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ORIGINAL ARTICLE

Virtual Screening, Molecular Docking Study, Characterization, and In-Vitro Antibacterial Evaluation of Piperazine Derivatives

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1Conception & Study Design, Data Collection & Processing, Data Analysis and/or Interpretation.
2Data Collection & Processing, Drafting of Manuscript.
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ABSTRACT

Background: Bacterial infections and the dilemma of increasing resistance against the available antibiotics demanded new medicines in this field without the wastage of time and material. Docking has been considered as an *In-Silico* technology that utilizes computer tools to find and screen most susceptible compounds (ligands) against the target proteins (enzymes) with less time and cost consumption.

Objective: This research plan presents derivatization as a potential and viable answer to explicate the issues of bacterial resistance by applying *In-Silico* tools to find new analogues with antibacterial activity.

Methodology: First of all a library of piperazine containing compounds was virtually scrutinized against three antimicrobial enzymes Dihydropteroate synthetase, Isoleucyl transfer RNA synthetase, and DNA gyrase having PDB IDs 2VEG, 1JZQ, and 3TTZ, respectively using Molecular Operating Environment (MOE version 2015.01) software. Five compounds were selected from the docking study which were then further obtained and explored for their antibacterial potential by Kirby Bauer method.

Results: It was observed that almost all the five compounds were effective in inhibiting the growth of tested bacterial strains. Compound C-1, C-3 and C-4 were proved to be the most active antibacterial agents who could be related to their docking score as well as to the binding of ligand at the binding pocket site.

Conclusion: The outcomes of the present study are encouraging enough to support the use of *In-Silico* tool and derivatization in the rapid discovery of antibacterial agents to overcome the problem of bacterial resistance.

Keywords: *Antibacterial activity, Docking study, In-silico study, Dihydropteroate synthetase, Isoleucyl transfer RNA synthetase, and DNA gyrase*

INTRODUCTION

Nowadays Computer Aided Drug Designing (CADD) has been frequently applied for the discovery of a lead molecule with reduced human sufferings. The traditional drug designing is a much time consuming process with high financial demands. Computational approaches and the use of software in drug discovery made this entire practice feasible by not only decreasing the financial burden but also evaded the late stage failures. CADD basically comprised of tools and techniques that are needed for the organization, examination and storage of the suitable structures used for drug discovery and hence aided in the establishment of virtual libraries to assess the chemical interactions (between ligand and target). Similarly, Computer aided drug designing has also guided pharmaceutical and medicinal chemists to demonstrate therapeutic profile of the structure (drug) before it is marketed and becomes available to the patient. [1-4]

In-silico study (computer assisted technology) has received wide acceptance in pharmaceutical research community in the past decades where researchers applied this information to improve and accelerate the process of drug designing. This tool aids in predicting the interaction pattern and binding affinities between ligand and target along with drug toxicity data and side effects. [5, 6]

Likewise, molecular docking can be regarded as an efficient *In-silico* tool for virtual optimization which explains the ideal conformation of the ligand (drug) to bind to its target (receptor/enzyme) to obtain desirable pharmacological activity. [7, 8]

This tool has also been used in combination with various algorithm to investigate libraries of compounds having potential to be used as therapeutics agents usually termed as “Hit”. Moreover, docking study helped researcher in finding a lead molecule for a target protein, its optimization and illustration of its binding models. [9]

Piperazine (Fig. **1**) is a heterocyclic organic molecule with two nitrogen atoms present para to each other. It was initially used to remove the worms but later on molecule became famous for multiple activities. In the past, various simple and complex molecules bearing piperazine moiety have been reported with activities other than anthelminthic [10-12]; some analogues have been effectively used as therapeutic agents and could serve as lead compounds for further optimization. [13]

The story of bacterial infections and resistance, the methods of prevention and treatment remained in progress since the beginning of human life. Several of the past researches have applied *in-silico* tools and docking studies to develop new antibacterial agents with improve efficacy with more accurate findings of their therapeutic and toxicological profiles without the loss of time and materials. This research study therefore aimed to apply *In-silico* tool to find compound(s) with better antibacterial activity.

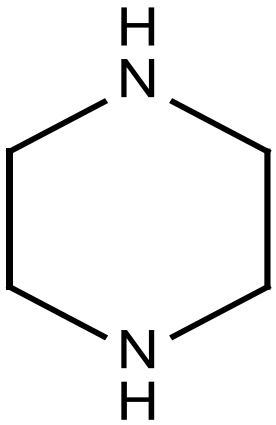


Figure. 1. Piperazine.

EXPERIMENTAL

Chemicals and General Requirements

Analytical grade chemicals were used in this research plan and were obtained from Sigma Aldrich. For *in-silico* study, Molecular Operating Environment (MOE version 2019.01) was used.

