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DESIGN, SYNTHESIS AND DOCKING STUDIES OF DERIVATIVES OF 1,4-DIHYDROPYRIDINE AS ANTI-BACTERIAL AGENTS

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ABSTRACT

The prime motivation of the present work is to design a drug in such a way that it can be used clinically to treat the bacterial disease by inhibiting the enzyme targets of the bacteria. Among heterocyclic compounds containing six membered rings, the important constituents that are usually found in biologically active natural products are 1,4-dihydropyridines. 1,4-dihydropyridine is a six membered aromatic ring containing N at the 1st position, which is saturated at the 1st and 4th position. The most feasible position for substitution is 4th of the heterocyclic ring which exhibit various pharmacological activities such as calcium channel antagonist, antihypertensive, anti-inflammatory, antifungal, analgesic and antimicrobial actions. It also shows anti-leishmanial, anticoagulant, anticonvulsant, anti-tubercular, antioxidant, antiulcer, antimalarial, HIV-1 protease inhibition and antifertility properties. Keeping in view of the above facts, a series of 1,4-dihydropyridine derivatives were prepared from three compounds by the condensation reaction of ethyl acetoacetate, aromatic aldehyde and ammonium acetate at 80°C. It is performed by Hantzsch synthesis. The synthesized 1,4-dihydropyridines were docked and investigated for its anti-bacterial activity against *Streptococcus pneumoniae*, *Staphylococcus aureus* & *E.coli*.

KEYWORDS: Heterocyclic compounds, 1,4-dihydropyridines, Hantzsch synthesis, Docking study, Anti-bacterial activity.

INTRODUCTION

Bacteria, singular bacterium, any of a group of microscopic single-celled organisms that live in enormous numbers in almost every environment on Earth, from deep-sea vents to deep below the Earth's surface to the digestive tracts of humans. Bacteria lack a membrane-bound nucleus and other internal structures and are therefore ranked among the unicellular life-forms called prokaryotes. Harmful bacteria that cause bacterial infections and diseases are called pathogenic bacteria. Bacterial diseases occur when pathogenic bacteria get into the body and begin to reproduce and crowd out healthy bacteria or to grow into tissues that are normally sterile. Harmful bacteria may also emit toxins that damage the body.[1]

Antibacterials are chemical substances derived from a biological source or produced by chemical synthesis that fight against pathogenic bacteria. Thus, by destroying or inhibiting the development or growth of bacteria, their pathogenic effect in the biological environments will be minimized.[2]

Drug discovery tools have been utilized now in designing new molecular entities which are safe and effective. Recent literatures shows that search of new drugs are now focused on designing the drugs to inhibit the enzyme targets.

DNA gyrases, topoisomerases are such a potential drug target in the development of new antibacterial agents. From the literature and virtual screening technique, 1, 4-dihydropyridine analogues possess promising DNA gyrase inhibiting action on bacteria.

Based on these reports an attempt was made here to design and develop new antibacterial agents by utilizing computational tools. The primary objective of the present work is to synthesize 4-Nitrobenzaldehyde, 4-Aminobenzaldehyde and Schiff base linked 1,4-dihydropyridines as promising antibacterial agents by inhibiting DNA gyrase enzyme.

In the present work, the focus is on the synthesis, docking study and antibacterial screening of 1,4-dihydropyridine derivatives and their potential to inhibit the growth of model bacteria. The experimental data indicate an increase in the antibacterial activity upon replacement of the C4-positioned substituent with different aldehyde groups.

MATERIALS AND METHODS

Materials

The chemicals and reagents were procured from ERODE SCIENTIFIC & CHEMICALS, Erode, Tamil Nadu. All the compounds procured were AR grade. Stannous chloride and Dinitrophenylhydrazine (DNPH) are the gift samples from CHEMKOVIL, RESEARCH INSTITUTE, Mettur, Salem, Tamil Nadu. Melting points were determined by melting point apparatus MR-VIS, visual melting range apparatus and corrected. Reactions were monitored by thin layer chromatography (TLC) on TLC plates using DNP and Ninhydrin reagents as visualizing agent. IR spectra on JASCO FTIR-420. NMR were recorded on the Bruker Ultra Shielded NMR-300MHz. MASS spectra were recorded on JEOL GC Mate GC-MS Spectroscopy. Docking studies was performed by AutoDock tools 4.2.6, Python, Molinspiration server, RCSB Protein data bank and online simile translator.

Methods

Phase I: Molecular Docking

From the virtual screening and literature review, the enzyme DNA gyrase was selected as the drug target for the present study. 2VEG.pdb, 1T2W.pdb, 3MZD.pdb were selected as the drug targets from RCSB Protein Data Bank, where the x-ray crystallographic structures were obtained and the docking studies were performed with the AutoDock 4.2 version.

Step 1: Selection from PDB

Streptococcus pneumoniae: PDB accession code: 2VEG.pdb

Staphylococcus aureus: PDB accession code: 1T2W.pdb

Escherichia coli: PDB accession code: 3MZD.pdb

Target proteins were downloaded from RCSB Protein Data Bank and docking studies were performed.

