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Penicillin Analogues as Inhibitors of Penicillin Binding Protein Screening Study Using Molecular Docking

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Abstract:
Antibiotics are medicines used to prevent and treat bacterial infections. They work by either killing bacteria or stopping their growth, helping the body's immune system fight infections more effectively. This discovery revolutionized medicine and has saved millions of lives by treating deadly diseases. Penicillin is a β-lactam antibiotic that kills bacteria by inhibiting cell wall synthesis. Penicillin binds to enzymes called penicillin-binding proteins. Here different Penicillin analogues are tested for their binding affinity to Penicillin binding protein. The role of organic chemistry was found to be crucial in determining the best possible lead compound. The effect of substituents was analysed and was related to binding energy. The aromatic ring as a substituent was found to form more stable complex when compared to aliphatic chain substituent. Longer chains are less effective than short chain of substituents. The study provides insight at molecular perspective.

Keywords: PBPs, β-lactam ring, Binding energy etc.

Introduction:

Penicillin was discovered in 1928 by Alexander Fleming. He observed that a mold (*Penicillium notatum*) growing on a culture plate inhibited the growth of *Staphylococcus* bacteria around it. Fleming named the antibacterial substance penicillin [1]. The discovery of Penicillin has revolutionised the world of medicines. Penicillin is effective against bacterial diseases. Penicillin remains an important antibiotic due to its high efficacy and safety, though careful use is necessary to prevent antibiotic resistance and allergic reactions [2]. Structurally Penicillin consists of fused bicyclic nucleus. One of the ring is four membered β-lactam ring and other is five membered Thiazolidine, Sulphur and nitrogen containing ring shown and an appropriate side chain. The antibacterial activity of penicillin depends on this core structure of

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penicillin. Penicillin acts by inhibiting bacterial cell-wall synthesis through a specific chemical interaction with enzymes required for peptidoglycan cross-linking [3]. The Bacterial cell walls are made of peptidoglycan [4]. The strength to these cell wall comes from cross-linking of peptide chains. This cross-linking is catalysed by enzymes called Penicillin-binding proteins (PBPs), mainly transpeptidase [5]. The highly strained β-lactam ring structurally mimics the D-Ala-D-Ala dipeptide of peptidoglycan [6]. Because of this similarity PBPs bind penicillin mistakenly. Once the PBPs become inactive and are unable to form strong cross-linking. This results in weak and defective cell wall formation [7]. It involves an interesting chemistry. The active-site serine (-OH) of transpeptidase attacks the carbonyl carbon of the β-lactam ring. The sensitive β-lactam ring undergoes a ring opening reaction and a stable covalent acyl-enzyme complex is formed. This reaction is irreversible in nature. The weak cell wall cannot withstand osmotic pressure; bacterial cell wall undergoes lysis and death. The human cells lack peptidoglycan and PBPs, therefore penicillin selectively targets bacteria [8].

