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Research Article
Novel Modified Piperacillin Inhibitors of Penicillin-Binding Protein 3 (PBP3) and Their Intermolecular Interactions

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Abstract

Background and Objective: Despite the rise of antibiotic resistance, penicillin and the broader group of β-lactams have continued to be the most crucial class of antibiotics. Penicillin-Binding Protein 3 (PBP3) in *Pseudomonas aeruginosa* is the specific molecule that β-lactam-based medicines target. The objective is to design and study several piperacillin derivatives to create novel antibacterial agents. **Materials and Methods:** Piperacillin derivatives were drawn using chem sketch and prepared using AutoDock 4.2.6 Tools. Molecular docking simulations were conducted on novel piperacillin derivatives and piperacillin (Control) against the 6r3x.PDB protein. The AutoDock log files were analyzed to determine the lowest energy of binding (LEB) values for each ligand. Consequently, the conformer with the most favorable binding energy may be identified. **Results:** All of the proposed piperacillin derivatives displayed improved binding energies when compared to the reference chemical piperacillin. This suggests the potential for stronger interactions between derivatives and proteins, resulting in an enhanced likelihood of biological effects. Compounds b, e and j, when used alongside piperacillin, showed similar binding sites inside the active site and have the potential for additional characterization. **Conclusion:** Compounds b, e and j are highly likely to exhibit inhibitory activity, indicating that they should be synthesized and tested for biological activity.

Key words: Piperacillin, antibacterial drugs, penicillins, *Pseudomonas aeruginosa*, PBP3, molecular docking

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics are substances, either naturally occurring or artificially created, that can impede the growth or cause the death of microorganisms[1]. The identification and medical application of antibiotics, such as penicillins, represent a remarkable accomplishment in modern human medicine. Nevertheless, the forthcoming antibiotic resistance crisis may lead us to a state where, effective antibiotics are failed to combat bacterial illnesses because of the rapid emergence of resistance to established categories of antibiotics. The ESKAPE family, consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter*, is recognized as a group of dangerous pathogens by the Infectious Diseases Society of America (IDSA) because they tend to escape the bactericidal effect of traditional antibiotics[2]. The identification of penicillin is acknowledged as a crucial occurrence in the history of 20th-Century healthcare. Although penicillin had a groundbreaking effect, it was not universally effective against all germs and the emergence of microorganisms that are resistant to it has remained a persistent issue. It has been chemically modified to create new classes within the penicillin family that have enhanced effectiveness and/or wider ranges of activity like piperacillin, oxacillin and ampicillin[3]. The structural integrity of bacterial cell shape is sustained by the essential peptidoglycan (PG) layer of the cell wall. Peptidoglycan (PG) is essential for the growth cycle of bacteria and serves as a protective barrier against cell bursting caused by pressure. This avoids cell lysis and ultimately, cell death. Bacteria have complex processes to preserve the structure and formation of the PG layer and to regulate the synthesis of the PG layer during the cell's life cycle. Peptidoglycan (PG) is a macromolecule that has a net-like arrangement consisting of glycan strands. The strands consist of alternating N-acetylglucosamine and N-acetylmuramic acid residues, which are linked by short peptides. Glycosyltransferases (GTases) facilitate the synthesis of glycan chains through polymerization. The DD-transpeptidases, specifically found in the Penicillin Binding Proteins (PBPs) family, are accountable for the formation of peptide cross-links[4-6].

The beta-lactams have a structural similarity to the terminal D-Ala-D-Ala dipeptide portion of the pentapeptide chain (natural ligand). The mechanism involves the attachment of β-lactams to a serine residue located in the active site of all functioning PBPs forming an ester linkage (a covalent acyl-enzyme complex) (irreversible inhibition)[7,8]. That was one of the reasons why I chose this protein. Penicillin-Binding Proteins (PBPs) are essential enzymes located in the periplasmic region of the bacterial cell membrane and they play a crucial role in the last steps of synthesizing the bacterial cell wall. The proteins have been categorized into a high-molecular-mass (HMM) group, consisting of members that are crucial for the survival of cells and a low-molecular-mass (LMM) group, consisting of members that seem to be unnecessary for proper cell growth. The HMM PBPs are categorized as class A and class B enzymes. The smaller LMM PBPs function as DD-carboxypeptidases, which means they remove the terminal d-alanine from the muramyl peptide. This action helps regulate the extent of peptidoglycan cross-linking[9]. Class A Penicillin-Binding Proteins (PBPs) possess both Glycosyltransferase (GTase) and transpeptidase (TPase) activities, while class B PBPs exclusively exhibit the monofunctional activity of peptidoglycan (PG) transpeptidases. Class C Penicillin-Binding Proteins (PBPs) cannot form DD-crosslinks, but instead demonstrate DD-endopeptidase or DD-carboxypeptidase activity. The GTase and TPase activities play a vital role in the bacteria's survival and inhibiting these activities leads to cell death[10]. Different bacteria display various numbers of Penicillin-Binding Proteins (PBPs) and these PBPs can perform distinct roles. Gram-negative bacteria, such as *Escherichia coli* or *Pseudomonas aeruginosa*, have more than eight Penicillin-Binding Proteins (PBPs), some of which are essential and well-established targets for β-lactam antibiotics with antibacterial properties[11]. The target-based inhibitor screening aims to identify new chemical scaffolds by focusing on PaPBP3 which is a specific protein of *Pseudomonas aeruginosa* (the second reason for choosing this protein for studying) which is an opportunistic pathogen that causes serious and life-threatening infections, particularly in people with chronic respiratory conditions like cystic fibrosis. The PaPBP3 possesses only transpeptidase activity and is encoded by the ftsI gene, it is crucial for the survival of *P. aeruginosa*, making it a prime candidate for the development and exploration of antibacterial drugs. This target has been clinically validated and its lack of a human homologue decreases the likelihood of drug-induced side effects. The catalytic domain of PBP3 class b is situated in the periplasm, making it susceptible to possible small molecule inhibitors[9,10].

The third reason for choosing this target is because it is a druggable target since it has a crystal structure in complex with piperacillin with 1.59 Å resolution (high resolution which can help in drug design and finding novel drugs), its PDB ID is (6R3X)[12] from *Pseudomonas aeruginosa* PAO1organism. The peptidoglycan D,D-transpeptidase FtsI (chain A) consists of 521 amino acid residues from *Pseudomonas aeruginosa* PAO1 wildtype. The main mechanism of resistance against β-lactams involves reducing the drug concentration using β-lactamases, increasing their removal from the cell (efflux) or in the case of gram-negative bacteria, preventing their entry into the

periplasm and creating mutations in the target[12]. The worldwide rise in bacteria that are resistant to multiple drugs (MDR) encouraged us to come up with new drugs. So, this study aimed to identify potential new antibacterial agents. To enhance the effectiveness of penicillin against Enterobacteriaceae and *Pseudomonas aeruginosa*, scientists created carboxypenicillins, ureidopenicillins and aminopenicillins. Piperacillin, often known as Pipracil, is a medication belonging to the "fourth generation" classification with IC_{50} = 166 nM. The ureido group of the penicillin family is not resistant to the action of penicillinase (function by breaking down the β-lactam ring), but it has a wide spectrum of activity[13].

