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RESEARCH ARTICLE

Synthesis, Characterization, Molecular Docking, and Molecular Dynamics Studies of Schiff Bases of 2-Phenoxy-1-Phenyl-Ethanone as Antibacterial Agents

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ARTICLE HISTORY

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Abstract: ***Background*:** The development of antimicrobial resistance and reduced discovery and commercialization of newer antibiotics point to the need for the discovery of novel antibacterial agents. Schiff bases are reported to possess a broad range of pharmacological activities, which also include antibacterial activity. With this background, novel Schiff bases were synthesized.

***Aim*:** The present study aimed to synthesize novel Schiff bases of 2-phenoxy-1-phenyl-ethanone with aryl amines and evaluate their antibacterial activity.

***Methods*:** The desired Schiff bases were synthesized by condensing various aryl amines with 2-bromo-1-phenylethanone and characterized by physicochemical and spectral methods. The antibacterial activity of the synthesized Schiff bases was determined by the cup diffusion method. Molecular docking with Staphylococcus aureus tyrosyl-t-RNA synthetase (Protein Data Bank ID: 1JIJ) was performed using Autodock. Molecular dynamics simulation studies were performed using GROMACS. ADME analysis and drug-likeliness of the synthesized compounds were predicted using SwissADME. Protein-ligand interactions were studied using BIOVIA Discovery Studio Visualizer.

***Results*:** Out of the synthesized compounds, two compounds, sb_04 and sb_05, exhibited fair antibacterial activity. The compounds sb_04 and sb_05 exhibited better binding energy values than the original ligand of the target enzyme and comparable binding energy with ampicillin. The Autodock program estimated the binding free energy and inhibition constant values of compound sb_05 as -9.18 kcal/mol and 187.33 nM and for the compound sb_04 as -8.82 kcal/mol and 341.59 nM, respectively. The RMSD values of the compound sb_04 were stable throughout the duration of the simulation; however, the compound sb_05 showed lesser stability in the docked complex. Interestingly, the compound sb_05 (-182.754 kJ/mol) exhibited stable interaction with a lower value of total interaction energy than sb_04 (-136.304 kJ/mol). The synthesized Schiff bases showed no violation of Lipinski's rule of five and a bioavailability score in the range of 55% to 85%.

***Conclusion*:** The synthesized Schiff bases present a scaffold for the development of novel antibacterial agents, in particular the compounds sb_04 and sb_05.

Keywords: Schiff base, antibacterial, molecular docking, molecular dynamics, autodock, GROMACS.

1. INTRODUCTION

The discovery of antibiotics may be considered as one of the major discoveries of the last century and can also be attributed to the increase in the average life span of a human. In the last 75 years, not only human society but also animal livestock have greatly benefitted from the use of antibacterial agents. However, the current scenario is of concern due to the emergence of antimicrobial resistance (AMR). The more we use, the more we lose can be well said about the antibiotics as the antibiotics are becoming ineffective on extensive use or inappropriate use due to the emergence of antimicrobial resistance. The reason for the development of AMR includes overuse in medicine, agriculture, animal farming, and the evolutionary processes [1].

Antimicrobial resistance occurs when the microbe develops the ability to bypass the effect of antimicrobial drugs. As a result of the development of AMR, the growth and survival of the microbe are not affected to the extent they should normally have in the presence of antimicrobial agents. The bacteria *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are amongst the microbes that exhibit resistance toward conventional antibiotics [2].

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World Health Organization in 2019 declared AMR as one of the top 10 global public health threats. As per an estimate, 700,000 deaths are caused globally by infections due to antibiotic-resistant microbes, and further, it is estimated that in 2050, this figure could be around 100,00000 [3]. Also, the annual economic burden of AMR in 2050 is supposed to lower the global gross domestic product by one trillion. Thus, AMR also presents a huge economic burden to the world [4].

On the other hand, it is worrying that the process of discovery and commercialization of newer antibiotics has declined since 1990. One of the reasons for the declining production of antibiotics may be the short life span of antibiotics due to the emergence of resistance due to the high cost incurred in research and production and time spent in the discovery [5].

The criticality of the need for the discovery of potent and novel antibacterial agents is thus evident. Tyrosyl-t-RNA synthetase is a key enzyme involved in bacterial protein synthesis and has been reported as an attractive target for the discovery of novel antibacterial agents [6].

Schiff bases (SBs) are organic compounds that have an imine group (> C=N-) in their chemical structure. SBs are reported to possess a broad range of pharmacological activities, which also include antibacterial activity, and are amongst the important compounds for the pharmaceutical industry [7]. The presence of the imine group has been reported as an important functionality for the antioxidant property in Schiff bases [8].

With this background, five novel Schiff bases of 2-phenoxy-1-phenylethanone with aryl amines were synthesized, characterized, and evaluated against Gram-positive and Gram-negative bacterial strains. The physicochemical parameters, ADMET analysis, and Lipinski's rule of five were estimated for the synthesized compounds by using the SwissADME web tool. A molecular docking study of the synthesized compounds with *Staphylococcus aureus* tyrosyl-t-RNA synthetase was also performed by using Autodock Tools and Autodock. Further, a molecular dynamics study of the best-docked pose of the compounds exhibiting better binding energy in the molecular docking study was also performed for further understanding of the interactions with tyrosyl-t-RNA synthetase by GROMACS.

2. MATERIAL AND METHODS

All the chemicals used were of analytical grade and purchased from commercial suppliers, viz. phenacyl bromide from Sigma and other chemicals from Central Drug House (CDH). Melting points of the synthesized compounds were determined by the open capillary method and were uncorrected. Thin-layer chromatography (TLC) using silica gel G was used to monitor the progress of the reaction and visualize it in an iodine chamber. Infrared (IR) spectra of synthesized compounds were recorded in potassium bromide discs on Perkin Elmer RX1 (4000 - 400 cm^{-1}). The ^1H NMR spectra were recorded on a Bruker DRX-300 spectrophotometer at 300 MHz in $CDCl_3$ with TMS as the internal standard. All chemical shift values were reported in ppm (δ).

Open Babel program was used to convert file formats used in molecular docking and molecular dynamics studies [9]. Autodock tools 1.5.6, along with algorithms autodock4 and autogrid4, were used to perform molecular docking of the compounds with selected target enzymes [10]. UCSF Chimera program (Chimera-1.17.3-linux_x86_64) was used for visualization of molecular structures and analysis [11]. The structures of the target enzymes were downloaded from the Protein Data Bank (PDB) [12]. The topology and parameters of the ligand were obtained by SwissParam, a web tool by the Molecular Modelling Group of the University of Lausanne and the SIB Swiss Institute of Bioinformatics [13, 14]. GROMACS version 2024.1 was used to perform molecular dynamics simulation and output analysis [15]. The computations were performed on a Linux system running Ubuntu 22.04 LTS. ADME parameters of the synthesized compounds were calculated by using SwissADME [16].

