DESIGN, SYNTHESIS AND SCREENING OF PYRAZOLE LINKED THIAZOLIDINONES, OXAZEPINES AND BENZOXAZEPINES AS *Pf*-ENR AND *E.Coli* FABI INHIBITORS

A Dissertation submitted to
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In partial fulfillment of the requirements for the award of the Degree of
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Submitted by
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OCTOBER 2017

CERTIFICATE

This is to certify that the M.Pharm dissertation entitled, **"Design, synthesis and screening of Pyrazole linked Thiazolidinones, Oxazepines and Benzoxazepines as *Pf*-ENR and *E.coli* FabI inhibitors"** being submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai was carried out by **Ms. Kokila Priya S (Reg. No.261515102)** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision and guidance to my fullest satisfaction.

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CONTENTS

DRUG DESIGN[1-4]

Drug design, often referred to as **rational drug design** or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Drug design for the knowledge of the three-dimensional structure of the biomolecular target is equant but not necessarily relies on computer modelling techniques. This type of modelling is sometimes referred to as **computer-aided drug design**.

Drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target known as **structure-based drug design**. In addition to small molecules, biopharmaceuticals and especially therapeutic antibodies are an increasingly important class of drugs and computational methods for improving the affinity, selectivity, and stability of these protein-based therapeutics have also been developed. The major aim is to find whether the given molecule bind to the target and causes pharmacological actions or not.

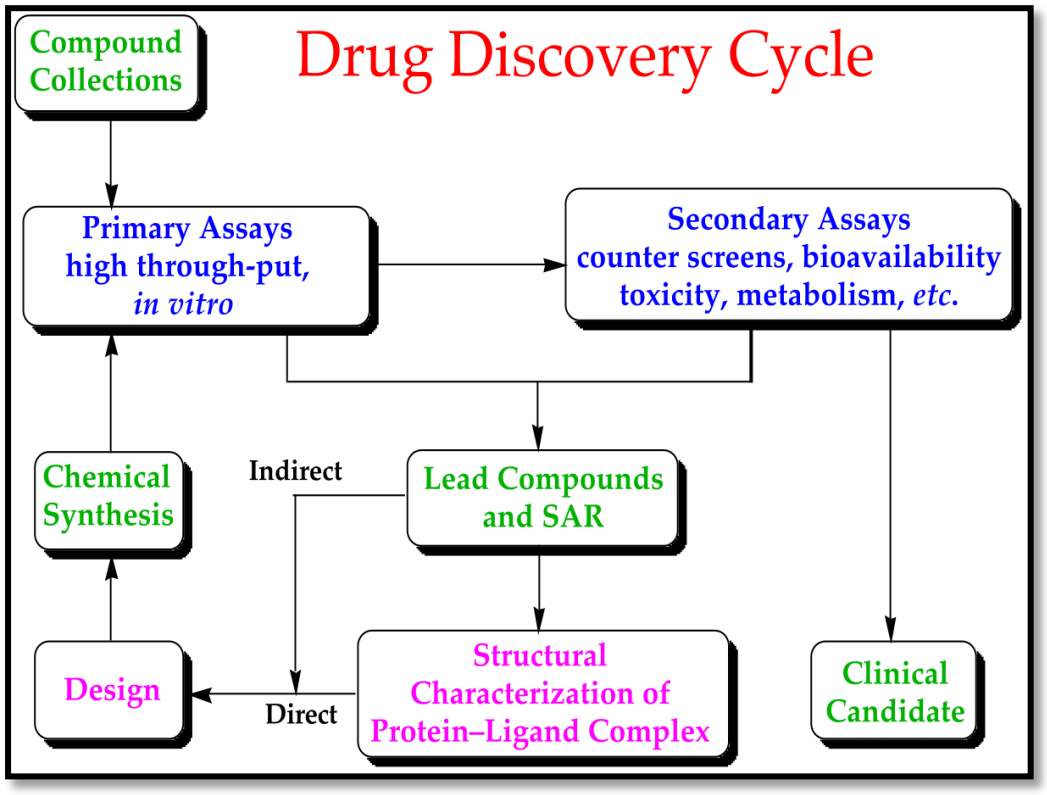


Fig.1: Flowchart of Drug Discovery

The basic steps involved in CADD are:

1. Hit identification using virtual screening (structure- or ligand-based design)
2. Hit-to-lead optimization of affinity and selectivity (structure-based design, QSAR, etc.)
3. Lead optimization of other pharmaceutical properties while maintaining affinity.

Types of drug design

- **Ligand-based drug design**
- **Structure-based drug design**

Ligand Based Drug Design (LBDD)

It is also known as indirect drug design. In the absence of the structural information of the target, ligand based method is used to know about inhibitors for the target receptor. Biologically active lead molecule is detected by using structural or topological similarity or pharmacophoric similarity properties. There are several criteria's for similarity comparisons such as structure as well as shape of individual fragment or electrostatic properties of the molecule. The generated lead molecules are ranked based on their similarity score or obtained by using different methods or algorithms.

Structure-based drug design (SBDD)

Structure-based drug design (or **direct drug design**) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as X-ray, crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates.

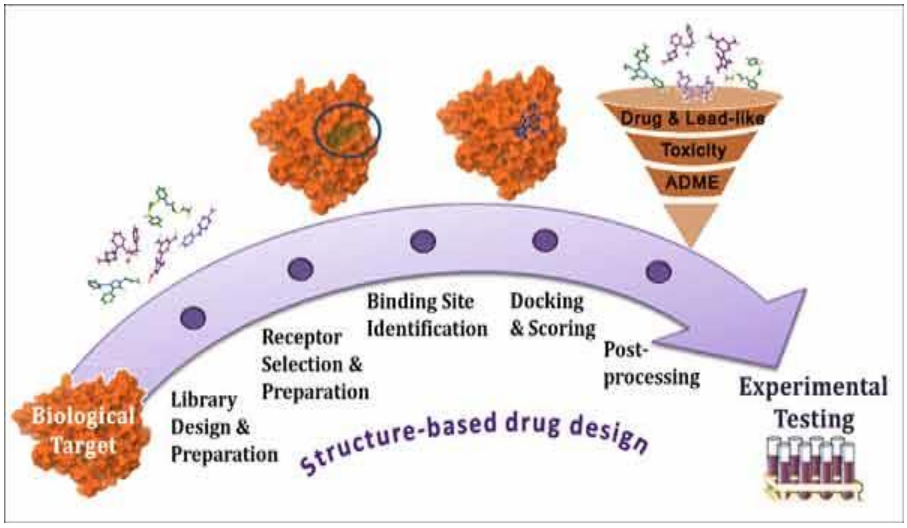


Fig.2: Structure Based Drug Design

Alternatively various automated computational procedures are used to suggest new drug candidates. Currently, use of SBDD has become a standard exercise as a part of drug discovery and development, both in academics and industry.