Step 2: Protein structure refinement

Proteins (2VEG, 1T2W, 3MZD) were refined to remove the water molecules and bound ligands if any.

Step 3: Docking study

After conversion of proteins to .pdb format and ligands into. pdbqt format, the docking was performed using AutoDock 4.2.

RESULTS AND DISCUSSION

The binding energies of the synthesized ligands and standard ligand Ciprofloxacin with 3MZD, 1T2W, 2VEG are given in Table-1.

Table 1: Binding energies of 2a, 3a, 4a & Ciprofloxacin with 3MZD, 1T2W, 2VEG.

S. No	Compound code	Binding energies (kcal/mol)		
		3ZMD.pdb	1T2W.pdb	2VEG.pdb
1	2a	-5.1	-4.61	-5.57
2	3a	-4.52	-4.71	-4.26
3	4a	-5.22	-6.49	-5.93
4	Ciprofloxacin	-6.04	-6.85	-7.03

The snapshots of the binding interactions are given in Fig. 1-9.

Binding interactions of 2a with *E.Coli* (3ZMD.pdb)

2a interacts with *E.coli* at Leu167, Arg174, Gly28, Lys294 and NAD^+. The binding energy was found to be **-5.1 kcal/mol**.

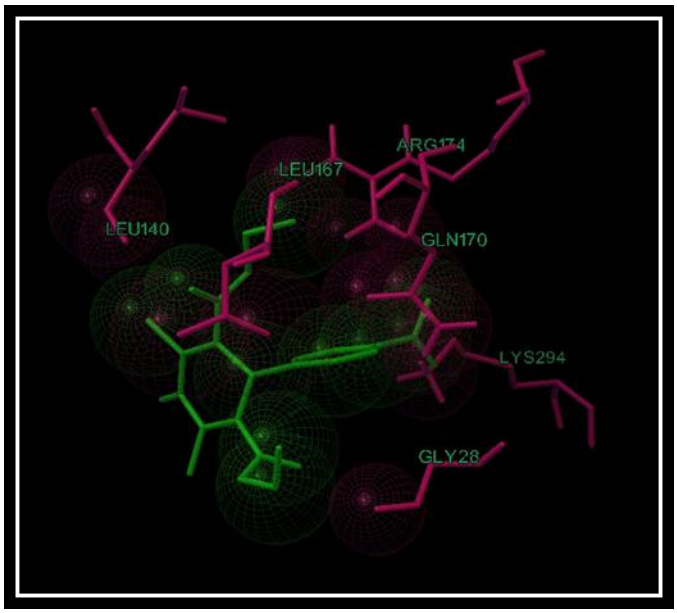


Fig. 1: Snapshot of 2a with *E.coli* (3ZMD.pdb)

Binding interactions of 2a with *S. aureus* (1T2W.pdb)

2a interacts with *S. aureus* at Ala184, Ile182, Arg197, Ala92, Glu105, Ala118, Trp194 and NAD^+. The binding energy was found to be **-4.61 kcal/mol**.

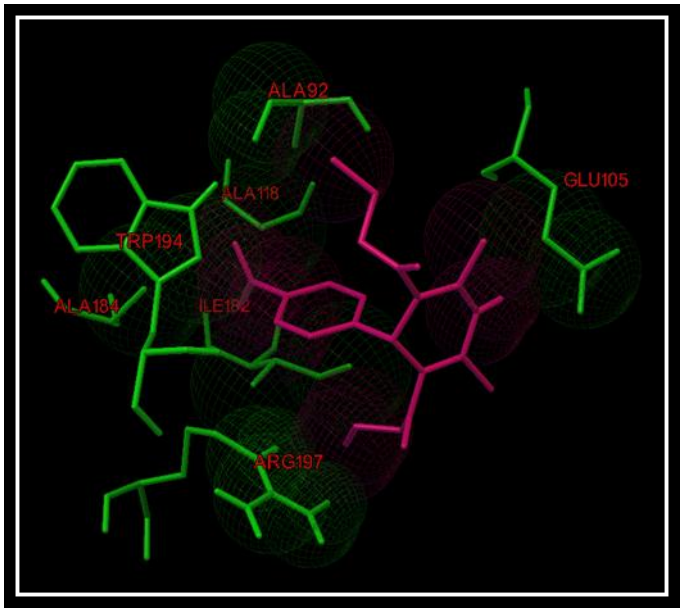


Fig. 2: Snapshot of 2a with *S. aureus* (1T2W.pdb).

Binding interactions of 2a with *S. pneumaniae* (2VEG.pdb)

2a interacts with *S. pneumaniae* at Glu55, Asn17, Arg282, Phe206, Phe154, Ile15 and NAD^+. The binding energy was found to **-5.57 kcal/mol**.