3. EXPERIMENTAL WORK

3.1. Synthesis of 2-phenoxy-1-phenyl-ethanone (3)

Equimolar amounts of 2-bromo-1-phenylethanone (**1**) (1.9g, 10 mmol), phenol (**2**) (0.94g, 10 mmol), and anhydrous K_2CO_3 (2.76g, 20 mmol) in dry acetonitrile was refluxed for about 6h. The mixture was filtered, and the solvent was removed under reduced pressure. The resulting solid was washed with excess water three times with 25 ml aliquots. The crude solid obtained was purified by recrystallization from ethanol to afford ethanone **(3)**. The reaction progress was monitored by TLC.

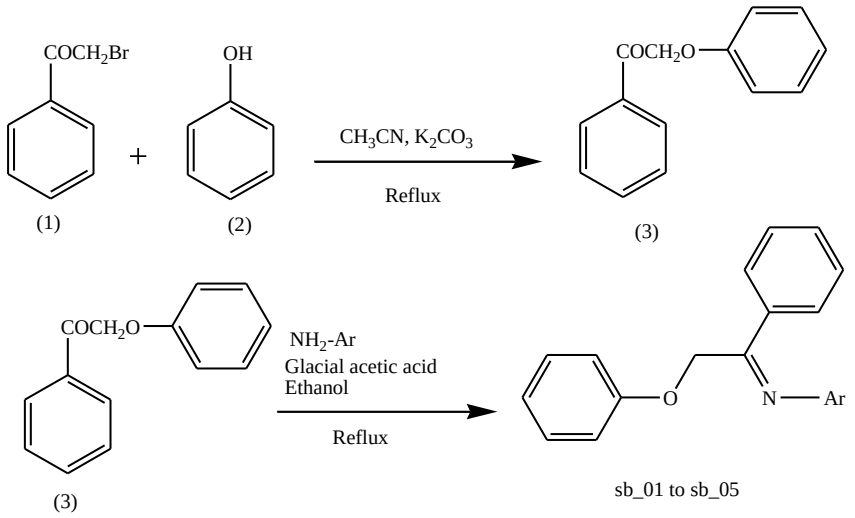
Yield: 72.00%, melting point: 61-64 ^oC, Rf value: 3:1 (n-hexane: ethyl acetate), FTIR (ν, cm^{-1}): 3064, 2900, 1705, 1580, 1093. ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 6.64-8.05 (m, Ar-H), 5.28 (s, 2H, $-OCH_2-$).

3.2. General Method for Synthesis of Imine Compounds (sb_01 to sb_05)

A mixture of compound **3** (0.01 mol), aryl amine **4** (0.01mol), and 1 ml of glacial acetic acid in ethanol was refluxed in the water bath for 10 h. The mixture was allowed to cool. The solid obtained in the reaction mixture was filtered. The separated solid was recrystallized from ethanol to afford the desired compounds **sb_01** to **sb_05**. The reaction progress was continuously monitored by TLC. The synthetic route for the synthesis of Schiff bases is outlined in Scheme **1**.

1-[4-(2-Phenoxy-1-phenyl-ethylideneamino)-phenyl]-ethanone (sb_01): Yield 55%, melting point: 60-64^oC, Rf value: 0.79 (n-hexane: ethyl acetate, 3:1), FT-IR (ν, cm^{-1}): 3060, 2900, 1702, 1593, 1495, 1430, 1246, 1090. ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 6.65-8.14 (m, Ar-H), 5.30 (s, 2H, $-OCH_2-$), 2.68 (s, 3H, $-CH_3$)

(2-phenoxy-1-phenyl-ethylidene)-[1,2,4]-triazol-4-yl)-amine (sb_02): Yield 58%, melting point: 63-65 ^oC, Rf value: 0.83 (n-hexane: ethyl acetate, 3:1), FTIR (ν, cm^{-1}): 3060, 2900, 1593, 1495, 1430, 1246, 1172, 1090. ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 6.79-8.24 (m, Ar-H), 5.3 (s, 2H, $-CH_2$).



Scheme 1. Synthesis of Schiff bases sb_01 to sb_05.

(2-phenoxy-1-phenyl-ethylidene)-pyridin-2-yl-amine (sb_03): Yield 60%, melting point: 58-62 ^{o}C, Rf value: 0.78 (n-hexane: ethyl acetate, 3:1), FTIR (ν, cm^{-1}): 3060, 2900, 1595, 1495, 1431, 1220, 1090. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 6.63-8.24 (m, Ar-H), 5.3 (s, 2H, -CH_2).

(5-methyl-pyridin-2-yl) -(2-phenoxy-1-phenyl-ethylidene)-amine (sb_04): Yield 62%, melting point: 62-65 ^{o}C, Rf value: 0.84 (n-hexane: ethyl acetate, 3:1), FTIR (ν, cm^{-1}): 3060, 2900, 1595, 1495, 1446, 1221, 1091. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 6.63-8.17 (m, 13H, Ar-H), 5.3 (s, 2H, -CH_2), 2.2 (s, 3H, Ar-CH_3)

2-(2-phenoxy-1-phenylethylideneamino)-benzenethiol (sb_05): Yield 54%, Rf value: 0.70 (n-hexane: ethyl acetate, 3:1), FTIR (ν, cm^{-1}): 3060, 2900, 1593, 1494, 1446, 1219, 1091. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 6.91-8.17 (m, Ar-H), 5.3 (s, 2H, -CH_2), 3.5 (s, 1H, -SH)

3.3. Molecular Docking

The binding of target compounds with the protein of interest was studied by using molecular docking. The molecular docking was performed using Autodock Tools 1.5.6 using the algorithm autodock 4.2 and autogrid 4.2 [16]. The 2D chemical structures of the target compounds were energy minimized prior to use for docking studies using a universal force field (UFF). The chemical structures of the synthesized compounds were converted to the required file format using the structural file converter Open Babel. The desired target antibacterial enzyme (PDB 1JIJ) was obtained from the Protein Data Bank (PDB). The unwanted ligands and water molecules were removed from the protein prior to docking analysis. The missing atoms of the protein were repaired, polar hydrogens and Kollmann charges were added to the protein molecule, and Gasteiger charges were added to the ligands. The grid box of 60 x 60 x 60 in X, Y, and Z, centred around the site of binding of the original ligand, was used to prepare the grid box, and subsequently, autogrid4 was used to generate the grid parameter file. The docking of the ligand onto the target enzyme was performed using a genetic algorithm (GA) with 25 runs and a population size of 300. Other parameters were kept at the default value of Autodock tools. The docking parameter file (*.dpf) was prepared using the Lamarckian Genetic Algorithm. The docking was performed using the autodock4 algorithm to obtain the output docking log file (*.dlg) file. Thereafter, the docking log file was used to obtain the estimated binding energy (kcal/mol) and inhibition constant (Ki) values (μM). All the synthesized compounds sb_01 to sb_05, the original ligand of the protein tyrosyl-t-RNA synthetase SB-239629 and ampicillin, a standard antibacterial drug, were subjected to molecular docking analysis.