The β-Lactam/β-Lactamase Inhibitor (BL/BLI) mixtures were created to remove the effects of β-lactamase enzymes. Commonly used combinations in clinical practice include piperacillin/tazobactam (P/T)[14]. Heterocyclic compounds are mostly of interest in the field of medicinal chemistry. Most of the synthetic heterocyclic compounds function as drugs and are utilized as anticonvulsants, hypnotics, antineoplastics, antiseptics, antihistaminics, antivirals, anti-tumor agents and antibacterials[15,16]. One of the primary objectives of the organic and medicinal chemistry community is to develop nitrogen-containing heterocyclic analogues such as triazine, diazine, oxathiazine, oxadiazine, thiazine, dithiazine and thiadiazine that have therapeutic benefits for humans and involves the process of designing, synthesizing and screening these compounds[17-22]. Therefore, in this work, piperacillin has been utilized as an inhibitor of PBP3. Consequently, a computational docking technique has been presented to study novel piperacillin derivatives (Fig. 2a-l) and piperacillin, a known inhibitor co-crystallized with PBP3. All the modifications made were by structure-activity relationship (SAR) rules. This work aimed to investigate the binding interactions and binding energies inside the active site of PBP3, with the expectation of gaining valuable insights for the development of more potent and less harmful medications to treat bacterial infections related to PBP3.

MATERIALS AND METHODS

Study area: The study area was situated in Arab American University, Jenin, Palestine. The duration of the study ranges from April to June, 2024.

Ligand and receptor preparation: The docking in this investigation was conducted using AutoDock 4.2.6, with piperacillin serving as the reference compound for comparison. The simulations were facilitated by utilizing the crystal structure of PBP3 in association with piperacillin (PDB ID: 6r3x) (Fig. 1) piperacillin was removed from its protein. All piperacillin derivatives illustrated in Fig. 2 which

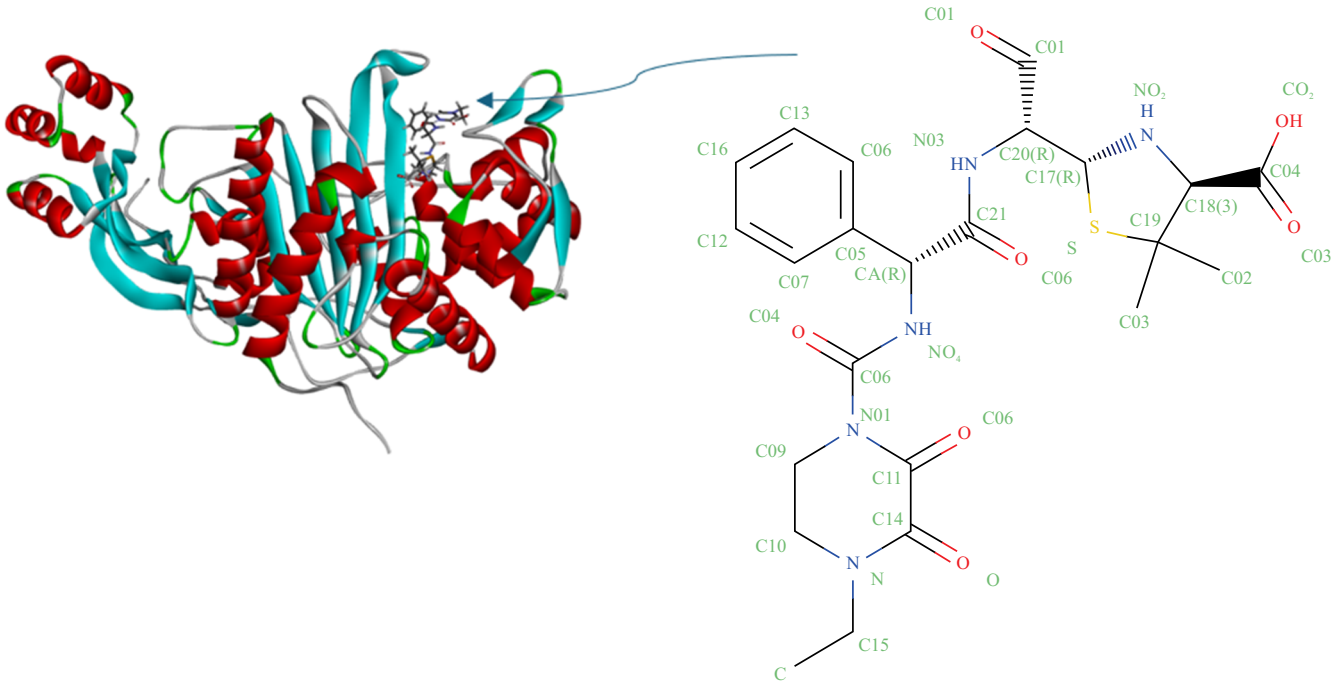

Fig. 1: Solid ribbon representation of the available crystal structures of Penicillin-Binding Protein 3 (PBP3) in complex with piperacillin
PDB ID: 6R3X[12]

