Synthesizes, characterization, molecular modelling studies and bioactivity of a novel bicyclic compound of δ-lactam with oxazepine ring containing-sulphur substitute using an economic method

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Abstract

Penicillin resistance is a commonly present and controversial matter due to the misuses by people for various reasons. However, little studies have been examined the bioactivity of the 5 and 6 membered rings. In this study, we aimed to synthesize a new compound contain 5- membered ring following a short and low cost method and combined it with oxazepine ring via Schiff bases to produce a bicyclic molecule (Lactozepine). In vitro examinations were implemented to assess the bioactivity of the prepared compound such as anti-bacterial, anti-fungal and anti-oxidant which has shown a wide zone of inhibition of lactozepine against *Streptococcus pneumoniae* but no inhibition was shown against *Kelbesilla pneumoniae* and *Staphylococcus aureus* except at a high concentration similar to the result of the anti-fungal assessment. Furthermore, lactozepine showed worthy anti-oxidant activity against free radical formation. The molecular modelling and docking assessment showed availability of lactozepine to bind to bacterial proteins and inhibit their growth with lowest free energy for the greatest and strong binding affinity with the PDB crystal structures 1VQQ, 2WAE, 1PYY and 1IYS were between − 6.5 and − 7.9 kcal/mol. Moreover, the molecular MD dynamic simulation showed that RMSF (root mean square fluctuation) for the assessed protein's amino acids remained consistent and tightly bound to lactozepine in the dynamic state. The novel compound of lactozepine having δ-lactam rings attached to oxazepine showed a bioactivity that are hopeful for in vivo studies in the future.

INTRODUCTION

The presence of bicyclic compounds involving a lactam ring with any other cyclic compound is reflected in various pharmaceuticals studies [1, 2]. A bicyclic δ-lactam containing oxazepine ring as a case in point. Penicillin resistance is the most common complicated matter that call for antimicrobial development in the last decades [3]. It is well-known that the main functional group in penicillin is the β-lactam ring (4-membered ring) which is a heterocyclic amide in where nitrogen atom is attached to the β-carbon atom comparative to the carbonyl group C = O [4]. However, antibiotics containing β-lactam struggle from resistance due to high prevalence of microbial infections. Therefore, recent years have shown a significant attention in the organic chemistry of six and seven heterocyclic rings due to their bioactivities [5, 6]. For example, 5 and 6-lactam ring contain similar functional group to β-lactam but with different number of carbons in the ring. Delta δ-lactam (6-membered ring) is a hexa-heterocyclic amide containing nitrogen atom next to carbonyl group [7, 8]. The only difference is the number of the carbon atom in the ring which could show a difference bioactivity δ-lactam is a subgroup of lactam family that widely used in and medicine and pharmaceutical chemistry [9]. On the other hand, oxazepine compounds have previously shown an inclusive range of applications in the pharmaceutical industry, including antidepressants, enzyme inhibitors, and palliative effects [10]. They are unsaturated heterocycles compounds comprising of seven atoms where two substituted by a nitrogen and oxygen atom respectively [3]. A previous study synthesised earlier a bicyclic β- lactam having an oxazepine circle, but the bioactivity has not been shown yet. Moreover, the study involved oxazepine ring without a substituted sulphur in both rings [5]. Various studies across the world tried to form C-S bonds as within the ring due

significant bioactivity of sulphur-containing compounds in multiple fields [11]. Practically, sulphonamide compounds contain sulphur are common antibiotics used in medicine [12, 13]. Therefore, it is very necessary to develop novel antimicrobial agents with similar bioactive properties but with combination of the three functional groups: δ-lactam and oxazepine ring containing sulphur atom.

The aim of this study is to synthesise and characterise in a novel economic procedure a combination of bicyclic compound of δ-lactam with oxazepine ring containing-sulphur substitute and study their biological activity computationally and experimentally. This will help addressing the bacterial resistance which is globally people suffer with using an economic procedure for synthesis the compounds. Furthermore, it utilizes the multiple properties of both compounds, sulphur and lactam groups, and combined the in one functional medication. The novel synthesised compound might change the behave of the pathways of the antibiotics against various types of bacterial and fungi infection but also overcome the resistance.

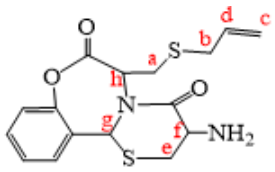
MARTIALS AND PROCEDURES

All used substances were obtained from Sigma Aldrich Company. The melting point was measured using Electro thermal (m.p) instrument-type Gallan Kamp by capillary tube. The measurement of ^{1}H-NMR spectroscopy of the synthesized Lactozepine was achieved at Gazi University, Turkey using devices: FT-IR/Thermo Electron Corporation, and an Ultra shield 300 MHz. Bruker NMR devices in the presence of DMSO solvent, respectively. A VITEK2 Compact-Biomerieux-USA bacterial activity tester was used for the bioactivity test. LC-Mass Spectra and CHN spectrum apparatuses were also used to assess the synthesized lactozepine in Gazi University. Moreover, all the intermediate and final products were confirmed by TLC method. Also, the bioactivity was assessed on newly prepared dishes which were grown up for at least 24 h at 37° C. The novel economic method to synthesise the suggested compound is demonstrated below.

Chemical synthesis of lactozepine

0.01 mole of S-Allyl-L-cysteine was dissolved in a combination of 1.0 mL of absolute EtOH and 12 mL of purified water (distilled water). The mixed with 0.01 mole of 2-hydroxy benzaldehyde in a round flask and refluxed for 3 hours. After, 0.01 mole of cysteine, an amino acid, was liquefied in 18 mL of purified water and then added to the reaction medium which were all refluxed for 5 hours. A thin layer chromatography was used to assess the products of each step during the interaction. The interaction mixture was left overnight in a 100 mL beaker. Golden yellow crystals appeared at the bottom of the beaker which were filtered, dried, and recrystallized from mixture of distilled water and 10% absolute ethanol (Table 1).

Table 1. Properties, structure, nomenclature, molecular weight of synthesized lactozepine

Name	Structure and nomenclature	Molecular Weight	Yield %	m.p. °C	Colour
Lactozepine	 6-((allylthio)methyl)-3-amino-2,3-dihydro-4*H*,12b*H*-benzo[*f*][1,3]thiazino[3,2-*d*][1,4]oxazepine-4,7(6*H*)-dione	350.45	70%	160-161	Golden Yellow

The examination of the biological activity of lactozepine

Anti-bacterial, anti-fungal and antioxidant activity

The anti-fungal activity of the synthesized lactozepine was examined in vitro as a growth inhibitor against *Candida albicans* and *Aspergillus niger* by the agar well distribution method at limited concentrations. The results were compared with the results of the other antibiotics (such as Fluconazole and Iitraconazole) under the selected concentrations (shown in Table 4) as a positive control and the solvent (DMSO) as a negative control [14, 15]. The anti-bacterial activity of lactozepine was evaluated against three types of pathogenic bacteria using the method of well diffusion on Mueller Hinton Agar dishes. The zones of inhibition diameter were recorded as anti-bacterial activity. The solutions were allowed to spread earlier than inoculated with the fungi, and then incubated at the temperature of 37 ˚C in the incubator for 24 hours. The dishes were examined for measuring the inhibition zone [16].

The antioxidant activity of lactozepine compound was tested in vitro method using the DPPH compound (2,2-diphényl-1-Picryl hydrazyle) as a reagent, this reagent allows for mensuration of the testability of the lactozepine to replace free radical scavenging effect, many concentrations of the measuring substance (citric acid) were used by preparing the main concentration.

Initially, the concentration of (0.8 mg/mL) of ascorbic acid was completed by dissolving 80 mg of ascorbic acid in 100 mL of absolute methanol to prepare the standard solution, other concentrations (0.05, 0.1, 0.15, 0.2, and 0.5 mg/mL) of ascorbic acid were completed by using the dilution law.

The concentration of (0.8 mg/mL) of lactozepine compound was completed by dissolving 80 mg of lactozepine compound in 100 mL of DMSO, other concentrations (0.05, 0.1, 0.15, 0.2, and 0.5 mg/mL) of lactozepine compound were completed by using the dilution law.

All concentrations prepared above were evaluated as antioxidants against the solution of 2, 2- di- phenyl-1-Picryl hydrazyle (control solution) [17].