3.4. Molecular Dynamics

Molecular dynamics (MD) simulation studies provide information about the structural behaviour of target enzymes and ligands over a defined period of time. The synthesized compounds sb_04 and sb_05 exhibited better binding energy than the original ligand of the antibacterial target enzyme and were thus selected for further computational studies. The MD studies of the best docking pose of the compound sb_04 and sb_05 with the target enzyme were performed using GROMACS 2024.1 on the Ubuntu 22.04 LTS operating system along with CUDA toolkit 12.4. The parameters of ligands required for molecular dynamics studies were obtained from the web server SwissParam. The structure was solvated with water model TIP3P in a periodic, triclinic box with 2Å distance from the edges. The system was neutralized by adding counterions. Verlet cutoff scheme was used for van der Waals and electrostatic interactions. The electrostatic interactions were calculated using the particle mesh Ewald (PME) method. The energy of the solvated system was minimized by the steepest descent minimization method. The equilibration of the system was performed first in NVT and then NPT conditions with a 100 ps run. The final production run was performed with a step size of 2 fs and a number of steps equivalent to a total duration of 20 ns MD simulation run. The visualization of trajectories of the MD simulation was done using the program Grace.

3.5. Study of Protein and Ligand Interactions

The interactions of the best-docked pose of the synthesized compounds with the target protein were estimated by BIOVIA Discovery Studio Visualizer. The analysis of the structural behaviour of target protein and compounds sb_04 and sb_05 over the duration of the MD simulation run was performed using GROMACS 2024.1 and UCSF Chimera 1.17.1 software. The molecular dynamics study of protein and the ligands was studied by analysing root mean square deviation (RMSD) and energy calculations. In particular, the short-range Coulombic and Lennard Jones interaction energies were calculated between the protein and the ligand using GROMACS.

3.6. ADMET Analysis

ADMET analysis and Lipinski's rule of five were estimated for all the synthesized compounds by using the SwissADME web server. The chemical structures of the synthesized compounds were converted to the format required as input by Open Babel.

3.7. Antibacterial Activity

The antimicrobial efficacy of the synthesized compounds was evaluated using the cup diffusion technique. The synthesized compounds, as 1 mg/ml solutions in dimethylformamide (DMF), were evaluated for activity against Gram-positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* by the cup diffusion technique. Dimethylformamide (DMF) was used as a negative control. Sterile nutrient agar was inoculated with the test organisms (each 100 ml of the medium received 1 ml of 24 h broth culture), and then seeded agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar, and each cup received 0.1 ml of the test compound solution. The plates were then incubated at 37°C for 24 h. The activities were estimated as zones of inhibition in mm diameter. Ampicillin (0.01%) was used as positive control.

4. RESULTS AND DISCUSSION

4.1. Characterization of Synthesized Compounds

The synthesized compounds were characterized by physicochemical methods and FT-IR and ^{1}H NMR spectral methods. The appearance of a peak at 1093 cm^{-1} in the FT-IR spectra of (**3**) assigned to -C-O-C stretching frequency indicated the presence of an ether bond. The FT-IR spectra of all the synthesized compounds showed a peak around 1595-1590 cm^{-1}, which was attributed to the imine (>C=N-) group, indicating the formation of Schiff bases. All the synthesized compounds exhibited peaks 1495-1430 cm^{-1} attributed to C=C stretching vibrations of the aromatic ring. The peaks around 1091-1090 $cm^{-1,}$ attributed to the -C-O-C stretching in ethers, were observed in all the synthesized Schiff bases.

The ^{1}H NMR peak of -OCH_2 in 2-bromo-1-phenylethanone (**1**) appeared as a singlet at 4.5 δ ppm, in the ^{1}H NMR spectrum of 2-phenoxy-1-phenyl-ethanone (**3**), a singlet peak was observed at 5.28 δ ppm, indicating the formation of (**3**). In the series of compounds synthesized (**sb_01** to **sb_05**), the -OCH_2 peak in the ^{1}H NMR spectra appeared as a singlet around 5.3 δ ppm, confirming the formation of >C=N bond in the Schiff bases. The peaks observed in the region 6.63-8.24 δ ppm were attributed to the presence of various aromatic protons. In addition to the characteristic peaks, as mentioned, all the synthesized compounds exhibited the characteristic peaks corresponding to their peculiar structural feature. In the compound **sb_01**, the appearance of a singlet peak at 2.68 δ ppm was attributed to methyl protons attached to the -CO group in the para position of the phenyl ring. The singlet peak observed at 2.1 δ ppm was attributed to the methyl group attached to the phenyl ring in **sb_04**. Similarly, a singlet peak observed at 3.5 δ ppm was attributed to the -SH group in **sb_05**. All these spectral characterizations confirmed the structure of synthesized compounds.

4.2. Molecular Docking

The molecular docking of the five compounds sb_01, sb_02, sb_03, sb_04, and sb_05 was performed on *S. aureus* tyrosyl-t-RNA synthetase. The three-dimensional structure of bacterial tyrosyl-tRNA synthetase was obtained from Protein Data Bank (PDB id 1JIJ). The tyrosyl-tRNA synthetase was involved in bacterial protein synthesis and has been reported as a target for finding novel antibacterial agents [6].

The docking of the target protein was performed for the five synthesized compounds, an original ligand of the target enzyme, and ampicillin. The energy of the ligands was minimized prior to docking with Universal Force Field (UFF). The free binding energy, a measure of the binding affinity of the protein and ligands expressed in kcal/mol, was estimated by the Autodock4 algorithm and shown in Table **1**. Higher negative values of free binding energy imply higher affinity of the ligands toward the target protein. The compounds sb_04 and sb_05 exhibited binding free energy values of -8.82 kcal/mol and -9.18 kcal/mol, respectively.

