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

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CHAPTER – 1

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 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 [12] .All living organisms on Earth are made up of one of two basic types of cells: eukaryotic cells, in which the genetic material is enclosed within a nuclear membrane, or prokaryotic cells, in which the genetic material is not separated from the rest of the cell. Traditionally, all prokaryotic cells were called bacteria and were classified in the prokaryotic kingdom Monera.

Bacterial infections:

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 in tissues that are normally sterile. Harmful bacteria may also emit toxins that damage the body. Common pathogenic bacteria and the types of bacterial diseases they cause include:

- ***Escherichia coli and Salmonella*** cause food poisoning.
- Helicobacter pylori cause gastritis and ulcers.
- ***Neisseria gonorrhoeae*** causes the sexually transmitted disease gonorrhea.
- ***Neisseria meningitides*** causes meningitis.
- ***Staphylococcus aureus*** causes a variety of infections in the body, including boils, cellulitis, abscesses, wound infections, toxic shock syndrome, pneumonia, and food poisoning.
- ***Streptococcal bacteria*** cause a variety of infections in the body, including pneumonia, meningitis, ear infections, and strep throat.

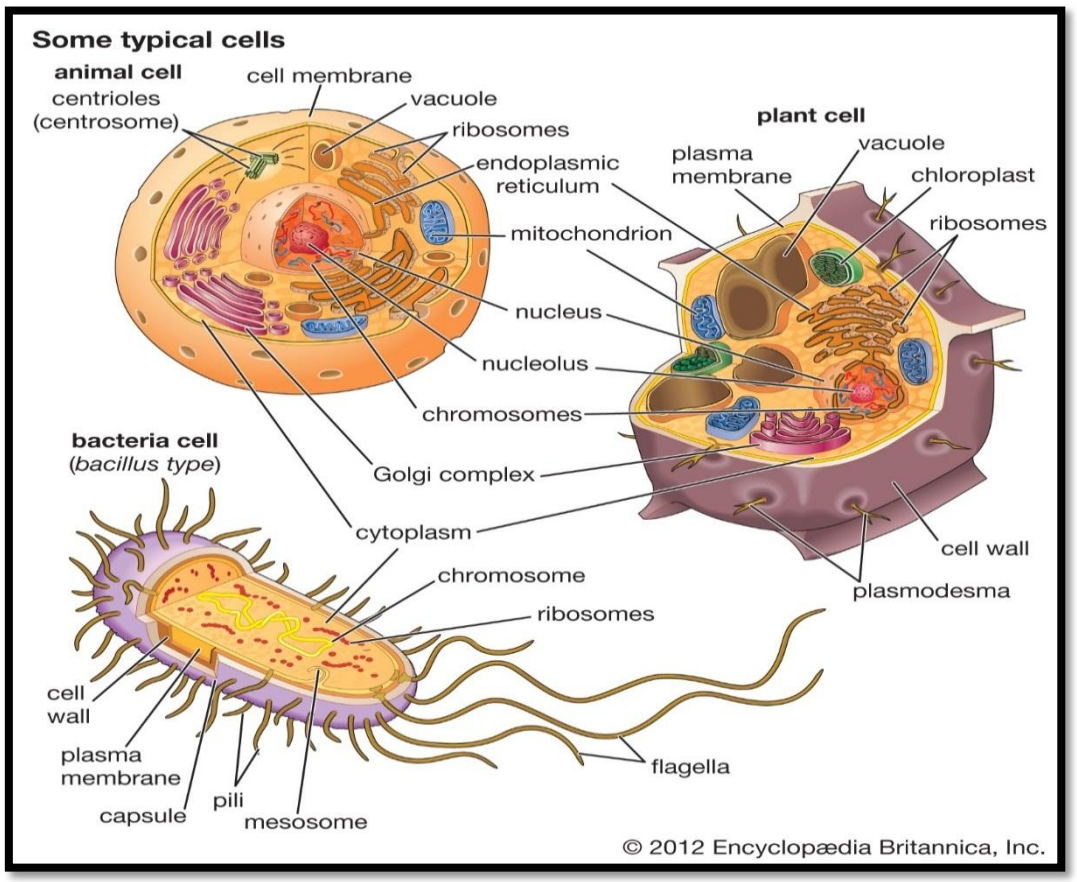


Fig.1: Types of bacterial cells

Bacterial diseases are contagious and can result in many serious or life-threatening complications, such as blood poisoning (b=acteremia), kidney failure, and toxic shock syndrome.

Pathogenic bacteria:

1. *Streptococcus pneumonia* [6] (pneumococcus) is a Gram-positive bacterium that is responsible for the majority of community-acquired pneumonia. It is a commensal organism in the human respiratory tract. Infection by pneumococcus may be dangerous, causing not only pneumonia, but also bronchitis, otitis media, septicemia, and meningitis. *S. pneumoniae* is alpha-hemolytic, that it can break down red blood cells through the production of hydrogen peroxide (H_2O_2). The production of $H_2O_2$by the bacterial infection can also cause damage to DNA, and kill cells within the lungs. Pneumococcal pneumonia causes fever and chills, coughs, difficulty in breathing, and chest

pain. If the infection spreads to the brain and spinal cord, it can cause pneumococcal meningitis, characterized by a stiff neck, fever, confusion, and headaches.

2. *Staphylococcus aureus[3]*or "staph" is a Gram-positive bacteria found on human skin, in the nose, armpit, groin, and other areas. It is the leading cause of skin and soft tissue infections. They can also cause more serious infections, such as pneumonia, bloodstream infections, endocarditis (infection of the inner lining of the heart chambers and heart valves), bone and joint infections. Over 30 different types of staphylococci can infect humans, but most infections are caused by *Staphylococcus aureus*.

3. Escherichia coli[5], also known as *E.coli*, is a Gram-negative, rod-shaped bacterium commonly found in the gut of warm-blooded organisms. Most strains of *E.coli*are harmless to humans. Some types of *E.coli* infection can lead to diarrhoea, nausea, vomiting, fever , urinary tract infections, respiratory illness, pneumonia, and other illnesses like meningitis.

Antibacterials:

Antibacterials are chemical substances derived from a biological source or produced by chemical synthesisthat 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.

Antibacterial agents:

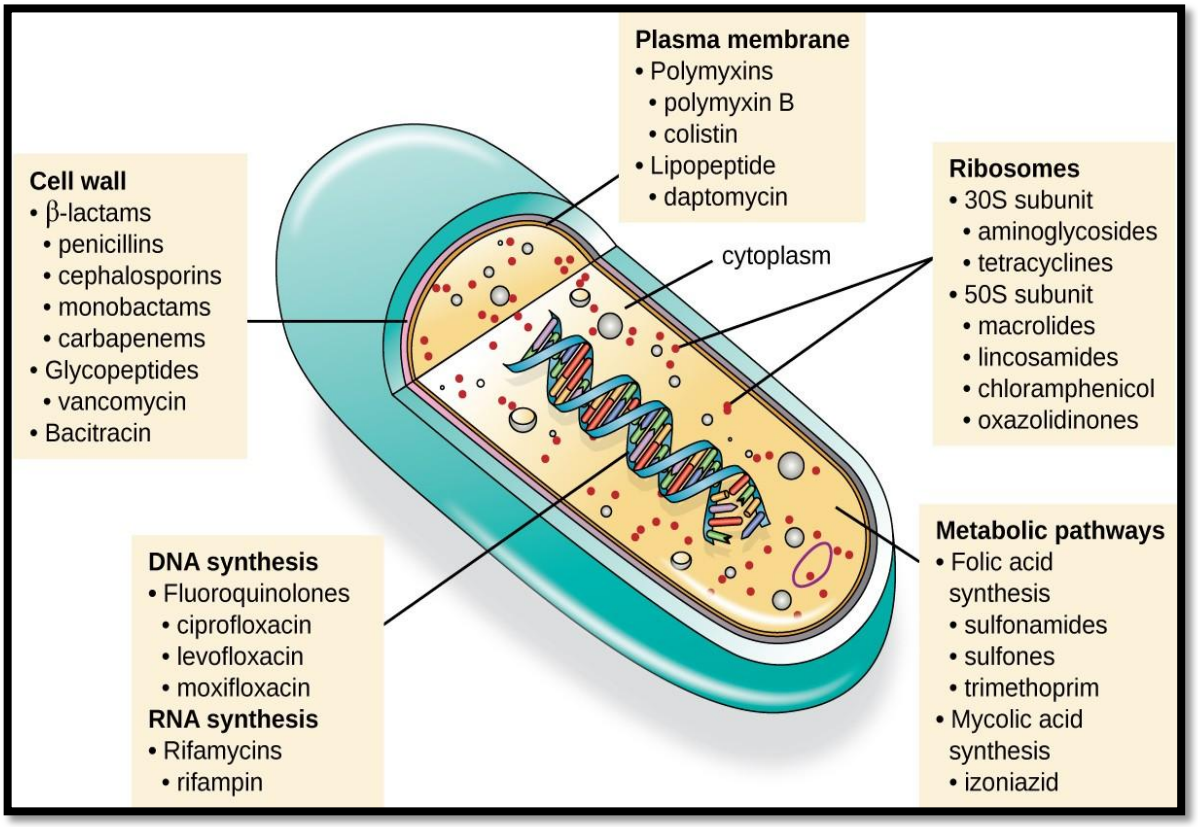


Fig.2: Atibacterial drugs

Mechanism of action:

- The antibacterial drugs are designed to have a complex mechanism of action by considering some essential factors which render to the obtained drug-specific features. One can classify antibacterial drugs by taking into account the following:
- The effect on bacterial growth
    - bacteriostatic
    - bactericidal drugs
- The targeted site

Drugs that inhibit bacterial wall synthesis or activate enzymes that destroy the cell wall

- Drugs that enhance cell membrane permeability (causing leakage of intracellular material)
- Drugs that determine lethal inhibition of bacterial protein synthesis
- Drugs that generate nonlethal inhibition of protein synthesis
- Drugs that inhibit bacterial synthesis of nucleic acids
- Antimetabolites (disruption of specific biochemical reactions---decrease in the synthesis of essential cell constituents)
- Inhibitors of viral enzymes

- The target specificity
  - The broad-spectrum drug affects a wide range of disease-causing bacteria, including both Gram-positive and Gram-negative bacteria
  - The narrow-spectrum antibacterial drug, which acts against specific families of bacteria. For example, ampicillin is a widely used broad-spectrum antibiotic.

Schematic diagram of mechanism of antibacterials:

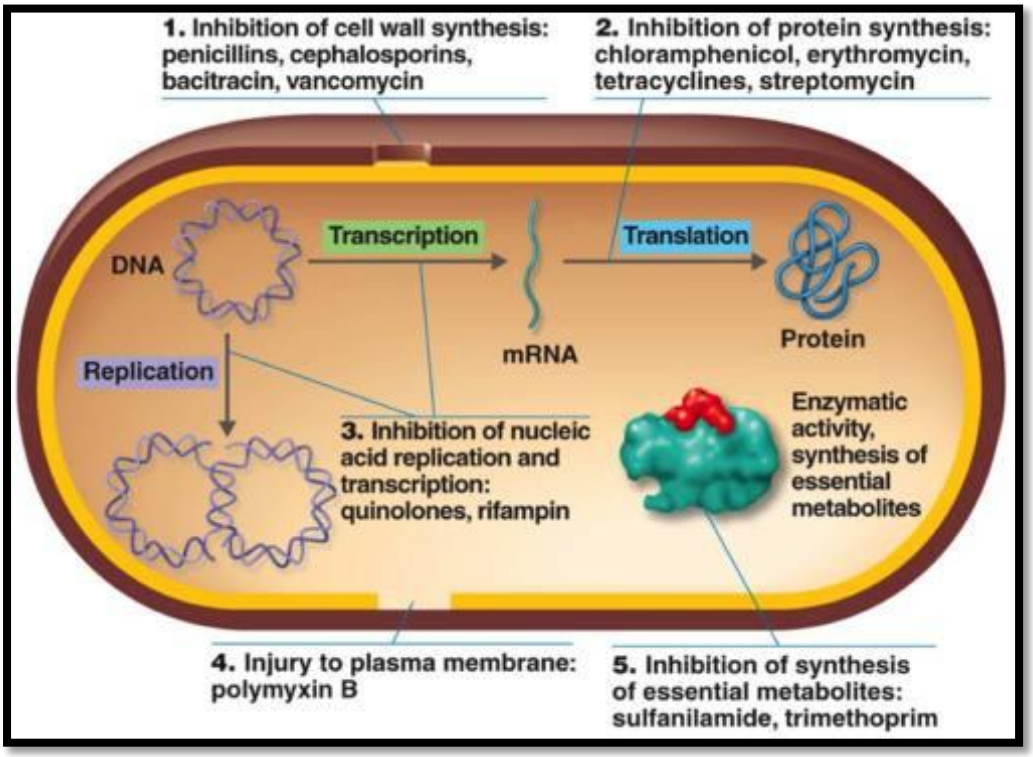


Fig.3: Mechanism of antibacterials

Antibacterial resistance:

The three fundamental mechanisms of antimicrobial resistance are (1) enzymatic degradation of antibacterial drugs, (2) alteration of bacterial proteins that are antimicrobial targets, and (3) changes in membrane permeability to antibiotics. Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome.

