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Synthesizes, characterization, molecular docking and in vitro bioactivity study of new compounds containing triple beta lactam rings

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ABSTRACT

Penicillin bioactivity is continuously decreased due to the misuses by people which resulted in an antibiotics resistance. However, B-lactam ring is still an effect group the attack bacterial infection. In this study, we aimed to synthesize compounds with triple B-lactam rings and study in vitro their bioactivity. Schiff bases were followed to form two effective ligands involving triple B-lactam rings that were also docked against Penicillin binding protein to show their abilities through computational simulation studies. The synthesis of the compounds was mainly performed in two major steps: the first step was to form three Schiff bases of the compound named as BTTP compound and the second step to perform a cyclisation to produce triple B-lactam rings named as BTTCDP compounds. The prepared compounds were then confirmed using various routinely spectroscopic methods such as FTIR, NMR and CHN techniques. In vitro study showed (MIC in μg ml^{-1}) ranged from (0.254 and 0.568), respectively for both ligands. However, no inhibition was shown against fungus which could be considered as specific antibacterial. The molecular docking study showed the pockets of the binding with minimum free energy about -8.9 and -9.0 kcal/mol. All to gather, the two novel compounds containing triple B-lactam rings showed an effective bioactivity that are promising for in vivo studies in future.

1. Introduction

Antibiotics have saved millions of people by treating or preventing bacterial infections. However, several peoples have shown resistance to antibiotics [1]. Therefore, this issue is still a matter of concern. Typically, the chemically synthesised antibiotics are based on the functional groups of antibiotics that are naturally appeared in environmental microorganisms [2,3]. One of these fundamental functional groups is beta-lactam group which is involved in Penicillin antibiotics. However, Penicillin becomes increasingly unfavourable due to the resistance that has shown due to the miss use. To address this issue, antibiotics structures need to be redesigned to achieve a better biological activity without or with less side effects and a high efficiency. However, this needs many efforts and trials but also it costs money. Recent studies have facilitated these obstacles and struggles by directing us to design a new type of medicines with a vital activity against bacteria using molecular docking simulation [4–7].

Azetidinone or β-lactam ring is a functional and an active compound that is involved in different castigates of antibiotics such as penicillin derivatives (penams), carbapenems and carbacephems, cephalosporins (cephems) and monobactams. β-lactam ring has shown a various vital activity, such as: anti-inflammatory antiviral antimicrobial anti-HCMV anticancer and anticonvulsant [8–12].

Chemically, β-lactam ring is a cyclic amide which can be readily hydrolysed easier than other liner amides or the large lactams. Furthermore, the main bioactivity of the β-lactam ring is due to the head and the tip, the tail, parts of the compound structure [13].

Various studies have shown a better improvement in the antibiotic's efficiency by a synthesis of compounds bearing two B-lactam rings in the same structure. However, as the best of our knowledge, an antibiotic bearing triple B-lactam rings is not published yet [2,14–16].

Therefore, previous studies tried to modify the tail or the head parts of the β-lactam ring which could result in more beneficial

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bioactive derivatives of Azetidinone. Other studies have used this feature to synthesize antifungal, antiviral and antibacterial agents [14–16]. In this paper, we aimed to synthesis new compounds of β-lactam derivatives using a simple and less expensive procedure with a better bioavailability. We hypothesised that the three aromatic episodes of β-lactam ring can be joined from their heads to produce a new bioactive structure with higher bioactivity. Technically, the newly prepared compounds are primarily tried in in vivo studies to compare their functions with the previous studies. Here, we show the synthesis, characterisation, and the bioactivity trials with conclusive scientific evidence for newly prepared compounds holding triple β-lactam rings at the same time. A computational simulation for docking two prepared ligands with Penicillin binding protein were also shown with promising results in this study.

2. Materials and methods

All the needed chemicals were purchased from Sigma and Aldrich. All synthesized compounds were characterised using melting point, HNMR, FT-IR, and CHN spectrum apparatuses. Also, all the biological activity was applied on prior freshly prepared dishes cultured for 24 h at 37 °C. All procedures for each compound mentioned below.

2.1. Chemical synthesis of the B-lactam derivatives

2.1.1. Synthesis of BTTP: N, N ', N' - (benzene -1,3,5-tri) Tris (1-phenylmethanamine)

This compound was prepared by adding 0.03 mol of 1,3,5- triamino benzene dissolved into 60 ml of absolute ethanol. Immediately, 5 ml of glacial acetic acid and 0.09 mols of benzaldehyde were added with continuous stirring overnight for 20-24 hours. The mixture was then distilled for 1 hour in a water bath until the reaction is completed. The procedure was repeated using different aldehyde compounds to end up with two products that were used to generate another reaction as in step 2. The prepared products were kept in chloroform.

Characterization of BTTP; Yield %: 85. Color: Yellow. m.p: 257 °C. FT-IR (KBr), ν (cm-1): 1720 (C=O), 1344 (C-N), 1461-1552 (C=C), 3051 (C-H_{aro}), 728 (C-Cl_{aza}), 637 (C-H_{aza}), 1442-1330 (N-O_2). ^{1}HNMR (25°C, CDCl3): 6.9-8.4 (m, C-H_{aro}), 3.1 and 3.8 (s, C-H_{aza}). CHN: ($C_{33}H_{18}Cl_8N_4O_5$) 47.52 (C_{theo}), 47.23 (C_{prac}), 2.18 (H_{theo}), 2.07 (H_{prac}), 34.0 (Cl_{theo}), 33.28 (Cl_{prac}), 6.72 (N_{theo}), 6.45 (N_{prac}). (Figures **S1** and **S2** in supplementary file).