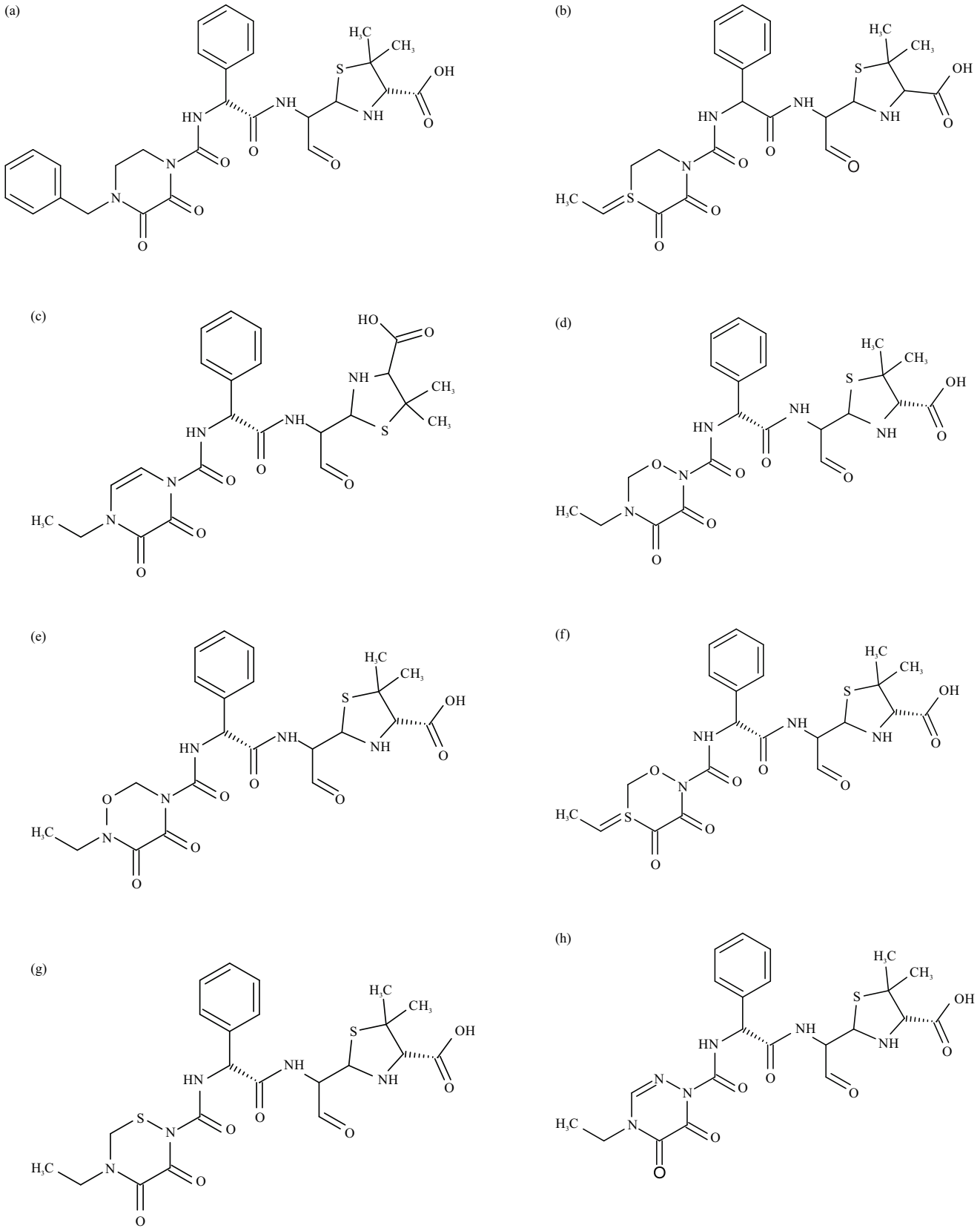

Fig. 2(a-l): Continue

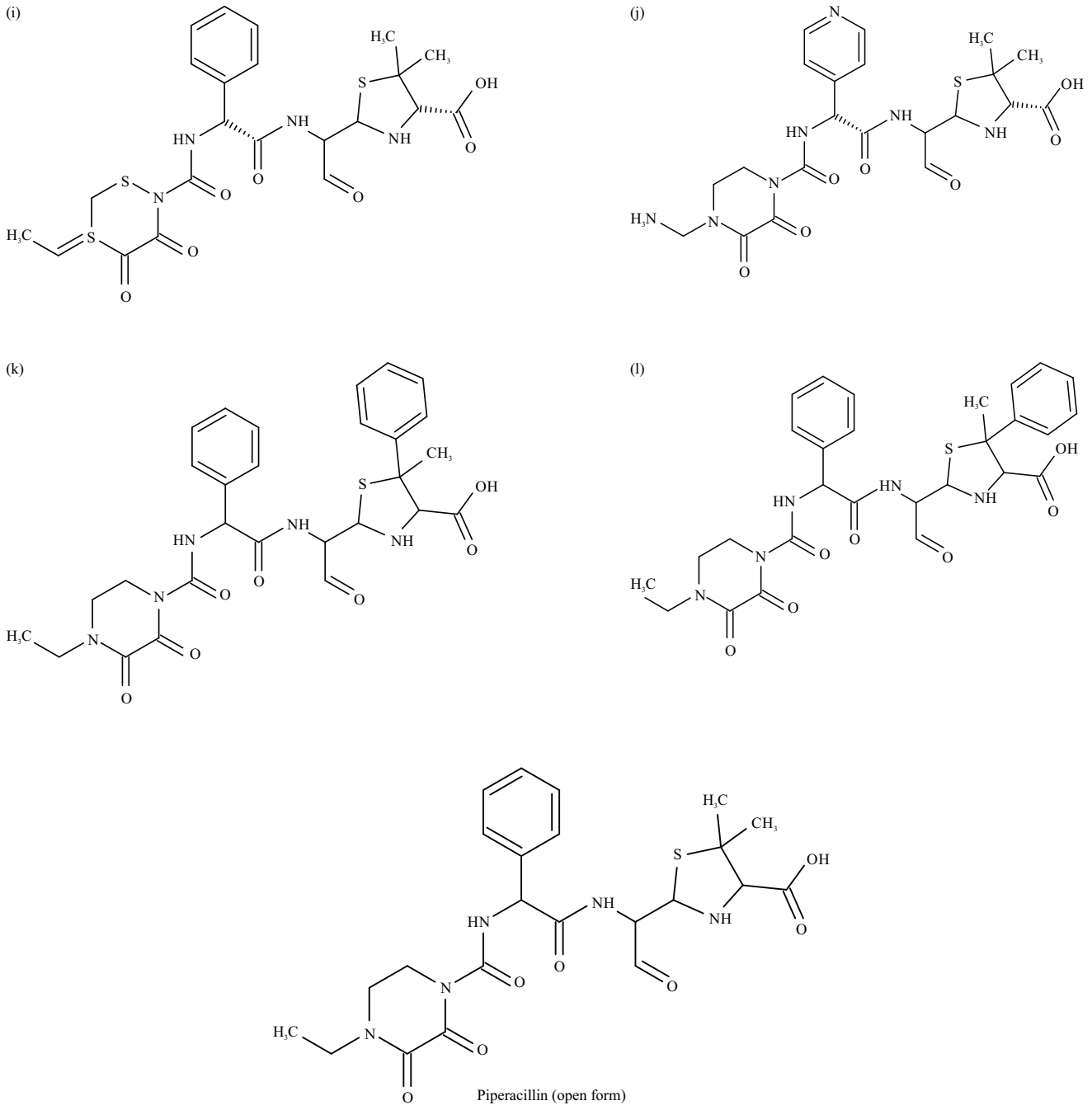

Fig. 2(a-l): 2D Structures of piperacillin and novel piperacillin derivatives

are subjected to the structure-activity relationship rules (Fig. 3) were sketched using ChemSketch(ACD/ChemSketch v.freeware 2021 2.0). The chemical structures were stored in MOL format and subsequently transformed into 3D structures as PDB files by avogadro program (Avogadro 1.90.0) using (GAFF) general amber force field to describe the ligands. The piperacillin, protein and ligands'' PDB files were prepared using AutoDock 4.2.6 tools. After being opened, polar hydrogens were introduced and Kollman charges were assigned, which improved the accuracy of the protein model. Afterward, each ligand molecule was loaded individually, all hydrogens were added and Gasteiger charges were assigned and each one was saved as PDBQT file.

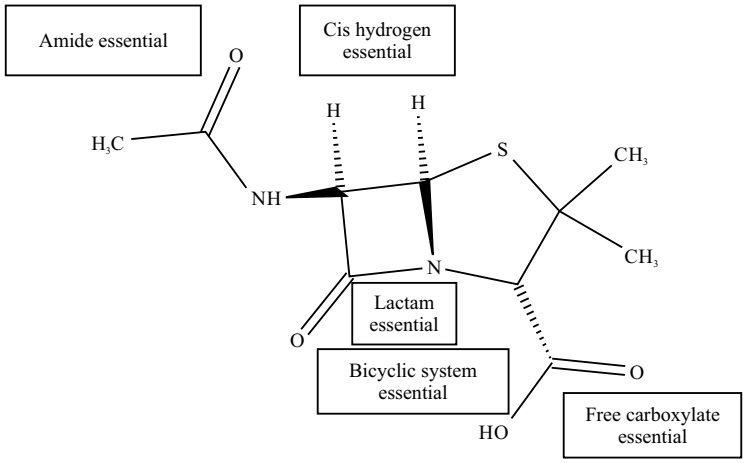

Fig. 3: Structures activity relationship of penicillins

Docking parameters: The docking process in this investigation utilized AutoDock 4.2.6 exclusively. Typically, the default values for the docking parameters of AD4 were used. Nevertheless, the quantity of AutoDock 4.2.6, GA runs was augmented from 10 to 100. The dimensions of the docking grid box were 15 A°×15 A°×15 A°, effectively containing the piperacillin structure and a default grid spacing of 0.375 angstroms (Å) was created and positioned at the coordinates 8.16, 40.718 and 15.919 for the x, y and z axes, respectively, with the center point being at these coordinates and saving as grid parameter file. The 100 separate genetic algorithm (GA) runs from AD4 were analyzed using the clustering analysis feature, which has a cutoff of 1.0 A°. while the other parameters were left at their default values. The Lamarckian genetic algorithm (LGA) was employed to optimize and minimize energy in the docking simulation and saving as a docking parameter file.