The absorbance reagent (DPPH) was measured instantly at 517 nm for measuring the control (1.5mL of DPPH), the solution was added to the test tubes containing lactozepine and concentrations of standard solutions both alone and then the samples were incubated in the dark for 30 minutes, the absorbance was recorded at 517 nm after 30 minutes using absolute methanol solvent as Planck's solution, the scavenging activities (%) were calculated using the below equation:

Antiradical activities %= ($A_{control} - A_{test} / A_{control}$) x 100%

Where:

$A_{control}$= Absorbance of control reaction (containing all reagents unless sample extract).

A_{test}= Absorbance of the lactozepine.

The free radical scavenging activity (RSA) of lactozepine was also tested by the DPPH method; lactozepine was mixed with the DPPH and permitted to interact for 0.5 h at 37°C, two compounds (n-Propyl gallate and 3-t-butyl-4-hydroxyanisole) were used in this evaluation method, after incubation absorbance was recorded by micro plate reader using the following equation [18].

$$RSA\ \% = \frac{100\text{- ([Absorbance of test compound (Lactozepine)]}}{\text{Absorbance control}} \times 100\ \%$$

The statistical analysis to calculate the antioxidant activity of lactozepine compound and Vitamin C was performed using a PRISM Graph Pad software version 8. The linear regression equation was applied to fit the points of the percentage of antioxidant activity % against serial dilution concentrations to calculate IC50 for lactozepine.

Docking and Molecular dynamic (MD) simulation study of the synthesised lactozepine

The synthesised compound that contains lactam and oxazepine functional groups was targeting various binding proteins and β-lactamase enzyme. All proteins involved in the cell wall of the bacteria which are usually targeted by β-lactam as in Penicillin. All the PDB crystal structures 1VQQ, 2WAE, 1PYY and 1IYS where downloaded from RCSB website to perform molecular docking to find the best active sites of the compound and the proteins that simulate where the chemical compound could fit. The main aim of targeting these are proteins and enzymes in this is study is due that penicillin and its derivatives have recently shown resistance due to many reasons one of them is misuse of the antibiotics. The results were presented using PyMol software. CB-docking and PILP database were used to perform the docking.

The MD simulations of lactozepine in complex with 1VQQ, 2WAE, 1PYY, and 1IYS proteins were performed to investigate the stability and conformational flexibility in a closely physiological

environment. The Desmond Schrodinger programme is utilised for input in building and running MD simulations by using fixed parameters of the OPLS3e force field [19, 20]. The MD simulations were accompanied for up to 100 ns for each system. For generating the simulations, the periodic boundary condition was implemented. The protein ligand complex system was placed to the SPC water model's water box. In the system builder, sodium and chloride ions were introduced to neutralise the charges, followed by 0.15 M NaCl salt concentrations to mimic human physiological conditions [21, 22]. Then the build system was minimized using a fixed parameter of the OPLS3e force field to remove electronic clashes between protein structures and align the protein structure inside the simulation boundaries appropriately. More information for the MD study (thermostat and barometer parameters, short- and long-range interaction calculations, etc.) can be found in previous studies because the same settings were used for the examined systems here [23–25].

RESULTS AND DISCUSSION

The chemical reaction procedure:

In this study, a novel compound named lactozepine was synthesized and characterised via various chemical appropriate techniques and instruments. The main object in this study was to prepare di-heterocyclic compounds contains lactam and oxazepine groups. The results confirmed that the synthesized compound is a combination of bicyclic compound of δ-lactam with oxazepine ring containing-sulphur substitute which hold two functional cyclo-groups.

Both δ-lactam and oxazepine derivatives' bioactivity are well-know and previously established. This results of the chemical reaction showed the synthesized compound named as lactozepine. It compresses of two fused rings, one of them is δ-lactam and the other cyclic ring is attached from C-N cyclic-ring involving C-O, C = O, and a chain containing methyl mercapto propene. A benzene ring is also shown in the structure. Briefly, this study assumed a formation of an efficient intermediate compound with a C = N group, which contributes in the production of the target compound by a chemical reaction with cysteine as a consequence of a ring-closing reaction.

The chemical reaction was performed via Schiff base reaction. However, no acid was employed as a catalysis to perform the Schiff base reaction which frequently used to prevent an aliphatic ester formation at the beginning of the reaction. Thus, the reaction could be performed in low-cost as the ratio of the ethanol solvent used in the reaction is 1 mL to 10 mL of distilled water. Once the compound obtained, it was then filtrated, purified, crystallised and then characterised using various chemical techniques. The following equation shows the proposed reaction to obtain the lactozepine compound. See Scheme 1.

Scheme 2 showed the suggested mechanism of obtaining the compound, it includes the following steps:

Characterisation of the synthesised lactozepine

FT.IR spectroscopic method was used to confirm the functional groups of heterogeneous cyclic molecule. The analysis of FT.IR charts results showed that the prepared compound involved a cyclo-closure process, which results in a cyclic ester inside the seven-ring of oxazepine which can be clearly observed from changing the carboxylic group in cysteine to ester and converting one of the primary amide to tertiary amide. Deeply, the disappearance of the following main bands was observed. For carboxylic acid, the broad band of hydroxyl (-OH) of the carboxyl group (-COOH) assumed to appear at (2500–3300 cm^{-1}), while the carbonyl (C = O) group of aldehyde assumed to appear at (1666 cm^{-1}). Regarding to aldehyde proton assumed to appear between (9580 – 2830 cm^{-1}). For the SH-group proton is supposed to appear between (2550–2600 cm^{-1}). The (-NH_2) group band was observed at (3489 and 3565 cm^{-1}). For (C = C) band of alkene, it was at (1560 cm^{-1}), regarding (= CH) group of alkene at 3101, the band of aromatic group (C = C) at (1489 cm^{-1}), for (= CH) Aromatic group at (3085 cm^{-1}), for lactam carbonyl group (-N-C = O) at (1618 cm^{-1}), a lactone carbonyl group (-O-C = O) at (1704 cm^{-1}). A C-S group of hexagonal ring at (750 cm^{-1}) and the single band of -(C-N) group between the heptagonal and hexagonal ring at (1160 cm^{-1}) and for the single band of (C-O) group of the seven-ring at (1236 cm^{-1}) [26, 27]. (The figure is shown in a supplementary file).

In the ^{1}H-NMR of the protons of the synthesised lactozepine, it can be noticed that the doublet signal for protons in carbon (a) is at 1.42 ppm and 1.43 ppm. The doublet signal for protons in carbon (b) is at 1.53 ppm and 1.55 ppm. A doublet signal for protons in carbon (c) at 1.80 ppm and 1.87 ppm and a triplet signal for protons in carbon d between 3.80 and 3.96 ppm, for the singlet signal for protons of the amine group NH_2 at 4.45 ppm were observed. The doublet signal for protons in carbon (e) at 4.85ppm, the triplet signal for protons in carbon (f) between 5.65–5.60 and the singlet signal for protons in carbon (g) at 8.97 ppm were observed. The triplet signal for proton in carbon (h) at 10.25 ppm and the multiple signal for protons of the aromatic rings at 6.10–7.90 ppm were observed [28, 29], The figure is shown in a supplementary file).

The reaction was followed by using TLC, where a mixture of two solvents was used consists of 5 mL ethyl acetate to each 1 mL normal hexane (results are not shown. The migration can be diagnosed by the Rf value, the calculation method can be found as below:

Rf = The distance moved by the solute (**Lactozepine**) / The distance moved by the mobile phase front which consists of 5 mL ethyl acetate to each 1 mL normal hexane.

Rf = 5 cm / 6.5 cm. The Rf was calculated to be 0.769. The results of the TLC confirmed the purity of the solubility of the compound. The compounds in the mass spectrum show signals representing the ratio of mass to charge (m/z). This happens by shedding a torrent of electrons on the compound entering the spectrum device. It is possible that molecular weights are higher than the compound under test, and this is due to the possibility of the interaction of fractures among them due to the presence of free radicals.

The results of the LC-Mass spectra technique showed the appearance of a signal at (m/z) equal to 356.87, representing the molecular weight of the synthesized compound “Lactozepine”, which has a

molecular weight of 350.45, and the slight difference is due to the difference in isotopes of some elements in the synthesized compound. In addition to the appearance of other fragments, some of which are shown in Table S 1. It confirmed the proposed formula of the compound. The results showed that there is one peak at 0.99 and this proves that the substance is pure, if there was more than one substance, peaks would appear in the number of substances. The Mass spectroscopy chart is shown in a supplementary file. The fragmentations of synthesized lactozepine are shown in scheme 3 [30–32].

