SAM DEPENDENT CobA PROTEIN AS A DRUG TARGET: COMPUTATIONAL APPROACHES

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LIST OF ABBREVIATIONS

AA	Amino Acid
ABC	ATP Binding Cassette
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
AMEs	Aminoglycoside Modifying Enzymes
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
ATP	Adenosine Triphosphate
BBB	Blood-Brain Barrier
BE	Binding Energy
CATs	ChloramphenicolAcetylTransferases
CDC	Centers for Disease Control and Prevention
CLEVER	Chemical Library Editing, Visualizing and Enumerating Resource
DNA	Deoxyribonucleic Acid
FBA	Flux Balance Analysis
FDA	Food and Drug Administration
FMN	Flavin Mononucleotide
FTIR	Fourier Transform Infrared Spectroscopy
HGT	Horizontal Gene Transfer
HTS	High Throughput Sequencing
LBVS	Ligand-Based Virtual Screening
MD	Molecular Dynamics

MDR	Multi-Drug Resistance
MFS	Major Facilitator Superfamily
NCBI	National Center for Biotechnology Information
PBP	Penicillin Binding protein
PDB	Protein Data Bank
PG	Peptidoglycan
PSA	Polar Surface Area
RBS	Ribosome Binding Site
RNA	Ribonucleic acid
RND	Resistance-Nodulation-Division family
SAM	S-Adenosyl Methionine
SBVS	Structure Based Virtual Screening
SMR	Small Multidrug Resistance
Tox	Toxicity
TPP	Thiamine Pyrophosphate
tPSA	Topological Polar Surface Area
VS	Virtual Screening
WHO	World Health Organization

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ABSTRACT

Emergence of multidrug resistant (MDR) has created a global public health risk. This rise of antimicrobial resistance among human pathogenic bacteria indicates the possible apocalypse in near future. This disastrous situation demands to be addressed and novel antibiotics need to be sought specially against the resistant strain recognized by World Health Organization. The present situation of ineffectiveness of antibiotics in practice because of rapid development of resistant by the pathogenic bacteria demands targeting essential proteins and early introduction of novel therapeutics. Computer aided drug discovery(CADD) has become a prominent and played a major role in the drug discovery for over three decades and has been established as successful methods in screening of the lead compounds against target protein within minimal time frame and with optimum resources. Computational approaches have been used in this study to identify putative drug candidates against the essential Uroporphyrinogen III methyltransferase (CobA) protein of *Psuodomonas*. Virtual screening of the different ligand libraries consisting of 205 natural products, 462 indole derivatives, 6449 kinase inhibitors and 654 nucleoside mimetics obtained from ADME/Tox and drug likeliness filter identified four lead candidates each from individual library. Ajmaline, 3-(4-fluoro phenyl)-3-(1H-indol-3-yl)-N-(2-morpholino ethyl) propanamide, 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one and 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol were identified as novel therapeutics from the natural products, indole derivatives, kinase inhibitors and nucleoside mimetics respectively. These compounds clearly showed the higher binding affinity than SAM in a protein-ligand complex. Also these compounds showed lower binding efficiency for human hMAT1A protein and larger binding energy for SAM utilizing target protein CobA. Protein ligand interactions from PyMOL, LiGPLOT^{+} and residual interaction map obtained using Discovery Studio showed strong bonds like π-alkyl bonding in all identified compounds indicating the stability of protein-ligand binding. Further DFT (Density Functional Theory) analysis of frontier molecular orbital studies, calculated parameters and vibrational spectrum of infrared region of studied compounds Ajmaline, 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide, 1-[6-(dimethylamino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol favoured the molecular docking results at PyRx interface. Thus results indicate that these drug candidates could potentially be used to develop putative drugs against CobA protein of pathogenic bacteria. Also these findings would help in exploring probable therapeutics against other SAM utilizing proteins as well.

Keywords: CADD, essential gene, Multidrug resistance, SAM, binding energy

1. INTRODUCTION

1.1 Background

Despite the fastest growing technologies that have revolutionized the medical field in many ways, the antimicrobial resistance (AMR) has emerged as one of the most serious global public threats in this century. About 700,000 deaths have been estimated around the globe due to AMR annually (WHO, 2014). However, the production rate of antibiotics and new drugs is far behind. The rate at which (MDR) multi-drug resistance (microorganism showing resistance to two or more categories of antibiotics) has been emerging indicates the possibility of post-antibiotic era in near future. The pathogenic microorganisms like *Escherechia, Pseudomonas, Klebsiella, Staphylococcus*, and others have become resistant to most of the existing drugs including even the fourth line of drugs (Mulvey and Simor, 2009). Antibiotics are one of the most successful therapies the medicine field has for treating bacterial infections. This also reflects the worth and need of the antibiotics for cure and treatment of different diseases of bacterial origins and conditions (Zhu *et. al*., 2013). Misuse of antibiotics, random mutations, horizontal gene transfers and are some factors that has fuelled the rapid emergence of AMR in recent years (Vargiu *et al*; 2016, Strachan and Davies, 2017, Jiang *et. al*; 2017). In this context the world needs the novel approaches in order to identify antimicrobials and act specifically.

The emergence of the carbapenem and/or colistin resistant pathogenic strains (Caniaux *et. al.,* 2017) is of great concern and WHO suggested for new drugs against the pathogens. WHO published global list of Critical and High priority resistant pathogens (Tacconell *et. al.,* 2018). However, the antibiotics market has witnessed only two noble classes of antibiotics, the oxazolidinones (Barbachyn and Ford, 2003) and cyclic lipopeptides (Kern, 2006) since 2000. This alarming rate of resistance in different types and groups of bacteria demands noble antibiotics within short time span. One of the approaches could be computer aided drug discovery (CADD) to select the target compounds, their proper identification for mechanism of action and relevancy to the context of particular study leading to the identification of novel leads and drug candidates. In silico drug design and discovery can be enhanced and uplifted with the help of chemoinformatic tools by integrating information through several levels that ensures the reliability and efficiency of data and outcomes of the studies and drug discovery researches (Katsila *et. al*., 2016). Computer aided drug design (CADD) has offered different valuable tools in the identification of compounds, minimizing the risk of later rejection of lead compounds. In past years CADD has played a significant role in high success rates of hit compound identifications. CADD is also very useful and has

been the efficient medium for the prioritization of HTS (Highthroughput screening) active compounds.

The physical screening of diverse and large libraries against a biological target (high-throughput screening is most used technique for the identification of new lead compounds in drug discovery. CADD has significantly decreased the number of compounds necessary to screen retaining the same level of lead compound discovery. Compounds that are inactive can be skipped, and those predicted to be active can be prioritized. This helps in reduction of the cost and workload of a full HTS screen without compromising lead discovery (Sliwoski *et. al*., 2013). Another approach called virtual screening computationally screens large libraries of chemicals for compounds that complement targets of known structure. Those compounds that bind well are selected and are preferred in drug discovery process. Different conformations of molecules under study, absolute binding energies and their interactions with cellular environment are predicted in an aqueous environment. Different novel ligands with hit rates have been identified successfully. It is based on receptor-ligand interaction and has been immensely studied, used in screening approaches and commonly termed as receptor-based virtual screening (Shoichet, 2004).

CADD has also been proved very useful in drug repurposing also. Drug repurposing has become an important branch of drug discovery. Drug repurposing involves discovering novel uses of already existing drugs. Several computational approaches have been used to uncover new repurposing opportunities and aid the discovery process. This repurposing also known as repositioning has been applicable in various ways in different aspects of drug discovery and seeking of hit compounds for different molecules under study (March-Vila *et. al*., 2017).

Computer aided drug design (CADD) also known as in-silico screening utilizes the two types of computer-aided drug design (CADD) approaches; Structure based drug design (SBDD) and ligand based drug design (LBDD). Structure-based drug design approaches include docking of ligands, ligand design methods to analyze macromolecular 3-dimensional structure. Structure based drug discovery screens the preferred ligands or inhibitors tightly to active sites and pass the ADMET properties. The key interacting sites play a major role in the functioning of the molecules (protein or RNA typically). During the screening procedure the similar ligands are thought to bind to molecule under study. This is also found in proteins, the similarity principle applies here also. Being similar in structure these compounds have very similar and common biological function so the similar ligands are end results as they are recognized ones. Proteins with similar structures are likely to have similar functions and to recognize similar ligands. In the field of drug repurposing, protein comparison is used as a method to identify secondary

targets of an approved drug can be identified by drug repurposing (Sliwoski *et. al*., 2013, Ehrt *et. al*., 2016).

Different parameters are used to define the target compound and meet the criteria under consideration. Various aspects regarding targets need to be contemplated before proceeding to further steps that include target selection, its druggability, structure, molecular flexibility, protonation state, and interacting water molecules (Cerqueira *et. al.*, 2009). The pharmacokinetic and Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of a compound would determine the fate of the compounds available in the database under study.

The human genome project that sequenced the whole human genes revealed a lot about human body on genetic level. And this has helped in discovery of the new therapeutic targets. The advancements in protein purification by incorporation of high-throughput technology, crystallographic techniques, and another structure based technique like spectroscopy has been proved to be of great value in drug discovery pathway. The insights into the protein-ligand complexities, interaction details has provided the understandings of underlying machineries and helped to create the vision of the compound or therapeutic target to be discovered (Meng *et. al*., 2011).

The protein is obtained or selected for the drug discovery process and this target protein is then further prepared for molecular docking. Molecular docking is an easy and fast method that is used to screen large libraries of both ligands and targets. It is a versatile tool that helps to predict the interaction between target proteins with preferred or specific ligand (Kitchen *et. al*., 2004). Molecular docking has also been extensively used in the repurposing of drugs (Li *et. al*., 2011). Different binding confirmations, scoring functions and most importantly binding energy is considered during selection of particular ligand. The specific ligand with higher binding energy than native ligand is considered for further analysis (Sgobba *et. al*., 2012, Hernndez-Santoyo *et. al*., 2013). Any target protein can be studied, processed and analyzed by molecular docking. The protein structure obtained from RCSB or predicted by using tools like phyre2, swiss-model, modeller and others can be used for screening of specific ligands from different ligand libraries depending upon the nature of protein and docking purposes (Bordogna *et. al*., 2013). Softwares like Datawarrior, AutoDock and PyRx are used for molecular docking, scoring purpose and determination of binding energy and finalize ligands with required functions and interacting modes for drug discovery and drug repurposing (March-Vila *et. al*., 2017, Hernndez-Santoyo *et. al*., 2013).

Molecular docking has been found to be effective in drug-based and target-based drug repurposing. a new connection was discovered between Cadherin-11, which was not targeted and studied before, implied in rheumatoid arthritis, and cyclooxygenase-2

(COX-2) inhibitor celecoxib and anti-parasitic drug has been successfully tested as an anti-angiogenic vascular endothelial growth factor receptor 2 (VEGFR2) inhibitor (Dakshanamurthy *et. al*., 2012). Although molecular docking has its own limitations, it has been established and experimentally validated approach for predicting new drug–target associations and interactions. For repurposing of drugs that includes specific disease, health conditions, or targeting certain proteins, docking can be very powerful tool when integrated with ligand-based methods and other available information about target–disease associations (March-Vila, *et. al*., 2017). Saquinavir (approved in 1995), used for the treatment of human immunodeficiency virus (HIV ritonavir and indinavir (both approved in 1996) carbonic anhydrase inhibitor dorzolamide (approved in 1995) (Vijayakrishnan, 2009), the angiotensin converting enzyme (ACE) inhibitor captopril (approved in 1981) as an antihypertensive drug (Talele *et. al*., 2010) are some therapeutics, in which CADD has played a significant and a notable role.

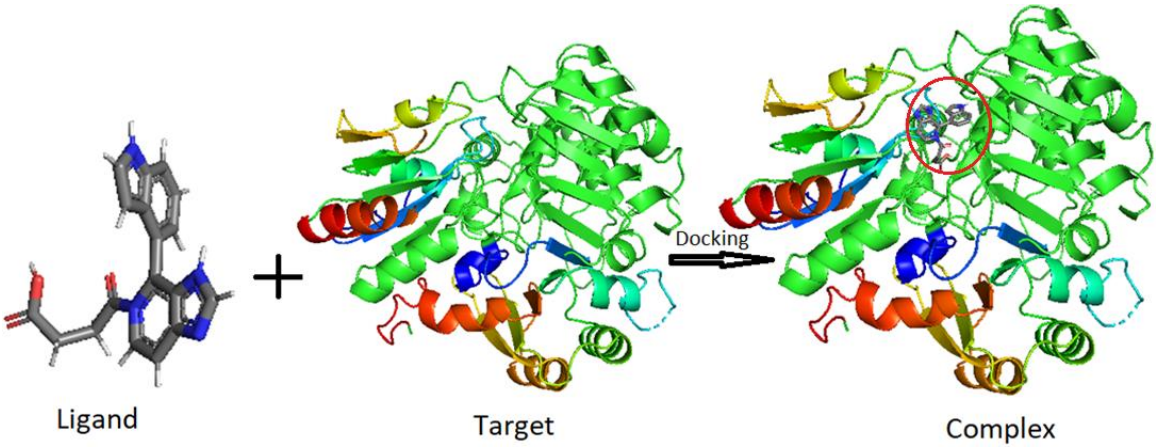


Fig: 1 Diagram depicting the docking procedure (Adapted from researchget.net)

Novel serotonin receptor subtype 1A (5-HT1A) agonists was designed using virtual screening tools. 5-HT1A agonists have been clinically found to be effective in treatment of anxiety and depression (Becker *et. al*., 2006). In 2003 search for novel transforming growth factor-β1 receptor kinase inhibitors presented the most striking examples of the possibilities of use of CADD extensively in the drug discovery and drug repurposing. . A traditional HTS was used by a group at Eli Lilly to identify a lead compound that was then improved by examination of structure-activity relationship by using in vitro assays (Sawyer *et. al*., 2003). A group at Biogen Idec used a CADD approach that involved virtual HTS based on the structural interactions between a weak inhibitor and transforming growth factor-β1 receptor kinase (Singh *et. al*., 2003). 87 hits were identified the best hit being identical in structure to the lead compound discovered through the traditional HTS approach at Eli Lilly (Shekhar, 2008).

Virtual screening has been initial phase in drug discovery with integration of computational approach and its extensive development. Virtual Screening (VS) is a computational technique used in drug discovery applicable in identifying the target compounds from the large ligand libraries which bind the molecule of interest with utmost efficiency. This is used as an important tool to guide the search for drug-like candidates. Virtual Screening reduces the time and effort in screening of compounds and their evaluation by High Throughput Screening (HTS) even in terms of cost (Trott and Olson, 2010). Virtual screening uses computer-based methods to discover new active compounds on the basis of biological structures (Shoichet *et. al*., 2004). There are two broad categories of Virtual Screening techniques: ligand based (LBVS) and structure based (often referred as docking) (SBVS).

S-adenosyl methionine (SAM) is a key methyl donor synthesized from methionine and ATP and is involved in the methylation of various macromolecules including DNA, RNA, proteins, phospholipids, hormones and neurotransmitters (Vance, 2014). There are many SAM dependent genes which play critical role in the metabolism of microorganisms. CobA is one of them. CobA is essential for corrin ring contraction for vitamin B12 biosynthesis and this is important in lipid biosynthesis thus plays significant role in the membrane stability in different bacteria. Targeting CobA for discovery of drug candidate thus can be a useful and significant in the development of antimicrobials.

1.2 Hypothesis

1.2.1 Null Hypothesis:

Compounds exhibiting inhibition against CobA protein couldn't be found by molecular docking approaches.

1.2.2 Alternative Hypothesis:

Compounds exhibiting inhibition against CobA protein could be found by molecular docking approaches.

1.3 Objectives

1.3.1 General Objective

- To identify novel therapeutics against the SAM utilizing proteins of pathogenic bacteria

1.3.2 Specific Objective

- To identify potential drug candidates against CobA from different databases
- To create ligand libraries for molecular docking

- To perform virtual screening against target protein
- To study antimicrobial properties and mechanisms of inhibition of selected compounds

1.4 Rationale

Emergence of multidrug resistant (MDR) has created a global public health risk. The demand of novel antibiotics and targeting essential gene has become the need of time. The present study is focused on the identification of compounds against the cobA gene and also finding the drug candidates which could be useful against inhibition of various metabolites that are involved in cobA translation, vitamin B12 synthesis and regulatory pathways through molecular docking and further analysis of selected compounds using different software.

2. LITERATURE REVIEW

2.1 Review of literature related to Antibiotics, history, their mechanisms of action and present situation

Antibiotics are the chemotherapeutic agents that have been used since 1940 for the clinical management of bacterial diseases. The very powerful tool that has a long history was first coined as antibiotics by the American microbiologist Selman Waksman and his colleagues. It described chemical substances produced by microorganisms having antagonistic effects on the growth of other microorganisms. Antibiotics were introduced into the clinical practice only in the middle of the last century however the history of using microorganisms for the management of microbial infections is found in ancient Egypt, Greece, China, and some other places of the world. The modern era of antibiotics started with the discovery of penicillin from the culture filtrate of a fungus, *Penicillium notatum* by Alexander Fleming in 1928.

Antibiotics which are used against microbial infections and available in the market are either produced by microbial fermentation or synthesized chemically. They are considered semi-synthetic as the pre-existing antibiotic can be used as backbone structure. Antibiotics are produced naturally by microorganisms as secondary metabolites at a concentration much lower than the therapeutic dose (Sengupta *et al*., 2013). On the basis of antimicrobial actions the antibiotics are commonly classified into bactericidal and bacteriostatic agents. Antibiotics are effective against gram- positive, gram-negative or both gram-positive and gram-negative bacteria. They are described as broad-spectrum and narrow spectrum antibiotics on the basis of efficacy against these gram-positive and gram-negative bacteria. Broad spectrum antibiotics have been found effective against both Gram's positive and Gram's negative bacteria while narrow spectrum antibiotic acts towards Gram's positive or Gram's negative bacteria only (Nemeth *et. al*., 2015).

Antibiotics target the bacterial cell and certain cellular structure and functions to inhibit its metabolism and cellular activities causing the death if cell. Destroying the bacterial cell by either preventing cell reproduction or changing a necessary cellular function or process within the cell is the pharmacology behind the working process of antibiotics. By understanding the target of the chemical compound in antibiotics the modification can be done by adding side chain or further chemical modifications to make it specific in a way that can ensure the safety of human health and target the bacterial cell as well (Calhoun *et al*., 2020). The basic mechanism of the antibiotics causing inhibition or death of bacterial cell has mechanism of actions as depicted in picture below.

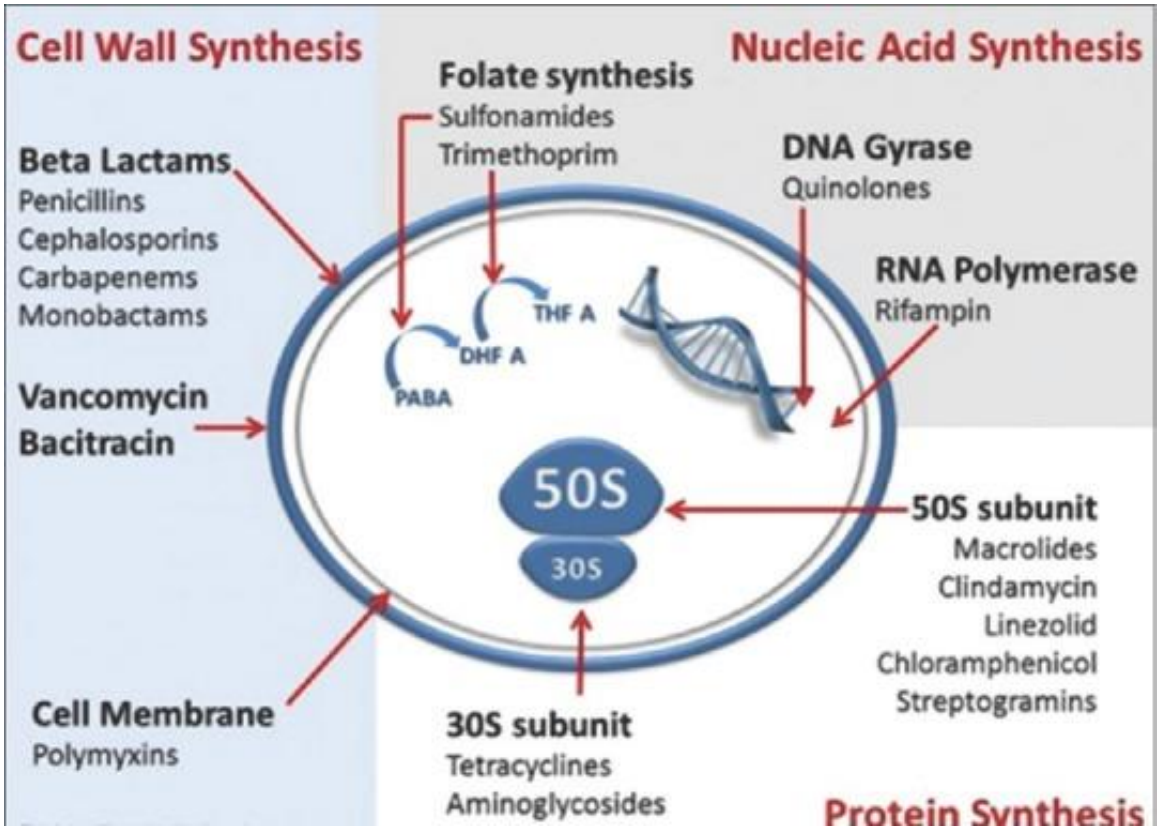


Fig: 2 Mechanism of actions of different antibiotics

Researchgate.net

2.1.1 Antibiotics targeting cell wall

Bacteria have a cell wall made of peptidoglycan, which surrounds the cell and forms outer layer and this layer consists of long sugar polymers. The peptidoglycan has cross-linking of the glycan strands by the action of transglycosidases, and the peptide chains extend from the sugars in the polymers and form cross links. One peptide is linked thus to another. The glycan chains are attached with peptides by penicillin binding protein (PBPs). The D-alanyl-alanine portion of peptide chain is involved in this cross linkage. This cross-linking helps in strengthening the cell wall (Kahne *et. al.,* 2005). β- Lactams and the glycopeptides inhibit the bacteial cell wall synthesis.

2.1.1.1 Beta-lactam antibiotics

The β-lactam ring mimics the D-alanyl D-alanine portion of peptide chain; this is bound by penicillin binding protein. The PBPs thus bind with the β-lactam ring. This makes them unavailable for the synthesis of new peptidoglycan layer. Thus the formation of peptidoglycan layer and cross linkage fails leading to the disruption and eventually lysis of bacterial cell.

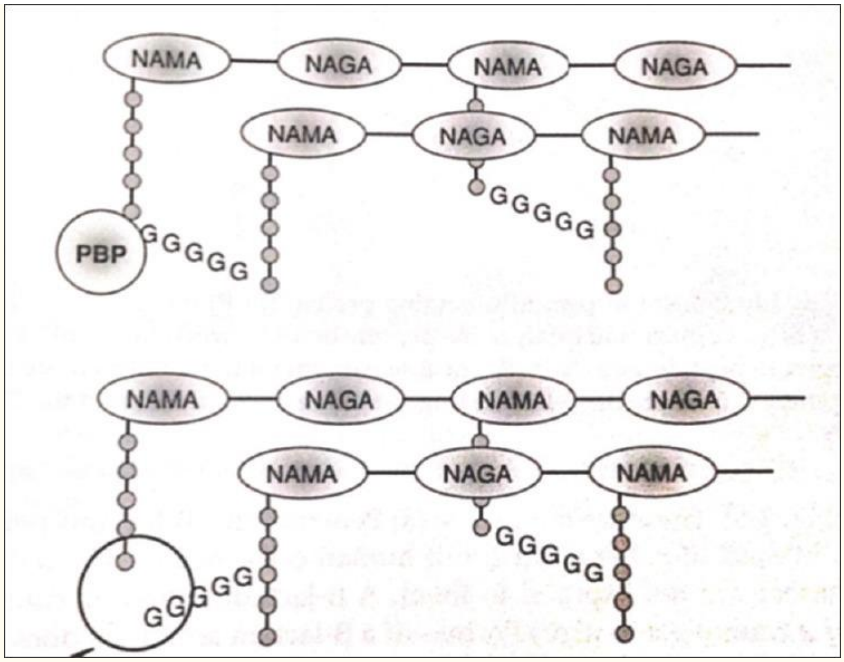


Fig: 3 Working mechanism of beta-lactam antibiotics (Kapoor *et. al*., 2017)

2.1.1.2 Glycopeptides

Glycopeptides are glycosylated non-ribosomal peptides. These are produced by a diverse group of soil actinomycetes and they target Gram-positive bacteria by binding to the acyl-D-alanyl-D-alanine (D-Ala-D-Ala) terminus of the peptidoglycan on the outer surface of the cytoplasmatic membrane. They are generally used to treat life-threatening infections caused by multidrug-resistant Gram-positive pathogens, such as Staphylococcus aureus, Enterococcus spp. and Clostridium difficile (Rossolini *et. al*., 2014). The binding of glycopeptides to D-alanyl D-alanine portion of peptide side chain of the precursor peptidoglycan subunit helps in growing the peptidoglycan chain as a mechanism of cell wall formation. Vancomycin prevents the binding of D-alanyl subunit with the penicillin binding protein (PBP) and the synthesis of cell wall is inhibited (Binda *et. al*., 2014)

2.1.2 Inhibitors of protein biosynthesis

RNA molecule referred as messenger RNA (m-RNA) is synthesized by using DNA as information to be coded. This process is known as transcription. Then the protein is synthesized in process called translation. Ribsomes synthesize the protein encoded in m-RNA. First the information in bacterial DNA is used to synthesize an RNA molecule referred to as messenger RNA (m-RNA) a process known as transcription. 70S ribosome of bacteria is composed of two ribonucleoprotein subunits, the 30S and 50S subunits. Antimicrobials target the 30S or 50S subunit of the bacterial ribosome and inhibit protein synthesis (Yoneyama *et. al*., 2006, Vannuffel *et. al*., 1996, Johnson *et. al*., 2002)

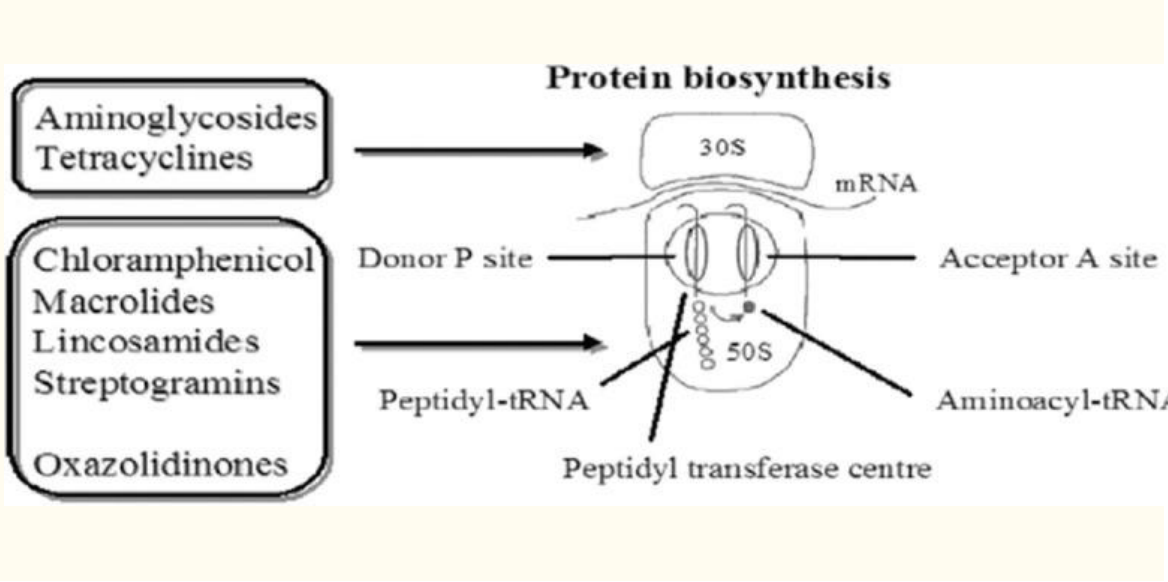


Fig 4: Action of antibiotics on protein synthesis (Kapoor *et. al*., 2017)

2.1.2.1 Inhibitors of 30S subunit

2.1.2.1.1 Aminoglycosides

The outer membrane (OM) of the bacterial cell is negatively charged thus the aminoglycosides being positively charged binds to it and the large pore are formed. The antibiotic molecule thus enters the cell. Aminoglycosides target the ribosome so it needs to be transported through energy dependent manner; it is transported through active transport mechanism. For this the oxygen and active proton motive force are required hence aminoglycosides are effective in aerobic conditions. Aminoglycosides show synergistic action with cell wall inhibiting antibiotics (such as β-lactam and glycopeptides). Aminoglycosides cause premature termination of translation of mRNA by interaction with 16s r-RNA of 30S subunit Aminoglycosides present near the A site through H-bonds (Kapoor *et. al*., 2017)

2.1.2.1.2 Tetracyclines

The tetracyclines inhibit the protein synthesis in bacteria; inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site. Tetracyclines are broad-spectrum antibiotics and act against a wide range of gram-positive and gram-negative bacteria. These antibiotics show inhibition against organisms such as chlamydia, mycoplasmas, and rickettsia, and protozoan parasites (Chopra and Roberts, 2001). Other antibiotics of this category such as tetracycline, chlortetracycline, doxycycline prevent binding of t-RNA to the A site by interacting with conserved sequences of the 16S r-RNA of the 30S ribosomal subunit (Yoneyama *et. al*., 2006, Wise 1999).