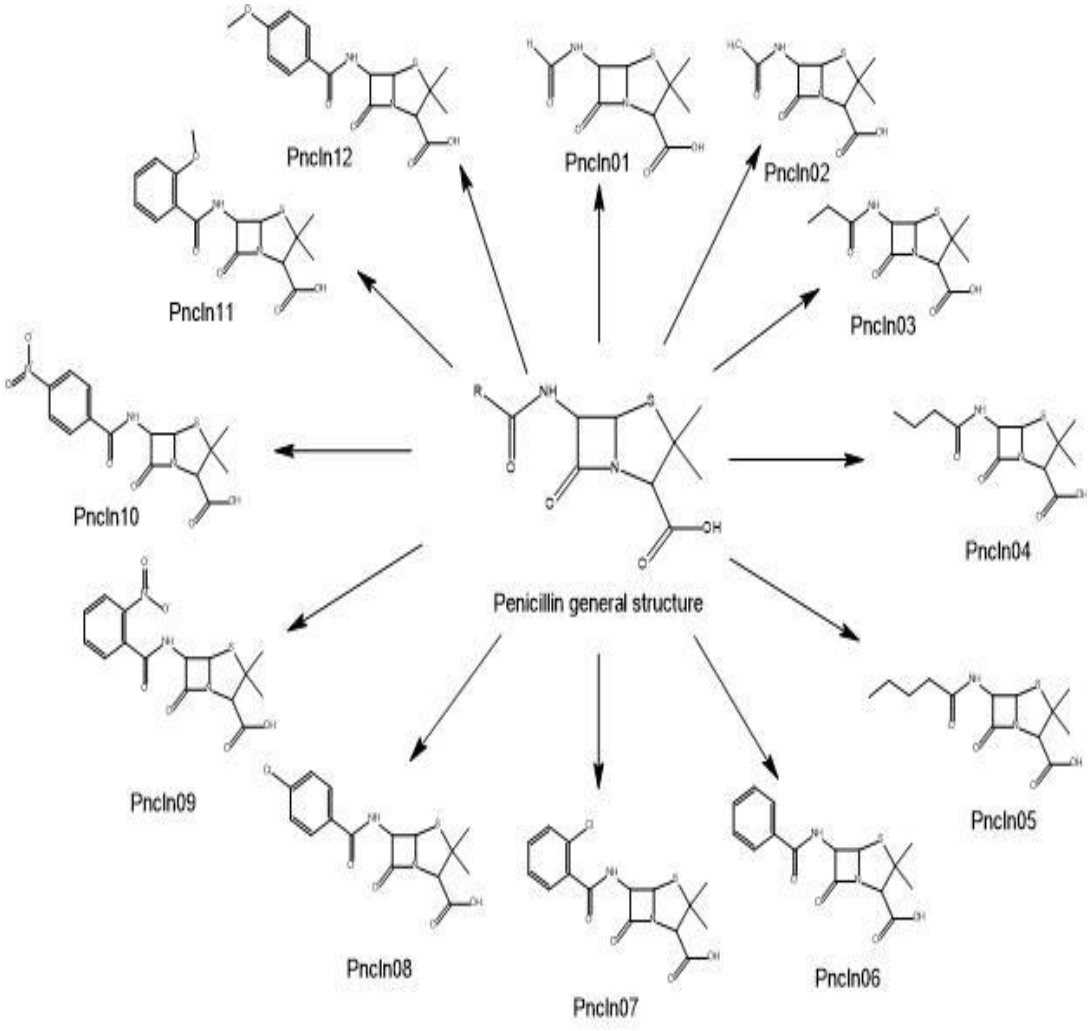

Fig. 1 General structure of Penicillin and Penicillin analogues

The PBPs are a group of bacterial enzymes located on the inner side of the cell membrane that play a key role in cell-wall (peptidoglycan) synthesis [9]. These are essential enzymes involved in bacterial growth and division and are the primary lethal targets of penicillin [10]. The PBPs are classified into class A PBPs, these are high molecular weight bifunctional enzymes which play important role in e.g. PBP1a, PBP1b, class B PBPs also high molecular weight monofunctional enzyme, e.g. PBP2, PBP3. The low molecular weight PBPs are not essential for survival but for maintenance of bacterial cell wall [11]. Here we have designed penicillin analogues **Fig.1** Keeping the core β-lactam ring constant the different analogues were designed by changing the -R substituent group. The docking study of these penicillin analogues was done with PBPs (PDB ID: 3UDI). The PBPs have a highly conserved active site. The highly conserved active site residue is Serine (Ser) amino acid. It forms a covalent acyl-enzyme complex with β-lactam ring [12]. The Lysine (Lys) stabilizes transition state. The different analogues of Penicillin namely Pncln1, Pncln2, Pncln3, Pncln4, Pncln5, Pncln6, Pncln7, Pncln8, Pncln9, Pncln10, Pncln11 and Pncln12 were docked with PBP to study effect of substituent on binding energy. The result of docking study was analysed. All penicillin analogues were found to form a stable complex with PBP.

Computational Methodology:

A. Protein preparation:

The 3-Dimentional Crystal structure of Acinetobacter baumannii PBP1a in complex with penicillin G (PDB ID: 3UDI) of resolution 2.60 A^0 was retrieved from RCSB Protein Data Bank. The total structure weight of the complex reported is 164.45 kDa containing 9726 number of atoms [13]. The protein structure was prepared by using AutoDocksTools-1.5.7 [14]. The chain A was retrieved. The ligand and water molecules were removed first. The Polar Hydrogen atoms were added to the structure followed by addition of Kollman charges. The protein molecule was saved as a .PDBQT file format after assigning AD4 field for it to be executable in AutodockTools.

