitor, ligand and modulator effects. All the derivatives scored above 0.20 as potent protease inhibitor and enzyme inhibitor except cefoxitin (Table 3b) against BSH of *E. faecalis*.

CONCLUSION

This work represents an advance in biomedical sciences in dentistry as there is a high possibility of orienting and scoring of the binding site of any target protein by molecular docking, which is a promising computer based drug design tool. The present investigation thus targets the BSH of *E. faecalis*, which is a potent virulent and pathogenic mechanism portraying challenge in the treatment strategies in *E. faecalis* associated endodontic diseases. In addition, BSH being an evolutionary analogue to penicillin V hydrolase, assessment of the best inhibitory beta lactam derivatives through molecular docking is promising to determine the potent drug of choice to combat resistant forms of *E. faecalis* in an alternate way by inhibiting the bile salt hydrolase. The present study is concluded by stating imipenem as the best inhibitory agent in targeting the BSH of *E. faecalis* towards arresting the bile resistance however requiring further in-vitro experimental validation.

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Conflict of Interest: None to declare

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