Docking study

Formation of compound Library

A Library of 2650piperazine based compounds having drug-like effects was obtained from Zinc database (http://zinc.docking.org) [14]

First, structures were made by applying MMFF99 charges, then protonation and energy minimization were carried out to simulate them with the biological system.

Formation of PDB

Three antimicrobial enzymes Dihydropteroate synthetase, Isoleucyl transfer RNA synthetase, and DNA gyrase (2VEG, 1JZQ, and 3TTZ) respectively were selected and their X-ray crystallographic structures were acquired from RCSB.com (Fig. **2**)[15].

The PDBs were minimized and charged by using Amber; force field applied MMFF99 with optimization algorithm steepest descent upto 500 steps and saved in PDB format. All ligands heteroatoms and other crystallographic agents were removed from the original proteins structure for ease of processing.

Docking calculations using MOE

For docking calculations, Molecular Operating Environment (MOE version 2019.01) software was used to virtually screen the prepared library and protein using default parameters. The grid box of optimal measurements was applied on the receptor binding pocket. In addition, the respective crystallographic ligand was re-docked over the active site to assess the accuracy of docking process. Afterwards, the docked poses with lowest binding affinity (Kcal/mol) and RMSD value between (0-3Å) observed. Although Chimera (1.11) was used initially to see the possible interactions between the ligands and the enzyme's active sites, but for their selection, thorough visual examination was carried out by observing ligand binding energies and interactions.

The selected compounds were then purchased from molportRegia, Latvia for experimental validation through *in-vitro* antibacterial testing.

In-Vitro Antibacterial Evaluation

Kerby Baeur method (or disc diffusion) [16] was used to examine the *in-vitro* antibacterial activity of the selected analogues against different gram positive and gram negative bacteria. For this, serial dilutions of all the tested compounds were prepared in Dimethyl sulfoxide (DMSO) to achieve a test concentration of 200µg/ml. To summarize the activity, first Muller Hinton agar and broth was prepared as per guidelines of the manufacturer, autoclaved and then cooled. To obtain agar plates, about 30ml of this freshly prepared media was added into sterile petri dishes, and cooled to get it solidified. 0.5M McFarland solution was prepared to achieve a turbidity of 1.5 x 108 cells/ml of E.coli suspension and maintained in air tight tubes (at 4-8°C). The inoculums of the collected clinical isolates were prepared in 5ml of nutrient broth media and incubated for 8-10 hours at 37°C. As soon as these prepared inoculums achieve turbidity of 0.5 McFarland's solution, their cultures were transferred into agar plates by uniform streaking. The susceptibility plates having antibiotic discs were incubated at 37±1°C for 18-24 hours. After this, the circular ring formed around the antibiotic discs was measured carefully using Vernier Caliper as zone of inhibition in mm.

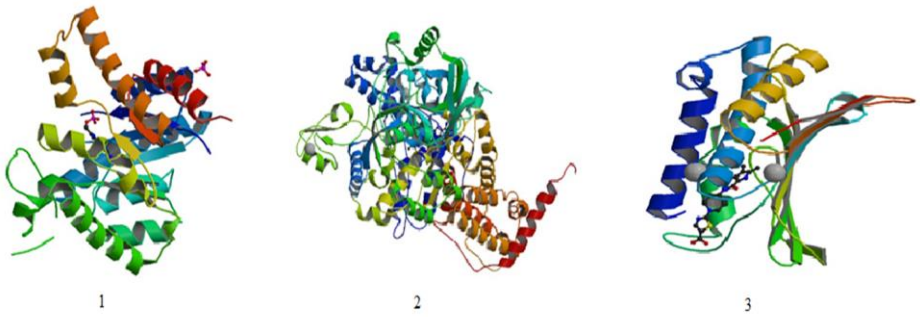


Figure 2. PDB structure of enzymes (Dihydropteroatesynthetase (2VEG) 1, Isoleucyl transfer RNA synthetase (1JZQ) 2, DNA gyrase having (3TTZ)