Biological and antioxidant activity of Lactozepine

Anti-bacterial and Anti-fungal activities

The prepared compound was examined against three types of bacteria; *Kelbesilla pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* to confirm its bioavailability to kill the bacteria or at least to overcome the lactamase resistance.

Figure 1 explain the inhibition zones of lactozepine compound against *Streptococcus pneumoniae*, where there is a high zone of inhibition of 19 mm for the 90% and 100% concentrations. It is noted that the prepared compound did not show activity against two types of bacteria; *Kelbesilla pneumoniae* and *Staphylococcus aureus* except at a high concentration of 100%, and the reason is due to the resistance of these two types of bacteria to the chemical composition of the compound lactozepine for the remaining concentrations. The effectiveness of the lactozepine is reasonable for all concentrations except at the 10% dilution, where it is believed that the high dilution show an increase of the resistance against bacteria, and thus the result was zero mm [16]. The concentrations of positive controls were considered at the (mg/mL) unit.

Another biological activity such as anti-fungal and anti-oxidant of Lactozepine were also estimated using the pathogenic fungi type *Candida albicans* and *Aspergillus niger*. In order to assess the MIC values of lactozepine compound, DMSO was used as a negative control and anti-biotic compounds (Iitraconazole and Fluconazole) as positive controls as opposed to fungi (C. Albian's and A. Niger), see (Tables 2 and 3).

The concentrations of Lactozepine compound and DMSO are taken at a percent unit (w/v %). The inhibition zone values of these determined concentrations of synthesized compound and DMSO were compared with the inhibition zone values of the concentrations of positive controls; Iitraconazole and Fluconazole. The best result of the anti-fungal was for concentration of 80% (13mm) (Fig. 2). The inhibition zone of 100% was less than the inhibition zone of 80%. This is due to the fact that the high concentration of 100% prevents the spread of the lactozepine well [33].

Table 2
The values of inhibition zone diameters (mm) of lactozepine against Fungi

Types of fungi	***Aspergillus niger***						***Candida albicans***					
Concentration%	10	20	40	60	80	100	10	20	40	60	80	100
Inhibition Zone Diameters (mm)	0	0	9	9	13	10	0	0	0	8	10	10
Negative Control (DMSO)	0	0	0	0	0	0	0	0	0	0	0	0

Table 3
The inhibition zone diameters (mm) for the positive controls

Type of Fungi	***Aspergillus niger***		***Candida albicans***	
Concentration mg/mL	15	30	15	30
Positive Control (litraconazole) in mm	4	6	5	9
Positive Control (Fluconazole) in mm	5	10	9	14

Antioxidant activity of the synthesise compound was also assessed in order to examine the ability of the compound to scavenge the free radicals using the DPPH reagent in a free radical scavenging assay. The DPPH reagent was characterized as a stable radical at the wavelength of 517 nm after 30 minutes using methanol solvent as Planck's solution. The activities of DPPH reagent in scavenging have been frequently used to approximate the anti- radical activities of lactozepine compound [34]. The percentage of antioxidant activity of lactozepine and Vitamin C was calculated using below equation. The percentage of antioxidant activity of the lactozepine in comparison to the percentage of antioxidant activity of Vitamin C are shown as in Table 4. Moreover, The IC50 was calculated which is a quantitative process that indicates how much of a particular inhibitory of lactozepine is needed to inhibit in vitro a given biological component by 50%. The calculation of IC50 for each compound were particular and displayed in the Table 5. Furthermore, the percent of free radical scavenging activity (RSA %) of lactozepine was determined, DMSO was used as a control group as seen in Fig. 3.

$$\% \text{ Antioxidant activity} = \frac{A_{\text{Control}} - A_{\text{Test}}}{A_{\text{Control}}} * 100$$

Table 4. Percentage of antioxidant activity of the lactozepine compound and Vitamin C.

Concentration (mg/mL)	Percentage of antioxidant activity %	
	Lactozepine	**Vitamin C** (Standard)
0.05	15.7	63
0.1	18.3	65.7
0.15	23	69
0.2	26.8	76.8
0.5	34.6	87.6

Table 5
IC_{50} values of antioxidant activity of lactozepine compound and Vitamin C.

Compound Name	IC_{50} (mg/mL)
Lactozepine	0.98
Vitamin C	0.205

Docking of the synthesized ligand named as Lactozepine with Penicillin binding proteins and β-lactamase:

The results were indicated that the lowermost free energy for the preeminent binding affinity with the PDB crystal structures 1VQQ, 2WAE, 1PYY and 1IYS were − 6.5 kcal / mole, -6.1 kcal / mole, -7.9 kcal / mole and − 6.8 kcal / mole respectively [35].

The interactions bonds with synthesized Ligand were all detected as presented in tables (6–8) and Figs. 4–7. The hydrogen bonds are shown in all figures in a red dashed line. The hydrophobic bonding is shown in all figures in a blue dashed line. Many of these interactions have shown better and firmer binding affinity to the directed ligand and adequate stability.

The online CB-DOCK website was used to perform the Ligand-protein docking and the PLIP website was used to show the binding types of Ligand-Protein interactions. However, PyMol software was used to visualize and present the docking results [36, 37].

Table 6

Shows the Hydrogen bonds interaction between amino acids and residues in the proteins with synthesized ligand. They are shown in the figures in blue dashed lines. The distance between amino acids and the ligand is measured by angstrom.

PDB	Residue	Amino acid	Distance H-A	Distance D-A
1VQQ	110A	ARG	2.47	2.94
2WAE	77A	LYS	2.35	2.80
2WAE	222A	ASP	2.65	3.13
1PYY	664A	GLY	1.95	2.85
1PYY	698A	ASP	2.11	3.01
1PYY	735A	ASN	3.99	3.99
1PYY	735A	ASN	2.93	2.93
1IYS	59B	ASN	2.4	3.3

Table 7
Shows the hydrophobic bonds interaction between amino acids and residues in the proteins with synthesized ligand. They are shown in the figures in red dashed lines. The distance between amino acids and the ligand is measured by angstrom.

PDB	Residue	Amino acid	Distance H-A
1VQQ	174B	VAL	3.99
1VQQ	209B	ASP	3.5
1VQQ	211B	PHE	3.71
1VQQ	110A	ARG	3.58
1VQQ	311A	HIS	4.47
2WAE	241A	LYS	3.37
1PYY	664A	THR	3.78
1PYY	694A	GLU	3.76
1PYY	695A	GLU	3.92
1IYS	63B	TRP	3.54
1IYS	98B	ILE	3.7
1IYS	107B	ALA	3.67
1IYS	109B	VAL	3.72
1IYS	109B	VAL	3.91

Table 8
Shows the Salt bridges and π-Cation interaction between some amino acids and residues in the proteins with synthesized ligand. They are NOT shown in the figures. The distance between amino acids and the ligand is measured by angstrom.

Specific interaction	PDB	Residue	Amino acid	Distance H-A
Salt bridges	2WAE	77A	LYS	5..07
Salt bridges	1VQQ	110A	ARG	3.58
π-Cation interaction	1PYY	420A	LYS	1.36

Zoomed in Fig. 4 illustrates the labelled residues with the contacts of the ligand with bacterial protein. The hydrogen bonds are shown in blue dashed lines whereas the hydrophobic bonds are shown in red dashed lines.

Zoomed in Fig. 5 illustrates the branded remains with the connections of the ligand with bacterial protein. The hydrogen bonds are shown in blue dashed lines whereas the hydrophobic bonds are presented in red dashed lines.

Zoomed in Fig. 6 displays the branded remains and the connections of the ligand with bacterial protein. The hydrogen bonds are shown in blue dashed lines whereas the hydrophobic bonds are presented in red dashed lines.

Zoomed in Fig. 7 shows the branded remains and the connections of the ligand with bacterial protein. The hydrogen bonds are shown in blue dashed lines whereas the hydrophobic bonds are presented in red dashed lines.

Molecular dynamic (MD) simulation

The docked complex was solvated in a frank solvent (SPC water model) PBC container for a 100-ns simulation in order to obtain a more realistic model of the interface pattern between lactozepine and the 1VQQ, 2WAE, 1PYY, and 1IYS proteins. The three basic parameters investigated to examine conformational stability are root-mean square deviation (RMSD) and root-mean square fluctuation (RMSF) and protein ligand contacts during the 100 ns run of MD.

