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Cheminformatics study: Homology modeling and molecular docking simulations study on milk proteins with most drugs used in dairy sector

Zabron Janes[*1], Daniel Shadrack[1,2], John Kyaruzi[3], Hulda Swai[3], Gabriel Shirima[1]

[1]*Department of Global Health and Biomedical Sciences, The Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania*

[2]*Directorate of Research, Innovation and Consultancy St John's University of Dodoma, Dodoma, Tanzania*

[3]*Tanzania Agricultural Catalytic Trust (TACT), Prime Minister's Office, Dar es salaam, Tanzania*

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Abstract

The widespread use of drugs in agriculture and husbandry poses a significant risk to human health through direct exposure via dairy products. In this study, the effects of drug interactions on the conformation, binding modes and affinities was investigated by employing in-silico methods, including homology modeling and molecular docking. Bovine milk proteins (PDB ID: ICE2, 3GC1, 7ER3, 4F5S), and drugs (oxytetracycline CID:54675779, enrofloxacin CID:2082, penicillin, CID: 5904 and albendazole CID:71188) were sourced from the RCSB protein data bank and PubChem database, respectively. Since Bovine β-casein crystal structure is experimentally not resolved and, its structure is absent in PDB bank, homology modeling was used to construct a 3D structure. MODELLER and I-TASSER were used to model the protein with an accuracy of 87.4% and 89.6%, respectively. Molecular docking simulations reveal that enrofloxacin and oxytetracycline, with Bovine lactoperoxidase (3GC1), showed a strong affinity of -8.4 kcal/mol and -8.3 kcal/mol, respectively. This study provides insights into molecular interactions pivotal for understanding milk quality. The implications extend to environmental, human health, and animal welfare, emphasizing the need for informed strategies in the dairy sector and in pharmaceutical industries during drug design and development.

* **Corresponding Author:** Zabron Janes ✉ janesz@nm-aist.ac.tz

Introduction

The dairy industry holds a pivotal role, serving as a key economic sector for small-scale dairy farmers, contributes significantly to food, security, employment, and energy production (Lemma *et al.*, 2018; Herrero *et al.*, 2013; FAO, 2018). Milk proteins serve as essential components in various dairy products, contributing to their nutritional value, functionality, and sensory properties (Fox, 2003; Davoodi *et al.*, 2016; Mizumachi and Kurisaki, 2005). Proteins such as caseins and whey proteins are abundant in milk and play crucial roles in processes like emulsification, foaming, and gelation, thereby influencing the texture and stability of dairy products (De Wit, 1998; Balivo *et al.*, 2024; Whitney, 1988). Moreover, milk proteins are also valued for their high-quality amino acid composition, making them valuable dietary sources of essential nutrients, particularly for infants, children, and individuals with specific dietary requirements (Gellrich *et al.*, 2014). Despite the advantages, the industry faces various challenges, including low milk production, inadequate infrastructures, diseases like mastitis and poor milk quality (Maleko *et al.*, 2018; Bandyopadhyay and Joshi, 2022). Milk quality encompasses a combination of factors, including chemical composition, physical characteristics, bacteriological aspects, and aesthetic properties that enhance the desirability of milk products (Merwan *et al.*, 2018; Vincent *et al.*, 2016).

Despite increased dairy cattle production in peri-urban and urban areas of Tanzania aimed at meeting the high demand for milk, there still exists a notable gap between supply and demand. To bridge this gap, intensified milk production systems are being adopted, necessitating the use of pharmaceuticals and antibiotics for preventive, control, or treatment purposes (Van Boeckel *et al.*, 2015). However, the misuse of antibiotics, including failure to adhere to withdrawal periods, has led to the presence of drug residues in animal products such as milk. Several studies conducted in Tanzania have reported antibiotic residues in milk, some of which exceed maximum residue limits (Azabo *et al.*, 2022). This misuse of antibiotics not only poses risks to human health due to potential ingestion of these residues but also contributes to the development of antimicrobial resistance (Kosgey *et al.*, 2018; Caneschi *et al.*, 2023; Bukuku *et al.*, 2020).

In the dairy sector, commonly drugs used to combat bacterial infections are such as penicillin, oxytetracycline, enrofloxacin, sulfamethoxazole, tylosin, and albendazoles for worms (Mdegela *et al.*, 2021). The primary objective of this study was to investigate the most commonly used drugs in the dairy sector, specifically, to explore the binding interactions and modes between the drugs and major milk proteins using homology modeling and molecular docking techniques (Genheden *et al.*, 2017). Traditional methods for assessing drug residues in milk include both quantitative and qualitative techniques such chromatography, mass spectrometry, and immunoassays (Kumar *et al.*, 2022). While these techniques offer sensitivity and accuracy, they may not comprehensively capture drug-protein interactions and are expensive.

In silico analysis, including homology modeling and molecular docking simulations, offers a complementary approach to traditional methods for studying drug-protein interactions in milk (Guntero *et al.*, 2021). Through computational models and algorithms, three-dimensional structures of milk proteins and binding drugs to these proteins were studied. This approach enables the exploration of molecular interactions at the atomic level, providing insights into binding affinities and binding sites. Results shows that oxytetracycline, the widely used antibiotic has strong affinity with the milk proteins used in this study this imply that, there is higher residues of such drug in milk and hence contributing to antibiotic resistance in human.

Materials and methods

Identification of potential templates for homology modelling

Potential templates for homology modeling were searched against the Protein Data Bank (PDB).