2.1.2. Synthesis of BTTDCP: 1,1′,1′′-(benzene-1,3,5-triyl) tris (3,3-dichloro-4-phenylazetidin-2-one)

The compound was prepared by adding 0.09 mol of 2,2,2-trichloroacetic acid (TCA) gradually to the compound that was prepared in the last step with a concentration of 0.03 mol of "BTTP" dissolved with 0.02 mol of diethylamine in 60 mL dioxane. The reaction was carried out using a reflex for 12 hours with continuous stirring in a water bath at 0-5 °C. The product was then crystallized and stored at 4 °C for 24 hours. In the next day, the product was filtered and recrystallized with ethanol, then left out to dry at room temperature. All the products were sent out for characterisation purposes.

Characterization of BTTDCP; Yield %: 79. Color: White crystals. m.p: 247 °C. FT-IR (KBr), ν (cm-1): 1716 (C=O), 1368 (C-N), 1651-1579 (C=C), 3059 (C-H_{aro}), 688 (C-Cl_{aza}), 633 (C-H_{aza}), 1304-1485 (N-O_2). ^{1}HNMR (25°C, CDCl3): 7.7-8.6 (m, C-H_{aro}), 3.4 and 4.5 (s, C-H_{aza}). CHN: ($C_{33}H_{18}C_{l6}N_6O_9$) 46.35 (C_{theo}), 45.96 (C_{prac}), 2.12 (H_{theo}), 2.01 (H_{prac}), 9.83 (N_{theo}), 9.56 (N_{prac}). (Figures **S3** and **S4** in supplementary file).

2.2. Biological activity investigation

Different concentrations of all the newly synthesized compounds were used to in vitro evaluate their bioactivity as antimicrobials against three-gram negative and two-gram positive bacterial strains which are Pseudomonas fluorescence type MTCC 2950, Staphylococcus aurous type MTCC 96. Pseudomonas aeruginosa type MTCC 687, Streptococcus epidermises type MTCC 435 and Escherichia coli type MTCC 443. Also, the prepared compounds were evaluated against two strains of M. fungi Candida's albinos type MTCC 227 and Aspergillums Niger type MTCC 1344. Ciprofloxacin was used as an antibacterial control and the fluconazole was used as an antifungal control under the same conditions. The prepared compounds were evaluated using the disk diffusion method and the minimum inhibition concentration was determined by the inhibition scale located at http://www.agardiffusion.com. It is a common database for theoretical measurement, and it provides higher accuracy for MIC [17].

2.3. Molecular docking simulation

Both synthesised products with triple B-lactam rings were used as ligands to target Penicillin binding protein 2B (chain A). It is well-known that B-lactam ring in penicillin antibiotics works as inhibitors for both transpeptidase and DD-carboxypeptidase activities. The mechanism of action of these two enzymes are ended by acylating the active-site serine. All chemical structures were drawn using Chem-Draw professional 16.0 software. The PDB file was downloaded from https://www.rcsb.org/ as crystal structure with name 2WAE which refers to a Penicillin binding protein 2B (chain A) from Streptococcus pneumoniae. Both ligands named as Ligand 1 and Ligand 2 were docked verses the Penicillin binding protein 2B (chain A) to examine the binding pocket, best binding sites and stability of the ligands binding to the mentioned protein. Molecular docking analysis was carried out using online database CB-DOCK[19]. Once the complex obtained and free binding energy was calculated for the best binding, another analysis was performed using PLPI online website to find the specific residues that bind to the ligand and particularly those within 5 °A bind length [18]. The visualisation of the binding was performed using last version of PyMol software. High quality pictures were obtained and presented using Microsoft Power Point version 16.0.

The B-lactam activity is well defined group accept the R groups that either increase the activity or decrease it or could even increase/decrease the toxicity of the drug. In the situation of (Q)SAR quantitate structure activity relationship, it should have been examined in such a work to enhance the bioactivity of the two synthesized structures and also eligible for medicinal chemistry work, however, using ChemDraw, the only software is available to do such a work, the log P be could not be calculated by Broto's method. Therefore, this work needs future development and modren software to perform it [13].

3. Result and discussion

In this research, two mainly compounds were prepared with triple rings of b-lactam. The two suggested steps of the reactions are as shown in Fig. 1, the preparation method was consisted mainly of two steps. Firstly, Schiff base reactions were performed by adding one mole of the primary polyamine compound with three moles of aromatic benzaldehyde with substitutions at para site with either NO_2 or Cl molecules to obtain BTTP compound. The last compound was then reacted with three moles 2,2,2-trichloroacetic acid chloride in a cyclisation reaction to obtain the final product that contains triple B-lactam rigs in a compound named as BTTBCP. See Fig. 1 and Table 1.