Scoring function: Molecular docking simulations were conducted on novel piperacillin derivatives and piperacillin (Control) against the 6r3x.PDB protein using AutoDock 4.2.6. Using AutoGrid 4.2, a series of grid maps were generated based on the atom kinds that were present. The AutoDock log files were analyzed to determine the lowest energy of binding (LEB) values for each ligand. This allowed for the identification of the conformer with the most favorable binding energy. Also, attention was paid to the higher population (the number of conformations in each cluster).

The selected conformers were later exported and visualized using BIOVIA Discovery Studio Visualizer 16.1 software to demonstrate the identification of ligands and amino acids in the PBP3 pocket through 2D and 3D docked visualization. This enabled us to get a more profound study and comparison of the binding interactions among the modified compounds, the original compound (piperacillin) and the PBP3 protein and search for essential interactions in the mechanism of action that should be found.

RESULTS AND DISCUSSION

Molecular docking simulations were performed to clarify the molecular mechanisms responsible for the antibacterial action of the chosen piperacillin derivatives on the PBP3 protein. To validate the docking process, the crystal structure of piperacillin was redocked, resulting in an RMSD value of 1.35 Å, as shown in (Fig. 4). The RMSD value, which is lower than the 2 Å threshold and has similar 2D interactions between piperacillin and PBP3 (Fig. 5-6) and its LBE was -7.5 kcal/mol verifies the reliability of the docking process for subsequent investigations.

When two molecules, which are located near each other, interact in a beneficial way, they join together to create a stable complex. The resulting complex is referred to as a receptor-ligand complex. Various noncovalent interaction forces facilitate the binding of the receptor and ligand, resulting in the formation of a stable complex. The interactions include torsional, hydrophilic, hydrophobic, van der Waals, electrostatic, hydrogen-bonding and desolvation. The primary objective was to identify the receptor-ligand complex that exhibited the highest stability, with optimal geometry and the lowest binding energy. The scoring (prediction of affinity) functions are used to calculate the energy score and energy terms of the binding pose. Scoring functions are utilized to rank the various conformations acquired during the search

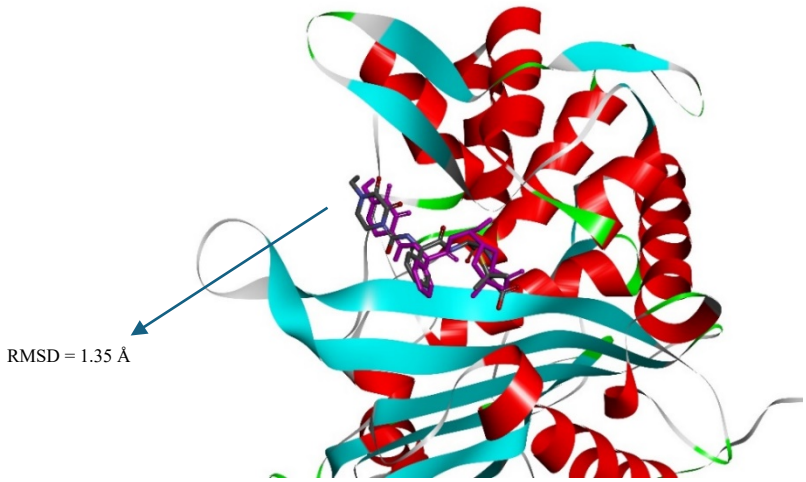

Fig. 4: Solid ribbon representation of PBP3 (PDB ID: 6r3x) (12) with cocrystal piperacillin (pink)

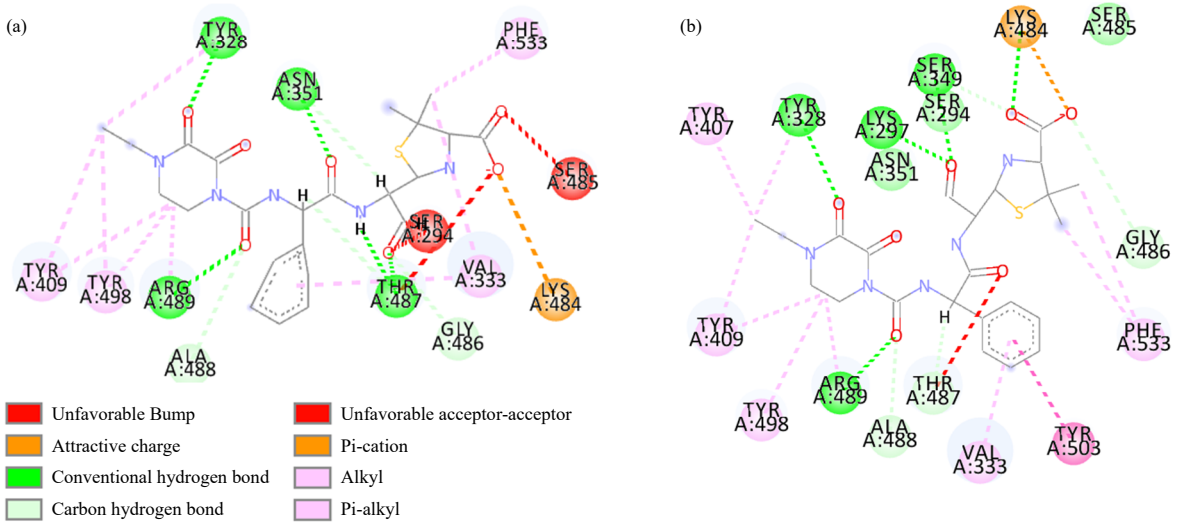

Fig. 5(a-b): 2D interactions with penicillin binding protein 3 target, (a) 2D interaction of piperacillin with PBP3 and (b) 2D interactions of cocrystal piperacillin with PBP3

method according to calculations performed using the MM force field[23]. The calculation of the net change in free energy in AutoDock is provided as follows:

$$\Delta G_{binding} = \Delta G_{VDW}\sum_{ij}\left[\frac{A_{ij}}{r_{ij}^{12}}-\frac{B_{ij}}{r_{ij}^{6}}\right]$$
$$+\Delta G_{HB}\sum_{ij}E(t)\left[\frac{C_{ij}}{r_{ij}^{12}}-\frac{D_{ij}}{r_{ij}^{10}}\right]$$
$$+\Delta G_{Estatic}\sum_{ij}\frac{q_i q_j}{\varepsilon(r_{ij})}$$
$$+\Delta G_{torsion}N_{torsion}$$
$$+\Delta G_{Solv}\sum_{ii}\left(S_i V_j + S_j + V_i\right)e^{\frac{-r_{ij}^2}{2\sigma}}$$

The summation is performed for every pair of ligand atom, i and receptor atom, j, as well as for every pair of atoms in the ligand that are separated by three or more bonds.