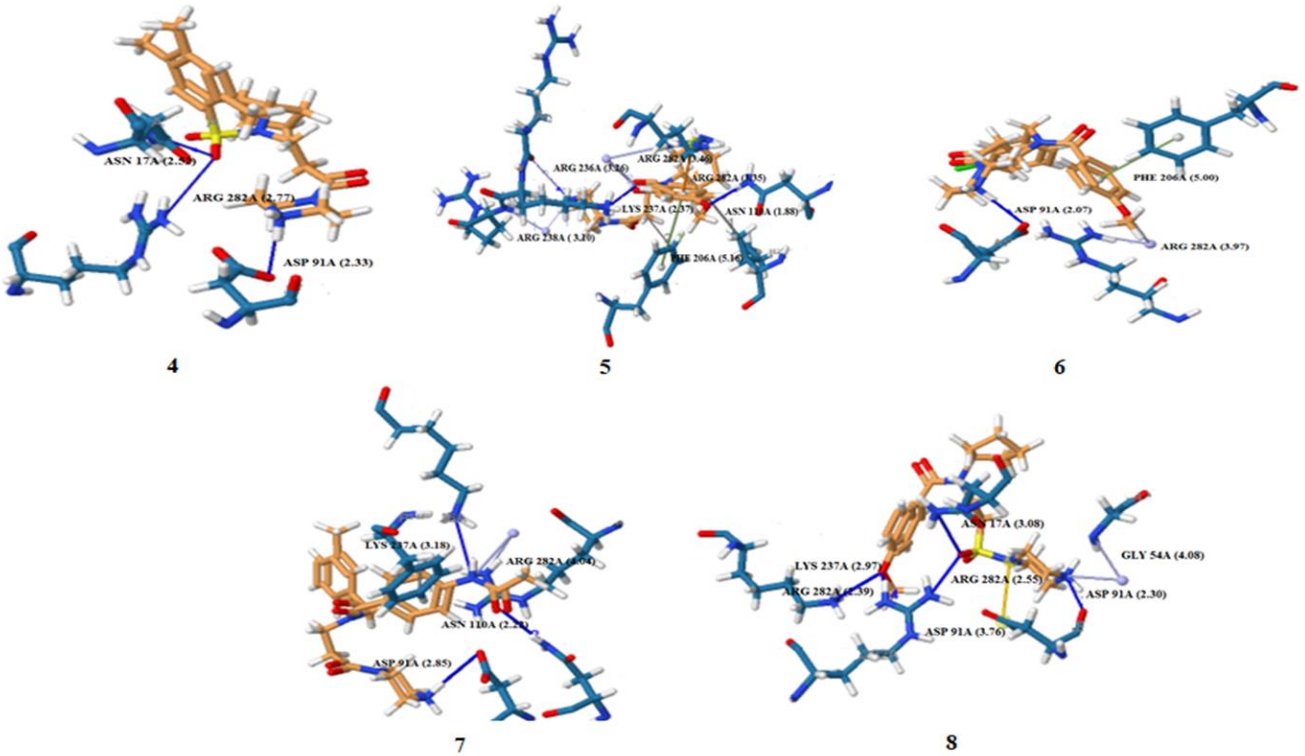


Figure 3. Ligand-protein interaction with 2VEG (C-1 4, C-2 5, C-3 6; C-4 7, C-5 8; Hydrogen bonds are indicated with blue lines and water bridges with silver lines)