Typically, the process involves

1. Selection and identification of the target.
2. Search for lead or lead identification.
3. Lead optimization.

Drug target

Drug discovery process begins with the identification of a possible therapeutic target. The selected drug target must be a key molecule involved in a specific metabolic or cell signalling pathway that is known or believed to be related to a particular disease state.

Important drug targets include:

- Enzymes (inhibitor- reversible or irreversible)
- Receptors (agonist or antagonist)
- Nucleic acid inter collators or modifiers
- Ion channels (blockers or openers)
- Transporters (uptake inhibitors)

The 3D structure of the protein target is usually obtained by X-ray crystallography [crystal structures of different macromolecules are available from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Database], Nuclear Magnetic Resonance (NMR) or homology modelling from a previously determined structure. Various other parameters like temperature factors, Vander Waals interactions, hydrogen bonding etc., in the region of interest on the target should be evaluated.

VIRTUAL SCREENING TECHNIQUES (VS) [5-7]

Virtual screening (**VS**) is a computational technique used in drug discovery to search libraries of small molecules in order to identify those structures which are most likely to bind to a drug target, typically a protein receptor or enzyme.

Virtual screening has been defined as the "automatically evaluating very large libraries of compounds" using computer programs. As this definition suggests, VS has largely been a numbers game focusing on how the enormous chemical space of over 10^{60} conceivable compounds can be filtered to a manageable number that can be synthesized, purchased and tested. Virtual screening has become an integral part of the drug discovery process.

Methods

These are two broad categories of screening technique: Ligand-based and structure based VS

Ligand-based virtual screening technique

It is further divided into **ligand alignment, Pharmacophoric approach and machine learning algorithms.**

In **Ligand alignment**, a single 3D structure of a biologically active ligand is used as a template by ligand alignment for the super positioning and scoring of other 3D molecular structures from chemical libraries with respect to similarity of their characteristics like shape, interaction possibilities or physicochemical properties.

In **Pharmacophoric approach**, by use of structurally diverse set of ligands that bind to the receptor, a coarse-grained 3D surrogate of receptor is generated in the pharmacophoric approach. These are usually done by calculating all of the possible superposition of predefined chemical groups which are recognized at the target binding site and are responsible for the biological activity. This pharmacophoric, serves as template for the selection of the molecules which fulfil the specified geometrical constraints in the VS queries.

Machine learning algorithms relays on QSARs which correlate biological data with molecular descriptors hence derives statistical models used to predict activity of novel compounds. Some of the examples of the machine learning techniques that are becoming popular tools of model building and VS are: self-organizing maps (SOM), Binary QSAR, k-nearest neighbor approach (kNN), aritificial neural network (ANN), etc.

Structure-based virtual screening technique [8, 9]

In silico or virtual screening (VS) of large compound collections to identify a subset of compounds that contains relatively many hits against the target. The compounds that are virtually screened can stem from corporate or commercial compound collections, or from virtual compound libraries. If a three-dimensional (3D) structure or model of the target is available, a commonly used technique is structure-based virtual screening (SBVS)

Structure-based virtual screening involves docking of candidate ligands into a protein target followed by applying a scoring function to estimate the likelihood that the ligand will bind to the protein with high affinity.

SEARCHING AND POSE PREDICTION

Searching for the correct binding mode (pose prediction) of a molecule is typically carried out by performing a number of trials and keeping those poses that are energetically best. It involves finding the correct orientation and, as most ligand molecules are flexible, the correct conformation of the docked molecule. The decision to keep a trial pose is based on the computed ligand–receptor interaction energy (score) of that pose. To identify and rank-order many different poses of a molecule during the search in a reasonable time, several programs calculate a 'dock score' (a crude score based on a simple energy function such as a force field with an electrostatic term and repulsive and attractive Van-der-Waals terms), which can be evaluated very rapidly during the docking process, while a more sophisticated function is used to calculate the final 'affinity score' for that molecule.

SCORING OR AFFINITY PREDICTION

Many of the scoring functions fall into one of two main groups. One main group comprises knowledge-based scoring functions that are derived using statistics for the observed interatomic contact frequencies and/or distances in a large database of crystal structures of protein–ligand complexes. Several such potentials to predict binding affinity have been developed (e.g., PMF, DrugScore, SmoG, and Bleep)

The other main group contains scoring schemes based on physical interaction terms. These so-called energy component methods are based on the assumption that the change in free energy upon binding of a ligand to its target can be decomposed into a sum of individual contributions:

$$\Delta G_{bind} = \Delta G_{int} + \Delta G_{solv} + \Delta G_{conf} + \Delta G_{motion}$$

The individual terms in this equation account for the main energetic contributions to the binding event, as follows: specific ligand–receptor interactions (ΔG_{int}), the interactions of ligand and receptor with solvent (ΔG_{solv}), the conformational changes in the ligand and the receptor (ΔG_{conf}) and the motions in the protein and the ligand during the complex formation (ΔG_{motion}). Many popular scoring functions have been derived this way (e.g., LUDI, ChemScore, Validate, GOLD score, PLP, FlexX score, ScreenScore, Autodock3). Various approaches to derive optimal coefficients for regression based scoring functions exist. Most of them aim to reproduce experimental binding affinities.

Advantages

- Time and cost reduction of screening process of millions of small molecules, compared to HTS (High Through-put Screening).
- There is no need for physically existing compounds to perform the screening process, unlike HTS.

- A large number of docking programs and scoring functions
- Different approaches of VS have been created for lead discovery depending
- Each time on the availability of experimental information (SBVS Ligand-Based VS, Fragment-Based VS, etc.)

Limitations

- Many VS tools are applicable and successful to specific case studies (based on the training set) and not in general cases.
- Compounds being identified by HTS are usually more bioactive than compounds identified by VS.
- Weakness in perfect inclusion of receptor structural flexibility and of water in docking computations due to computational-cost and high complexity of its modelling.
- Scoring is still challenging in predicting accurately the correct binding pose and ranking of the compounds due to the difficulties in parameterizing the complexity of the ligand-receptor binding interactions and the approximations in calculating desolvation and entropic terms.

By using various methods described above, lead moiety is identified by using software's like **iGEMDOCK v.2, AUTODOCK**, **DOCK** etc. The molecules which are docked well will be superimposed on one another. The ligands which are not docked well will be in different places. This lead molecule is being subjected to docking to know the interaction between the protein of interest and the lead. A graphical-automatic drug discovery system, called **iGEMDOCK v.2** is used for integrating docking, post-analysis, screening and visualization. To our best knowledge, **iGEMDOCK v.2** is the first system which combines structure-based virtual screening and post-screening analysis.