Schematic diagram of mechanism of antibacterial resistance:

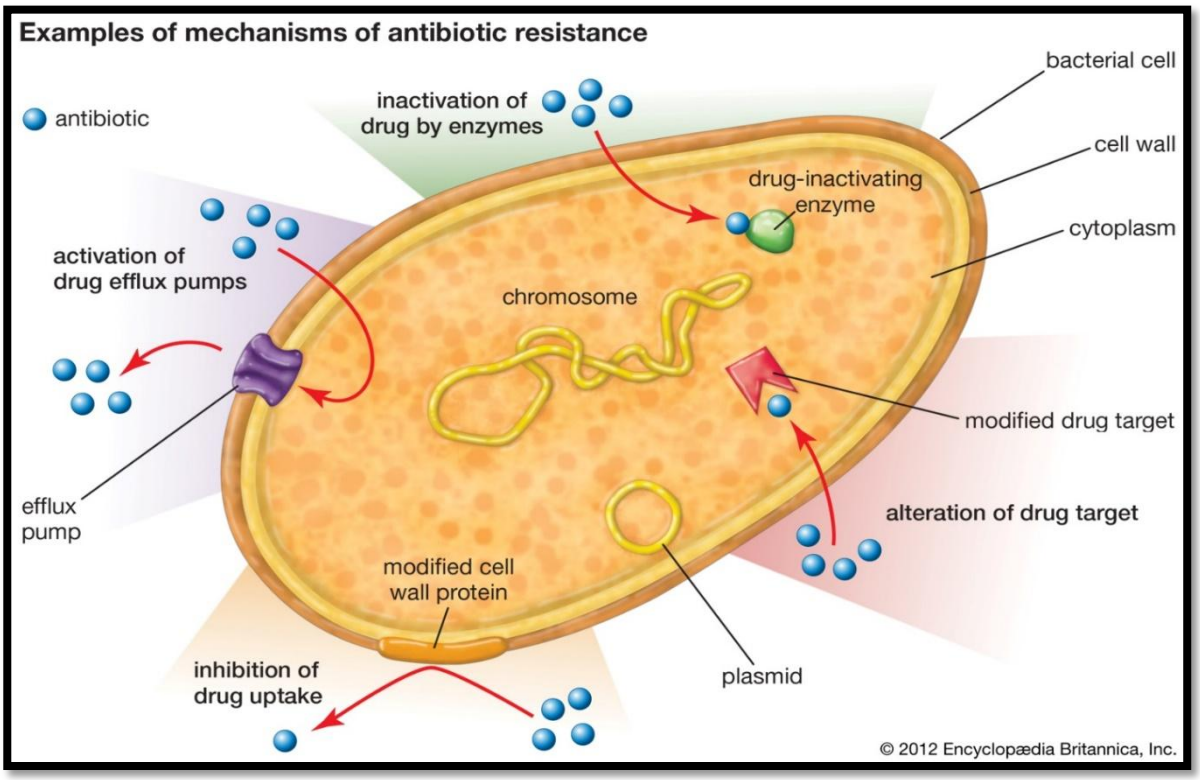


Fig.4: **Mechanism of antibacterial resistance**

Computer-Aided Drug Design

Computer-aided drug design (CADD) is a summarizing term for the methodology to design and develop new ligands with *in silico* techniques. It is used mainly for finding hit compounds and for lead optimization. CADD does not replace *in vitro* testing of ligand activities, but potentially saves on time and resources required by high-through put screening.

Molecular docking:

Nowadays, researchers have focused much interest towards an inventive process of finding new medications based on knowledge of biological target and novel drug delivery system. Drugs can be designed specifically to interact with the target molecule in such a way as to disrupt the disease. In the field of molecular modeling, **docking** is a method which predicts the preferred orientation of one

molecule to a second when bound to each other to form a stable complex.The goal of a docking experiment is an accurate *in silico*prediction of the ligand's binding mode. Molecular docking tools employ algorithms to generate and score reasonable binding poses of a selected ligand to a specific binding site in a target protein. *In silico* experiments are advantageous in terms of time and material consumption.

As 1,4-dihydropyridine derivatives exhibit various pharmacological actions which include calcium channel antagonist property, antihypertensive, anti-inflammatory, antifungal, analgesic, antimicrobial, anti-thrombotic actions , it is subjected and docked for anti-bacterial activity against *Streptococcus pneumoniae, Staphylococcus aureus*&*E.coli* using various substituted aromatic aldehydes in this research.

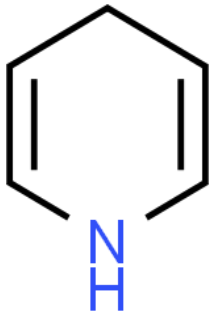
1, 4 DIHYDROPYRIDINES:

- Among heterocyclic compounds containing six membered ring, 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 1^{st} position, which is saturated at 1^{st} and 4^{th} position.

- The most feasible position for substitution is 4^{th} of the heterocyclic ring which exhibit various pharmacological activities such as calcium channel antagonist, antihypertensive, anti-inflammatory, antifungal, analgesic, antimicrobial, anti-thrombotic actions.

- It also shows vasodilation, anticonvulsant and stress protective effect by binding to L and N channels, antibacterial, anti-leishmanial, anticoagulant, anticonvulsant, anti-tubercular, antioxidant, antiulcer, antimalarial, neuroprotection properties, HIV-1 protease inhibitors, antifertility activities.



1,4dihydropyridine

Keeping in view of above facts, an efficient and versatile methods are developed for synthesis of 1,4-dihydropyridines. In the present study, the goal is to synthesize and dock 1,4-dihydropyridine derivative by modifying the aromatic aldehyde group, and the objective behind the study is to investigate the effect of docked 1,4-DHP derivative for its anti-bacterial activity against *Streptococcus pneumonia, Staphylococcus aureus&E.coli.*

In the chart below, five of the best-known docking tools are listed together with their operating principle.

Table 1: Five popular docking methods with their operating principles..

Docking Method	**Working methodology**
AutoDock [Osterberg, F, 2002]	Flexible docking into a rigid receptor.
DOCK [Venkatachalam, C. M., 2003]	Rigid, shaped-based docking, key-into-lock principle.
FlexX [Rarey, M., 1996]	Fragment-based docking approach.
GOLD [Jones, G., 1997]	Genetic algorithm to dock flexible ligand into a semi-rigid receptor.
Glide [Friesner, R., 2004; Friesner, R., 2006]	Systematically docking a ligand into a receptor; offers induced-fit docking.

CHAPTER - 2

LITERATURE REVIEW

1. **Kanji Meguro et al., 1984**[1]attempted to synthesize long acting 1,4 - dihydropyridine derivatives by introducing functional groups that have both high lipophilicity and high affinity for the vascular tissues. Piperazine moieties with lipophilic substituents were chosen. A variety of piperazinylalkyl esters and related compounds were prepared and tested for antihypertensive activity.
2. **N. SrinivasaRao et al., 2013**[9]prepared a new series of 1,4dihydropyridine derivatives via reaction with α-naphthayl amine using the condensation method. The synthesized compounds were confirmed by IR, 1 H NMR, 13C-NMR and elemental analyses. The synthesized compounds were screened for antimicrobial properties against the bacteria S*taphylococcus aureus, Bacillus subtillus, Escherichia coli*&*Vibreocholerae*and fungi *Trichoderma Sp., A. Niger, A. Parasitica, ChrysosporiumSp*
3. **Vrushali H Jadhav et al., 2015[13]revealed that** Carbonaceous solid acid catalyst was used for convenient and efficient synthesis of 1,4-dihydropyrine (DHP) derivatives under solvent free conditions. He searched for a better catalyst for the synthesis of DHP derivatives using less hazardous solvents or solvent free conditions is of prime importance. He further explored the carbonaceous catalyst on various substituted aldehydes possessing either electron donating or electron withdrawing substituents with ethyl acetoacetate and ammonium acetate using 10 wt% of the catalyst. The products were characterized by IR, 1H NMR and GC Mass.

4. **ShardaGoel et al., 2018**[16]investigated the catalytic activity of tetrabutylammonium hydrogen sulfate on Hantzschdihydropyridine synthesis by the condensation of ethylacetocetate, ammonium acetate and substituted arylaldehydes in the presence of TBAHS at 70°C in 2:1.5:1 ratio under solvent free conditions. The prepared products were characterized by spectral analysis, comparison of the melting points and TLC.
5. **Petra Olejníkováet.al., 2014**evaluated the antimicrobial activity of 3-methyl-5-isopropyl (or ethyl) 6-methyl-4 nitrophenyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives for bacteria and filamentous fungi. The antimicrobial activities of various 1,4-DHP derivatives against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, *Proteus vulgaris,* as well as *Mycobacterium tuberculosis as well as* against the fungi *Aspergillus fumigates* and *Candida albicans*was investigated.
6. **G.Swarnalathaet.al.,2011**[4]reviewedthe highlights of the various synthetic routes with specified structural activityfeatures with corresponding pharmacological activities.
7. **H.M.Patelet.al., 2017**[15]reported A novel green and efficient one-pot multicomponent reaction of dihydropyridine derivatives having good to excellent yield. In the presence of the catalyst ceric ammonium nitrate (CAN), different 1,3-diones and same starting materials as 5-bromothiophene-2-carboxaldehyde and ammonium acetate were used at room temperature under solvent-free condition for the Hantzsch pyridine synthesis within a short period of time.

8. **Dong Fang et.al.,2013** [20]established a clean and efficient procedure to synthesize 1,4-dihydropyridines via one-pot Hantzsch reactions in aqueous medium without the use of a catalyst and/or organic solvent. The reaction with stoichiometric molar ration could be carried out in a sealed vessel with a water steam, air, or nitrogen atmosphere to afford Hantzsch esters in good to excellent yields and purities.
9. **Mauro Cataldiet.al., 2012** examined how changes in the DHP structure can modify the pharmacological properties of these drugs and how some of these chemical manipulations have been exploited to obtain clinically more effective molecules.Special emphasis is given to the evidence that L-type Ca^{2+} channels are an heterogeneous family and that DHPs with different pharmacological properties differ in their affinity for the different isoforms of this class of channels.
10. **Petra Olejnikovaet.al., 2014** evaluated the antimicrobial activity of 3-methyl-5-isopropyl (or ethyl) 6-methyl-4-nitrophenyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives.In this work, our effort was focused on the antimicrobial screening of 3-methyl 5-isopropyl (or ethyl) 6-methyl-4-nitrophenyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives and their potential to inhibit the growth of model bacteria and filamentous fungi.
11. **Prabha Mehtaet.al., 2012** [31]synthesized some halo- and nitrophenyldihydropyridines and evaluated their antimicrobial activity. The minimum inhibitory concentration (MIC) was determined by microdilution technique in Mueller Hinton broth. The MICs were recorded after 24 hours of incubation at 37°C. These results showed that these compounds exhibited

significant to moderate activities against both Gram-(+) and Gram-(−) organisms.

12. **F.MatloubiMoghaddam et.al., 2009** described aone-pot four-component reaction of aldehydes, ethyl acetoacetate/5,5-dimethyl-1,3-cyclohexanedione, ethyl acetoacetate and ammonium acetate in the presence of 10 mol% of ZnO as a heterogeneous catalyst for the synthesis of corresponding 1,4-dihydropyridineand polyhydroquinoline derivatives *via* the Hantzsch condensation.
13. **Soo-Jeong Choi et.al., 2010**[33]synthesized 1,4-dihydropyridine (DHP) scaffold as a inhibitor of BACE-1 by modifying the known BACE inhibitor **2** containing a hydroxyethylamine (HEA) motif. Using structure-based drug design based on computer-aided molecular docking, the isophthalamide ring of **2** was replaced with a 1,4-dihydropyridine ring as a brain-targeting strategy. Several of the new dihydropyridine derivatives were synthesized and their BACE-1-inhibitory activities were evaluated using a cell-based, reporter gene assay system that measures the cleavage of alkaline phosphatase (AP)-APP fusion protein by BACE-1.
14. **MelikaHadjebiet.al.; 2011,** [34] reportedthe four-component reaction of dimethyl acetylenedicarboxylate (=dimethyl but-2-ynedioate; DMAD), aromatic aldehydes, and malononitrile (=propanedinitrile) leads to polyfunctionalized 1,4-dihydropyridine derivatives. The reaction proceeds at room temperature and in the presence of a catalytic amount (20%) of $(NH_4)_2HPO_4$ as a base in aqueous media.

15. **Debache et al., 2008**, reported a simple, inexpensive, and efficient one-pot synthesis of 1,4- dihydropyridine derivatives of good yields via the three-component reaction of aromatic aldehydes, ethyl acetoacetate, and ammonium acetate using PhB(OH)2 as catalyst.
16. **Yao et al., 2005**, [34] claimed that a simple, inexpensive and efficient one-pot synthesis of 1,4- dihydropyridine derivatives at room temperature was being developed by them using catalytic amount of iodine with excellent product yields. An easy access to various substituted 1,4- dihydropyridine derivatives were realized quantitatively using commercially available iodine as the catalyst.
17. **Debache et al., 2009,** reported an efficient one-step synthesis of 1,4-dihydropyridines in good to excellent yields via the triphenylphosphine-catalyzed Hantzsch three-component reaction of an aromatic aldehyde, ethyl acetoacetate and ammonium acetate.
18. **Sabitha et al., 2003**,[27] reported that the synthesis of various substituted Hantzsch 1,4- dihydropyridine can be realized using the classical Hantzsch procedure and modified Hantzsch conditions at room temperature in the presence of iodotrimethylsilane (TMSI) generated in situ in CH3CN, in excellent yields
19. **Sridhar et al., 2006**,reported that one-pot condensation of β-dicarbonyl compounds with aldehydes and ammonium acetate could be feasible in the presence of HClO4–SiO2 at 80 oC under solvent-free conditions with good to excellent yields. The catalyst is easily prepared, stable, reusable and efficiently used under reaction conditions

20. **Menéndez et al., 2007**, reported that cerium ammonium nitrate (CAN) capable of catalyzing the three-component domino reaction between aromatic amines, α, β-unsaturated aldehydes, and ethyl acetoacetate, providing an efficient new entry of 1, 4-dihydropyridines. This new reaction requires very mild conditions. Since water appears to be the only side product and is complementary to the classical Hantzsch synthesis in that it is well suited to the preparation of Naryl-5,6-unsubstituted dihydropyridines. Experiments in the presence of a radical trap suggest 78 that a one-electron oxidative mechanism can be excluded and that CAN acts as a Lewis acid.
21. **Ghosh et al., 2013**,reported that a highly efficient environment-friendly Hantzsch 1,4- dihydropyridine synthesis under visible light in aqueous ethanol can be feasible in excellent yield via a one-pot three component reaction of various types of aliphatic and aromatic aldehydes with ethyl acetoacetate and ammonium hydroxide solution.
22. **Makone et al., 2013**, described that Sodium perchlorate catalyzes the synthesis of Hantzsch 1,4- dihydropyridine derivatives using aldehydes, methyl acetoacetate and ammonium acetate in an aqueous media at room temperature . This method offers several advantages including high yields, an environmentally friendly procedure, mild reaction conditions and economic viability.
23. **DavoodHabibi et al., 2013**[35]synthesized a good range of 1,4-dihydropyridines bearing a carbamate moiety on the 4-position from the primary reaction of different hydroxyaldehydes with phenyl isocyanates and the subsequent reaction of the obtained carbamates with methyl acetoacetate in the presence of ammonium fluoride.