Interestingly, these compounds exhibited better binding free energy than the original ligand (SB-239629) of the protein tyrosyl-tRNA synthetase, having a value of -8.72 kcal/mol and closest to the binding free energy of ampicillin (-9.94 kcal/mol). In the series of compounds synthesized, sb_03 exhibited comparable binding energy to the original ligand of the protein PDB id 1JIJ. However, the compounds sb_01 and sb_02 exhibited lesser binding affinity than the original ligand. Further, the inhibition constant (Ki) values (nanomolar, nM), which are a measure of the dissociation constant of the ligand-protein complex, were estimated by the molecular docking. Lower values of inhibition constant (Ki) indicate higher inhibition of the ligand-protein complex. The values of inhibition constants are in line with the binding energy results and are shown in the table. A molecular docking study indicates that the compound sb_05 has the highest affinity towards the target protein, followed by the compound sb_04 amongst the synthesized compounds. The standard drug ampicillin exhibited better binding energy values (-9.92 kcal/mol) than the synthesized compounds; however, the difference was less than around 1 kcal/mol when compared to synthesized compounds sb_04 (-8.82 kcal/mol) and sb_05 (-9.18 kcal/mol).

Table 1. Estimated binding free energy and inhibition constants of the ligands with target protein.

Compounds	Binding Free Energy (kcal/mol)	Inhibition Constant (Ki) Values (nM)
sb_01	-7.72	2180
sb_02	-8.06	1240
sb_03	-8.70	- 420.32
sb_04	-8.82	341.59
sb_05	-9.18	187.33
SB-239629	- 8.72	408.96
Ampicillin	-9.94	52.04

The interactions between the best-docked pose of the ligands with tyrosyl-t-RNA synthetase protein were further analysed with respect to the formation of hydrogen bonds and other non-bonded interactions. The best-docked pose of the ligands was extracted by UCSF Chimera, and the interactions between the protein and ligands were predicted using the BIOVIA Discovery Studio Visualizer. The original ligand of the target protein showed Pi-alkyl interactions with Leu70 and conventional hydrogen bond interactions with Asp80, Gly38, Arg88, Lys84, and Asp40; further Pi-Donor hydrogen bonds with Tyr36 and Asp195 were also exhibited. Antibacterial drug ampicillin exhibited conventional hydrogen bond interactions with Asp80, Asp40, Thr75, and pi-alkyl interactions with His50, Ala39, and Leu70. The common residues thus involved in the interaction of the original ligand and ampicillin with the target protein are Asp80 and Asp40. The interactions exhibited by the compound sb_05 include conventional hydrogen bond interaction with Asp40 and the hydrogen bond of thiol (-SH) with Asp 195 in addition to Pi-alkyl interactions with Leu70 and van der Waals interaction with several residues, which include Thr75 and Gly38, the residues involved in the interaction of original ligand and the target protein. These interactions of the sb_05 and the target protein thus seem important for the binding free energy value exhibited and also the experimental values of antibacterial activity shown by the compound. The known antibacterial ampicillin exhibited the highest number of interactions with the target protein, and these interactions seem to be responsible for its better binding energy values when compared to the synthesized compounds. The interactions of all the synthesized compounds, original ligand, and ampicillin with the target protein are shown in Fig. (**1**).

4.3. Molecular Dynamics Simulations

The best docking poses of the sb_04 and sb_05, which had the highest negative binding free energy values with the target protein, were subjected to molecular dynamics study. The structural behaviour of the compounds and protein were analysed for a duration of 20 ns using GROMACS.

The best-docked conformation of sb_04 was subjected to an MD simulations study, and Root Mean Square Deviation (RMSD) was calculated to determine the stability of sb_04 in the binding site. The RMSD plot of sb_04 with protein showed stabilized conformation of the ligand in the binding site of protein throughout the duration of the MD simulation run, probably due to strong forces involved in the interaction of the sb_04 with the target protein. The backbone deviation of sb_04 with reference to protein indicates slight destabilization in the first 5 ns of the MD simulation, which thereafter stabilizes and remains constant throughout the duration of the simulation. The stability of the ligands in the binding site was inferred from the low deviation in the RMSD values during the duration of the simulation. This stability of the ligand in the binding site may have resulted from the stable interactions of the ligands within the active site. Such interactions may include hydrogen bonds, hydrophobic, and other interactions. The RMSD plot of sb_05, which exhibited better binding free energy values in the molecular docking experiment, showed lesser stability of the docked complex. The docked complex remained stable for 6 ns but exhibited large deviations from 6 ns to 11 ns and smaller deviations thereafter till 20 ns. The behaviour exhibited by sb_05 with the protein suggests that the forces involved in its interaction with the protein are not strong enough to provide stability to the docked complex. The RMSD plots of sb_04 and sb_05 and the protein during the MD simulation run are shown in Fig. (**2**).

Further, interactions of sb_04 and sb_05 with the target protein and stability of the docked complex were also studied by analysis of interaction energy during the duration of the MD simulation. Coulombic Short Range (Coulombic SR) and Lennard-Jones Short Range (LJ-SR) interaction energy were computed by the GROMACS, and the sum of Coulombic SR and L-J SR provided the total interaction energy of the complex as shown in Table **2**. The compound sb_05 exhibited stable interaction with a lower value of total interaction energy than sb_04, in contrast with the RMSD values, wherein sb_04 exhibited better stability in the complex. The reasons for the contrasting behaviour observed in the RMSD values and total interaction energy need to be explored further. The Coulombic-SR interaction energies and Lennard-Jones SR interaction energies of the compound sb_04 and sb_05 throughout the duration of MD simulation are shown in Fig. (**3**). The variation in total interaction energy through the duration of MD simulation is shown in Fig. (**4**). As is reflected, the compound sb_05 exhibited better values of interaction energy through the duration of MD simulation run when compared to the compound sb_04. The total interaction energy of both the compounds sb_04 and sb_05 remained stable throughout the duration of the MD simulation.

Table 2. Interaction energy (kJ/mol) of the synthesized compounds with target protein during 20 ns MD simulation.