B. Ligand Preparation:

The two-dimensional structures of Penicillin molecule was drawn using software program ChemDraw Ultra 12.0 [15]. All structures were saved as Pncln1, Pncln2, Pncln3, Pncln4, Pncln5, Pncln6, Pncln7, Pncln8, Pncln9, Pncln10, Pncln11 and Pncln12 in the .cdx file format. The all-Penicillin analogues were then drawn in software program Discovery studio 2025 and saved as .PDB file format [16]. These structures were opened in open-source software program Avogadro to obtain three-dimensional structures [17]. The energy minimization of all these structures was carried out by applying MMFF94 force field and selecting steepest descent algorithm. The all energy minimized structures were saved as .PDB file format. The saved ligand files were opened one by one in AutoDockTools-1.5.7. and saved as .PDBQT AutoDock executable files.

C. Molecular docking study:

The molecular docking study of all designed penicillin analogues with PBP was performed using AutoDockTools-1.5.7. The already prepared. PDBQT protein file was opened in AutoDockTools-1.5.7. The grid dimensional box was chosen to be at coordinate X = 34.097478, Y = -0.693739 and Z = 12.514522. The box cantered around 60 x 60 x 60 with grid point setting at 0.500 A^0. The Lamarkian

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Genetic algorithm was used for docking study keeping rest parameters default. The lowest binding energy conformation was analysed and visualized using Pymol and Discovery studio software program. The docking result is given in Table 1.

Table 1

Sr. No.	Name of Penicillin analogue	Penicillin analogue substituent (R =)	Binding Energy (Kcal/mol)
1	Pncln01	-H	-6.0
2	Pncln02	$-CH_3$	-6.6
3	Pncln03	$-CH_2-CH_3$	-6.6
4	Pncln04	-CH2-CH2-CH3	-6.3
5	Pncln05	-CH2-CH2-CH2-CH3	-6.3
6	Pncln06	-Ph	-7.2
7	Pncln07	-ortho-Cl-Ph	-7.5
8	Pncln08	-para-Cl-Ph	-7.3
9	Pncln09	-ortho-Nitro-Ph	-7.5
10	Pncln10	-para-Nitro-Ph	-7.2
11	Pncln11	-ortho-methoxy-Ph	-7.3
12	Pncln12	-para-methoxy-Ph	-6.9

Result and discussion:

The molecular docking study of Penicillin analogues with PBPs was performed. The binding energy study is reported in Table 1. The binding energy is calculated in units of Kcal/mol. All the Penicillin derivatives show negative binding energy. The negative binding energy means favourable binding which means strong and stable complex formation. This suggests that all the Penicillin analogues are forming stable complexes with PBP protein.

When analysed carefully the first five penicillin analogues with aliphatic chain substituent Pncln01, Pncln02, Pncln03, Pncln04 and Pncln05 have less binding energy compared with remaining penicillin analogues with aromatic ring substituents Pncln06, Pncln07, Pncln08, Pncln09, Pncln10, Pncln11 and Pncln12.