CHAPTER - 3

AIM AND OBJECTIVE

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 disease. Drug discovery tools have been utilized now in designing new molecular entities which are safe and effective without consuming much of the research hours. Recent literatures shows that search of new drugs are now focussed on design of drugs as inhibitors of enzyme targets.

DNA gyrases, topoisomerases is such a potential drug target in the development of new antimicrobial agents. From the literature and virtual screening technique 1,4dihydropyridine analogues, etc., 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 identify and synthesize 4-Nitrobenzaldehyde and 4-Aminobenzaldehyde linked 1,4dihydropyridines as promising and antibacterial agents by the inhibition of DNA gyrase enzyme.

In this work, the focus is on the antibacterial screening of 1,4dihydropyridine 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.

Objectives of the present work:

- The present study is about the synthesis of DHP derivatives without the use of solvents using a carbonaceous solid catalyst.
- Use of carbonaceous catalyst under solvent free conditions is likely to result in greater selectivity, enhanced reaction rates, cleaner reaction products, operational simplicity and eco-friendly.
- Computational approaches in medicinalchemistry.
- Molecular docking of hit molecules obtained from SimilaritySearch.
- Docking is subjected to predict the Ligand's binding mode.

CHAPTER - 4

PLAN OF WORK

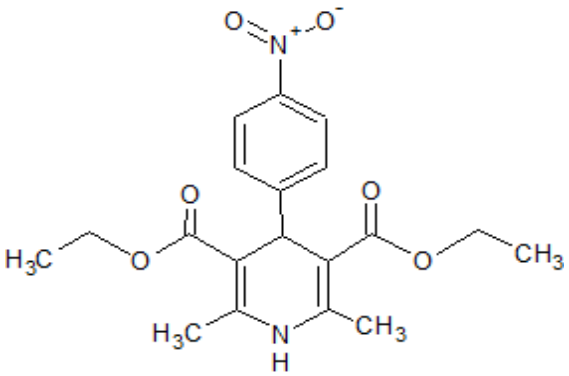
The present work has been carried out under the following sections.

Phase I - *In-silico*studies

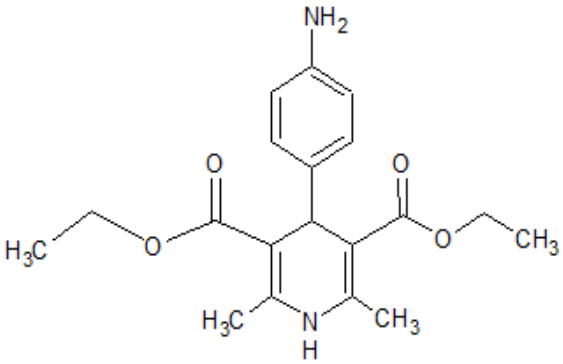
- Selection of Target & Lead Molecule by virtual screening.
- Lead Optimization.
- Docking of the Lead molecules.

Phase II - Synthesis

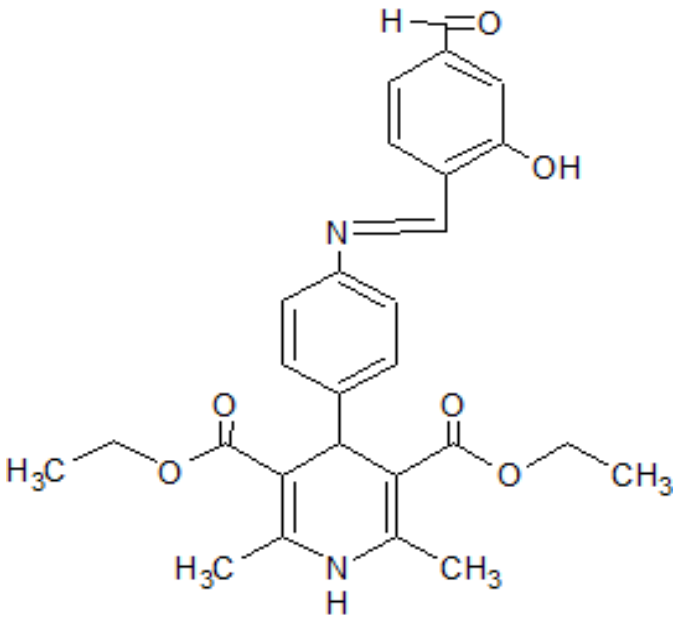
- **2,6-dimethyl-3,5-dicarbethoxy-4-(4-nitrophenyl)-1,4-dihydropyridine (2a)**



- **2,6-dimethyl-3,5-dicarbethoxy-4-(4-aminophenyl)-1,4-dihydropyridine (3a)**



- **Schiff base (4a)**



PHASE III - Biological studies

- Antibacterial Studies

- *Streptococcus pneumonia*e.
- *Staphylococcus aureus.*
- *Escherichia coli.*

CHAPTER - 5

EXPERIMENTAL WORKS

MATERIALS AND METHODS

PHASE I :*INSILICO* STUDIES

Software and databases used

- Zinc database
- Cygwin
- Mgltools 1.5.6
- Autodock tools 1.5.6
- Python 3.4.3
- Discovery studio visualizer
- Molinspiration server
- RCSB Protein data bank
- Online simile translator

Virtual Screening

- Identification of drug target.
- Selection of the lead.

From the virtual screening and literature review I have selected 2VEG.pdb, 1T2W.pdb, 3MZD.pdbas the targets for the present study. The enzyme have been selected from **RCSB Protein Data Bank** where the x-ray crystallographic structures were obtained and the docking studies were performed with the AutoDock 4.2version.

Various steps involved in docking are :

Step 1:SELECTION FROM PDB

- Stephylococcusaureus : PDB accession code: 1T2W.pdb
- Escherichia coli : PDB accession code: 3MZD.pdb
- Streptocoocus pneumonia : PDB accession code: 2VEG.pdb

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

Step 2: **PROTEIN STRUCTURE REFINEMENT**

Proteins(3MZD, 1T2W, 2 VEG) were downloaded from protein data bank as such cannot be used for docking process. It has to be refined before docking. Refinement of downloaded protein involves the removal of water and bound ligand if any.

The steps involved are :

1. Open Discovery studio viewer.
2. File → Open → Protein(downloaded fromPDB).
3. View → Hierarchy.
4. Click water molecule.
5. Ctrl + shift and click the last water molecule (select all the water molecule)
6. Give right click and cut.
7. Select ligand, which is unnecessary. Give right click and cut.
8. Save the molecule in our desiredarea.

The 3ZMD.pdb, 1T2W.pdb and 2VEG.pdb were refined by the above method.

Step 3: **LIGAND FILE FORMAT CONVERSION**

The ligands were drawn in Chemsketch.

1. Tools → Generate →SMILES notation [Simplified Molecular Input Line Entry System, which is a fileformat].
2. Save the SMILES in a worddocument.
3. Open the online smile translator – catus.nci.nih.gov/services/translate/
4. Upload theSMILES.
5. By choosing the required file format we can save the file. Here, we are saving it as pdb format inCygwin/usr/local/bin.

Online smile translator allows the user to convert SMILES format into PDB, MOL, SDF and smile text file format. Thus the selected ligand molecule of canonical smile formats was converted to pdb formats. The protein and ligand files which are prepared by above said procedures were taken fordocking.

Step 4: **DOCKING**

Docking was performed using AutoDock and requires a refined protein and the ligand in PDB format and files like autogrid4 andautodock4.

Docking process is done with AutoDock 4.2 Steps involved are :

- Conversion of refined enzyme into .pdbformat.
- Conversion of pdb format of ligand into .pdbqtformat.
- Preparation of grid box by setting grid parameters at 60, 60 &60.
- Docking process by setting dockingparameters.
- Saving the docked result as .dlgfile.
- Viewing the docked conformation.
- Taking snapshots of the interactions.
- Docking studies for all the ligands were carried out in the samemanner.

RESULTS AND DISCUSSION

The docking results of 3MZD, 1T2W, 2 VEG with the ligands are reported below. The binding sites and the active sites are represented in the snap shots.

The results have been tabulated in the Table followedby the snapshots.

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

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

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

2a interacts with *E.coli* at Leu 167, Arg 174, Gly 28, Lys 294 and NAD^+. The binding energy was found to be -5.1 kcal/mol.

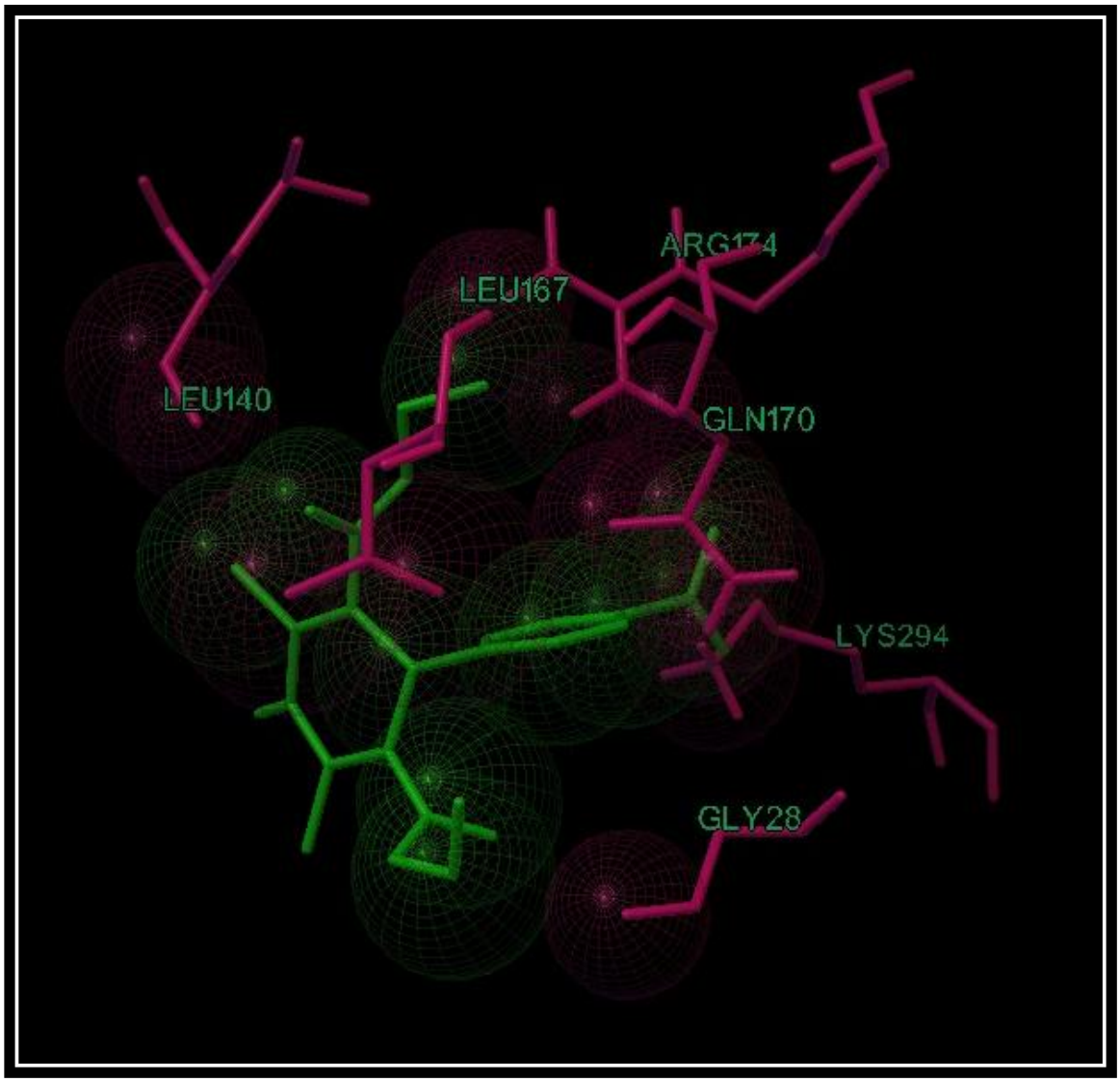


Fig.5: Snap shot of 2a with *E.coli*(3ZMD.pdb)

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

2a interacts with*S.pneumania*at Glu 55, Asn 17, Arg 282, Phe 202, Phe 154 and NAD^+. The binding energy was found to -5.57 kcal/mol.