The lead molecule which is identified by virtual screening are taken for lead optimization by knowing ADME data and drug likeliness properties.

ADME data [10]

The high-throughput screening in drug discovery for absorption, distribution, metabolism and excretion (ADME) properties has become the norm in the industry. Only a few years ago it was ADME properties that were attributed to more failure of drugs than efficacy or safety in the clinic trials. With the realization of new techniques and refinement of existing techniques better projections for the pharmacokinetic properties of compounds in humans are being made, shifting the drug failure attributes more to the safety and efficacy properties of drug candidates. There are tremendous numbers of tools available to discover scientists to screen compounds for optimization of ADME properties and selection of better candidates. However, the use of these tools has generally been to characterize these compounds rather than to select among them. This report discusses applications of the available ADME tools to better understand the clinical implication of these properties, and to optimize these properties. It also provides tracts for timing of studies with respect to the stage of the compound during discovery, by means of a discovery assay by stage (DABS) paradigm. The DABS provide the team with a rationale for the types of studies to be done during hit-to-lead, early and late lead optimization stages of discovery, as well as outlining the deliverables (objectives) at those stages. DABS has proven to be optimal for efficient utilization of resources and helped the discovery team to track the progress of compounds and projects. Various medium and high throughput *in vitro* ADME screens are therefore now in use. In addition, there is an increasing need for good tools for predicting these properties to serve two key aims first, at the design stage of new compounds and compound libraries so as to

reduce the risk of late-stage attrition; and second, to optimize the testing and screening by looking at only the most promising compounds. Various software's and servers are available; one such is *pharma algorithm* server.

DRUG LIKELINESS [11,12]

Druglikeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. A druglike molecule has properties such as:

- Solubility in both water and fat, as an orally administered drug needs to pass through the intestinal lining after it is consumed, be carried in aqueous blood and penetrate the lipid-based cell membrane to reach the inside of a cell.
- Potency at the biological target.
- Several scoring methods can be used to express druglikeness as a function of potency and physicochemical properties, for example ligand efficiency and lipophilic efficiency.
- Since the drug is transported in aqueous media like blood and intracellular fluid, it has to be sufficiently water-soluble in the absolute sense (i.e. must have a minimum chemical solubility in order to be effective). Solubility in water can be estimated from the number of hydrogen bond donors vs. alkyl side chains in the molecule.
- Molecular weight: the smaller the better, because diffusion is directly affected. The great majority of drugs on the market have molecular weights between 200 and 600 Daltons
- A traditional method to evaluate drug likeness is to check compliance

of Lipinski's Rule of Five, which covers the numbers of hydrophilic groups, molecular weight and hydrophobicity. There are many online servers available for calculating druglikeness score, one such is molinspiration server.

LIPINSKI'S RULE OF FIVE[13]

Lipinski's rule of five also known as the **Pfizer's rule of five** or simply the **rule of five** (**RO5**) is a rule of thumb to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules.

Lipinski's rule says that, in general, an orally active drug has not more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors (sum of OHs and NHs)
- Not more than 10 hydrogen bond acceptors (sum of Ns and Os)
- Molecular weight not greater than 500 Daltons
- An octanol-water partition coefficient, log *P*, not greater than 5.
- Number of violations less than 5

Improvements

To evaluate druglikeness in a better way, the rules have spawned many extensions by Ghose *et al.* in 1999.

- log *P* : -0.4 to +5.6 range
- Molecular refractivity : 40-130

- Molecular weight : 160-480
- Number of atoms : 20-70
- Polar surface area must not be greater than 140 Å.

Over the past decade Lipinski's profiling tool for drug likeness has led to further investigations by scientists to extend profiling tools to lead-like properties of compounds in the hope that a better starting point in early discovery can save time and cost.

DOCKING [14, 15]

Docking entails predicting the protein-ligand complex structure and is followed by scoring in SBVS in order to rank the compounds. Docking programs utilize various methods of conformational search in order to explore the ligand conformational space; these are categorized as following:

a) Systematic methods, which place ligands in the predicted binding site after considering all degrees of freedom.

b) Random or stochastic torsional searches about rotatable bonds, such as Monte Carlo and genetic algorithms to "evolve" new low energy conformers.

(c) Molecular Dynamics simulation methods and energy minimization for exploring the energy landscape of a molecule.

Molecular docking may be defined as an optimization program, which would describe the 'best-fit' orientation of a ligand that binds to a particular protein of interest. The focus of molecular docking is to computationally stimulate the molecular recognition process.

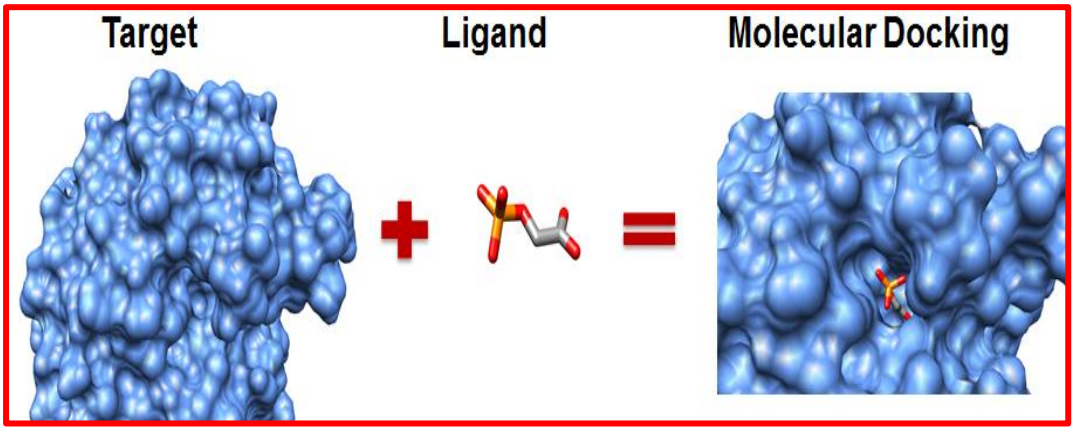


Fig.3: Docking

The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized.

A molecular docking calculation consists of the following steps:

- ✓ Optimization of the ligand geometry, calculation of pH-dependent partial charges, and identification of rotatable bonds.
- ✓ Calculation of electrostatic properties of the protein of interest and defining the ligand –binding region.
- ✓ Calculation of ligand-protein interaction by a scoring function that includes terms and equations that describe the intermolecular energies.

Docking produces plausible candidate structures. These candidates must be ranked by using scoring functions and to identify structures that are most likely to occur in nature.