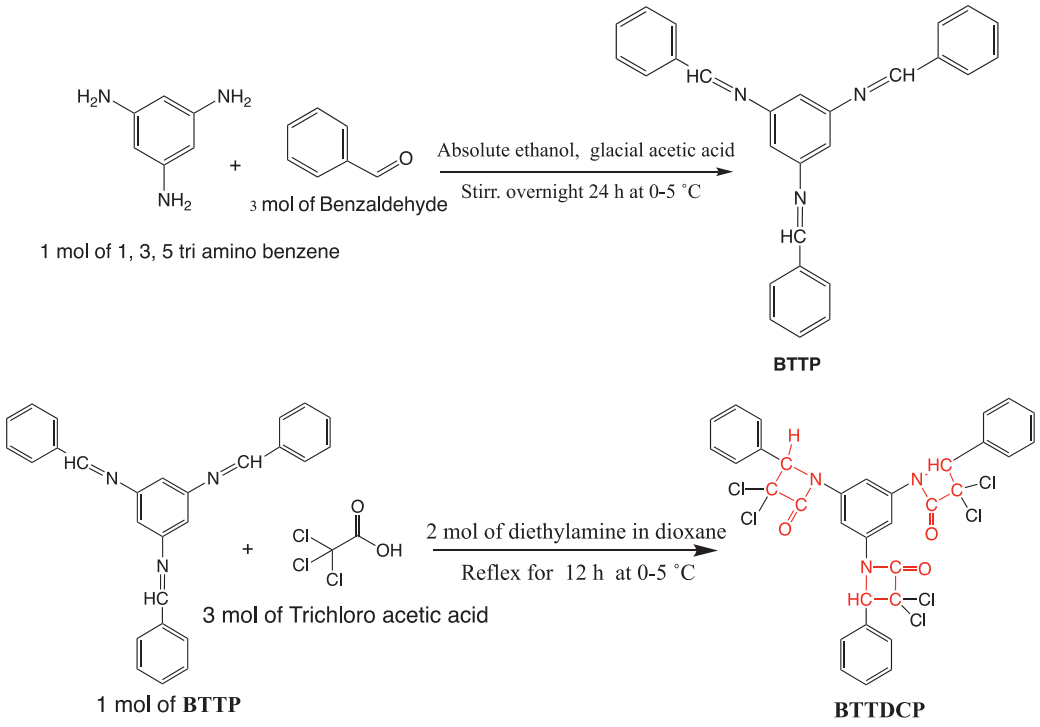

Fig. 1. Shows the two steps reactions that followed to obtain the final products involving the cyclisation reaction in the second step. B-lactam groups are labelled in red colour.

Table 1
Shows the final products that are used later as ligands for docking with Penicillin binding protein. It also shows the aldehyde compounds that were involved in the first step of the reaction to obtain the intermediate compounds BTTP.

Compound's name	Aldehyde compound	Structure
BTTDCP1 named as Ligand 1	1 mole of: para nitrobenzaldehyde And 2 moles of: para chlorobenzaldehyde	
BTTDCP2 named as Ligand 2.	3 moles of para nitrobenzaldehyde	

3.1. Mechanism of synthesis the BTTP and BTTDCP compounds

The Schiff's bases are typically produced by a condensation of primary amines and carbonyl groups involving a nucleophilic addition which result in a hemiaminal formation. This step is then followed by a dehydration to generate an imine. As it is clearly seen in Fig. 2, the nitrogen atoms in the triple amine groups acts as a nucleophile which attack the electrophilic carbonyl carbon of benzaldehydes resulting in a C=N and a water molecule as side product. The cyclisation reaction is generated by another Schiff's base reaction preformed when a nitrogen atom of C=N that was produced previously in step 1 as a nucleophilic additive, reacts with the carbonyl group as a nucleophile of trichloro acetic chloride resulting in a Azetidinone compound, B-lactam ring, releasing one

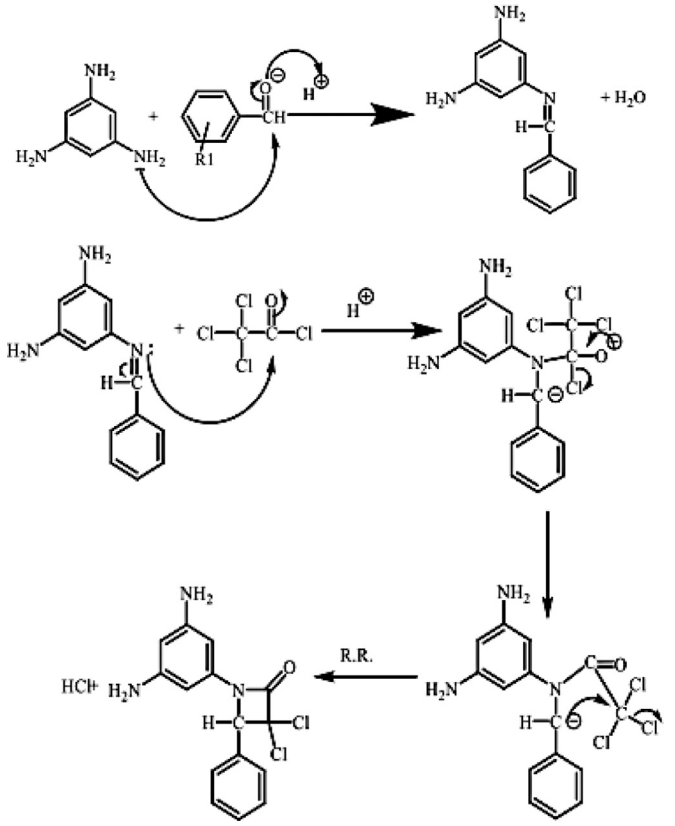

Fig. 2. a schematic reaction mechanism shows the two main steps of synthesis of BTTP and BTTCDP compounds.

HCl molecule. In other words, the first Schiff's base product was considered as a new nucleophile to perform another Schiff's bases reaction to form the cyclic compound.

3.2. Spectroscopic characterisation

The prepared compounds have been exposed to different spectrophotometric methods such as FTIR, CHNO and NMR spectra to confirm the assumed structures formula. The FT-I.R spectra confirmed the presence of functional groups in the prepared compounds as follow; FT.IR v (cm -1): 1870-1710 belongs to stretching vibration of C=O. 1690-1650, 1595-1520 belongs to C=C group and 1250-1360 belongs to C- N group. Therefore, these spectra confirm the composition of the azetidinone ring (see figures S1). 1H-N.M.R spectrum (ppm) showed multiplexed beam at the site 6.5-9.9 refers to the aromatic ring and 3- 4.5 refers to the C-H azetidinone ring Table 3. (See Figures S2). CHN data showed the quantitative analysis of the elements in the compounds. All the atoms numbers were close to those calculated in theory. However, there are small differences which could be due to the lack of measurement of the amount of oxygen element in the compound by the device itself.