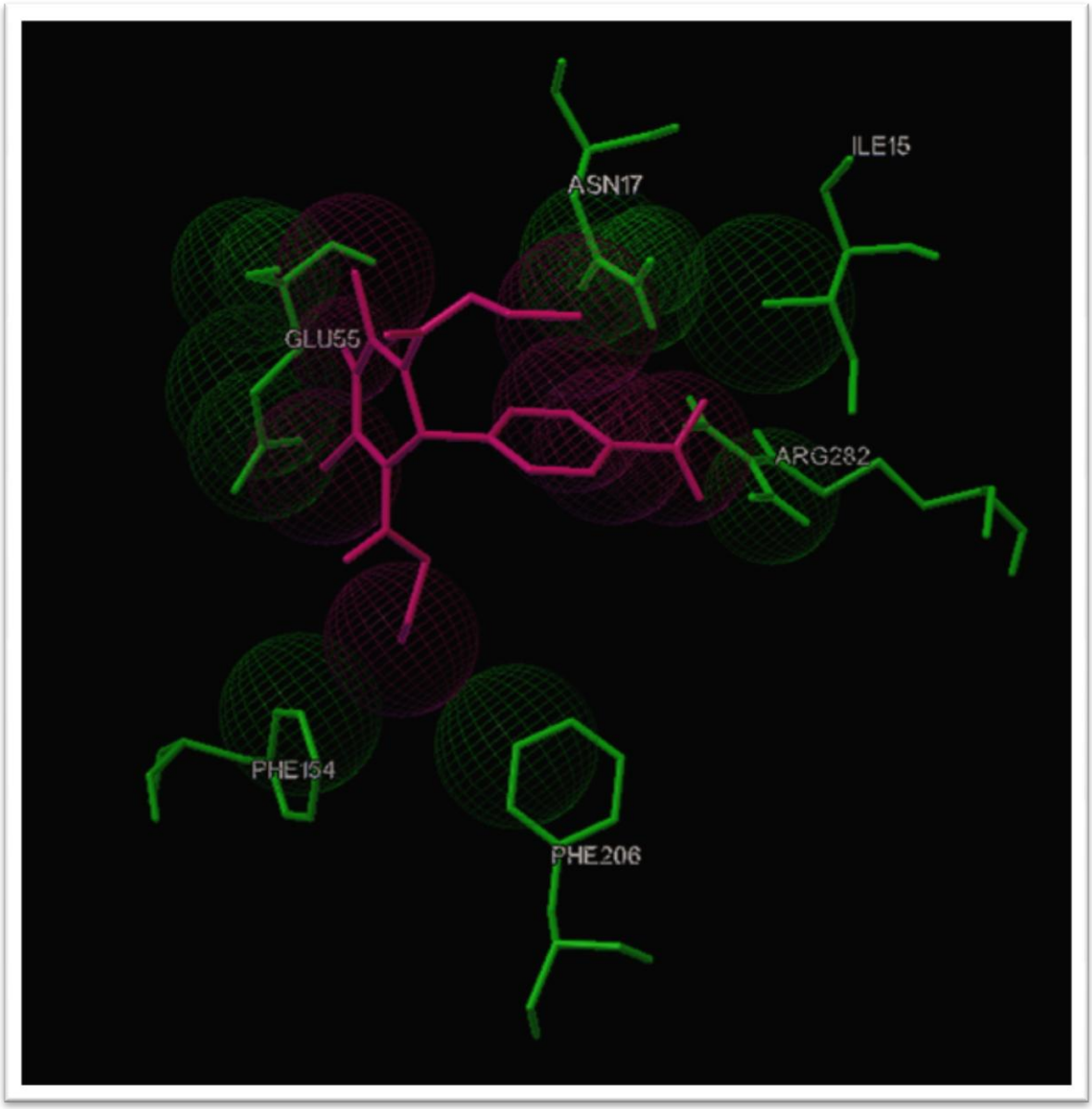


Fig.6: Snap shot of 2a with *S.pneumania***(**2VEG.pdb)

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

2a interacts with *S.aureus* at Ala 184, Ile 182, Arg 197, Ala 92, Glu 105 and NAD^+. The binding energy was found to be -4.61 kcal/mol.

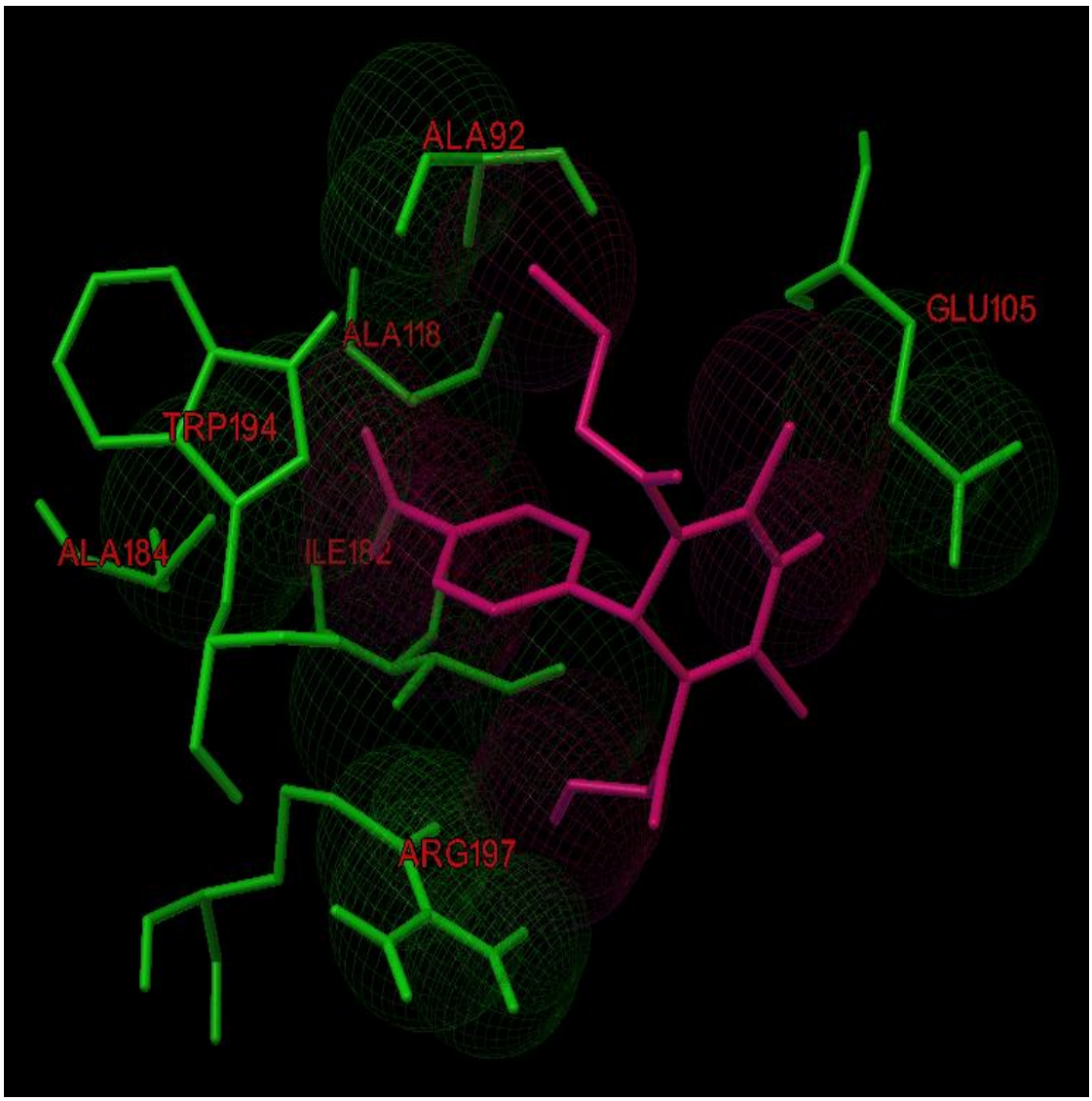


Fig.7: Snap shot of 2a with *S.aureus*(1T2W.pdb)

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

3a interacts with *E.coli* at Gly 28, Arg 174, Leu 167, Leu 140, Gln 170 and NAD^+. The binding energy was found to be -4.52 kcal/mol.

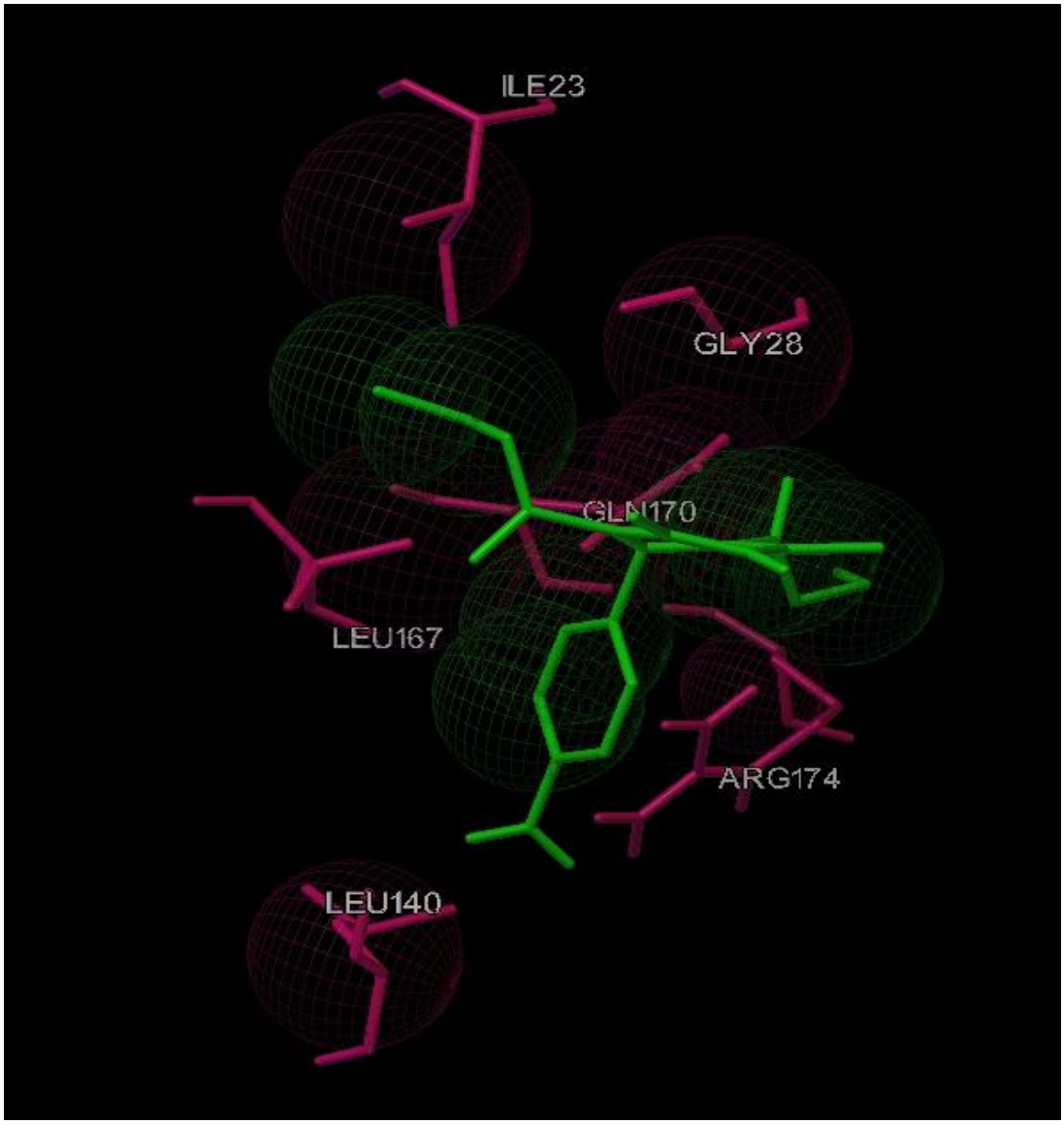


Fig.8: Snap shot of 3a with *E.coli*(3ZMD.pdb)

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

3a interacts with *S.pneumania* at Ala 118, Ala 92, Glu 105, Ser 116, Ile 182 and NAD^+. The binding energy was found to be -4.26 kcal/mol.

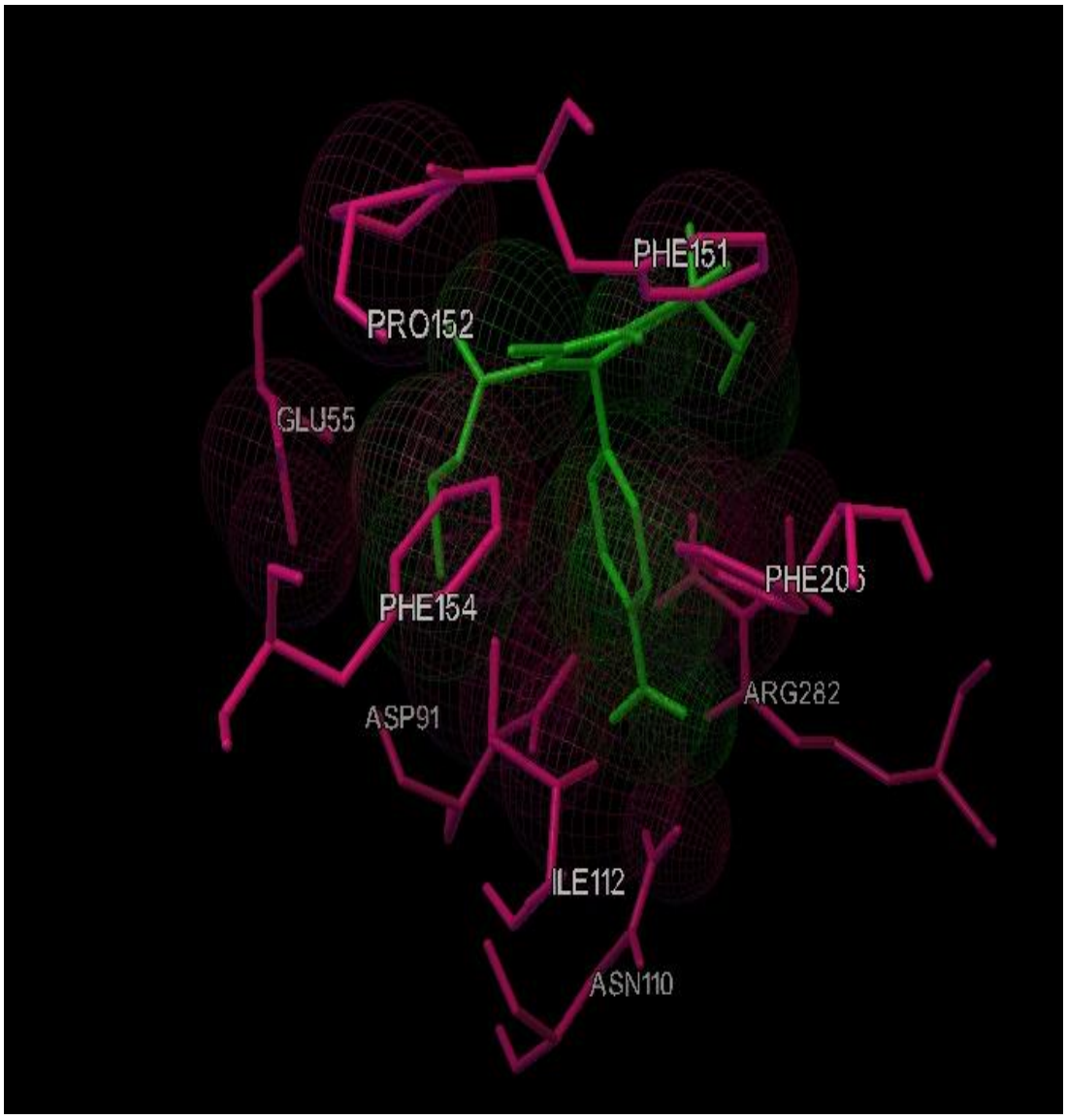


Fig.9: Snap shot of 3a with*S.pneumania*(2VEG.pdb)

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

3a interacts with *S.aureus* at Ala 118, Ala 92, Glu 105, Ser 116, Ile 182 and NAD^+. The binding energy was found to be -4.71 kcal/mol.