2.1.2.2 Inhibitors of 50S subunit

2.1.2.2.1 Chloramphenicol

Chloramphenicol is an old broad-spectrum antibiotic which specifically inhibits bacterial protein synthesis (Dinos *et. al*., 2016). It prevents the binding of t-RNA to the A site of the ribosome by interacting with the conserved sequences of the peptidyl transferase cavity of the 23S r-RNA of the 50S subunit (Vannuffel *et. al*., 1996; Yoneyama *et. al*., 2006)

2.1.2.2.2 Macrolides

Macrolides target conserved sequences of the peptidyl transferase center of the 23S r-RNA of the 50S ribosomal subunit and affect translocation, during protein synthesis. This causes the premature detachment of incomplete peptide chains (Kapoor *et. al*., 2017)

2.1.2.2.3 Oxazolidinones

Oxazolidinones interfere with protein synthesis by binding to 23Sr RNA of the 50S subunit and suppresses 70S inhibition and it interacts also with peptidyl-t-RNA (Bozdogan and Appelbaum, 2004)

2.1.3 Inhibitors of DNA replication

2.1.3.1 Quinilones

The fluoroquinolones (FQ) introduces negative supercoils and then reseals the nicked ends. It inhibits the enzyme bacterial DNA gyrase, which nicks the double-stranded DNA. It prevents excessive positive supercoiling of the strands when they separate to permit replication or transcription. The fluoroquinolones bind to A subunit of DNA gyrase with high affinity and interfere with its strand cutting and resealing function. Fluoroquinolones have higher affinity for gram-positive bacteria so it has greater potency for topoisomerase IV in gram-positive bacteria which nicks and separate's daughter DNA strand after DNA replication.

2.1.4 Folic acid metabolism inhibitors

Sulfonamides and trimethoprim Sulfonamides and trimethoprim inhibit different steps in folic acid metabolism. They have synergistic actions and act at distinct steps on the same biosynthetic pathway. Sulfonamides inhibit dihydropteroate synthase in a competitive manner and shows higher affinity for the enzyme than the natural substrate, p-amino benzoic acid. Trimethoprim acts at a later stage of folic acid synthesis and inhibit the enzyme dihydrofolate reductase (Yoneyama *et. al*., 2006). Folic acid is essential for nucleic acid and amino acid metabolism so alteration in the biosynthetic pathway causes the destruction of bacterial cells.

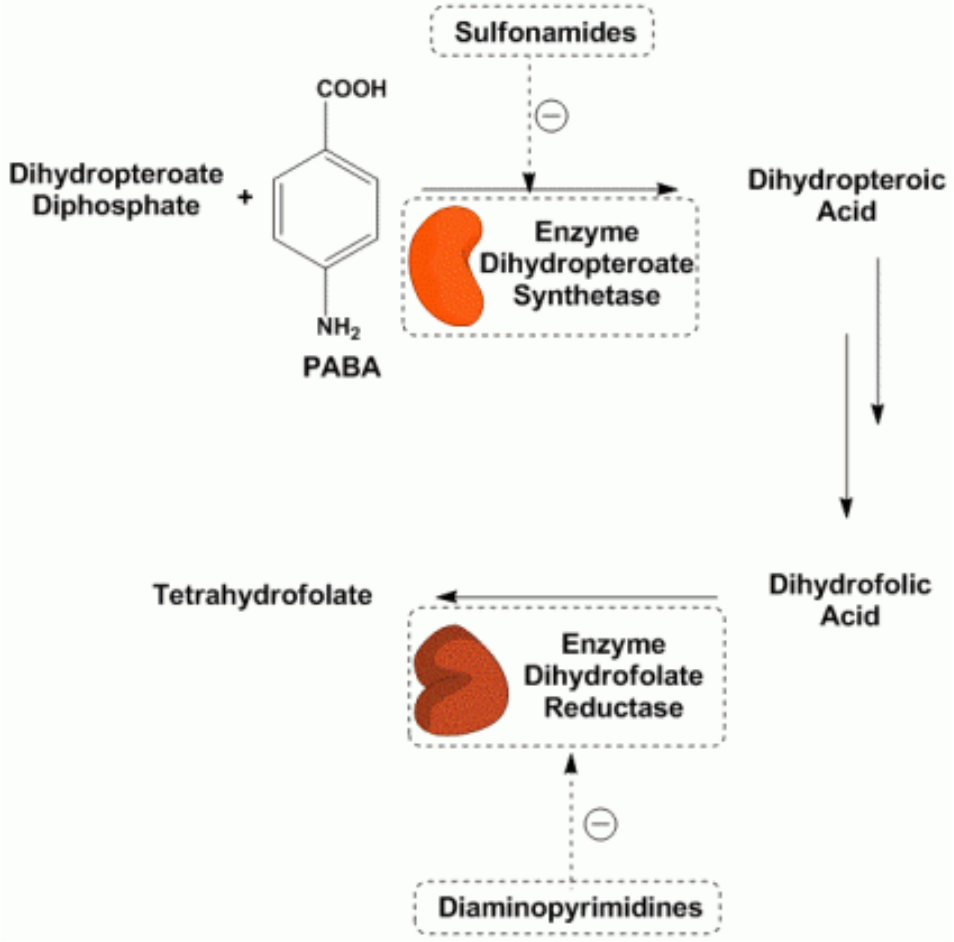


Fig: 5 Biosynthesis of tetrahydrofolate and mechanism of action of sulfonamides and diamonipyrimidines (https://www.google.com/pharmaxchange.info)

2.1.5 Free radical induced killing of bacterial cells

Any species that contains one or more unpaired electrons is called free radical. These include reactive oxygen species(ROS) such as superoxide(O_2^{--}), hydroxyl (OH^-), peroxyl (RO_2^-) and non-radical oxidizing agents such as hydrogen peroxide(H_2O_2), ozone(O_3) (Bayr, 2005). In mammalian cells, variety of enzymatic and non-enzymatic process can generate ROS in DNA and Protein Damage Hydroxyl radicals are induced by the bactericidal drugs. These ions are toxic to cellular components they readily damage proteins, membrane lipids, and DNA within bacterial cells (Farr and Kogoma, 1991). The treatment with norfloxacin has been found to increase SOS activity significantly. Similarly beta-lactams have been shown to induce expression of the SOS response mediator of filamentation, sulA, through the DpiBA two-component system (Miller *et. al*., 2004).

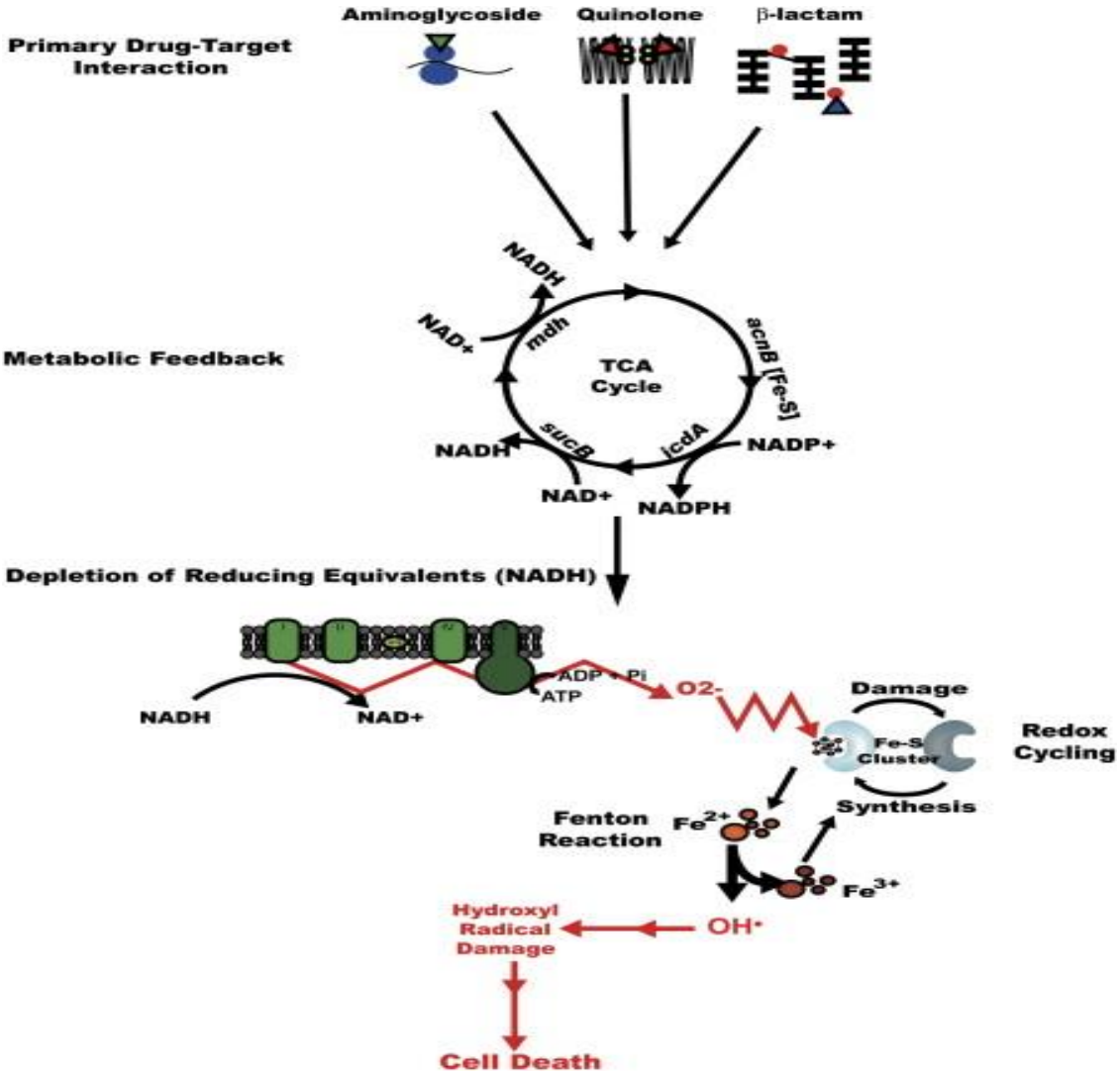


Fig: 6 Mechanism leading to ROS mediated Antibiotic killing of bacteria (Kohanski *et. al*., 2007)

2.2 Review of literature related to Antibiotic Resistance

In spite of being used in treatment of different bacterial diseases and successful cure of many infections, these lifesaving agents have lost their magical power with widespread emergence and dissemination of antibiotic-resistant strains. Excessive and imprudent use of antibiotics significantly contributes to the emergence of resistant strains. The antibiotic biosynthetic genes and resistance conferring genes both have been found to evolve long before the discovery of the antibiotics. Antimicrobial resistance (AMR) is mechanism by which the microorganisms exhibit resistance against at least three existing antimicrobial drugs (antibiotics, antifungals and antivirals). The rapid emergence of resistant bacteria that has been observed worldwide has been a major concern among health and medicine authorities. The microorganisms involved as referred as "superbugs" which denotes microbes with higher morbidity and mortality rate increased due to different mutations which provide resistance to various classes of antibiotics

(Aslam *et. al*., 2018).

In 2019, WHO considered antibiotic resistance as top five most important public health threats of the 21st century. The world is about to face the worst consequences which has been called to be the post-antibiotic era, the duration when antibiotics would be ineffective and new antibiotics will not be developed soon. The news and research papers mentioning the continuous discovery of bacterial and fungal pathogens against existing drugs indicate towards the alarming situation in near future. The intrinsic and extrinsic factors and different parameters have caused the increasing resistance to the pathogens. Different important organizations like Centers for Disease Control and Prevention (CDC), Infectious Diseases Society of America, World Economic Forum, and the World Health Organization (WHO) have declared antibiotic resistance as a "global public health concern. This has caused great loss of economy and at the same time health severities especially in developing countries as the bacterial infections have been proved lethal as the existing antimicrobials have been found to be ineffective (Spellberg *et. al*., 2006, Michael *et. al*., 2014, Rossolini *et. al*., 2014)

The health and clinical personnel, authorities, organizations along with WHO has shown serious concern towards the growing impacts of antimicrobial resistance in the society. WHO has asked and called upon the need for an improved and coordinated global effort to contain AMR. To slow the rapid emergence and reducing the spread of antimicrobial resistant microorganisms the WHO Global Strategy for Containment of Antimicrobial Resistance has provided a framework of interventions to introduce different measures in 2001. According to The US Center for Disease Control and Prevention (CDC) estimated that, in the US, more than two million people every year are affected with antibiotic-resistant infections, and about 23,000 death case has been shown due to the infection. Similarly in Europe each year, the number of infections and deaths due to the most frequent multidrug-resistant bacteria (*S. aureus, Escherichia coli, Enterococcus faecium, Streptococcus pneumoniae, Klebsiella pneumoniae and Pseudomonas aeruginosa*) was estimated at ∼400 000 and 25,000, respectively, in 2007 (Prestinaci *et al*., 2014). High incidence of infectious diseases, inappropriate use of antibiotics in treatment, use of antibiotics as growth promoters and lack or poor implementation of legislation to antimicrobial resistance has been found to drive the antimicrobial resistance in developing countries like Nepal (Levy *et. al*., 2004, Thakur *et. al*., 2014, McEwen *et. al*., 2002). Antibiotics are easily available from pharmacies without any prescriptions (Shrestha, 2016).

Physicians are also responsible for this increasing antimicrobials resistance (AMR), highly unsafe drugs categorized as "Group A" and "Group B" are prescribed in an irrational way, which aids the development of resistance further (Basnyat *et. al*., 2015).

Similarly the misuse of broad-spectrum antibiotics and prescribing these antibiotics over narrow-spectrum unnecessarily has even made the condition worse. The different resistance mechanisms against various pathogens is emerging rapidly (Ferri *et. al*., 2015) in every part of the world. According to WHO about 558,000 new cases of MDR-TB showing were found resistance against rifampicin (first line drug), which accounts for nearly 50 % of the global TB cases (WHO). 8.5% of MDR-TB was extensively drug–resistance TB (XDR-TB) (resistant to isoniazid, rifampicin plus any Fluoroquinolone in 2017 (CDC, 2017). According to surveillance report of European Centre for Disease Control (ECDC, 2018), about 33,000 people die each year due to multidrug resistant *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and others.

This continuity of emergence of resistance against the different bacteria has led to the serious discussions among concerned authorities and meanwhile WHO has updated the list of bacterial infections for which urgent need of antibiotics is there. WHO divided the pathogens into three categories to guide research, discovery, and development of new antibiotics.

Priority 1: CRITICAL

-*Acinetobacter baumannii*, carbapenem-resistant

-*Pseudomonas aeruginosa*, carbapenem-resistant

-*Enterobacteriaceae**, carbapenem-resistant, 3rd generation cephalosporin-resistant

Priority 2: HIGH

-*Enterococcus faecium*, vancomycin-resistant

-*Staphylococcus aureus,* methicillin-resistant, vancomycin intermediate and resistant

-*Helicobacter pylori,* clarithromycin-resistant

-*Campylobacter*, fluoroquinolone-resistant

-*Salmonella* spp., fluoroquinolone-resistant

-*Neisseria gonorrhoeae*, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

-*Streptococcus pneumoniae*, penicillin-non-susceptible

- *Haemophilus influenzae*, ampicillin-resistant

-*Shigella* spp., fluoroquinolone-resistant

Source: WHO (February 27, 2017)

WHO has also published a list of bacteria for which new antibiotics are urgently needed. WHO divided the bacteria into three categories according to the urgency of need for new antibiotics: critical, high and medium priority.

Critical

The most critical group of all includes multidrug resistant bacteria which particularly have been great threat in hospitals, nursing homes, and especially among patients whose care requires devices such as ventilators and blood catheters. They include *Acinetobacter, Pseudomonas* and various Enterobacteriaceae (*Klebsiella, E. coli, Serratia, and Proteus*). They have been found to cause severe and often deadly infections such as bloodstream infections and pneumonia. These bacteria show resistance against large number of antibiotics including carbapenems and third generation cephalosporins (these are considered best for treatment of multi-drug resistance bacteria among antibiotics available now in the market.

The high and medium priority categories

The second and third tiers in the list and contain other increasingly drug-resistant bacteria that has been the cause of more common diseases such as gonorrhoea and food poisoning caused by *salmonella*.Source: WHO (February 27, 2017)

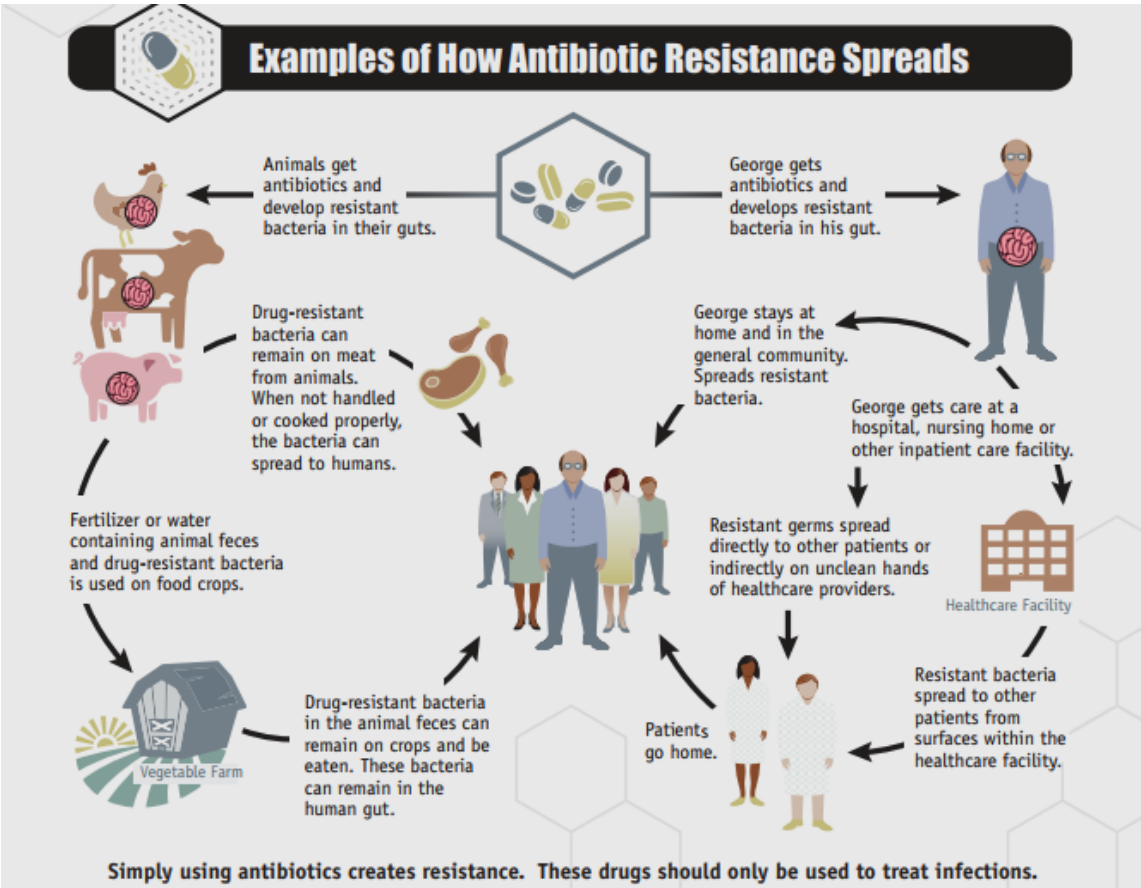


Fig: 7 Illustration of how antibiotics spread among different hosts (CDC, 2013)

2.2.1 Mechanism of antibiotics resistance

Bacteria have developed different resistance mechanism over a period of time. As an evolutionary mechanism against the detrimental effects of the antimicrobials, microorganisms have been found to develop methods to survive and adapt in the environment. At present there are wide varieties of antimicrobial agents targeting the microorganisms and one has numerous options to choose from different ranges for treatment and for potential infection therapy. However the medicine field has been facing the great challenges as resistance has been documented and antimicrobial resistance is observed soon after the drug comes into consideration for use particularly. The bacterial community is however not uniformly resistance to all drugs. Minimum Inhibitory concentration is one factor that determines the resistance and susceptibility of microorganisms towards certain antibiotics. Susceptibility is average of range of Minimum inhibitory concentration (MIC) of certain drug towards some microorganisms and if average MIC for any microorganism is in range of resistant part the microorganism is considered to be intrinsic resistant to that drug. The microorganism might acquire some resistant gene from other species and in such situation the resistance and its level depends upon the gene acquired and different other factors like the species and environment. So on the basis of origin of resistance the resistance exhibited by microorganism can be described as; natural resistance and acquired resistance.

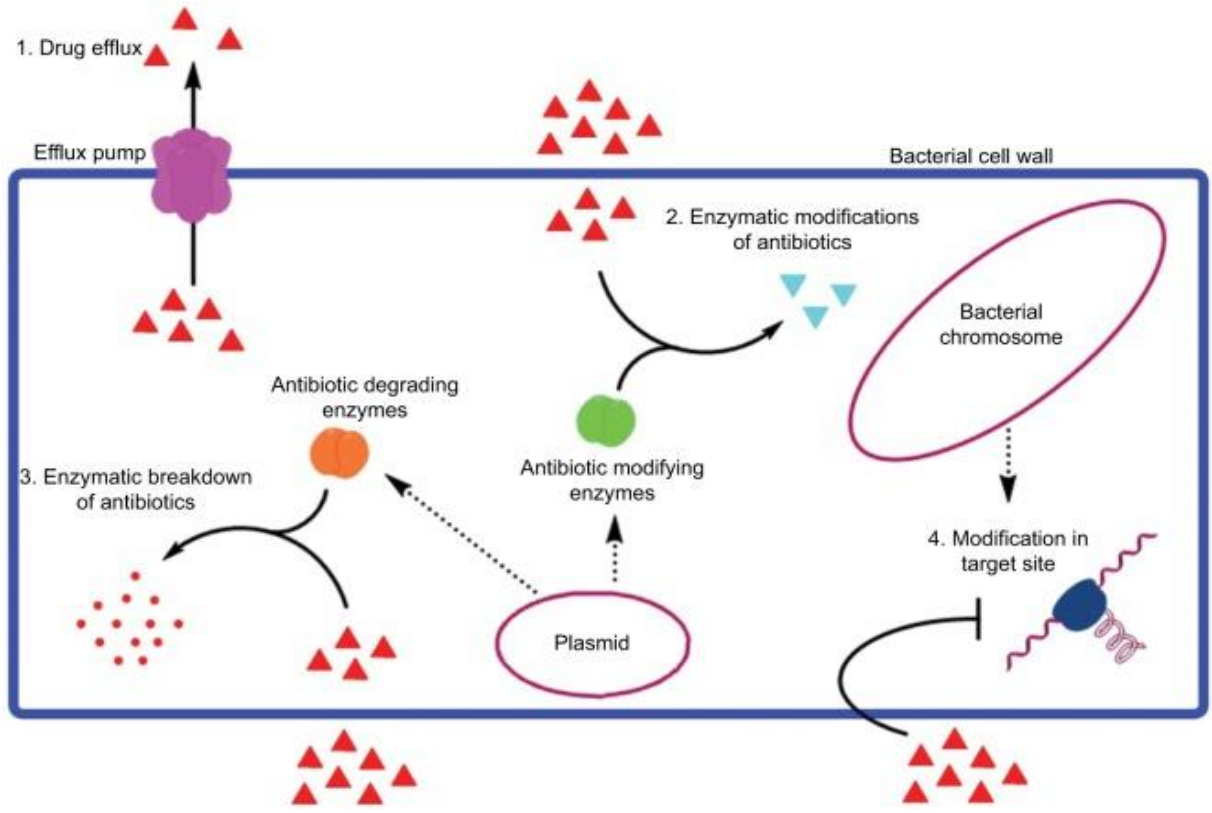


Fig: 8 Different mechanisms of antimicrobial resistance

Natural resistance

Natural resistance is presence of natural resistance mechanism or biological efficiency of bacterial community and not adapted characteristics. Natural resistance can be intrinsic or induced one. Intrinsic resistance is a trait and not related to horizontal gene transfer in anyway. It is shared within a bacterial species and independent of prior application of antibiotics. Reduced permeability of outer membrane in gram negative bacteria and the activity of efflux pump are involved in intrinsic resistance (Cox and Wright 2013; Fajardo *et. al*., 2008; Marinez *et. al*., 2014)

Acquired resistance

Bacteria can acquire genetic material through all the methods of horizontal gene transfer—HGT; by transformation, transposition, and conjugation (all termed horizontal gene transfer—HGT). This gene confers the resistance to bacterial community against certain antibiotics. The mutations in the original chromosome of their DNA are also responsible for the resistance shown by the microorganisms. Mutations occurring in certain genes; especially those encoding drug targets, those encoding drug transporters, those encoding regulators that control drug transporters and those encoding antibiotic-modifying enzymes make the microorganism resistance (Coculescu, 2009; Martinez, 2014; Davies and Davies , 2010).

1) Mechanistic basis of antimicrobial resistance

i) Decreased antibiotics uptake by microorganisms and Efflux pumps

Decreased uptake by microorganisms due to change in permeability:

Those antibiotics that need to cross the barrier to reach designated region and act particularly are affected by change in membrane permeability. Some drugs use porins to cross the barriers across the cellular environment. These antibiotics have intracellular targets and in gram-negative bacteria located in cytoplasmic membrane. So the antibiotics need to be penetrated through cytoplasmic membrane to reach the target and act specifically. By decreasing the uptake of antibiotics and preventing it from reaching intracellular target bacteria develops resistance against these antimicrobials. In gram-negative bacteria particularly, the outer layer act as the first line of defense against penetration of antimicrobials. Antibiotics such as β-lactams, tetracyclines and some fluoroquinolones are particularly affected by changes in permeability of the outer membrane as they often use water-filled diffusion channels known as porins to cross the barrier (Pagès *et. al*., 2008). The changes in porin that can limit the uptake of drug is either decrease in the number of porins or mutations that brings the changes in the selectivity of porins. In Enterobacteriaceae resistant has been shown against carbapenem due to the reduction in number of porins (Cornaglia *et. al*., 1996; Chow and

Shlaes 1991). And in in *Neisseria gonorrhoeae* mutations cause changes within the porin channel which makes them resistant and which then become resistant to β-lactams and tetracycline (Gill, 1998; Thiolas *et. al*., 2004)

Porins can be descried on the basis of their selectivity and the regulation of their expression. They can be classified according to their structure as trimeric, monomeric. Three major proteins produced by *E. coli* known as OmpF, OmpC and PhoE and the *P. aeruginosa* OprD are some of classical examples of porin-mediated antibiotic resistance. Further alterations of porins that affects the behavior of microorganisms towards antimicrobials involve, i) a shift in the types of porins that are expressed across the membrane barrier ii) a change in the level of porin expression, and iii) impairment of the porin function. However these changes have been related to comparatively low level of resistance and often include the other resistance mechanism such as increase in expression of efflux pumps (Nikaido, 2003). The treatment after results i.e. post-therapy results were when compared exhibited a shift in porin expression from OmpK35 to OmpK36. OmpK36 showed the decrease in channel size. The susceptibility towards the β-lactums was found to be reduced due to the alteration in the type of porin expressed (Doménech-Sánchez *et. al*., 2003).

Efflux pump

Some pathogens are able to flush out the antibiotics of their system with the help of the proteinaceous transporters called efflux pump that can extrude a wide range of substrates including antibiotics. Efflux pumps are complex bacterial machineries that are capable to extrude a toxic compound out of the cell can also responsible for antimicrobial resistance. Both gram-positive and gram-negative bacteria have been observed exhibiting resistance to certain drugs by using the efflux pump to remove the harmful toxic substances from the cell. The efflux system that can pump out the tetracycline from E. coli was first described efflux system in 1980 (McMurry *et. al*., 1980). These pumps are mostly encoded on the chromosome although they can also be plasmid-encoded. Bacterial drug efflux pumps can be categorized into five families, i.e., the ATP-binding cassette (ABC) superfamily (Lubelski et al., 2007) the major facilitator superfamily (MFS) (Pao *et. al*., 1998), the multidrug and toxic compound extrusion (MATE) family (Kuroda *et. al*., 2009), the small multidrug resistance (SMR) family (a subgroup of the drug/metabolite transporter superfamily (Jack *et. al*., 2001) and the resistance-nodulation division (RND) superfamily (Tseng *et. al*., 1999; Seeger *et. al*., 2008). In gram-negative bacteria RND exporters have been found to play a key role and are involved in most relevant clinical resistance shown by theses group of bacteria (Li and Nikaido, 2009). These efflux systems may be substrate-specific such as tet determinants for tetracycline and mef genes for macrolides in pneumococci or may have

broad substrate specificity, which is usually common in MDR bacteria (Poole, 2005).

RND efflux systems function as drug antiporters and are particularly found among Gram-negative bacteria and are capable of catalyzing the active efflux of a wide variety of antibacterial substrates including many antibiotics and chemotherapeutic agents. RND transporters have large periplasmic domains. They form tripartite complexes with the periplasmic adaptor proteins or membrane fusion proteins (MFPs) and outer membrane (OM) channels. The adaptor proteins such as AcrB, MexB and membrane fusion proteins such as AcrA and MexA found in gram-negative bacteria are the proteins involved in efflux system f gram-negative bacteria. Crystallographic revealed that the AcrB has asymmetric trimer structure whereas each AcrB protomer in the trimeric assembly goes through a cycle of conformational changes during drug export (Murakami *et. al*., 2006; Seeger *et. al*., 2008s; Sennhauser *et. al*., 2007).

This mechanism of resistance affects a wide range of antimicrobial classes including protein synthesis inhibitors, fluoroquinolones, β-lactams, carbapenems and polymyxin are affected by this resistance mechanism. Several MDR efflux pumps like AcrAB-TolC in Enterobacteriaceae and MexAB-OprM in *Pseudomonas. aeruginosa* are able to extrude MDR pumps belonging to the RND family are often found in the chromosome of clinically relevant gram-negative bacteria \ AcrAB-TolC system is best studied system which is found in *E. coli,* which is composed of a transporter protein located in the inner membrane (AcrB), a linker protein located in the periplasmic space (AcrA), and a protein channel located in the outer membrane (TolC) (Dijun Du, Cell 2015). AcrB has been found to have two binding pockets with different substrate preferences and that compounds move out of the cell through a series of conformational changes in a functionally rotating mechanism that ends with the substrate being extruded via TolC.