3.3. Biological activities

In vitro bioactivity examinations were performed to detect the effects of prepared compounds against one type of E.coli bacteria which were *Staphylococcus aureus and also against* fungal strains. However, the bioactivity of the compounds against fungal strains was not promising and not encouraging. The disk diffusion method of Kirby and Bauer were followed to examine antibacterial activity [19]. Through the data obtained from measuring the diameter of inhibition of the effectiveness of the compounds prepared against bacteria, as was expected when starting to prepare these compounds, they have a high and distinctive inhibition against bacteria but have not shown inhibition against fungal (Tables 2–6). in Table 2, it can be clearly seen that both compounds, Ligand 1 and Ligand 2 have shown a remarkable effect on inhibiting Escherichia coli bacteria, and the compounds with the value of (MIC in μg ml^{-1}) ranged from (0.254 and 0.568), respectively. The main reason is probably due to the free presence of active groups on aromatic rings with triple B-lactam rings. However, both compounds showed less efficacy against *Staphylococcus aureus* than that of Escherichia coli. Despite that, they have shown enough inhibition against *Staphylococcus* aureus which depends on the concentrations of the compounds. In general, the higher concentrations used the better bioactivity was shown. Ciprofloxacin was used as a control regarding to the inhibition zoon's dimeters which were equal, close, or sometimes higher than the reference. (As seen in Tables 2-6).

3.4. Molecular docking

The main repressing objective for β-lactam antibiotics in S. aureus is the bifunctional transglucosylase-transpeptidase PBP2 [20,21]. Molecular docking analysis was performed to identify the variation of binding affinity for both ligands obtained in the current study that contain triple B-Lactam rings. Due to the high bioactivity of one B-lactam as a penicillin antibiotic, we therefore assume that triple B-lactam rings could show a better inhibitor for Penicillin binding protein in *Streptococcus pneumoniae bacteria* as a case of report.

3.5. Docking of ligand 1 with Penicillin binding protein 2B (chain A)

The result of docking showed that the lowest free energy for the best binding affinity was (-8.4 kcal/mol). The RMSD value for this binding for both lower l.b and upper u.b bound was zero. The interactions bonds with Ligand 1 were all observed as shown in table (1) and Figs. 3 and 4. ATP molecules pockets were observed interacting with Mg molecules inside the protein at Ala 501 and 502. The bonds showed average length to most of the residues, Lys 77, Pro 196, Glu 240, Lys 241 and Lys 241 that within 5 ˚A are 3.0 +- 0.3 ˚A. The pi-cationic interaction with (NO_2 bound to the benzene ring) was shown with Lys 77 has bond's length 3.67 ˚A. Other interactions with halogen bonds were shown bound to Ser 107 and Tyr 176 with bonds length 3.71 and 3.77 ˚A respectively. Most of these interactions show strong binding affinity to the targeted ligand and enough stability.

3.6. Docking of ligand 1 with Penicillin binding protein 2B (chain A)

The docking of the second ligand has showed better results even than the first ligand due to the three times repeated NO_2 groups bounds to the benzene rings. The result showed that the lowest free energy value for the best binding affinity was (-9.0 kcal/mol). The RMSD value for this binding for both lower l.b and upper u.b bound was zero as well.

This ligand showed various interactions bonds with Ligand 2 with Penicillin binding protein 2B (chain A) such as hydrogen bonds, water bridges, salt bridges and p—stacking bonds. Significantly, the distance between Hydrogen bond and the residues in chain A were very short with range (1.88-3.26) ˚A. Also, more residues have shown various interactions with ligand in the pocket. Collectively, this ligand showed better binding due to functional groups attached to the terminal ring of benzene. All the information about the bond's lengths is provided in the Table 7.

Table 2
MIC value, and inhibition values, for the synthesized against *Escherichia coli.*

Compounds	Zone of inhibition (diameter) in mm									g ml^{-1}
	400 μg ml^{-1}	200 μg ml^{-1}	100 μg ml^{-1}	50 μg ml^{-1}	25 μg ml^{-1}	15 μg ml^{-1}	10 μg ml^{-1}	5 μg ml^{-1}	1μg ml^{-1}	
L1	16.3	11.9	11.3	9.4	10.2	-	7.3	-	-	0.681
L2	16.0	15.2	14.2	12.9	9.9	8.6	7.1	7.9	0.9	0.568
Ciprofloxacin	16.3	15.7	15.0	13.6	10.8	9.8	8.11	8.2	7.7	0.799

Table 3
MIC value, and inhibition values, for the synthesized *Staphylococcus aureus.*

Compounds	Zone of inhibition (diameter) in mm									MIC in μg ml^{-1}
	400 μg ml^{-1}	200 μg ml^{-1}	100 μg ml^{-1}	50 μg ml^{-1}	25 μg ml^{-1}	15 μg ml^{-1}	10 μg ml^{-1}	5 μg ml^{-1}	1 μg ml^{-1}	
L1	14.2	10.5	11.7	12.9	9.2	10.1	7.5	7.1	7.9	1.246
L2	11.9	11.1	9.4	8.5	6.3	11.5	8.2	-	8.4	0.254
Ciprofloxacin	16.8	16.1	15.4	14.8	11.8	10.2	8.1	7.9	7.2	0.886

Table 4
MIC value, and inhibition values, for the synthesized against *Psuedomonas Aeruginosa.*

Compounds	Zone of inhibition (diameter) in mm									MIC in μg ml^{-1}
	400 μg ml^{-1}	200 μg ml^{-1}	100 μg ml^{-1}	50 μg ml^{-1}	25 μg ml^{-1}	15 μg ml^{-1}	10 μg ml^{-1}	5 μg ml^{-1}	1μg ml^{-1}	
L1	15.2	11.1	11.4	10.6	8.9	8.1	7.4	-	-	2.154
L2	16.1	13.4	13.8	11.2	9.9	7.1	8.4	7.9	7.9	0.367
Ciprofloxacin	16.8	15.7	14.5	12.4	10.9	9.7	8.5	8.0	7.8	0.587

Table 5
MIC value, and inhibition values, for the synthesized against *Staphylococcus epidermidis.*

Compounds	Zone of inhibition (diameter) in mm									MIC in μg ml^{-1}
	400 μg ml^{-1}	200 μg ml^{-1}	100 μg ml^{-1}	50 μg ml^{-1}	25 μg ml^{-1}	15 μg ml^{-1}	10 μg ml^{-1}	5 μg ml^{-1}	1 μg ml^{-1}	
L1	15.9	13.8	11.5	11.9	8.8	7.7	8.9	7.9	6.8	1.698
L2	16.1	15.2	14.9	10.3	10.5	9.1	8.3	7.3	7.1	0.548
Ciprofloxacin	16.7	15.3	14.1	12.5	10.8	9.7	9.2	8.6	7.1	0.734