Rigid-body docking and flexible docking

If the bond angles, torsion angles and bond lengths of the components are not modified at any stage of complex generation, then they are known as rigid body docking. A rigid-body docking is sufficiently good for most docking, when substantial change occurs within the components at rigid-body docking. Docking procedures which permit flexible docking procedures or conformational change, must intelligently select small subset of possible conformational changes for consideration.

Mechanics of docking

To perform a docking screen, the first requirement is a structure of interested protein. Usually the structure has been measured using a biophysical technique such as X-ray crystallography or NMR spectroscopy. The protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program is based on two components:

Search algorithm

The search space includes all possible orientations and conformations of the protein paired with ligand. With present computing resources, it is impossible to exhaustively explore the search space; which involves enumerating all possible distortions of each molecule (molecules are dynamic and exist in an ensemble of conformational states) and all possible rotational and translational orientations of ligand relative to the protein at a given level of granularity. Most docking programs account for a flexible ligand, and several are attempting to model a flexible protein receptor. Each “snapshot” of the pair is referred to as a pose.

There are many conditions for sampling the search space. Here are some examples:

- Use a coarse-grained molecular dynamics simulation to propose energetically reasonable poses stimulation. (direct search-simplex method; gradient-based search-steepest descent, Fletcher-Reeves method, Newton-Raphson method; least square methods-Marquardt method)
- Simulated annealing (Monte Carlo search of the parameter space)
- Use a "linear combination" multiple structures determined for the same protein to emulate receptor flexibility
- Use a genetic algorithm to "evolve" new poses that are successively more fragment-based construction.

Scoring function

The scoring function takes a pose as input, returns a number indicating the likelihood that the pose represents the favourable binding interaction.

Most scoring functions are physics based molecular mechanics force fields that estimate the energy of the pose; a low (negative) energy indicates a stable system and thus likely for a binding interaction. It is an alternative approach to derive a statistical potential for interactions from a large database of protein-ligand complexes, such as the Protein Data Bank. This evaluates the fit of the pose according to this inferred potential.

There are a large number of structures from X-ray crystallography for complexes between proteins and high affinity ligands. It is comparatively fewer for low affinity ligands as the later complexes tend to be less stable and therefore more difficult to crystallize. Scoring function trained with this data can dock hits (ligands predicted to bind to the protein and actually do not, when placed together in a test tube).

Autodock 4.2

Autodock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Autodock uses *Monte Carlo method* and *simulated annealing* in combination with *genetic algorithm* for building the possible conformations. The genetic algorithm is used for global optimization. Autodock works in Linux platform. Cygwin is used as a user friendly interface. The local search method is energy minimization and Amber "force field" model helps in the evaluation of binding positions compatible with several scoring functions based on the free energy. The atomic affinity grids can be visualized. This is helpful to guide organic synthetic chemists to design better binders. Autodock consists of two main programs:

- AutoGrid pre-calculates the grids.
- AutoDock perform the docking of the ligand to a set of grids describing the target protein.

It also has got capabilities to visualize atomic affinity grids and its graphical user interface, thus to support the analysis of docking results. It has an advantage of getting free academic license, at the same time parallel computation is not supported.

ANTIMALARIAL AGENTS [16,17]

Antimalarial agents are chemotherapeutic agents which are used for the prevention and treatment of malaria. Malaria is a mosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the Plasmodium type. The disease is most commonly transmitted by an infected female *Anopheles* mosquito.

The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood. The parasites travel to the liver where they mature and reproduce. Five species of Plasmodium can infect and be spread by humans. Most deaths are caused by *P. falciparum* because *P. vivax, P. ovale,* and *P. malariae* generally cause a milder form of malaria. The species *P. knowlesi* rarely causes disease in humans. Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests. Methods that use the polymerise chain reaction to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity.

LIFE CYCLE

In the life cycle of *Plasmodium*, a female *Anopheles* mosquito (the definitive host) transmits a motile infective form (called the sporozoite) to a vertebrate host such as a human (the secondary host), thus acting as a transmission vector. A sporozoite travels through the blood vessels to liver cells (hepatocytes), where it reproduces asexually (tissue schizogony), producing thousands of merozoites. These infect new red blood cells and initiate a series of asexual multiplication cycles (blood schizogony) that produce 8 to 24 new infective merozoites, at which point the cells burst and the infective cycle begins anew.

Other merozoites develop into immature gametocytes, which are the precursors of male and female gametes. When a fertilized mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut. The male and female gametocytes fuse and form an ookinete a fertilized, motile zygote. Ookinetes develop into new sporozoites that migrate to the insect's salivary glands, ready to infect a new vertebrate host. The sporozoites

are injected into the skin, in the saliva, when the mosquito takes a subsequent blood meal.

Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar and do not transmit the disease. The females of the *Anopheles*genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk and will continue throughout the night until taking a meal. Malaria parasites can also be transmitted by blood transfusions, although this is rare.

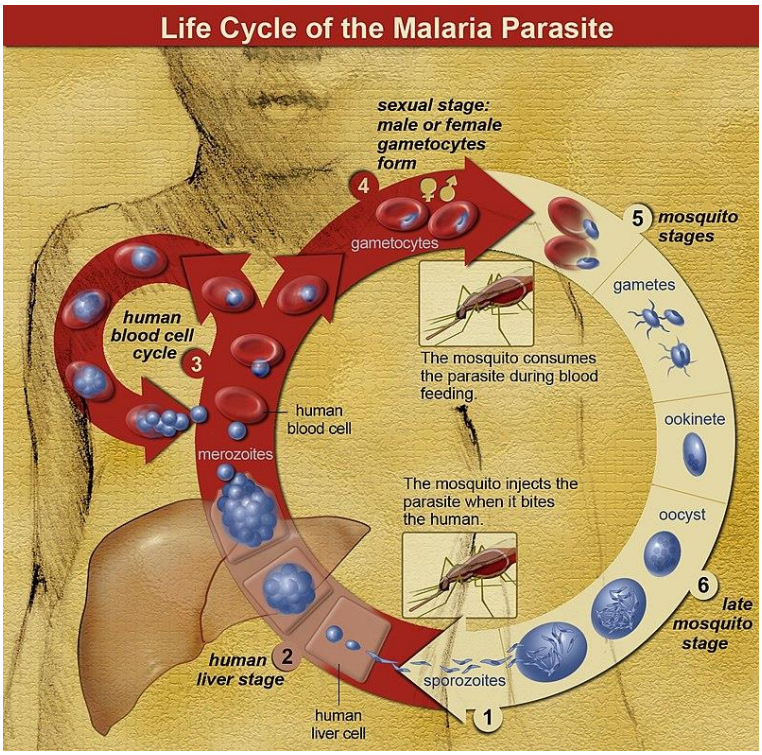


Fig.4: Life Cycle of Malarial Parasite

Classification of Antimalarial drugs[18]