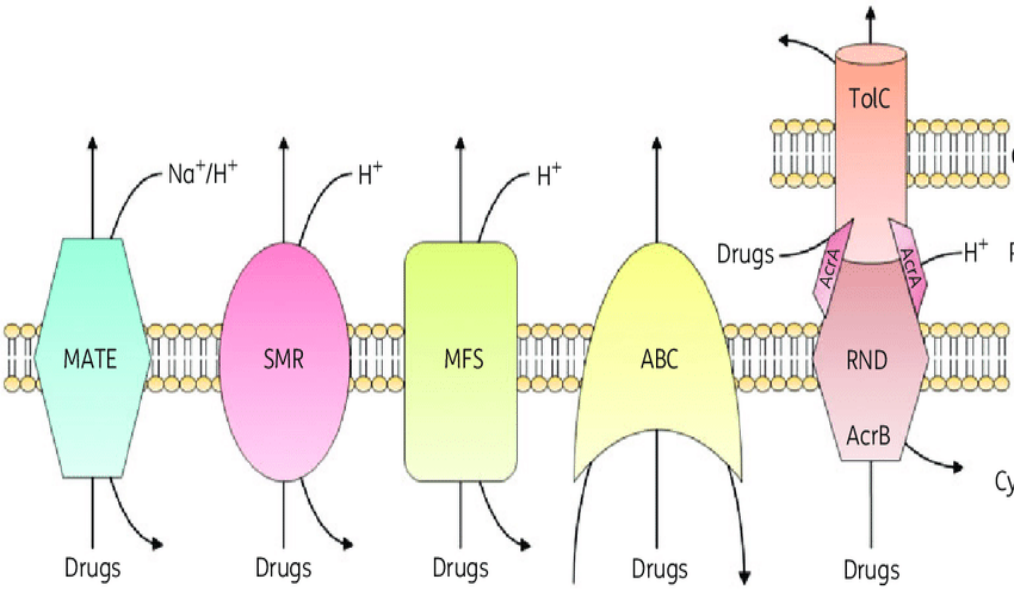


Fig: 9 Efflux pumps found in bacteria and their energy-coupling mechanism

ii) Modification of antibiotics

Chemical or enzymatic alteration of antimicrobials

Chemical modifications of the antibiotics are one of the strategies used by a bacterium that is responsible for the resistance to certain antibiotics. The bacterial community produces the enzymes which are capable of introducing and bring about the chemical changes in antibiotics molecule. This has been shown as well known acquired resistance in both gram positive and gram-negative bacteria. Most of the antibiotics that exert antimicrobial effect by inhibition of protein synthesis at ribosome level are affected by enzymatic modification (Wilson, 2014).

Different biochemical reactions bring about the alteration such as acetylation (aminoglycosides, chloramphenicol, and streptogramins), phosphorylation (aminoglycosides, chloramphenicol), adenylation (aminoglycosides, lincosamides. This modifies the antibiotic molecule and also its original capability. Even slight changes or modification makes the microorganism resistant to the certain drug molecule. The presence of aminoglycoside modifying enzymes (AMEs) that covalently modify the hydroxyl or amino groups of the aminoglycoside molecule is best examples of resistance via modification of the drug.

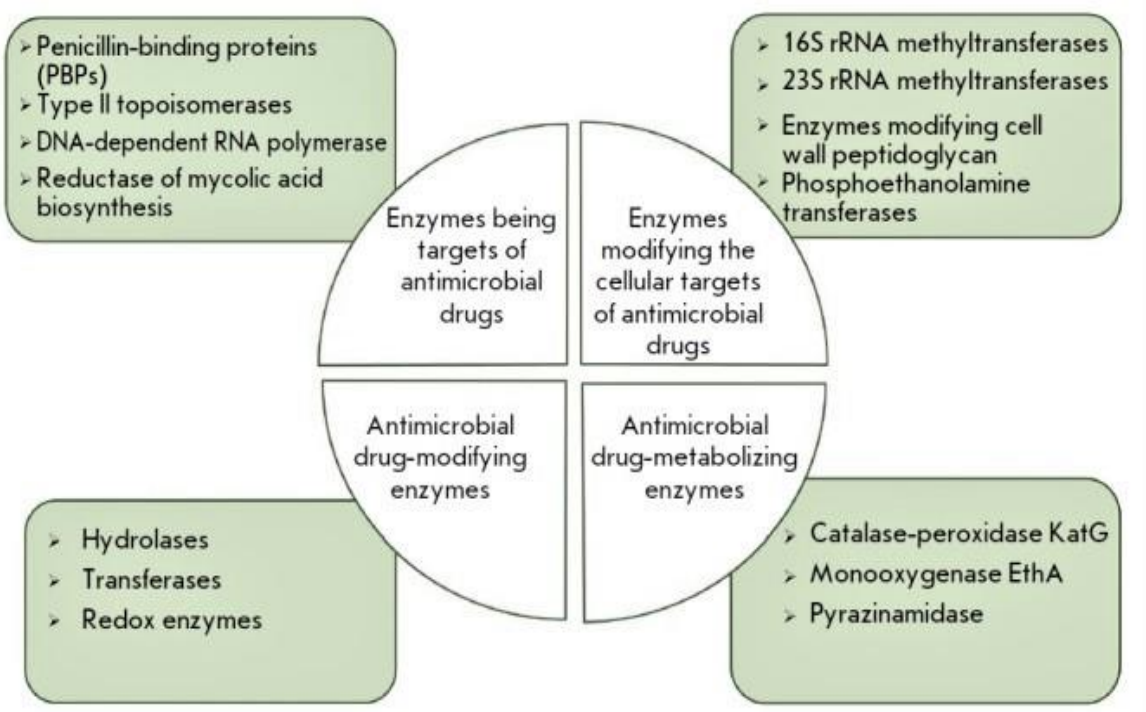


Fig: 10 Classes of enzymes involved in various mechanisms of resistance to antimicrobial drugs (Wright 2005)

Modification of chloramphenicol is another example of enzymatic alteration of antibiotics. It inhibits protein synthesis by interacting with the peptidyl-transfer center of the 50S ribosomal subunit. Expression of acetyltransferases known as CATs (chloramphenicol acetyltransferases) brings about the chemical modification of chloramphenicol. Multiple cat genes have been observed in both gram-positives and gram-negatives. They can be classified in two main types. Type A, results in high-level resistance, and type B confers low-level chloramphenicol resistance. These determinants are usually harbored plasmids and transposons and part of the chromosome of certain bacteria (Munita and Arias, 2016).

Destruction and inactivation of the antibiotic molecule

The enzymatic degradations lead to the inactivation and inhibition of functional drug molecule thus destructing or inactivating it. Bacterial enzymes add chemical groups to vulnerable sites on the antibiotic molecule thus preventing the antibiotic from binding to its original target. The hydroxyl and amides groups can be changed easily within the structure of an antibiotic, by hydrolysis (Blair *et. al*., 2015; Mehta *et. al.,* 2016). Similarly acetyl, phosphate and nucleotide groups can be added to the antibiotics thus making them ineffective or functionless Mingeot-Leclercq and Decout, 2016). β- Lactamases destruct the β-lactam antibiotics making them non-functional. The amide bond of the β-lactam ring is destroyed hence antimicrobials become ineffective (Egorov *et. al*., 2018). Staphylococcus aureus has been reported to show resistance to penicillin which is mediated by the blaZ gene that codes for β-lactamase (Ali *et. al*., 2018).

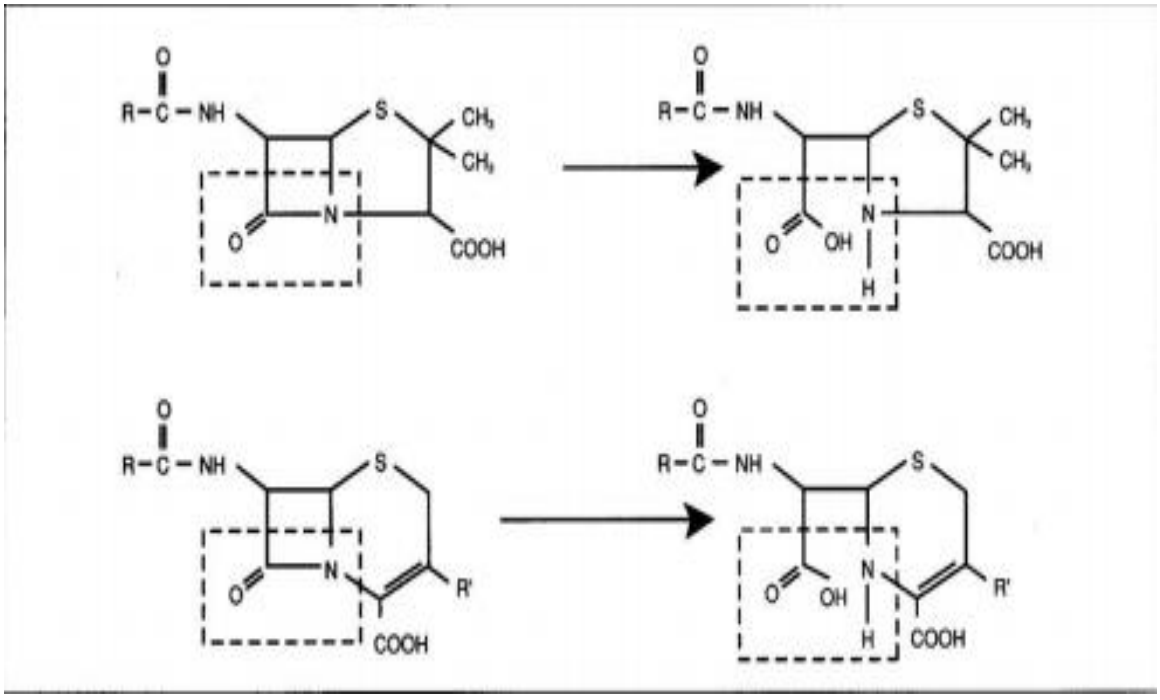


Fig: 11 Reactions catalyzed by β-lactamases which resulted into the degradation of penicillins (top) and cephalosporins (bottom) (Dever and Dermody, 1991).

A plasmid-encoded penicillinase inactivates the penicillin one of the earliest used and popular antibiotics. Infections caused by penicillin-resistant *Staphylococcus aureus* became clinically relevant and that was readily transmitted between S. aureus strains, resulting in rapid dissemination of the resistance trait (Bush, 2013). Ampicillin which came later in use a beta-lactum antibiotics however faced the same consequences and many studies revealing resistance against this antibiotics have been published lately. A new plasmid-encoded β-lactamase capable of hydrolyzing ampicillin was found among gram-negatives (termed TEM-1) (Paterson and Bonomo, 2005).

iii) Change in target site

The antimicrobials have specific targets they interact with and perform their action to inhibit the bacterial systems. Thus interference with these targets is one of the strategies bacterial communities adapt and show resistance to the antibiotics. This includes interference with the targets to prevent for effective actions of antibiotics and even protection of targets and stops the antibiotics from binding with the target. The modification in target has also been seen which decreases the affinity or renders the antibiotics non-functional.

Antibiotic class	Resistance type	Resistance mechanism	Common example
Aminoglycoside	Decreased uptake	Changes in outer membrane	*P. aeruginosa*
	Enzymatic modification	AGE's	Gram-negative bacteria
Beta-lactams	Altered PBP	PBP 2a	Mec A in *S. aureus*, CONS, *S. pneumoniae*
	Enzymatic degradation	Penicillinase which are classified as per ambler classification	Gram-negative bacteria
Glycopeptides	Altered target	D-alanyl-alanine is changed to D-alanyl-D-lactate	Vancomycin resistance in *E. faecium* and *E. faecalis*
Macrolides	Altered target	Methylation of ribosomal active site with reduced binding	*erm*-encoded methylases *in S. aureus*, *S. pneumoniae*, and *S. pyogenes*
	Efflux pumps	Mef type pump	*S. pneumoniae* and *S. pyogenes*
Oxazolidinones	Altered target	Mutation leading to reduced binding to active site	*E. faecium* and *S. aureus*
Quinolones	Altered target	Mutation leading to reduced binding to active site(s)	Mutations in gyr A in enteric Gram-negative bacteria and *S. aureus*
	Efflux	Membrane transporters	Mutations in gyr A and par C in *S. pneumoniae*. Nor-A in *S. aureus*
Tetracyclines	Efflux	New membrane transporters	*tet* genes encoding efflux proteins in Gram-positive and Gram-negative bacteria
	Altered target	Production of proteins that bind to the ribosome and alter the conformation of the active site	*tet* (M) and *tet* (O) in Gram-positive and Gram-negative bacteria species
Chloramphenicol	Antibiotic inactivation	Chloramphenicol acetyl transferase	CAT in *S. pneumonia*
	Efflux pump	New membrane transporters	cml A gene and flo gene efflux in *E. coli*
Sulfa drugs	Altered target	Mutation of genes encoding DHPS	*E. coli*, *S. aureus*, *S. pneumoniae*

DHPS=Dihydropteroate synthase, P aeruginosa=Pseudomonas aeruginosa, S. aureus=Staphylococcus aureus, S. pneumoniae=Streptococcus pneumoniae, E. faecium=Enterococcus faecium, E. faecalis=Enterococcus faecalis, S. pyogenes=Streptococcus pyogenes, E. coli=Escherichia coli, PBP=Penicillin binding protein, AGE's=Aminoglycoside modifying enzymes. CAT=Chloramphinecol acetyl transferases

Fig 12: Resistance mechanism of antibiotics (Kapoor *et. al*., 2017).

Mutation of target site

A mutation in the target sites of antibiotics is also responsible for the resistance shown by bacteria. This has been seen in many antimicrobials. Different genetic changes, amino acid substitutions bring about the changes in target sites. The genes coding target site may be different than original thus different amino acid is coded making a different end product. Development of Rifampicin resistance is one of such examples (Campbell *et al*., 2001). Single step point mutations resulting into the amino acid substitutions in the rpoB gene (it encodes RIF binding pocket) (Floss and Yu, 2005). The resistance to Fluoroqinolones is also related to the mutation in target site. The DNA replication is altered by this antibiotic by inhibition of enzymes DNA gyrase and Topoisomerase IV. Chromosomal mutations in the genes encoding subunits of these enzymes (gyrA-gyrB and parC-parE for DNA gyrase and topoisomerase IV, respectively) is the common mechanism of acquired resistance to these compounds (Hooper, 2002).

Enzyme alterations of target sites

Enzymatic modification of target sites also makes the bacterial community resistance to certain drug molecule. Resistance to macrolide is the best example of this. The methylation of the ribosome catalyzed by an enzyme encoded by the erm genes (erythromycin ribosomal methylation) develops resistance against macrolide. 30 different erm genes have been described, which are distributed among different genera, including aerobic and anaerobic gram-positive and gram-negative bacteria. In MRSA (methicillin resistant *Staphylococcus aureus*) the most important erm genes are ermA genes are mostly distributed in a transposon.

2) Genetic basis of antimicrobial resistance

Microorganisms have remarkable genetic manipulation that helps them to prevent the detrimental effects of chemical compounds. They have the ability to respond to a wide range of environment threats including the antibiotics. They have developed the evolutionary mechanisms that enable them to survive the presence of antibiotics. There are two major strategies that enable the microorganisms to adapt and overcome the effects of presence of antimicrobials in its ecological niches. They are: i) Mutation in genes encoding the compounds or target proteins of antimicrobials. And ii) Horizontal gene transfer (HGT), through which they acquire resistant coding foreign genes from same niches (Ali *et. al*., 2018)

Mutation in gene(s)

The mutation that takes place in the genome of the susceptible population makes them resistance to certain antimicrobials (Munita and Arias, 2016). In presence of antimicrobials the cell population can survive with changes in response to the drug

molecule. This mutation sometimes renders the antibiotics ineffective because of the changes in the native structures or machineries within bacterial cell. Several types of mutation including random mutation can even enable the microorganism to survive in presence of antibiotics. Due to the mutations within themselves multidrug resistance (MDR) bacteria have been the reason of clinically relevant and life-threatening diseases and infections since mid-20th century. Random point mutation has recently been documented in in *Helicobacter pylori* (Klesiewicz *et. al*., 2014; Caliskan *et. al*., 2015; Eghbali *et al*., 2016). Within 23s rRNA the most common binding sites for antibiotics are present thus mutation within this region affects the inhibition capability of those drugs for which common mechanism of action is inhibition of transcription and translation. The mutation within 23s rRNA of *H. Pylori* prevents the Clarithromycin to act effectively. Similarly mutations within 23s rRNA of Staphylococcus has been shown related to the resistance to linezolid (Alonso *et. al*., 2014; Dong *et. al*., 2014; Ontsira Ngoyi *et. al*., 2015; Gu *et. al*., 2013). Mutations in bacterial gene brings about the change in antibiotic action via i) Decrease in drug uptake ii) modifications in the antimicrobial target eventually reducing the affinity for the drug iii) Enhancing efflux mechanisms, iv) Changing the metabolic pathways by modulating the regulatory network.

Horizontal gene transfer

Horizontal gene transfer also known as lateral gene transfer is the process by which bacteria acquires foreign DNA material. This genetic material which gets transferred is inserted into the genome. Thus it is one of the mechanisms by which bacteria acquires genetic materials that are responsible for development of resistance in bacteria (Burmeister, 2015). These genes then evolve along with pathogen producing new resistant strains which then show resistant to certain drugs exhibiting completely different behavior from the original population which is susceptible to those particular antibiotics (Harris *et. al*., 2010; Lindsay, 2013; McCarthy *et. al*., 2014; Stanczak-Mrozek *et. al*., 2015). This genetic material favored by natural selection, virulence determinants further evolve producing a whole new population of resistant bacterial community making the antibiotics ineffective(Burmeister, 2015). The AMR is associated with mobile genetic elements such as mobile introns; loss of genetic loci of the insertion site in the host; and acquiring an AMR gene through gene transfer (Lindsay, 2014; Mullany *et. al*., 2015).

The antimicrobials are derived product of the environment and bacteria also share the same environment and bacteria harbor intrinsic genetic determinants of resistance. Thus these “environmental resistome” acts as the prolific source that enables the clinically relevant bacteria to acquire antibiotic resistance genes in (Munita and Arias, 2016). These resistant genes are when present in multiple numbers of copies the

bacteria has high chances of surviving the presence of antibiotics. In gram-positive bacteria six copies of the gene 23s rRNA provides the ability to survive the harmful effects of linezolid (Marshall *et al*., 2002). Bacteria acquire external genetic material through three main strategies, i) Transformation, ii) Transduction and, iii) Conjugation (Munita and Arias, 2016)

Transformation is the genetic alteration in a cell as a result of the direct absorption, incorporation, and expression of exogenous DNA between closely related species bacteria, which is mediated by encoded proteins Lorenz and Wackernagel, 1994). This genetic material is naked and taken up by cell when it is in competent state. The DNA material is transferred to the surface then to cytoplasm through a array of layer and channels (Shintani, 2017). In transduction the transfer of genetic material is facilitated by the bacteriophages. The segment of host DNA gets packed up in capsids and then injected into the new environment during cell lysis. This genetic material gets recombined with core genome creating lytic or lysis cycle (Arber, 2014).

Other mobile genetic elements of their genomes, such as pathogenicity or genomic islands, and transposons can be transferred within the genome of bacteriophases from other bacterial species with the action of specialized enzymes. They have specific genetic topology which makes this possible (Juhas, 2015). Congugation is the main mechanism responsible for genetic material transfer in bacteria. It is considered major mode of transmission of antibiotics resistance genes and for the emergence of multi-drug amongst others (Lerminiaux and Cameron, 2019; Guglielmetti *et. al*., 2009). Conjugation is one of the most active ways of gene transfer, and it has been found responsible for the propagation of different antibiotic resistance genes in the Enterobacteriaceae family. Conjugative plasmids upon close contact between cells with the interaction of a highly specialized structure called pilus transfer the genetic materials. Thus the plasmid carrying structures, such as transposons, integrons, or insertion sequences that have antimicrobial resistance encoded in them, helps in propagation of resistance genes facilitating multi-drug resistance further (Bello-López *et. al*., 2019).

3) Other mechanism of resistance

Quorom Sensing

Quorum sensing is a cell-to-cell communication system which is is related to cell density. And the high-density colony populations accumulate or come together in a sufficient number and form a layer. Large number of small molecule signals is generated, activating a variety of downstream cellular processes, that includes virulence and drug resistance mechanisms, enabling the microorganisms to tolerate antibiotics, survive its detrimental effects. This is harmful to the host and causes the severities. Microbial

quorum-sensing systems are coordinated and capable of regulating microbial resistance mechanisms. It regulates different mechanisms such as drug efflux pump and microbial biofilm formation making the microorganism resistant to the drug molecule (Zhao *et. al*., 2020). Quorum sensing exists both in gram-positive and gram-negative bacteria.

The behavior of the entire bacterial population is controlled by synthesizing and secreting signal molecules. Many pathogenic and clinically relevant bacteria form a dense biofilm which makes them highly resistance. Bacterial QS systems can be divided into three types; QS system with acyl-homoserine lactone (AHL) as the self-inducible molecule, which exists in Gram-negative bacteria, the oligopeptides QS systems that are self-inducing molecules and exist in Gram-positive bacteria. Some bacteria use furan borate diesters as self-inducing molecules and exist in Gram-negative and Gram-positive bacteria. The increased cell density helps the bacteria to synthesize a large number of virulence factors, which increases the pathogenicity. This is a result of a response made by oligopeptide signal molecules that regulate gene expression and stimulate cells. The signal molecule oligopeptide secreted to the outside when reaches a certain concentration, it binds to the receptor protein on the cell membrane and pass the phosphorylation/dephosphorylation cascade to pass the oligopeptide to the intracellular binding promoter. This initiates the transcription mechanism and post-translational modifications that activate or inhibit the expression of the gene of interest (Monnet and Garden, 2015).

Multi drug resistance is achieved through QS by two ways - Regulation of biofilm associated EPS matrix and regulation of efflux pump genes. There is upregulation of efflux pump, which is controlled by QS. For example: in *E. coli*, overexpression of luxR homologue SdiA, a quorum sensing regulator, leads to overexpression of AcrAB efflux pump which positively affects the multidrug resistance (Rahmati *et. al*., 2002).

Biofilm formation

Biofilm is a group of microorganism and constitute of a complex assembly of protein, polysaccharide, and DNA in a self-produced extracellular polymeric matrix. It can be found on various surfaces, living tissues, medical devices, aquatic systems (Donlan, 2002). Biofilm communities have special structures that allow diffusion of nutrients, gasses, and antimicrobial agents through the biofilms. The quorum sensing molecules are exchanged along with the plasmids giving rise a heterogeneous community within biofilm layer. There is genetic exchanges, and other specific regulatory mechanisms which form the exclusive cell envelope composition and non-susceptible proteins within bacteria rendering the antibiotics ineffective (Singh *et. al*., 2017).

Biofilm formation contributes to the resistance by formation of capsule and through enzyme mediated actions. This capsule layer uses electrostatic, Van Der Waal and

hydrogen bonds forces for cohesion and helps in adhesion of the biofilm with the solid surface (Peña *et. al*., 2011) and this helps biofilm maturation (Sutherland, 2001; Abee *et al*., 2011). Anti-bacterial material are accumulated to the outer side, the adsorption sites of matrix limits the transportation, and exoenzymes prevents the mobility of these particles and provide resources for substrate of biocide metabolite degradation that slows down the activity of drug molecule (Singh *et. al*., 2017). The transformation of bactericides particle are transformed into nontoxic form for bacteria. This is enzyme mediated procedure that provides resistance to biofilm. Few species of bacteria reported for Degradation of the toxic compounds such as aromatic, phenolic and other heavy metals (nickel, cadmium, mercury etc) takes place in some bacteria. (Brown *et. al*., 1995) The reduction of ions and metals by enzymatic actions act as detoxification mechanism.

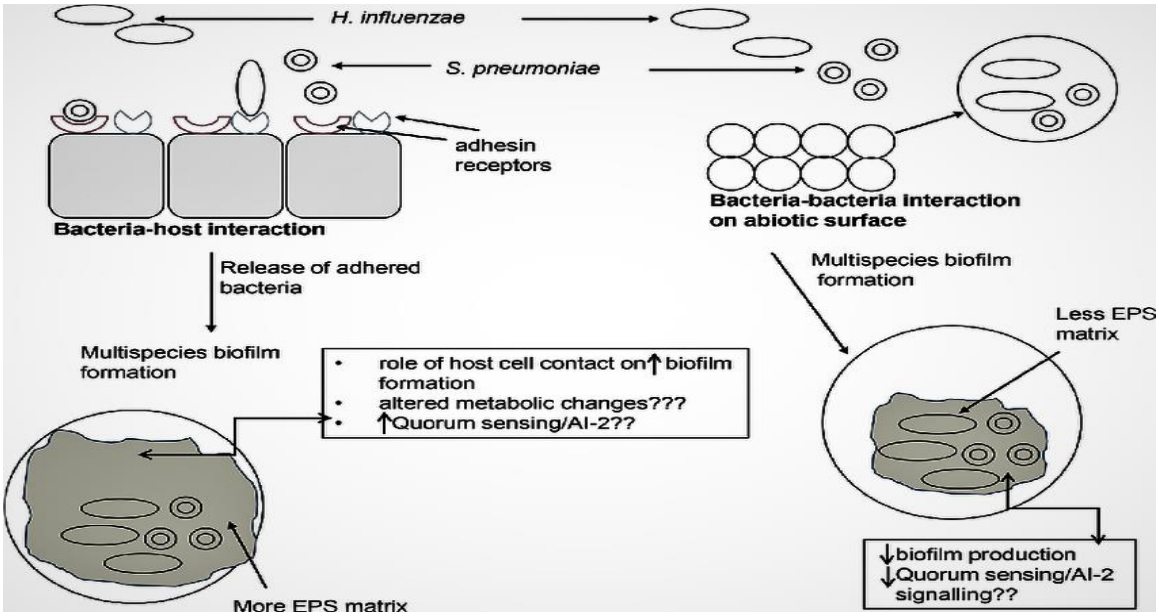


Fig: 13 Mechanisms involved in biofilm formation. The increase and decrease in biofilm production has been shown here in favorable and constrained situation (presence of antibiotics) (Krishnamurthy *et. al*., 2016).

2.2 Review of literature related to cob*A* gene (Uroporphyrinogen III methyltransferase)

Essential genes are the genes that are critical for the survival of organisms. The deletion or inhibition of these genes has lethal effects on microorganisms. Basic cell activities are severely influenced leading to the death of the cells so genes are considered as foundation of cellular life. These essential genes present in microorganisms are of great value for biomedical and clinical field. These genes can be targeted and inhibition system can be understood by studying the role of particular genes in microorganism and drug

candidate can be developed on this foundation. Pharmaceutical industries and drug discovery studies targets these genes and their products to develop antimicrobials for the treatment of diseases (Zhang *et. al.,* 2015). Uroporphyrinogen III C-methyltransferase (CobA) was identified as essential protein hence its inhibition is fatal to an organism and thus it can be targeted for drug discovery and can be used as potential therapeutic target (Tha *et. al*., 2018)

Structure of *cob*A gene

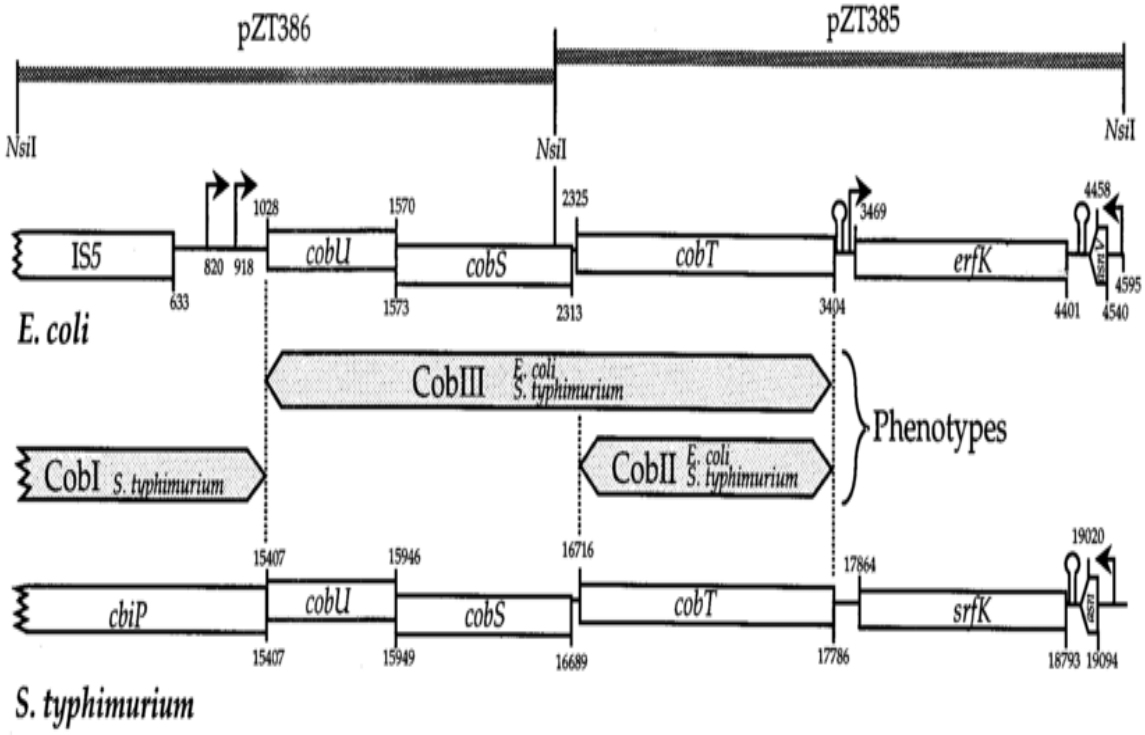


Fig: 14 Structure of the cob operon and the promoter-distal portion (Lawrence and Roth, 1995)

CobA is a member of methyltransferase family that catalyzes the two successive C-2 and C-7 methylation reactions which are involved in the conversion of uroporphyrinogen III to precorrin-2 via the intermediate formation of precorrin-1. It is a necessary step in the biosynthesis of both cobalamin (vitamin B12) and siroheme (Blanche *et. al*., 1989). CobA acts as a rate limiting enzyme which encodes S-adenosyl-L-methionine-dependent uroporphyrinogen III methyltransferase (SUMT), and converts the uroporphyrinogen to precorrin-2(dihydrosirohydochlorin) and later on forms cobalamin after incorporation with DMB(dimethyl Benzimidazole) (Piwowarek K *et. al*., 2018; Sattler *et. al*., 1995, Martens *et. al*., 2002). SUMT is a homodimer with around 280 amino acid residues of about 28 kDa molecular weight, has kidney like shape and consists of two domains connected to each other by a linker. N-terminal domain consists of a five stranded parallel β-sheet with topology 3-2-4-1-5 which is associated with four α- helices. C-

terminal domain is a five stranded β-sheet with topology 1-2-5-3-4 with three α- helices wrapped around it (Vévodová *et. al.,* 2004). At least 22 *Cob* genes have been identified and studied that are involved in the biosynthesis of vitamin B12 (Roth *et. al.,* 1996).