The time evolution changes in all atoms' Cα-RMSD to reveal the conformational stabilization of the lactozepine - protein complex, as shown in Fig. 8. The degree of fluctuation in RMSD is inversely connected to the stability of a complex: the lesser the variation, the better the stability [38]. It can be observed that all trajectories were substantially equilibrated during the simulation time. There is no significant fluctuation observed in any protein-ligand complex except the lactozepine-1IYS complex. The protein backbone of the lactozepine-1IYS complex was shown to undergo minimal changes across a simulation duration of roughly 28 ns. Following then, the RMSD remained stable in the 3.56 Å to 4.08 Å range. After 75 ns, the RMSD declines with little fluctuation until the simulation ends. A similar trend of RMSD variations has been observed in MD simulation in the cases of lactozepine-1VQQ and lactozepine-1PYY complexes. On the other hand, the lactozepine-2 WAE complex, exhibits stability between 1.83 and 4.43 Å, with a rising RMSD trend. The average RMSD for protein Cα atoms of 1VQQ, 2WAE, 1PYY, and 1IYS bound with lactozepine has been found to be 3.00, 2.95, 2.84, and 3.30 Å, respectively. Overall, monitoring the protein Cα atom's RMSD value provides insights into its structural conformation throughout the simulation run, indicating that the assessed protein has not undergone any such large or observable conformational change through the 100 ns MD simulation.

Additional, in order to analyse the dynamic nature of each amino acid remainder during simulations, the RMSF is also measured. In the protein RMSF graph, the α-helical and β-strand areas are shown in red and blue colour, correspondingly, while the loop area is depicted in white. Protein α- helical and β- strands domains are firmer than unstructured protein areas and consequently fluctuate fewer than loop regions [39, 40]. The minimal fluctuation of the energetic site and main chain atoms revealed that the

conformational shift was modest, demonstrating that the reported lead chemical was securely bound inside the hole of the assessed protein binding pocket.

The RMSF of protein α-carbon atoms of all system were analysed and represented in Fig. 9. The average RMSF values of Lactozepine-1VQQ, lactozepine-2WAE, and Lactozepine-1PYY, Lactozepine-1IYS are 1.77,1.59, 1.34, and 1.50 Å, respectively. These values indicated that all the lactozepine–protein complexes experienced relatively less conformational fluctuation during the simulation time. According to the RMSF plot, it was observed that lactozepine contacted 19 amino acids of 1VQQ protein, namely, Asp82 (2.22 Å), Lys84 (2.62 Å), Gln98(1.85 Å), Asp109(1.63 Å), Arg110(1.60 Å), Asn111 (1.90), His311 (1.25 Å), Thr312 (1.17 Å), Leu313(1.19 Å), Val174 (2.33 Å), Lys176 (3.31 Å), Asn177 (3.10 Å), Asp209 (2.54 Å), Phe211(2.06 Å), His232 (2.48 Å), Leu233 (1.85 Å), Thr234(1.71 Å), Thr235(1.69 Å), Asn236(1.68 Å). In the case of 2WAE protein lactozepine interacted with 16 amino acids including Lys77 (1.38 Å), Ser105 (2.85 Å), Ile106 (2.73 Å), Ser107 (3.10 Å), Ser108 (2.78 Å), Asn110 (2.28 Å), Tyr176 (2.09 Å), Ala193 (1.39 Å), Thr194 (1.32 Å), Asp195 (1.50 Å), Pro196 (1.45 Å), Asp222 (1.23 Å), Lys224 (1.30 Å), Ser239 (1.44 Å), Glu240 (1.26 Å), Lys241 (1.30 Å). In this higher fluctuation is observe with Ala133 (5.33 Å), Leu152 (6.47 Å), Glu151 (6.68 Å), Ser150 (7.16 Å), Leu134 (7.30 Å), and Pro135 (8.80 Å) which is lies in the loop region and end terminals. In Lactozepine-1PYY complex 21 amino acids shows interactions which are as Asn417 (0.86Å), Lys420 (0.78Å), Val423 (0.80Å), Pro424 (0.72Å), Thr425 (0.70Å), Arg426 (0.78Å), Arg463 (0.61Å), Glu476 (0.74Å), Val499 (0.79Å), Pro646 (0.88Å), Gly647 (0.84Å), Ala650 (0.67Å), Arg654 (0.83Å), Ile661 (0.62Å), Val662 (0.66Å), Gly664 (1.21Å), Gly666 (1.30Å), Ser690 (1.02Å), Pro697 (1.10Å), Asp698 (1.00Å), Asn735 (1.35Å), while in Lactozepine-1IYS complex 23 Amino acids shows interactions including Trp28 (1.01Å), Ala31 (1.43Å), Glu35 (1.89Å), Asn46 (2.30Å), Asp48 (2.93Å), Ser50 (2.09Å), Asp52 (1.34Å), Leu56 (0.84Å), Gln57 (1.07Å), Ile58 (1.05Å), Asn59 (1.33Å), Arg61 (1.61Å), Trp62 (1.46Å), Trp63 (1.28Å), Ala95 (0.73Å), Ile98 (1.23Å), Val99 (1.28Å), Asp101 (2.66Å), Asn103 (2.48Å), Gly104 (1.72Å), Asn106 (1.81Å), Ala107 (2.09Å), Trp108 (1.90Å). All this interacted residue is labelled in green - coloured perpendicular bars which showing Stability of lactozepine in the assessed protein target.

The protein ligand contacts histogram indicates that lactozepine forms major hydrogen bonds with Gln98, Asp109, Asn111, Asn177, Phe211, and Thr234 in 1VQQ protein. In the Lactozepine-2WAE complex, amino acids Tyr176 and Ala193 are involved in hydrogen bonding, while Ser105, Ser107, Ser108, Glu240, and Lys241 are interacted through hydrogen bonding, which correlates with the docking interaction mode. Water-mediated hydrogen bonding was involved in the lactozepine-1PYY complex, along with hydrogen-bonding amino acids Pro424, Arg426 and Arg463. Lactozepine systems some hydrophobic connections and mostly water bridges with **1IYS**. Lactozepine interacts with Gln57 and Asn59, which is an important residue contact observed, *via* hydrogen bonding and water refereed interactions. Based on the RMSD and RMSF results, it is plausible to conclude that the assessed protein's amino acids remained consistent and tightly bound the lactozepine in the dynamic state.

Conclusions

A combination of 5- membered ring so-called δ-lactam with oxazepine ring by a new and short suggested chemical synthesis involved Schiff bases formation to produce a bicyclic effective molecule has shown a remarkable and promising medication. Since the affinity binding of lactozepine was validated using docking and molecular simulation studies, the synthesized molecule was assessed for its in vitro inhibitory activity for further support to computational studies. The lowest free energy for the greatest and strong binding affinity with the PDB crystal structures 1VQQ, 2WAE, 1PYY and 1IYS were found between − 6.5 and − 7.9 kcal/mol. These bacterial proteins were shown inhibition by the lactozepine in different binding affinity which is measured by the lowest free Gibbs energy values. Therefore, Molecular simulation was performed to assess the time where the degree of fluctuation in RMSD that is inversely connected to the stability of a lactozepine –protein complex, where the lesser the variation, the better the stability. The complex of lactozepine − 1IYS has only shown a significant fluctuation due to binding affinity. Altogether, the in-silico results confirmed in vitro assessment as follow.

In vitro examinations results showed that there was a wide zone of inhibition of 19 mm for the 90% and 100% concentrations of lactozepine against *Streptococcus pneumoniae* but no inhibition was shown against the other two types of bacteria; *Kelbesilla pneumoniae* and *Staphylococcus aureus* except at a high concentration. The best result of the anti-fungal assessment was shown for concentration of 80% (13mm). Furthermore, lactozepine showed worthy anti-oxidant activity against free radical formation. Moreover, the computational studies on molecular docking results indicated that the lowermost free energy for the greatest binding rapprochement with the PDB crystal structures 1VQQ, 2WAE, 1PYY and 1IYS were between 6.5 and − 7.9 kcal/mol. Most of the interactions of the lactozepine have shown frim binding affinity to the docked ligand and sufficient stability. Moreover, RMSF (root mean square fluctuation) was assessed for above bacterial proteins by running a molecular MD dynamic simulation to examine the conformational flexibility of the complex which showed that the assessed protein's amino acids remained consistent and tightly bound to lactozepine in the dynamic state. In conclusion, the novel compound of lactozepine involving δ-lactam rings attached to oxazepine revealed an effectual bioactivity that are encouraging for farther upcoming studies.