Homology modeling was employed to generate a 3D model for bovine β-casein due to the unavailability of experimentally resolved crystal structure. The β-casein sequence, initially obtained from the Uniprot database (entry P02666), underwent sequence alignment using the Basic Local Alignment Search Tool (BLAST) within the UniProt database's post-translational modifications (PMT) section. A BLAST search with a query length of 209 amino acids (aa 16-224) yielded 228 hits with known protein structures matching the sequence. Two templates, α/β-gliadin MM1 UniProtKB (entry: P08079) and Bel602-DQ8.5-glia-γ1 complex Uniport (entry: P18573), were selected through this process (Zhou *et al.*, 2019). To compare and assess the similarities between the obtained fasta file of the target sequence and the selected templates, a sequence alignment was performed using Clustal W Omega for further analysis and visualization of secondary structures in ESpript. MODELLER (Modeller10.4) played a crucial role in template determination, extracting spatial restraints by aligning sequences with available crystal structures (Jalily Hasani and Barakat, 2017). The aligned sequences of both templates and target were then employed to generate 100 potential homology models. The selection of the best model was based on criteria such as Discrete Optimized Protein Energy (DOPE) and Root Mean Square Deviation (RMSD) concerning the template structure.

For comparison, I-TASSER was employed to construct separate 3D models of β-casein. I-TASSER contributed by identifying structural templates from the Protein Data Bank and assembling models. The quality of the model was predicted by C-score and checked by local accuracy via I-TASSER server (Yang *et al.*, 2015). Typically, C-scores range between -5 and 2, to assess the quality of its predicted models but based on the model generated the C-scores was 1.06 which was selected indicated good quality. To refine the models, the SAVES6.0 web server, incorporating tools like Verify 3D, PROVE, ERRAT, and PROCHECK, was utilized, ensuring comprehensive structural validation. PROCHECK analysis focused on stereochemical parameters, contributing to the overall assessment of model precision through Ramachandran plots (Dastmalchi *et al.*, 2016).

Molecular docking simulation

Protein preparation

In this study, 3D structures of various milk proteins, including β-lactoglobulin (PDB ID: 7ER3), chymosin (PDB ID: 4AA8), Bovine lactoperoxidase (PDB ID: 3GC1), lactoferrin (PDB ID: 1CE2), and Bovine serum albumin (PDB ID: 4F5S), were acquired from the RCSB Protein Data Bank (PDB) (Burley *et al.*, 2023). 3D structure of β-casein was modelled using I-TASSER and MODELLER tools. Initially, the proteins were prepared by removing all crystallographic water, ions, and co-crystallized ligands. Additionally, any missing side chain residues were added, and polar hydrogens were added at a physiological pH of 7.4 using the Auto Dock Vina tool as described by (Huey and Morris, 2008). Gasteiger charges were assigned to all proteins following the methodology outlined by (Gasteiger and Marsili, 1978). To facilitate grid-based molecular docking, the binding pockets of the proteins were identified utilizing co-crystallized ligands. In addition, blind docking experiments were conducted; the entire proteins were utilized as the receptor, allowing ligands to autonomously identify potential binding pockets within the protein structure (Torres *et al.*, 2019).

Ligand selection and preparation

The ligands, including oxytetracycline (CID: 54675779), penicillin (CID: 5904), albendazole (CID: 2082), and enrofloxacin (CID: 71188), were selected for the study. Their 3D structures were obtained from the PubChem Database (Kong *et al.*, 2020; Jagadeesh *et al.*, 2023). The ligands selected for investigation were prepared to ensure their suitability for docking simulations within a physiological context, characterized by a pH of 7.4. Initially polar hydrogen was added to the structures. Gasteiger charges (Gasteiger and Marsili, 1978) were added to account for electrostatic effects. Structures were energy minimized using MMFF94 force field and converted into the pdbqt file format using Open Babel (Kumar *et al.*, 2021) ready for docking calculation as required by Auto Dock Vina 11.2v tool (Trott and Olson, 2010).

Docking calculations

During docking simulations, proteins were positioned at the following coordinates, the dimensions for β-lactoglobulin (X = -13.658, Y= -0.878, Z= 0.477), lactoferrin (X = 10.832, Y= 12.521, Z= 68.967) bovine albumin serum (X = 10.720, Y= -13.963, Z= -11.393) Chymosin (X = 17.456, Y= 33.675, Z= 42.342), bovine lactoperoxidase (X = 9.542, Y= -14.012, Z= -12.201) and β-casein (X = 31.651, Y= 37.174, Z= 39.729). The docking boxes surrounding each protein were defined with dimensions ranging from 20 to 100 units along each axis (X, Y, and Z) with 8 set of exhaustiveness (Attique *et al.*, 2019).

Throughout the process, the proteins remained rigid, while the ligands were allowed to flexibly interact. Default docking parameters were applied consistently across all experiments. Each ligand was docked independent to all proteins, with 10 iterations conducted for each protein-ligand pair. For post-docking simulation, Discovery Studio tools was employed for analysis of the resulting interactions between the milk proteins and ligands, offering visualization of their binding configurations and intermolecular contacts.

Results and discussion

Homology modeling of bovine β-casein protein

To address the lack of experimental resolution for β-casein, a 3D structure was constructed based on its amino acid sequences using homology modeling. A template search with BLAST homology was conducted, and the most effectively identified template were γ-gliadin (PDB ID: 5KSA, E-value 5.6 × 10-6 resolved at 3.05Å) and α/β gliadin MM1(PDB ID: 2NNA, E-value 1.1×10-2 resolved at 2.0Å) and selected as templates with 29.8% and 32.4% sequences identity and the sequence coverage was 83% and 68% with that β-casein, respectively, (Zhou *et al.*, 2019). The homology modeling process started with the use of sequence search tools to identify sequences showing evolutionary similarity and identity. In selecting templates for homology modeling, a criterion was set that the chosen sequences must exhibit ≥ 30% identity Fiser (2010). This threshold ensured that the selected templates shared a substantial degree of similarity with the target β-casein, laying the foundation for accurate and meaningful homology modeling.