The genes involved in this whole reaction process are referred as cob I, Cob II and Cob III genes. Cob I genes encode the enzymes which catalyze the reactions taking places upto the formation of tetrapyrrole derived corrin ring. Genes encoding the enzymes involved in the formation of DMB are referred as cob II genes and final attachment of corrin ring and coordination catalyzing enzymes are coded by cob III genes (Martens *et. al.,* 2002). The biosynthesis of SAM requires methionine synthase which depends on Vitamin B12 in order to carry out the metabolic activities thus biosynthesis of nucleic acid and proteins are correlated (Fenech *et. al*., 2001).

Vitamin B12 also known as cobalamin is a water soluble vitamin and is synthesized by some archaebacteria and few other bacteria. Microbial biosynthesis of vitamin B12 occurs mainly through two routes: the aerobic or anaerobic pathway that takes place in bacteria and archaea, respectively (Yin *et. al.,* 2013). The coenzyme form of Vitamin B12, adenosyl cobalamin requires 30 enzymatic steps along with respective enzymes catalysing each step. The cobalamin formation takes place with initial formation of tetrapyrrole ring derived corrin structure which is originated from uroporphyrinogen as a result of 8 S-adenosyl-L-methionine-dependent methylations, ring contraction, cobalt chelation, decarboxylation, amidations, and 1-amino-2-propanol attachment. Dimethylbenzimidazole (DMB) nucleotide forms loop with central cobalt ion. Corrin ring attachment to DMB and addition of coordinating ligand for cobalt completes the complex structure of cobalamin formation.

The genetic complexity of this pathway via aerobic and/or anerobic pathway is present according to the nature of organism and its genetic constitution. Besides cob genes as mentioned above the hem BCD, Cbi (genes analogous to Cob I genes present in *Salmonella typhi* and others are involved depending upon the organisms involved. In *Pseudomonas* Vitamin B12 is produced only in aerobic condition. *E*. *coli* over producing strains constructed by deletion of the upstream region and by incorporation of a consensus RBS by site-directed mutagenesis and PCR to allow chemical and biochemical studies of the various intermediates on the cobalamin pathway showed the high levels of Cbi A proteins (Lanois *et. al.,* 1996).

The comparative studies performed with *Propionibacterium shermanii* and other species revealed that there is more than one pathway for corrin biosynthesis. The basic difference is timing of the cobalt insertion and the method of ring contraction (Balachandran *et. al*., 1994).

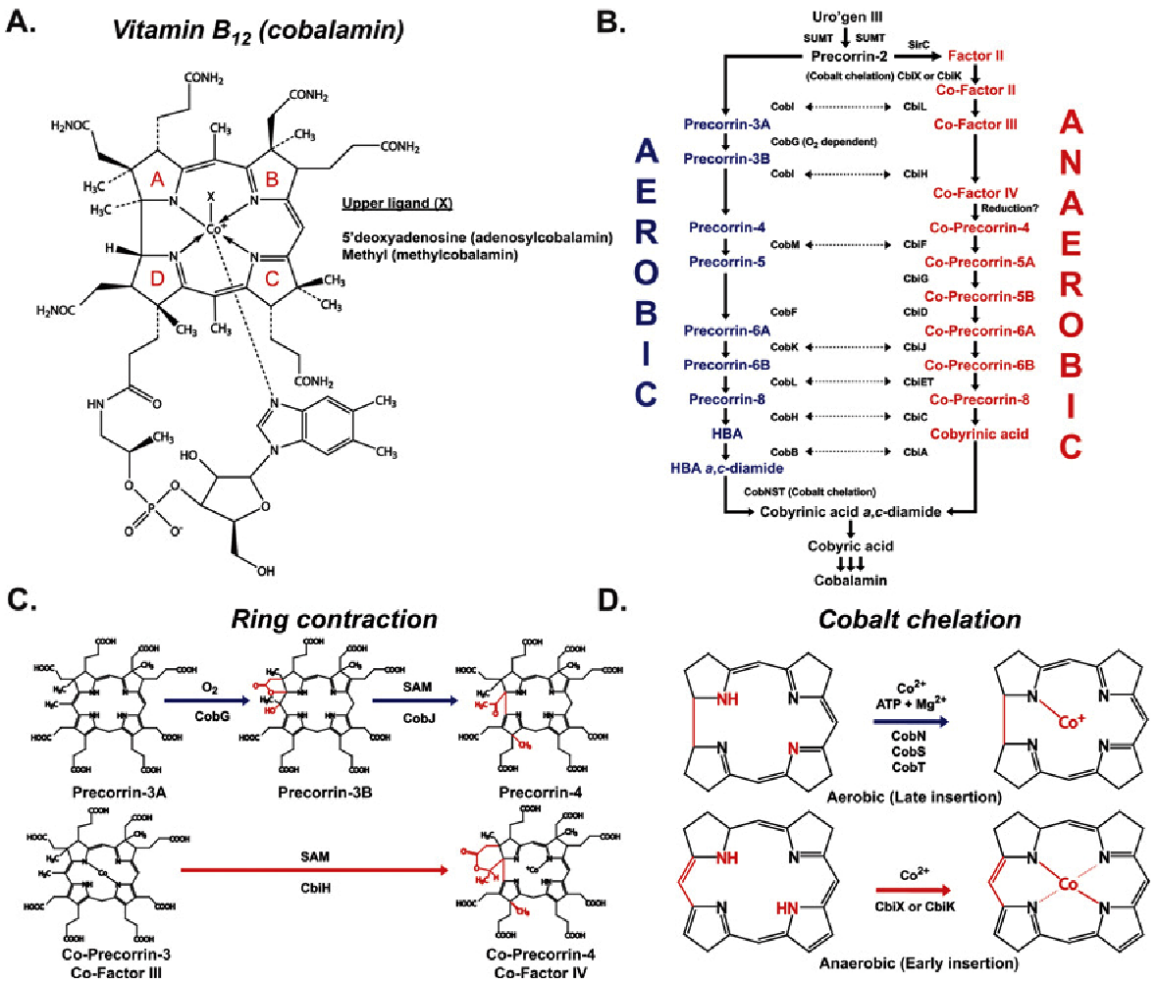


Fig: 15(A) Structure of vitamin B12, pyrrole rings labelled A–D in red (B) Aerobic and anaerobic pathway of Vitamin B12 synthesis. (C) Ring contraction. (D) Simplified diagram of cobalt chelation (Moore and Warren, 2012).

2.3 Literature review on Computer aided drug discovery (CADD)

Computer aided drug discovery also known as in silico drug design has gained popularity in recent years. Changing lifestyles food habits, environmental issues cause the outbreak of deadliest diseases time to time. Meanwhile there is growing emergence of resistance against different viruses and bacteria. So the need of novel antibiotics is felt all the time by the pharmaceutical and health fields. The concept of computer aided drug design was accepted in 20th century by the scientific society since then use of combinatorial chemistry and merging with various bioinformatics tools have been the major part of drug discovery process now (Talambedu *et. al.*, 2017). Drug development is a complex and very time consuming and costs billions. Moreover the chances of failure are always there from initial to preclinical and clinical phases. It takes about 10-16 years to introduce certain drug into the pharmaceutical practice and there involves wholesome

of a strategic and well implemented procedure with various steps to make it fruitful including identification of target, validation, lead identification, synthesis, candidate optimization, preclinical studies and researches, clinical trials (phase I, II and III), evaluation and getting approval by the FDA l and Phase IV studies (Paul *et. al*., 2010). Computational approach has thus been part of interdisciplinary drug discovery approach and considered more sophisticated. It is less laborious, cost-effective and less time consuming. So the pharmaceutical world has been widely using computational tools for the design and discovery of therapeutic products for different life threatening diseases (Talambedu *et. al*., 2017, Sliwoski *et. al*., 2014).

Computational strategies like high through put screening of molecules prioritize and identify small molecules for screening (Leelananda and Lindert, 2016) Further toxicity testing can be done in optimized conditional while saving cost and time, minimizing experimental testing, optimization of overall resources including the large number of animals used for clinical trials (Gillespie and McHugh, 1997). High throughput screening (HTS) has been used for very long time and is a traditional approach of discovering novel therapeutics. Virtual screening (VS) has emerged as a powerful complementary tool of CADD to HTS which is less expensive and gives hit compound within less time. The number of ligands can be significantly reduced making the screening procedure much convenient. New compounds or ligands can be discovered based on biological structure using computational tools by virtual screening methods (Shoichet *et. al*., 2004).

CADD provides a virtual platform in the journey of drug discovery process. The lead compound is identified for testing, its effectiveness, side effects and bioavailability is predicted and is thus a virtual shortcut in lengthy procedure of traditional drug discovery pathway. CADD has played a major role in the discovery of many pharmaceutical drugs available that have obtained FDA approval (Talele *et. al*., 2010; Clark 2006; Kitchen *et. al*., 2004). The carbonic anhydrase inhibitor dorzolamide (1995) (Talele *et. al*., 2010; Clark 2006) angiotensin-converting enzyme (ACE) inhibitor captopril (1981), a fibrinogen antagonist tirofiban (1998) and therapeutic agents for the treatment of human immunodeficiency virus (HIV): saquinavir (1995), ritonavir (1996) and indinavir (1996) are some successful drug discovered and approved novel therapeutics developed by using the computational tools of CADD (Talele *et. al*., 2010).

Computer Aided Drug Discovery (CADD) can be broadly studied into two categories; structure based and ligand based drug discovery approaches.

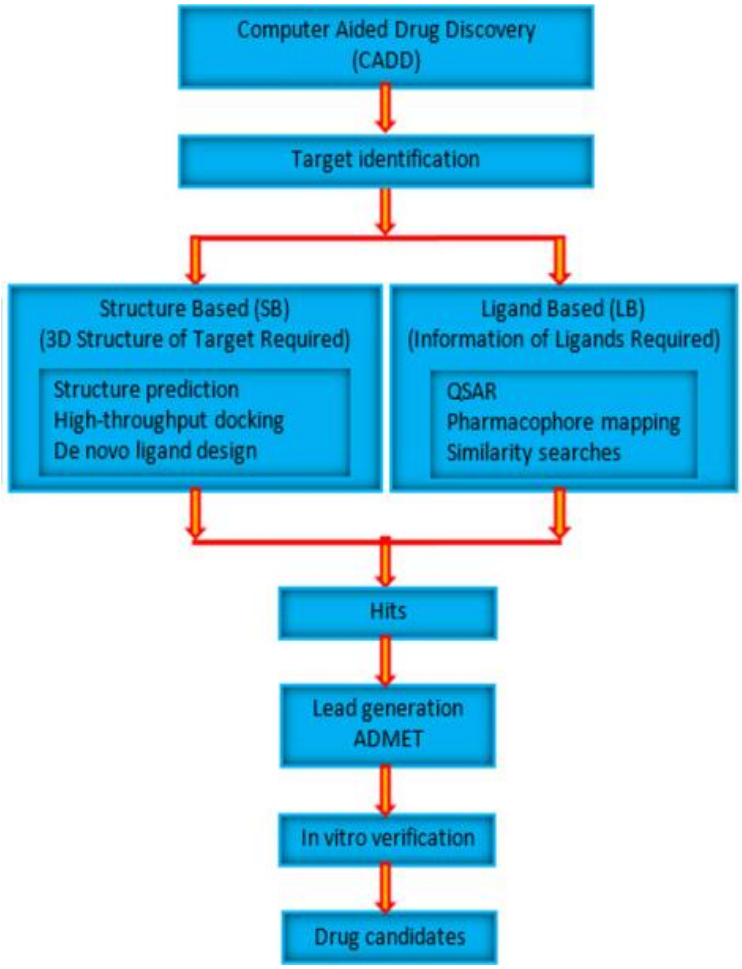


Fig: 16 Schematic representation of drug discovery using Computer Aided Drug Discovery (Leelananda and Lindert, 2016).

2.3.1 Ligand Based drug discovery (LBDD)

LBDD is the approach of drug discovery which is used when the 3D structure of the target is not available. The 3D structure of the receptor molecule or protein is not present and the ligands binding to it act as pivotal in the drug discovery procedure. In ligand based drug designing path the 3D quantitative structure activity relationships (3D QSAR) and pharmacophore modeling are most widely used tools (Aparoy *et. al*., 2012). QSAR is the method which correlates molecular structure with properties like in vitro or in vivo biological activity. This method can also be applied to toxicological data and it is called quantitative structure toxicological relationship (QSTR). QSAR deals with the physiochemical properties of the compounds (Winkler, 2002). The physical, chemical and biological properties of a molecule depend upon the geometric, steric and electronic properties that are responsible for the structure of any molecule. QSAR is based on this assumption assumption(Eleni *et. al*., 2003) and it analyzes the set of similar compounds and proceeds by correlating works by correlating the structural molecular properties of a

particular molecule with functions (i.e. physicochemical properties, biological activities, toxicity, etc.) (Merz *et. al*., 2010)

2.3.2 Structure Based drug discovery (SBDD)

The three dimensional structure of the target protein related to the disease is known in structure based approach of drug discovery. It is the most commonly used approach of CADD and has been able to play important role in the discovery of some FDA approved drugs available in market. SBDD methods study and analyze the information related to the structure of 3-dimensional macromolecular target. This may include proteins or RNA, the key sites within the macromolecule are identified and interactions that are important for their respective biological functions are studied in details. This information is very important and plays key role in the structure based drug discovery (Yu *et. al*., 2017).

Structure based drug discovery uses two methods in the procedure of drug discovery. They are molecular docking and de novo designing of ligands. De novo designed ligands can be inhibitors, antagonists, agonists. The X-ray or NMR structures of such molecules are used and these can be retrieved from different structural databases such as PDB (https://www.rcsb.org/pdb/home/home.do) and others. Different online tools and software available are utilized to generate the experimental structure through homology modeling if the structure is not found in PDB. Different strategies are utilized to gain the required structure by homology modeling. Some of them are very crucial and needs to be taken care of for better results. General plan for generation of protein model includes selection of a suitable template; align the target sequence to the template and finally building a model using the 3D co-ordinates of each atom of the template (Usha *et. al*., 2017; Sonnhammer *et. al*., 1998).

SBDD is comparatively effective and advantageous in the journey of drug discovery pathway. Hundreds of thousands of ligands can be studied, described and virtually screened as potential drug leads. The prior purchasing or synthesizing is not required which makes it relatively rapid in comparison to in vitro screening and is cost effective also. The large scale drug discovery programs also get benefited and SBDD is efficient for this as well. The computational strategies and software identify optimal binding modes of small-molecule ligands in the structure of a target and these binding modes are then scored for their non-covalent interactions (McInnes, 2007; Moitessier *et. al*., 2008). The molecular basis of a disease can be understood by analyzing and utilizing the knowledge of the three-dimensional (3D) structure of the biological target in the process. Similarly using different computational methods and the information from 3D structure of the protein target, the underlying molecular interactions involved in ligand-protein binding can be investigated which helps in the interpretation of experimental results in atomic-

level detail (Lionta *et al*., 2014).

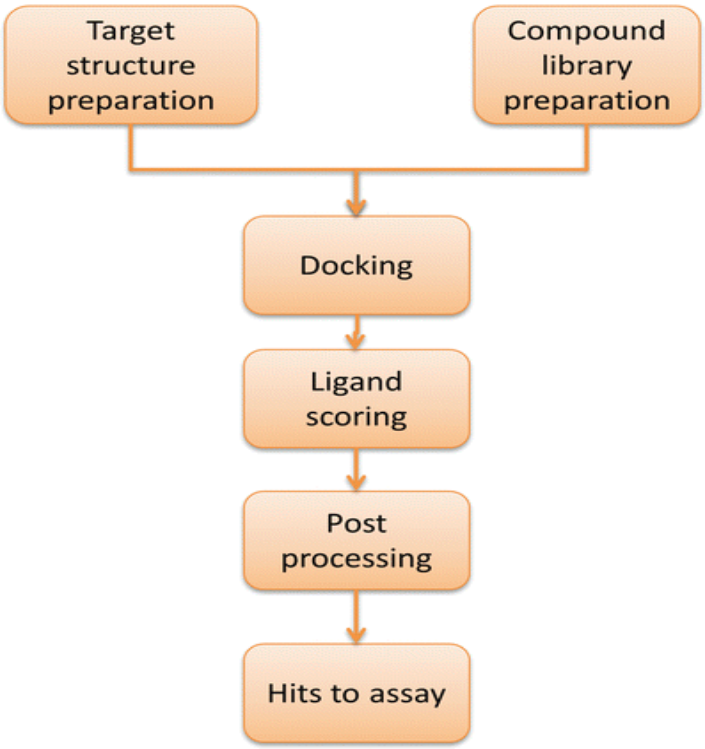


Fig: 17 General plan in the drug discovery pathway through structure based drug discovery (Cheng *et. al*., 2012)

The screening of compounds and finalization of specific compound during the course of drug discovery by SBDD initially focuses on the determination of protein structure or target. With the proper defining and understanding of target protein, numerous aspects are addressed including establishment of database library and the application of filters along with structural issues. After this docking and post processing is carried out which has post filters and other experimental assays to maintain accuracy (Reddy *et al*., 2007; Cheng *et al*., 2012; Lavecchia *et. al*., 2013)

2.3.2.1 Defining target protein (or preparation of drug target)

A well-defined drug target structure with quality 3D structure is the good starting for SBDD. The structure which is experimentally determined through X-ray crystallography or NMR techniques and deposited in the PDB is considered ideal for docking. Target structures are determined with greater efficiency by the application of structural genomics. Comparative models of target proteins are generated and have been used successfully in many docking and drug discovery process when experimentally determined structure are not available (Becker *et. al*., 2006; Budzik *et. al*., 2010).

Usually a structure present in repository like PDB contains water molecules, cofactors, activators, ligands, and metal ions as well as several protein subunits. However the detailed information regarding the information on bond orders, topologies, or formal

atomic charges is generally not given. Ionization and tautomeric states might not be assigned. Because of low resolution of a particular protein area and steric clashes may exist. Thus the structure preparation for target protein can address these issues (Pitt *et al*., 2013; Cheng *et. al*., 2012; Sastry *et. al*., 2013). Comparative modeling is used to predict target structure on the basis of a template with a similar sequence. Homology modeling is a specific type of comparative modeling which uses the template and target proteins with the same evolutionary origin. With advancement in computer technologies many web server and computer programs have been introduced which automates the comparative modeling procedure (Silowski *et. al*., 2014). MODELLER is one of the computer programs that help in the generation of target model (Marti-Renom *et. al*., 2000).

2.3.2.2 Binding site identification

The possible binding sites can be predicted from the interaction of natural or non-natural ligand with 3D crystal structure of target protein or such similar protein structures (Silowski *et. al*., 2014). The binding of ligand within specific area is associated with increase or decrease in its activity within a protein molecule (Leelananda and Lindert, 2016). This interaction determines the drug activity. Protein-ligand interaction is a prerequisite for drug activity (Laurie and Jackson, 2006; Henrich *et. al*., 2010). Active site water molecules also play important roles in ligand-target binding (Thilagavathi and Mancera, 2010). Accounting for water molecules has been quite a challenge in docking and different studies have focused on this issue recently (Abe *et. al*., 2008). Computational methods have been using geometric algorithms, methods based on energetic consideration and methods considering dynamics of protein structures (Silowski *et. al*., 2014)

2.3.2.3 Compound database selection and library preparation

The construction of compound databases is also the important step in the SBDD process. More diverse and structured database provides the efficiency in the screening process. Ligand libraries are constructed by enriching ligands for drug likeness or certain desirable physiochemical properties that are suitable for the target of interest (Silowski *et. al*., 2014) Some of the commonly used databases are ZINC (with 230 million compounds available in 3D format) (http://zinc15.docking.org/), PubChem (with 30 million compounds available in 3D format) (http://pubchem.ncbi.nlm.nih.gov). Databases are freely available or available via purchase or synthesis, and these are often drug-like small molecules with desirable characteristics such as stability and solubility in aqueous media and possess appropriate functional groups to interact with biological targets along with other important characters like absence of toxic and undesirable moieties. Sometimes a custom-made library is to be created by the users from the vast

databases available (Lionta *et. al*., 2014).

2.3.2.4 Filters

Physicochemical properties of the selected drug candidates, ligands and hits, affect their absorption, distribution, metabolism, elimination, and toxicity (ADMET) and, consequently, their drug-like properties which is very important for effectiveness of final product (W.P *et. al.,* 2011). The huge library needs to be screened for probable candidates thus to facilitate the decision-making process, and to increase the speed and probability of rapidly finding and developing high quality compounds, a variety of multi parametric guidelines, also named rules, have been developed, including the Lipinski's rules ('rules of five', Ro5) (Lipinski *et. al.*, 1997), extended Ro5 (eRo5), and beyond Ro5 (bRo5) (Doak *et. al*., 2016).

Similarly other rules like ligand-binding thermodynamic and kinetic profiles (Pan *et al*., 2014), and, more recently, the use of LE indices, also known as LE metrics have been introduced for this purpose (Abad-Zapatero and Metz, 2005). Similarly monitoring of molecular weight (MW) and lipophilicity during optimization also simplify drug-likeness prediction during the multipara metric optimization (Mignani *et. al*., 2018) process as pharmacological compound has to penetrate through various physiological barriers, such as blood-brain barrier (BBB) gastrointestinal barrier, and microcirculatory barrier. Similarly determining the potency and safety drug profiles of compounds in humans is also analyzed (Gardiner, 2006).The in silico modeling of ADMET properties predict the in vivo deposition behavior of potential drug molecules in the human body and gathers all the ongoing kinetic processes which helps to picture and gives understanding of how the predicted drug compound acts and gives the tentative idea of response by the human body towards the drug molecule (Mignani *et. al*., 2018). Most commonly followed in the drug discovery purpose for screening of suitable compound is Lipinski's rule of five (Lipinski, 2004). According to this rule the orally active drug has to follow given criteria for certain physico-chemical properties (with no more than violation of one criterion mentioned below):

Molecular weight < 500 Daltons

Calculated logP < 5

Hydrogen bond donors < 5

Hydrogen bond acceptors <10

An extension of the Ro5, bRo5, et al. and is also considered which is based on 475 complex drugs and clinical candidates against difficult targets that are outside the Ro5 space (Doak *et. al.,* 2016). This rule has an appropriate balance between rigidity and

flexibility to bind to the considered targets, and can be defined as follows: MW >500, cLogP 7.5, HBD >5, HBA >10, PSA >200 Å2, and NRB >20. The main targets involved in the bRo5 space include G-protein-coupled receptors (GPCRs), proteases, hydrolases, transferases, isomerases, as well compounds such as enzyme regulators (Mignani *et al*., 2018).

Online tools for library design such as CLEVER (Chemical Library Editing, Visualizing and Enumerating Resource) helps in chemical library manipulation, combinatorial chemical library enumeration using user-specified chemical components, chemical format conversion, as well as chemical compound analysis and filtration with respect to druglikeness, lead-likeness, and fragment-likeness based on the physicochemical properties computed from the derived molecules. It also provides an integrated property-based graphing component that visually depicts the diversity, coverage and distribution of selected compound collections (Lionta *et. al*., 2014). Knowledge of the binding kinetics between a drug and its target is also taken as a powerful parameter of drug-likeliness filter for drug discovery with good-quality compound selection criteria during the hit and lead optimization phase. Slow offset, slow off-rate, slow dissociation, insurmountable antagonism, ultimate physiological inhibition, tight binding, and non-equilibrium blockade are the main binding kinetic profiles which are considered during drug discovery for optimization steps (Keighley, 2011).

2.3.2.5 Docking and scoring

Molecular docking predicts the protein-ligand complex structure and is followed by scoring in virtual screening procedure in SBDD in order to rank the compounds. Docking programs utilize different methods of conformational search and explore the ligand conformational space; these can be categorized as follows: a) Systematic methods, which place ligands in the predicted binding site considering all degrees of freedom, b) Stochastic torsional searches about rotatable bonds, such as Monte Carlo and genetic algorithms to determine low energy conformers, (c) Molecular Dynamics simulation methods and energy minimization for exploring the energy landscape of a molecule (Guido *et. al*., 2008).

Large number of docking programs have been developed and used for docking purpose. AutoDock (Morris *et. al*., 2008), Dock (Ewing *et. al*., 2001), Gold (Jones *et. al*., 1997), FlexX (Rarey *et. al*., 1996), Surflex (Jain, 2003), and eHiTS (Zsoldos *et. al*., 2003) are some of the programs used for docking.

Autodock uses an interaction grid to account for receptor conformations and Monti Carlo simulated annealing, evolutionary genetic and Lamarckian genetic algorithm methods to account for ligand conformations (Morris *et. al*., 2009). It is also used with

AutoDock Tools (ADT) (Cosconati *et. al*., 2010). Details will be discussed further.

GOLD (Genetic Optimization of ligand docking) uses genetic algorithms. It allows partial protein flexibility and explores the full range of ligand conformational flexibility with partial flexibility of the protein. It can work even in presence of water and ion molecules in the active site of proteins (Jones *et. al*., 1997).

FlexX uses incremental construction for ligands (Rarey, 1996).

Docking programs utilize scoring functions in order to rank compounds and estimate the free energy of binding of a ligand to a specific target and is based on a docked pose generated after docking of protein with different ligands of a database. Several scoring functions have been developed. They can be categorized as follows: (a) Force field-based functions which estimate the binding free energy by adding the strength of intermolecular van der Waals, electrostatic interactions and hydrogen bonding between all the atoms of the protein and ligand involved in complex along with the Solvation and entropy. (b) Empirical scoring functions that are based on counting the number of various types of interactions between hydrophobic contacts, number of hydrogen bonds and number of rotatable bonds immobilized in complex formation. (c) Knowledge-based functions that use statistical observations of intermolecular contacts in receptor-ligand complexes with known structural conformations (Lionta *et al*., 2014).

2.3.2.6 Compound selection after docking (post processing)

In SBDD the expert chemist is required for compound selection and post processing after the virtual screening to finalize the compounds for experimental trial and is considered the rate limiting step. Simplified scoring functions and the inadequate sampling of the conformational space for the ligand lead to unrealistic poses, intra-ligand steric clashes, twisted amides, E/Z esters, imperfect hydrogen bonding network, and poses based on shape complementarity. This result into an unreasonably high score so such compounds need to be discarded. Therefore, visual inspection and careful analyzing of thousands of docking poses and interatomic contacts between docked ligands and target is done for efficient and reliable outcomes (Athanasiadis *et. al*., 2012; Waszkowycz, 2008).

2.3.2.7 Sotwares used in this study

i) Auto Dock

Auto dock is a suite of automated docking tools used to design to predict how small molecules such as substrates or drug candidates, bind to a receptor of known 3D structure. This software is very fast, provides high quality predictions of ligand conformations, and is able to show good correlations between predicted inhibition constants and experimental ones. Auto Dock can also be used in blind docking when the

binding site is unknown (http:// autodock.scripps.edu/). It was developed in the 1990s at the Scripps Research Institute. It has been used widely in number of researches, studies and for educational purpose (Morris *et al.*, 2009). The graphical user interface (GUI) of AutoDock provides the opportunity for students to view proteins in three dimensions. This allows the user to understand tertiary structures more effectively. Further students can view surface representations of protein crystal structures to demonstrate that finite ligand binding regions exist. This helps in understanding the mechanisms involved in protein–ligand interactions and provides the unique opportunity for students to explore protein structures in three dimensions (Helgren *et al*., 2017).

This docking tools tests and ranks the geometry of the drug compounds or the ligands under study perhaps based on modifications of an existing lead compound, or to screen entire databases of available molecules, searching for novel compound (Goodsell *et al*., 1996). Ligands are regarded as rigid bodies or with torsional flexibility; scoring is made possible by shape complementarity or with detailed energetic models; employing exhaustive searches or more limited search techniques (Lybrand, 1995; Rosenfeld *et al*., 1995). It uses simulated annealing for searching conformations, allowing several torsional degrees of freedom in a flexible ligand to be searched. A grid-based technique is used for energy evaluation at each step of the simulation (Goodsell and Olson, 1990).

Autodock Tools (ADT) is a graphical user interface which helps to set up which bond is to be treated as rotatable in the ligand and to analyze dockings. It has improved graphical front-end for Autodock and AutoGrid. It runs on Linux, Mac OSX, SGI IRIX and Microsoft Windows. With this software we can viewew molecules in 3D, rotate & scale in real time, add all hydrogens or just non-polar hydrogens, assign partial atomic charges to the ligand and the macromolecule (Gasteiger or Kollman United Atom charges), merge non-polar hydrogens and their charges with their parent carbon atom, Set up rotatable bonds in the ligand using a graphical version of AutoTors, set up the AutoGrid Parameter File (GPF) using a visual representation of the grid box, and slider-based widgets, set up the AutoDock Parameter File (DPF) using forms, Launch AutoGrid and AutoDock,read in the results of an AutoDock job and graphically display them and view isocontoured AutoGrid affinity map (Morris, 2007). Autodock Tools-1.5.6 was used in this study. It was developed by the Scripps Research Institute in python programming language. The release of MGL tools 1.5.6 (ADT) was announced in 2012 February 2 (http://mgltools.scripps.edu/News/mgltools-1-5-6-release-announcement).

Ii) Osiris datawarrior

Datawarrior is an open source data visualization and analysis program Data visualization and analysis software with sufficient chemical intelligence. It runs on all major operating system and was developed in Java programming language by Actelion Pharmaceuticals

Ltd. In 1998 which has been updated and added with novel features time to time at Actelion/Idorsia Pharmaceuticals Ltd. DataWarrior combines dynamic graphical views and interactive row filtering with chemical intelligence. Numerical and categorical data along with chemical information as shared scaffolds and compound substitution patterns is visualized by scatter plots, box plots, and bar or pie charts visualize numerical or category data along with. Chemical descriptors independently encode various aspects of chemical structures, e.g. the chemical graph, chemical functionality and 3-dimensional pharmacophore features. ADME/Tox properties are used as filtration measure to generate library of preferred compounds with drug like properties. Different kinds of molecular similarities is calculated and used used in graphical views or for row filtering and other purposes. Special features are inbuilt which support different stages of drug discovery from the screening of compounds through structure activity analysis to the statistical interpretation of animal experiments (Sander *et al*., 2015). In this study Osiris datawarrior version 5.2.1 ©2002-2020 Idorsia Pharmaceuticals Ltd. was used. It is highly integrated into the platform, connected to databases and other tools.