The SER 294 is essential in the interaction of penicillin and active site. The C4 carboxylic acid group is firmly attached to the hydroxyl groups of SER 485 and THR 487 through two hydrogen bonds. Several amino acids, specifically TYR 328, ARG 489, THR 487 and ASN 351, established conventional hydrogen bonds with piperacillin. The PHE 533, TYR 489 and TYR 498 form alkyl-alkyl hydrophobic interactions. Residues, ASN 351, ARG 489 (H-bond interactions) and VAL 333 (pi-alkyl interaction) are believed to contribute to the

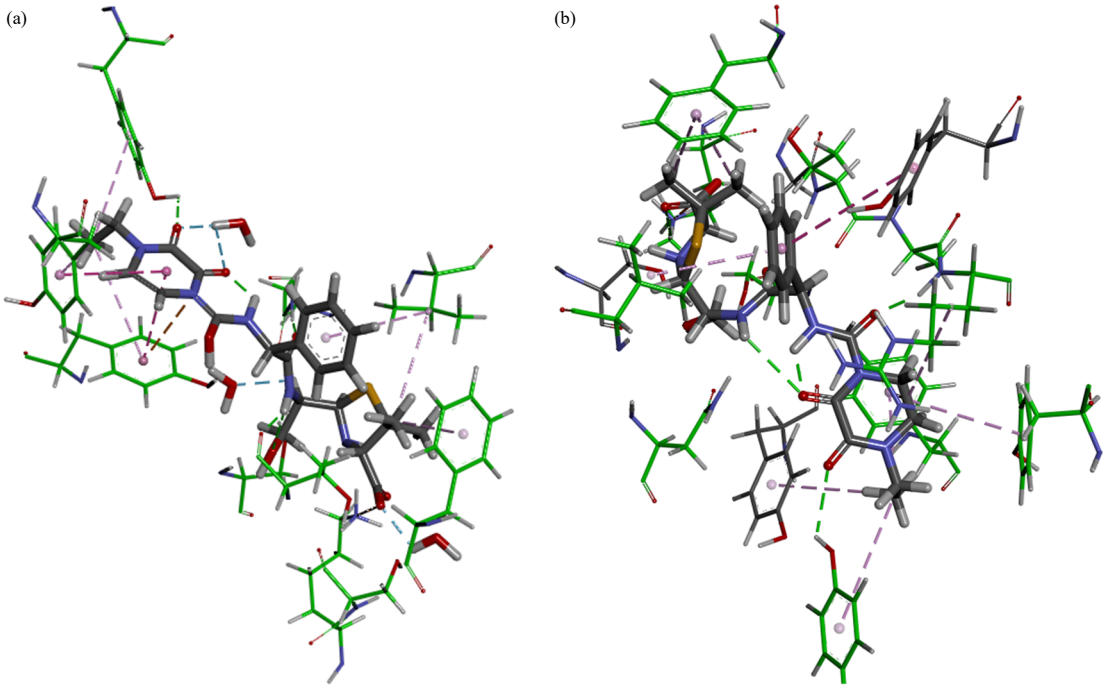

Fig. 6(a-b): 3-D structure of peptidoglycan D,D-transpeptidase amino acids (green carbon stick) with a-piperacillin b-cocrystal (in grey carbon sticks) interactions

Table 1: Minimum binding energies derived from AutoDock 4.2 and the interacting amino acids

Compound	Lowest binding energy (kcal/mol)	Interacting amino acids
Piperacillin	-7.50	SER294, THR 487, AGR 489, TYR 328, ASN 351, VAL333, PHE 533, TYR 498, TYR 409 and LYS 484
a	-9.11	TYR 328, TYR 498, TYR 409, VAL 333, TYR 503, ARG 489, ASP 332, THR 487 and ASN 351
b	-10.00	SER 334, SER 349, VAL 333, ASN 351, ARG 489, TYR 409 and TYR 498
c	-8.76	ARG 335, ARG 331, THR 487, TYR 409, ARG 489 and SER 294
d	-9.06	SER 294, SER 349, LYS 297, ASN 351, THR 487, ARG 489, TYR 498, TYR 409 and ARG 335
e	-10.40	TYR 324, TYR 498, ARG 489, TYR 409, ASN 351, SER 294, SER 349, VAL 333, SER 334 and PEH 533
f	-9.35	THR 487, ASN 351, VAL 333, ARG 489, ARG 331, THR 329 and TYR 328
g	-9.19	TYR 503, VAL 333, ARG 489, ASN 351, THR 487, TYR 328, THR 329 and ARG 331
h	-9.11	ARG 335, THR 329, TYR 407, TYR 498, TYR 409, ARG 489 and VAL 333
i	-9.29	TYR 503, ARG 489, THR 329, ARG 331, TYR 328, ASN 351 and THR 487
j	-9.05	TYR 328, ASN 351, VAL 333, SER 349, SER 334, PHE 533, THR 487, SER 294, TYR 409, TYR 498 and ARG 489
k	-8.53	THR 487, PHE 533, VAL 333, TYR 407, ARG 331, TYR 328 and ARG 489
l	-10.30	TYR 498, ARG 331, ARG 489, THR 487, ASN 351, SER 294, SER 349, LYS 297 and VAL 333

effective inhibition of PaPBP3[24,25]. The twelve piperacillin derivatives that were suggested were effectively subjected to docking analysis against the PBP3 crystal structure. The outcomes of this analysis are presented in (Table 1). According to the docking results, all the suggested piperacillin derivatives exhibited favorable binding energies within the range of -8.53 to -10.4 kcal/mol compared with the reference compound piperacillin -7.5 kcal/mol, as seen in (Table 1). This implies the possibility of stronger interactions between molecules and protein, leading to increased potential for biological effects.

An in-depth investigation was conducted on the ligand-binding interactions of the piperacillin derivative. Piperacillin, a well-established control compound, was also included in the analysis for comparison. The compounds a, c, d, f, g, h, i, k and l have extra hydrogen bonds and extra other types of interaction (Fig. 7) but lose the serine 294 interaction with beta-lactam and this contradicts the mechanism of action of beta-lactam, so they were excluded from further study. Additional hydrogen bond interactions enhance the binding of many β-lactams[26]. Compounds b, e and j were tightly attached to the active site, establishing several binding