4-Aminoquinolines	Chloroquine (CQ), Amodiaquine (AQ), Piperaquine
Quinoline-methanol	Mefloquine
Acridine	Mepacrine
Cinchona alkaloid	Quinine, Quinidine
Biguanides	Proguanil(Chloroguanide)
Diaminopyrimidines	Pyrimethamine
8-Aminoquinoline	Primaquine, Tafenoquine, Bulaquine
Sulfonamides and Sulfone	Sulfadoxine, Sulfamethopyrazine Dapsone
Anitibiotics	Tetracycline,Doxycycline, Clindamycin
Sesquiterpine lactones	Artesunate, Artemether, Arteether
Phenanthrene methanol	Halofantrine
Naphthoquinone	Atovaquone

Pharmacological classification of Anti-Malarial drugs

Classification	Stages	Drugs
Blood schizonticidal drugs	Erythrocytic phase	Chloroquine, Artemissin. Quinine, Atovaquone
Tissue schizonticidal drugs	Tissue form of plasmodium	Primaquine, Pyrimethamine, Proguanil, Tetracycline
Gametocidal drugs	Destroy sexual forms of parasite prevent transmission to mosquitoes	Primaquine, Quinine
Hypnozoiticidal	Destroy persistent liver stage of P.Vivax, P. ovale	Primaquine

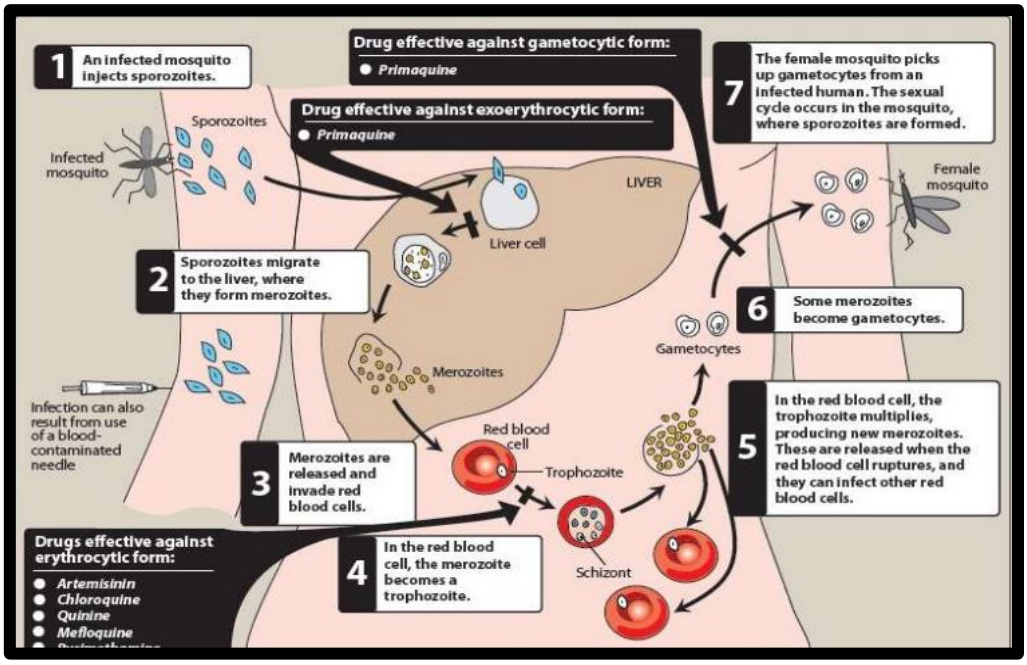


Fig.5: The Site of action of Antimalarial drugs

Plasmodium Falciparum Enoyl-acyl carrier protein Reductase (PfENR)[19-22]

Fatty acids plays a critical role in providing metabolic precursors of biological membranes and also represent an important form of metabolic energy, making their biosynthetic pathway an excellent target for antimicrobial agents. The fatty-acid biosynthesis pathway is localized in the parasite apicoplast, an essential organelle ancestrally related to cyanobacteria. In brief, the fatty acid synthesis starts with the formation of malonyl-acyl carrier protein (ACP) by malonyl-CoA ACP transacylase (MAT). Malonyl-ACP undergoes decarboxylative condensation with acetyl-CoA by the enzyme β-ketoacyl-ACP synthase III (KS) or acyl-malonyl-ACP condensing enzyme (FabB or FabF), respectively. This reaction is followed by nicotinamide adenine dinucleotide phosphate reduced (NADPH) dependent reduction, which is catalyzed by β -

ketoacyl-ACP reductase (KR) forming β-hydroxy butyryl-ACP. β-Hydroxy butyryl- ACP is then dehydrated to trans-2-butyryl-ACP (crotonoyl- ACP) by 3-hydroxy acyl-ACP dehydratases (DH).

The last step is the nicotinamide adenine dinuleotide reduced (NADH) dependent reduction, of the enoyl-ACP catalyzed by enoyl-ACP reductase (FabI) forming butyryl-ACP. Repetition of the elongation cycle 6–7 times yields a long acyl chain covalently linked to ACP. Plasmodium enoyl-ACP reductase (PfENR) catalyses the deterministic step of the elongation cycle and therefore, has emerged as an important drug target.