(https://openmolecules.org/datawarrior/index.html).

iii) PyMol

PyMOL is a Python-enhanced molecular graphics tool which is open source visualization software that can be used in drug discovery process for 3D visualization of proteins, small molecules, density, surfaces, and trajectories. It also allows molecular editing, ray tracing, and movies (https://fossies.org/linux/privat/pymol-open-source-2.4.0.tar.gz/index_tp.html). This has many features including the active sites determination within protein and protein sequence visualization. It can be extended to python plugins because of the language it is written in. PyMOL is one of the most widely used macromolecular visualization tools. This software can be used not only in macromolecular visualization but also for macromolecule editing (Yuan *et al*., 2017). PyMOL has been successfully used in the discovery of new drug candidates for various targets such as in the discovery of a potent small molecule inhibitor for gankyrin (Thakur *et al*.,2011) lead optimization for Cytochrome P450 enzymes (Danielson *et al*., 2011) and VS of new drug candidates for the tumor suppressor protein P53 (Pereira *et al*., 2016).

PyMOL was created by Warren Lyford Delano and commercialized initially by DeLano Scientific LLC. In 2010, after an agreement was done with Schrödinger Inc. Since then PyMOL is developed, maintained, supported and sold and all the current subscription is regulated by Schrödinger Inc (Yuan *et al*., 2017). The PyMol Molecular Graphic System Version 2.4.0 Copyright Schrödinger, LLC was used in this study.

iii) Discovery Studio Visualizer

The BIOVIA Discovery Studio Visualizer is a suite of software, free, feature-rich molecular modeling application which allows viewing, sharing and analyzing protein and small molecule data. It offers an interactive environment for viewing and editing molecular structures, sequences, X-ray reflection data, scripts, and other data relevant to life science researchers. Discovery studio visualizer supports a range of stereo graphical options (E.g., split screen, hardware stereo). It is designed to enable hardware graphics acceleration which supports advanced visualization options such as depth cueing, blur and shading capabilities. Some other features include visualization capabilities such as:

- Sequences, including Chain view support for multi-domain proteins (E.g., Antibodies)
- 2D and 3D Charting, Histograms, Heat maps and Data tables
- Map interactions using a comprehensive set of favorable, unfavorable and unsatisfied non-bond monitors
- Interactivity between multiple graphical views on the same data

(https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/visualization/).

It is developed and distributed by Dassault Systemes BIOVIA. Discovery Studio Visualiser v16.1.0.15350.2015 by Dassault Systemes Biovia Corp. was used in this study.

iv) PyRx

PyRx is open sourcse Virtual Screening software that can be used to screen libraries of compounds against potential drug targets in Computational Drug Discovery approach. Virtual Screening of compounds is possible from any platform and it helps users in every step throughout this process. PyRx wizard features facilitate easy-to-use user interface and chemical spreadsheet like functionality that makes it a valuable tool for Rational Drug Design in the drug discovery process. It is widely common for structure based drug discovery process as it includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for structure based virtual screening (https://sourceforge.net/projects/pyrx/).

PyRx is written in python programming language. It is a GUI (Graphic User Interface) that uses a large body of established open source software such as:

- AutoDock 4 and AutoDock Vina are used as docking software.
- AutoDockTools, used to generate input files.
- Python as a programming/scripting language.

- Python for cross-platform GUI.
- The Visualization ToolKit (VTK) by Kitware, Inc.
- Enthought Tool Suite, including Traits, for application building blocks.
- Opal Toolkit for running AutoDock remotely using web services.
- Open Babel for importing SDF files, removing salts and energy minimization
- matplotlib for 2D plotting

(https://cac.queensu.ca/wiki/index.php/HowTo:pyrx)

AutoDock Vina is open source software used for docking, designed and implemented by Dr. Olegg Trott in the Molecular Graphics Lab at The Scripps Research Institute. It is easy to use and flexible. It only needs the structure of molecule to be docked and specification of search space including the binding sites. Vina uses the PDBQT file format for both input and output as used by AutoDock (http://vina.scripps.edu/).

Open babel is an established software within PyRx generates PDBQT file format. It uses Lipinski rules of five for filtering molecules and converting the different format files and energy minimization is performed with the universal force field (UFF) (Aissouq *et al*., 2021) using the conjugate gradient algorithm. It can be done in interactively or in batch. It can read write and convert it to about 110 file formats (http://openbabel.org/wiki/Main_Page)

vi) Ligplot^{+}

LigPlot^{+} is a successor to the original **LIGPLOT** program which is used for automatic generation of 2D ligand-protein interaction diagrams. It runs from an intuitive java interface which allows on-screen editing of the plots. It is an interface to the LIGPLOT and DIMPLOT (a program for plotting protein-protein or domain-domain interactions) programs which generates schematic diagrams of protein-ligand and protein-protein interactions respectively. Users can flexibly select the interface of interest can be selected and **DIMPLOT** generates a diagram showing the residue-residue interactions across the interface. The residues in one of the interfaces can be optionally displayed in sequence order for further interpretation (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). LigPlot^{+} v.1.4.5 by Roman Laskowski, 2009 was used in our study.

vi) Gaussian

Gaussian is an electronic structure program. It is a computational chemistry software package. It was initially released in 1970 by John People (Pople, 2004) and his research group at Carnegie Mellon University as Gaussian 70 (Hehre *et al.,* 1970*).* It is widely used by chemists and scientists for analysis of computational data. Gaussian 03 version 6.0 by

Gaussian Inc. was used in this study.

vii) Chemcraft

Chemcraft is a graphical program for working with quantum chemistry computations. It is a convenient tool for visualizing computed results and preparing new jobs for a calculation. Chemcraft is mainly developed as a graphical user interface for the GAMESS (US version and the PCGamess/Firefly) and Gaussian program packages.

3. MATERIALS AND METHODOLOGY

3.1 Protein or target structure retrievation

Completion of human genome project provided the knowledge of human genome and understanding the genetic depth within human body the scope of therapeutic targets in drug design and discovery has broadened. Meanwhile the advanced strategies such as excessive-throughput crystallography, protein purification, and nuclear magnetic resonance (NMR) spectroscopy have been providing structural information of protein complexes, protein-ligand interactions. This has helped in the development of computer aided drug design and molecular docking (Bajorath, 2002).

Structure based drug discovery methods of docking system is used when the structure of target protein is present. And there is prior knowledge and information about the target structure which can be employed through calculation of interaction energy for all compounds tested.

Methyl transferases have been found to be essential for bacterial survival (Tha *et. al*., 2018). CobA being one of them was used as major antimicrobial target in this study. The crystal structure of CobA protein (PDB ID: 1S4D) encoding S-adenosylL-methionine-dependent uroporphyrinogen III methyltransferase (SUMT), from *Pseudomonas denitrificans* complexed with native ligand S-adenosyl -L-homocysteine was retrieved from Protein Data Bank (PDB)(Vevoda *et. al*.,2004). The crystallographic structure with 2.7 A^0 resolutions was obtained in PDB format. Vitamin B12 is very essential for bacterial survival thus it can be used as a probable therapeutic target for drug discovery and drug repurposing.

3.2 Ligand database/ drug structure selection

Many studies have shown that the similar compounds exhibit similar physico-chemical and biological properties so chemical database with structural diversity is always considered an ideal for lead identification in drug discovery. For this study natural products library was obtained from Zinc 15 database. It is provided by Irwin and Shoichet laboratories in the department of pharmaceutical chemistry at the University of California, San Fransisco (UCSF). It is a database of commercially available compound in ready-to-dock, 3D formats for virtual screening (https://zinc15.docking.org/).A ligand database containing 205 molecules was prepared that included natural products. Similarly the ligand library of kinase Inhibitors was obtained from the UORSY database in sdf format. UkrOrgSyntez Ltd. is a leading global provider of quality screening libraries and building blocks for small molecule drug discovery. It was founded in 2001 (https:// www uorsy.com/). In this study a ligand database of 6449 kinase inhibitors was obtained from UORSY was prepared and processed for ADME/Tox filters for docking through

OSIRIS. A ligand library containing 1685 molecules was prepared. Further Nucleoside Mimetics from ASINEX database was also used for docking. ASINEX is actively providing screening library of lead-like molecules since 1994. Nucleoside like core intermediates (with adenosine moiety) has been prepared with addition of varous long chain amines, acids and amino acids (http://www.asinex.com/). Ligand library of about 654 molecules containing Nucleoside Mimetics were prepared and used for docking in this study.

10342 indole derivatives were obtained and a library was prepared from different random sources including https://www.chemdiv.com/screening-libraries11/, Pubchem. The ligand database consisting of 462 indole derivatives was prepared and used for docking purpose.

S- Adenosylmethionine (Compound CID: 34755**)** and S-adenosyl -L-homocysteine (Compound CID: 439155) were also obtained from Pub Chem. Pub Chem is an open chemistry database at the National Institutes of Health (NIH). PubChem mostly contains small molecules, but also larger molecules such as nucleotides, carbohydrates, lipids, peptides, and chemically-modified macromolecules along with information on chemical structures, identifiers, chemical and physical properties, biological activities, patents, health, safety, toxicity data, and many others (https://pubchem.ncbi.nlm.nih.gov/).

3.3 Preparation of protein for docking

Pyrx virtual screening software was used for screening that has open source established software Autodock vina that uses PDBQT file format for both the input and output files. The crystal structure of CobA protein retrieved from RCSB (PDB ID: 1S4D) encoding S-adenosylL-methionine-dependent uroporphyrinogen III methyltransferase (SUMT) was in PDB format. The structure obtained was huge with identical 12 chains (A,B,D,E,F,G,H,I,J,K,L,M) with sequence length of 280. Each chain has 3 molecule of glycerol and a SAH attached to them. The PDB file obtained was opened with PyMol. Then the sequence was displayed by clicking the S option in the lower right corner. And all the chains except A and B were removed. Then the ligands and water molecules were also removed and the file was saved in PDB format. The PDB file saved earlier was then opened with Autodock Tools (MGL tools 1.5.6) by clicking on file area and choosing the file saved. Under ‘edit’ option, add hydrogen, merge non-polar and finally gasteiger charge was computed under ‘edit’ option and ‘charges’ subheading. Then under grid option macromolecules was chosen a dialog box with file name appeared which was selected by clicking it and then the select molecule option at bottom thus file was saved in PDBQT format, the readable file format by PyRx interface used for docking.

3.4 Preparation of ligand library

The ligand libraries obtained from different sources were in .sdf format. To make the efficient selection of ligands these need to have drug like properties. This is called drug likeliness. The drug likeliness selection criteria for ligands ensure the discovery of the promising lead compounds by dealing with certain pharmacokinetic properties as well as toxicity issues. Sometimes we need to set the parameters more than one time similar as above. After this under file option visible as option was clicked and file was saved.

OSIRIS data warrior program predicts the drug likeliness and toxic profiles of the ligands based on the binding energy and helps in the preparation of ligand library consisting of ligands which encompass the filter within OSIRIS as a measure of selection and rejection in final prepared library (Toepak and Tambunan, 2017). OSIRIS calculate various drug like properties like molecular weight, cLogP, cLogS, Drug likeness, and toxicities like mutagenicity, tumorigenicity, reproductive effects and irritant effects are calculated in the lead molecules (Sander *et. al.*, 2015). For this the ligand library were obtained in sdf format and opened with OSIRIS program installed in computer. Under chemistry at menu bar chemical structure was clicked, under which one find the calculate properties option where different properties are set as per our criteria. For this we set the parameters as below:

Screening of ligands (ADME/Tox)

Total Molecular weight: 200-500 Daltons

cLogP: -3 to +6

cLogS: -2-4

Hydrogen bond acceptor : 0-10

Hydrogen bond donors : 0-5

Topological surface area : 0-120

Drug likeliness : positive value (as default)

LE/TOx/S criteria

Mutagenesis : None

Reproductiveness : None

Tumorogenicity : None

Irritant : None

Rotable bonds: 0-10

This saves the file in data warrior-SD-file(.dwr) format, thus saved file was opened again with OSIRIS and under file option on menu bar, save special with sdf was selected and 2D on the dialogue box made 3D and file was finally saved in (.sdf) format. OSIRIS calculates and converts ligand library with large number of ligands at once into required file format (.sdf). The ligands can be appended together and properties can be calculated for drug likeliness and toxicity profile when they are present as a single file of many ligands. Initially one file is opened under file option append file is selected then the folder containing the data warrior-SD-files to be appended is selected. In existing data set we keep the first compound name then click ok and then another file is added in the similar way and calculated properties, parameter is set and file is saved.

3.5 Active binding sites prediction with PyMol

Active site prediction is necessary to find out the specific functional part of the protein therefore, the prediction of protein domains is crucial to understand the protein function (Adi *et. al*., 2016). Thus, active site residues within 3D structural protein were identified by using PyMol. For this the protein was loaded in PyMol window along with native ligand (the PDB file). Under sele option at the right A was selected then, under rename option protein was named. Then in command section of PyMol window, command was given as: show lines byres all within 5 of name of protein (the one we named earlier with). Under hide option hide cartoon was clicked. Ligand was zoomed out by selecting it and clicking Zoom option. After this colour option was selected from ligand, under this by element was chosen. This makes the ligand distinct from protein. Ligand was then deselected and every side chain of the protein (not ligand) was selected and active sites was clicked under rename in sele under subheading A (action). Under active sites selection of option names of active residues L followed by the residues shows active binding sites within the protein-ligand interaction. This method was used to determine the binding sites between protein and native ligand SAM and used in docking later.

3.6 Virtual screening and docking

PyRx was used for virtual screening. It includes AutoDock 4 and AutoDock Vina that are used as docking software. PyRx has GUI which has established open softwares. Obabel is also one of them. By using Lipinski rules of five it filters and minimizes energy and converts the file into PDBQT or readable form By Auto Dock. Auto dock Vina converts the ligand library to be docked into PDBQT format.

Native ligand (SAM) was used as reference for selection of ligand after the docking. The ligands having binding energy higher than that of the negative ligand is searched and

used for further procedure of drug screening. The binding energies of all the docked ligands in the target proteins are computed in KJ/mol. More negative the value of binding energy with which the ligands bind, the stronger is the bond between ligand and active site. HMAT1A was also used for this purpose.

For docking initially all the contents of PyRx workspace was deleted. The ligand library was copied. PyRx was opened on the computer and ligands were loaded in ligand option of PyRx workspace that we emptied earlier. Under load molecule option from file at top of PyRx window Protein prepared in PDBQT format was added. Protein was selected and with right click Auto Dock was selected under which make macromolecule option was clicked and then start option was selected. The ligands loaded were selected, and refresh option was selected after right click. Then forward option at the bottom was selected the grid box appeared. This grid box was made as small as possible while keeping active sites inside it. Under coordinates option X, Y and Z coordinates of center and dimensions were noted. This is used further for docking with other ligand library. The number of chains in the protein can be used for docking according to the need of experiment. With left click plus option inside box active residues can be selected for the docking purpose. Under parameters option exhaustiveness and number of modes were added. Then under Vina Search Space on the Maximize button was clicked and then clicking on the forward button at right bottom starts AutoDock Vina and docks each ligand one by one.

3.7 Visualization of protein-ligand interaction

3.7.1 Visualization of protein ligand interaction using PyMol

The preferred ligand and protein were opened in the same PyMol window saved in pdb format. This file was reopened with PyMol and amino acids involved in protein –ligand interaction were observed along with polar interactions and interacting amino acid residues. Same protocol was followed as mentioned in active bindig site prediction. After active residues appeared, ligand was selected and from find option polar contacts to any atoms was selected to find out polar contacts formed in the protein-ligand interaction.

3.7.2 Visualization of protein ligand interaction using Ligplot

The PDB file of protein and ligand was opened with ligplot from PDB option under open from file located at upper right corner. Then browse opton was selected and by selecting run under default condition Ligplot visualization map was observed for hydrophobic interactions along with possible hydrogen bonding in protein-ligand interaction.

3.8 Density Functional Computations (DFT) using Gaussian 03

DFT calculations were measured on GAUSSIAN 03 platform to interpret the atomic arrangement of studied compound, and were optimized using B3LYP/ 631G basis set to establish the geometry theoretically and study further. Frontier molecular orbital studies and molecular electrostatic potential map (MEP) was prepared and visualized using Gauss view and the compounds under study were analyzed using the information obtained.

Gauss input file was prepared initially. For this the pdb file of docked ligand structure was prepared in PyMol by adding hydrogen. Then it was opened in Gauss view and parameter was set for calculate Gaussian calculator option, under this further parameters were selected in job type option as Optimization + frequency and option for Raman computation with yes option. Similarly under in method option DFT was set as computation method with B3LYP/631G basis set. Another in title option has to be filled for file name and under link zero checkpoint file and read write file was kept default name and memory limit specified as 1GB. Then retain option was selected and file was saved. File name has to be saved without any space in between and specification has to be given under file type as Gaussian input file.

Gaussian input file was then opened in Gauss 03 and the content of in % selection was copied upto slash sign (/) and this was pasted in % rwf section. Below this under in route section option genome+ connectivity and space before it was removed and finally in molecule specification section all numbers and spaces below C and H was removed then under file option exit and run was selected for Gauss run. For visualization and preparation of MEP and observation of vibrations chk file obtained after Gauss run was used. Frontier orbital studies was done to obtain highest occupied molecular orbital (HOMO) and lowest occupied molecular orbital (LUMO) and HUMO-LUMO energy gap. Similarly Gaussian output file was studied for observing charge distribution. This gives us the regions of positive electrostatic potential and negative electrostatic potential.

4. RESULTS AND DISCUSSION

The extensive variety of computational tools used in drug discovery has broadened the dimensions of the computer aided drug discovery and has made possible the discovery and development of number of novel therapeutics. The emergence of multidrug resistant pathogens has been a challenge so there has been an increasing interest in the search for novel and effective antimicrobials. Implementation of computational tools in the drug discovery process and lead molecule identification has saved both time and expense unlike traditional method of drug discovery which was expensive, quite laborious and seemed a lengthy procedure (Meng *et. al*., 2011)

4.1 Target protein selection

Humans do not biosynthesizes vitamin B12 and consumed through food, thus vitamin B12 biosynthesis could be one of the avenues to develop drug against MDR pathogens The CobA was used as a potential target protein in this insilico screening study. Based on earlier work *cob*A was found to be essential gene for vitamin B12 biosynthesis and survival of bacteria in *Salmonella typhimurium* (Tha *et. al*., 2018). The proteins whose deletion causes inhibition of bacterial growth are valuable from pharmaceutical prospects. The crystal structure of CobA protein (PDB ID: 1S4D) encoding S-adenosylL-methionine-dependent uroporphyrinogen III methyltransferase (SUMT), from *Pseudomonas denitrificans* was used for virtual screening. SUMT is a branchpoint enzyme and plays a key role in the biosynthesis of modified tetrapyrroles. It is responsible for controlling flux to compounds such as vitamin B12 and sirohaem, and catalyses the transformation of uroporphyrinogen III into precorrin-2(Martens *et. al*., 2002)

SAM utilizing protein CobA (Uroporphyrinogen III methyltransferase) has critical role in vitamin B12 biosynthesis pathway (Sattler *et. al*., 1995) and thus cell membrane formation in bacteria through lipid biosynthesis (Romine *et. al*., 2017). SAM acts as a methyl group donor indifferent biological events and biochemical pathways thus biological substrates like RNAs, proteins, lipids and secondary metabolites (Chiang *et. al*., 1996). S-adenosyl methionine is considered to be the common co-substrate involved in methyl group transfers (Gao *et. al*., 2018), transsulfuration and aminopropylation (Lu, 2000) and is synthesized from methionine and ATP (Parkhito *et. al*., 2019). During these biochemical reactions S-adenosyl-L-homocysteine (SAH) is released (Roth *et. al*., 1996). Apart from playing important role in homeostasis and metabolic energy transduction, the bacterial membrane houses a third of cell proteins, which participate in regulation of many crucial pathways, so it is essential independently of the metabolic status of the cell (Hurdle *et. al*., 2011). Thus, the development of resistance becomes incredibly difficult

for bacterial cell as cell envelope ultrastructure cannot easily change without substantial loss of function. Thus, cell membrane is essential and can be targeted for development of antibiotics

DNA methylation is critical for gene expression regulation; similarly the phospholipid methylation is must for retaining of the membrane receptors and fluids. Thus the ligands that can compete with SAM at SAM binding pocket in target protein are relevant and can be studied for probable therapeutics.

4.2 Selection of CobA and vitamin B12 biosynthesis pathway as a potential therapeutic target

The CobA is essential for corrin ring contraction for vitamin B12 biosynthesis and this is important in lipid biosynthesis. Hence, vitamin B12 can be considered highly valuable for the survival of bacteria.

The transformation of uroporphyrinogen III into tetrapyrrole containing vitamin B12 passes through a number of peripheral C-methylations, ring contraction and there loss of the C-20 meso-position. Similarly cobalt chelation, amidation of the majority of the carboxylic acid side chains, decarboxylation of the acetic acid side chain on ring C, aminopropanol attachment, adenosylation and attachment of the lower base dimethylbezoimidazole(DMB) with the central corrin ring are critical steps in vitamin B12 biosynthesis (Warren *et. al*., 2002). It is very expensive in terms of energy as it involves most complex biosynthetic pathway among all vitamins (Crofts *et. al*., 2013).

Numerous transmethylations and biochemical reactions utilize the cobalamin as a crucial cofactor. Enzymes including diol dehydratase (Toraya, 2002), ethanolamine ammonia lyase – a key element of glycerophospholipid metabolism (Mori *et. al.*, 2004), ribonucleotide reductase that catalyzes the formation of deoxyribonucleotides from ribonucleotides (Taga and Walker, 2010) are involved in many important biosynthetic pathways in archaea and bacteria.

Thus the cobalamin dependent enzymes play major role in bacterial survival. Internal metabolic pathways were targeted taking into the probability of and can used to screen new inhibitory ligand molecules as a potential therapeutic agent as a solution to the increasing resistance problems exhibited by microorganism to many antibiotics.

4.3 Ligand database preparation and ADME/Tox tests for molecular docking

4.3.1 Ligand database selection

Virtual screening for identification of novel therapeutics and drug repurposing has been proved to be an established method in drug discovery. However this requires the prior

knowledge of protein target along with the ligand database and the molecules used for screening. The chemical collection must be containing the potential lead compounds against our protein target because the presence of undesirable chemical or lead compounds is time consuming and adds expenses only. For this the filtering of the desired compounds and performing molecular docking with compounds finalized under certain criteria is likely to screen the lead molecule which could be therapeutically suitable.

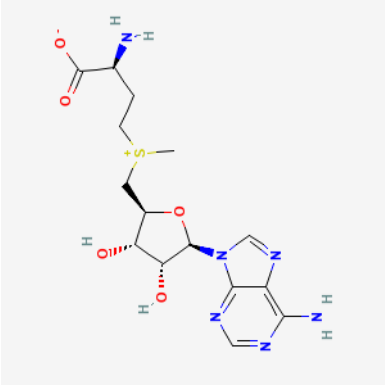
Physicochemical properties of clinical candidates affect their absorption, distribution, metabolism, elimination, and toxicity (ADMET) profiles (Mignani *et al*., 2018). The distribution coefficient of a drug strongly affects how easily the drug reaches its intended target in the body, how strong an effect it will have once it reaches its target, and how long it will remain in the body in an active form. Thus, the molecules selected for making library was subjected to filtration through "Lipinsky Rule of Five" (Lipinski *et. al*., 2001) where the molecules were filtered on the basis of parameters set as mentioned in materials and methods using OSIRIS Data Warrior software (Brito, 2011)

Natural products (NP) have been good source of drug candidates in past (Newman and Cragg 2020). In drug discovery process natural products have been the major molecular structural resources for a very long time. Most of the compounds present in this library are found in many plants There are many successful examples such as Antibiotics penicillin, drug huperzine A, analgesic and antipyretic drug aspirin, anti-cancer drug taxol, are the typical successful examples(Grabley and Sattler,2003; Bai *et al*.,2000). Natural product ligands have enormous scaffold diversity and structural complexity. They also have a higher molecular mass, a larger number of carbon atoms and oxygen atoms but fewer nitrogen and halogen atoms. Similarly higher numbers of H-bond acceptors and donors (Feher & Schmidt, 2003) that supports in stronger binding, lower calculated cLogP value for greater hydrophilic nature of the molecule.

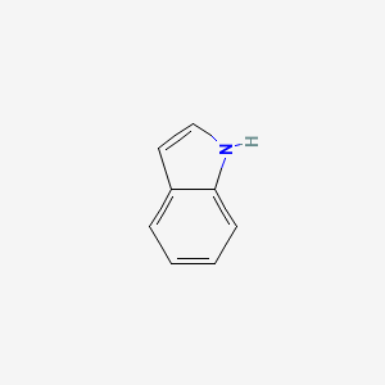
The study of physico-chemical and biological properties of such compounds, which makes them probable drug target, can be exploited. This might be helpful in the development of new drug substance and their availability is natural sources (plants) which in itself is a very eco-friendly and a great source to obtain the chemical compounds easily. Thus natural products can be used as a probable therapeutic target to study inhibition of bacterial gene such as cob*A.* A natural product ligand library containing 20,000 natural products was obtained. Ligand library with 205 molecules was prepared finally from Zinc database for this study.

In addition, since CobA protein uses SAM as the substrate for methylation, a ligand library of indole derivatives, Nucleoside mimetics and Kinase inhibitors were developed

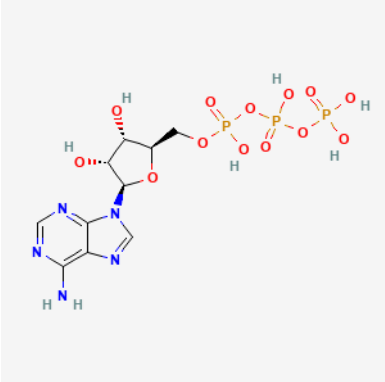
for virtual screening of ligand molecule against target protein CobA because the ATP moiety of SAM makes it appropriate for to look in these groups.



a) S-adenosyl-methionine (SAM)

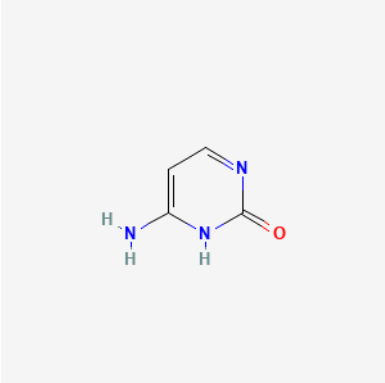


b) Indole

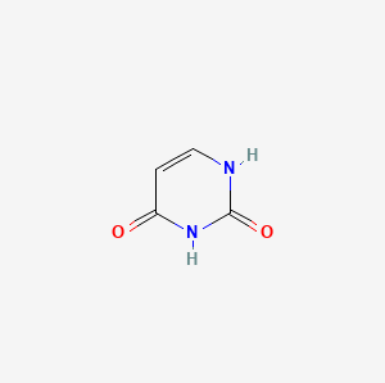


c) Adenosine triphosphate (ATP)

d) Thymine



e) Cytosine



f) Uracil

Figure 18: Chemical structure of a) S-adenosyl-methionine (SAM), b) Indole c) Adenosine triphosphate (ATP), d) Thymine, e) Cytosine and f) Uracil

Moreover, indole derivatives were also used as the probable therapeutic targets in this against and screened against cob*A*. Indole derivatives have been used as drug targets in many drug sought for a very long time. The scaffold of indole derivatives which shows structural similarity with the adenosine moiety of SAM makes it probable target for SAM

utilizing bacterial gene cob*A.* In addition it is found that pyrazole and imidazole have shown broad spectrum of antimicrobial activity (Badgujar *et. al*., 2018). Indirubin (a di-indole alkaloid) has been found to be kinase inhibitor (Wu *et. al*., 2005). Some other indole derivatives studied also were shown to be potent kinase inhibitor (for potent therapeutic agent (Kiliç *et. al*., 2009). It is the only moiety in drug discovery with the sole property of resembling different structures of the protein. A ligand library of 462 molecules including indole derivatives was prepared after passing a library of 10342 molecules through OSIRIS ADME/Tox filter.

Nucleoside analogues (Nucleoside like core intermediates with adenosine moiety) have been shown to have antibacterial activity. A significant number of clinically approved derivatives of both pyrimidines and purines including halogenated, thiolated, and azolated compounds have shown promising results. Many nucleoside analogues have been developed that works as antibacterial agent which primarily target cell-wall biosynthesis (Winn *et. al*., 2010; Niu and Tan, 2015). The adenosine moiety in these compounds makes them similar in core to that of SAM. A ligand library of nucleoside mimics containing 3118 molecules were obtained from ASINEX and final library of 654 molecules was prepared after passing through OSIRIS filter.

Kinase inhibitors could be potential therapeutic targets as they can compete with SAM because of adenosine moiety. In addition, kinase inhibitors can act as potential inhibitors because of the ATP binding pocket in the cleft between the C- and N-lobes of kinases mimic the hydrogen bond interactions normally formed by the adenosine ring of ATP (Zhang *et. al*., 2009) so these can be the probable inhibitors of CobA protein utilizing SAM with adenosine molecule in its structure In this study a ligand database of 6449 kinase inhibitors was obtained from UORSY and was prepared and processed for ADME/Tox filters for docking through OSIRIS. A ligand library containing 1685 molecules was prepared which included kinase inhibitors and used for molecular docking in this study.

4.3.2 In-silico ADME/Tox tests*:*

The prior passing or testing of ligands for certain essential parameters of drug molecules enables to make the ligand library that is not only dock-able but also has higher chances of clearing further steps in drug discovery, including clinical trials and addressing toxic issues as well. The toxic profiles and drug likeness of the ligands were predicted using OSIRIS. ORISIS program analyzed druglikeness and pharmacokinetics properties using a list of parameters including Lipinski's rule of five (Sander *et. al.*, 2015) as mentioned in material and methodology section.

Drug molecules need to be absorbed properly for their optimal functionality. Drug molecules are absorbed via passive diffusion and convective volume flow through water

filled intercellular spaces. These are drug particles with lower molecular weight small molecules.

The cLogP value of a compound, is the logarithm of its partition coefficient between n-octanol and water log ($c_{octanol}/c_{water}$), is a measure of the compound's hydrophilicity. Thus lower hydrophilicity and higher LogP values decreases the absorbance and permeation of drug molecule. It has been shown that compounds must not have clogP greater than 5.0 to have a reasonable probability of being well absorbed.