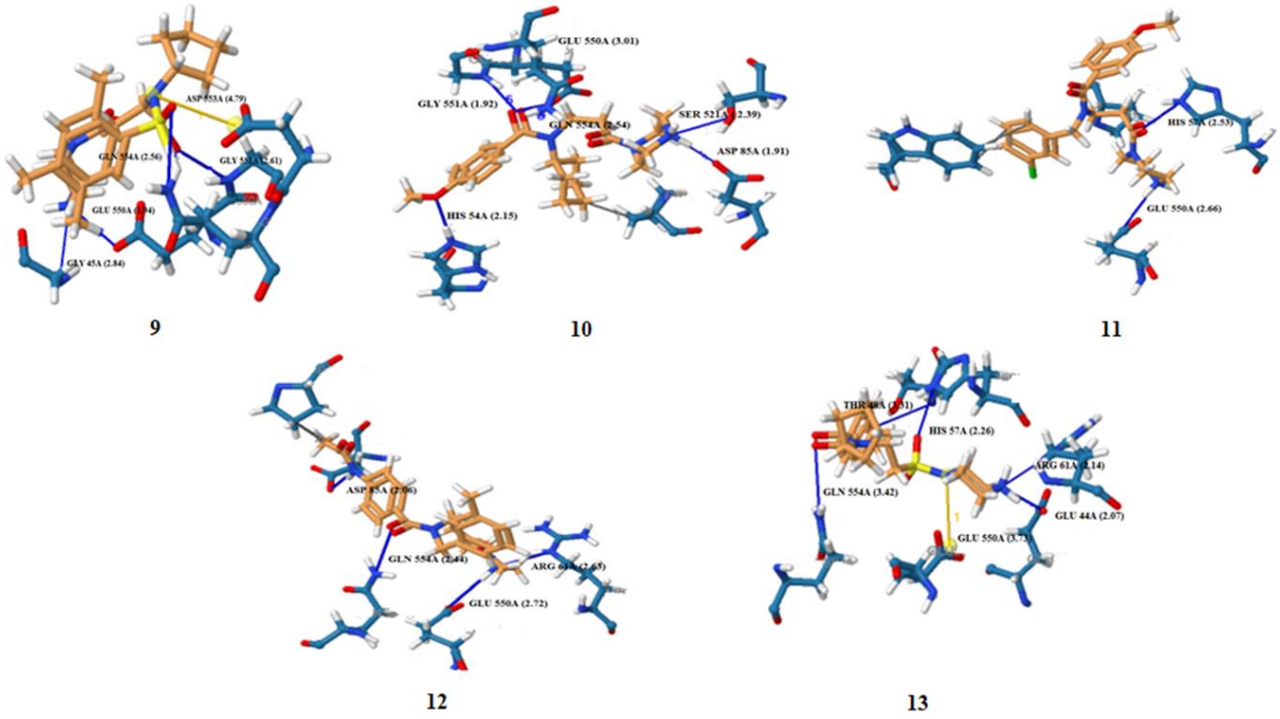


Figure 4. Ligand-protein interaction with Isoleucyl RNA transferase PDB ID 1JZQ (C-1 9, C-2 10, C-3 11; C-4 12, C-5 13; Hydrogen bonds are indicated with blue lines and water bridges with silver lines)

RESULTS

Docking study

From docking studies, five compounds were chosen and characterized to screen their *in-vitro* antibacterial potential after characterization. The chemical structures, IUPAC names, physical properties and LCMS of all the five compounds were given in TABLE-**1** and Fig.**3**.The docking scores of the standard and selected compounds against 2VEG, 1JZQ and 3TTZ were given in TABLE-**2-4** respectively.

Table 1. IUPAC names. Structures and Physical properties of C-1 – C-5.

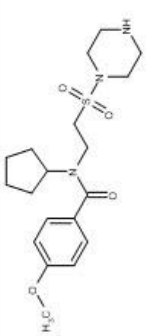
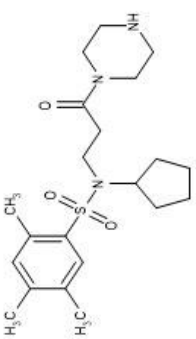
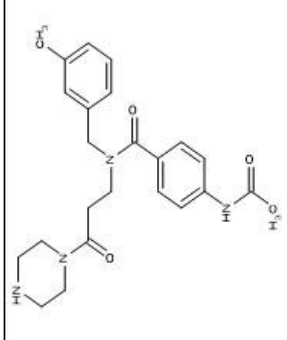
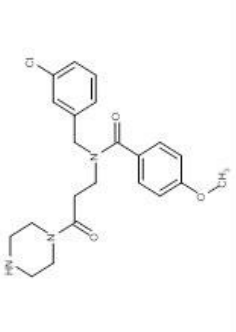
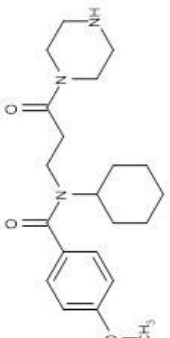
S.No.	Compound structure	IUPAC Name	State	Molecular Formula	Weight (g/mol)	Solubility	H- bond donor count	H- bond acceptor count	Heavy atom count
1		N-cyclopentyl-4-methoxy-N-(2-piperazin-1-ylsulfonylethyl)benzamide.	Solid	$C_{19}H_{29}N_3O_4S$	395.51	DMSO 20%	1	6	27
2		N-cyclopentyl-2,4,5-trimethyl-N-(3-oxo-3-piperazin-1-yl(propyl)benzenesulfonamide	Solid	$C_{21}H_{33}N_3O_3S$	407.573	DMSO 20%	1	5	28
3		4-acetamido-N-[(3-methylphenyl) methyl]-N-(3-oxo-3-piperazin-1-ylpropyl)benzamide	Solid	$C_{24}H_{30}N_4O_3$	422.529	DMSO 20%	4	7	31
4		N-[-[(3-chlorophenyl)methyl]-4-methoxy-N-[3-oxo-3-(piperazin-1-yl)propyl]benzamide	Solid	$C_{22}H_{26}ClN_3O_3$	415.92	DMSO 20%	4	7	31
5		N-cyclohexyl-4-methoxy-N-[3-oxo-3-(piperazin-1-yl)propyl]benzamide	Solid	$C_{21}H_{31}N_3O_3$	373.497	DMSO 20%	4	7	31