Compounds	Coulombic-SR	Lennard-Jones-SR	Total Interaction Energy
sb_04	-21.0994	-115.205	-136.304
sb_05	-33.931	-148.823	-182.754

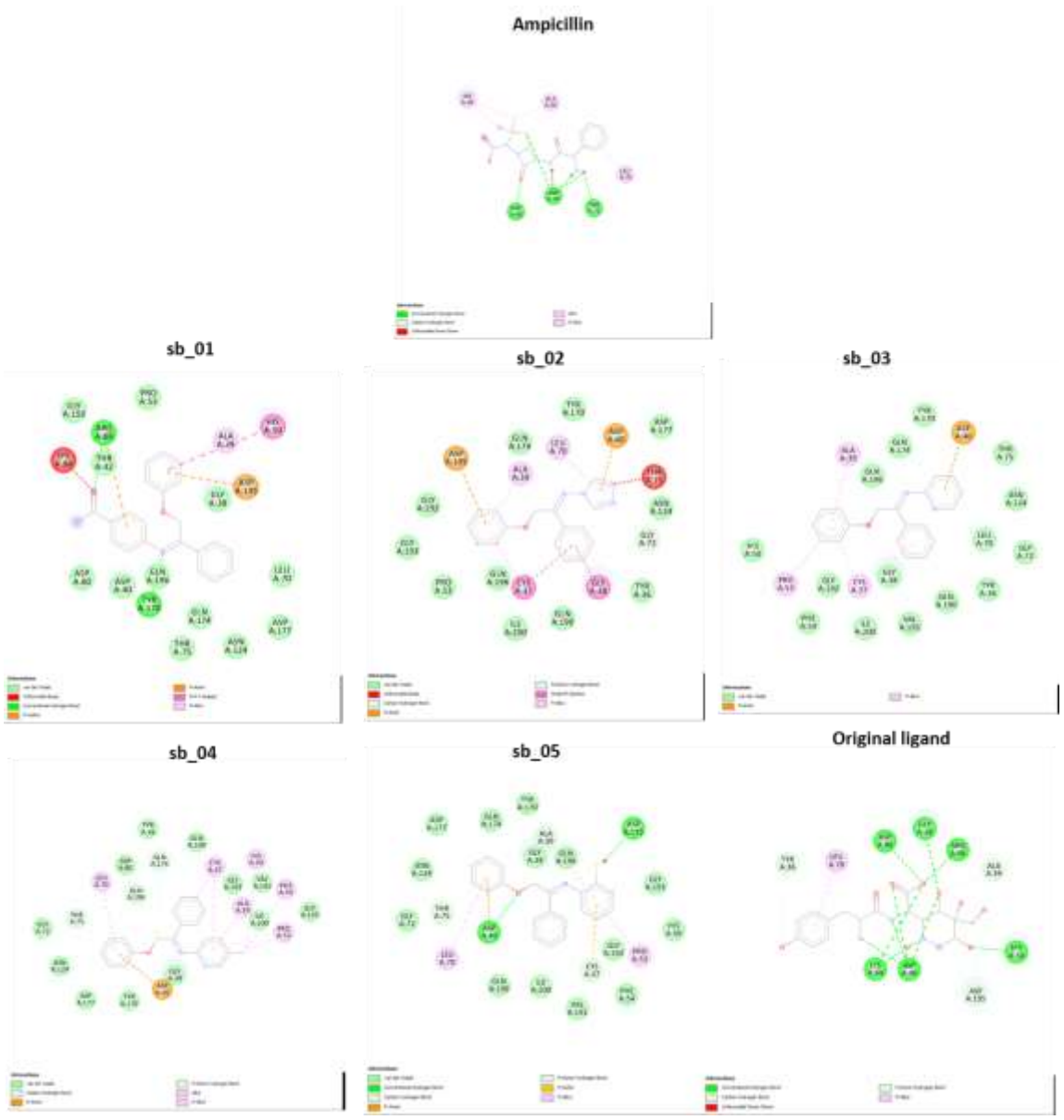


Fig. (1). 2D interactions of the synthesized compound, original ligand and ampicillin with the target protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

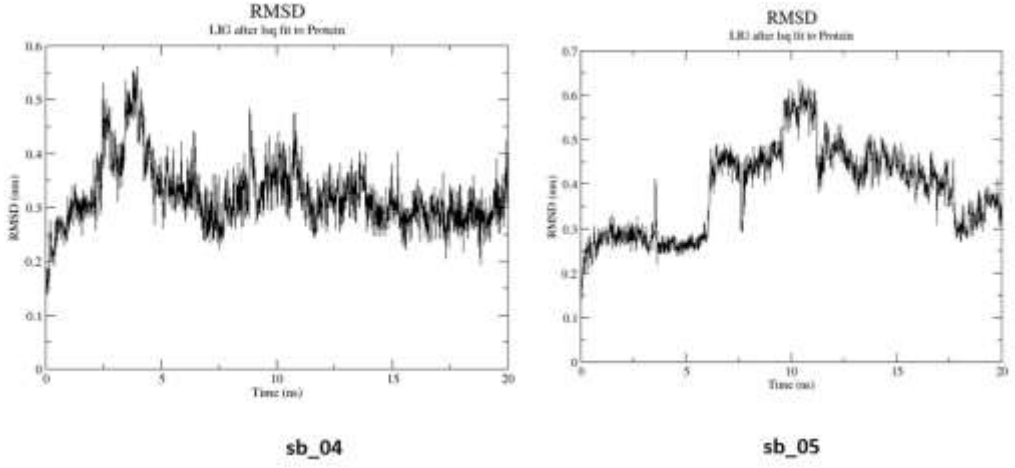


Fig. (2). Results of MD simulations of the best docked poses of compounds sb_04 and sb_05 with target protein.

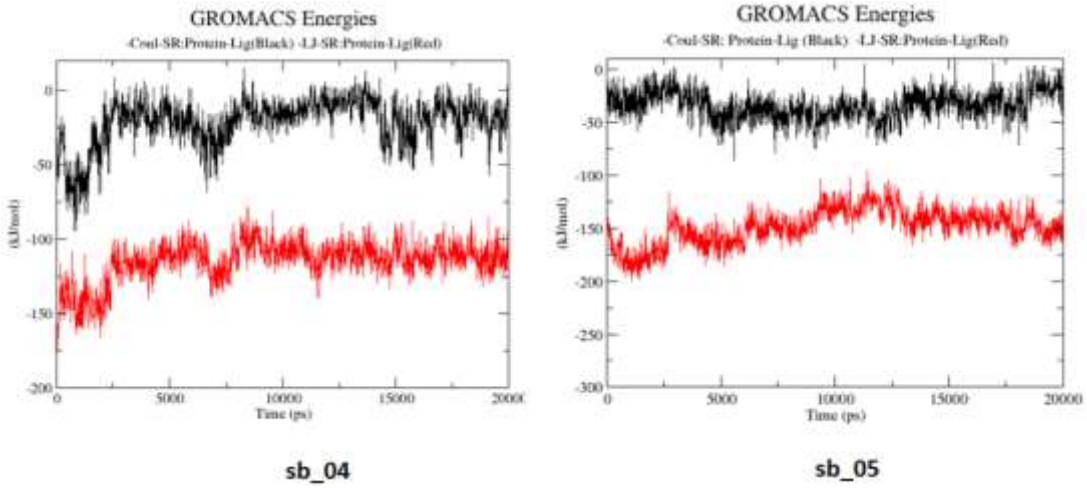


Fig. (3). Coulombic SR (Black) and Lennard Jones SR (Red) interaction energies of synthesized compounds during 20 ns MD simulation. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