In addition, cLogS correspond to 10-based logarithm of the solubility of a molecule measured in mol/L and correlates to water solubility of a drug. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. It plays important role in drug uptake and elimination. A drug molecule needs to be absorbed optimally to exhibit pharmacological activity. Poorly soluble drugs get eliminated before entering circulation and thus do not exhibit pharmacological activity.

ORISIS calculated the other essential features of drugs like druglikeness and toxicities like mutagenicity, tumorigenicity, reproductive effects and irritant effects. And the compounds with no such effects were filtered from the inbuilt parameters of OSIRIS.

Hydrogen bonding affects the membrane transport and distribution of drug within the biological systems. The number of hydrogen bonds affects the absorption as well. It is unfavorable for a compound to have more hydrogen bonds. These bonds need to be broken for the drug molecule to pass across the aqueous environment within the cells.

Topological polar surface area (TPSA) makes use of functional group contributions based on a large database of structures. It is considered a convenient measure of the polar surface area that avoids the need to calculate ligand 3D structure or to decide which is the relevant biological conformation or conformations (Prasanna and Doerksen, 2009). Low polar surface area is found to be important predictors of good oral bioavailability.

Druglikeness is a qualitative concept that is used in drug designing to determine how "drug like" a substance is in terms of bioavailability. It is estimated from the molecular structure before the substance is synthesized and tested. It is calculated by using the sum of all drug scores in OSIRIS. Parameter is set as 0 to positive value for druglikeliness. A positive value states that our molecule contains predominantly fragments which are frequently present in commercial drugs.

Ligand molecule with rotable bonds between 0-10 is taken as a selection parameter. Increase in number of rotable bonds decreases permeation. This is important criteria that need to be addressed in drug screening for the oral bioavailability of drugs.

The compounds were also screened for mutagenicity, tumorogenicity irritant properties and effect in reproductiveness by OSIRIS. These parameters were set at null value to

make the ligand library including the ligands non-mutagenic, non-tumorigenic non-irritant and no any adverse effect in reproductiveness. Thus, eliminating the ligand molecules which fail to meet the basic criteria set for compound screening in this study.

Thus ligand libraries were prepared which included the compounds which were filtered through ADME/Tox screening. Finally, ligand library is converted into pdbqt file format after. Energy minimization was performed with the universal force field (UFF) using the conjugate gradient algorithm in Openbabel GUI available in PyRx interface. This is the useable file format for molecular docking in PyRx.

4.4 Binding site analysis in target protein and grid box generation for molecular docking

Total gasteiger charge added was -0.9965. Gasteiger (-Marsili) charge was added. For the given molecular system when partial charges are not present in the protein. Gasteiger method assumes an overall net neutral state for the respective molecular system. Polar hydrogens are hydrogen atoms that are bonded to electronegative atoms like oxygen and nitrogen. Auto Dock tools assumes that non-polar hydrogen is hydrogen bonded to carbon atoms. It creates cellular environment by charge equilibration.

Active binding sites were determined by taking the amino acid residues lying within 5 A^0 of protein and native ligand interaction sites.

Table 1: Active binding sites obtained by PyMol in uroporphyrinogen III methyltransferase (*P. denitrificans* PDB: 1S4D)

Residues	Amino acid	Residues	Amino acid
24	PRO	131	ALA
25	GLY	182	MET
50	VAL	183	TYR
100	GLY	184	MET
101	GLY	210	VAL
102	ASP	211	CYS
103	PRO	212	ASP
105	VAL	213	ALA
106	PHE	215	THR
107	GLY	240	PRO
129	ILE	241	ALA
130	THR	242	ILE

The binding site has to lie within the gird box in PyRx interface used for molecular docking. The grid box size is defined and determined as mentioned in material and methodology section. Calculation of binding energies was performed using AutoDock vina PyRx. Initially, a grid box was set to cover the active site of crystal structure of experimental protein within the grid box and grid size was determined. This was kept

constant for each docking process with all the ligand libraries studied and used in virtual screening in this study. This helps to maintain similar experimental rooms for each ligand library under study and the outcomes can be comparatively studied as unbiased results

The following dimension were fixed in Å: Center(X, Y, Z) = (80.362, 88.807,-0.487), Dimensions(X, Y, Z) = (32.983, 37.708, 31.204) with an exhaustiveness of 8 and move 16. PyRx automatically advances to results page, where results of virtual screening computation can be viewed.

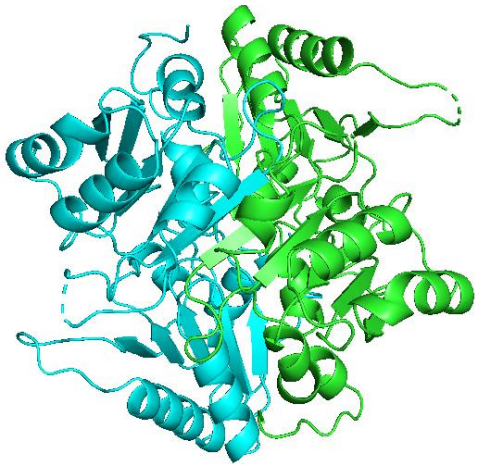


Figure 19: 3D Structure of Uroporphyrinogen III Methyltransferase (CobA) (*P. denitrificans*) as given by PyMol

4.5 Screening of ligand library against hMAT1A and its significance

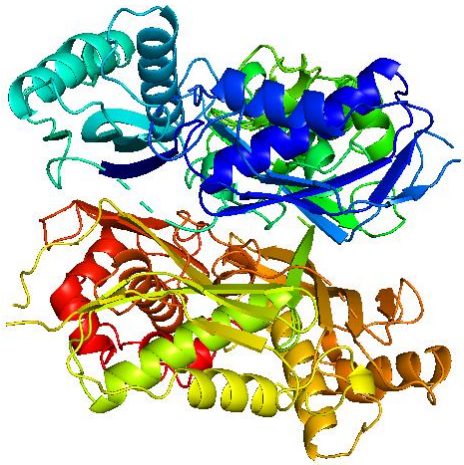


Figure 20: 3D Structure of S-Adenosylmethionine Synthetase (hmat1A) prepared from PyMol (*E. coli*) (PDB id: 6sw5)

S-adenosylmethionine (SAM) is formed through methionine catabolism which occurs mostly in the liver in a reaction catalyzed by methionine adenosyltransferase (MAT). MAT is product of two different genes, MAT1A and MAT2A, respectively. Decrease in SAM has shown adverse effects in liver leading to many hepatic complexities including

production of tumor necrosis factor (TNF). Thus SAM is critical for human. In human body, hepatic hMAT1A and the extra hepatic MAT, hMAT2A biosynthesize SAM (Lu *et al*., 2002). It is necessary to protect the interference with hepatic SAM biosynthesis pathway by preventing the inhibition of hMAT1A while screening the ligand library through molecular docking. However the SAM transporters could also be used in some extreme conditions.

This is crucial because the drug targets discovered shouldn't degrade human health and must be safe for human consumption. Therefore, to ensure the hepatic safety of the screened compound, the ligand libraries were screened against hMAT1A also. The 3D structure of hMAT1A (6SW5) was retrieved from PDB. It was processed in Auto dock tools and converted into pdbqt file format a readable file format for docking in PyRx interface later. The ligands with equal or lower binding affinity to hMAT1A were screened and studied further.

Table 2: Active binding sites obtained by PyMol in hMAT1A (PDB: 6SW5)

Residues	Amino acid
55	ALA
70	GLU
112	GLN
114	SER
117	ILE
133	GLY
134	ASP
289	LYS
291	ASP

The following dimension were fixed in Å: Center(X, Y, Z) = (31.096, -0.571, 24.983), Dimensions(X, Y, Z) = (27.625, 57.151, 31.622). The ligand molecules having binding energy higher or similar than native ligand SAM were selected from docking of ligand library against cob*A* and those having lower or similar binding energy than SAM from docking results with hMAT1A were selected. Thus lead molecules were identified based on the comparison of binding affinity of those compounds with target protein cobA and with human hepatic hMAT1A.

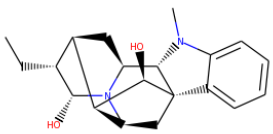
4.6 Virtual Screening of the ligand libraries and analyzing the protein-ligand interactions

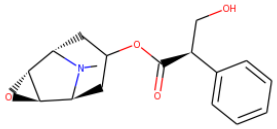
The 3D structure-based VS approach, also known as molecular docking has become most important and popular method of drug discovery recent years. In docking screens, large libraries of probable drug targets are docked into receptor structures and ranked by the calculated binding affinities.

4.6.1 Virtual Screening of the natural products

Docking was performed using AutoDock Vina in Virtual Screening Software, Pyrx interface. Natural products were docked with CobA and hMAT1A. Ligands showing higher or similar binding affinity with target protein cobA were considered. The compounds with higher negative value of binding energy were selected for further study. This higher value of negative binding energy indicates that that the screened ligands can block the activity of Cob*A* thus inhibiting the SAM mediated methylation. This affects the release of SAM eventually affecting the methyl incorporating steps within biosynthetic pathways; such as cobalamin (vitamin B12) biosynthesis that requires methylations at various steps. Methylations at C-2 and C-7 of uroporphyrinogen converts it into precorrin-2 which is the precursor of cobalamin (Martens *et. al*., 2002). SAM also serves as cofactor in different enzymatic reactions. Methyltransferases uses SAM to transfer methyl group to different substrates from small molecules to proteins, RNAs and DNAs.

A toatal of 205 natural products were finalized after the ADME/Tox filter rom OSIRIS datawarrior. After molecular docking, the ligands showing higher binding energy with CobA and lower binding affinity with hMAT1A than native ligands were screened. Of them 5 ligands met our criteria. However ligand molecule with Zinc Id, ZINC000106400715 was common in man and the world type. Similarly the ligand molecule with Zinc Id, ZINC000100057318 was also found common in world and trial type also in type *in vivo*. So one compound was only considered.





a) b)

Figure 21: Molecular structures of molecules that could be developed as putative antibacterial drugs a) ZINC000106400715 and b) ZINC000100024907

Table 3: Summary of results of molecular docking of natural products against CobA analysis of top hits (Binding energy as KJ/mol)

Natural Products	Molecular characters						Binding energy	
Database ID	cLogP	cLogS	H-Acceptors	H-Donors	Polar Surface Area	Druglikeness	CobA	hMAT1A
ZINC000106400715	1.791	-3.484	4	2	46.94	3.4513	-8.3	-7.9
ZINC000100024907	0.779	-2.295	5	1	62.3	2.875	-8.3	-7

Among the two top scoring compounds, ZINC000106400715 is identified by WHO as ajmaline (https://zinc15.docking.org/substances/ZINC000106400715/).

It is used for prevention of cardiac arrhythmias. Anti-arrhythmia drugs may affect the polarisation-repolarisation phase of the action potential, its excitability or refractoriness, or impulse conduction or membrane responsiveness within cardiac fibers.

(http://purl.obolibrary.org/obo/CHEBI_38070)

Ajmaline is cardiovascular drug used as anti-hypertensive drug for the treatment of acute or chronic vascular hypertension regardless of pharmacological mechanism.

(http://purl.obolibrary.org/obo/CHEBI_35674)

Ajmaline is found in most species of the genus *Rauvolfia* (bark and stem) as well as *Catharanthus roseus* (Roberts and Wink, 1998). In addition, antibacterial activity of indole alkaloid, ajmaline has been shown to have eight fold reduction in the MIC of NAL (Nalidixic acid) against the *E. coli* DH5α and four- to eightfold reduction against CA8000. It also reduced MIC of, tetracycline up to 8-folds, against the MDREC-KG4, a multidrug-resistant clinical isolate of *E. coli*. The overexpression of membrane porin forming protein genes ompA and ompX was observed in *E. coli.* (Dwivedi *et. al*., 2015).A similar study showed the potent antibacterial activity of alkaloid extracts from *Rauvolfia* against *Staphylococcus* (Deshwal and Kavita, 2012).

4.6.2 Virtual Screening of the indole derivatives

A ligand library of Indole derivatives consisting of 10,345 compounds subjected to ADME/T analysis in OSIRIS which narrowed these compounds to 462 ligands and were then virtually screened for binding affinity with CobA protein using AutoDock Vina in PyRx. Native ligand SAM showed B.E. -8.4 with CobA and -7.4 with hMAT1A. Thus the ligands having higher binding affinity than SAM to CobA and lower binding affinity than native ligand SAM to hMAT1A were considered. And top hits were selected and their name and molecular characteristics were derived from the respective database. The top hit compounds which have high affinity to target protein than native ligand are tabulated in table 4. The compound having highest binding energy with target protein CobA and meeting our criteria of drug screening was selected and studied and analyzed further.

Among indole derivatives top scoring compounds is 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide (v029-4195). Indole is an interesting heterocyclic aromatic compound and a planar bicyclic molecule with the benzene ring fused through 2 and 3 positions of N-containing pyrrole ring. Indole scaffolds have been used in most of the synthetic drug molecules available at present (Kumari and Singh, 2019). Their successful use in the medicine field for a really long time has left a remarkable place and faith in the way of drug development.

Table 4: Summary of results of molecular docking of indole derivatives against CobA analysis of top hits (Binding energy as KJ/mol) Binding energy (KJ/Mol)

Indole derivatives	Molecular characters						Binding energy	
Database ID	cLogP	cLogS	H-Acceptors	H-Donors	Polar Surface Area	Druglikeness	CobA	hMAT1A
v029-4195	2.8875	-3.334	5	2	57.36	3.1666	-9.6	-7.7
s233-0061	2.4249	-3.513	5	2	65.2	1.7581	-9.5	-7
v029-3401	2.8098	-3.793	6	2	71.94	2.9258	-9.5	-7.3
l259-0946	3.3907	-3.679	4	1	70.63	2.5567	-9.3	-7.7
m378-0067	3.4957	-3.777	3	0	25.24	3.2444	-9.2	-7.8

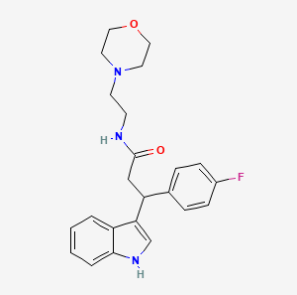
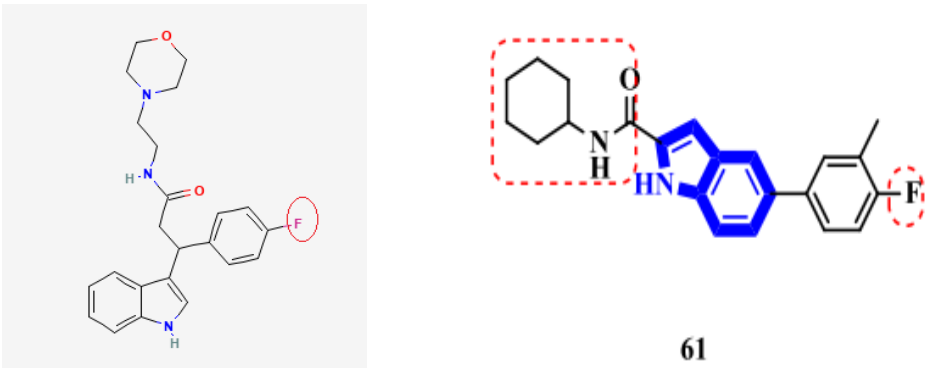


Figure 22: Molecular structure of the top scoring indole derivative (v029-4195) that could be developed as putative antibacterial drugs

Indoles and their derivatives have shown antibacterial activities and their scaffolds have been used in many antibacterial antibiotics. They have also shown promising results in their potential as anti-oxidants, anticancer, anti-inflammatory, anti-diabetic, anti-tuberculosis and anti-viral agents (Kumari and Singh 2019). Indole derivatives showed potential antibacterial activities against MRSA (Methicillin resistant *Staphyloccus aureus*). The synthesized indole derivatives showed moderate to excellent antibacterial activities with MIC values ranging from 20 to 40 mg/ ml (Qin *et. al*., 2020). According to Mane et al, 2016, indole derivatives have shown promising results against. *K. pneumonia, E. coli, P. aeruginosa, S. typhi, C. albicans, C. neoformans, A. fumigates, C. parapsilosis.* SAR studies suggested that the alkyl and halogen substituted phenyl and cyclohexyl carboxamide derivatives are favourable for their antibacterial potency. Top scoring indole derivative compound in this study has fluorinated phenyl group as this is reported to be responsible for antibacterial properties exhibited by compound 61(among many other indole derivatives) prepared by Mane et al., 2016 and had shown maximum anti-microbial activity (MIC < 6.25 µg/mL) compared with standard drug gentamicin(MIC < 3.0 µg/mL). Thus this compound could be further studied as a putative antibacterial compound.



61

a) b)

Fig: 23 Comparative study of a) Top scoring indole derivative in this study and b) Compound 61 synthesized by Mane et al., 2016

Although the reason isn't explained about the compound being able to exhibit maximum anti-microbial activity, rendering the fact that the compound contains indole in its structure resembling adenosine moiety and SAM also resembles in structure due to adenosine in its molecular structure, microbial killing might be due to competitive inhibition of SAM binding. Similarly the selected ligand contains morpholine in its molecular structure which has been used in number of drugs as well as bioactive compounds (ref). The heterocyclic compound with versatile scaffold makes it promising structure for synthetic drugs through substituted reactions. Many *in vivo* studies has demonstrated that morpholine moiety not only increase potency but also provide drug compounds with desirable drug-like properties and improved pharamacokinetics (Kourounakis *et al*.,2020). It is used as anti-diabetic, anti-emetic, growth stimulant, anti-depressant, bronchodilator and anticancer agent. Thus the screened indole derivative can be suggested as potential drug target and can be studied for drug development as antibiotics.

4.6.3 Virtual Screening of the kinase inhibitors

A ligand library consisting of 1685 kinase inhibitors was prepared after narrowing down 6449 ligands using ADMET/Tox filter from OSIRIS datawarrior. This ligand library was then docked with CobA in PyRx interface after being converted into readable pdbqt format by in built AutoDock Vina. Among them 20 ligands met our criteria. Top 10 hits were studied and their name and molecular characters was derived from the database. Top scoring compound 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one (pb28339681) had binding energy -9.8 for cobA and -7.4 for hMAT1A. Native ligand SAM showed -8.4 and hMAT1A had binding energy -7.4 with SAM, suggesting that this compound could be a lead for further study.

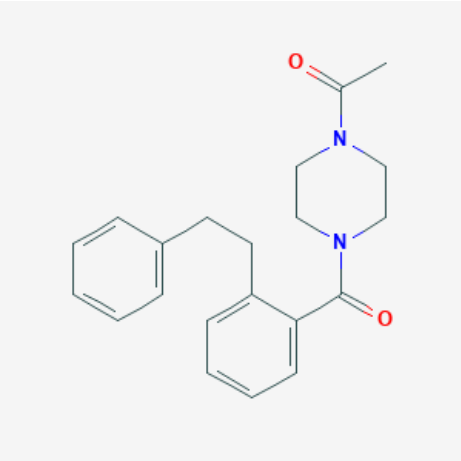


Figure 24: Structure of the top scoring kinase inhibitor (pb28339681) that could be developed as putative antibacterial drugs

Table 5: Summary of results of molecular docking of kinase inhibitors against CobA analysis of top hits (Binding energy as KJ/mol) Binding energy (KJ/Mol)

Kinase inhibitors	Molecular characters						Binding energy	
Database Id	cLogP	cLogS	H-Acceptors	H-Donors	Polar Surface Area	Druglikeness	CobA	hMAT1A
pb28339681	3.7014	-2.748	4	0	40.62	5.8046	-9.8	-7.3
pb57990117	2.6744	-2.607	3	1	42.23	2.3823	-9.4	-7.4
pb166708306	3.0884	-3.652	5	0	51.13	3.3074	-9	-7.2
pb208269008	3.4323	-3.996	5	0	51.13	3.3074	-9	-7.2
pb1694871815	2.2913	-3.484	5	1	59.81	4.4849	-8.9	-6.8

Study of molecular structure of 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one (pb28339681) indicates that it consists of piperazine molecule which has been used as important component in many synthetic drugs available today. Piperazines are 6-membered nitrogen containing heterocycles and present in the core structure of many important commercial fluoroquinoline antibiotics such as: Norfloxacin, Ciprofloxacin, Gatifloxacin, Grepafloxacin, Sparfloxacin, and Levofloxacin (Zhanel *et. al*., 1999).

Piperazine derivatives have shown potent antibacterial activity against *Escherichia. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), *Klebsiella pneumoniae* (ATCC 1705), *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) and resistant strains of *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. hemolytic*. One of the derivative even showed inhibition activity against both type of tested strains (MIC ¼ 0.5 mg/mL for Gram-positive and 1 mg/mL for Gram-negative strains (Tahir *et. al*., 2019). Thus the screened kinase inhibitor can be suggested as potential drug target for drug development as antibiotics.

4.6.4 Virtual Screening of the nucleoside mimics

654 molecules were screened according to binding affinity against cobA. Binding energy of native ligand SAM was -8.4 for cobA and -7.9 for hMAT1A. Top scoring compounds are enlisted in table.

Table 6: Summary of results of molecular docking of nucleoside mimics against CobA analysis of top hits (Binding energy as KJ/mol)

Nucleoside mimics	Molecular characters						Binding energy	
Database ID	cLogP	cLogS	H-Acceptors	H-Donors	Polar Surface Area	Druglikeness	CobA	hMAT1A
LAS52137907	2.1069	-2.973	8	1	68.2	5.3332	-8.7	-7.7
LAS52141662	1.9905	-3.142	7	2	73.75	3.8818	-8.6	-7.5
BDF32183476	1.6538	-2.623	4	1	47.04	0.4745	-8.6	-7.8
BDG34071518	-0.0996	-2.023	8	2	98.66	7.0812	-8.5	-7.7
LAS52136296	2.1069	-2.973	8	1	68.2	5.3332	-8.5	-7.7

(1-[6-(dimethylamino)-pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-1-yl-ethoxy) phenyl] methyl] amino] methyl] pyrrolidin-3-ol) is the top scoring compound from the nucleoside mimetics ligand library. Total of 654 moleculescwerecscreeed according to binding affinity against CobA.Binding energy of native ligand SAM was -8.4 for CobA and -7.9 for hMAT1A. The molecular structure of top scoring compound has important druglike molecules which have been used in many synthetic drugs. Piperidine, pyrimidin and pyrrolidin all make a promsing component in many synthetic drugs. Piperidin is saturated heterocycle which is considered to have better solubility as drugs and enhance their metabolism than corresponding N-aromatic heterocycles. It is used as antibacterial, anti-hypertensive (Zhou *et. al*.2006), anti-inflammatory (2011), anti-convulsant (Hoa *et. al*., 2001), antiproliferative, antitubercular and antioxidant (Alluri *et. al*., 2018).

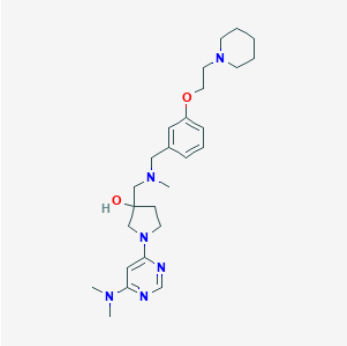


Figure 25: Structure of the top scoring nucleoside mimetics (LAS52137907) that could be developed as putative antibacterial drugs.

According to Arslan et al., 2006 some piperidine and pyrrolidin substituted compounds demonstrated antibacterial activity against the standard strains: *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 as Gram positive, *Yersinia enterocolitica* ATCC 1501, *Escherichia coli* ATCC 11230 *and Klebsiella pneumoniae* as Gram negative, at MIC values of 32-512 µg/ml.

Similarly piperidine derivatives also showed the potent antibacterial activity against *Pseudomonas aeuroginosa* ATCC 27853 with MIC upto 1 µg/ml, E. coli ATCC 25922 at MIC value upto 4 µg/ml, gentamicin was used as control which showed MIC <0.5 µg/ml(Zhou *et al*., 2005). Thus nucleoside mimics like aminoglycoside analogues with piperidine and pyrrilodine as core structure or their scaffolds with addition groups forming different backbones can act as potential anti-bacterial compounds and can be studied further for drug discovery.

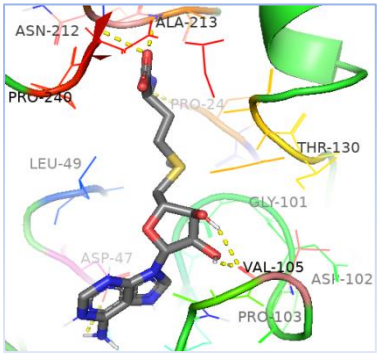
4.7 Analysis of the protein-ligand interactions

4.7.1 Study of protein-ligand interactions using PyMol and Discovery studio

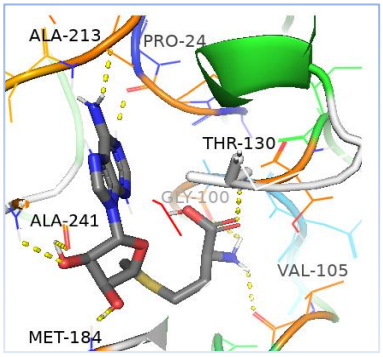
The lead compounds obtained from all ligand libraries were studied for protein-ligand interaction. The lead compounds finalized on the basis of their binding affinity were studied for their binding interactions with protein. Different amino acids are involved in such interactions and interact via possible bonding. The interactions of different amino acid residues involved and type of interactions were observed in protein-ligand complexes. H-binding interaction, hydrophobic interactions, electrostatic interactions were visualized and studied. Hydrogen binding interactions were observed in protein-ligand complex using PyMol. Four lead compounds(c, d, e and f) shown in figure 20 were considered best from docking results. Interaction of native ligand SAM and SAH were also observed (a and b respectively). SAM showed six polar contacts with five residues, PRO-24, ASN-212, ALA-213, VAL-105 and ASP-47, it formed two polar contacts with VAL-105. Similarly SAH formed polar contacts with Val-105, GLY-100, THR-130 ALA-213, two polar contacts with ALA-241 and MET-184.

Polar contacts play a significant role and provide specificity by forming hydrogen bonds, especially with charged residues. These charges can be either positive or negative and opposite charges attract whereas like charges repel, and interactions between charged residues can confer specificity (Zhou and Pang, 2018). Charge−charge interactions can be strong even at a long distance (e.g., of 5−10 Å) and “long-range” interactions can play a vital role in determining the rate constants of proteins binding with small and macromolecular partners (Pang and Zhou, 2017). Native ligand as well as the lead compounds studied revealed the hydrogen bonds in their interactions. Some of them

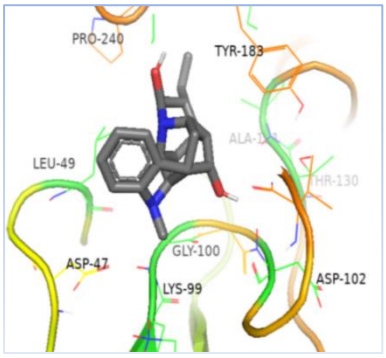
being interactions between charged residues and polar group could be accounted for the specificity with SAM as a native ligand where H-bond was formed between NH group of indole moiety and charged residue ASP 47 by accepting proton from by accepting proton.



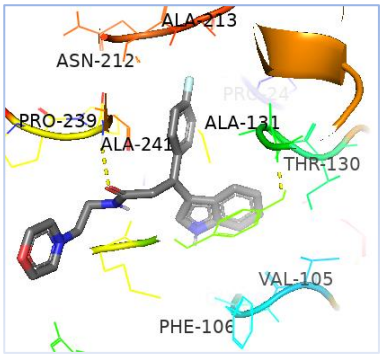
a)



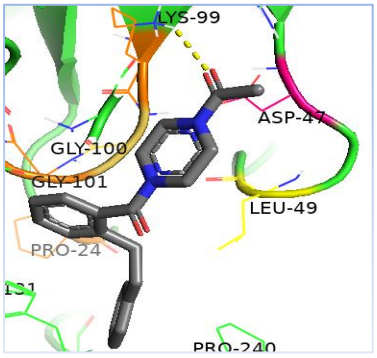
b)



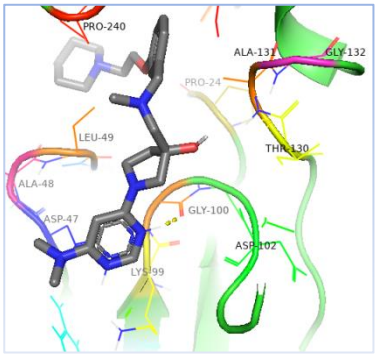
c)



d)



e)



f)

Fig: 26 PyMoL visualization of ligand binding interaction between lead compounds and CobA

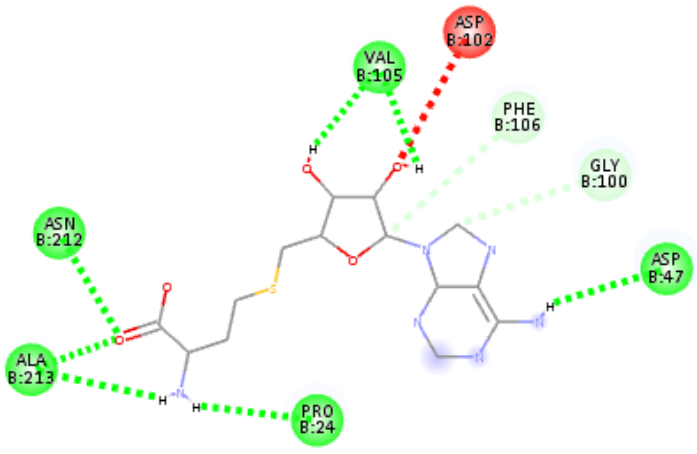
a) Ligand binding interaction between CobA and SAM, b) Ligand binding interaction between CobA and SAH, c) Ligand binding interaction between CobA and Ajmaline, d) Ligand binding interaction between CobA and 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide, e) Ligand binding interaction between CobA and 1-{4-

[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one and f)1-[6-(dimethylamino)pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy)phenyl]methyl]amino]methyl] pyrrolidin-3-ol.