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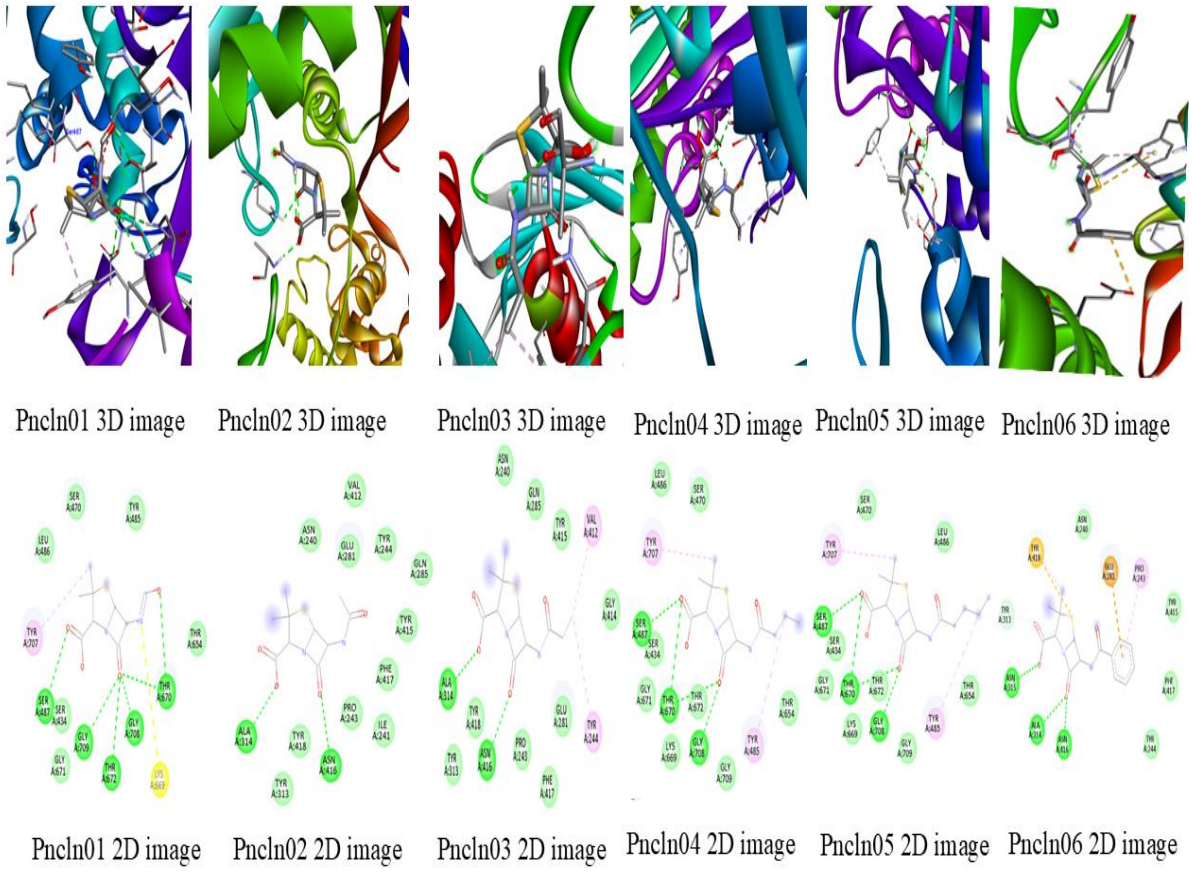

Fig. 2 Showing three-dimensional and two dimensional docking poses of Penicillin analogues 1 to 6 with PBP.

The comparison of binding energy of first five analogues Pncln01, Pncln02, Pncln03, Pncln04 and Pncln05 found to be -6.0, -6.6, -6.6, -6.3, and -6.3 respectively. This suggests that short aliphatic carbon chain containing 1 to 2 carbon atoms shows better binding energy than longer C3 and C4 chains. Means increasing chain length decreases the binding energy.

The comparison of binding energies of penicillin analogues Pncln06, Pncln07, Pncln08, Pncln09, Pncln10, Pncln11 and Pncln12 suggests that aromatic substituent is favourable for stable complex formation. Particularly the ortho substitution is more effective than para-analogue. -ortho-Chloro has higher binding energy -7.5 than para-Chloro substituent -7.3. Similarly -ortho-Nitro has higher binding energy than -7.5 than para-Nitro substituent. Also -Ortho-Methoxy has -7.3 compared to -para-methoxy substituent -6.9 Kcal/mol of binding energy. The docking poses and best docked conformer three dimensional and three-dimensional images are shown in Fig 2.

Conclusion:

The highest binding energy among all penicillin analogues is obtained for -ortho-Chloro and -ortho-Nitro analogue -7.5 Kcal/mol to both. This suggests that Pncln07 and Pncln09 have better binding affinity for

PBP. The world of microorganisms is continuously evolving which results into development for antibiotic resistance. So there has always been a thrust for novel modified antibiotic molecule. The present study aims to screen new possible penicillin structures for better binding to target and effective efficacy. The present study suggests that Pncln07 and Pncln09 could be the best lead compounds which further need to be investigated clinically.

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