Table 6
MIC value, and inhibition values, for the synthesized against *Pseudomonas fluorescens.*

Compounds	Zone of inhibition (diameter) in mm									MIC in μg ml^{-1}
	400 μg ml^{-1}	200 μg ml^{-1}	100 μg ml^{-1}	50 μg ml^{-1}	25 μg ml^{-1}	15 μg ml^{-1}	10 μg ml^{-1}	5μg ml^{-1}	1 μg ml^{-1}	
L1	13.9	14.1	14.7	11.1	9.9	8.7	-	-	-	4.235
L2	14.9	12.9	14.1	12.7	10.3	10.8	7.6	7.2	5.4	0.459
Ciprofloxacin	16.4	15.8	14.8	12.4	11.9	10.8	9.8	8.5	7.3	0.66

Most of these interactions show strong binding affinity to the targeted ligand and enough stability. These would confirm the *in vitro* experiments that were observed against the *Streptococcus pneumoniae bacteria* suggesting that multiple B-lactam rings would be beneficial for bacterial synthesis inhibition. It is wealth mentioning that these simulation studies always confirm the practical studies in the lab which could reduce the cost and the effort of experimental performance through short computational work.

3.7. Molecular dynamics simulation

Molecular dynamics (MD) simulation is an imperative and useful method for investigating the dynamic behavior of the protein-ligand complex and its relative stability. The compounds with the maximum binding affinity scores, ligands **1** and **2**, with Penicillin binding protein 2B protein (PDB ID: 2WAE), were chosen for further MD simulation study. The Schrödinger Desmond MD simulation programme (version 2021-1) was used to perform MD simulation, installed on a Z2 TWR G4 workstation with the configuration Ubuntu 18.04.3 LTS 64-bit, Intel Core i7-9700 and NVIDIA Quadro P620/PCIe/SSE2 graphics processing unit [22,23]. In an orthorhombic box, protein ligand complexes were solvated with a minimal distance of 10 Å from the protein surface to the edges of the box using the Simple Point Charge (SPC) water model. At a salt concentration of 0.15 mol/L, sodium and chloride counter-ions were introduced to make each system electrically neutral, mimicking human physiological circumstances [23,24]. Following system preparation, the model system was subjected to energy minimization using a fixed parameter of the OPLS3e force field to remove electronic conflicts between protein structures and properly align the protein structure inside the simulation boundaries [25]. Minimization tasks relax the system into a local energy min-

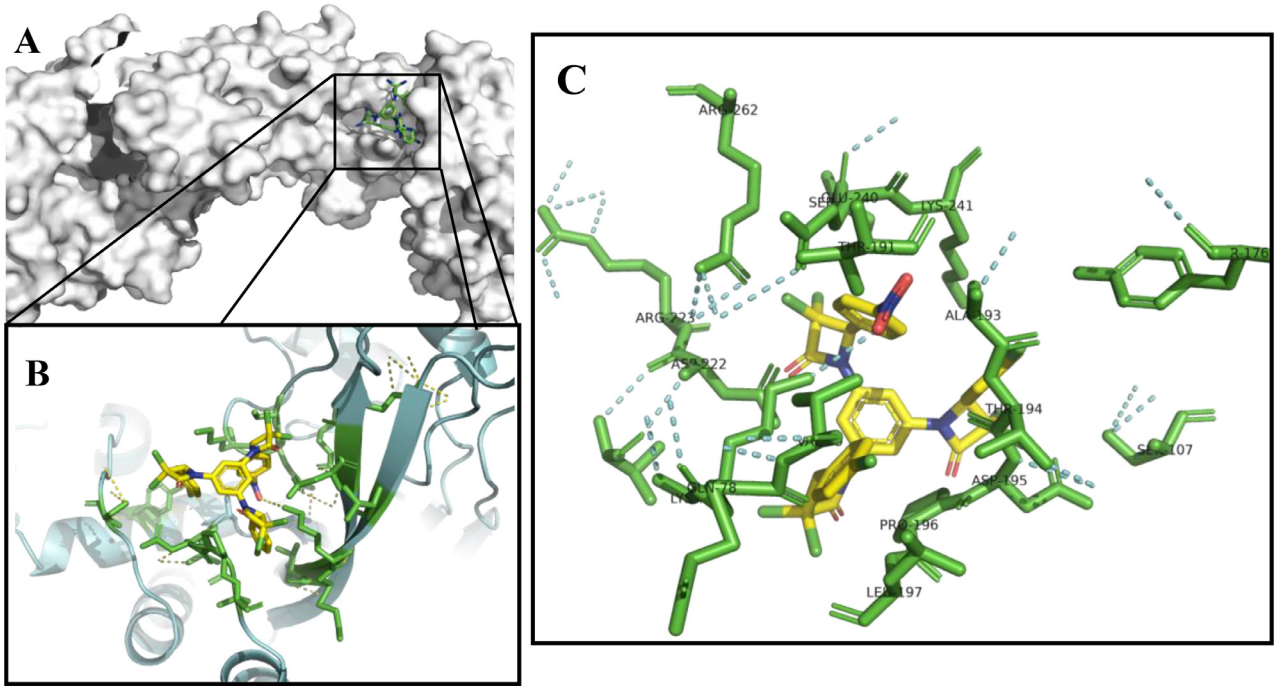

Fig. 3. Molecular visualizations using PyMol software of the CB-docking of ligand 1 with Penicillin binding protein 2B (chain A) 2WAE PDB. A shows the binding pocket of ligand 1 in the PDB crystal structure of the protein. B shows some contacts in zoomed in section. C shows the labelled residues and the contacts of the ligand with bacterial protein.