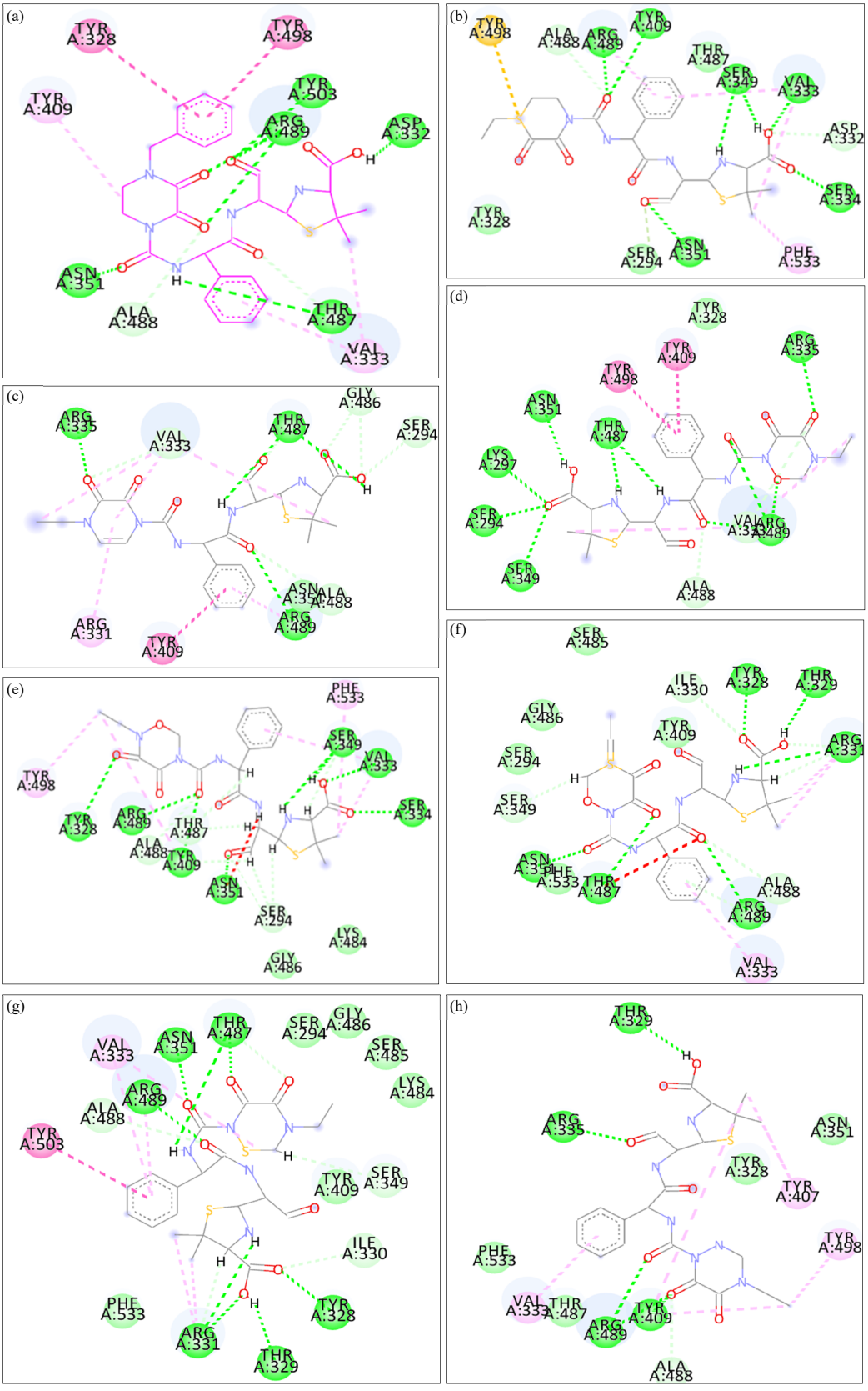

Fig. 7(a-l): Continue

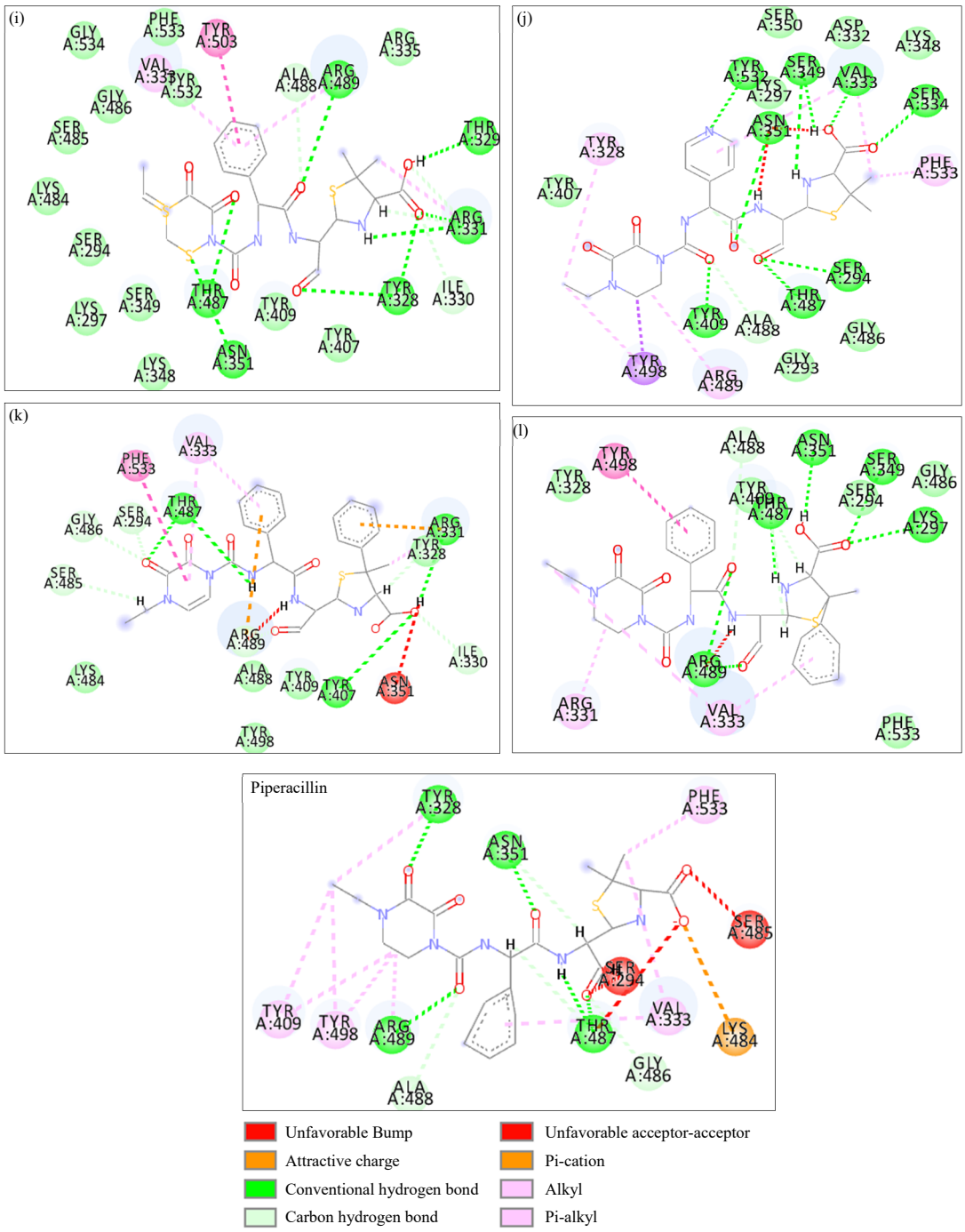

Fig. 7(a-l): 2D intermolecular interactions between the docked drugs (a-j and piperacillin) and the PBP3 protein are being studied
Amino acids that are colored orange, green and pink reflect their relative contributions in ionic bonding, hydrogen bonding and hydrophobic interactions

contacts with specific amino acids (Fig. 7). These interactions involve the SER 294 which is important in the interaction of penicillin and active site. The mechanism of inhibition is thought to be due to serine 294 attack on beta lactam ring which competes with pentapeptide chain. The hydroxyl of the nucleophilic SER 294 forms a covalent ester-linkage with the β-lactam. This observation is consistent with other PBP3-β-lactam interactions. So SER 294 is an essential amino acid that should be found in all effective compounds. The ASN 351, ARG 489 (H-bond interactions) and VAL 333 (pi-alkyl interaction) which are believed to contribute to the effective inhibition of PaPBP3 are still found in these compounds.

