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Original Article

Identification of small molecule inhibitors of penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus* for the therapeutics of bacterial infection

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Abstract

The penicillin-binding protein 2a (PBP2a) is a key enzyme associated with bacterial cell wall synthesis and bacterial infection. Therefore, targeting PBPa2 offers a promising approach for the therapeutics of bacterial resistance and infection. This study presents a comprehensive analysis of alpha-mangostin as a potential inhibitor of PBPa2. Molecular docking simulations revealed a robust binding affinity between alpha-mangostin and PBP2a, with an affinity score of -6.01 kcal/mol. Notably, alpha-mangostin formed a preferential hydrogen bond with THR216 of PBP2a, alongside several other polar and hydrophobic interactions. ADME and Toxicity predictions indicated that alpha-mangostin possesses favorable pharmacokinetic properties, suggesting its potential as a therapeutic agent. PASS analysis further highlighted its broad range of good biological properties. SwissTargetPrediction analysis reinforced these findings, indicating alpha-mangostin's association with various biological processes. Cell toxicity assays demonstrated that alpha-mangostin had no significant impact on the viability of HEK-293 cells, suggesting its potential safety for further development. The IC50 value for alpha-mangostin was found to be 33.43µM. Fluorescence-based binding assays showed that alpha-mangostin effectively inhibited PBP2a activity in a concentration-dependent manner, supporting its role as an inhibitor. In conclusion, the results suggest alpha-mangostin as a promising candidate for inhibiting PBP2a. Further, extensive studies are warranted to explore its clinical applications.

Keywords: Alpha-mangostin, Bacterial resistance, Inhibition, Molecular docking, HEK-293, Cell toxicity and binding assays.

1. Introduction

The discovery of antibiotics revolutionized modern medicine and significantly extended the human lifespan by enabling the effective treatment of bacterial infections. However, over time, bacteria have developed various mechanisms to resist the action of antibiotics, posing a significant threat to public health [1]. One such mechanism involves the production of penicillin-binding protein 2a (PBP2a), which plays a pivotal role in antibiotic resistance, particularly in the case of methicillin-resistant *Staphylococcus aureus* (MRSA) [2,3]. Penicillin-binding proteins (PBPs) are enzymes that synthesize bacterial cell walls [4]. They are named after their affinity for penicillin and related β-lactam antibiotics. PBP2a, a member of the PBP family, is distinctive in that it exhibits a low binding affinity for β-lactam antibiotics, allowing it to continue catalyzing cell wall synthesis even in the presence of these drugs [5]. The mecA gene encodes PBP2a, commonly found in *Staphylococcus aureus* strains, including MRSA [6]. Its molecular structure differs from other PBPs due to amino acid substitutions, particularly in the active site [7,8]. These substitutions reduce its binding affinity for β-lactam antibiotics, rendering traditional antibiotics ineffective against bacteria expressing PBP2a [9].

The bacterial cell wall is a crucial structural component that provides rigidity and protects the cell from environmental stresses [10]. It consists of peptidoglycan, a polymer composed of sugar chains and peptide cross-links. PBPs catalyze the cross-linking of peptidoglycan strands, contributing to the integrity of the cell wall [4] PBP2a is specifically responsible for the transpeptidation reaction, which forms the cross-links between peptidoglycan chains [6].

Understanding the mechanisms of PBP2a-mediated antibiotic resistance is essential to combatting infections caused by MRSA and other PBP2a-producing bacteria. Antibiotics work by inhibiting PBPs, preventing the formation of cross-links in the bacterial cell wall, and ultimately leading to cell lysis [11]. In MRSA and related strains, PBP2a provides a unique mechanism of resistance. Its reduced binding affinity for β-lactam antibiotics allows the bacteria to maintain cell wall integrity even when exposed to these drugs [12]. As a result, the antibiotics cannot effectively disrupt cell wall synthesis, rendering them ineffective against PBP2a-producing bacteria [13]. PBP2a-mediated antibiotic resistance presents significant clinical challenges. MRSA infections, for example, are

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notoriously difficult to treat with traditional β-lactam antibiotics [1,2,3]. Healthcare providers must resort to alternative antibiotics, such as vancomycin, which can have limitations due to factors like drug resistance and adverse effects.