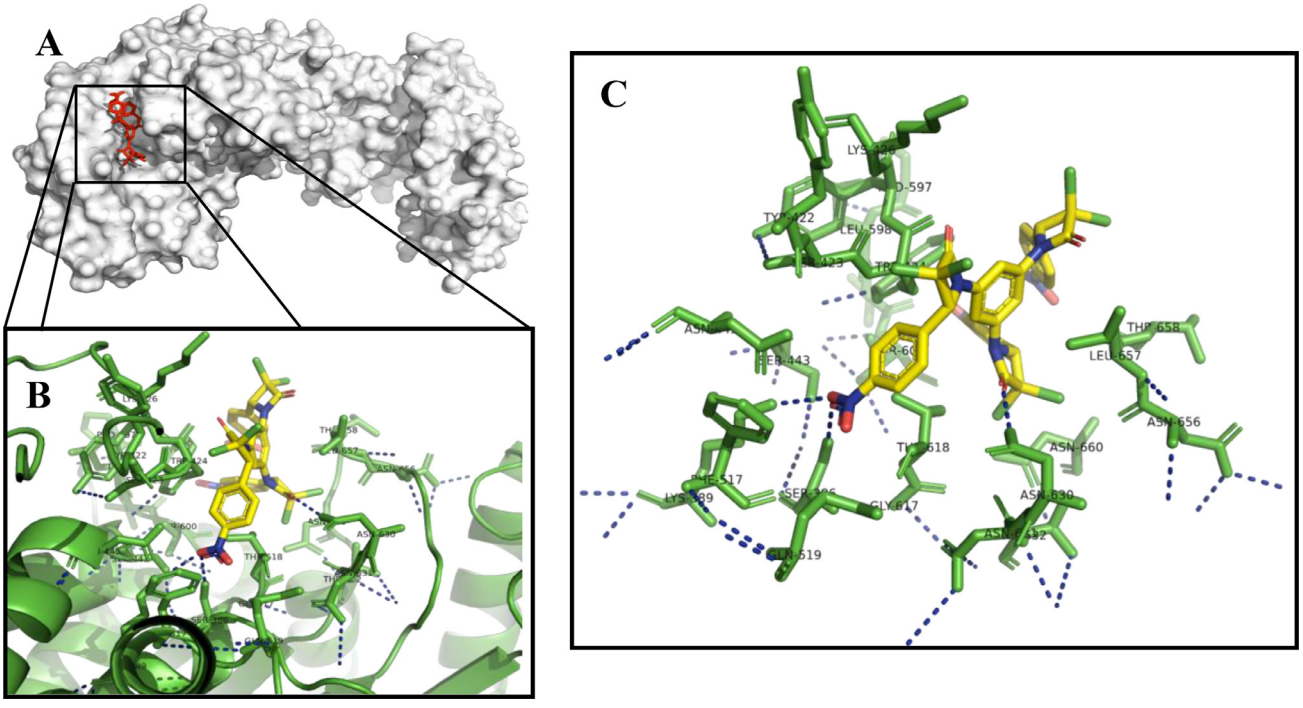

Fig. 4. Molecular visualizations using PyMol software of the CB-docking of ligand 1 with Penicillin binding protein 2B (chain A) 2WAE PDB. A shows the binding pocket of ligand 1 in the PDB crystal structure of the protein. B shows some contacts in zoomed in section. C shows the labelled residues and the contacts of the ligand with bacterial protein.

imum, using a hybrid method of the steepest decent and the limited-memory Broyden-Fletcher- Goldfarb-Shanno (LBFGS) algorithms with a maximum iteration of 2000 and a convergence threshold of 1.0 kcal/mol/A°. To distribute the solvent and ions evenly throughout the protein-ligand complex, the complete system was equilibrated using canonical NVT followed by isothermal-isobaric NPT ensemble methods [26]. Following the import of the constructed minimized system (.cms file) into the molecular dynamics module, the simulation was conducted for 100ns with simulation snapshots acquired at 100 ps intervals.

3.8. Molecular dynamics (MD) simulation

Proteins undergo a major conformational alteration when in contact with a drug molecule, and dynamics simulations are required to understand the internal motions, conformational

Table 7
shows the details of the docking Ligand 2 with 2wae PDB using online software.

Hydrogen bonds Residue name	Residue number	H-Chain A distance	Ligand2-ChainA distance
Tyr	58	3.26	4.03
Ile	59	1.91	2.75
Ile	61	1.87	2.85
Glu	86	2.03	2.91
lys	87	2.81	3.15
Val	88	2.09	3.04
Asn	92	1.92	2.90
Arg	111	2.46	3.23
Arg	111	1.88	2.81
Water bridges **Residue name**	**Residue number**	**A-W distance**	**D-W distance**
His	89	2.93	2.97
Thr	91	3.97	3.30
Arg	111	3.65	2.58
Lys	307	2.70	3.15
Lys	307	3.95	3.15
Arg	309	4.06	2.95
Arg	309	2.95	3.27
Arg	309	3.79	3.27
Arg	309	3.40	3.55
Salt bridges **Residue name**	**Residue number**	**Distance**	
Lys	87	3.74	
Lys	87	4.03	
Arg	111	4.92	
Lys	307	3.55	
Arg	309	4.83	
Arg	309	4.80	

changes, and stability of protein-ligand complexes. The root-mean square deviation (RMSD), root-mean square fluctuation (RMSF), and protein ligand contact mapping were estimated using the MD trajectories to investigate their structural stabilities, binding modes, and binding strengths.