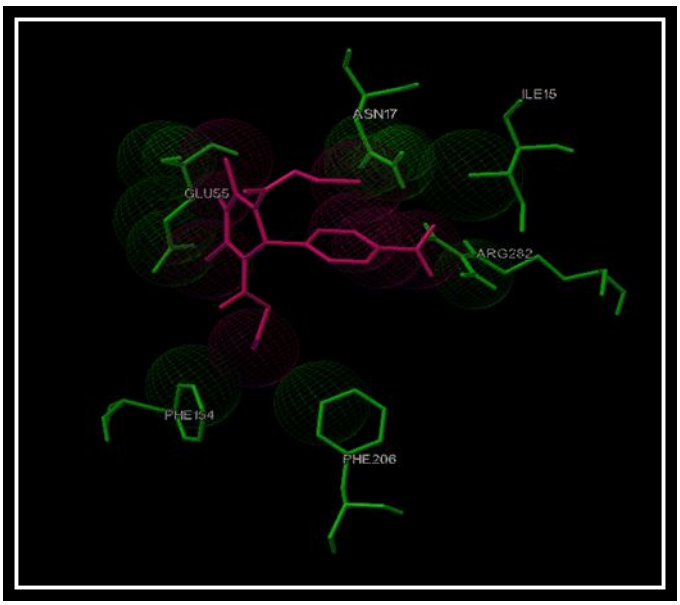


Fig. 3: Snapshot of 2a with *S. pneumaniae* (2VEG.pdb).

Binding interactions of 3a with *E.coli* (3ZMD.pdb)

3a interacts with *E.coli* at Gly28, Arg174, Leu167, Leu140, Gln170, Ile23 and NAD^+. The binding energy was found to be **-4.52 kcal/mol**.

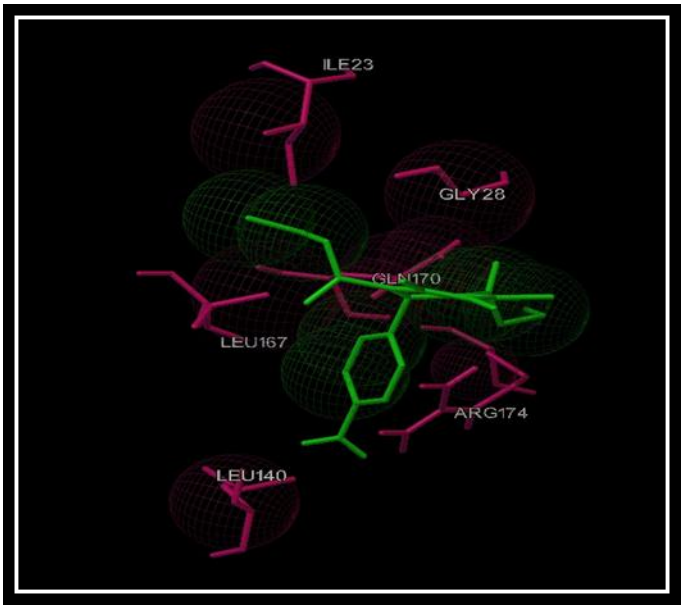


Fig. 4: Snapshot of 3a with *E. coli* (3ZMD.pdb).

Binding interactions of 3a with *S. aureus* (1T2W.pdb)

3a interacts with *S. aureus* at Ala118, Ala92, Glu105, Ser116, Ile182, Ala104 and NAD^+. The binding energy was found to be **-4.71 kcal/mol**.

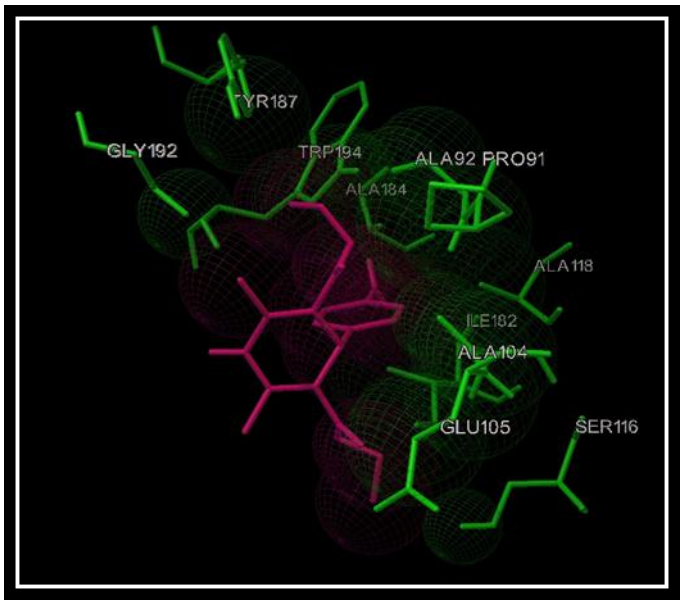


Fig. 5: Snapshot of 3a with *S. aureus* (1T2W.pdb).

Binding interactions of 3a with *S. pneumaniae* (2VEG.pdb)

3a interacts with *S. pneumaniae* at Asn110, Asp91, Pro152, Phe154, Phe151, Ile112, Arg282 and NAD^+. The binding energy was found to be **-4.26 kcal/mol**.