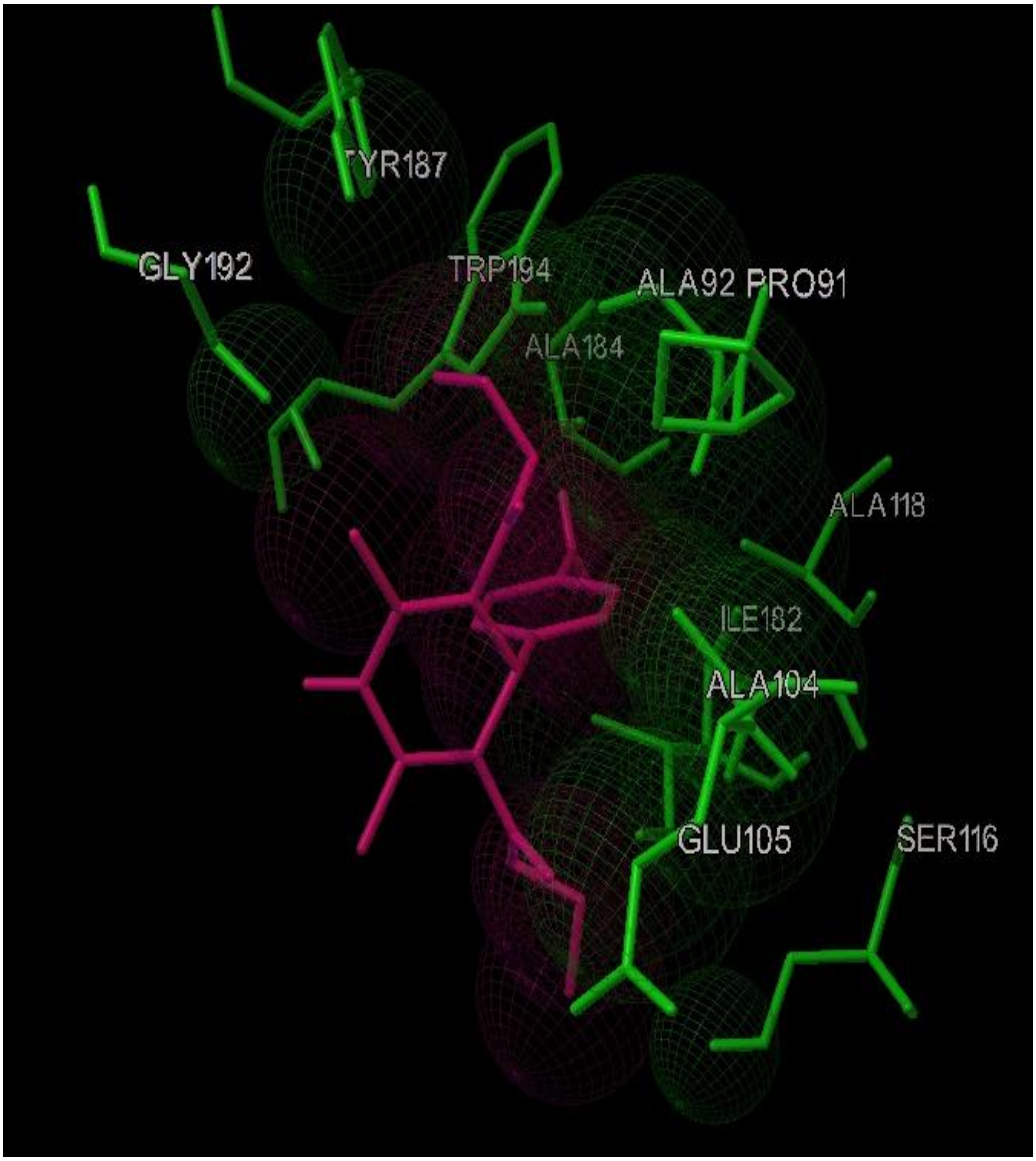


Fig.10: Snap shot of 3a with *S.aureus*(1T2W.pdb)

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

4a interacts with *S.pneumania*at Gly 205, Asp 91, Glu 55, Lys 237 and NAD^+. The binding energy was found to be -5.93 kcal/mol.

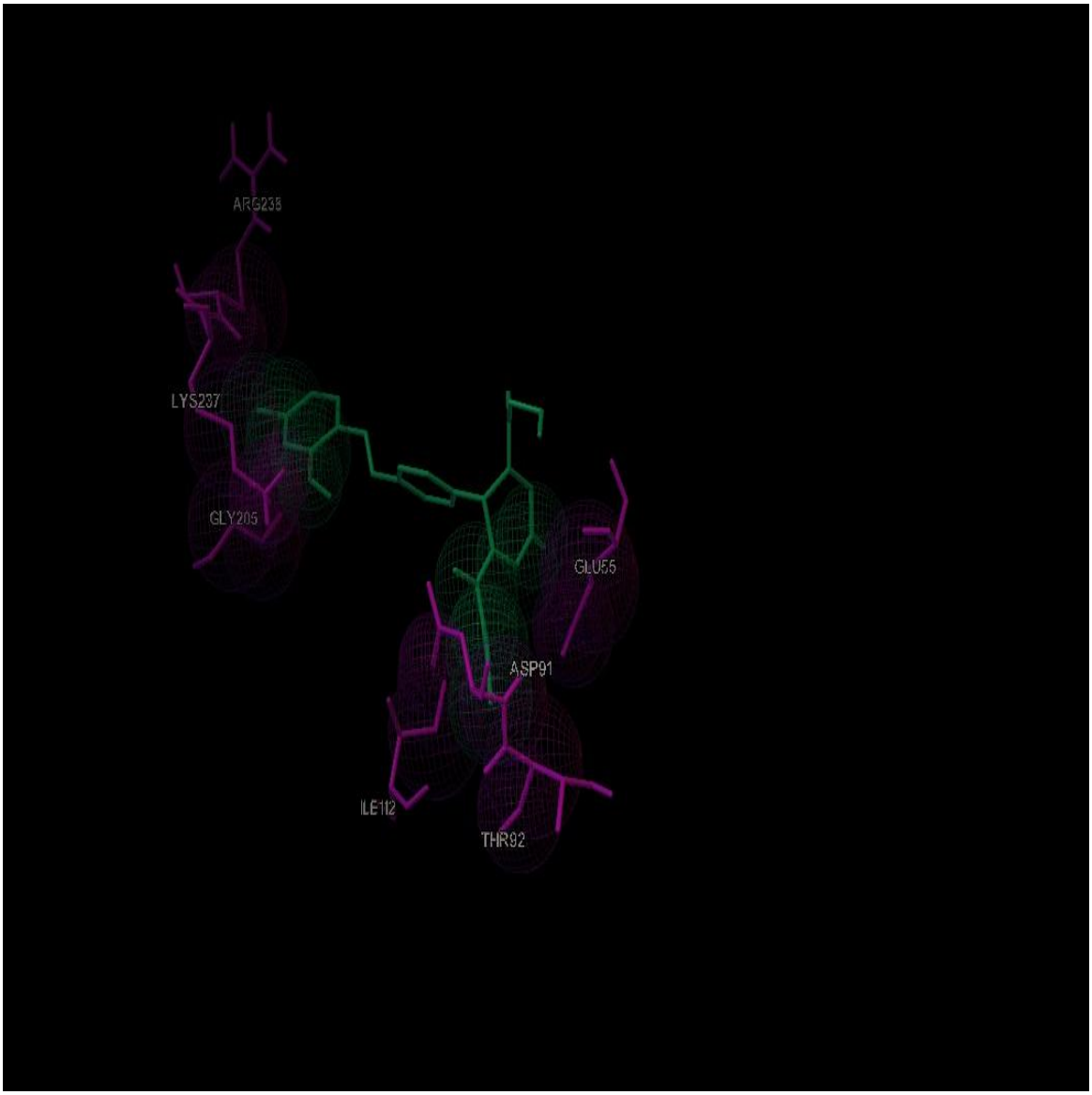


Fig.11: Snap shot of 4a with *S.pneumoniae* (2VEG.pdb)

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

4a interacts with *S.aureus*at Arg 197, Asp 91, Glu105, Ala104, Asn 114and Ala 134 NAD^+. The binding energy was found to be -6.49 kcal/mol.

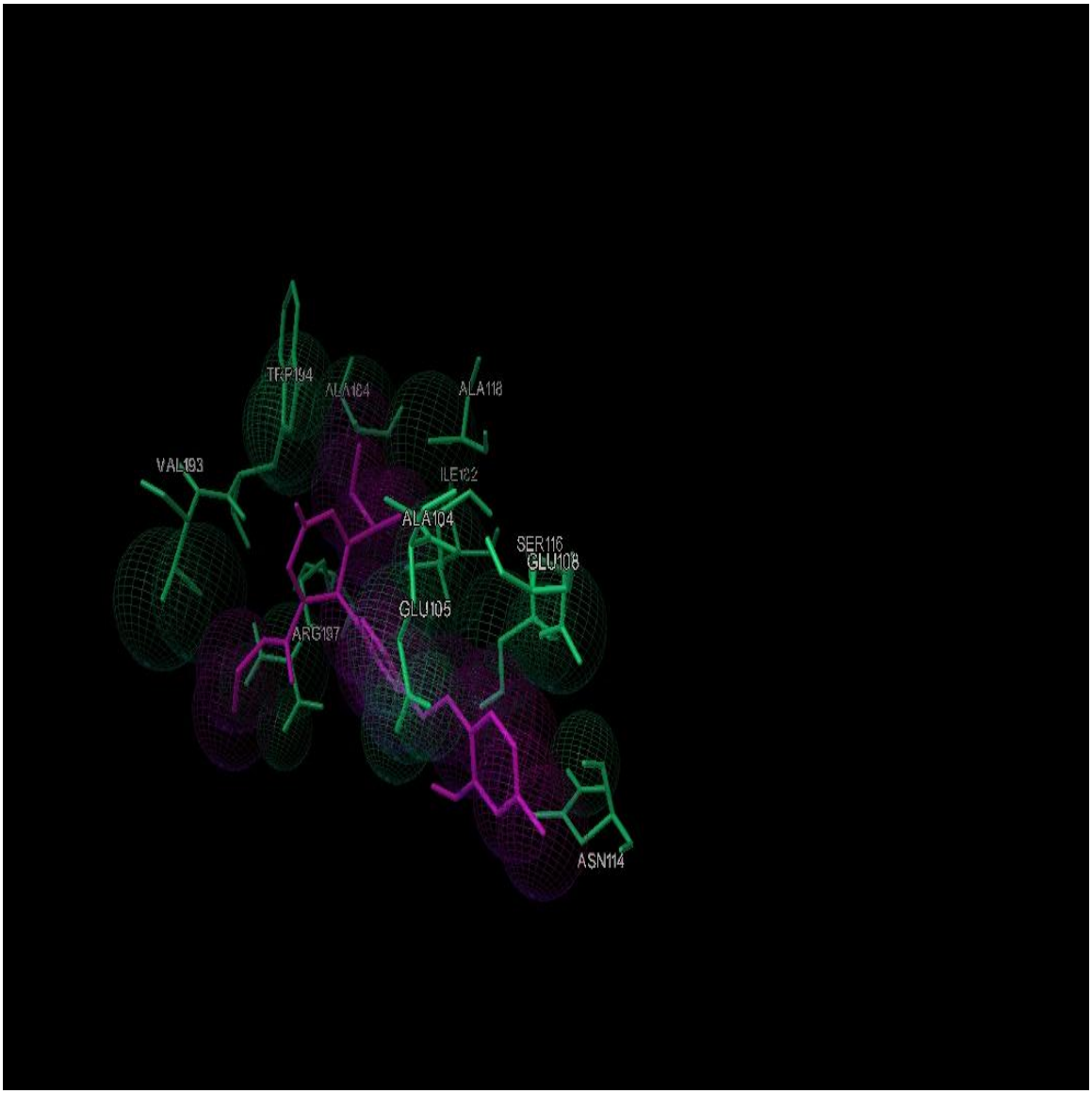


Fig.12: Snap shot of 4a with *S.aureus* (1T2W.pdb)

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

4a interacts with *S.aureus*at Gln56, Asp 175, Arg174, Gln170, Leu 140, Gly 141 and Leu 140NAD^+. The binding energy was found to be -5.22 kcal/mol.