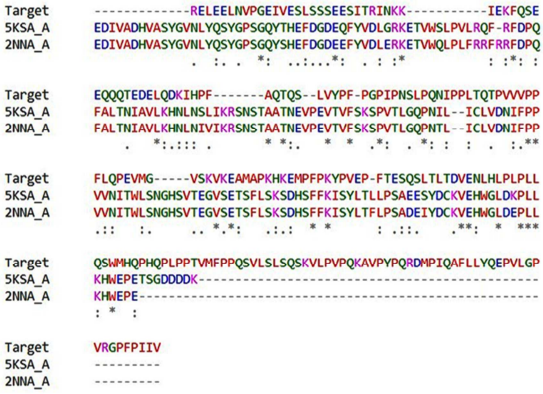

Fig. 1. The aligned sequence conveys into the functional and evolutionary relationships on target (Bovine β-casein) with templates γ-gliadin (5KSA) and α/β-gliadin (2NNA)

Key: *Residues or nucleotides in that column are identical in all sequences in the alignment.

:Conserved substitutions have been observed, according to the COLOUR

:Semi-conserved substitutions are observed, i.e., amino acids having a similar

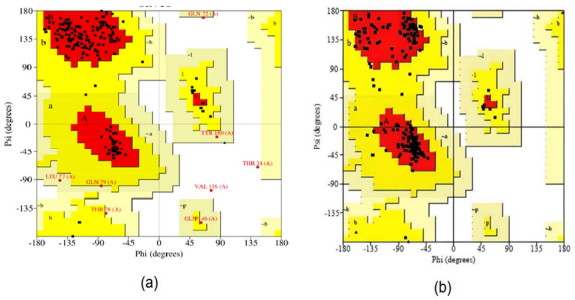

Fig. 2. Ramachandran Plot of the modelled protein from the (a) MODELLER and (b) I-TASSER tools

Sequences alignment of target-templates

Amino acid sequences of target (bovine β-casein was aligned using Clustal W Omega tools with sequences of templates (γ-gliadin (5KSA) and α/β-gliadin (2NNA). The conserved regions in both target (bovine β-casein) with templates γ-gliadin (5KSA) and α/β-gliadin (2NNA), are indicated by the same color in the column (Fig. 1). Conserved residues play a crucial role in maintaining the protein 3D structure, which is essential for its proper function (Bianchetti *et al.*, 2005).

Table 1. The summary of the Ramachandran plot for statistical analysis of modelled β-casein from MODELLER and I-TASSER

Ramachandran plot regions and number of residues	MODELLER		I-TASSER	
	No. residues	%	No. residues	%
Residues in most favored regions [A, B, L]	146	87.4	146	89.4
Residues in additional allowed regions[a,b,l,p]	13	7.8	17	10.4
Residues in generously allowed regions [~a, ~b,~l,~p]	5	3.0	0	0.0
Residues in disallowed regions	3	1.8	0	0.0
Number of glycine and non-proline residues	167		163	
Number of end residues (ex. Gly and Pro)	2		7	
The number of Glycine residues shown as triangles	5		5	
Number of proline residues	35		4	
Total number of residues	209	100	209	100

The Ramachandran plot was utilized to characterize the modeled protein structure, offering insights into the dihedral angles of the polypeptide chain and the distribution of phi (ϕ) and psi (ψ) angles of residues within the protein. These angles govern the rotation of peptide bonds between consecutive residues and are pivotal in determining the overall conformation of the protein backbone. Residues falling within the favored regions on the plot indicate accurately modeled regions, while outliers may suggest potential errors or inaccuracies in the modeled structure. Both Modeller and I-Tasser assessments indicate the quality of the modeled protein at 87.4% and 89.6%, respectively, with residues predominantly situated in the favored regions (Fig. 2, Table 1). The favored regions depicted on a Ramachandran plot represent combinations of phi and psi angles that are both energetically allowed and sterically favored. Residues positioned within these favored and allowed regions signify well-modeled backbone conformations, suggesting higher-quality structures, particularly when the percentage of residues in favored regions equals or exceeds 90% Zhang (2008).

Table 2. Experimental binding free energy and calculated binding energy values, and root mean square deviation (RMSD)

PDB code	Calculated energy	Experimental energy	RMSD (Å)
2ZC9	-38.911	-35.4	0.26
2ZF0	-37.656	-34.8	0.87
2ZHQ	-37.234	-37.8	1.01
2ZIQ	-37.237	-37.4	1.12
2ZI2	-33.890	-32.8	1.21
2ZFP	-33.472	-31.3	1.17
2ZDA	-44.003	-46.1	1.22

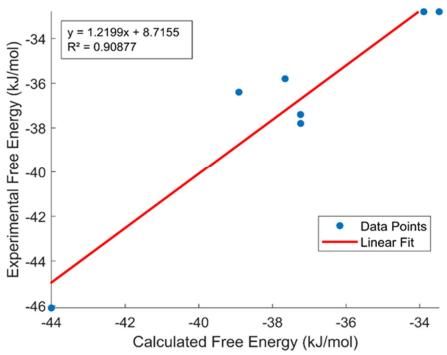

Fig. 3. Correlation of experimental binding energy values with corresponding calculated binding energy in kJ/mol ($r^2 = 0.91$)

Molecular docking benchmarking and validation

Molecular docking benchmarking and validation are essential prerequisites for any docking calculations for ensuring the accuracy, reliability, and predictive power of docking methodologies (Torres *et al.*, 2019). The docking protocol underwent validation using an experimental dataset reported by (Bauman et. al 2009) serving as a benchmark for evaluation. The validation results, presented in Table 2 and Fig. 3, demonstrate the tool capability to reproduce experimental binding affinities. Additionally, Root Mean Square Deviation (RMSD) analysis was employed, a metric known for its ability to replicate experimental binding modes. This study revealed that the re-docked complexes exhibited an RMSD value of less than 2 Å, a threshold consistently reported in literature to accurately replicate experimental binding modes (Gohlke *et al.*, 2000). Such meticulous validation instilled confidence in the reliability of the protocol and tool, thereby enabling the subsequent docking calculations with assurance.