Declarations

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Schemes

Scheme 1 to 3 are available in Supplementary Files section.

Figures

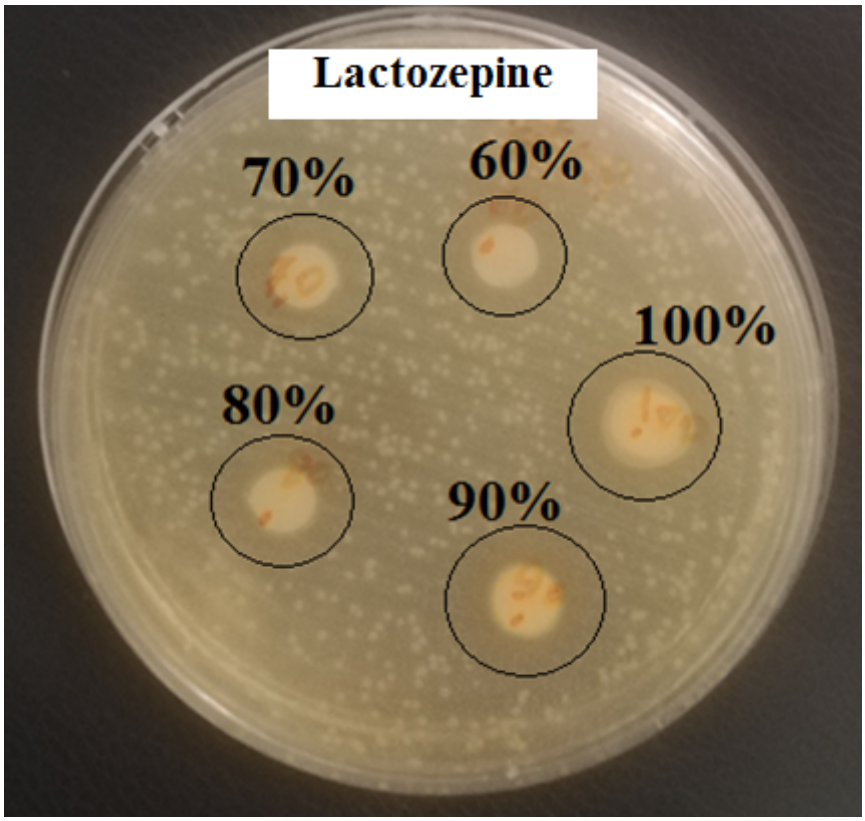

Figure 1

The inhibition zones of lactozepine against *Streptococcus pneumoniae* at 60 %, 70 %, and 80 % 90% and 100 % concentrations using by the agar well diffusion method at various concentrations.

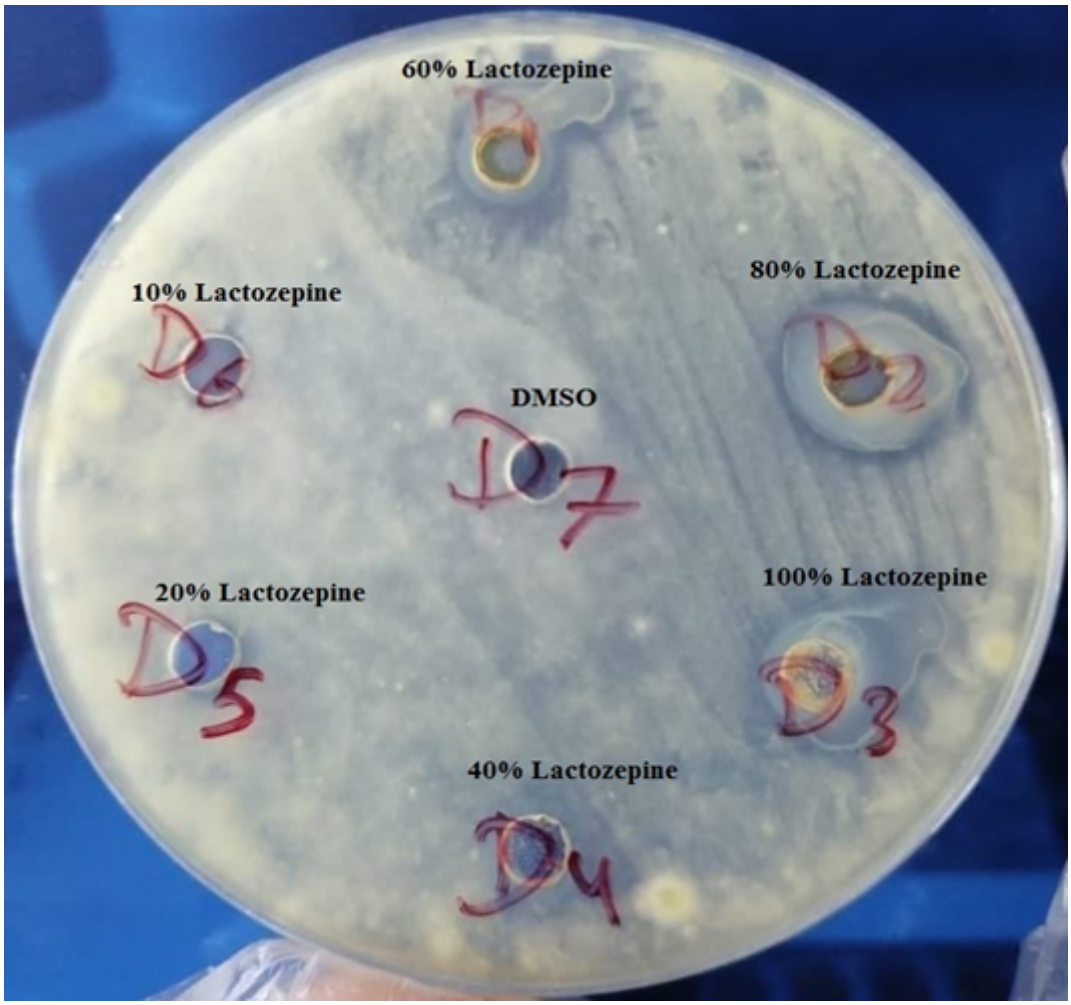

Figure 2

Inhibition zones of lactozepine against *Aspergillus niger*

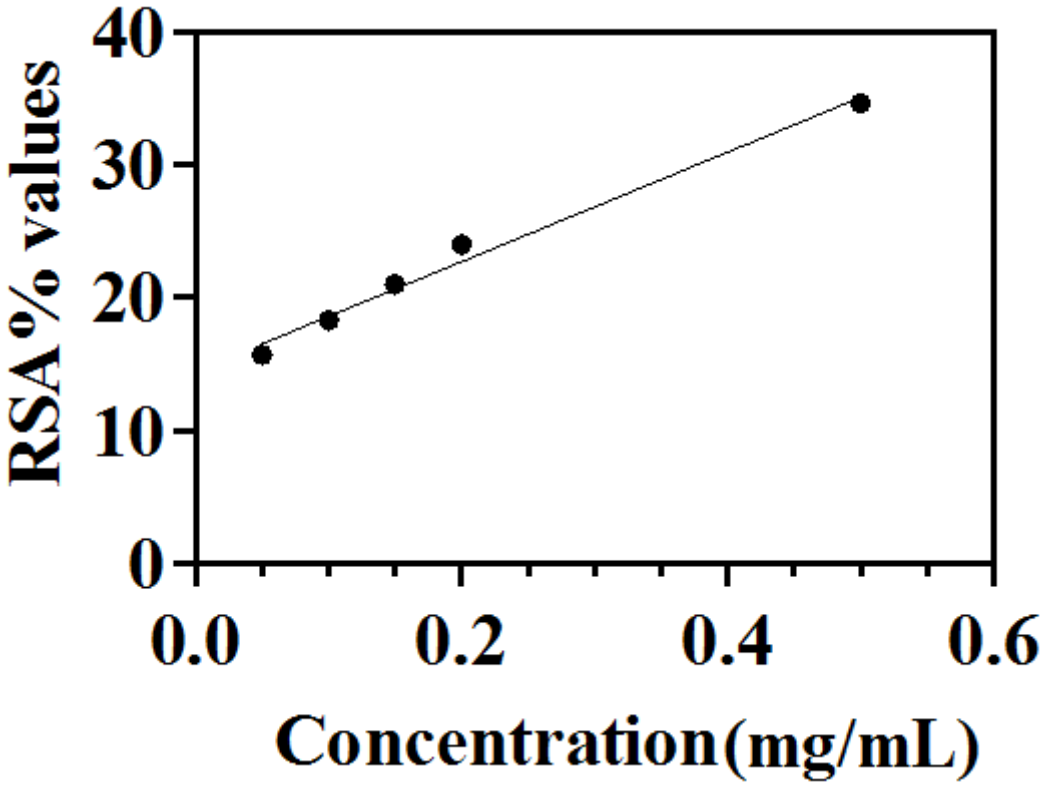

Figure 3

Shows the calculation of the linear parameters is a percentage values against concentrations of the synthesised lactozepine and vitamin C. The IC_{50} for lactozepine. R^2 =0.98.