Efforts to combat PBP2a-mediated antibiotic resistance involve a combination of approaches, including drug development, combination therapy, and infection control measures. Researchers are actively investigating and designing molecules that either inhibit PBP2a directly or overcome its resistance mechanisms. These inhibitors block PBP2a's role in cell wall synthesis, again making the bacteria susceptible to β-lactam antibiotics. The potential for therapeutics targeting PBP2a offers hope for more effective treatments against antibiotic-resistant bacterial infections, ultimately improving patient outcomes and public health.

2. Material and Methods

2.1. Prepare the target and perform molecular docking.

The crystal structure of penicillin-binding protein 2a (PBP2a) from MRSA was retrieved from the Protein Data Bank (PDB ID: 4CJN) [14]. This structure was resolved at 1.95 Å and was in a complex with quinazolinone ligand molecule. Further NPACT database [15], containing 1574 entries, was screened and alpha-mangostin was selected as lead molecule. Further, we retrieved the 2D structure of the ligand molecule, alpha-Mangostin (Compound CID: 5281650), from PubChem with (https://pubchem.ncbi.nlm.nih.gov/#query=alpha-Mangostin). Figure 1 represents the structure of alpha-mangostin. To ensure the target protein's suitability for docking studies, a standard receptor preparation protocol was applied [16,17]. Docking calculations were carried out using DockingServer [18]. Gasteiger partial charges were added to the ligand atoms. Nonpolar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on alpha-Mangostin;protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [19]. Affinity (grid) maps with a spacing of 0.375 Å were generated using the Autogrid program [19]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [20]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

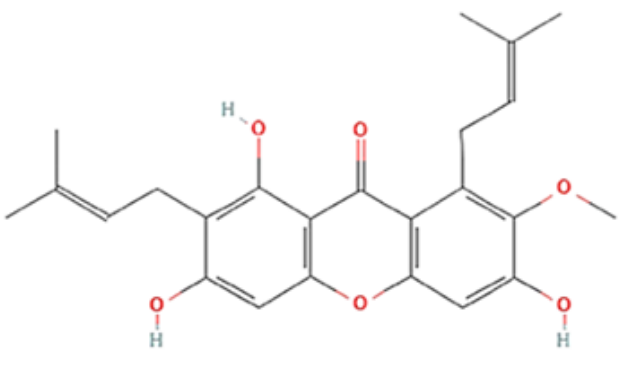


Fig. 1. Structure of alpha-mangostin.

2.2. ADME & Toxicity properties and expectation of activity spectra for alpha-mangostin using PASS online and Swiss Target Prediction tool

The assessment of the ADME & Toxicity (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics of the selected alpha-mangostin molecule was conducted through the utilization of the pkCSM online tool. The SMILE ID corresponding to alpha-mangostin was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/#query=alpha-Mangostin) and subsequently employed in the pkCSM server to elucidate its ADME and Toxicity properties.

In the realm of chemoinformatics and drug discovery, the Prediction of Activity Spectra for Substances (PASS) serves as a computational instrument for forecasting the biological activities inherent to chemical compounds. For the execution of the PASS analysis, the chemical structure of alpha-mangostin, delineated in the standardized "SMILES" format, was submitted to the online server accessible at http://www.pharmaexpert.ru/passonline/ to ascertain the likelihood of biological activities associated with this compound.

Furthermore, to explore the potential associations of alpha-mangostin with various biological properties, the SwissTargetPrediction online tool, accessible via http://www.swisstargetprediction.ch/, was also employed in this investigation.

2.3. Preparation of test compound HEK-293 cell line

Alpha-mangostin was acquired from Sigma, USA, and then solubilized in dimethyl sulfoxide (DMSO) (Sigma, USA) to prepare a stock solution with a concentration of 100 µg/mL. The HEK-293 cell line was sourced from NCCS, Pune, and was cultured in DMEM media with 10% FBS and 1% antibiotics.