Table 2. Docking score of C-1 – C-5 against 2VEG

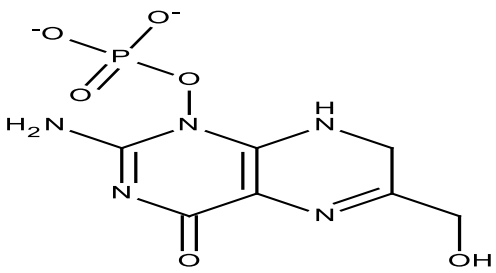
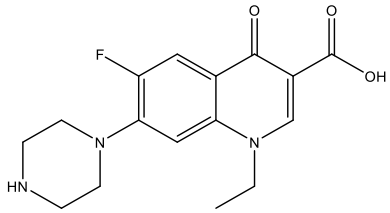
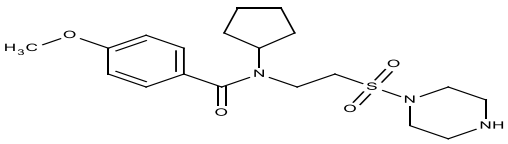
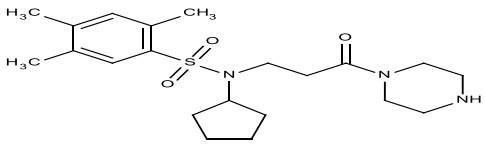
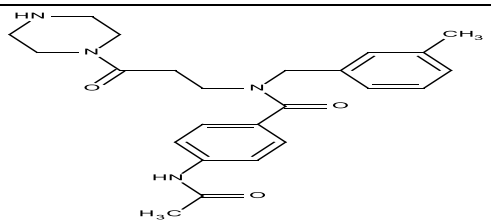
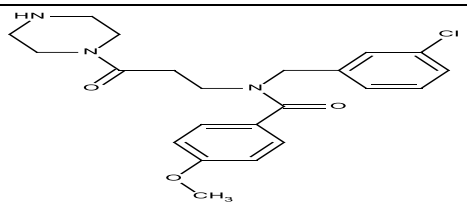
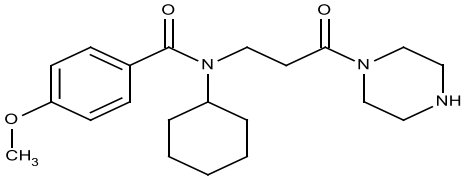
Compound	Structure	Docking Score	Interactions	
			Number of Hydrogen bonds	Amino acids involved in Hydrogen bonding
Standard (6-hydroxymethyl-7,8-dihydropterin monophosphate)		-6.1131	4	ASN 17A, ASN 110A, ASN 110A, LYS 237A
Norfloxacin		-5.9	3	LYS 210A, ASN 213A, PHE 239A
C-1		-4.8964	3	ASN 17A, ASP 91A, ARG 282A
C-2		-5.8882	3	ASN 110A, ARG 236A, LYS 237A
C-3		-6.2043	1	ASP 91A
C-4		-6.6138	3	ASP 91A, ASN 110A, LYS 237A
C-5		-5.9832	5	ASN 17A, ASP 91A, LYS 237A, ARG 282A, ARG 282A

Table 3. Docking score of C-1 – C-5 against 1JZQ

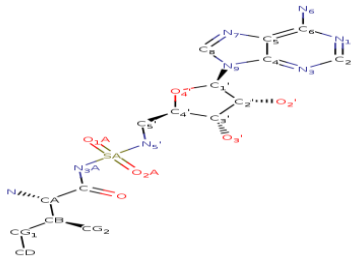
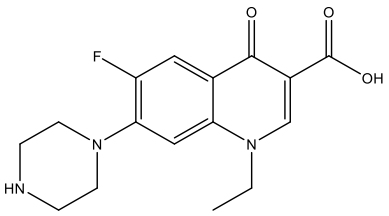
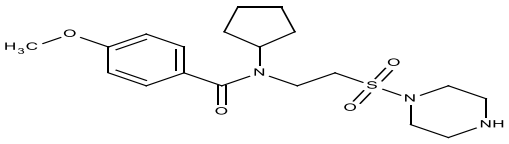
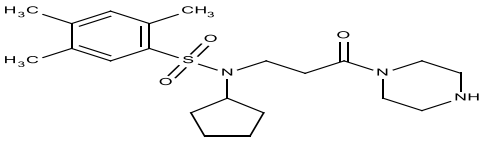
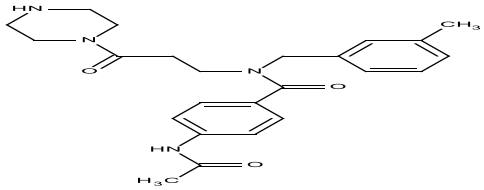
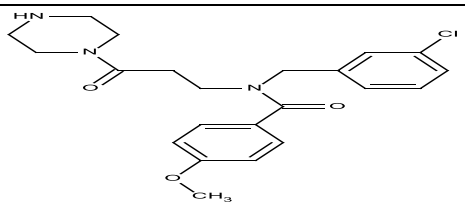
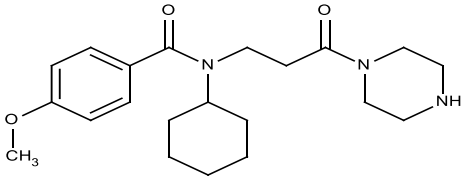
Compound	Structure	Docking Score	Interactions	
			Number of Hydrogen bonds	Amino acids involved in Hydrogen bonding
Standard (N-[ISOLEUCINYL]-N'-[ADENOSYL]-DIAMINOSUFONE)		-5.2012	8	ASP 85A, ASP 85A, GLU 550A, GLY 551A, ASP 553A, GLN 554A, ILE 584A
Norfloxacin		-6.6	3	GLU 135A, TRP 140A, ASP 142A
C-1		-6.8685	4	GLY 45A, GLU 550A, GLY 551A, GLN 554A
C-2		-6.0415	6	HIS 54A, ASP 85A, SER 521A, GLU 550A, GLY 551, GLN 554A
C-3		-6.6862	2	HIS 57A, GLU 550A
C-4		-6.1938	4	ARG 61A, ASP 85A, GLU 550A, GLN 554A
C-5		-5.9255	5	GLU 550A, THR 48A, HIS 57A, ARG 61A, GLN 554A