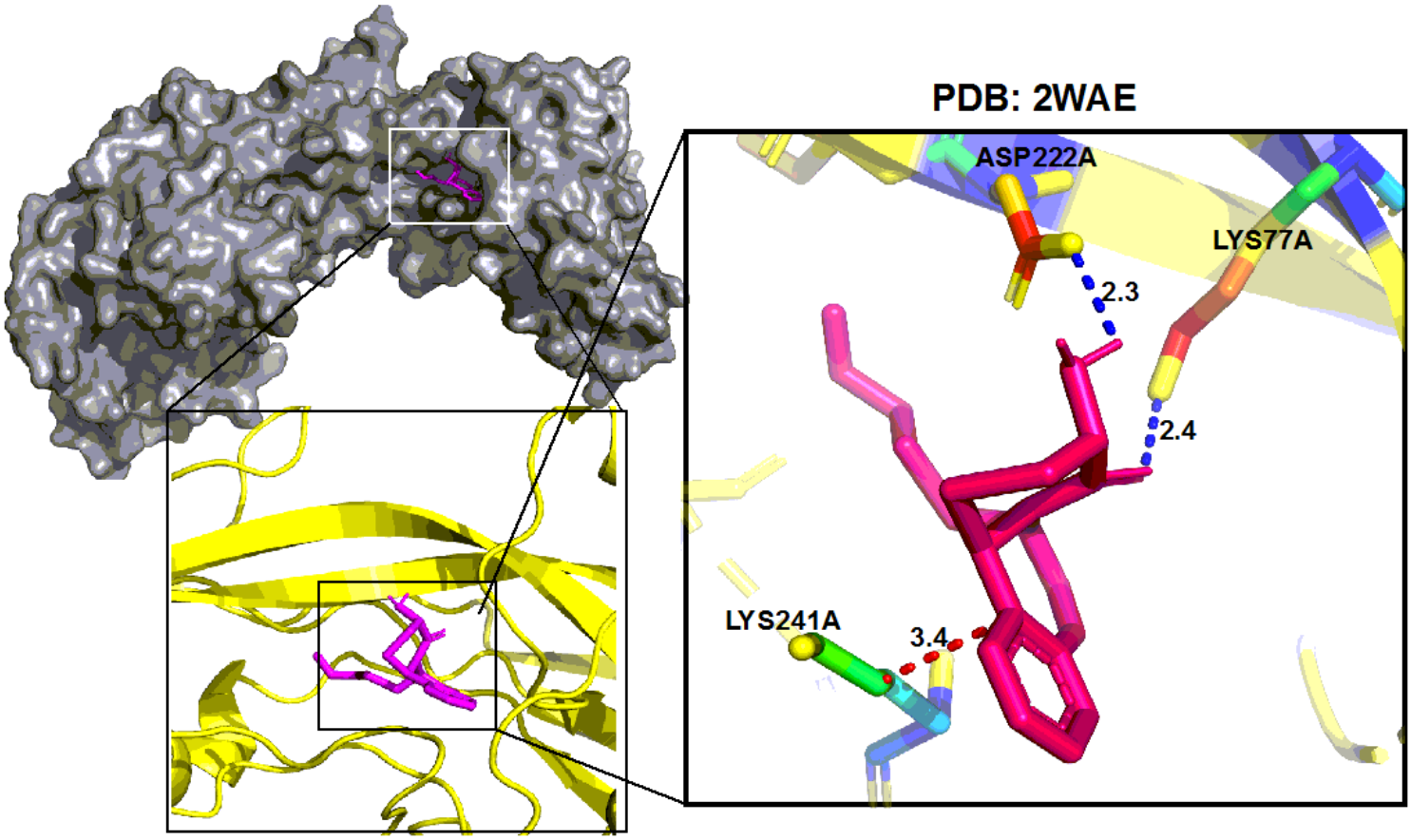

Figure 4

Molecular docking presentation of the synthesized ligand named as lactozepine with beta-lactamase protein 2B (class A) 2WAE PDB, coloured in grey.

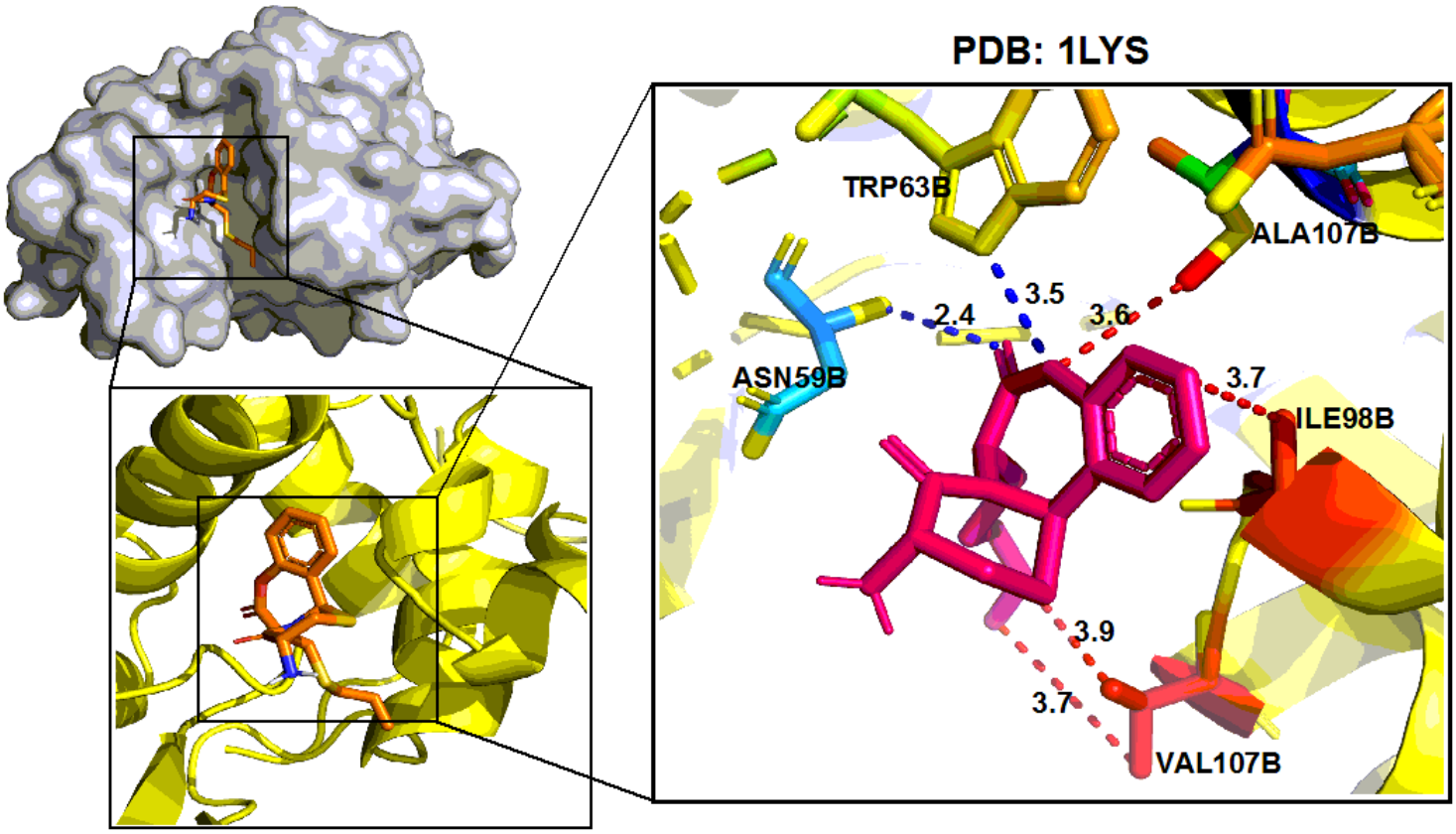

Figure 5

Molecular docking presentation of the synthesized ligand named as lactozepine with beta-lactamase protein 2B (class A) 1LYS PDB, coloured in grey.