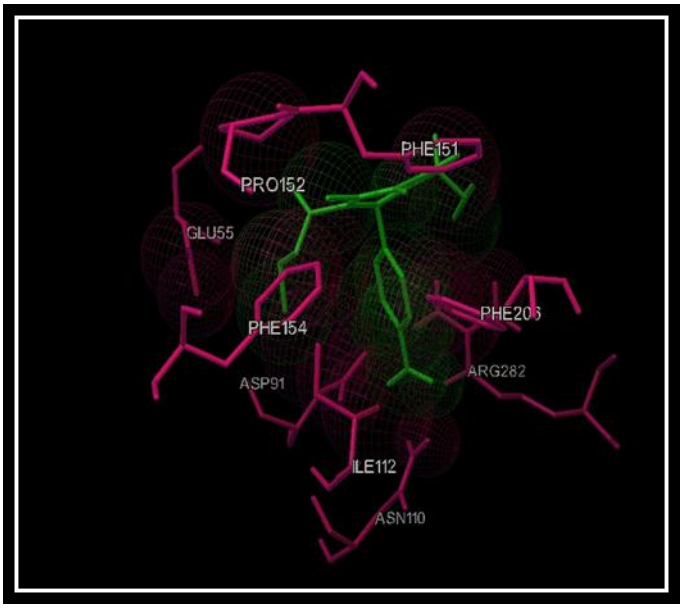


Fig. 6: Snapshot of 3a with *S. pneumaniae* (2VEG.pdb).

Binding interactions of 4a with *E.coli* (3ZMD.pdb)

4a interacts with *E.coli* at Gln56, Asp175, Arg174, Gln170, Leu140, Gly141 and Leu140 NAD^+. The binding energy was found to be **-5.22 kcal/mol**.

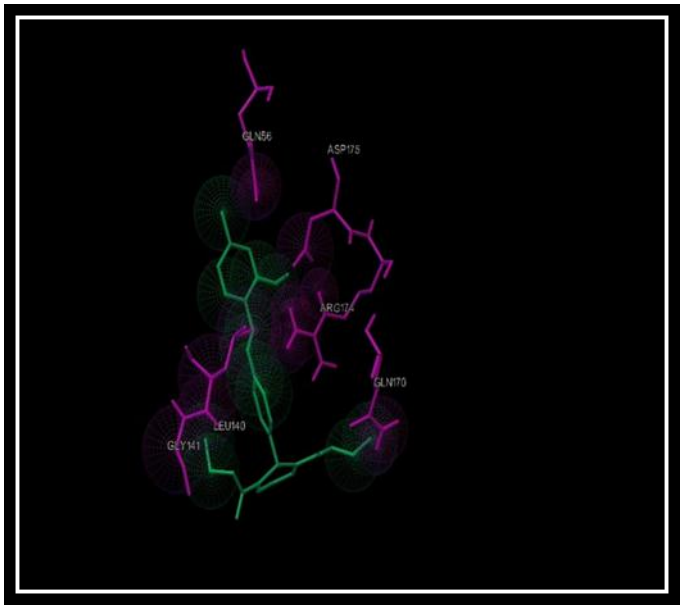


Fig. 7: Snapshot of 4a with *E.coli* (3ZMD.pdb).

Binding interactions of 4a with *S. aureus* (1T2W.pdb)

4a interacts with *S. aureus* at Arg197, Ala118, Glu105, Ala104, Asn114 and Val193 NAD^+. The binding energy was found to be **-6.49 kcal/mol**.

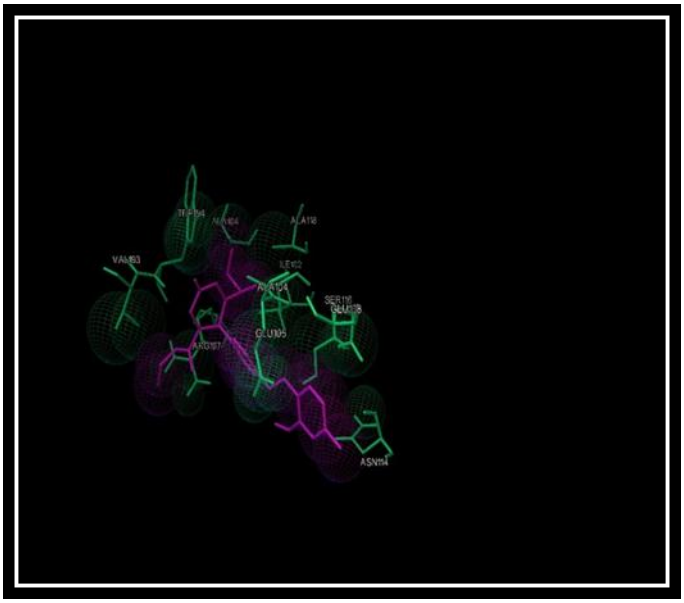


Fig. 8: Snapshot of 4a with *S. aureus* (1T2W.pdb).