Table 4. Docking score of C-1 – C-5 against 3TTZ

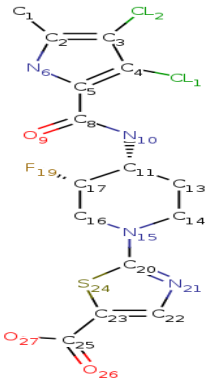
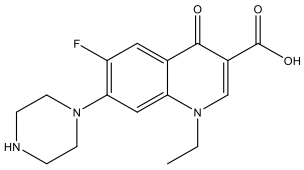
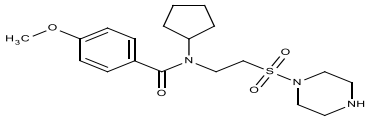
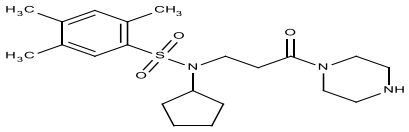
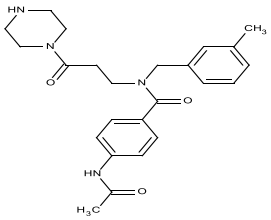
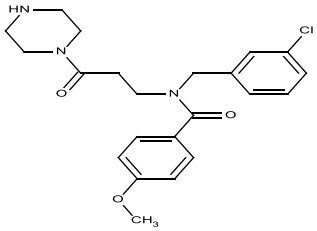
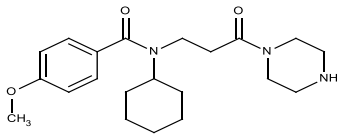
Compound	Structure	Docking Score	Interaction	
			Number of Hydrogen bonds	Amino acids involved in Hydrogen bonding
Standard (2-[(3S,4R)-4-{[(3,4-dichloro-5-methyl-1H-pyrrol-2-yl)carbonyl]amino}-3-fluoropiperidin-1-yl]-1,3-thiazole-5-carboxylic acid		-4.776	3	THR 173A, ARG 84A, THR 173A
Norfloxacin		-4.9	4	ASN 54A, ASN 54A, SER 55A, GLY 85A
C-1		-5.2965	3	ARG 84A, ARG 84A, THR 173A
C-2		-4.8625	2	ASN 54A, GLU 58A
C-3		-5.0378	2	ARG 84A, ARG 144A
C-4		-5.8682	4	ARG 84A, ARG 144A, ARG 144A, THR 173
C-5		-5.0111	2	ARG 84A, THR 173A

Table 5. Antibacterial activity of all compounds C-1 – C-5 in zone of inhibition (mm)

S. No.	Zone of Inhibition (mm) / Bacteria	C1	C2	C3	C4	C5	Norfloxacin
1	*Staphylococcus aureus*8862	13	10	10	12	15	7.8
2	*Pseudomonas aeruginosa 9352*	14	11	12	16	18	7.8
3	*Pseudomonas aeruginosa*9353	10	10	16	16	17	7.8
4	*Pseudomonas aeruginosa*9355	12	12	10	12	17	7.8
5	*Escherichia coli*8885	12	14	12	15	17	7.4
6	*Escherichia coli*8888	12	12	12	14	16	7.4
7	*Escherichia coli*8887	12	15	14	13	15	7.4
8	*Klebsiellapneumoniae*	12	13	10	11	18	4.7
9	*Klebsiella pneumonia* 8834	10	11	9	9	14	4.7
10	*Klebsiella pneumonia* 8837	10	10	8	9	13	4.7