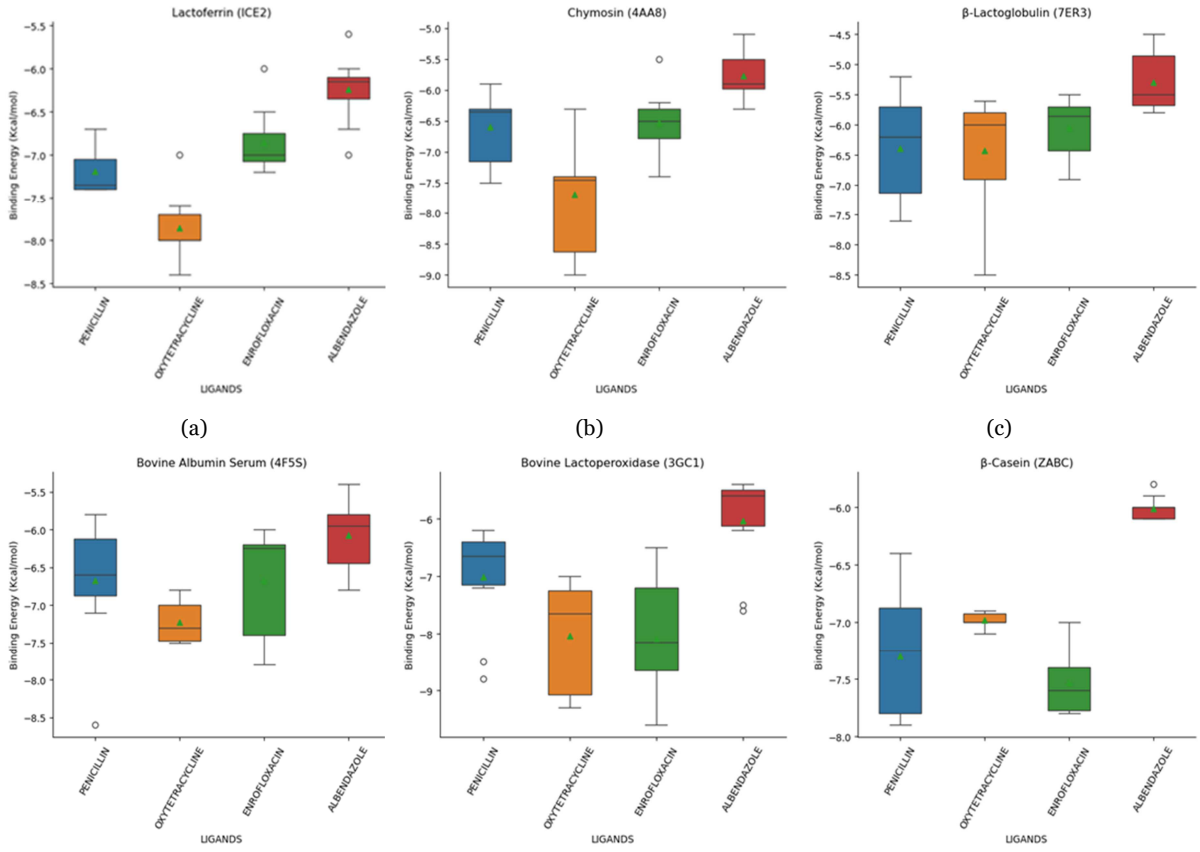

Fig. 4. The binding affinity between drugs and milk proteins (a) Lactoferrin (b) Chymosin (c) β-lactoglobulin (d) Bovine albumin serum (e) Bovine lactoperoxidase (f) β-casein

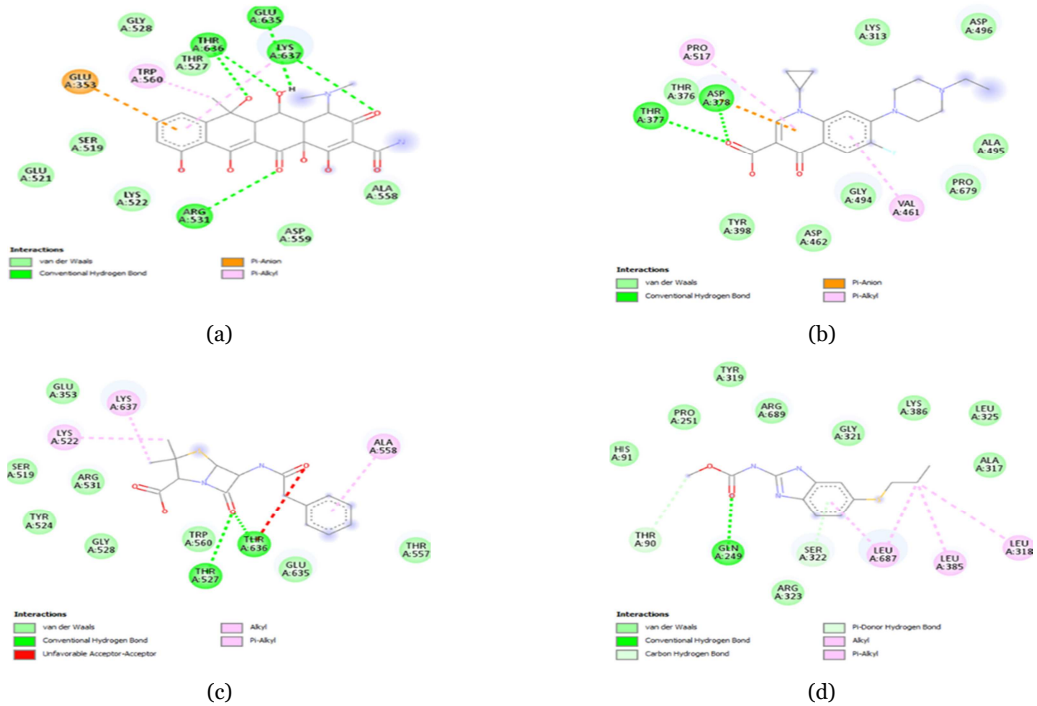

Fig. 5. The binding interactions of lactoferrin (1CE2) (a) oxytetracycline (b) enrofloxacin (c) penicillin and (d) albendazole