Binding interactions of 4a with *S. pneumaniae* (2VEG.pdb)

4a interacts with *S. pneumaniae* at Gly205, Asp91, Thr92, Ile112, Glu56, Lys237 and NAD^+. The binding energy was found to be **-5.93 kcal/mol**.

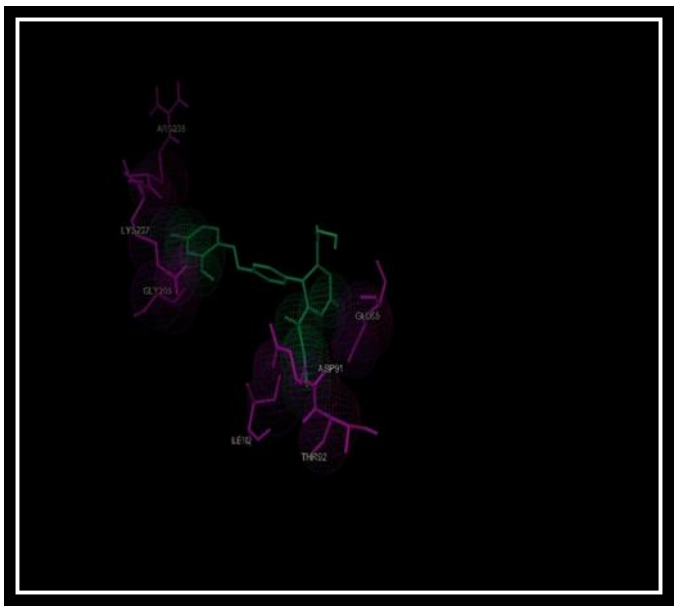


Fig. 9: Snapshot of 4a with *S. pneumoniae* (2VEG.pdb).

Binding interactions of Ciprofloxacin with *E.coli* (3ZMD.pdb)

Ciprofloxacin interacts with *E.coli* at Gly28, Ser27, Glu342, Lys29, Arg344, Lys294 and NAD^+. The binding energy was found to be **-6.04 kcal/mol**.

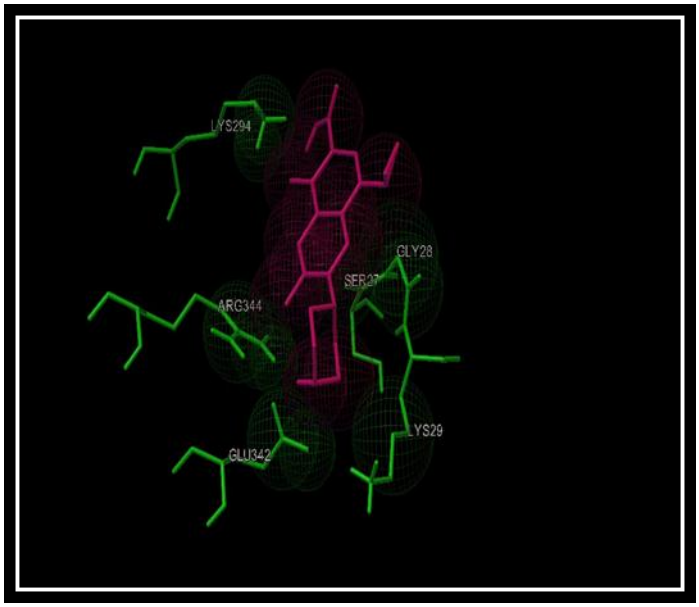


Fig. 10: Snapshot of Ciprofloxacin with *E. coli* (3ZMD.pdb).

Binding interactions of Ciprofloxacin with *S. aureus* (1T2W.pdb)

Ciprofloxacin interacts with *S. aureus* at Ala92, Tyr187, Trp194, Ile182, Arg197, Gly192 and NAD^+. The binding energy was found to be **-6.85 kcal/mol**.

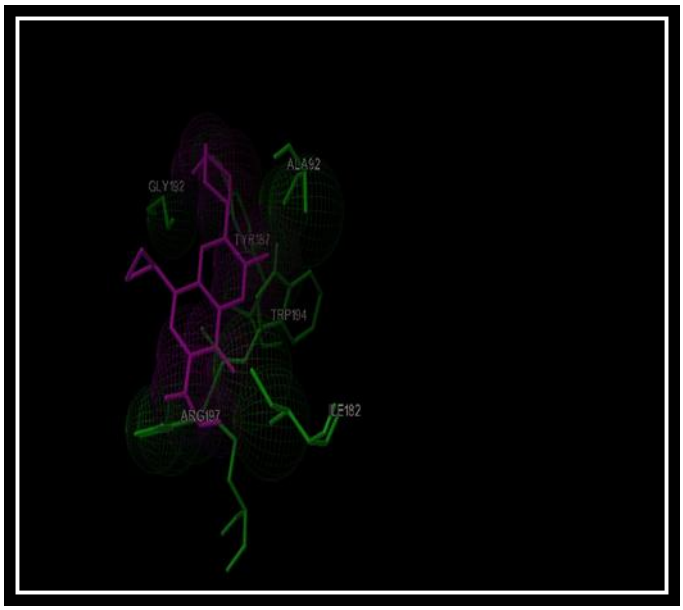


Fig. 11: Snapshot of Ciprofloxacin with *S. aureus* (1T2W.pdb).