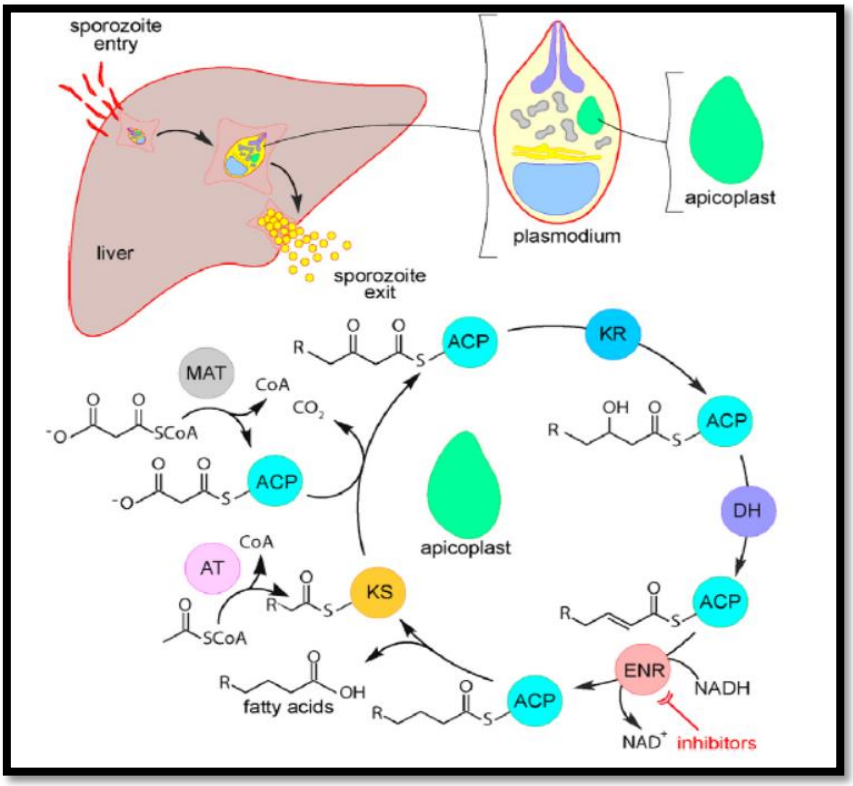


Fig.6: Pathway for fatty acid synthesis

Abbreviations are as follows: acyl carrier protein (ACP), malonyl transferase (MAT), β-ketoacyl acyl carrier protein reductase (KR), 3-hydroxyacyl ACP dehydrase (DH), enoyl-acyl carrier protein reductase (ENR), β-ketoacyl acyl carrier protein synthetase (KS), and coenzyme A (CoA).

The substrate-binding loops of *Pf*ENR have a maximum number of interactions with NAD^+ and they have the best binding affinities with triclosan with inhibition constants in the picomolar range. Triclosan binds non-covalently in this loop such that the ether linkage of triclosan is oriented like the enoyl group of the fatty acyl substrate. The ring A of triclosan nestles in a hydrophobic pocket lined by the residues, Tyr267, Tyr277, Gly313, Pro314, Ile323, Phe368, Ile369, and Ala372, while ring B of triclosan is surrounded by the nicotinamide ribose and the phosphates of NAD^+, Met281 and the substrate-binding loop residues Ala319, Ala320, and Ile323 and another loop with conserved residues Ala217, Asn218, Ala219, and Val222.14 While the interactions between the substrate-binding loop of the protein and NAD^+ dictate, to an extent, the affinity of the protein for triclosan, there are direct interactions, too, between ENR and triclosan. The phenolic OH of triclosan makes hydrogen bonds with NO^+_2 of nicotinamide ribose and with Tyr277 OH in PfENR which are conserved in other pathogenic organisms too, thus making it very critical for the inhibitory activity. Therefore, the most suitable positions for enhancing the biological activity of diphenyl ether class of compounds can be the substitutions at position 4 in ring A and position 20 and 40 in ring B. Examination of the PfENR:NAD^+ triclosan structure has earlier revealed that the 40 chloro group is in vanderwaals contact with the hydrophobic sidechains of Val-222 and Met-281. The halogen is directed toward the sidechain of Asn-218 and the backbone carbonyl of Ala-219, and is approximately 4 A° from the solvent accessible surface, therefore the 40-position could serve to

append functionality to alter the physiochemical properties of the inhibitor. 16 The 20-chloro group, on the other hand, is in close proximity to Ala-217 and atoms of the pyrophosphate moiety of NAD+. So the inhibitory activity against *Pf*ENR Series of pyrazolyl linked thiazolidinone, oxazepine and benzoxazepine was determined.

ANTIBACTERIAL AGENTS [25-29]

Bacteria are unicellular organisms. Under a microscope, they look like balls, rods or spirals. Some bacteria help to digest food, give the needed vitamins for body and destroy disease-causing cells. Bacteria are used in making healthy foods like yogurt and cheese.

But infectious bacteria can cause infections. They reproduce quickly in body. Many produce chemicals called toxins, which damages tissue and make you sick. Examples of infections causing bacteria include *Escherichia coli*, *Streptococcus* and *Staphylococcus*.

Bacteria role in disease

Most *E.coli* strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and gram-negative pneumonia. There is one strain, *E. coli* 0157:H7 that produces the Shiga toxin (classified as a bioterrorism agent). This toxin causes premature destruction of the red blood cells, which then clog the body's filtering system, the kidneys, causing Hemolytic-Uremic Syndrome (HUS). Uropathogenic *E.coli* (UPEC) is one of the main causes of urinary tract infections. Certain strains of *E.coli* are a major cause of foodborne

illness. The outbreak started when several people in Germany were infected with enterohemorrhagic *E.coli* (EHEC) bacteria, leading to hemolytic-uremic syndrome (HUS), a medical emergency that requires urgent treatment.

Antibacterial drugs

Antibacterial agents are agents that are 'selectively' toxic to bacteria, either killing them (bactericidal) or inhibiting their growth (bacteriostatic) without causing harm to the patient. These compounds act on structure found in bacteria and not in the host.

Antibacterial agents may be either antibiotics, which are natural substances produced by certain groups of microorganism or chemotherapeutic agents which are chemically synthesized. A semi synthetic antibiotic produced by the chemist by chemically modifying a molecular version, produced by the microorganism to impart desired properties.

Antibacterial agents specific to *E.coli*

- Amoxicillin along with other semi-synthetic penicillins
- Cephalosporins
- Carbapenems
- Aztreonam
- Trimethoprim-sulfamethoxazole
- Ciprofloxacin
- Nitrofurantoin
- Aminoglycosides