Binding free energy analysis

In the docking process, the binding affinity values were scrutinized, where more negative values indicate favorable and energetically stable interactions between drugs and the proteins. On the other hand, the observed strong affinity provides suggestive drug residues in milk proteins as observed in lactoperoxidaese and enrofloxacin and oxytetracycline Fig. 4. The general observation is that, albendazole, a molecule used to treat worms, generally ranked the least, (with poor binding affinity) across all the proteins (Fig. 4 a-f). Enrofloxacin, is another molecular that showed least binding affinity across the proteins, except two protein PDB IDs: 3GC1 and ZABC (Fig. 4 e, f). Penicillin shows an appreciable strong affinity across milk proteins. The most ligand that consistently ranked high across all proteins was oxytetracycline. The general trends of the binding affinity are enrofloxacin > oxytetracycline > penicillin > albendazole. These findings suggest that, oxytetracycline has high affinity with milk proteins, and could have higher residues in milk, a factor that could contribute to antibiotic drug resistance in human. The findings, collaborates well with (Liang *et al.*, 2024) who reported the high oxytetracycline residues in milk protein (Mohamed *et al.*, 2020).

Binding interaction analysis

To gain further insights and establish the origin of the difference in binding affinity, interaction analysis of drugs and their receptor was carried out. The interaction of drugs with lactoferrin (PDB ID: ICE2) (Fig. 5 a-d) displays diverse interaction patterns that resulted in difference binding affinities. For example, oxytetracycline which displayed a binding affinity of -7.9 kcal/mol interacted with Thr636, Arg531, Glu635, and Lys637 forming hydrogen bonds (Fig. 5a). However, drugs such as albendazole which showed poor affinity only interacted with lactoferrin by forming a single hydrogen bond with Gln 249 (Fig. 5d), such residue was not observed to form a hydrogen bond with oxytetracycline or even other drugs that showed a strong affinity with the receptor lactoferrin (Fig. 5c-d). This suggests that albendazole exhibited a slightly different binding mode in the active site of lactoferrin which resulted in a weaker interaction and affinity.

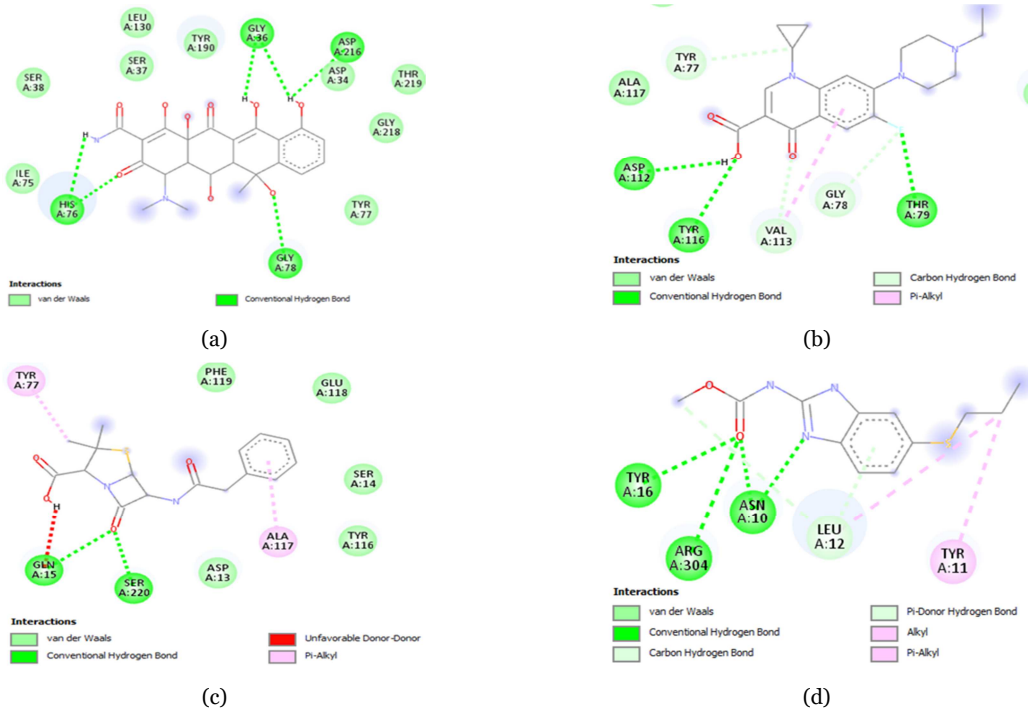

Fig. 6. The binding interactions Chymosin (4AA8) with molecule (a) oxytetracycline, (b) enrofloxacin, (c) penicillin, and (d) albendazole

The interaction of chymosin (PDB ID: 4AA8) with various drugs provides insights into the observed differences in binding affinities (Fig. 6a-d). For instance, oxytetracycline (-7.7 kcal/mol) which showed the strongest affinity than albendazole (-5.8 kcal/mol), interacted with chymosin by forming hydrogen bonds with His76, Gly78, Gly36 and Asp216. Some van der Waals interactions with Ile75, Ser38, Ser37, Ty190, Tyr77 and Leu130 which stabilized the interaction are also observed in oxytetracycline complex. Enrofloxacin forms hydrogen bonds with Asp112, Thr116, and Thr79. It also shows van der Waals interactions with Ala117, Tyr77, and Phe119, π-alkyl interactions with Val113, and π-sigma interactions with Phe113. Penicillin only forms a single hydrogen bond with Ser220, however, van der Waals interactions with Gly78, Phe119, Glu118, Ser14, Tyr116, Asp13, plays an important role in stabilization of the complex. Albendazole forms hydrogen bonds with Tyr16, Asn10, and Arg304. It also forms π-alkyl interactions with Tyr11, and Alkyl interactions with Leu12 that favors hydrophobic interaction. Albendazole, with a binding energy of -5.8 kcal/mol, has a less negative value compared to oxytetracycline. This suggests a relatively weaker binding affinity between albendazole and chymosin.