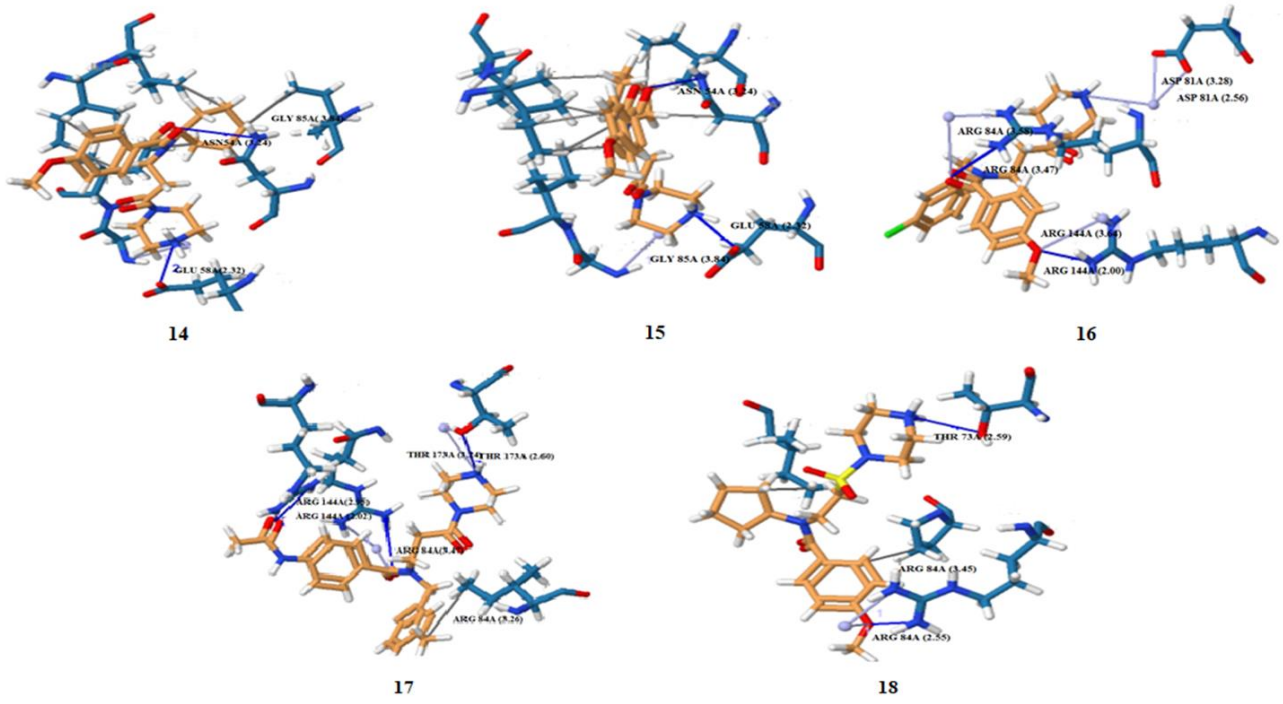


Figure 5. Ligand-protein interaction with DNA Gyrase PDB 3TTZ; (C-1 14, C-2 15, C-3 16; C-4 17, C-5 18;Hydrogen bonds are indicated with blue lines and water bridges with silver lines)

According to TABLE-2, it was noticed that C-4 and C-3 displayed docking scores -6.61381 and -6.2043 in contrast to the co-crystal ligand (-6.1131) and standard that was norfloxacin (-5.9) against 2VEG respectively. In case of 1JZQ and 3TTZ (TABLE-**3 &4**), it was interesting to note that all of the five compounds presented equal and better docking values as compared to the co-crystal ligand and standard. Compounds C-1 and C-3 with scores -6.8685and -6.6862in case of 1JZQ and C-4 and C-1 with values -5.8682 and -5.2965 in case of 3TTZ evolved out as more favorable structures to bind to these target proteins respectively.

All the short listed structures (C-1, C3 and C4) were profoundly visualized in selected target proteins (enzymes) (Fig.**4-6**) and established valuable interactions for binding and bioactivity.

In-vitro Antibacterial Activity:

The results of antibacterial activity have been displayed in TABLE-**5**. All the five compounds produced more zone of bacterial growth inhibition as compared to the standard Norfloxacin used during the study. It was noticed that compound C-1, C-4 &C-5 exhibited good antibacterial effects against *Staphylcoccus aureus* while moderate activity was also found with Compound C-2 and C-3. Almost all compounds were evaluated as good antibacterial agents against *Pseudomonas aeruginosa.* Interestingly, all of the tested compounds possessed good inhibitory potential for different strains of *Escherichia coli.* The tested compounds were also succeeded in inhibiting the growth of tested strains of *Klebsiella pneumoniae*

DISCUSSION

As mentioned earlier, molecular docking served as a significant technique in selecting drug candidates on a rational basis. The way ligand interacts and binds to the target, could provide essential information in developing effective therapeutic selections for experimentation.