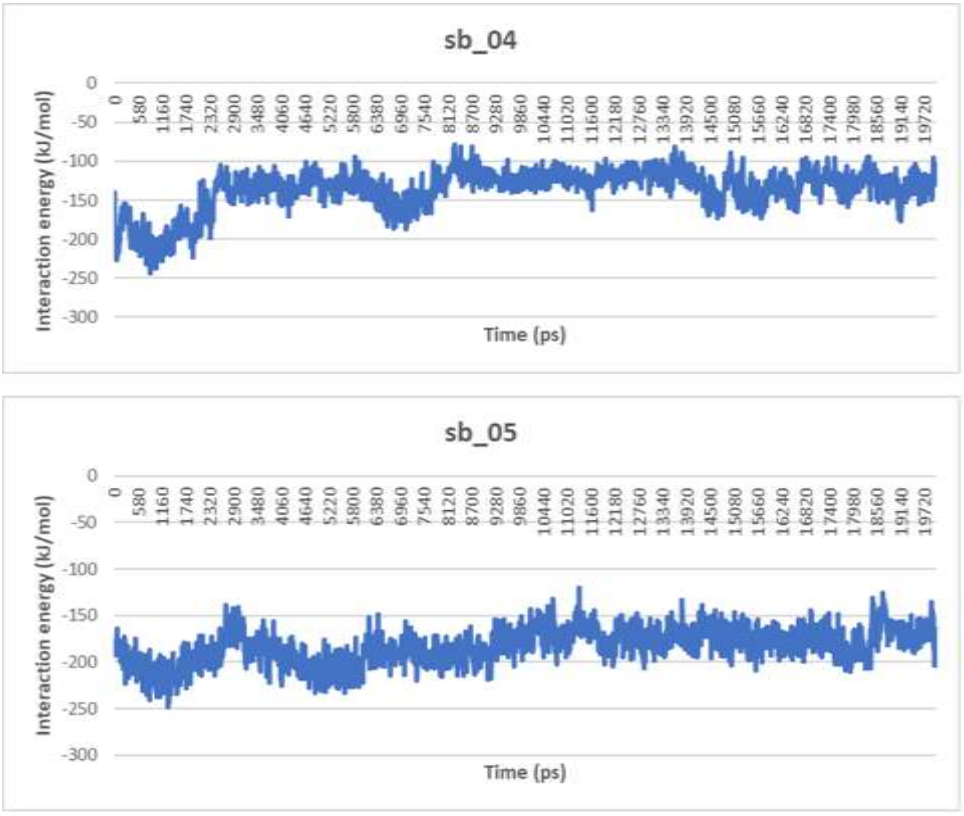


Fig. (4). Total Interaction energy(kJ/mol) of the synthesized compounds during 20 ns MD simulation. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The binding modes of the sb_04 during the MD simulation run of 20 ns were also analysed to provide information regarding the stability of interactions during MD simulation. The binding modes of the MD simulation run were thus taken at an interval of 500 ps. The interactions between protein and the compounds sb_04 and sb_05 at different time intervals during the course of MD simulation of 20 ns are shown in Fig. (**5**) and Fig. (**6**). The interactions as predicted by BIOVIA Discovery Studio Visualizer from the frame extracted at different time intervals during the course of MD simulation of 2000 ps suggest that the screened compounds exhibited mostly the non-bonded interactions with the target protein. Some new interactions were formed, and some interactions that were present at the start of the MD simulation were broken down during the course of the MD simulation. Further, the formation and breaking of non-bonded interactions between the compounds and protein provides insight into the fluctuations observed during the MD simulation of compound sb_04 and,

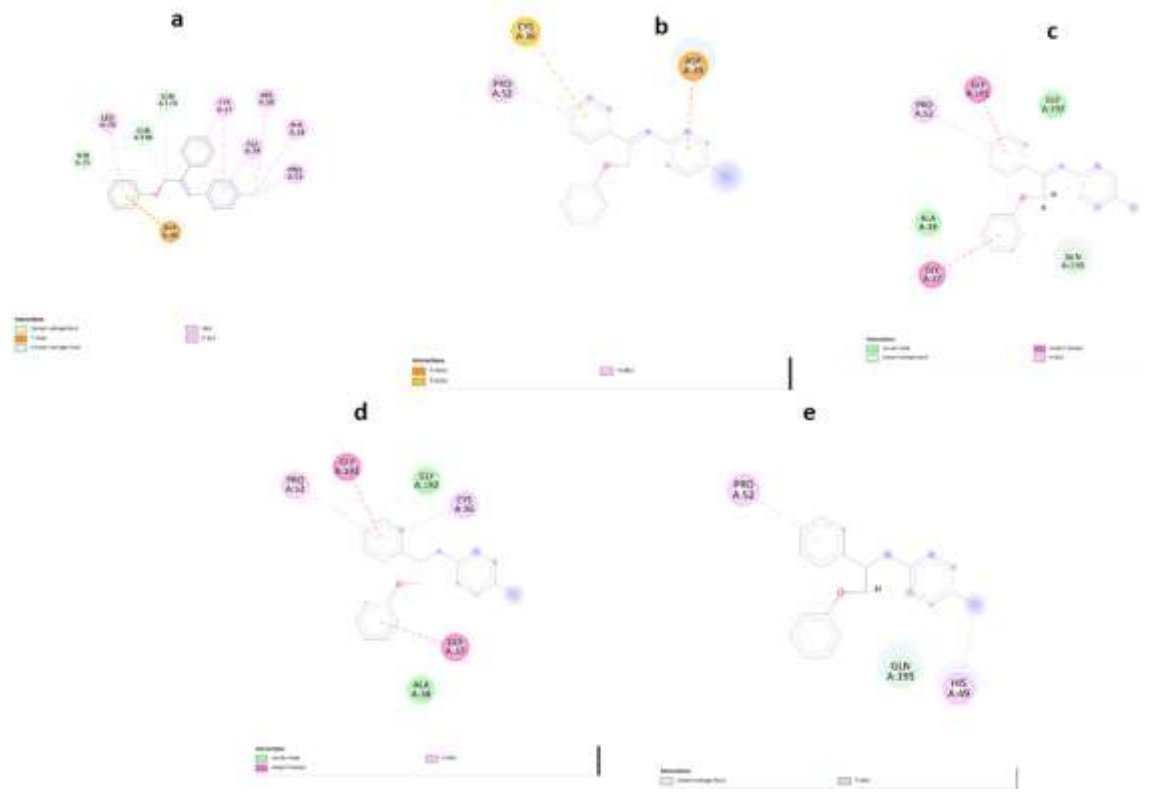


Fig. (5). Interactions of sb_04 with protein over different time during MD simulation run at 0 ps (**a**), 500 ps (**b**), 1000 ps (**c**), 1500 ps (**d**) and 2000 ps(e). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