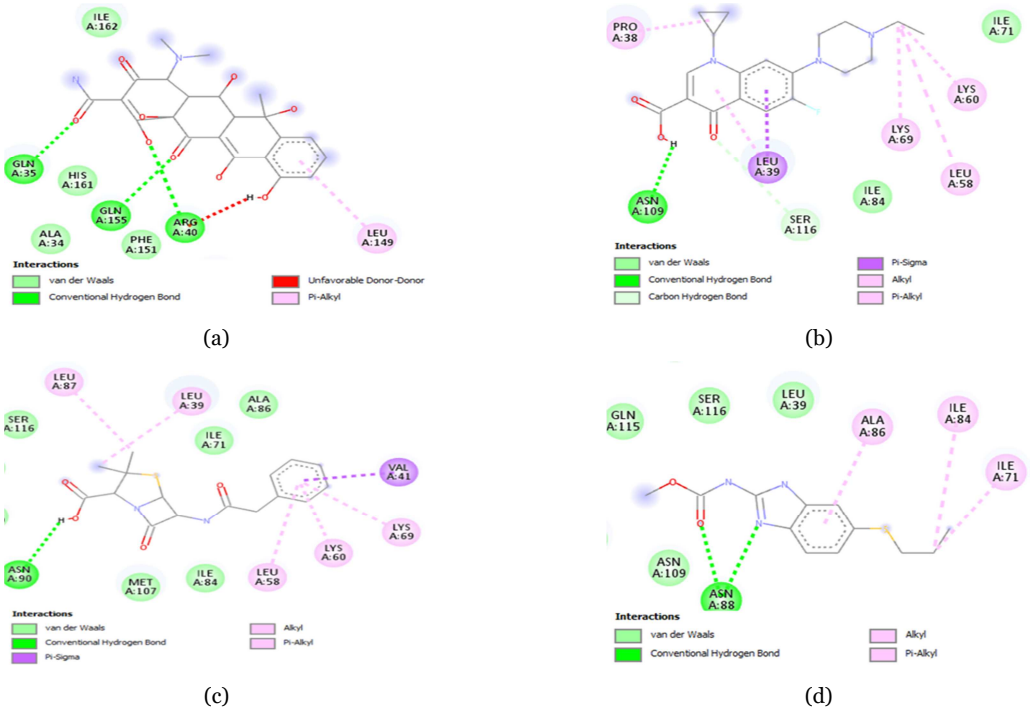

Fig. 7. The binding interactions β-lactoglobulin(7ER3), (a) oxytetracycline, (b) enrofloxacin, (c) penicillin, and (d) albendazole

The interaction of β-lactoglobulin (PDB ID: 73R3) with various drugs provides insights into the observed differences in binding affinities (Fig. 7a-d). For instance, oxytetracycline demonstrates a higher binding affinity of -6.5 kcal/mol with β-lactoglobulin and formed a hydrogen bond with Arg40, Gly155, and Gly35. Additionally, van der Waals interactions are observed with His161, Phe151, and Phe162, contributing to stabilization. Furthermore, a π-alkyl interaction with Leu149 enhances the interaction between oxytetracycline and β-lactoglobulin complex. Enrofloxacin, on the other hand, presents a moderate binding affinity, reflected in its binding energy of -6.1 kcal/mol. It creates hydrogen bonds involving Ser116 and Asn109 and exhibits van der Waals interactions with Leu87, Val41, Ile71, and Ile84. Moreover, it demonstrates π-alkyl interactions with Leu39, π-sigma interactions with Leu39, and Alkyl interactions with Pro38, Leu58, Lys69, and Lys60 as in (Fig. 7a). Lastly, albendazole demonstrates a weaker binding affinity, with a binding energy of -5.3 kcal/mol (Fig. 7d). Hydrogen bond formed with Asn88 and engages

in van der Waals interactions with Asn109, Leu39, Leu31, Ser116, and Gly115 and π-alkyl interactions with Ala86 and alkyl interactions with Ala86, Ile84, and Ile71. The aromatic rings of oxytetracycline engage in π-alkyl interactions with aliphatic hydrocarbon chains found in amino acid residues like leucine, isoleucine, and valine within beta-lactoglobulin (Makwana and Mahalakshmi, 2015). Lastly, pi-anion interactions occur between the aromatic rings of oxytetracycline and negatively charged side chains, such as aspartate and glutamate, within β-lactoglobulin, contributing to the electrostatic component and further stabilizing the complex as reported by (Habibian-Dehkordi *et al.*, 2022).

Bovine Serum Albumin (PDB ID: 4F5S) interacts with oxytetracycline and provides the binding affinities of -7.2 kcal/mol (Fig. 8a), the oxytetracycline, forms hydrogen bonds with Ser109, Asp111, and Arg185, while engaging in van der Waals interactions with Asp108, Arg144, His145, Lys114, and Pro110 that stabilizes bovine serum albumin the complex. Enrofloxacin and penicillin forms hydrogen, van der Waals interactions, π-alkyl interactions and Pi-cation interactions (Fig. 8 a-d). Albendazole forms a -6.1kcal/mol and formed hydrogen bond with Ser191, engages in van der Waals interactions with Tyr156, Glu152, Tyr149, Arg198, and Arg194. Furthermore, it demonstrates π-alkyl interactions with Lys187, π-sigma interactions with Thr290, and π-alkyl interactions that destabilizes the complex. The hydrogen bonds formed by oxytetracycline contribute to the stability of the drug-protein complex by forming strong electrostatic interactions. In addition to hydrogen bonding, oxytetracycline also engages in van der Waals interactions with other amino acid residues within the binding pocket, including Asp108, Arg144, His145, Lys114, and Pro110. These van der Waals interactions enhance the overall binding affinity of oxytetracycline by promoting close contact and favorable interactions between the drug molecule and the protein. Oxytetracycline fit more into the binding pocket compared to other drugs, allowing for more extensive interactions and a higher binding affinity. The chemical structure of oxytetracycline possess functional groups or motifs that are particularly complementary to the binding pocket of the bovine serum albumin, enabling strong and specific interactions that contribute to its high binding affinity (Chi *et al.*, 2010).