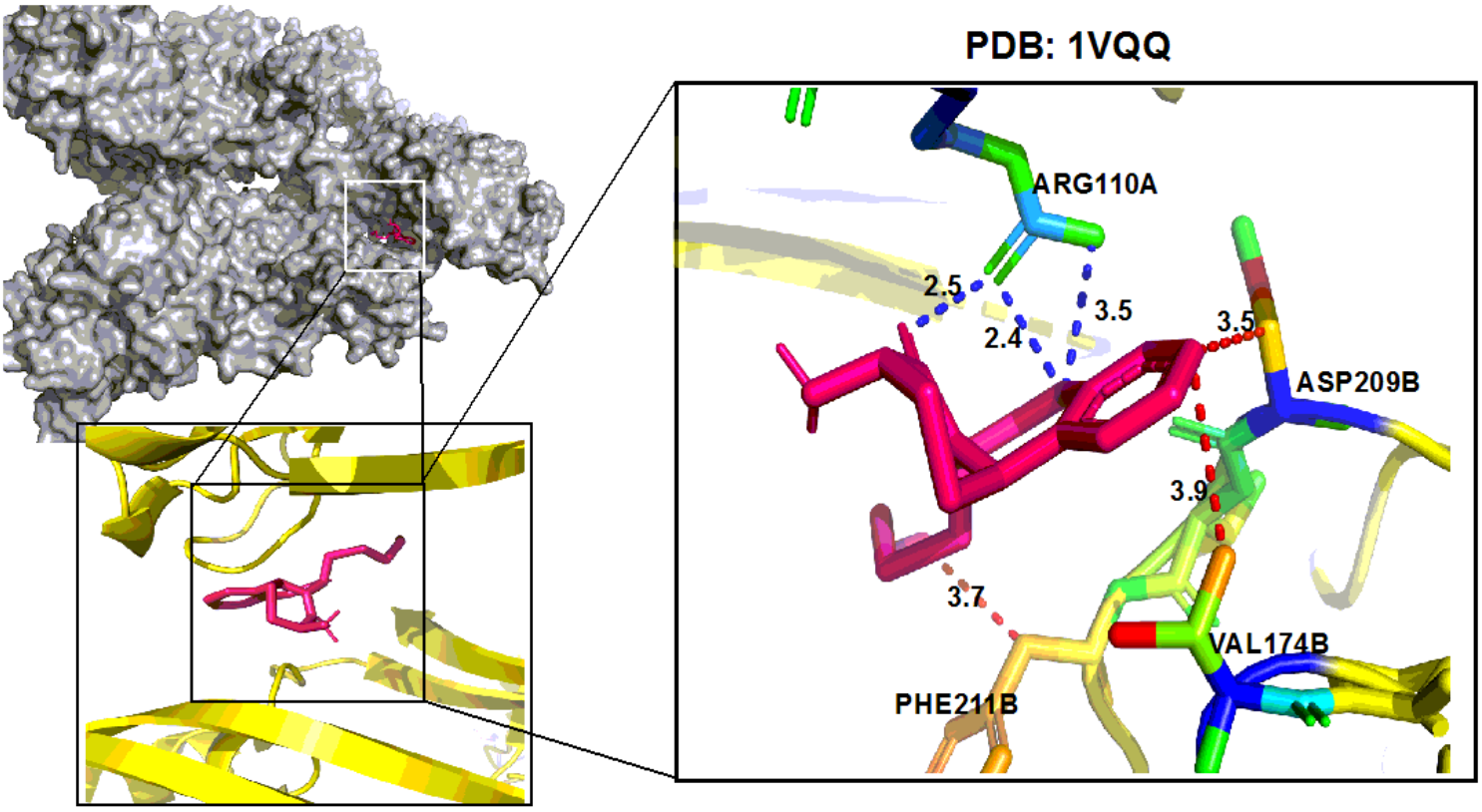

Figure 6

Molecular docking presentation of the synthesized ligand named as lactozepine with Penicillin binding protein 2B (chain 2A) 1VQQ PDB, coloured in grey.

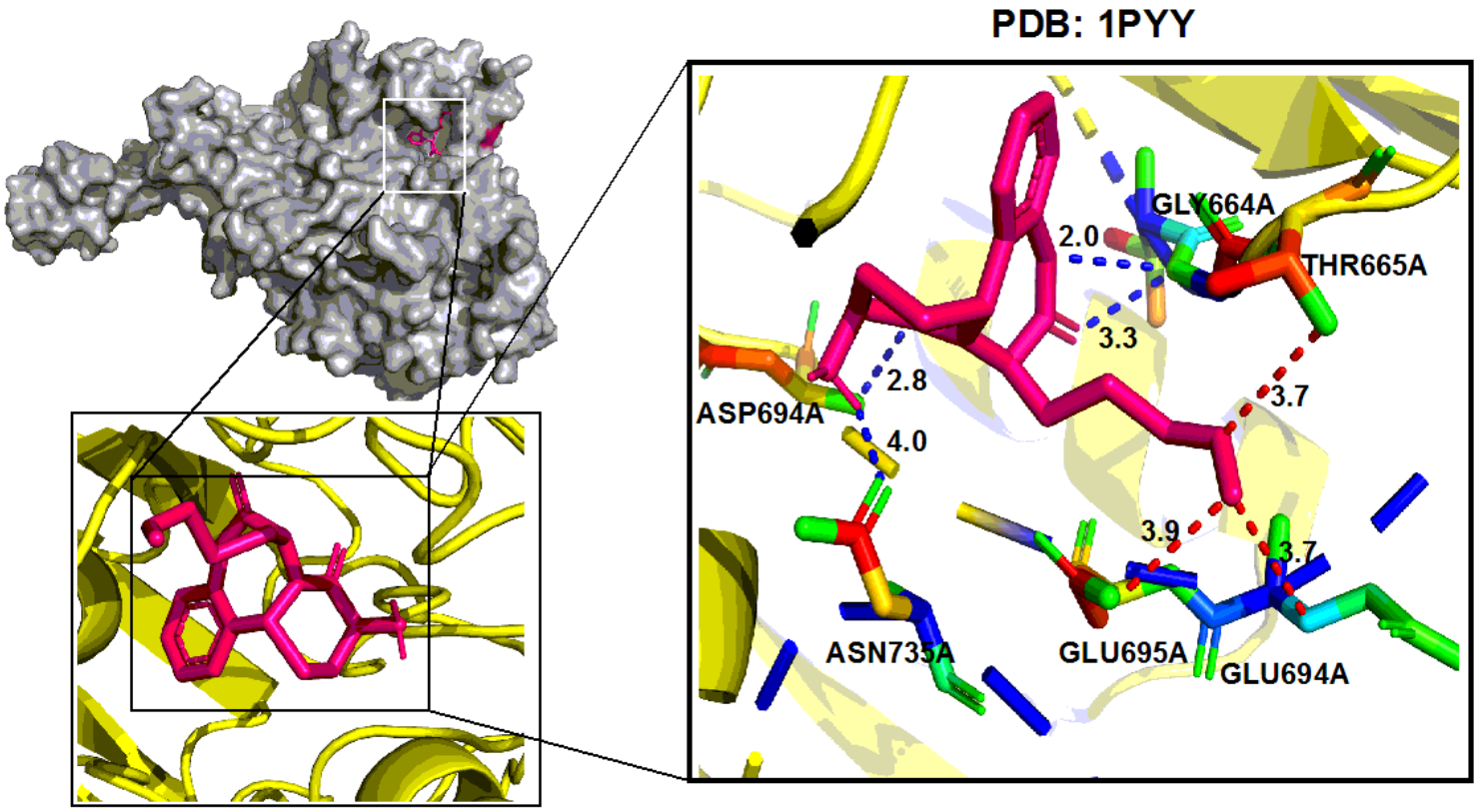

Figure 7

Molecular docking presentation of the synthesized ligand named as lactozepine with Penicillin linking protein 2X (chain A) 1PYY PDB, coloured in grey.