2.4. Cell culture & cytotoxicity assay

In this experimental procedure, HEK-293 cells were cultured within a 96-well microplate at a seeding density of 5,000 cells per well, each containing 100 µL of DMEM medium. Subsequently, the microplate was placed inside a CO2 incubator under 37°C temperature and controlled humidity of 5% for 24 hours. Following this initial incubation period, the cells were subjected to treatment involving varying concentrations of alpha-mangostin (0 to 1000 M) for an additional 24 hours. The next step entailed replacing the culture medium in each well with 100 µL of fresh medium containing the MTT reagent, which was added to achieve a final concentration of 0.5 mg/mL. The cells were then subjected to a further incubation period lasting 4 hours at 37°C. After this 4-hour incubation, the medium containing the MTT reagent was carefully aspirated from the wells, and to each well, 100 µL of dimethyl sulfoxide (DMSO) was introduced. This addition of DMSO was necessary to facilitate the dissolution of the purple formazan crystals produced by the MTT reagent. To ensure complete dissolution, the microplate underwent gentle agitation

for 10 minutes. To quantify the outcome, the absorbance of each well was measured at 570 nm using a dedicated plate reader. The calculation of net absorbance involved subtracting the absorbance values of blank wells, which contained solely culture medium and MTT reagent, from the absorbance values of the test wells. Finally, to determine the percentage of cell viability, the net absorbance of the test wells was divided by the net absorbance of the control wells (comprising cells without any treatment), with the resulting quotient being multiplied by 100. Similarly, a time-dependent cell viability assay (for 24 hrs, 48 hrs, and 72 hrs) was conducted to investigate the effect of IC50 concentration of alpha-mangostin on HEK-293 cells.

2.5. *In vitro* inhibition assay

The recombinant penicillin binding protein, which had been subjected to affinity purification exceeding 90% purity as verified by SDS-PAGE, was procured from Abcam (Product ID: ab168007). Fluorescence binding studies involving the interaction between penicillin binding protein and alpha-mangostin were carried out utilizing a JASCO 6300 spectrofluorometer equipped with a 1 cm quartz cell. The generation of fluorescence spectra for both penicillin binding protein and the protein-ligand complex was achieved through the following sequential steps: (i) Excitation of the protein was initiated at a wavelength of 280 nm; (ii) Emission spectra emanating from the protein were recorded across the range of 300-500 nm and; Parameters for excitation and emission slit widths were set at 10 mm, and the response setting was configured to medium sensitivity. Subsequently, the fluorescence data acquired from the protein and the protein-ligand complex were subjected to meticulous analysis. This analysis was undertaken with the primary objective of extracting various binding parameters pertinent to the protein-ligand complex, thus elucidating the inhibitory effects of alpha-mangostin on penicillin binding protein.

3. Results

3.1. Molecular docking revealed alpha-mangostin as potential inhibitor of PBP2a

The molecular docking results of the PBP2a:alpha-mangostin complex are presented in Table 1 & Table 2 and Figure 2a-d. The docking analysis yielded affinity scores and docked poses, revealing that alpha-mangostin exhibited a notable binding affinity score of -6.01 kcal/mol with PBP2a (Table 1). Figure 2a represents the cartoon presentation of PBP2a:alpha-mangostin complex. Figure 2b presents the 2D plot and Figure 2c represents the amino acid residues of PBP2a making different interactions with alpha-mangostin. Figure 2d represents the surface view of PBP2a:alpha-mangostin complex. The docking pose exhibited the successful docking of alpha-mangostin into the deep binding cavity of PBP2a. Furthermore, the docking results demonstrated a preferential hydrogen bond interaction of alpha-mangostin with the THR216 amino acid residue of the PBP2a (Table 2).