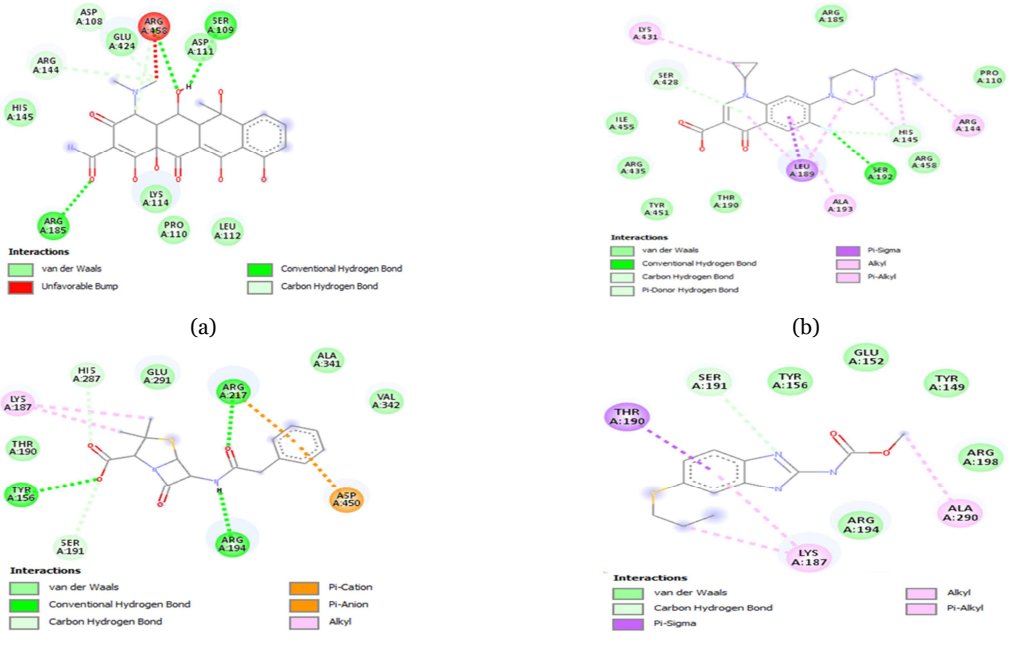

Fig. 8. The binding interactions Bovine albumin serum (4F5S) with ligands: (a) oxytetracycline, (b) enrofloxacin, (c) penicillin, and (d) albendazole

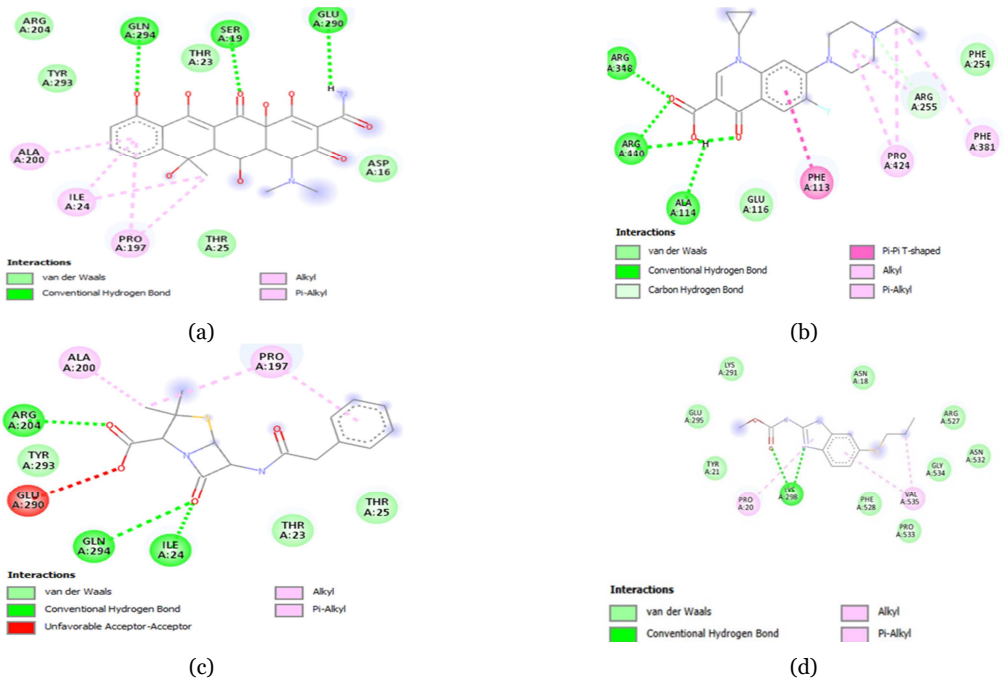

Fig. 9. The binding interactions between bovine lactoperoxidase (3GC1), with(a) oxytetracycline, (b) enrofloxacin, (c) penicillin, and (d) albendazole

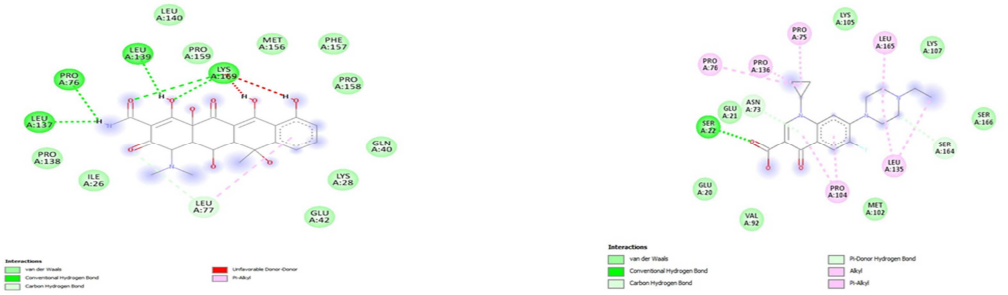

Fig. 10. Illustrates the binding interactions β-casein (ZABC) with (a) oxytetracycline, (b) enrofloxacin