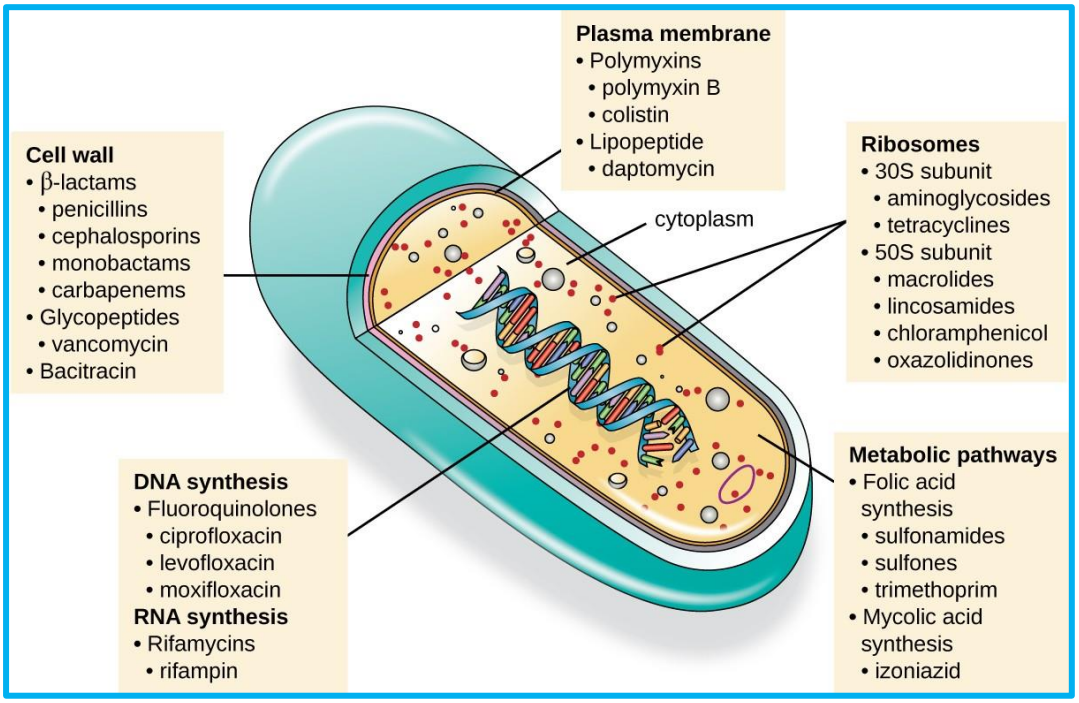


Fig 7: The site of action of Antibacterial drugs

***Escherichia coli* Enoyl-acyl carrier reductase protein Reductase (*E.coli* FabI)**

Owing to the development of new antibacterial agents which are non-resistant and site active, the enzyme inhibitory drug targets have been the main focus for the researchers. Enzymes that compromise the fatty acid synthetase (FAS) complex responsible for fatty acid biosynthesis are considered ideal targets for designing new antibacterial agents. Enoyl ACP reductase is a key regulatory in fatty acid elongation. Virtual screening and the databases revealed the importance of triclosan based *E.coli*- ENR inhibitors which act as the antibacterial agents by inhibiting the enoyl ACP reductase (ENR) enzymes.

The fatty acid synthase system of *Escherichia coli* is the paradigm for the type II or dissociated fatty acid synthase systems. Distinct genes encode each of the individual enzymes, and the same basic chemical reaction is often catalysed by multiple isozymes. There are four basic reactions that constitute a single round of elongation. The first step is the condensation of malonyl-ACP with either acetyl-CoA to initiate fatty acid synthesis (FabH) or with the growing acyl chain to continue cycles of elongation malonyl-ACP (FabB or FabF). The β-ketoacyl-ACP (FabH) is reduced by an NADPH-dependent β-ketoacyl-ACP reductase (FabG). There are two β-hydroxyacyl-ACP dehydratases (FabA or FabZ) capable of forming *trans*-2-enoyl-ACP. The product of the FabA gene is specifically involved in the introduction of a *cis* double bond into the growing acyl chain at the β-hydroxydecanoyl-ACP step and most efficiently catalyzes dehydration of short-chain β-hydroxyacyl-ACPs, whereas the FabZ dehydratase has a broader substrate specificity. The last reaction in each elongation cycle is catalysed by enoyl-ACP reductase (FabI).

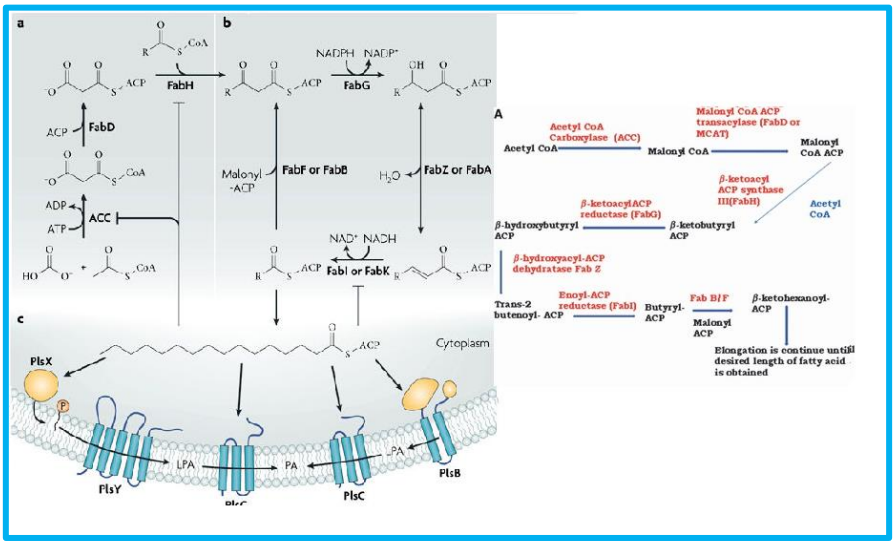


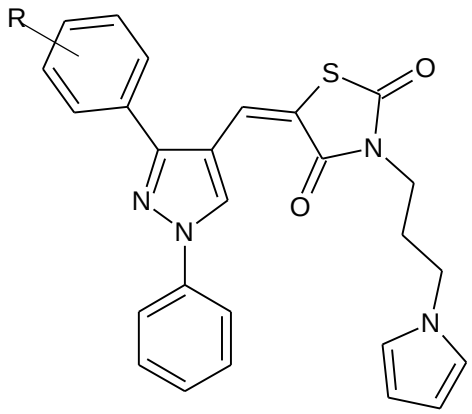
Fig.8: Biosynthetic pathway for fatty acid synthesis

Reviews reveal that the main chains of **Gly93–Ala95** involve vander Waals contacts with the bound NAD^+. Hydrophobic interaction with **Leu100, Tyr156, Met159, Ala196** and **Ile200** and a couple of water molecules are been observed in the binding domain **Gly 93, Ala 95, Tyr 146, Pro 191, Ile192, Tyr 194, Ala 196, Ala197, Phe 203**and **Met 206** forms the binding pocket.

LITERATURE REVIEW

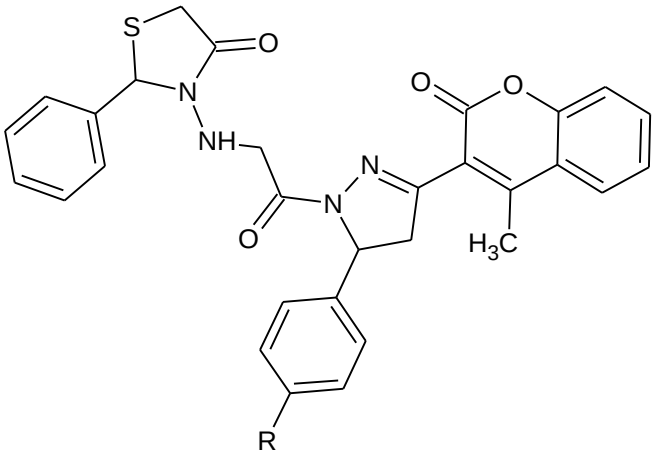
2.1 PYRAZOLE

- Nisheet et al., synthesized N-substituted thiazolidinone containing pyrazole(1) and screened for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. [30]