Additionally, alpha-mangostin formed several polar, hydrophobic and other types of the interactions with PBP2a (Table 2). Further the interaction of alpha-mangostin with the amino acid residues of PBP2a was also confirmed

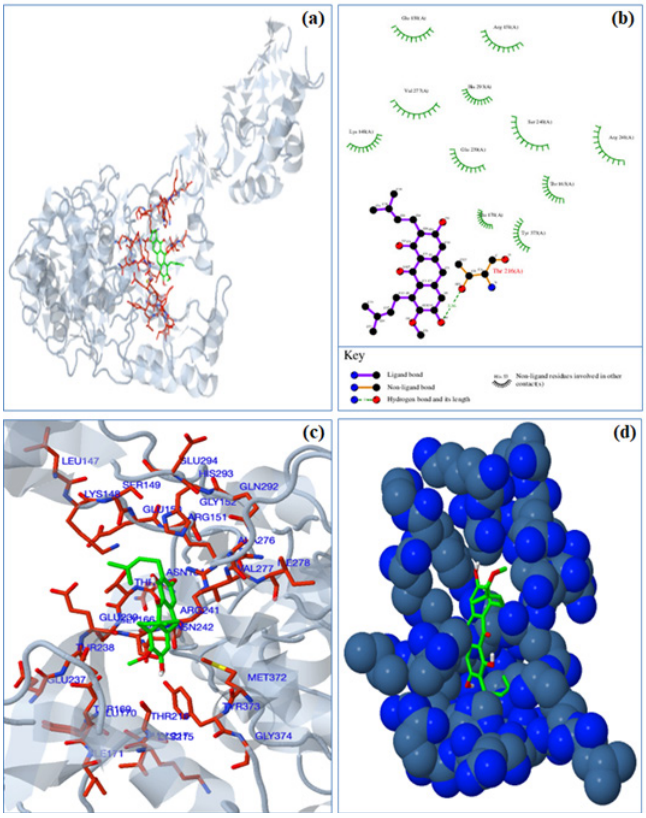


Fig. 2. Molecular docking analysis PBPa2:alpha-mangostin complex. (a) represents the cartoon presentation of PBP2a:alpha-mangostin complex; Grey: protein backbone; Red: Interacting side chains of the protein; Green: Ligand molecule. (b) represents the 2D plot. (c) represents the amino acid residues of PBP2a making different interactions with alpha-mangostin. (d) represents the surface view of PBP2a:alpha-mangostin complex.

Table 1. Binding affinity and estimation of inhibition constant (Ki) of Penicillin-binding protein 2a (PBP2a) and alpha-mangostin complex.

Compound	Target protein	Binding affinity (kcal/mol)	Est. Inhibition Constant, Ki
Alpha-mangostin	Penicillin-binding protein 2a	-6.01	39.47 uM

Table 2. Decomposed Interaction Energies in kcal/mol.

Hydrogen bonds	Polar	Hydrophobic	Other
THR216 (-0.4488)	ARG241 (-1.0232)	VAL277 (-1.056)	SER240 (-1.0635)
	ARG151 (-0.8893)	HIS293 (-0.4414)	GLU150 (-0.7992)
	LYS148 (-0.623)		GLU239 (-0.7454)
			TYR373 (-0.5489)
			THR165 (-0.5071)
			GLU170 (-0.2194)

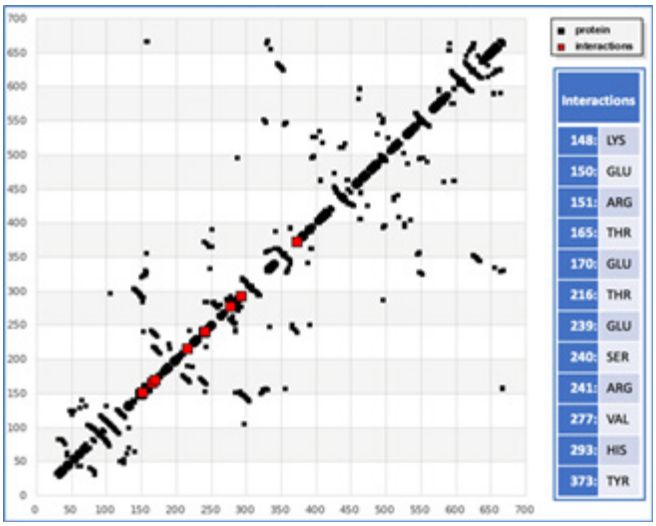


Fig. 3. HBPlot analysis of alpha-mangostin: PBP2a complex. This figure is representing interaction of alpha-mangostin with the amino acid residue number of PBP2a.