Binding interactions of Ciprofloxacin with *S. pneumaniae* (2VEG.pdb)

Ciprofloxacin interacts with *S. pneumaniae* at Lys237, Arg282, Asn110, Asp91, Glu65, Ile112, Phe206 and NAD^+. The binding energy was found to be **-7.03 kcal/mol**.

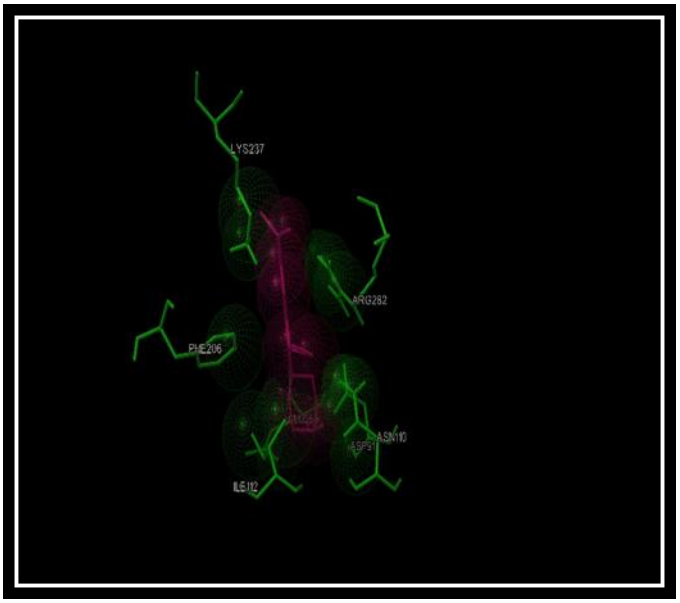
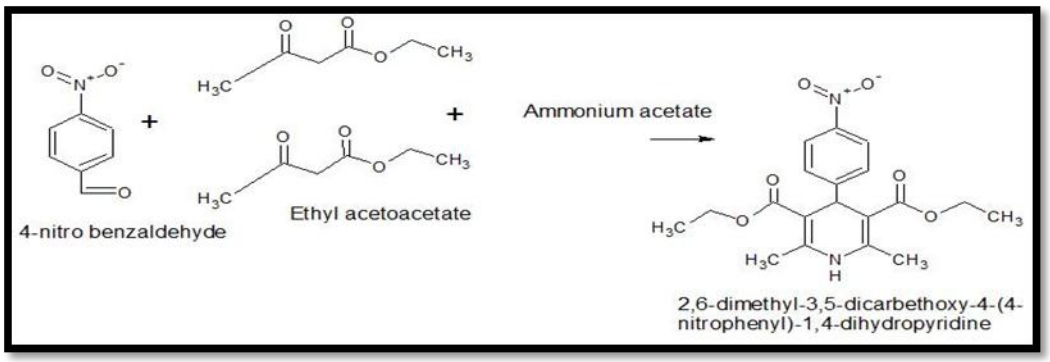


Fig. 12: Snapshot of Ciprofloxacin with *S. pneumoniae* (2VEG.pdb).

Phase II: Synthesis

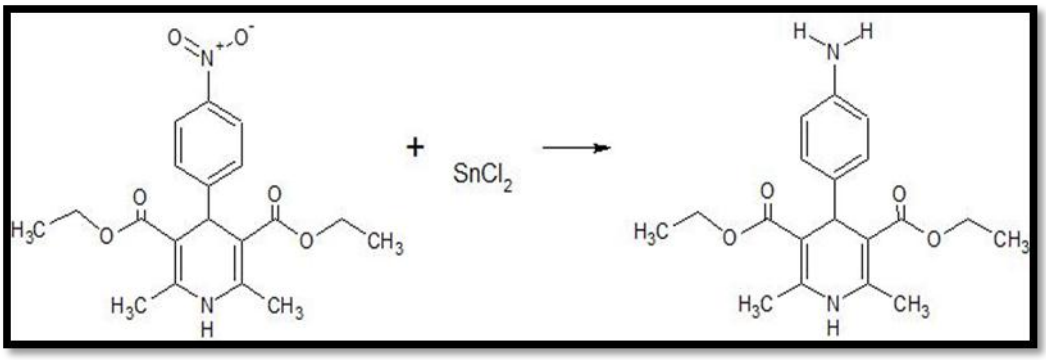
Step 1: Synthesis of 2,6-dimethyl-3,5-dicarbethoxy-4-(4-nitrophenyl)-1,4-dihydropyridine (2a)

A mixture of 4-nitrobenzaldehyde (3g) [1eq], ethyl acetoacetate (5.1g) [2eq], ammonium acetate (1.53g) [1eq] and catalyst β-cyclodextrin (2.25g) [10% wt] was taken in a 100 mL multi neck round bottom flask, mixed well and stirred on a magnetic stirrer at 80°C for 8 hrs. After completion of the reaction monitored by TLC, the contents were cooled. The solid catalyst was removed by filtration, washed with ethanol and kept aside for reuse. The filtrate was concentrated under reduced pressure to obtain crude product and was further purified from ethyl acetate and the formation of nitro group was identified by dinitro phenyl hydrazine reagent (DNPH)