The deviation of the protein Cα atoms associated with drug molecules may be explained by the RMSD parameter from the MD trajectory, where minimal variations indicate attainment of a stable conformation and vice versa [27]. The RMSD of each frame of the Penicillin binding protein 2B Cα atoms associated with ligands **1** and **2** was determined and is shown in Fig. 5. It can be noticed that the RMSD value of the Penicillin binding protein 2B Cα atoms bound with both ligands **1** and **2** was steadily raised initially. The maximum RMSD value can provide information regarding the stability of the protein Cα atoms throughout simulation. The maximum RMSD of the Penicillin binding protein 2B Cα atoms was found to be 5.4 Å and 5.6 Å bound with ligands **1** and **2**, respectively. As a result, less RMSD and constant Cα atom fluctuation clearly explained the stability of protein-ligand interactions. It was observed that ligand **1** RMSD varied from 2.4 Å to 4.8 Å. ligand **1** showed very little fluctuation and stayed intact throughout the simulation, and no significant fluctuation was observed. Except for a slight variation around 60 ns of simulation time, the RMSD of ligand **2** remained steady throughout the simulation. The difference between the maximum and mean RMSD can explain the molecules' deviation from their mean positioning. The difference between the maximum and mean RMSD values for ligands **1** and **2** was observed to be 1.124 Å and 1.351 Å, respectively. The results presented above clearly indicate that both molecules stayed inside the pocket without significant conformational changes.

In the MD simulation study, proteins individual amino acid residues are particularly important for determining the relative stability of the protein-ligand complex. The fluctuation of each and every amino acid residue was explored through the RMSF parameter, which represents the average deviation of each amino acid from the reference position, and it describes the fluctuation of each amino acid. A high RMSF value suggests loose bonding or the existence of loops, as well as the flexibility of the protein structure; on the other hand, a lower RMSF value shows stability, as well as the presence of secondary structures like sheets and helices [28–31]. Loop regions are represented by a white backdrop in the RMSF graph, whereas β-sheets and α-helices are represented by pink and blue backgrounds, respectively. From the MD simulation trajectory, the RMSF of each individual amino acid of Penicillin binding protein 2B associated with ligand **1** and ligand **2** was computed, and it is shown in Fig. 6. The contribution of interaction residues between the active site of protein chains and ligand **1** and ligand **2** is depicted by the green vertical lines on the X-axis of the graph. It is observed that ligand **1** and ligand **2** interacted with 20 and 41 amino acids of the Penicillin binding protein 2B, respectively. With the exception of the loop areas and the C terminus, most residues have RMSF values of less than 3 Å, suggesting that the residue conformation is reasonably stable during the simulation.

The existence of a significant number of inter-molecular interactions is critical for the stability of a protein-ligand complex. There are four types of protein-ligand interactions: amino acid mediated water bridges, hydrogen bonds, hydrophobic interactions, and ionic interactions. In the **ligand 1-2WAE** complex, the hydrophobic amino acid Trp424 interacted 41% of the simulation time with the central phenyl ring of ligand **1**. Protein ligand contact mapping shows that, the residues ala603, and ala660, exhibited more than 10% hydrogen bond interactions with the lead compound ligand 1. According to protein ligand contact mapping, the residues Ala603 and Ala660 have more than 10% hydrogen bond interactions with the lead chemical ligand 1 (Fig. 6A). In the ligand 2-2WAE complex, Ser418, Ala419, Val466, Asn508, and Asn511 were identified as interacting with ligand 2 through hydro-

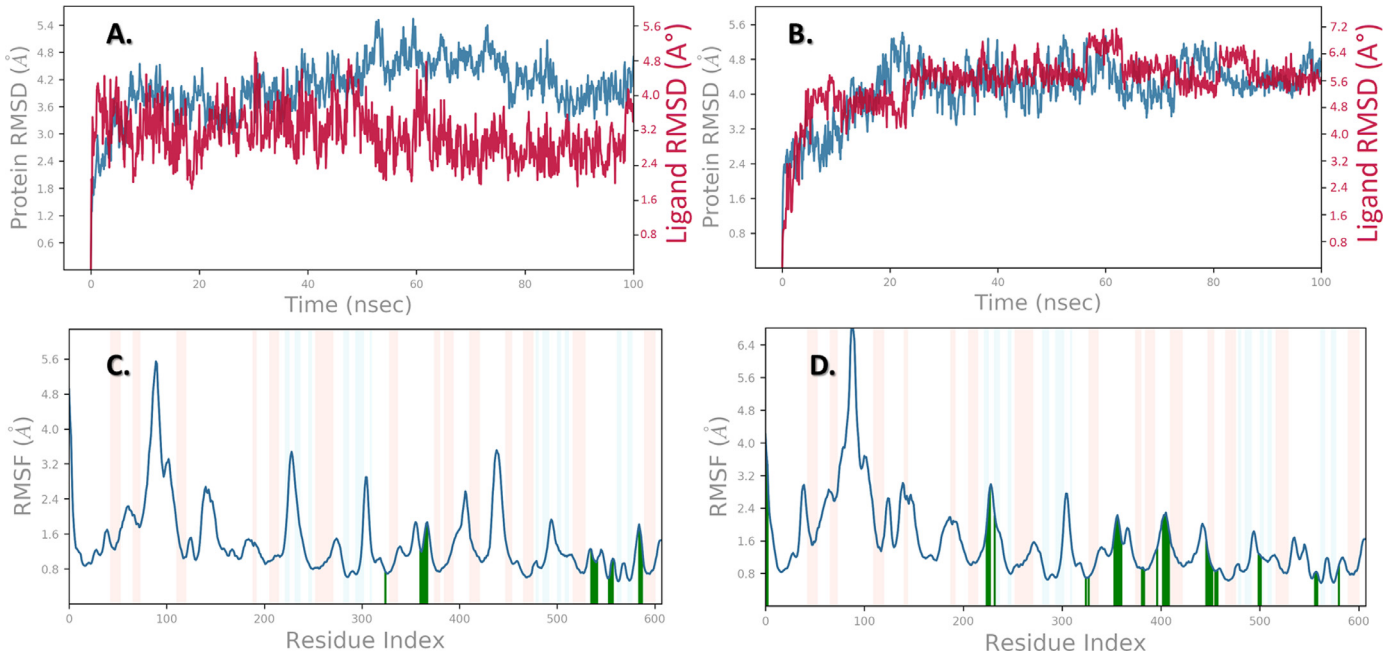

Fig. 5. RMSD and RMSF of Penicillin binding protein 2B (PDB ID: 2WAE) relative to the starting complexes during 100 ns MD trajectory for Ligand 1 (A&C) and ligand 2 (B&D).