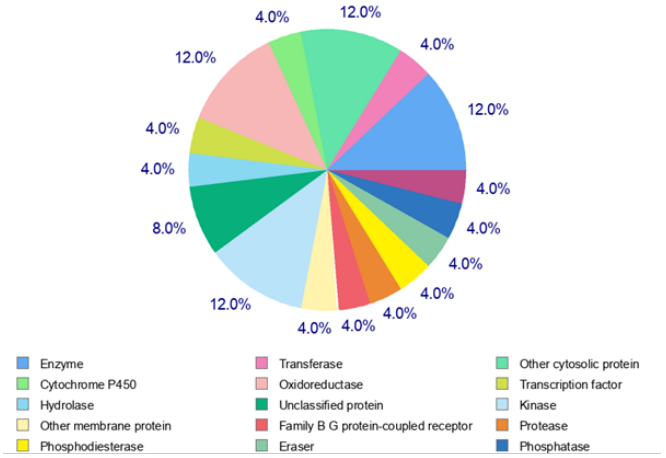


Fig. 4. SwissTargetPrediction analysis. This figure is representing association of alpha-mangostin with different biological properties.

by the HBPlot analysis of alpha-mangostin:PBP2a complex (Figure 3).

3.2. ADMET predictions and PASS analysis showed that alpha-mangostin has drug like properties

The outcomes derived from the ADMIT predictions are presented comprehensively in Table 3. The assessments made through ADMET predictions affirm that alpha-mangostin meets all the essential pharmacokinetic criteria. Consequently, alpha-mangostin emerges as a promising candidate, meriting validation and subsequent development as a therapeutic molecule aimed at inhibiting the enzymatic activity of PBP2a, which is intricately linked to bacterial cell wall synthesis and the occurrence of bacterial infections. Furthermore, the results obtained from the PASS Analysis unveil a plethora of favorable biological properties attributed to alpha-mangostin, as elucidated in Table 4. These findings underscore alpha-mangostin's association with various biological processes of significance. The SwissTarget prediction analysis also corroborates and further explains the manifold physical properties linked with alpha-mangostin, as depicted in Figure 4.