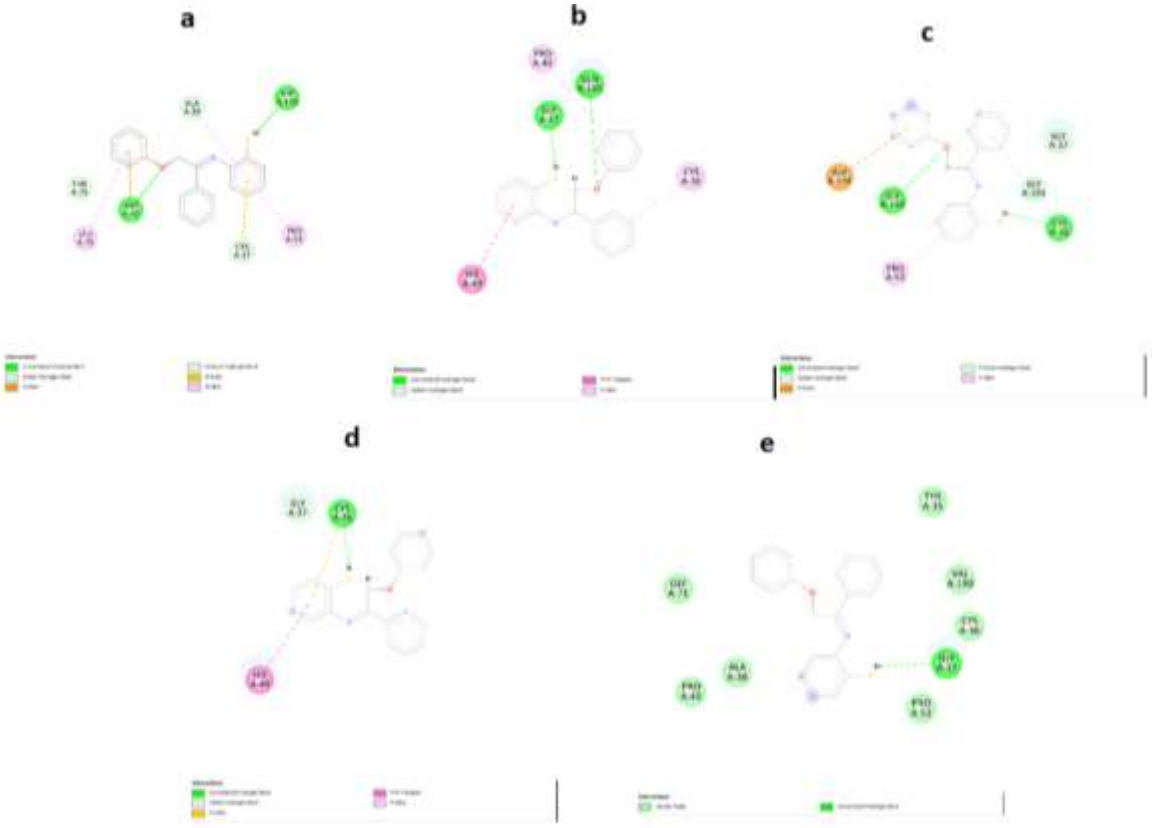


Fig. (6). Interactions of sb_05 with protein over different time during MD simulation run at 0 ps (**a**), 500 ps (**b**), 1000 ps (**c**), 1500 ps (**d**) and 2000 ps(e). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 3. Molecular properties of the compounds.

Molecule	sb_01	sb_02	sb_03	sb_04	sb_05	Ampicillin
Molecular weight	315.28	278.31	288.34	302.37	331.43	349.4
Rotable bond	6	5	5	5	3	5
H-bond acceptors	3	4	3	3	2	5
H-bond donors	0	0	0	0	0	3
MR	84.86	80.28	88.75	93.72	102.45	92.56
TPSA	38.66	52.3	34.48	34.48	60.92	138.03
Consensus Log P	3.94	2.59	3.88	4.17	4.87	0.08
ESOL Class	Moderately soluble	Soluble	Moderately soluble	Moderately soluble	Moderately soluble	Very soluble
GI absorption	High	High	High	High	High	Low
BBB permeant	Yes	Yes	Yes	Yes	Yes	No
Pgp substrate	No	No	No	No	Yes	No
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	Yes	No
CYP2D6 inhibitor	No	No	Yes	Yes	No	No
CYP3A4 inhibitor	Yes	No	No	No	Yes	No
log Kp (cm/s)	-4.79	-5.81	-4.93	-4.76	-4.45	-9.23
Lipinski #violations	0	0	0	0	0	0
Bioavailability Score	0.85	0.55	0.55	0.55	0.55	0.55

more so, the variation in the RMSD values of compound sb_05. However, as the overall RMSD of the compounds sb_04 and sb_05 with respect to protein remained more or less stable throughout the duration of MD simulation, it indicates that the new bonds formed during MD simulation run imparted sufficient stability to the complex. This stability is the docked complexes is also indicated by the total interaction energy.

4.4. ADMET Analysis

Various physicochemical properties of the synthesized compounds were estimated by SwissADME web server, and some of the important parameters are shown in Table **3**. The molecular properties of the synthesized compounds were compared with the known antibiotic, ampicillin. The number of rotatable bonds in the compounds ranged from a maximum of 6 in sb_01 to a minimum of 3 in sb_05. The molecular weight of all the synthesized compounds was less than ampicillin. The predicted values of various parameters of the synthesized compounds showed that none of the synthesized compounds exhibited violations of the Lipinski rule of five. The number of H-bond acceptors in sb_02 is 4, interestingly, analysis of ligand protein interaction as predicted by BIOVIA Discovery Studio Visualizer showed no H-bond formation between sb_2 and the target protein. All the synthesized compounds did not have H-bond donors as predicted by the SwissADME. The consensus log P, which gives an arithmetic mean of five different computational methods for the determination of log P, was also computed by SwissADME. The values of consensus log P for the synthesized compounds ranged from 2.59 for sb_02 to 4.87 for the compound sb_05.