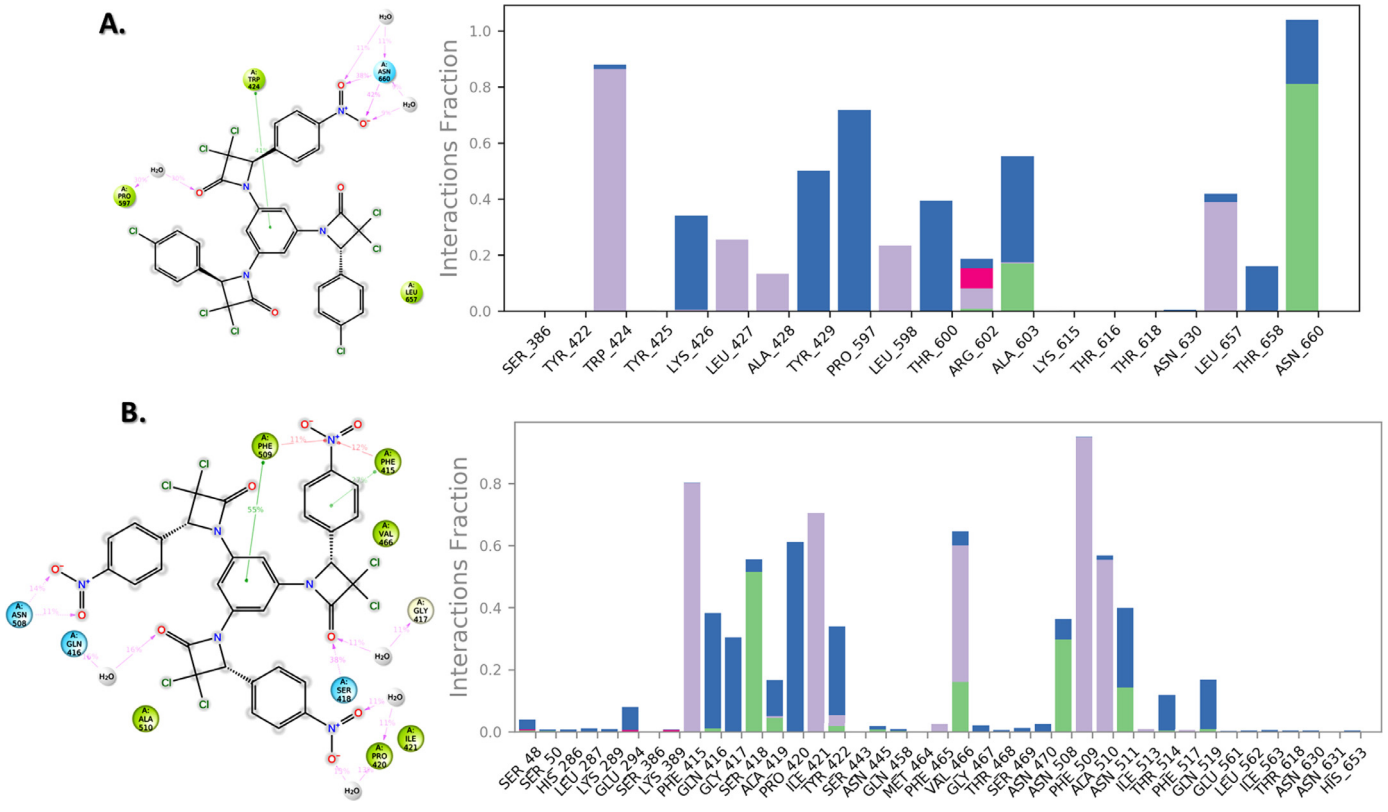

Fig. 6. Protein–ligand contact analysis of 100ns MD trajectory; A. ligand 1-2WAE complex and B. ligand 2 -2WAE compl.

gen bonds, whereas many water bridges were also noticed with Gln416, Gly417, Ala419, Pro420, Tyr422, Asn511, Thr514, and Gln519 (Fig. 6B). Overall, the simulation revealed more hydrophobic interaction, amino-acid mediated water bridges stabilized the compound ligand **1** and ligand **2.**

4. Conclusion

B-lactam ring is a well-known effective compound that has exceptional bioactivity against different microbials, vials and fangs. However, Penicillin bioactivity has substantially decreased due to the uncontrolled use of it by people which resulted in a antibiotics resistance. We have synthesized novel compounds holding triple B-lactam rings and examined their biological activity in vitro. In prior, a molecular docking simulation was performed to examine the binding pockets inside the Penicillin binding protein that is one of the bacterial proteins targeted by antibiotics using online docking databases. Consequently, two compounds holding triple B-lactam were synthesized and confirmed using various routinely spectroscopic methods such as FTIR, NMR and CHN techniques. In vitro study of these two ligands showed a remarkable bioactivity (MIC in μg ml^{-1}) ranged from (0.254 and 0.568), respectively. However, no inhibition was shown against fungus which could be considered as specific antibacterial. On the other hand, the molecular docking study showed the pockets of the binding with minimum free energy about -8.9 and -9.0 kcal/mol. It can be stated that the two novel compounds containing triple B-lactam rings that showed an effective bioactivity are promising for in vivo studies in future which probably use as antibiotics medicine.

Data availability

The authors do not have permission to share data.

Declaration of Competing Interest

None declared.

CRediT authorship contribution statement

Marwan Mohammed Farhan: Conceptualization, Validation, Formal analysis, Investigation, Project administration. **Manaf A Guma:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Muwafaq A Rabeea:** Methodology, Software, Validation, Investigation. **Iqrar Ahmad:** Software, Writing – original draft, Writing – review & editing. **Harun Patel:** Software, Project administration.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.133781.

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