In this research activity, interactions obtained by the binding of co-crystal ligand and norfloxacin were taken as standard to compare the binding of compounds with each protein. The binding sites of compounds with more docking values were considered on priority. In case of Dihydropteroate Synthetase (2VEG), co-crystal ligand was deeply noticed where interactions such as hydrogen bonds (ASN 17, ASP 91, ASN 110, ARG 282, LYS 237 etc.) and pi interactions (PHE 206) etc. were observed. Similarly, the standard drug, norfloxacin, was also produced 3 hydrogen bond with LYS 210A, ASN 213A, and PHE 239A. According to Fig.**4**, compound C-3 interacted through one hydrogen-bond ASP91 (2.07 Å), whereas C-4 showed four interactions, three hydrogen bonds ASP 91(2.85Å), ASN 110 (2.22Å), LYS 237 (3.18Å)and one water bridge ARG 282A (4.04Å), respectively.

To investigate the antibacterial potential of the said ligands against multiple targets, Isoleucyl transfer RNA synthetase and DNA Gyrase were also selected. Compounds were tested against Isoleucyl transfer RNA synthetase(1JZQ) using their co-crystal structure as reference. The bound ligand targeted this enzyme through HIS 54, GLY 45, Glu 550, GLN 554 etc. and norfloxacin created three hydrogen bond with GLU 135A, TRP 140 A, and ASP 142A. Hence, these interaction patterns were then used to examine the binding capability of designated five compounds. According to Fig.**5**, C-1 displayed five interactions i.e. four H-bonds GLY 45 (2.84Å), Glu 550 (1.94Å), GLY 551 (2.61Å) and one Salt bridge GLN 554 (2.56Å). However, C-3 displayed only two H-bonds interactions i.e. HIS 57 (2.53Å) and GLU 550A (2.66Å).

The ligand attached to DNA Gyrase (3TTZ) showed numerous interactions like Hydrogen bonds (ARG 144, ARG 84, ILE 86, ASP 81, SER 55) essential for activity. The standard compound that was norfloxacin was show 3 hydrophilic interaction with GLU 135A, TRP 140A, and ASP 142A. The selected compounds instead of showing similar contacts with the reference residues, they paralleled the hotspot residues with same docking poses against this enzyme. As per Fig.**6**, C-1 displayed good interactions in terms of hydrogen bonds [ARG 84 (3.41Å), (2.47Å), THR 173 (2.85Å)] to aid molecule binding potential. Similarly, hydrogen bonds such as ARG 84A (3.47 Å), ARG 144A (2.95 Å), (2.02 Å) and THR 173A (3.24 Å) showed excellent interactions in compound 4. Similar studies have reported these results [17,18].

From this research study, it was established that nearly all structures had comparable binding pockets as that of reference against the enzymes DNA gyrase, dihydropteroate synthetase and Isoleucyl transfer RNA synthase. In addition, all five compounds demonstrated moderate to good antibacterial potential as compared to the standard Norfloxacin against the tested strains of gram positive and gram negative bacteria. Especially antibacterial activity in C-3, C-4 and might be related to the fact that these structures produced additional interactions with the enzymes as compared to the reference with improved affinity. It could be said that the electronegativity pattern of moieties such as halogen, sulfate and amide carbonyl supported the placement of these structures within the binding pockets in a most suitable manner. Similarly, the compounds with larger structures and more lipophilicity were assessed as better choices among other molecules. The hydrogen bonds that were result of good binding of NH…O, indicated strong interaction between ligand

and the target proteins at that particular point. In the same way, water bridges were also pictured in the binding pockets of some ligands. Interactions such as H, C=0 and N—H fragments between the protein and ligand, helped the compounds in better fitting into the active pocket site and therefore render them apposite to be used as antibacterial agents.

CONCLUSION

Docking studies of some piperizine analogues was performed that showed good interactions of these compounds at the binding pocket site of three antimicrobial enzymes Dihydropteroate synthetase, Isoleucyl transfer RNA synthetase, and DNA gyrase. Kirby Bauer method also displayed moderate to good antibacterial activity for these compounds which suggest that these derivatives could be offered for further development in this field.

Since, C-1, C-3 and C-4 all are binding to the said enzymes with considerable affinity, this highlighted the structural needs of therapeutic candidate to be active against different strains of bacteria. However the chemical interaction seen in other ligands would also be fruitful in designing antibacterial compounds

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