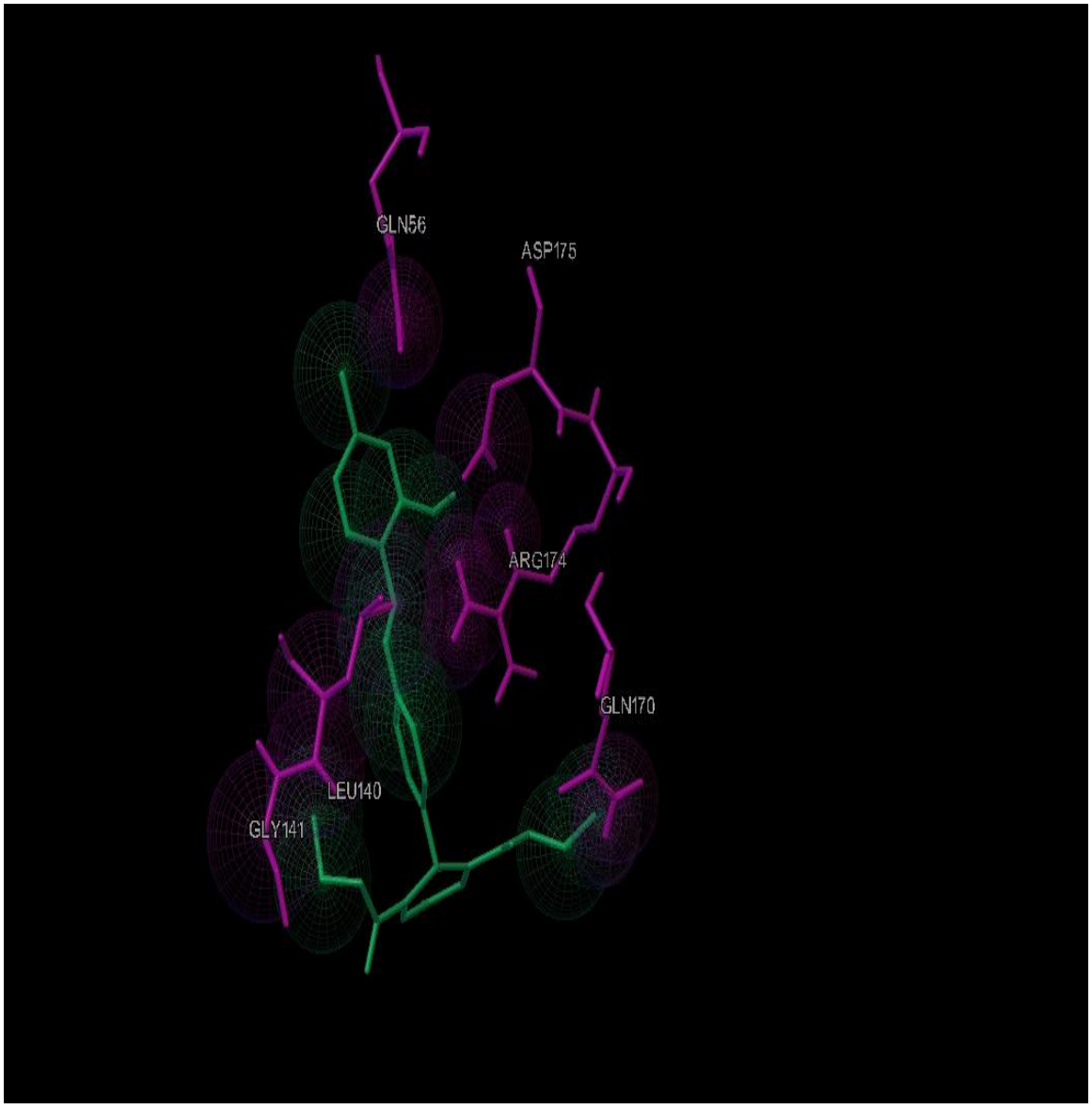


Fig.13: Snap shot of 4a with *E.coli* (3ZMD.pdb)

5.1.**PHASE II : SYNTHESIS**

CHEMICALS AND REAGENTS USED

4- nitrobenzaldehyde, ethyl acetoacetate, ammonium acetate, beta-cyclodextrin, ethylacetate,stannous chloride, sodium hydroxide, 1N HCl, hexane, chloroform, DNPH, Ninhydrine reagent.

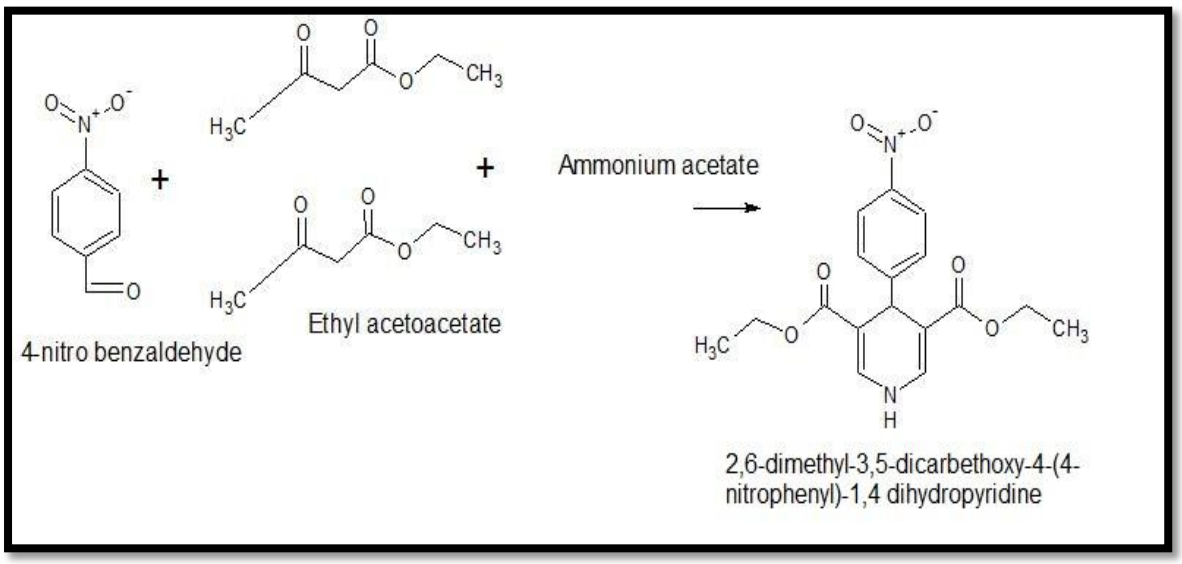
All the chemicals and reagents were procured from Sisco Research Laboratories Pvt. Ltd. All the compounds procured were AR graded.

Analytical work

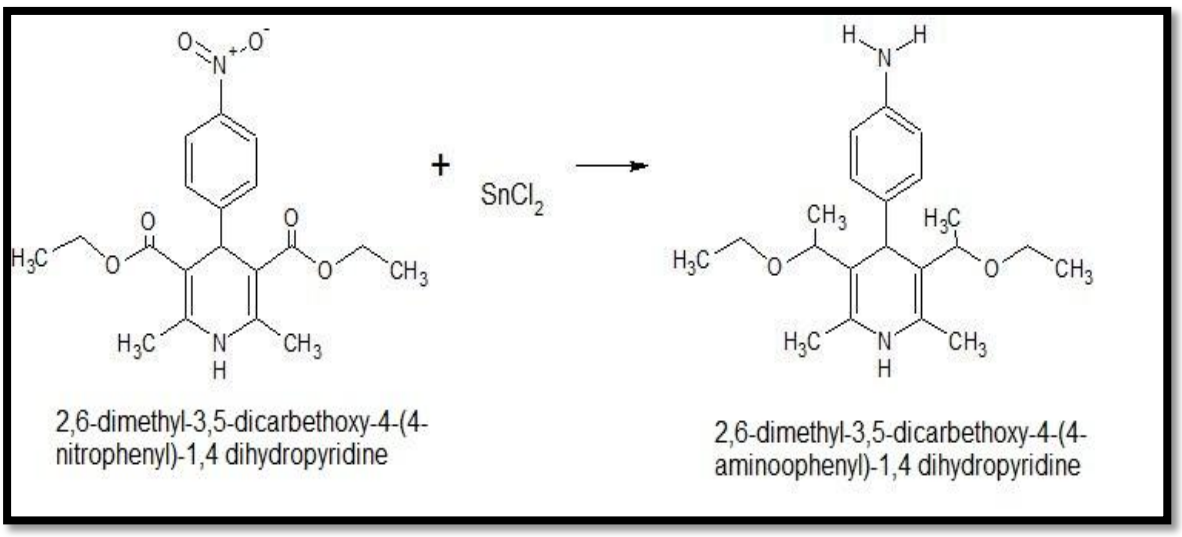
- Melting point were determined by using melting point apparatus MR-VIS, visual melting range apparatus and corrected.
- Reactions were monitored by thin layer chromatography (TLC) on TLC plates using DNP and Ninhydrine reagents as visualizing agent.
- IR spectra on JASCO FTIR-420 in the Department of Pharmaceutical AnalysisNandha College of Pharmacy, Erode,.
- NMR were recorded on the Bruker Ultra Shielded NMR-300MHz.
- MASS spectra were recorded on JEOL GC Mate GC-MS Spectroscopy.

SCHEME

STEP-1

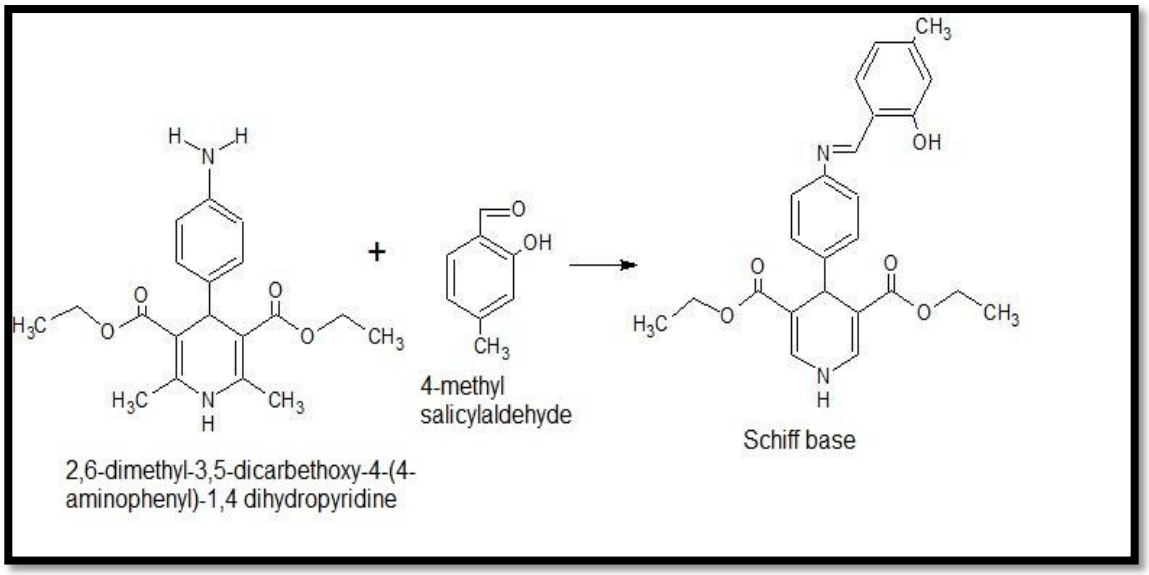


STEP-2



STEP-3

(Schiff base)



Procedure

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

A mixture of 4-nitrobenzaldehyde (3g) [1eq], ethylacetoacetate (5.1g) [2eq], ammonium acetate (1.53g) [1eq] and in presence of β-cyclodextrin (2.25g) [10%wt] was taken in a 100mL multineck round bottom flask, mixed well and stirred on magnetic stirrer at 80°C for 8hrs. 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 ethylacetate and the formation of nitro group was identified by dinitrophenylhydrazine 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 (1.6g), methanol (3ml) was taken in 100mL pyrex beaker, mixed well and stirred on magnetic stirrer for 30min. After completion of the reaction, the mixture was neutralized by 40% sodium hydroxide solution andmonitored by TLC. The formed amino derivative was further identified by ninhydrine 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)

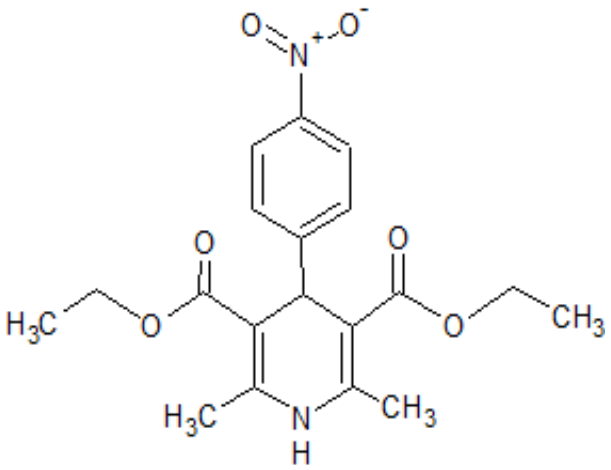
A mixture of 2,6-dimethyl-3,5-dicarboethoxy-4-(4-aminophenyl)-1,4-dihydropyridine (0.50g), glacial acetic acid (2.6g), 4-methyl salicylaldehydealdehyde(2gm) and water was taken in 100mL pyrex beaker, mixed well and stirred on magnetic stirrer for 30min. After completion of the reaction, the mixture was monitored by TLC. The mixtrure was filtered andthe filtrate was concentrated under reduced pressure to obtain crude product.