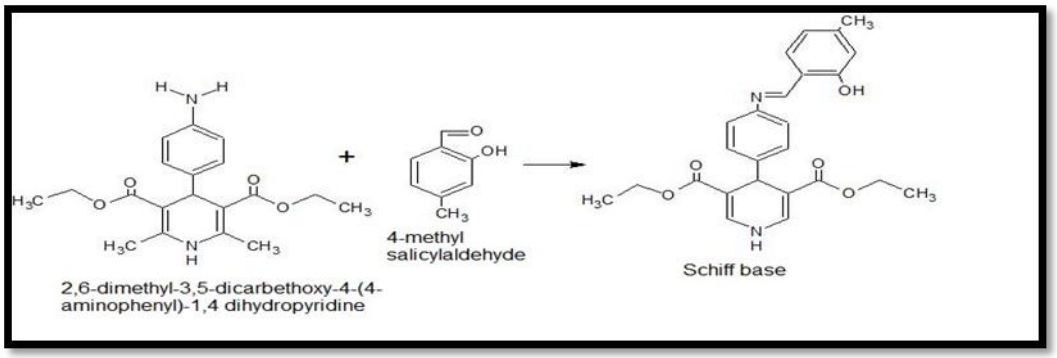
Step 2: Synthesis of 2,6-dimethyl-3,5-dicarbethoxy-4-(4-aminophenyl)-1,4-dihydropyridine (3a)

A mixture of 2,6-dimethyl-3,5-dicarboethoxy-4-(4-nitrophenyl)-1,4-dihydropyridine (0.50g), stannous chloride (1.6 g), methanol (3ml) was taken in 100mL pyrex beaker, mixed well and stirred on a magnetic stirrer for 30min. After completion of the reaction, the mixture was neutralized by 40% sodium hydroxide solution and monitored by TLC. The formed amino derivative was further identified by ninhydrin reagent. To filter the product, saturated sodium chloride is added. The filtrate was concentrated under reduced pressure to obtain crude product.



Step 3: Synthesis of Schiff base (4a)

A mixture of 2,6-dimethyl-3,5-dicarboethoxy-4-(4-aminophenyl)-1,4-dihydropyridine (0.50g), glacial acetic acid (2.6g), 4-methyl salicylaldehyde (2gm) and water was taken in 100 mL Pyrex beaker, mixed well and stirred on a magnetic stirrer for 30 min. After completion of the reaction, the mixture was monitored by TLC. The formed imino derivative was further identified by using DNP on TLC plates and ninhydrin reagent as visualizing agents. The mixture was filtered and the filtrate was concentrated under reduced pressure to obtain the crude product.



After completion of the reactions, the synthesized compounds were subjected to various analytical tests such as melting point determination, IR, NMR and Mass spectra for the confirmation of the compounds.

Phase III: Biological screening

Test Microorganisms

Gram-positive organisms *Streptococcus pneumoniae, Staphylococcus aureus*, Gram-Negative organism *Escherichia coli* were chosen based on their clinical and pharmacological importance. They were maintained in Mueller-Hinton Agar medium.

Experimental Procedure

Mueller Hinton agar plates were prepared and dried at 37°C before inoculation. The organisms were inoculated in the plates. The inoculated medium was allowed to dry at room temperature, with the lid closed. The well was made in the plates by using a cork borer. By using micropipette, the test sample and standard were added into the well and were refrigerated for one hour to facilitate uniform diffusion of the drug. It was then incubated for 18-24 hours at 37°C. The diameter of the zone of inhibition around the drugs were measured and compared with that of the standard. Ciprofloxacin was used as a standard drug at 100 µg ml^{-1}. All the synthesized compounds were tested for antibacterial activity against *Streptococcus pneumoniae, Staphylococcus aureus and Escherichia coli* bacteria. The results were interpreted in term of diameter (mm) of zone of inhibition.

The bacterial zone of inhibition values (mm) are given in Table 2-4

- The Compounds were screened on *Streptococcus pnemoniae*.

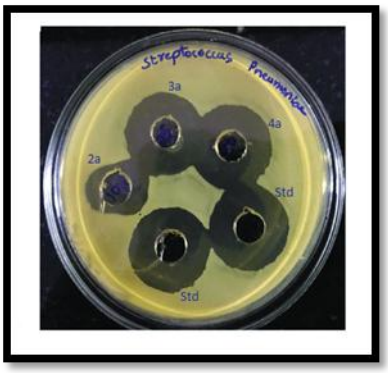


Fig. 13: Antibacterial activity on *Streptococcus pneumonia.*

Table 2: Zone of inhibition on *Streptococcus pneumonia.*

Compound	Zone of inhibition (mm)
2a	6
3a	9
4a	10
Ciprofloxacin	14

❖ The Compounds were screened on *Staphylococcus aureus*.