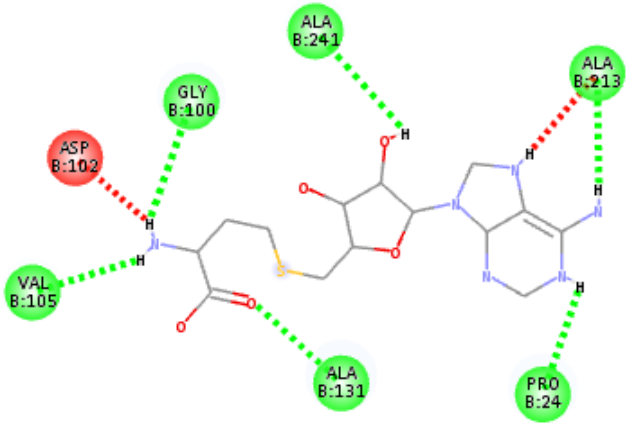
Ajmaline(a) didn't show any polar contacts with residues in the binding site of cobA. Steric hindrance near binding sites might be the reason. Indole derivative, 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide(d) showed polar contact with ALA-241. Similarly Kinase inhibitor, 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one (e) formed polar contact with LYS99. Nucleoside mimetic, 1-[6-(dimethylamino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl]amino]methyl] pyrrolidin-3-ol(f) showed polar contact with GLY-100.

The protein-ligand interactions were further analyzed by using Discovery studio and Ligplot software. Residual interaction map were then generated for detailed information, groups involved, type of bonds with such groups and distance of these bond. The ligand-receptor interaction plays important role in the efficiency of a drug molecule. These interactions and their results are termed pharmacodynamics.

2D and 3D interaction maps generated by Discovery Studio gives clear details on the chain of the protein involved in protein-ligand interaction, types of bond being formed, distance of such binding interactions and groups involved can also be easily visualized. Some of the amino acids were found to interact more often and these residues were found in almost all of the protein-ligand interactions. The interactions of individual amino acid and their orientations depend on the stereochemistry and orientation of individual groups involved in interactions.



a)


ALA
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ALA
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GLY
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ASP
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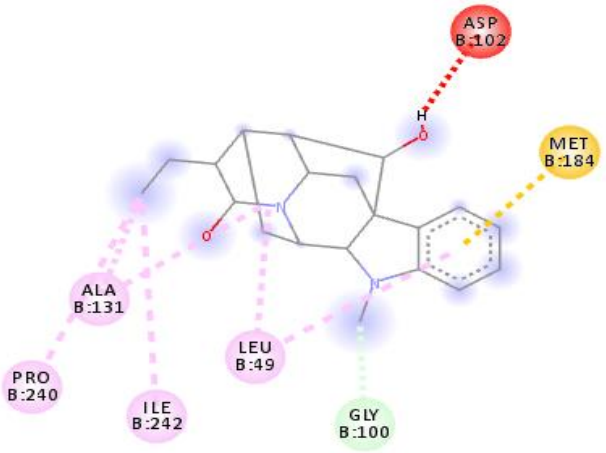
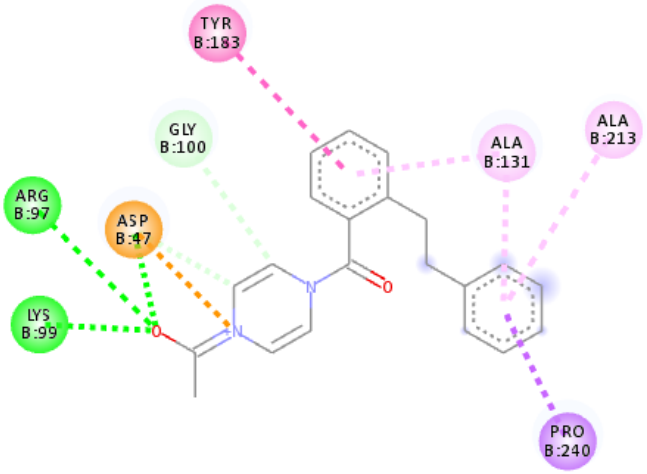
VAL
B:105
ALA
B:131
PRO
B:24

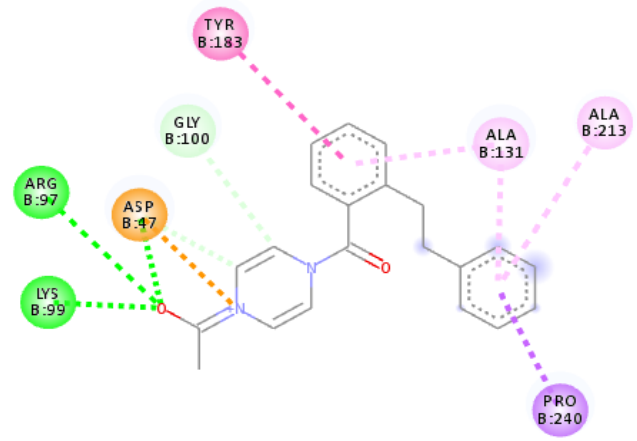
b)


ASP
B:102
MET
B:184
ALA
B:131
LEU
B:49
PRO
B:240
ILE
B:242
GLY
B:100

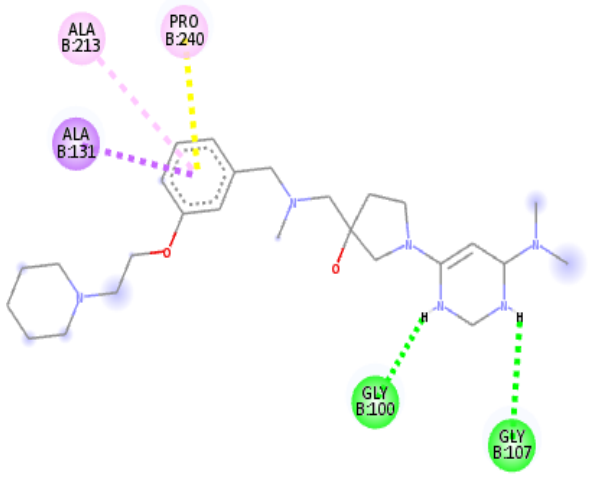
c)


TYR
B:183
GLY
B:100
ALA
B:131
ALA
B:213
ARG
B:97
ASP
B:47
LYS
B:99
PRO
B:240

d)



e)



f)

Fig: 27 Discovery Studio visualization of ligand binding interaction between lead compounds and CobA

a) SAM

b) SAH

c) Ajmaline

d) 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl)propanamide

e) 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one

f) 1-[6-(dimethylamino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl]amino] methyl] pyrrolidin-3-ol

When protein - ligand docking is performed, we check the conformation of ligand which binds to the target protein and quantify the binding energy between them using various force field equations. 3D visualization map of top scoring compounds of each ligand library was prepared from the PDB file of protein and ligand interaction generated in PyMol. Thus the details of the protein-ligand interactions was studied for bonds formed

during interactions and amino acid residues of the CobA protein and respective groups of the ligands involved in such bindings were obtained.

Table 7: Summary of results of protein-ligand interactions of Ajmaline and CobA

Chain	Amino acid	Bond type	Bonded with	Bond length
B	MET184	π-alkyl	Benzene	4.99
B	ASP102	Unfavourable Donor-donor	Hydrogen	2.04
B	ALA131	Alkyl	Methyl	3.67
B	ALA131	π-alkyl	Cyclohexenol	4.92
B	PRO240	π-alkyl	Methyl	4.74
B	ILE242	Alkyl	Methyl	4.63
B	LEU49	π-alkyl	Benzene	5.29
B	GLY100	C-H bond	Diazahexacyclo ring (aminogroup)	3.19
B	LEU49	π-alkyl	Diazahexacyclo ring	4.20

Table 8: Summary of results of protein-ligand interactions of 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide and CobA

Chain	Amino acid	Bond type	Bonded with	Bond length
B	MET184	π-alkyl	Morpholine	4.92
B	PRO239	π-alkyl	Morpholine	5.24
B	ALA241	Conventional H-bond	Amide	2.38
B	ASN212	Halogen	Fluorine	3.58
B	PRO240	π-alkyl	Benzene	4.42
B	ALA213	Halogen	Fluorine	4.63
B	ALA131	π-alkyl	Benzene	3.51
B	LEU49	π-alkyl	Indole	5.38
B	TYR183	π- π Tshaped	Indole	3.19
B	ALA131	π-alkyl	Indole	4.86
B	LEU49	π-alkyl	Benzene	5.50

Table 9: Summary of protein-ligand interactions between 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one and CobA

Chain	Amino acid	Bond type	Bonded with	Bond length
B	TYR183	Pi-Pi T shaped	Benzene	5.30
B	ALA131	π-alkyl	Benzene	4.57
B	GLY100	C-H bond	Piperazine	3.50
B	ASP47	C-H bond and	Piperazine	3.35
B	ASP47	Attractive charge	Amino group of Piperazine	3.79
B	ARG97	Conventional H-bond	Carbonyl group	3.51
B	ALA213	π-alkyl	Amino group	5.16
B	PRO240	π-sigma	Benzene	3.19
B	LYS99	Conventional H –bond	Carbonyl group	2.34
B	ASP47	Conventional H-bond	Carboxy group	3.36
B	ALA131	π-alkyl	Benzene	3.57

Table 10: Summary of results of protein-ligand interactions of 1-[6-(dimethylamino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino] methyl] pyrrolidin-3-ol and CobA

Chain	Amino acid	Bond type	Bonded with	Bond length
B	GLY107	Conventional H-bond	Amino group	2.90
B	GLY 100	Conventional H-bond	Amino group	1.96
B	PRO240	π-alkyl	Benzene	4.20
B	ALA213	π-alkyl	Benzene	5.12
B	ALA131	π-sigma	Benzene	3.84

The protein-ligand interaction electrons are involved in formation of covalent or non-covalent bonding. Pi-alkyl, pi-pi T shaped and a pi-Sulphur interaction comes in the category of non-covalent interactions. In pi-alkyl interactions there is interaction of pi-

electron cloud over an aromatic group and electron group of any alkyl group. In pi-pi T shaped interaction there is interaction of pi- electron cloud between two aromatic groups but in a T shaped manner. In pi-sulphur interaction pi electron cloud of aromatic ring interact with lone pair of electron cloud of Sulphur atom.

π-alkyl bond was found in most of the interactions between benzene moiety of the ligand framework and amino acid residues of the binding sites of protein. This is due to interaction of pi- electron cloud over an aromatic group (benzene) and electron group of alkyl group in amino acid residues of the target protein cobA. Native ligand SAM was found forming two hydrogen bonds with GLY100 and PHE106 residues of the protein cobA, similarly five conventional hydrogen bonds were observed with ASN212, ALA213,PRO24, ASP47 and two such bonds with VA105 residues respectively and one unfavorable donor-donor type bond was observed with ASP102 . In the same manner , discovery studio visualization of cobA and SAH revealed six conventional hydrogen bonds with PRO24, ALA213, ALA241, GLY 100, VAL 105 and ALA131 residues and two unfavorable donor-donor bonds were formed with ASP102 and ALA213.

PRO240 residue was found to be involved in π-alkyl binding in Ajmaline, 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl)propanamide and 1-[6-(dimethylamino)pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy)phenyl]methyl]amino]methyl] pyrrolidin-3-ol with bond length greater than 4. As expected most of the residues involved in protein-ligand interaction were found to be hydrophobic. Electron rich aromatic moieties show high preference of CH-Pi interactions with side chains (Sepay *et al*., 2021). As discussed earlier benzene and benzene containing framework was found to be involved in π-alkyl interaction in all the lead compounds with various amino acids. Nucleoside mimetics, 1-[6-(dimethylamino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy)phenyl] methyl] amino] methyl] pyrrolidin-3-ol showed lesser number of interactions however stability could be accounted due to π-interactions thus binding affinity is similar.

MET 184, ASP102 ALA131, PRO240, ILE 242, LEU49 and GLY100 were involved in protein ligand interactions of Ajmaline and cobA as shown in table 7. C-H bond was formed between amino group of ligand and carbonyl group of amino acid residue. Pi-sulfur bond was formed between sulfur of methionine and methyl group of benzene. ASP102 was found to be involved unfavourable donor-donor bond by accepting proton. Similarly ALA131, LEU49 formed π-alkyl bond and ALA131 and ILE242 were involved in alkyl bond. GLY100 was found exhibiting C-H bond formed between its carbonyl group and amino group of diazacyclo ring.

Interactions of 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide and cobA involved PRO239 and MET184 forming π-alkyl bonds, with Morpholine ring,

PRO240 and ALA131 forming π-alkyl bonds with benzene. ALA213 and ASN212 were found involved in halogen bonding with fluorine of ligand moiety. Similarly PI-Pi T shaped bond formed between TYR183 and Indole. LEU49 exhibited two π-alkyl bonds with Indole ring and benzene with bond length of 5.38 and 5.50 respectively. Conventional-H bond was observed with hydrogen of amino group of ALA241 and oxygen of the amide group of the ligand.

1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one and cobA exhibited three π-alkyl interactions, involving ALA131 (two bonds) and ALA213. C–H bond was formed between carbon atom of GLY100 and hydrogen of Piperazine ring and between ASP47 and benzene. Conventional hydrogen bond was observed between hydrogen of ARG97 and oxygen of carbonyl group of the ligand. Similarly ASP47 bonded due to presence of attractive charge with Piperazine ring of the ligand. Another bond was observed between TYR183 and Benzene forming PI-PI T shaped bond because of exchange of pi-electrons in T-shaped manner. Pi-sigma bond was formed between PRO240 and benzene moiety providing stability to the complex. 1-[6-(dimethylamino)pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-yl-ethoxy) phenyl] methyl]amino]methyl] pyrrolidin-3-ol showed five interactions. Conventional hydrogen bond was observed between amino group ligand and GLY107.

Additionally π-alkyl bond was observed between PRO240 and benzene, ALA213 and benzene. Pi-sigma bond was observed between ALA 131 and Benzene. π–alkyl interactions, and simultaneous presence of hydrogen bonds in the binding pose provides stability in the binding sites. Inspite of being weak interaction, C-H bonds helps in stabilization of protein-ligand complex (Mora Lagares *et al*., 2020). These types of interaction have been found in all the lead compounds identified hence the protein-ligand complex formed could be considered stable and bind competitively with target protein and block the activity of cobA protein by inhibiting normal interaction with native ligand as these compounds have already shown higher binding affinity than native ligand.

4.7.2 Study of protein-ligand interactions using Ligplot

Ligplot analysis was performed to analyze hydrogen bond interaction and hydrophobic interactions. Hydrogen-bonds play a crucial role in determining the specificity of ligand binding with the target protein. Hydrophobic interactions are short-range attractive interactions; these interactions make an important contribution to ligand–receptor binding affinities. The hydrophobic interactions of nonpolar residues help in the folding stability of proteins. They also contribute to specificity but in a less geometrically constrained way than hydrogen-bonding interactions. Hydrophobic interactions are also regarded as the main driving force for conformational changes of the receptor

upon ligand binding. Medicinal chemists increase the hydrophobicity of compounds as a common approach to improve binding affinity, since this leads to an increase in the total amount of hydrophobic surface area burial (Motiejunus and Wade, 2007). The interactions between native ligand SAM and SAH and target protein cobA were also studied by using ligplot. Similarly protein-ligand interactions of lead compounds were also observed via Ligplot.

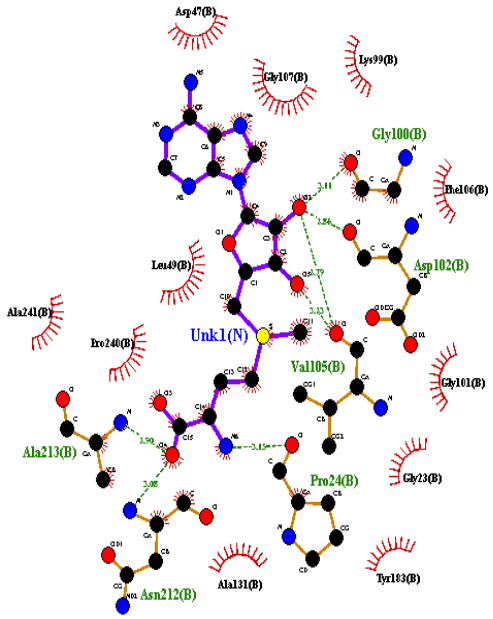


Fig: 28 Ligplot visualization of ligand binding interaction between native ligand SAM and CobA

Protein-ligand interaction of target protein CobA and native ligand SAM, exhibited both hydrophobic interactions and H-bonds. ALA131, TYR183, GLY23, GLY101, PHE106, LYS99, GLY107, ASP47, LEU49, PRO240 AND ALA241 showed hydrophobic interactions. Similarly GLY100, ASN212, ALA213, PRO24, VAL105 and ASP 102 were found to be involved in hydrogen bonds with bond length of 3.11, 3.05, 3.50, and 3.15, two bonds with bond length 3.83 and 3.79 and 3.36 respectively.

Similarly, the protein-ligand interaction of the cobA and SAH revealed hydrophobic interactions between GLY100, THR130, GLY101, LEU49, ASN212, PRO240, PHE 106, and TYR183. H-bond were observed between ALA 213, PRO240, ALA241, MET184, VAL1053, ALA131 and ASP 102 and SAH with bond lengths 3.93, two H-bond with bond length 3.85 and 3.79, 3.05 3.51,3.01,3.11 and 3.9 respectively.

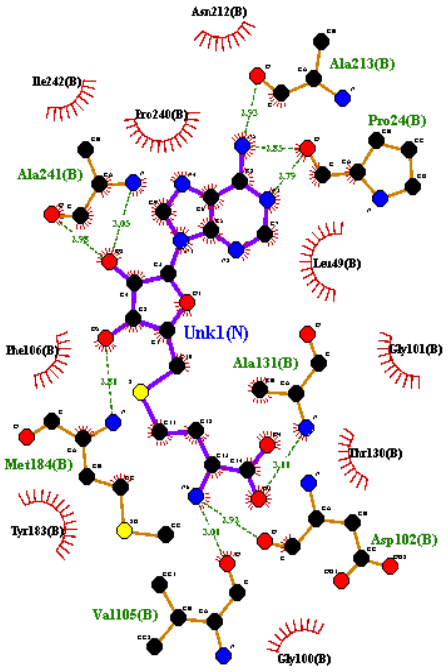


Fig: 29 Ligplot visualization of ligand binding interaction between native ligand SAH and CobA

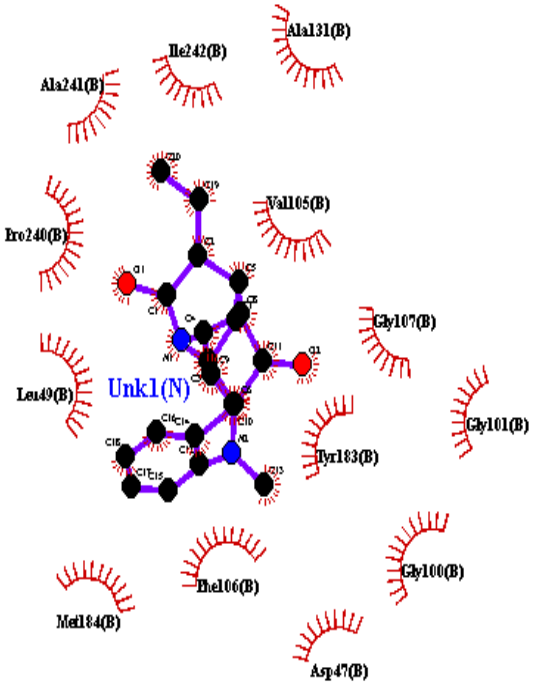


Fig: 30 Ligplot visualization of ligand binding interaction between CobA and Ajmaline

The ligplot vsualisation map of of the protein-ligand interaction between CobA and Ajmaline didn't show any hydrogen bonds. The hydrophobic interactions were observed between 13 amino acid residues of the B chain of target protein and studied compound Ajmaline. Amino acid residues involved in such interactions are ASP47, PHE106, MET184, LEU49, PRO240, ALA241, ILE242, ALA131, VAL105, GLY107, GLY101 and GLY100.

Similarly, The ligplot map of protein-ligand interaction between CobA and top scoring indole derivative 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one was prepared and studied for probable hydrogen bonds formed, their bond length and other amino acid residues involved in hydrophobic interactions. Ligplot visualization of protein-ligand interaction didn't show any hydrogen bond being formed. And 16 aminoacid residues were found to be involved in hydrophobic interactions. They are LYS187, ALA241, MET184, LEU49, ILE242, ALA131, PRO240, GLY101, VAL105, THR130, ASP102, GLY100, TYR183, PRO239, HIS158 and MET186.

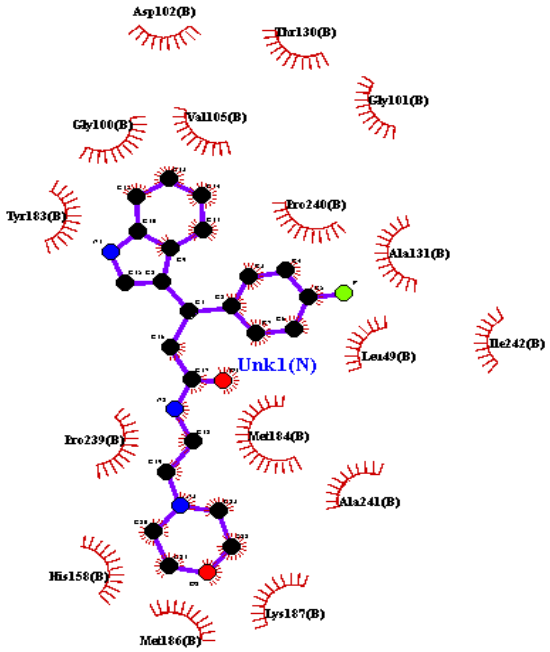


Fig: 31 Ligplot visualization of ligand binding interaction between CobA and 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide

The ligplot visualization of top scoring compound among kinase inhibitors 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one showed one hydrogen bond being formed involving LYS99 amino acid residue of target protein CobA with bond length of about 2.85 A^0. 14 amino acid residues were found to be involved in hydrophobic interaction in the protein ligand complex. They are ALA213 (B), LEU49 (B), GLY101 (B), THR130 (B), PRO240 (B), ALA131 (B), GLY100 (B), ILE242 (B), ASP 102(B), GLY107 (B), ASP47 (B), VAL105 (B), PHE106 (B) and ALA241 (B).

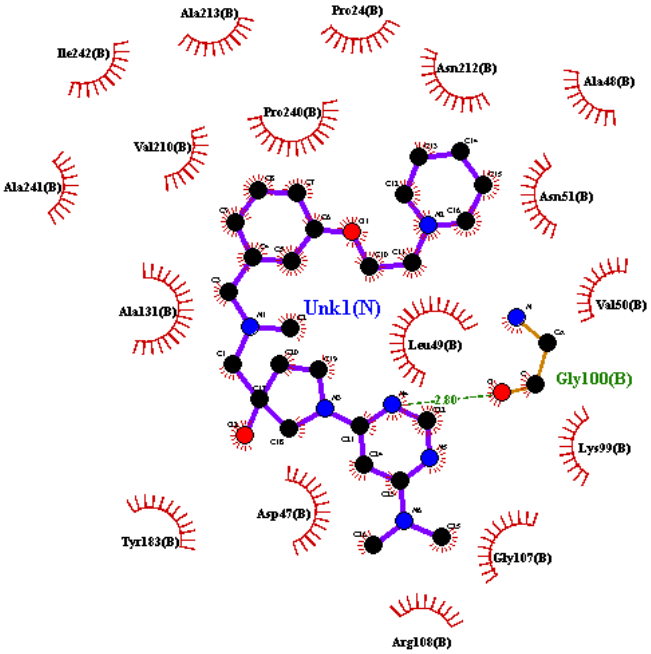


Fig: 32 Ligplot visualization of ligand binding interaction between CobA and 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl}

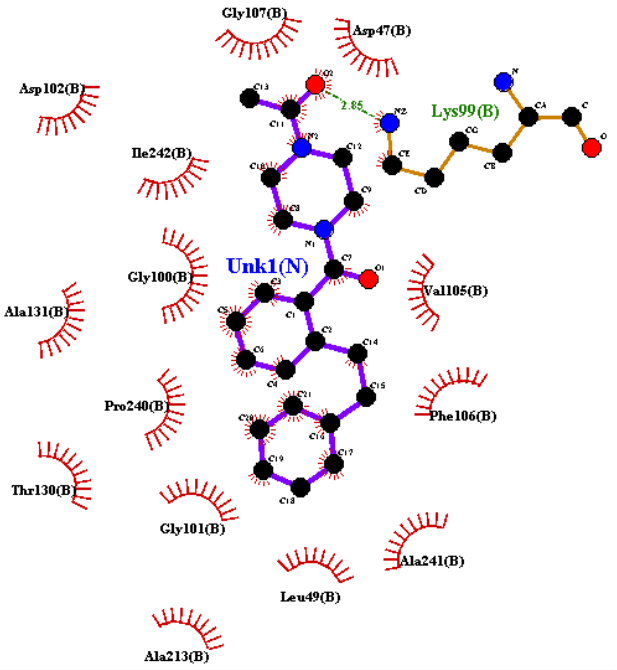


Fig: 33 Ligplot visualization of ligand binding interaction between CobA and of 1-[6-(dimethyl lamino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino] methyl] pyrrolidin-3-ol

The ligplot visualization of protein-ligand interaction of CobA and top scoring nucleoside mimics 1-[6-(dimethyl lamino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino] methyl] pyrrolidin-3-ol

4.8 DFT analysis (Density Function Theory)

The 3D structure of top scoring compound from natural product library, Indole derivatives and nucleoside mimetics was prepared using Density Function Theory(DFT) and calculations were carried out using B3LYP method (Becke3-Lee-Yang-Parr) with 631G basic set using Gaussian 03 program. DFT is a computational quantum mechanical modeling method used to examine the electronic structure structure of atoms, molecules, and solids (van Mourik *et al*., 2014) and also to investigate the interactions involved between the receptors and the ligands(Jordaan *et al*.,2020). A basis set is a set of wave functions that describes the shape of atomic orbitals (AOs). These basis sets are also called Pople basis sets and allow us to specify the number of GTO's(Gaussian type orbital) to use for core and valence electrons separately (size adjustable). These are double Zeta (2 functions per AO) or triple Zeta. Basis set are employed for organic compounds under study and 631G indicates 6 GTOs for inner shell, 3 GTOs for inner valence, 1 GTO for outer valence. G represents the use of GTOs. The molecular structure, vibrational spectra, frontier molecular orbital were calculated as a part of quantum calculations using Density Function Computation method.

4.8.1 Computational analysis (DFT)

DFT calculations were performed on Gaussian 03 platform to interpret the atomic arrangement of compounds under study and optimized using B3LYP/631G(6D,7F) basis set for establishment of theoretical geometry. The optimized structure parameter of studied compound had singlet spin, zero charge and parameters were calculated by RB3LYP method with Optimization+ Frequency job type. The calculated parameters of Ajmaline(a), 3-(4-fluoro phenyl)-3-(1H-indol-3-yl)-N-(2-morpholino ethyl) propanamide (b) and 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino] methyl] pyrrolidin-3-ol(c)are tabulated in table 11.

Table 11: Calculated parameters of the compounds studied

Compound	Total Energy (in a.u.)	Dipole moment (in Debye)	RMS Gradient norm (in a.u)
a)	-1037.40707392	2.2088	0.00000414
b)	-1303.84553671	5.2507	0.00000242
c)	-1494.45166322	1.7713	0.00000173

The study of dipole moment of the compounds clearly indicates that all the compounds possess stronger dipole-dipole interactions. Dipole moment shows the molecular charge distribution and is given as a vector in three dimensions and is used as descriptor to depict the charge movement across the molecule depending upon the centers of positive and negative charges. Dipole moments are strictly determined for neutral molecules3-(4-

fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide was found to have highest dipole moment with 5.2507 thus highest dipole-dipole interactions. A molecule is more stable if there are more attractive forces and less repulsive forces. Attractive forces lower the potential energy of the molecule and repulsive forces increase the potential energy of the molecule. Therefore molecules with lower energy are more stable. All compounds exhibited negative value of energy indicating the stability of complexes. Root Mean Square (RMS) Gradient is calculated as a root mean squared gradient, sqrt (mean (F_i dot. F_i)**,** where F_i is the force on atom i and mean () takes an average over all atoms. RMS Gradient norm is the RMS of individual variables.

4.8.2 Frontier molecular orbital study and chemical reactivity descriptor analysis

Frontier molecular orbitals (FMO) are most important orbitals and play a vital role in the charge transfer interactions with the binding site of target protein. FMO analysis helps in understanding the reactions and active site conjugations. These are highest occupied molecular orbital (HOMO) and lowest unoccupied molecule orbital (LUMO). The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are the main orbital's that are responsible for the chemical stability of the complex. The difference in bond energy distinctly explains the chemical reactivity and chemical stability of the active molecules (Govindarajan *et al*., 2012). HUMO energy indicates the ability or character of donating electrons and LUMO energy is responsible for accepting the electron. These regions are located over a protein-ligand complex at different locations indicating the charge transfer in LUMO regions. Energy gap between HUMO and LUMO energy is called energy gap values. The calculated energy gap between the LUMO and HOMO energies of Ajmaline(a), 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide (b) and 1-[6-(dimethylamino)pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy)phenyl]methyl]ami-no]methyl]pyrrolidin-3-ol(c) (c) is given in table 12. Lower the gap energy higher the chances of transition of electron and thus compound is reactive. Wide energy gaps negatively affect the electron to move from the HOMO to the LUMO subsequently leading to a weak affinity of the ligand for target protein.

Table 12: Calculated energy differences (LUMO-HOMO) of the compounds

Compound	HOMO(eV)	LUMO(eV)	(eV)
a)	-0.18752	0.00326	0.1908
b)	-0.16036	-0.01187	0.1485
c)	-0.13051	0.00494	0.1355

.

The values for the band energy and energy difference (LUMO-HOMO) describe the charge transfer (CT) interaction and thus reflect the chances of possible reactions or reactivity of the compounds studied.