Various pharmacokinetic parameters were predicted for the synthesized compounds. The aqueous solubility predicted by SwissADME ranged from moderately soluble to soluble for all the synthesized compounds; however, the predicted aqueous solubility of ampicillin is highly soluble. All the synthesized compounds exhibited high gastrointestinal absorption and blood-brain permeation ability, in contrast to ampicillin, which exhibited low gastrointestinal absorption and did not exhibit blood-brain barrier permeation.

The toxicology profile of the synthesized compounds was also computed for the synthesized compounds. Except for the compound sb_04, none of the compounds showed ability to inhibit the P-glycoprotein. SwissADME also predicted the ability of the compounds to inhibit various isoforms of cytochrome P-450 enzyme. All the synthesized compounds inhibited the CYP1A2 isoform of the enzyme. The compounds sb_03 and sb_04 exhibited CYP2D6 inhibition, while the compounds sb_01 and sb_05 exhibited CYP3A4 inhibition. The drug-likeness of the synthesized compounds was also predicted, wherein all the compounds showed no violations of the Lipinski rule. The Abbot Bioavailability score, a measure of oral bioavailability, was also computed for all the compounds

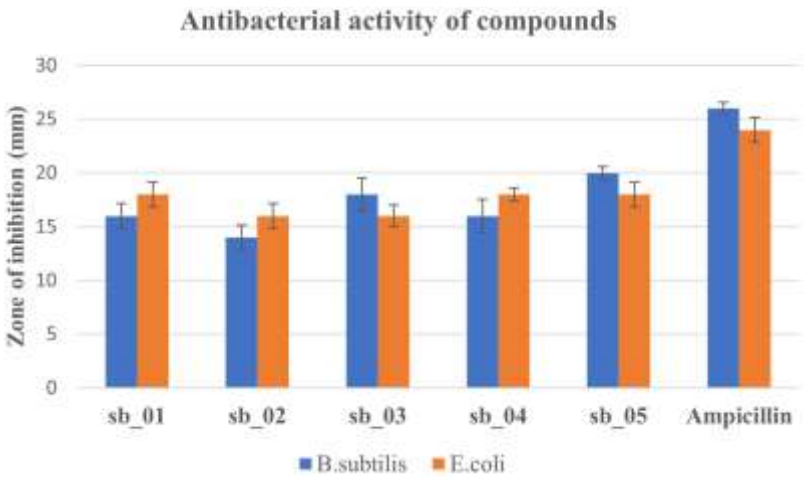


Fig. (7). Antibacterial activity of the synthesized compounds. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

and predicted all of the compounds to have 55% oral bioavailability except the compound sb_01, which was predicted to have 85% oral bioavailability.

4.5. Antibacterial Activity

The synthesized compounds were evaluated for antibacterial activity against Gram-positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* by cup-diffusion method. The antibacterial activity exhibited by the synthesized compounds and ampicillin is shown in Fig. (**7**). The compounds showing inhibitory zones of 18 mm were categorized as active antibacterial agents. Ampicillin was used as positive control and DMF as negative control. The negative control group did not exhibit any zone of inhibition. The compound sb_05 exhibited the best antibacterial activity against Gram-positive *B. subtilis* with a zone of inhibition of 20 mm, while the compound sb_04 exhibited the best activity against Gram-negative bacteria *E. coli* with a zone of inhibition of 19 mm. The compound sb_02 was the least effective antibacterial amongst the series of compounds synthesized. Also, none of the synthesized compounds exhibited better antibacterial activity than the known antibiotic ampicillin.

The synthesized compounds could not be categorized as to be more effective on Gram-positive or Gram-negative strains as some of the compounds exhibited more efficacy against the Gram-positive bacterial strain while some of the compounds showed higher efficacy for Gram-negative strains. Gram-positive and Gram-negative bacteria differ in the composition of the cell wall and, hence, may be a contributing factor to the difference in the spectrum of antibacterial activity. The nature of the activity of synthesized compounds may be the result of their diverse chemical structures, which varied from phenyl, substituted phenyl, triazole, pyridine, and substituted pyridine.

Out of the synthesized compounds, the compounds sb_03 and sb_05 exhibited the highest efficacy against the Gram-positive strain, while the compounds sb_04 and sb_05 exhibited the highest efficacy against the Gram-negative bacterial strain. The pyridine, substituted pyridine, and the phenyl ring with thiol group thus appeared to be the structural fragments responsible for better antibacterial activity of these compounds.

The analysis of the molecular docking of the compounds into the target bacterial tyrosyl-tRNA synthetase enzyme revealed that the compounds sb_04 and sb_05 exhibited the highest binding affinity to the target enzyme. The results of the molecular docking suggest inhibition of bacterial protein synthesis by inhibition of bacterial tyrosyl-t-RNA synthetase enzyme as a possible mechanism of action of the compounds sb_04 and sb_05. The results of the molecular docking are also consistent with the experimental values of antibacterial activity determined for the synthesized compounds.

CONCLUSIONS

Schiff bases of 2-phenoxy-1-phenyl-ethanone and various aryl amines were synthesized and evaluated for antibacterial activity. The Schiff bases having benzenethiol (sb_05) and 4-methyl-2-pyridyl (sb_04) structural fragments exhibited the best antibacterial activity against the Gram-positive and Gram-negative bacteria. The molecular docking of the synthesized compounds with the bacterial tyrosyl-t-RNA synthetase, an enzyme involved in bacterial protein synthesis, was also done to further explore the mechanism of antibacterial activity of the synthesized compounds. The compounds sb_04 and sb_05 exhibited better binding energy values than the original ligand of the target enzyme and comparable binding energy with standard ampicillin. Molecular dynamic simulation studies indicated the structural change in the docked complex throughout the duration of the simulation. The RMSD values of the compound sb_04 were stable throughout the duration of the simulation; however, the compound sb_05 showed lesser stability in the docked complex. Interestingly, the compound sb_05 exhibited more stable interaction with a lower value of total interaction energy than sb_04. The computational studies indicate that the inhibition of bacterial protein synthesis may be the reason for the antibacterial activity of the compound, in particular for the compounds sb_04 and sb_05. These compounds also exhibited the drug likeliness properties and thus provide a scaffold for the discovery of novel antibacterial agents. Further, more analogues based on this structural

scaffold need to be synthesized and evaluated for antimicrobial activity.

AUTHOR'S CONTRIBUTIONS:

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

LIST OF ABBREVIATIONS

AMR	=	Antimicrobial Resistance
SBs	=	Schiff Bases
CDH	=	Central Drug House
TLC	=	Thin-Layer Chromatography
IR	=	Infrared
PDB	=	Protein Data Bank
UFF	=	Universal Force Field

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used in this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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