Recrystallisation solvent : Ethanol

Solvent system : n-Hexane : Ethyl acetate (8:2)

SPECTRAL CHARACTERIZATION OF COMPOUND

The structures of synthesized compounds were established on the basis of the IR, NMR, and Mass spectral data.



Chemical name :2,6-dimethyl-3,5-dicarbethoxy-4-(4-nitrophenyl)-1,4 dihydropyridine.

Yield : 57%

Melting point : 125°C

IR (KBr, cm^{-1}) : 3318 (N-H str), 2980(Ar-H), 2979 (C-H str of CH_3), 1710 (C=O,ester), 1636 (C-NO_2), 882 (Ar-H).

^{1}H-NMR data : (300 MHz, DMSO-d6, δ / ppm): 8.13–7.44 (4H, m, Ph--ring), 8.14 (1H, s, NH of pyridine ring), 4.78 (1H, s, C4–H), 4.28 (4H, q, C3 –OCH2CH3 and C5 –OCH2CH3), 2.33 (6H, s, C2–CH3 and C6–CH3), 1.31 (6H, t, C2–OCH2CH3 and C6–OCH2CH3).

^{13}C-NMR (300 MHz, DMSO-d_6, δ / / ppm):	144.8, 123.6, 126.9, 152.0 (Ph–NO2), 153.2 (C2,6), 44.9 (C4), 103.2 (3,5-COOCH2CH3), 61.8 (3,5-COOCH2CH3), 14.5 (3,5-COOCH2CH3), 18.9 (2,6-CH3).
MS (m/z (relative abundance, %)):	372.22 (M^++1, 21.2), 281.26, 185.26, 171.23, 138.21, 330.39 (M++1, 38.9), 285.33, 241.28, 185.26, 171.23, 157.21, 81.11, 68.11.

^{1}H-NMR SPECTRAL ANALYSIS:

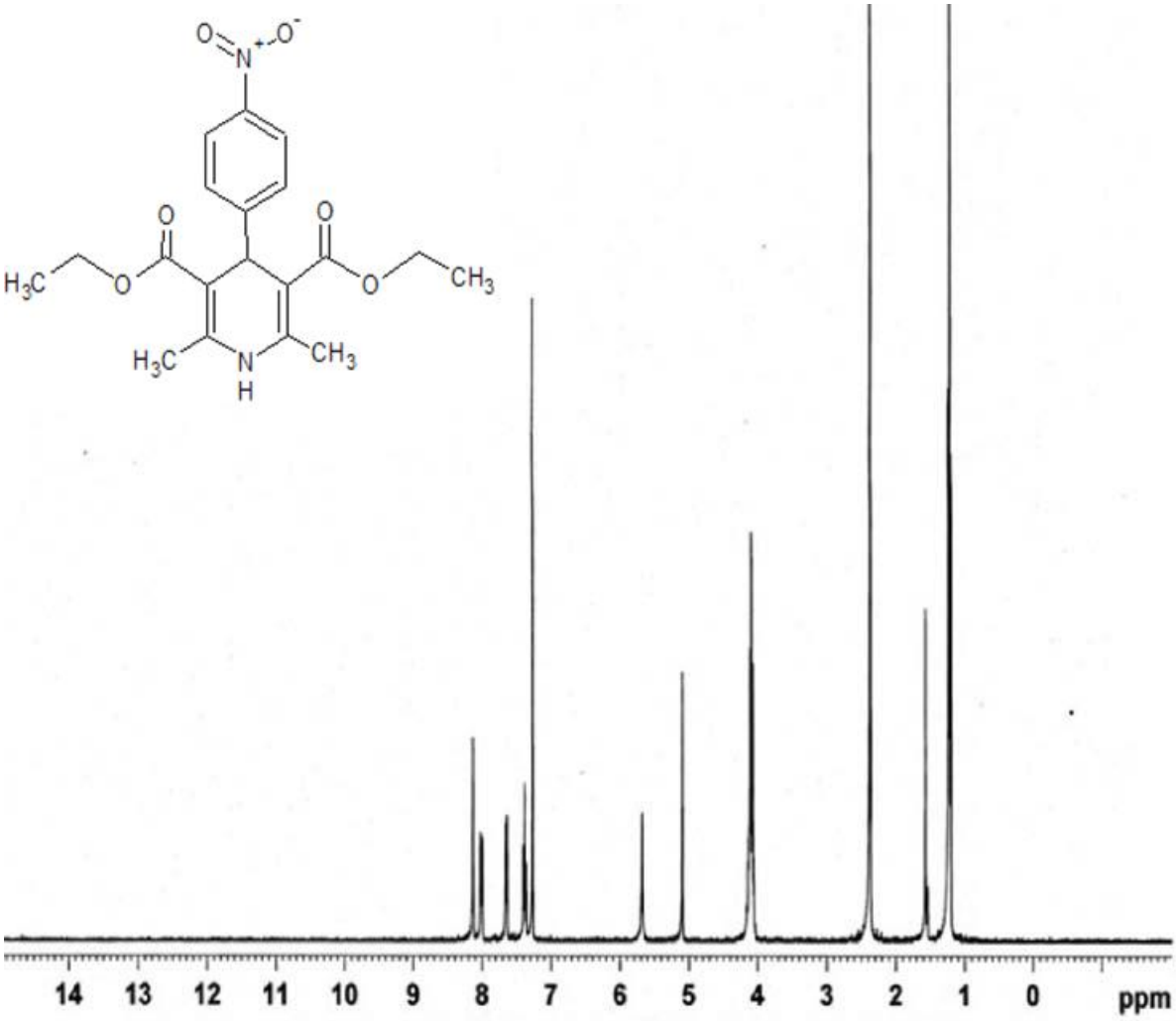


Fig.14:^{1}H-NMR spectrum of 2,6-dimethyl-3,5-dicarbethoxy-4-(4-nitrophenyl)-1,4 dihydropyridine

^{13}C-NMRSPECTRAL ANALYSIS:

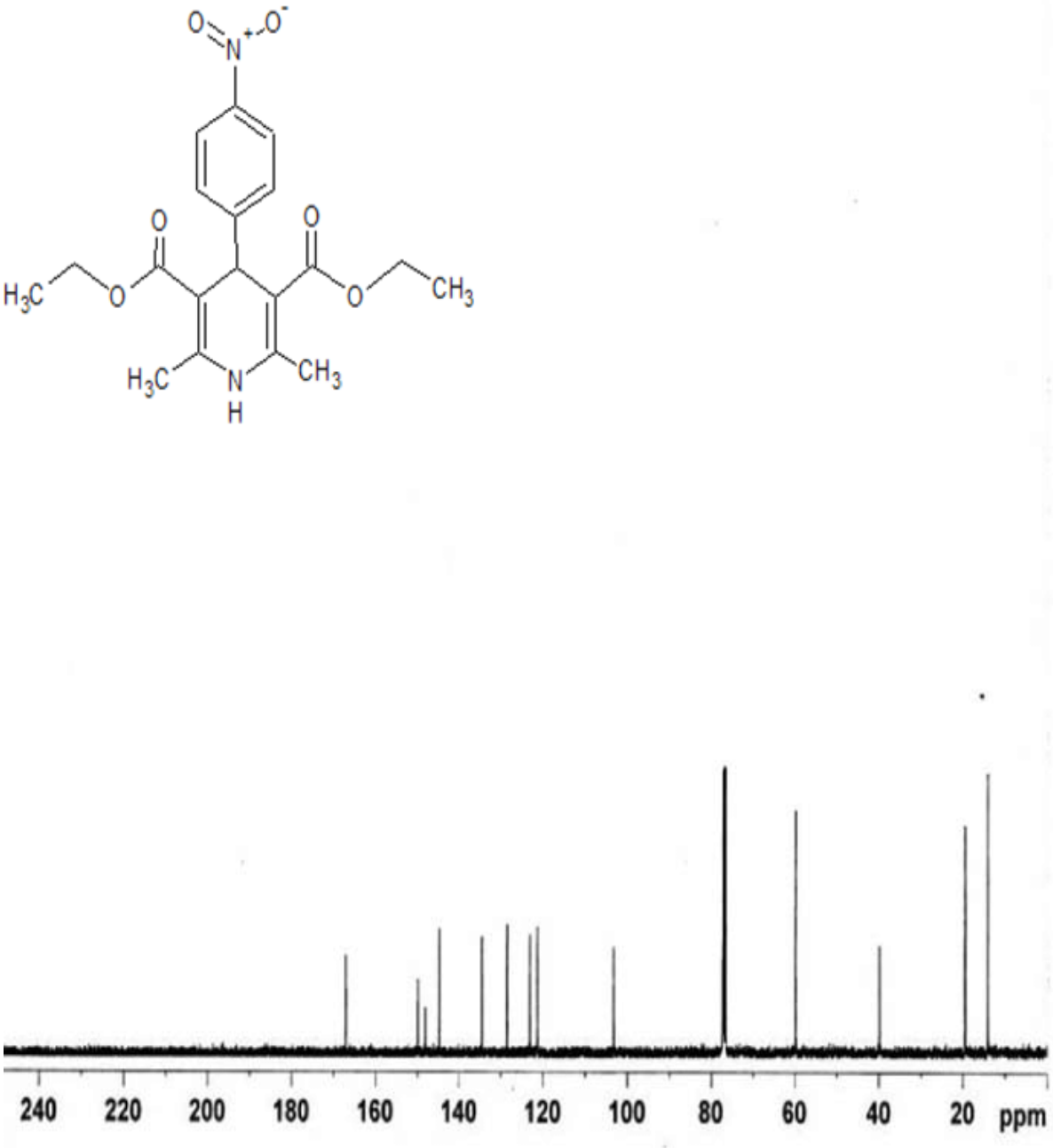


Fig.15: ^{13}C-NMR spectrum of 2,6-dimethyl-3,5-dicarbethoxy-4-(4-nitrophenyl)-1,4 dihydropyridine.

IR SPECTRAL ANALYSIS

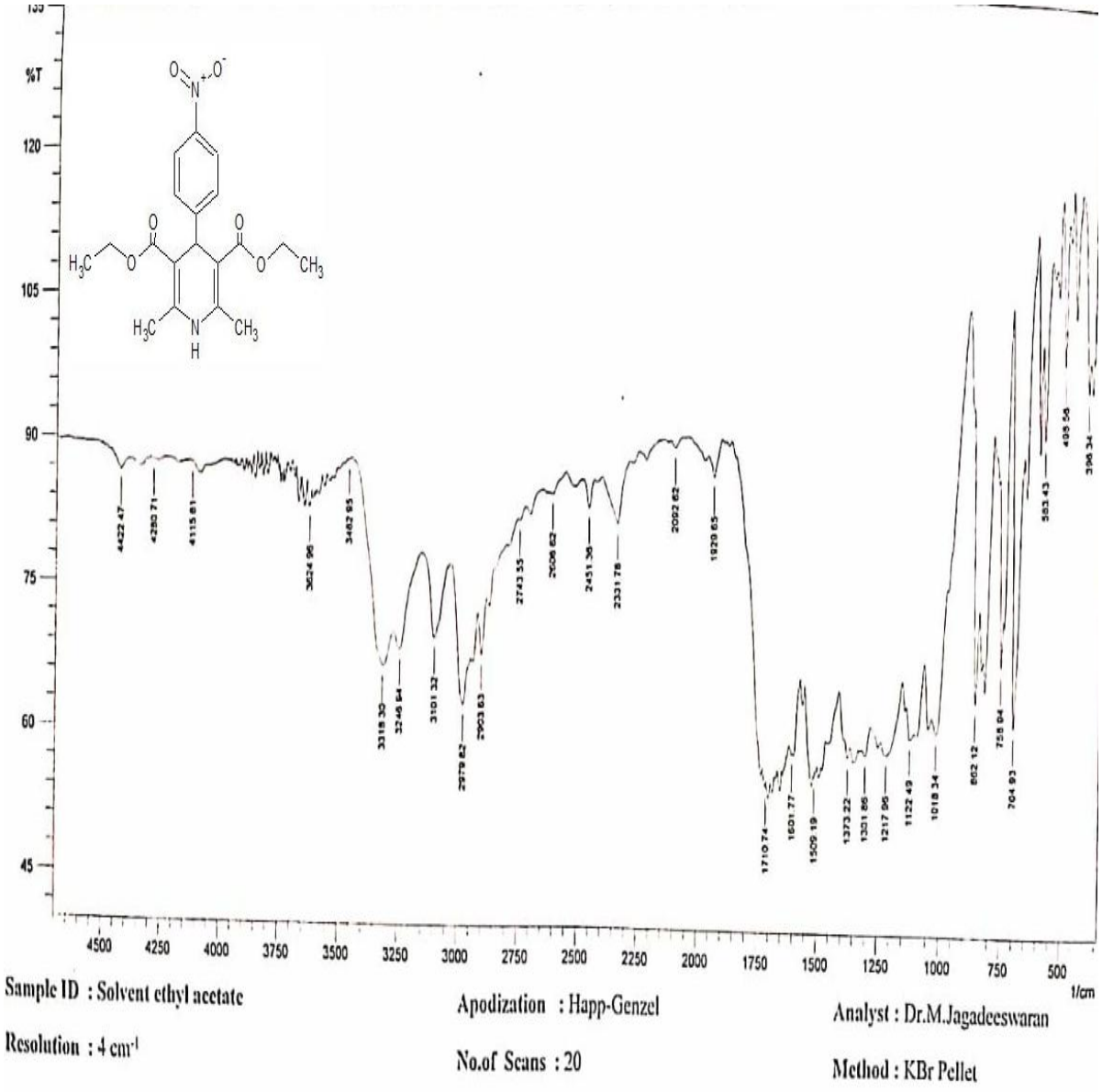
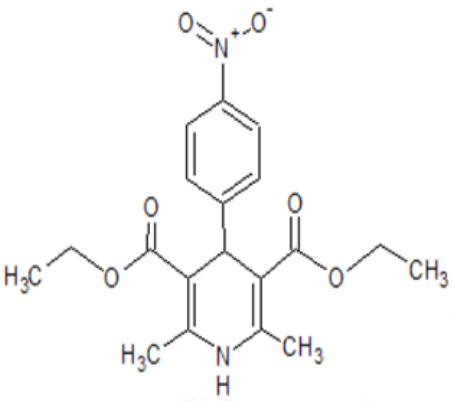


Fig.16 :IR spectral analysis of2,6-dimethyl-3,5-dicarbethoxy-4-(4-nitrophenyl)-1,4 dihydropyridine.