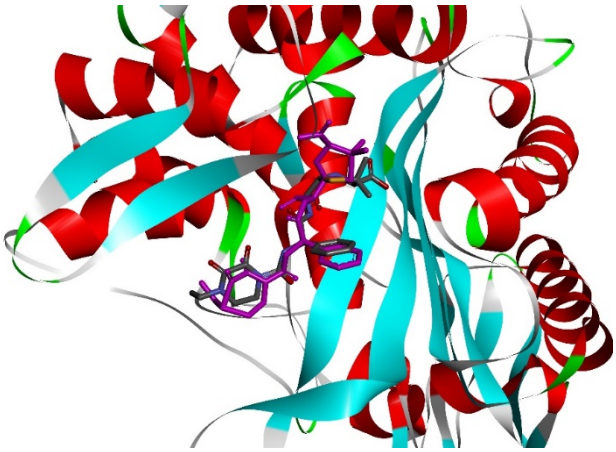

Fig. 8: Solid ribbon representation of PBP3 docked with crystal structure of piperacillin (gray color) and inhibitor b (pink color) in the active site

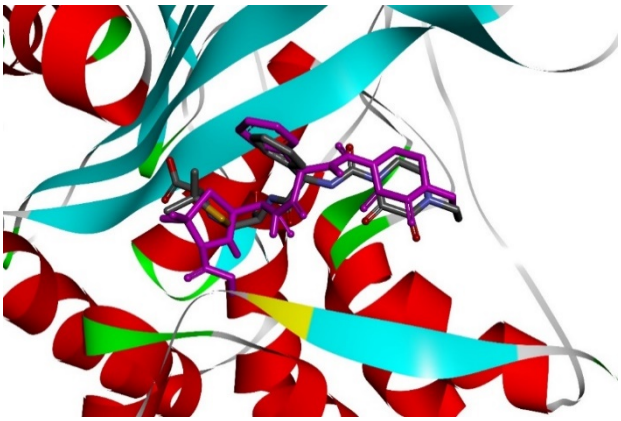

Fig. 9: Solid ribbon representation of PBP3 docked with crystal structure of piperacillin (gray color) and inhibitor e (pink color) in the active site

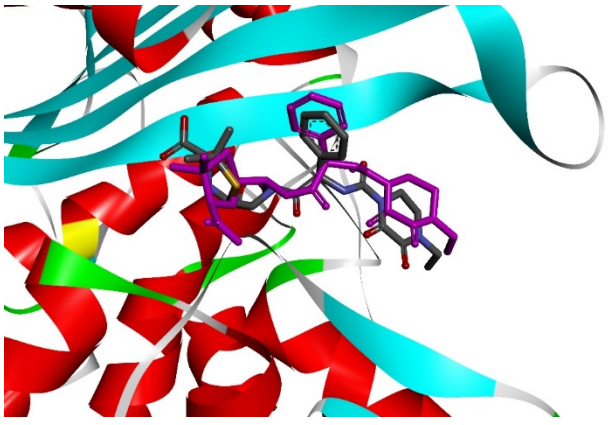

Fig. 10: Solid ribbon representation of PBP3 docked with crystal structure of piperacillin (gray color) and inhibitor j (pink color) in the active site

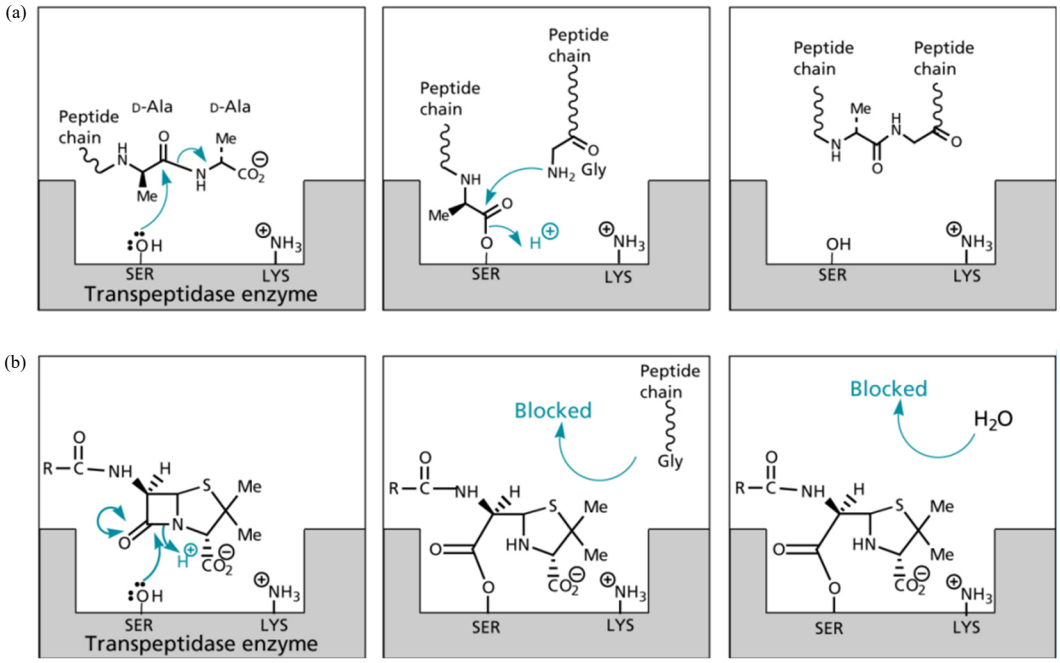

Fig. 11(a-b): Mechanism of actions of (a) Transpeptidase cross-linking and (b) Penicillin inhibition

(a) Bacterial cell wall synthesis. The DD-transpeptidases, also known as TPases, derived from the Penicillin-Binding Proteins (PBPs) family, are responsible for the formation of peptide cross-links. (b) Beta-lactams exhibit a structural similarity to the terminal D-Ala-D-Ala dipeptide segment of the pentapeptide chain. The mechanism involves the binding of β-lactams to a serine residue situated in the active site of all functional PBPs, resulting in the formation of an ester bond (8)

Table 2: Extra intermolecular interactions in compounds b, e and j formed by modified part and whole compound

Compound	H-bond interactions in the whole compound	Hydrophobic interactions in the whole compound	Modified part and its extra bond
Piperacillin	4	9	-
b	7	4	Thiazine ring make pi-sulfer interaction with TYR 498
e	7	4	Oxadiazine ring didn't make extra bond
j	9	7	Pyridine ring make extra H-B interaction with TYR 532

Thiazine ring in compound b gives extra pi-sulfer interaction and other extra intermolecular interaction compared to a reference, in compound e oxadiazine ring increases the hydrogen bond interactions to 7 compared to 4 in the reference, finally in compound j, the pyridine ring makes extra hydrogen bond interaction besides the other extra intermolecular interaction in the rest of the molecule (Table 2).

Compounds b, e and j in alongside piperacillin, exhibited comparable binding locations inside the active site, as depicted in Fig. 8-10. These interactions focus on the SER 294 residue, which plays a crucial role in the interaction between penicillin derivatives and the active site (Fig. 11).

CONCLUSION

In this study, the molecular framework of the piperacillin inhibitor was employed to identify and evaluate candidate inhibitors that specifically target the PBP3 protein. The examination of PBP3 from *Pseudomonas aeruginosa*, when combined with various β-lactam antibiotics such as penicillins, carbapenems and cephalosporins, reveals that a specific group of amino acids, including the serine residue in the active region that undergoes acylation, play a crucial role in the binding process. A total of twelve candidates underwent testing and exhibited greater binding affinities to PBP3 compared to the control molecule and three molecules among them have the potential for additional characterization. The compounds displayed a variety of interactions, such as important covalent connections, hydrogen bonds and hydrophobic contacts. Additional optimization focused on specific interactions formed with SER 294 (such as compound b, e and j) could result in the development of new and powerful antibacterial compounds that target PBP3. There is a strong likelihood that compound b, e and j will demonstrate inhibitory activity, suggesting that it should be synthesized and subjected to biological activity tests.