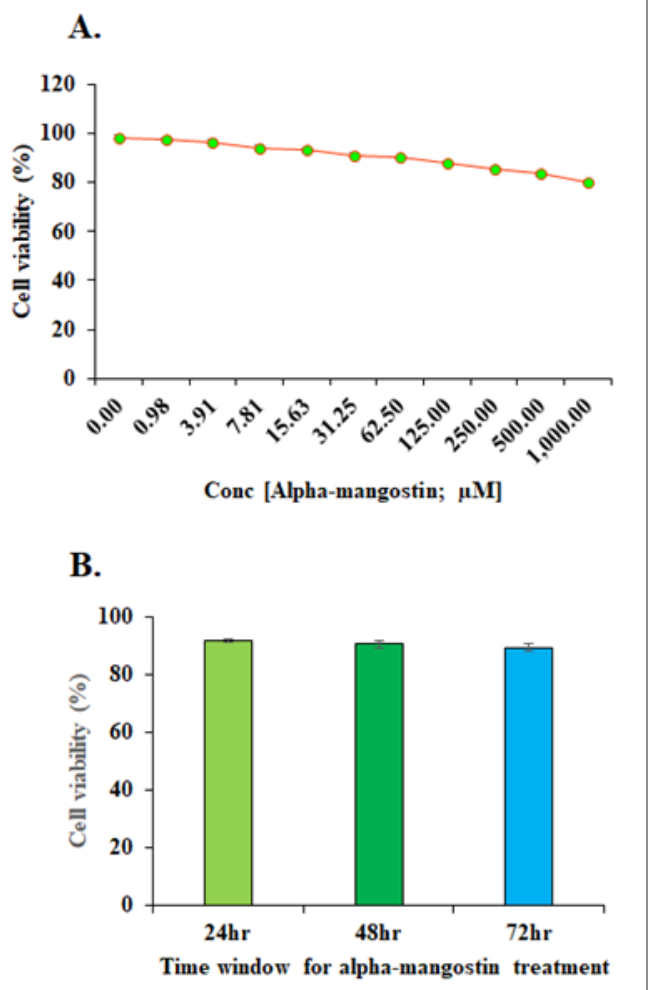


Fig. 5. SwissTargetPrediction analysis. This figure is representing association of alpha-mangostin with different biological properties.

3.3. Concentration dependent cytotoxicity assay revealed no significant effect of alpha-mangostin on the viability of HEK-293 cells

The results of the concentration-dependent cell toxicity assay are presented in Figure 5A. The MTT assay results revealed that the increasing concentrations of alpha-mangostin did not significantly affect the viability of HEK-293 cells. Further, the IC50 value for alpha-mangostin was found to be 33.43 µM.

3.4. Time dependent cytotoxicity assay revealed no significant effect of alpha-mangostin on the viability of HEK-293 cells

The results of the time-dependent cell toxicity assay are presented in Figure 5B. The MTT assay results revealed that the increasing exposure time (24 hrs, 48 hrs, and 72 hrs) of alpha-mangostin did not significantly affect the viability of HEK-293 cells.

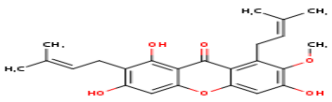
3.5. Fluorescence-based binding assay revealed alpha-mangostin as a potential inhibitor of PBP2a

The fluorescence emission spectra of PBP2a, acquired at different alpha-mangostin concentrations spanning from 0 to 24 µM, are illustrated in Figure 6. Examination of the data disclosed a consistent trend: with the elevation of alpha-mangostin concentration, a concurrent decline in the fluorescence emitted by PBP2a was observed. This empirical evidence strongly supports the assertion that alpha-mangostin functions as an inhibitor of PBP2a, impe-

Table 3. In silico prediction of ADME & Toxicity properties for alpha-mangostin (AM). These properties represent pharmacokinetics and safety profile of a potential drug candidate. The results reflect that AM qualifies the ADME & Toxicity properties test.

	Properties												
	Absorption	**Distribution**			**Metabolism**							**Excretion**	**Toxicity**
Models	Intestinal absorption (human)	VDss (human)	BBB permeability	CNS permeability	CYP Substrate		Inhibitor					Total clearance	AMES toxicity Hepatotoxicity
					2D6	3A4	1A2	2C19	2C9	2D6	3A4		
Unity	Numeric (% absorbed)	Numeric (log L/kg)	Numeric (Log BB)	Numeric (Log PS)	Categorical (yes/no)							Numeric (log ml/min / kg)	Categorical (yes/no)
Predicted values													
AM	93.43	-0.012	-0.467	-1.124	NO	NO	NO	NO	NO	NO	NO	0.04	NO

Table 4. PASS (Prediction of Activity Spectra for Substances) analysis of alpha-mangostin. Probability "to be active" was set at Pa>0,7.