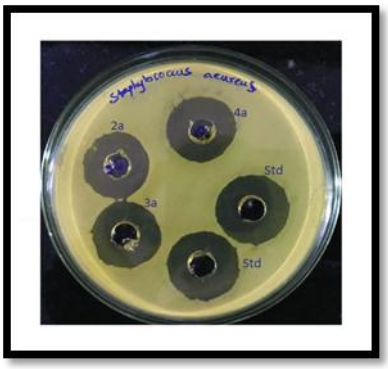


Fig. 14: Antibacterial activity on *Staphylococcus aureus.*

Table 3: Zone of inhibition on *Staphylococcus aureus.*

Compound	Zone of inhibition in (mm)
2a	7
3a	7
4a	8
Ciprofloxacin	10

❖ The Compounds were screened on *Escherichia coli.*

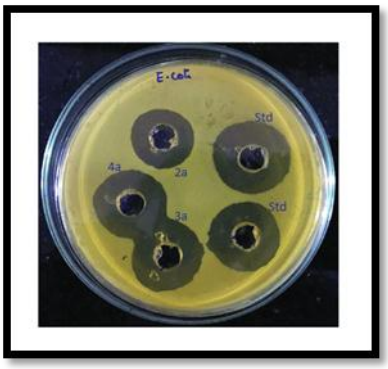


Fig. 15: Antibacterial activity on *Escherichia coli.*

Table 4: Zone of inhibition on *Escherichia coli.*

Compound	Zone of inhibition (mm)
2a	6
3a	7
4a	7
Ciprofloxacin	10

RESULTS AND DISCUSSION

The present work was focused on the design, docking, synthesis and evaluation of antibacterial activities of 1, 4-dihydro pyridine series as possible DNA gyrase inhibitors.

Phase I - In-Silico studies

Selection of the target

The enzymes involved in the formation of DNA and replication of *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* i.e., DNA gyrase was selected as the drug target of the study. 3MZD, 1T2W, 2VEG are the drug targets selected for the present study. The corresponding enzymes were obtained from the protein data bank.

Selection of lead by virtual screening

From the virtual screening, the lead molecules were identified which can be optimized by chemical modification to produce drug candidates.

Lead optimization

The three modified ligands **2a, 3a** and **4a** were subjected to *in-silico* lead optimization. The ligands were optimized for evaluating oral bioavailability by utilizing the Molinspiration server. Lead optimizations revealed that **4a** derivative possess good drug likeness score than 2a and 3a derivatives.

Docking

The optimized leads were subjected to docking studies using AutoDock 4.2 and the interactions of the derivatives with the active sites of the enzymes were studied. The derivatives were subjected to interact with 3MZD (*E.Coli)*, 1T2W (*Staphylococcus aureus*) and 2VEG (*Streptococcus pneumoniae).*

Phase II – Synthesis

Step 1: Synthesis of 2,6-dimethyl-3,5–dicarbethoxy-4-(4-nitrophenyl)-1,4-dihydropyridine (2a)

A mixture of 4-nitrobenzaldehyde, ethyl acetoacetate, ammonium acetate with β-cyclodextrin catalyst form nitro derivative which is subjected to further derivative.

Step2: Synthesis of 2,6–dimethyl-3,5–dicarbethoxy-4-(4-aminophenyl)-1,4-dihydropyridine (3a)

A mixture of 2,6-dimethyl-3,5-dicarboethoxy-4-(4-nitrophenyl)-1,4-dihydropyridine, stannous chloride and methanol form amino derivative which is subjected to further derivative.

Step 3: Synthesis of Schiff base (4a)

A mixture of 2,6-dimethyl-3,5-dicarboethoxy-4-(4-aminophenyl)-1,4-dihydropyridine, glacial acetic acid, 4-methyl salicylaldehyde with water form Schiff base.

Phase III - Biological activity

Antibacterial activity

Antibacterial activity was performed by agar well diffusion method on *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. All the derivatives were screened for antibacterial activity. Among 1,4-dihydropyridine series, **2a** and **3a** (nitro and amine) has shown moderate sensitivity with a zone of inhibition and **4a** (imine) has shown good zone of inhibition respectively. Thus the series of compounds synthesized can be utilized for antibacterial activity by the mechanism of action of inhibiting the DNA gyrase enzyme.

CONCLUSION

In the present study, virtual screening was utilized for filtering the compounds and selecting the lead compounds. From the binding energies obtained from docking studies, Ciprofloxacin has shown high binding energies than 2a, 3a and 4a. Whereas **4a** shows good binding energies than 2a and 3a against ***S. pneumoniae, S. aureus*** and ***E.coli***. Using the schemes, a new series of 1,4-dihydropyriodine derivatives **2a, 3a** and **4a** were synthesized. The synthesized compounds were screened for their antibacterial activity, whereas compound **4a** was found to be quiet comparable with the standard screened under similar conditions and was more active against ***S. pneumoniae*** **and** ***S. aureus***.

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