The enrofloxacin and oxytetracycline exhibits exceptional binding affinities with bovine lactoperoxidase (3GC1) of -8.4, and -8.3 kcal/mol (Fig. 9b). Enrofloxacin demonstrates a remarkable binding energy of -8.4 kcal/mol, establishing hydrogen bonds with key amino acids such as Arg440, Arg348, Ala114, and Arg255. It also engages in significant van der Waals interactions with His109, Pro236, Phe254, and Glu116. Moreover, it showcases unique interactions, including π-alkyl interaction with Pro424 and π-π interaction with Phe113, contributing to its high binding affinity (Fig. 9b). Oxytetracycline exhibits a robust binding energy of -8.3 kcal/mol, forming hydrogen bonds with Gln294, Ser19, and Glu290. It also engages in substantial van der Waals interactions with Arg204, Tyr293, Arg22, Asp16, and Thr25, as well as alkyl and π-alkyl interactions with Ile24, Pro197, and Ala200 (Fig. 9a). Conversely, albendazole shows a comparatively lower binding energy of -6.0 kcal/mol, indicating a weaker interaction profile with bovine lactoperoxidase. While it forms hydrogen bonds and van der Waals interactions, the interaction profile reveals significant Van der Waals interactions with amino acids Lys291, Glu295, Tyr21, Phe528, Asn18, Arg527, Asn532, Pro533, Gl534, and Phe528. These interactions contribute to the stabilization of the complex formed between the protein and ligands. Additionally, a notable hydrogen bond is formed with Lys298, further enhancing the binding affinity (Fig. 9d). However, the absence of additional strong interactions like π-π interactions may contribute to its

reduced binding affinity compared to enrofloxacin and oxytetracycline.

Enrofloxacin form specific interactions with the amino acid residues within the binding site of bovine lactoperoxidase that result in stronger binding compared to oxytetracycline. These interactions include hydrogen bonding, van der Waals forces, and electrostatic interactions, which contribute to the stability of the drug-protein complex (Fig. 9a-b). The chemical structure of enrofloxacin possess specific functional groups such as quinolone core, fluoro group, carboxylic acid group, piperazinyl ring and substituted amino groups or motifs that enhance its ability to bind to the protein with higher affinity. Enrofloxacin may have better access to the binding site of bovine lactoperoxidase to penetrate deeper into the binding pocket, allowing for stronger interactions with Arg440, Arg348, Ala114, and Arg255 residues. This increased accessibility could contribute to its higher binding affinity compared to oxytetracycline. Enrofloxacin exhibit greater conformational flexibility, allowing it to adopt optimal orientations within the binding site of bovine lactoperoxidase and establish stronger interactions with key residues. This flexibility enables enrofloxacin to adapt to the structural features of the protein, enhancing its binding affinity (Kalin *et al*., 2022).

The oxytetracycline and amino acid residues of β-casein involves a diverse array of interaction with molecular forces influencing their respective binding energies. These forces encompass π-alkyl interactions, hydrogen bonding, van der Waals forces, and π-anion interactions (Fig. 10a-b). The disparities in binding energies among these antibiotics may arise from the involvement of specific amino acid residues, geometric intricacies in the interactions, or the distinctive chemical characteristics inherent to each antibiotic. Considering their interaction with Enrofloxacin -7.5 kcal/mol exhibit a robust binding energy, these variations are likely attributed to the unique nature of interactions and the three-dimensional configuration of enrofloxacin within the β-casein binding site. The favorable alignment of hydrogen bonding, π-alkyl interactions, van der Waals forces, and π-anion interactions in the penicillin-beta-casein complex could contribute to the higher observed binding energy (Dantas *et al*., 2020).

Conclusion

Through molecular docking study, oxytetracycline and enrofloxacin demonstrating strong binding affinity to milk proteins, alongside the characterization of their binding modes and the key amino acid residues such as alkyl (Ala200, Ile24, Pro197), hydrogen bond (Gln294, Ser19, Glu290), Van Der Waals (Arg204, Tyr293, and Thr25) and hydrogen bond (Arg348, Arg440, Ala114), Van der Waals (Phe254, Glu116), π-π T-Shaped (Phe113) And Π-Alkyl (Pro424) involved in these interactions respectively. This investigation further assessed how such drug-protein interactions could potentially impact the structure and function of milk proteins. Understanding these interactions becomes paramount not only for ensuring the efficacy of drug therapies in dairy animals but also for minimizing any adverse effects on milk composition and properties. Consequently, there is need for additional research efforts and regulatory measures aimed at evaluating the risks associated with drug residues in milk. Researchers who are dealing with studying on how antibiotics engage with biological systems to design more targeted and effective antibiotics, minimizing potential side effects and optimizing therapeutic outcomes. This ongoing exploration contributes to the continuous improvement of antibiotic therapies, enhancing their precision and safety in medical applications.

Recommendation(s)

Rigorous testing of milk for antibiotic residues is standard practice before it is brought to market. Farmers are advised to observe appropriate withdrawal periods and adhere to good agricultural practices to ensure that milk remains free from antibiotic residues. Further research to be conducted in vitro and in vivo studies to validate the predicted interactions and assess their impact on drug distribution, absorption, bioavailability and efficacy.

In molecular docking, the goal is to investigate on interaction and binding mode and affinity of milk proteins with drugs, which can have various applications in various fields such as structural biology, biochemistry, biomedical research, and the development of food products.

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Conflict of interests

Authors declare no conflict of interests.

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