Compound	Structure	Pa	Pi	Activity
Alpha-mangostin		0,950	0,004	Membrane integrity agonist
		0,875	0,005	Apoptosis agonist
		0,865	0,003	UGT1A9 substrate
		0,855	0,003	Lipid peroxidase inhibitor
		0,858	0,010	Chlordecone reductase inhibitor
		0,854	0,018	CDP-glycerol glycerophosphotransferase inhibitor
		0,831	0,008	Antineoplastic
		0,824	0,003	Monophenol monooxygenase inhibitor
		0,817	0,002	CYP1A1 inhibitor
		0,799	0,002	NOS2 expression inhibitor
		0,796	0,003	MMP9 expression inhibitor
		0,795	0,003	Free radical scavenger
		0,785	0,004	Antiulcerative
		0,781	0,002	Anti-Helicobacter pylori
		0,782	0,005	UGT1A6 substrate
		0,783	0,008	UDP-glucuronosyltransferase substrate
		0,770	0,004	Histamine release inhibitor
		0,767	0,005	Histidine kinase inhibitor
		0,775	0,014	TP53 expression enhancer
		0,762	0,004	Beta-carotene 15,15'-monooxygenase inhibitor
		0,790	0,036	Ubiquinol-cytochrome-c reductase inhibitor
		0,747	0,005	Hepatoprotectant
		0,742	0,003	Plastoquinol-plastocyanin reductase inhibitor
		0,777	0,040	Aspulvinone dimethylallyltransferase inhibitor
		0,739	0,005	Chemopreventive
		0,738	0,004	UGT1A1 substrate
		0,733	0,004	CYP1A inhibitor
		0,719	0,002	Transcription factor NF kappa B inhibitor
		0,717	0,005	Antimutagenic
		0,715	0,005	Cell adhesion molecule inhibitor
		0,713	0,004	Transcription factor inhibitor
		0,703	0,004	UGT1A3 substrate
		0,702	0,005	Antineoplastic (breast cancer)
		0,707	0,012	Kinase inhibitor
		0,711	0,019	HIF1A expression inhibitor
		0,706	0,023	Anaphylatoxin receptor antagonist

Pa - Probability "to be active"; Pi - Probability "to be inactive"

ding its enzymatic activity. These findings unmistakably establish a pronounced reduction in the activity of PBP2a as a consequence of escalating alpha-mangostin concentrations, thereby underscoring its inhibitory potency on PBP2a.

4. Discussion

Penicillin-binding protein 2a (PBP2a) plays a crucial role in bacterial resistance to beta-lactam antibiotics like penicillin and methicillin [7]. Understanding its function is essential for developing effective therapeutic strategies against antibiotic-resistant bacterial infections, particularly those caused by methicillin-resistant *Staphylococcus aureus* [1]. The current study's findings provide compelling evidence suggesting that alpha-mangostin exhibits strong potential as an inhibitor of PBP2a, an enzyme associated with bacterial cell wall synthesis and bacterial infection. Several other studies have shown the antibacterial effect of alpha-mangostin [21, 22,23]. The study employs a multi-

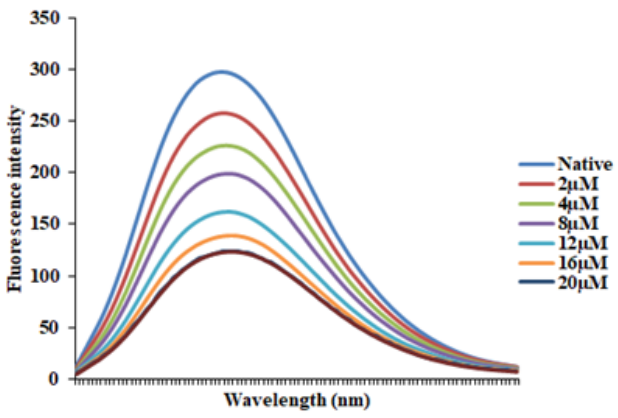


Fig. 6. Fluorescence emission spectra of PBPa2 under in the absence and presence of different concentrations of alpha-mangostin. The PBPa2 was subjected to excitation at 280 nm, and its emission was recorded within the range of 300-500 nm. 20µM concentration was found as an effective concentration to inhibit the actvity of PBPa2.

faceted approach combining molecular docking, ADMET predictions, PASS analysis, cytotoxicity assays, and fluorescence-based binding assays to evaluate the inhibitory properties of alpha-mangostin on PBP2a comprehensively. These results hold significant promise for developing novel therapeutic molecules for combating bacterial infections.