MASS SPECTRAL ANALYSIS



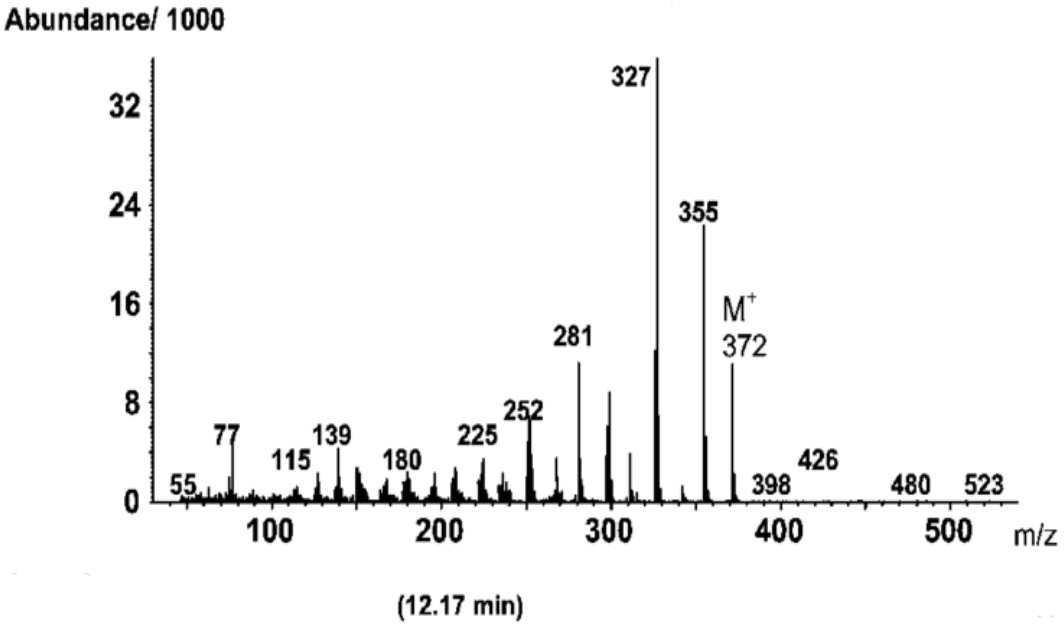


Fig.17 : Mass spectrum of 2,6-dimethyl-3,5-dicarbethoxy-4-(4-nitrophenyl)-1,4 dihydropyridine

5.2. PHASE III – BIOLOGICAL SCREENING

INVITRO ANTIBACTERIAL SCREENING

Mueller Hinton agar plates were prepared aseptically to get a thickness of 5-6mm. The plates were allowed to solidify and inverted to prevent condensate falling on the agar surface. The plates were dried at 37°C before inoculation. The organisms were inoculated as per the following method in the plates prepared earlier. The sterile swab was dipped in the previously standardized inoculums and excess of inoculums was removed by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid.

The swab was then streaked all over the surface of the medium three times, rotating the plates through an angle of 60^{o} after each application. Finally, the swab was pressed round the edge of the agar surface. The inoculated medium was allowed to dry at room temperature, with the lid closed. Cork borer was sterilized by using flame and well was made by using 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. This was then incubated for 18-24 hrs at 37°C.

The diameter of the zones of inhibition around the drugs were measured and compared with that of the standard. All the synthesized compounds were tested for antibacterial activity against *Streptococcus pneumoniae*, *Staphylococcus aureus and Escherichia coli* bacteria.The results were interpreted in terms diameter (mm) of zone of inhibition.

The bacterial zones of inhibition values (mm) are given in Table 2. Ciprofloxacin was used as a standard at 100 µg ml^{-1}.

- The Compounds were screened for *Streptococcus pnemoniae*.

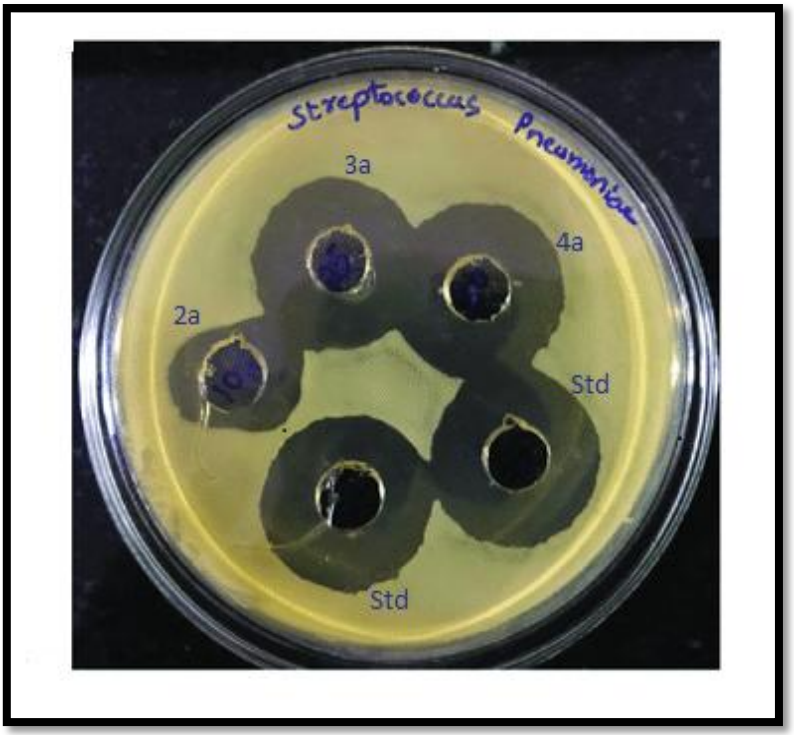


Fig.18: Antibacterial screening of *Streptococcuspneumoniae*.

Compound	Diameter of zone of inhibition in mm
2a	6
3a	10
4a	10
Ciprofloxacin	14

Table 3: Antibacterial data for*Streptococcus pneumoniae*.

- The Compounds were screened for *Staphylococcus aureus*.

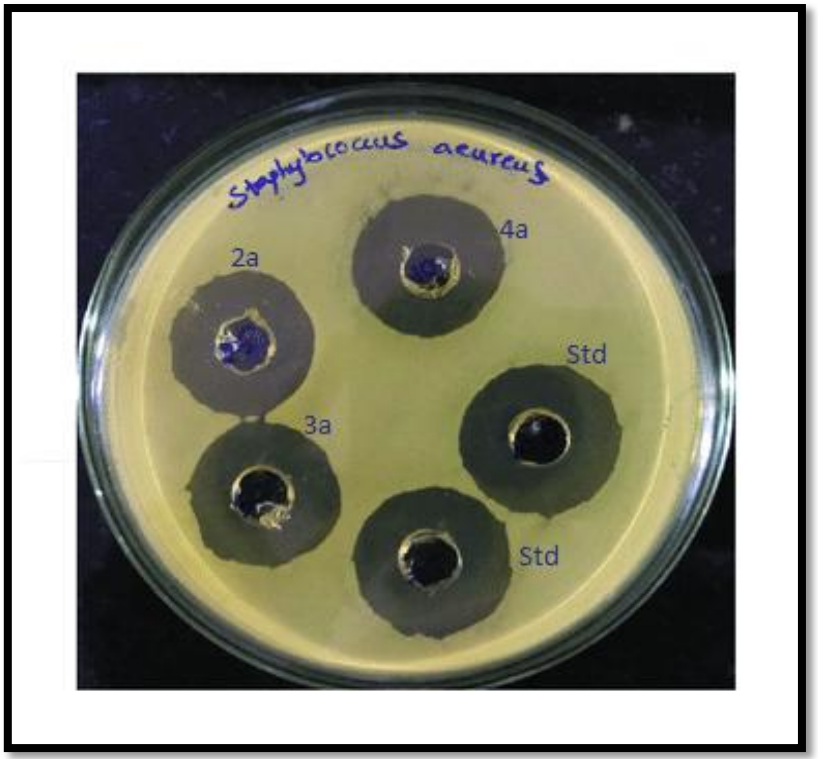


Fig.19: Antibacterial screening of *Staphylococcus aureus*

Compound	Diameter of zone of inhibition in mm
2a	7
3a	7
4a	8
Ciprofloxacin	10

Table 4 : Antibacterial data for *Staphylococcus aureus*

- The Compounds were screened for *E.Coli*.

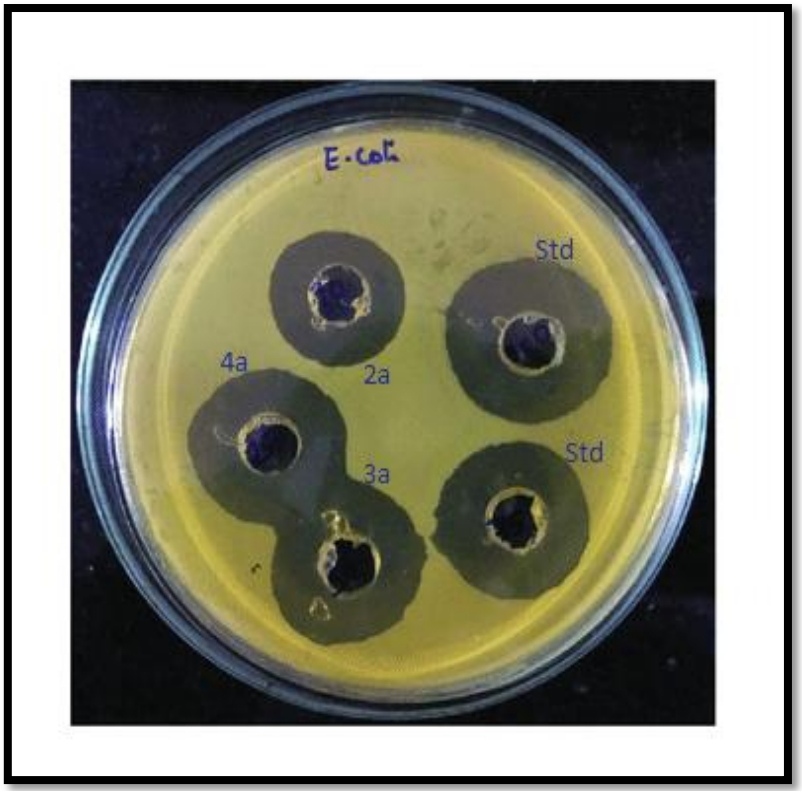


Fig.20 : Antibacterial screening of *E.Coli*

Compound	Diameter of zone of inhibition in mm
2a	6
3a	7
4a	7
Ciprofloxacin	10

Table 5 : Antibacterial data for *E.Coli*

CHAPTER - 6

RESULTS AND DISCUSSION

The present work was focused on the design, docking, synthesis and evaluation of antibacterial activities of 1,4- dihydropyridineseries as possible DNA gyraseinhibitors.

Phase I - *In-silico* studies

- **Selection of the target**

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

- **Selection of lead by virtual screening**

From the virtual screening 2VEG.pdb, 1T2W.pdb, 3MZD.pdbare selectedas the targets for the present study. The enzyme have been selected from **RCSB Protein Data Bank** where the x-ray crystallographic structures were obtained and the docking studies were performed with the AutoDock 4.2version.

- **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 optimization revealed that 4a derivative possess good drug likeness score than 2a and 3a derivatives.

- **Docking**

The optimized leads were subjected to docking studies using Autodock4.2 and the interactions of the derivatives with active sites of the enzymes werestudied. The derivatives were subjected to interactions with 3MZD (*E.Coli)*, 1T2W (*Staphylococcus aureus*) and 2VEG (*Streptococcus pneumonia)*

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, ethylacetoacetate, ammonium acetate with β-cyclodextrincatalyst form nitro derivative with good yield which is subjected to furtherderivative.

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, stannous chloride and methanol form amino derivative with good yield 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 salicylaldehydewithwater form Schiff base with good yield.

Characterization

Melting point of all newly synthesised compounds were determined. Rfvalues were determined by fixing various suitable solvent system on TLC plates. The solvent system used was ethyl n-Hexane : Ethyl acetate (8:2). The structure was finally characterized by IR, Mass, 1H and 13C NMR spectra.

Phase-III - Biological activity

Antibacterial activity

Antibacterial activity was performed by agar well diffusion method by using Streptococcus pneumonia, Staphylococcus aureus and Escherichia coli. All the derivatives of three different series were screened for antibacterial activity. Among 1,4dihydropyridine 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 inhibition of DNA gyrase enzyme.

CHAPTER 7

CONCLUSION

- The present study establishes that computational tools help in minimizing the tedious process of drug discovery and delivers new drug candidate more quickly.
- Virtual screening was utilized for filtering the compounds and selecting the lead compounds.
- The drug likeness score established the compounds to be pharmacokinetically active.
- The binding energy obtained from docking study further confirmed the possibility of the affinity of the selected leads towards the enzyme,DNA gyrasefrom *Streptococcus pnemoniae, Staphylococcus aureus*and *Escherichia coli*.
- Using the schemes various 1,4dihydropyridines were synthesized with good yield.
- Structure of the synthesized compoundis confirmed by Melting point, TLC, NMR and MASS spectra.
- The compounds were screened for antibacterial activity.

CHAPTER 8

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