SIGNIFICANCE STATEMENT

This work was important due to the increasing concern regarding antibiotic resistance especially that targeting Penicillin-Binding Protein 3 (PBP3), an essential contributor to the process of bacterial cell wall production and trying to increase the potency compared with piperacillin control drug. In this study novel modified piperacillin inhibitors were designed. The intermolecular interactions between these inhibitors and PBP3 were clarified and the binding energies were estimated and compared with the reference. The results demonstrated enhanced binding affinity using molecular docking simulations. Proposing that three compounds that have been studied need to be prepared and undergo biological activity testing and the possibility of designing similar compounds to develop antibiotic properties and further understand the principle of protein binding inhibitor mechanism.

REFERENCES

1. Fymat, A.L., 2017. Antibiotics and antibiotic resistance. Biomed. J. Sci. Tech. Res., Vol. 1. 10.26717/BJSTR.2017.01.000117.
2. Wang, Z., X. Liu, Y. Duan and Y. Huang, 2022. Infection microenvironment-related antibacterial nanotherapeutic strategies. Biomaterials, Vol. 280. 10.1016/j.biomaterials.2021.121249.
3. Miller, E.L., 2002. The penicillins: A review and update. J. Midwifery Womens Health, 47: 426-434.
4. Egan, A.J.F., J. Errington and W. Vollmer, 2020. Regulation of peptidoglycan synthesis and remodelling. Nat. Rev. Microbiol., 18: 446-460.
5. Vollmer, W., B. Joris, P. Charlier and S. Foster, 2008. Bacterial peptidoglycan (murein) hydrolases. FEMS Microbiol. Rev., 32: 259-286.
6. Vollmer, W., D. Blanot and M.A. de Pedro, 2008. Peptidoglycan structure and architecture. FEMS Microbiol. Rev., 32: 149-167.
7. Bush, K. and P.A. Bradford, 2016. β-Lactams and β-lactamase inhibitors: An overview. Cold Spring Harbor Perspect. Med., Vol. 6, No. 8. 10.1101/cshperspect.a025247.
8. Patrick, G.L. and J. Spencer, 2009. An Introduction to Medicinal Chemistry. 4th Edn., Oxford University Press, Walton Street, Oxford, ISBN: 9780199234479, Pages: 752.
9. Sainsbury, S., L. Bird, V. Rao, S.M. Shepherd and D.I. Stuart *et al*., 2011. Crystal structures of penicillin-binding protein 3 from *Pseudomonas aeruginosa*: Comparison of native and antibiotic-bound forms. J. Mol. Biol., 405: 173-184.
10. López-Pérez, A., S. Freischem, I. Grimm, O. Weiergräber and A.J. Dingley *et al*., 2021. Discovery of pyrrolidine-2,3-diones as novel inhibitors of *P. aeruginosa* PBP3. Antibiotics, Vol. 10. 10.3390/antibiotics10050529.
11. Sauvage, E., F. Kerff, M. Terrak, J.A. Ayala and P. Charlier, 2008. The penicillin-binding proteins: Structure and role in peptidoglycan biosynthesis. FEMS Microbiol. Rev., 32: 234-258.
12. Bellini, D., L. Koekemoer, H. Newman and C.G. Dowson, 2019. Novel and improved crystal structures of *H. influenzae*, *E. coli* and *P. aeruginosa* penicillin-binding protein 3 (PBP3) and *N. gonorrhoeae* PBP2: Toward a better understanding of β-lactam target-mediated resistance. J. Mol. Biol., 431: 3501-3519.
13. Wright, A.J. and C.J. Wilkowske, 1987. The penicillins. Mayo Clinic Proceed., 62: 806-820.
14. Gálvez-Benítez, L., J.M.O. de la Rosa, A. Rodriguez-Villodres, C.S. Casimiro-Soriguer and I. Molina-Panadero *et al*., 2023. Role of bla_{TEM} and OmpC in the piperacillin-tazobactam resistance evolution by *E. coli* in patients with complicated intra-abdominal infection. J. Infect., 87: 220-229.
15. Gomtsyan, A., 2012. Heterocycles in drugs and drug discovery. Chem. Heterocycl. Compd., 48: 7-10.
16. Abd El-Salam, N.M., M.S. Mostafa, G.A. Ahmed and O.Y. Alothman, 2013. Synthesis and antimicrobial activities of some new heterocyclic compounds based on 6-chloropyridazine-3(2*H*)-thione. J. Chem., Vol. 2013. 10.1155/2013/890617.
17. Liu, H., S. Long, K.P. Rakesh and G.F. Zha, 2020. Structure-activity relationships (SAR) of triazine derivatives: Promising antimicrobial agents. Eur. J. Med. Chem., Vol. 185. 10.1016/j.ejmech.2019.111804.
18. Akbar, S., S. Das, R.P. Dewangan, A. Joseph and B. Ahmed, 2024. Review on the potential of 1,3,4-oxadiazine derivatives: Synthesis, structure-activity relationship, and future prospects in drug development. Eur. J. Med. Chem. Rep., Vol. 11. 10.1016/j.ejmcr.2024.100152.
19. Asif, M., M. Imran and Abida, 2022. Antimicrobial activities of various thiazine based heterocyclic compounds: A mini-review. Mini-Rev. Org. Chem., 19: 166-172.
20. Hui, X.P., H.S. Dong, P.F. Xu, Z.Y. Zhang, Q. Wang and Y.N. Gong, 2000. Heterocyclic systems containing bridged nitrogen atom: Synthesis and antibacterial activity of 3-(2-phenylquinolin-4-yl)/3-(1-p-chlorophenyl-5-methyl-1,2,3-triazol-4-yl)-s-triazolo[3,4-b]-1,3,4-thiadiazine derivatives. J. Chin. Chem. Soc., 47: 1115-1119.
21. Maffuid, K.A., M. Koyioni, C.D. Torrice, W.A. Murphy and H.K. Mewada *et al*., 2021. Design and evaluation of 1,2,3-dithiazoles and fused 1,2,4-dithiazines as anti-cancer agents. Bioorg. Med. Chem. Lett., Vol. 43. 10.1016/j.bmcl.2021.128078.

22. Majchrzak-Stiller, B., M. Buchholz, I. Peters, J. Strotmann and J. Möhrke *et al*., 2023. Oxathiazinane derivatives display both antineoplastic and antibacterial activity: A structure activity study. J. Cancer Res. Clin. Oncol., 149: 9071-9083.
23. Mohanty, M. and P.S. Mohanty, 2023. Molecular docking in organic, inorganic, and hybrid systems: A tutorial review. Chem. Mon., 154: 683-707.
24. Chio, H., E.E. Guest, J.L. Hobman, T. Dottorini, J.D. Hirst and D.J. Stekel, 2023. Predicting bioactivity of antibiotic metabolites by molecular docking and dynamics. J. Mol. Graphics Modell., Vol. 123. 10.1016/j.jmgm.2023.108508.
25. Han, S., R.P. Zaniewski, E.S. Marr, B.M. Lacey and A.P. Tomaras *et al*., 2010. Structural basis for effectiveness of siderophore-conjugated monocarbams against clinically relevant strains of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U.S.A., 107: 22002-22007.
26. Ren, J., J.E. Nettleship, A. Males, D.I. Stuart and R.J. Owens, 2016. Crystal structures of penicillin-binding protein 3 in complexes with azlocillin and cefoperazone in both acylated and deacylated forms. FEBS Lett., 590: 288-297.