Molecular docking is a powerful computational technique that provides critical insights into the binding affinity and interaction patterns between a ligand (alpha-mangostin) and a target protein (PBP2a) [16, 17]. The impressive binding affinity score of -6.01 kcal/mol suggests a strong interaction between alpha-mangostin and PBP2a. This affinity is crucial since it reflects the strength of the ligand-protein interaction, with lower scores indicating higher binding affinity. Furthermore, the successful docking pose of alpha-mangostin within the deep binding cavity of PBP2a implies that the compound can effectively block the active site of the enzyme, potentially inhibiting its function. The preferential hydrogen bond interaction between alpha-mangostin and the THR216 amino acid residue of PBP2a is particularly noteworthy. Hydrogen bonds are crucial for ligand-protein binding specificity and stability [24]. The additional polar, hydrophobic, and other interactions further emphasize the diverse range of forces contributing to the binding affinity between alpha-mangostin and PBP2a.

ADME and Toxicity predictions play a pivotal role in drug discovery by assessing the pharmacokinetic properties of potential drug candidates [25]. The positive results indicating that alpha-mangostin satisfies all requisite pharmacokinetic criteria are highly encouraging. These predictions suggest that alpha-mangostin could be a viable candidate for further development as a therapeutic molecule targeting PBP2a. Successful translation from in silico predictions to in vivo applications will depend on comprehensive preclinical and clinical studies. Moreover, the PASS analysis offers valuable insights into the potential biological properties associated with alpha-mangostin. The multitude of favourable biological processes associated with alpha-mangostin underscores its potential as a therapeutic agent. The SwissTargetPrediction analysis further supports these findings by highlighting the compound's associations with various biological properties [26].

We also talk about extracts here; the ethanolic extracts of Emblica officinalis, Bacopa monnieri, and Boerhavia diffusa influenced the anti-UTI action. These extracts found a significant zone of inhibition against the UTI pathogen *K. cowanii* (OQ 073698) Still, the conventional antibiotics ampicillin 1.6 mm, penicillin 1.7 mm, and chloramphenicol 1.8 mm were effective. The maximum zone of inhibition against *K. cowanii* was demonstrated *by E. officinalis* (OQ 073698) [27].

Vancomycin antibiotic resistance is a severe issue when treating illnesses caused by resistant forms of the drug. Thus, it is essential to refrain from giving antibiotics without a prescription and from misusing readily available medicines to stop the emergence of resistance to the listed antibiotics and other common antibiotics [28].

Ensuring the safety of potential drug candidates is paramount. The cytotoxicity assay results indicate that alpha-mangostin does not significantly affect the viability of HEK-293 cells, suggesting a degree of selectivity for bacterial targets rather than indiscriminate cell toxicity [29]. This is a crucial finding, as it indicates a potential therapeutic window for alpha-mangostin in treating bacterial infections without harming human cells. Further investigations into its safety profile and potential side effects will be necessary.

The fluorescence-based binding assay provides experimental confirmation of alpha-mangostin's inhibitory effect on PBP2a. The observed decrease in fluorescence intensity of PBP2a with increasing concentrations of alpha-mangostin strongly supports the hypothesis that alpha-mangostin hinders the enzymatic activity of PBP2a. This assay is a pivotal bridge between computational predictions and experimental validation, lending credibility to the potential therapeutic application of alpha-mangostin [30,31].

5. Conclusion

In conclusion, the findings of this research article collectively demonstrate the potential of alpha-mangostin as an inhibitor of PBP2a. The multi-pronged approach, encompassing computational analysis, experimental assays, and safety assessments, provides a comprehensive and robust foundation for future investigations. The development of alpha-mangostin or similar compounds could significantly contribute to the arsenal of antibacterial agents and represents a promising avenue for further research and development in infectious diseases. Nevertheless, other studies must validate these findings and assess the compound's efficacy and safety.

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Conflict of interest

Author declare no conflict of interest.

Consent for publications

The author read & approved the final manuscript for publication.

Ethics approval and consent to participate

There is no use of human or animals in the present research.

Informed consent

The author declare not used any patients in this research.

Author's contribution

RA: Design the experiment, analysis, and draft manuscript.

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