The gap energy value was found to be in decreasing order as: Ajmaline(a)> 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholin oethyl) propanamide(b)>1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino] methyl] pyrrolidin-3-ol(c). Gap energy decreasing in this order indicates the reactivity of 1-[6-(dimethylamino) pyrimidin-4-yl]-3-[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino] methyl] pyrrolidin-3-ol to be highest followed by 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide and then Ajmaline. Significant chemical reactivity parameters, such as electronegativity (χ), chemical potential (μ), global hardness (η), global softness (σ) and global electrophilicity (ω) can be calculated by using HOMO and LOMO energies on the basis of chemical reactivity (Pearson 1989; Padmanabhan *et al*., 2007). The chemical reactivity values are calculated as:

$$\chi = -\frac{(E_{LUMO}+E_{HOMO})}{2}$$

$$\mu = -\chi = -\frac{(E_{LUMO}+E_{HOMO})}{2}$$

$$\eta = \frac{(E_{LUMO}+E_{HOMO})}{2}$$

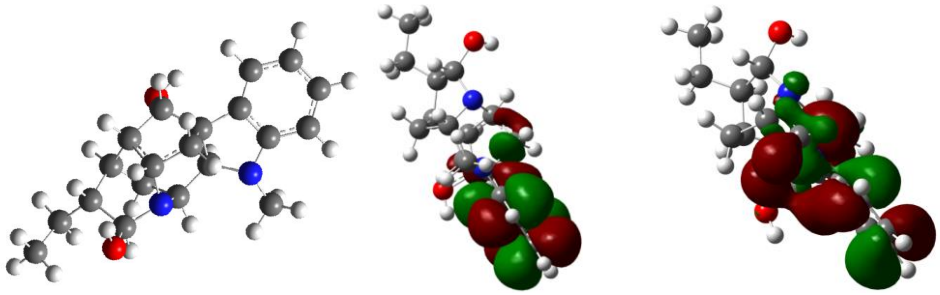
$$\sigma = \frac{1}{\eta}$$

$$\omega = \frac{\mu^2}{2\eta}$$

Table 13: Calculated chemical reactivity values of the compounds

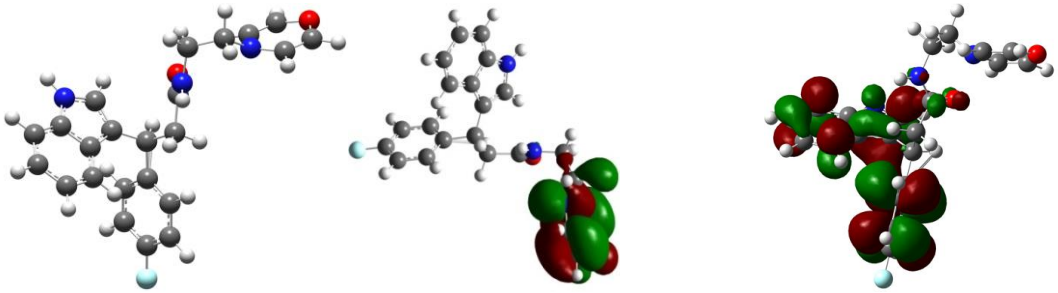
Compounds	χ (Pauling)	η(eV)	σ	μ (eV)	ω(eV)
a)	0.091	0.0970	10.31	-0.091	0.021
b)	0.092	0.0683	14.64	-0.092	0.062
c)	0.060	0.0702	14.24	-0.060	0.026

Compound a (Ajmaline) showed lower value of softness than other studied compound indicating the chemical stability than other compounds. The lower value for chemical softness indicates the low energy gap and thus easy polarization and easy transfer of electron and distribution of charge showing possibility of reactivity. The entire studied compound had positive electrophilicity (ω) value indicating tendency to gain electron from the surrounding. Ajmaline has lower electrophilicity value indicating being most stable among lead compounds studied for DFT analysis through chemical reactivity measures.



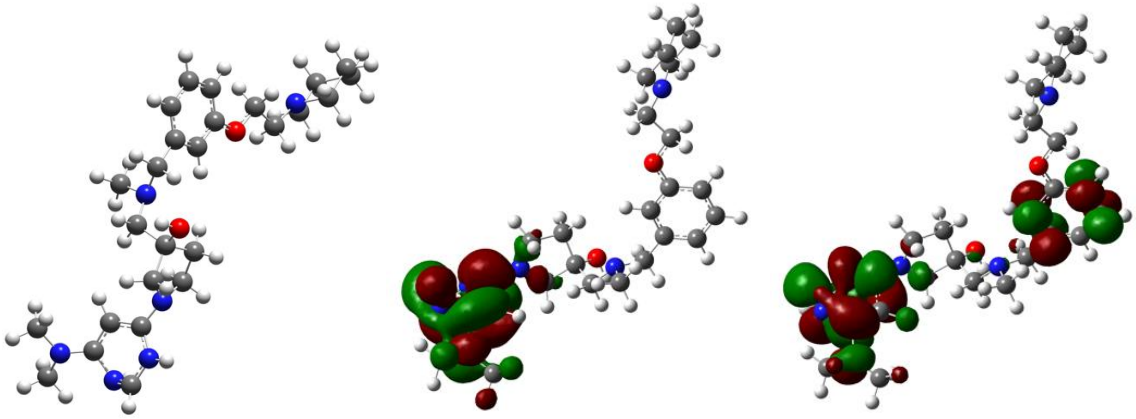
a) b) c)

Figure: 34 Optimized structure (a) and Frontier molecular orbitals; HUMO (b) and LUMO (c) plot of plot of of Ajmaline.



a) b) c)

Figure: 35 Optimized structure (a) and Frontier molecular orbitals; HUMO (b) and LUMO (c) plot of 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide.



a) b) c)

Figure: 36 Optimized structure(a)and Frontier molecular orbitals; HUMO (b) and LUMO (c) plot of 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol.

Analysis of HUMO plot of Ajmaline shows that the electrons are localized on the upper part of the ligand including diazahexacyclo ring and one alcohol group and and are not localized on nonadeca-2, 4, 6-triene and one of alcohol group. LUMO structure shows

that the electron is not localized in whole diazahexacyclo ring and in both alcohol groups as well. This shows that the whole diazahexacyclo ring, one of the alcohol groups can donate the electron to the interacting groups of amino acids in the ligand binding site. Similarly study of HOMO structure of 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide indicates the localization of electrons on the morpholinoethyl group and LUMO structure shows the electrons localizedin Indole moiety, fluorophenyl group and propanamide group.

From this we can conclude that the morpholino ethylene group can easily donate electrons to interacting groups of amino acid residues in the ligand binding site. The HUMO and LUMO structures of 1-[6-(dimethylamino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino] methyl pyrrolidin-3-ol reveals the localization of electron in pyrimidine ring in HUMO structure. LUMO structure shows that electrons are not localized here hence pyrimidine ring can donate electrons to the groups of amino acids involved in interaction in the ligand binding site.

4.8.3 Molecular electrostatic potential maps (MEP)

The molecular electrostatic potential map (MEP) is a reactivity map of the compound. It shows the regions for the electrophilic attack of charged groups. Molecular electrostatic potential is responsible for different chemical reactivity in a chemical reaction and its spatial distribution reflects the probable interactions that take place in the active sites in a strong binding condition. MEP describes the atomic charges of the protein-ligand complex using a visualized method. The relative polarity can be clearly understood in MEP plots as different colours represent different electrostatic potential. The red colour in the map shows the most negative region or most negative electrostatic potential, blue represents as most positive electrostatic potential and green coloured region indicates zero potential or neutral region. Thus red region is electron rich and contains negatively charged groups; blue region is electron deficient and partially positively charged. Slightly blue colour shows slightly electron deficient region and yellow or fade red indicates slightly electron rich region. MEP plots and 3D surface of molecular electrostatic potential shows the red around the oxygen indicating the electron rich (nucleophilic sites) region and blue around nitrogen showing electron deficient region or (electrophilic sites).

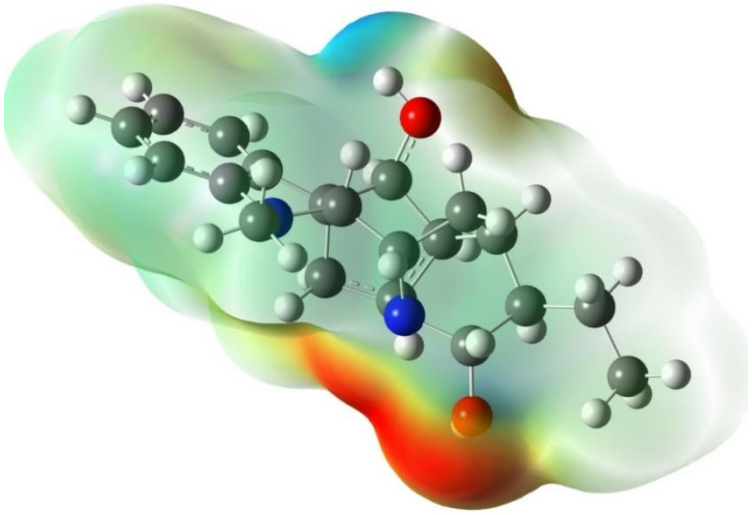


Figure 37: Electrostatic potential map of Ajmaline

In Ajmaline (figure 37) the charge distribution shows the negative charge and thus negative electrostatic potential around oxygen atom of diazahexacyclo ring and oxygen atoms of ethanol group. It also represents the interactions of hydrogen bonding. Similarly the blue region around nitrogen indicates positive electrostatic potential as usual.

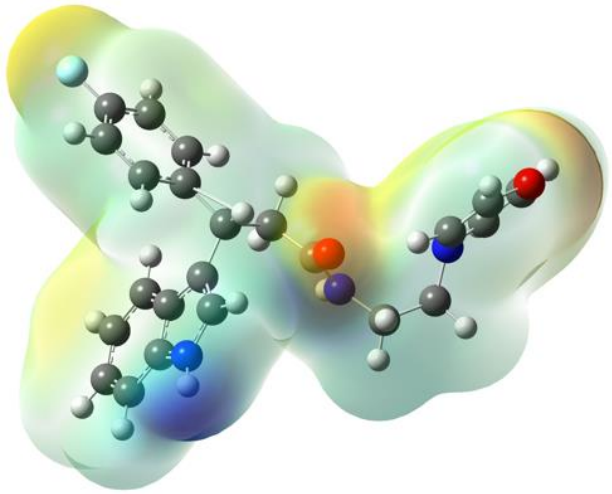


Figure 38: Electrostatic potential map of 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(morpholinoethyl) propanamide

Electrostatic potential map of the 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide shows the red region with faded red or yellow after certain distribution of negative charges around oxygen atom of propnamide group, oxygen atom of morpholine ring . Similarly yellow region represents slightly electron rich region around fluorine. And blue region around nitrogen of indole ring, morpholine ring

and propanamide shows the positive potential site. Slightly blue colour around carbon and hydrogen atoms reflects the slightly electron deficient region or slightly electrophilic region.

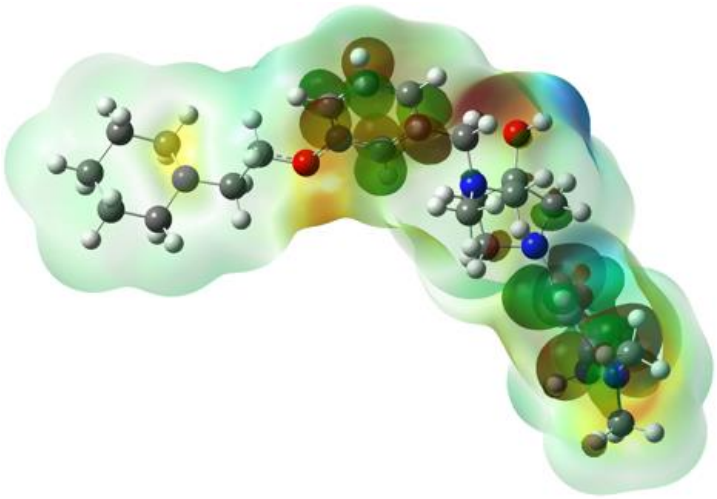


Figure 39: Electrostatic potential map of 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol

Similarly, the molecular electrostatic map of 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol shows the negative electrostatic potential around oxygen atom of alcohol, ethoxy group. Andelectropositive potential around nitrogen of pyrimidine, pyrrolidin, piperidin with blue colour indicates the active sites of electrophilic region.

4.8.4 Infrared spectrum analysis

IR spectrum and Raman was studied for possible frequencies of C-H,C-C,C-O set vibrations for all the compounds studied using Gauss 03 using B3LYP/631G basis set and Chemcraft software was also used to analyze and observe numerical values of intensity and frequencies clearly from output file obtained from Gaussian files of particular compounds. Vibrations observed only within the regions for particular set obtained from experimental values (adapted from other researches) were noted from the intensity-frequency plot obtained from Gauss 03. For Ajmaline IR spectrum observed from 3650-50 cm^{-1}. For 3-(4-fluorophenyl)-3-(1H-indol-3-yl)- (2-morpholinoethyl) propanamide IR spectrum was observed from 3600-12 cm^{-1}. Similarly 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol showed IR spectrum in the region 3608-7.84 cm^{-1}. The C-H stretching frequencies appear in the range of 3100– 3000 cm cm^{-1}. The ring C=C and C-C stretching vibrations, known as semicircle stretching occurs in the region 1625-1400 cm^{-1}. C-O vibrations occur at 1600-1850. N-H vibrations appear in the range of 3500-3800. O-H vibrations can be observed at 3500 cm^{-1}.

C-H, C-C, C-O, vibrations

Vibrational spectrum of Ajmaline

C-H vibrations of Ajmaline were observed at 3000, 3025 and 3100cm^{-1}. C-C vibrations observed at 1450, 14100, 1475, 1500, 1525 and C-O vibrations were observed at 1650 cm^{-1}.

Vibrational spectrum of 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-(2-morpholinoethyl) propanamide

C-H stretching vibrations of 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-(2-morpholinoethyl) propanamide were observed at 3063, 3062 and 3041. Similarly C-C vibrations were obtained at 1434 and 1570. And C-O vibrations were obtained at 1690.

Vibrational spectrum of 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol

C-H streching vibrational frequencies were observed at 3025, 3050 and 3083. Similarly C-C and C-O stretching vibrational spectrum were observed at 1400, 1537, 1627 and 1627 and 1638 respectively.

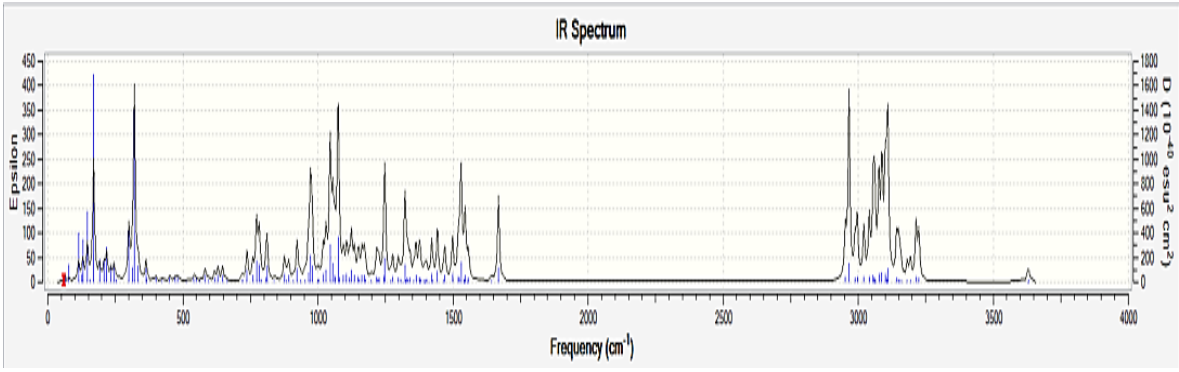


Fig: 40 FTIR spectrum of Ajmaline

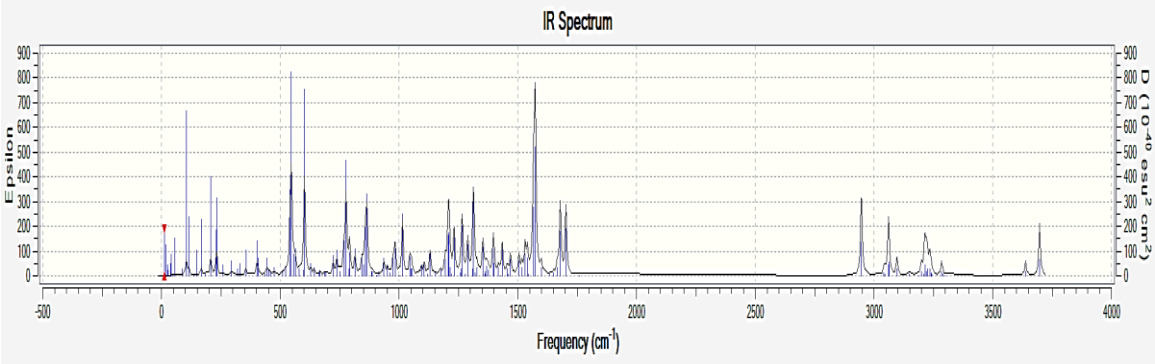


Fig: 41 FTIR spectrums of 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide

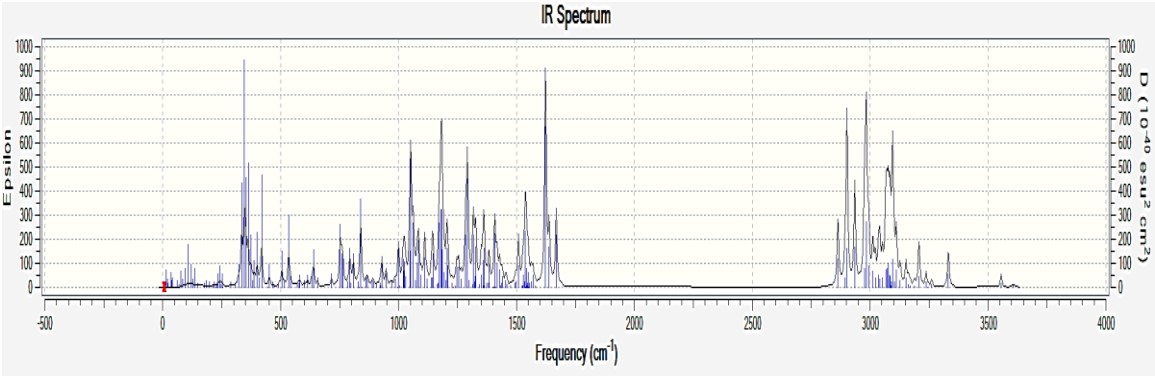


Fig: 42 FTIR spectrum of 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol.

5. SUMMARY

Many researches are ongoing for the discovery of probable therapeutics which could be proved as a permanent solution to the emergence of MDR and emergence of new disease. Failure of chemotherapeutics has been a burning issue among drug researches and concerned people because of growing emergence of resistance against currently used antibiotics. So world is in need of new antibiotics at present time to tackle the health issues that has emerged in recent times. Virtual screening based computational techniques have gained popularity in drug discovery now because of successful prediction of new ligands. Computational approach has been used in this study too. Molecular docking was used for physical screening of different ligand libraries to predict potential inhibitors of target protein CobA. Natural product library, Indole derivatives, kinase inhibitors, nucleoside mimetic libraries were used for predicting probable drug candidates. The lead compounds were selected on the basis of binding affinity with CobA. To ensure the safety of human health human hMAT1A was also used in the molecular docking to find out the compounds which have low affinity to this protein and binds to CobA with higher affinity. Thus ligands were finalized through comparative study of docking results of ligand library with CobA and molecular docking results of particular ligand library with hMAT1A. Ajmaline from natural products library, 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide among indole derivatives, 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one of kinase inhibitor ligand library and 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol among nucleoside mimetics were found to inhibit CobA and showed highest binding energy for CobA in comparative study of all the ligands from particular ligand library. These compounds also showed low binding energy against hMAT1A hence showing competitive binding with cobA. Further protein-ligand interaction study of these selected compounds using PyMOL, Discovery studio, LIGPLOT^{+}, Gauss 03 revealed the strong interactions indicating the stability of the complexes. Lead compound Ajmaline could be used as potential drug target and can be used as important core structure for development of other drugs as well. It is approved drug which has been a common name in medicine study. Simialrly indole derivative has been used as drug candidates itself and it has been found to be metabolized into other indirubin derivatives showing even with stronger activity against the target proteins. And lead compounds 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol also have potential to be used as drug targets in their present state or modifications. owing to their excellent character. Our findings suggest that the in silico approaches of drug discovery is really helpful in

narrowing down the bulk libraries. Meanwhile it is advantageous in comparison to traditional screening methods in terms of requirement of resources and important factor like time needed to screen the potential antibiotics. Thus computational approaches can be used to cope with the present situation of emergence of MDR strains and to discover effective inhibitor or antibiotics within minimal time frame.

6. CONCLUSION

In present situation of emergence of resistance against available antibiotics and emergence of MDR in many bacterial strains, computational method of drug discovery could be of great aid to meet the need of time. Pharmacokinetic properties of compounds are closely associated with their chemical structure. And these characteristics could be understood and compared with physiochemical properties of the compound using different computational software. It was hypothesized that the lead compounds screened could be developed as putative competitive inhibitors of SAM utilizing protein CobA and might inhibit other SAM utilizing proteins as well. All of the lead compounds Ajmaline, 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide, 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one and 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol were narrowed down on the basis of ADME/Tox screening, and other drug like properties and also showed better virtual binding efficiency than native ligand SAM in molecular docking studies. Further these compounds have shown lower binder affinity than native ligand SAM in molecular docking with hMAT1A. Also the compound structure as they are or their modifications have been studied and found to be used successfully as potential drug target. The compounds screened in this study could be considered harmless and could inhibit CobA protein and rendering the pathogenic activity difficulty in survival of pathogens owing to the fact that this gene is critical for survival of some bacteria like *Pseudomonas*, *Salmonella* and some other pathogenic bacteria as well.

RECOMMENDATION

Lead compounds Ajmaline, 3-(4-fluorophenyl)-3-(1H-indol-3-yl)-N-(2-morpholinoethyl) propanamide, 1-{4-[2-(2-Phenylethyl) benzoyl] piperazin-1-yl} ethan-1-one and 1-[6-(dimethyl amino) pyrimidin-4-yl]-3-[[methyl-[[3-(2-piperidin-ylethoxy) phenyl] methyl] amino-methyl] pyrrolidin-3-ol could be further studied for enzyme kinetics, physio-chemical pathways and tested invitro for further verifications and analytical measures to develop drug. Through animal testing and toxicity testing as additional steps these compounds could be developed as future antibiotics.

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8. APPENDICES

8.1 List of target proteins with sources

Proteins	Pdb Ids	Ligand	Organisms	References
hMAT1A	6SW5	SAM	*E. coli*	Komoto *et al.*,2003
CobA (Uroporphyrin-III C-methyl transferase)	1S4D	SAH	*Pseudomonas denitrificans*	Vevodova *et. al* 2004

8.2 Indole derivatives with higher binding potential in CobA than SAM

Energy minimized ligands	B.E in CobA(B.E of native ligand SAM=-8.4)
v029-4195_uff_e=873.46	-9.6
s233-0061_uff_e=1438.91	-9.5
v029-3401_uff_e=883.41	-9.5
l259-0295_uff_e=616.37	-9.3
l259-0946_uff_e=681.43	-9.3
m378-0751_uff_e=522.04	-9.3
m378-0067_uff_e=536.86	-9.2
p935-1673_uff_e=2029.55	-9
v027-9818_uff_e=419.17	-9
m286-1470_uff_e=724.70	-8.9
m378-0051_uff_e=589.43	-8.9
p935-1675_uff_e=1971.55	-8.9
y031-2293_uff_e=353.65	-8.9
l345-0030_uff_e=406.17	-8.8
m286-1234_uff_e=596.15	-8.8
m378-0544_uff_e=533.49	-8.8
m378-0710_uff_e=568.36	-8.8
p090-0612_uff_e=465.90	-8.8
p095-0030_uff_e=440.80	-8.8
t841-2578_uff_e=453.00	-8.8
v030-3008_uff_e=705.12	-8.8
v031-0915_uff_e=304.23	-8.8
p091-0461_uff_e=398.16	-8.7
p095-0088_uff_e=345.18	-8.7
s219-0054_uff_e=425.32	-8.7
v029-3984_uff_e=832.67	-8.7
v029-4005_uff_e=379.94	-8.7

m378-0540_uff_e=545.74	-8.6
m378-0599_uff_e=595.21	-8.6
p090-0026_uff_e=294.44	-8.6
p090-0252_uff_e=315.38	-8.6
p091-0167_uff_e=306.98	-8.6
p091-0215_uff_e=318.26	-8.6
p970-1066_uff_e=1367.46	-8.6
s219-0182_uff_e=1710.75	-8.6
s223-0043_uff_e=1890.91	-8.6
v027-1429_uff_e=488.97	-8.6
v029-1342_uff_e=462.00	-8.6
y020-1434_uff_e=303.25	-8.6
y040-5608_uff_e=728.94	-8.6
m378-0556_uff_e=546.95	-8.5
m770-0450_uff_e=498.46	-8.5
p090-0008_uff_e=292.04	-8.5
p091-0103_uff_e=298.66	-8.5
p091-0449_uff_e=279.71	-8.5
p091-0573_uff_e=292.34	-8.5
p095-0430_uff_e=314.92	-8.5
p095-0552_uff_e=327.51	-8.5
v029-5685_uff_e=621.82	-8.5
y031-2200_uff_e=321.57	-8.5

8.3 Top 50 Kinase inhibitors with higher binding potential in CobA than SAM

Energy minimized ligands	B.E in CobA(B.E of native ligand SAM=-8.4)
pb28339681_uff_e=314.61	-9.8
pb745171168_uff_e=437.77	-9.8
pb371217768_uff_e=1323.33	-9.7
pb1152158302_uff_e=851.95	-9.6
pb30981308_uff_e=311.63	-9.6
pb71472525_uff_e=183.39	-9.6
pb118017112_uff_e=448.59	-9.5
pb1272578125_uff_e=327.92	-9.5
pb1577054334_uff_e=321.73	-9.5
pb223891616_uff_e=646.93	-9.5
pb52208263_uff_e=552.53	-9.5
pb73358996_uff_e=632.45	-9.5
pb117523362_uff_e=312.01	-9.4
pb16280414_uff_e=532.68	-9.4
pb211365232_uff_e=382.38	-9.4

pb223902150_uff_e=541.88	-9.4
pb229388928_uff_e=490.65	-9.4
pb26680168_uff_e=196.47	-9.4
pb27841254_uff_e=445.61	-9.4
pb49586811_uff_e=449.93	-9.4
pb57990117_uff_e=355.15	-9.4
pb1103092085_uff_e=879.65	-9.3
pb116884756_uff_e=439.24	-9.3
pb1218025461_uff_e=666.49	-9.3
pb1873286139_uff_e=1860.46	-9.3
pb217319254_uff_e=514.37	-9.3
pb223398070_uff_e=938.64	-9.3
pb56008237_uff_e=316.13	-9.3
pb99153948_uff_e=458.79	-9.3
pb126120442_uff_e=957.74	-9.2
pb223891344_uff_e=538.40	-9.2
pb223924630_uff_e=556.55	-9.2
pb237657070_uff_e=340.59	-9.2
pb237809020_uff_e=669.40	-9.2
pb25725116_uff_e=776.92	-9.2
pb274679024_uff_e=661.35	-9.2
pb318196090_uff_e=314.25	-9.2
pb1232249244_uff_e=423.09	-9.1
pb1735152738_uff_e=282.94	-9.1
pb199695682_uff_e=314.77	-9.1
pb27820490_uff_e=544.43	-9.1
pb27847178_uff_e=401.92	-9.1
pb31409419_uff_e=458.18	-9.1
pb46298794_uff_e=1921.97	-9.1
pb57188840_uff_e=407.76	-9.1
pb85957376_uff_e=209.30	-9.1
pb109782786_uff_e=414.53	-9
pb1122807348_uff_e=551.81	-9
pb1277365952_uff_e=298.73	-9
pb134379490_uff_e=603.96	-9

8.4 Top 50 nucleoside mimetics with higher binding potential in CobA than SAM

Energy minimized ligands	B.E in CobA(B.E of native ligand SAM=-8.4)
las_51495177_uff_e=542.83	-11
bde_26099348_uff_e=2109.20	-10.7
las_51495213_uff_e=541.33	-10.6
bdf_33392217_uff_e=1916.87	-10.5
las_34154544_uff_e=542.35	-10.5
las_73739917_uff_e=1524.65	-10.4

bdh_33631060_uff_e=577.10	-10.2
las_33565829_uff_e=1687.09	-10.2
las_51495167_uff_e=632.70	-10.2
las_51499314_uff_e=2707.95	-10.2
bdh_33628646_uff_e=1613.77	-10.1
las_51115305_uff_e=1451.14	-10.1
bdd_30344181_uff_e=653.02	-10
bdd_30344195_uff_e=633.22	-10
las_34151640_uff_e=1434.47	-10
las_34154490_uff_e=563.25	-10
las_51499235_uff_e=2643.27	-10
las_34151399_uff_e=380.47	-9.9
las_51497393_uff_e=1544.91	-9.9
las_51500258_uff_e=1693.08	-9.9
bdd_30344179_uff_e=652.24	-9.8
bdd_30344214_uff_e=500.26	-9.8
bdd_30344219_uff_e=512.10	-9.8
bdf_34039702_uff_e=1546.50	-9.8
las_51499056_uff_e=1776.99	-9.8
bdd_30344188_uff_e=747.24	-9.7
bde_33184620_uff_e=733.14	-9.7
bdg_34096651_uff_e=1259.74	-9.7
bdg_34151363_uff_e=383.62	-9.7
bdh_30950331_uff_e=618.46	-9.7
bdh_33729083_uff_e=1370.23	-9.7
las_34151538_uff_e=1472.74	-9.7
las_34151643_uff_e=1347.41	-9.7
las_51495635_uff_e=463.18	-9.7
bdd_30344209_uff_e=510.81	-9.6
bde_30714286_uff_e=587.00	-9.6
bde_33857748_uff_e=454.24	-9.6
bde_34023420_uff_e=483.07	-9.6
bdg_34042386_uff_e=1467.94	-9.6
bdh_34040990_uff_e=1234.43	-9.6
las_51495645_uff_e=378.61	-9.6
las_51501716_uff_e=1457.53	-9.6
bdd_30344184_uff_e=672.60	-9.5
bdd_30344185_uff_e=651.92	-9.5
bdd_30344186_uff_e=746.45	-9.5
bdd_30344216_uff_e=498.47	-9.5
bde_30875662_uff_e=500.01	-9.5
bde_33184110_uff_e=655.82	-9.5
bdf_33508663_uff_e=469.91	-9.5
bdg_34079164_uff_e=444.01	-9.5
las_34151625_uff_e=450.75	-9.5