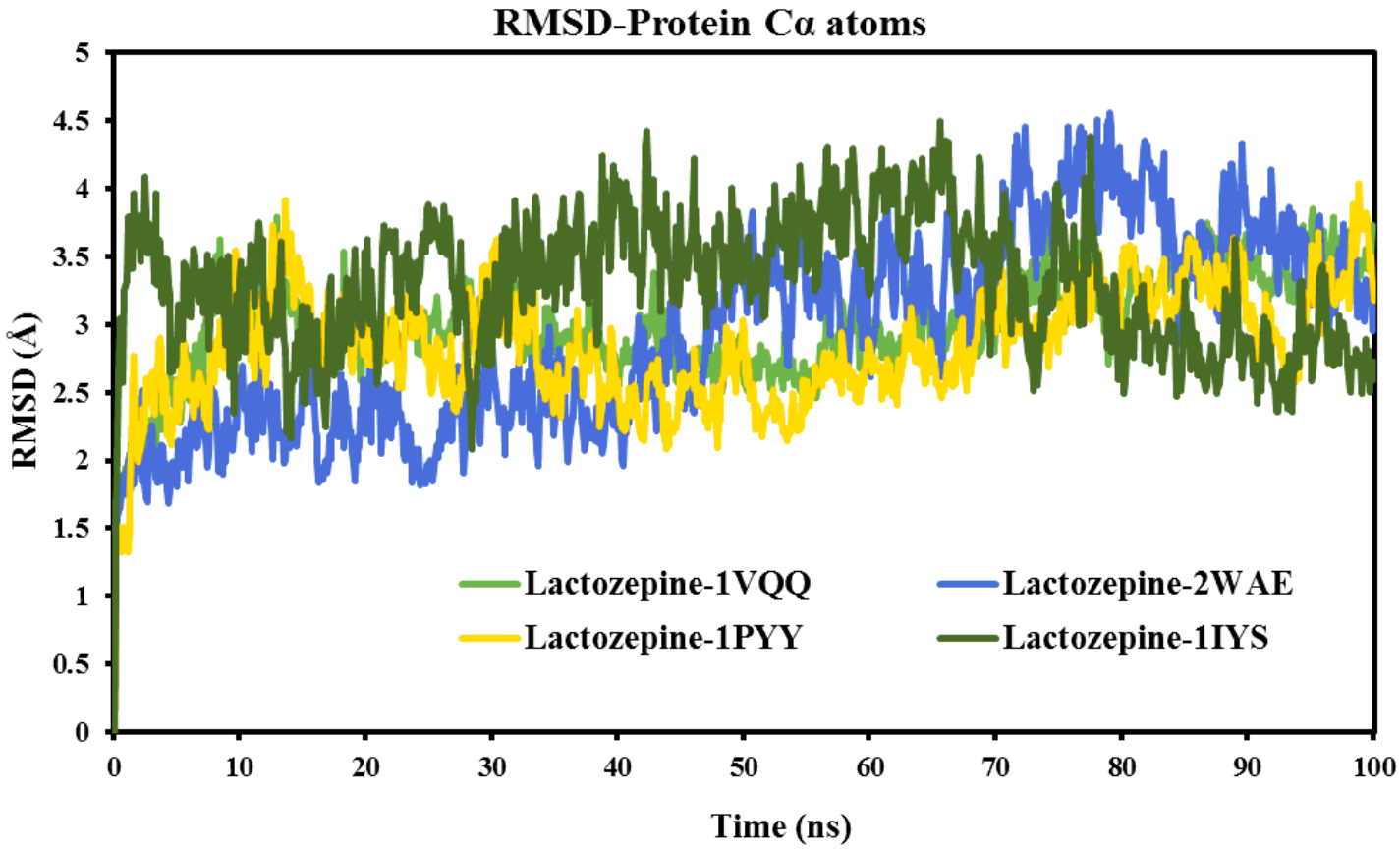

Figure 8

RMSD plot of lactozepine in complex with 1VQQ, 2WAE, 1PYY and 1IYS vs. time of the simulation

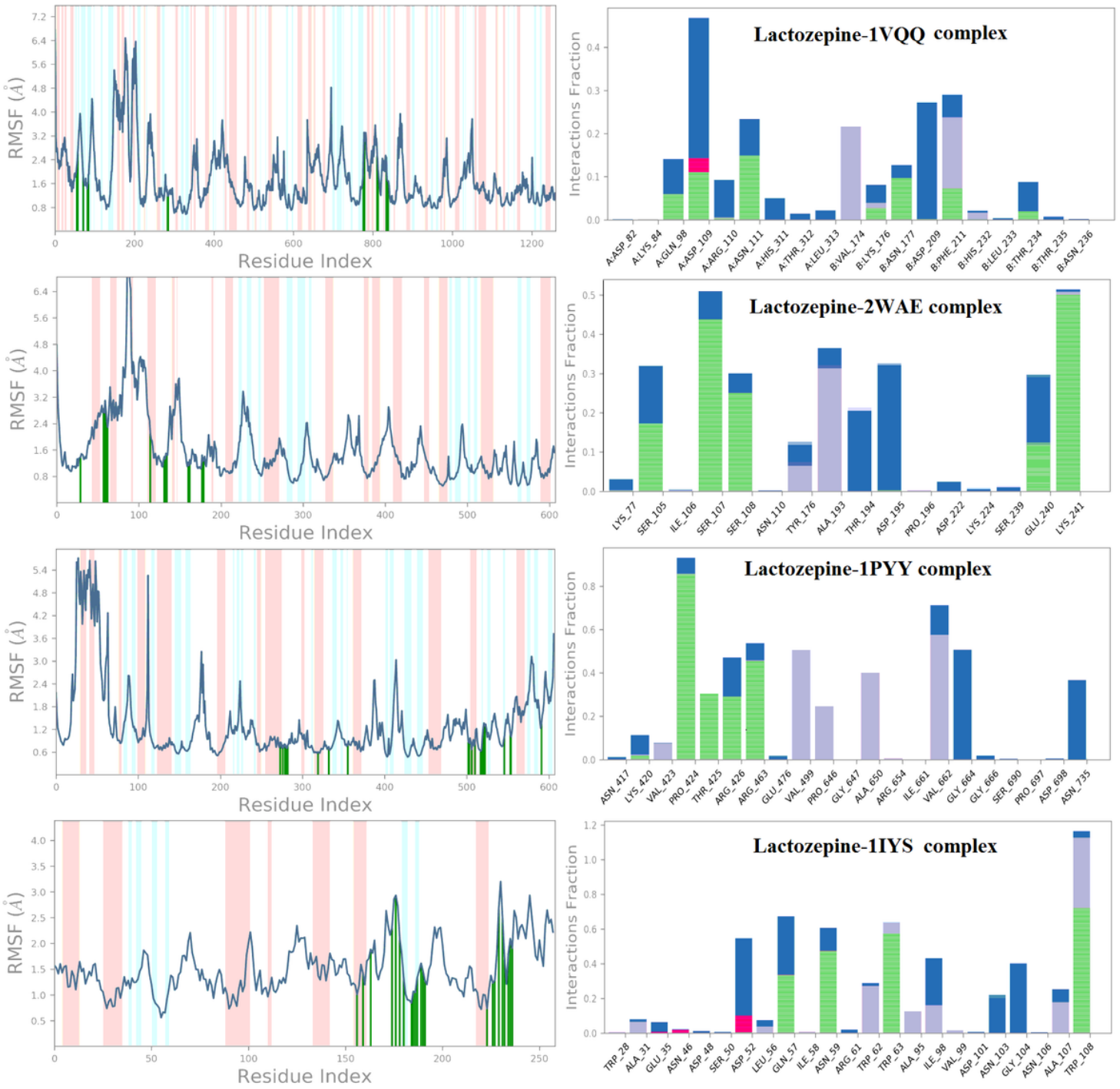

Figure 9

Individual amino acids RMSF and Protein ligand contact analysis of lactozepine in complex with 1VQQ, 2WAE, 1PYY and 1IYS vs. time of the simulation

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.jpg
- Scheme2.jpg
- Scheme3.jpg
- Supplenemtryfile.docx