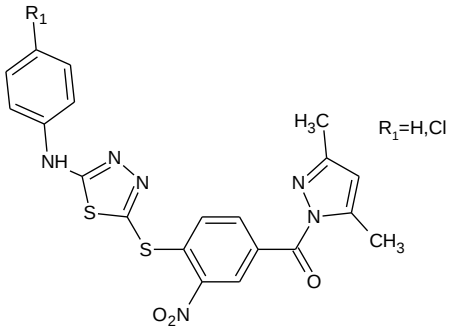
(1)

- Pawar et al., synthesized some pyrazole, thiazolidinone derivatives (2) and the compounds were screened for their antibacterial activity. [31]



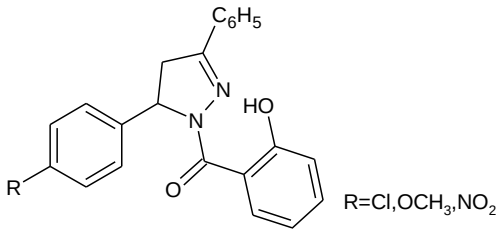
(2)

- Sah et al., reported the synthesis of substituted pyrazoles bearing 2-aryl amino -5- mercepto-1, 3, 4- thiadiazole (3) nuclei as possible antimicrobial agents. [32]



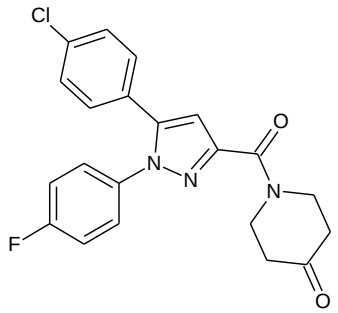
(1)

- Garkwad et al., synthesised certain N-1-[2-hydroxy benzoyl]- 5 – substituted phenyl-3- phenyl 4,5- dihydro pyrazoles (4) and screened for their antimicrobial activity. [33]



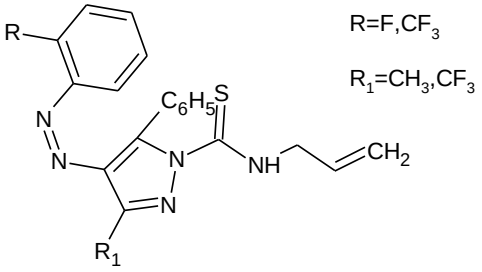
(2)

- Vijaykumar et al., synthesised novel 1,5-diaryl pyrazole (5) derivatives which exhibited good antibacterial and antifungal activities. [34]



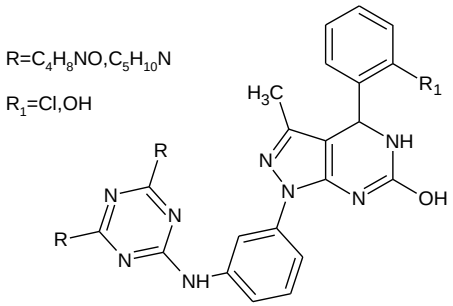
(3)

- Garg et al., synthesised 1-thiocarbonyl 3-trifluoro methyl-5- phenyl-4(2-fluro phenyl azo) pyrazoles (6). These compounds showed significant antibacterial activity against *S.aureus*, *S.typhi* and *E.coli.* [35]



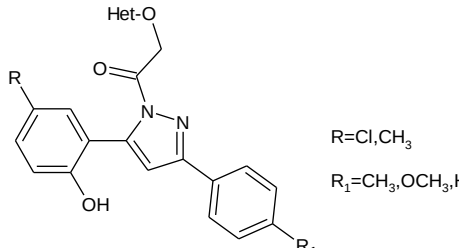
(4)

- Mistry et al., succeeded in the synthesis of a series of pyrazolo (5,4-d) – pyrimidine (7) derivatives which exhibited antimicrobial activity. [36]



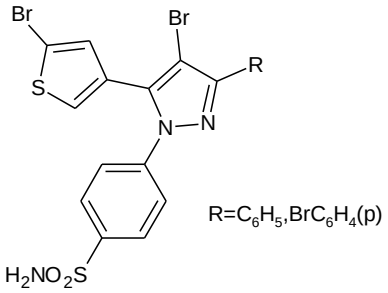
(5)

- Bhawsar et al., synthesised some new coumarino pyrazoles (8) and were evaluated them for antimicrobial activity. [37]



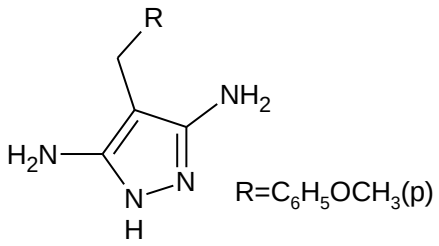
(6)

- Faidallah et al., synthesised 3,5-disubstituted pyrazoles (9)from chalcones. Their antimicrobial activities have been examined successfully. [38]



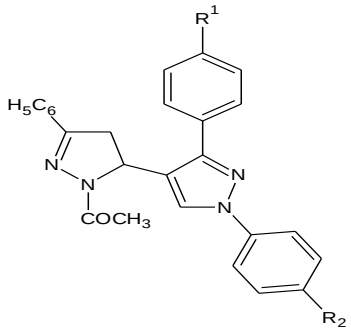
(7)

- Vishnu et al., reported the synthesis of a few pyrazoles (10) and these compounds were screened for antimalarial activity. [39]



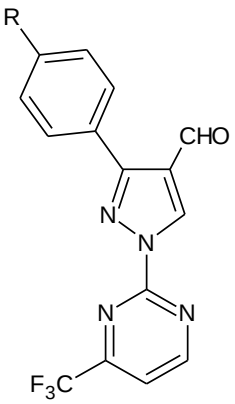
(8)

- Bekhit et al., synthesized new heterocyclic hybrids of pyrazole and its bioisosters(11).They were screened for antimalarial-antileishmanial activity. [40]



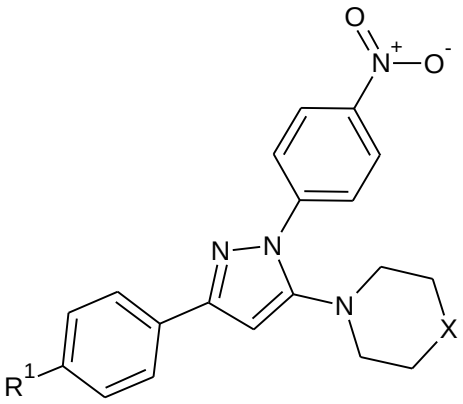
(9)

- Sanjay et al., synthesis of substituted pyrazole (12). They were screened for potential antimalarial target of the Enoyl-ACP Reductase inhibitor of *Plasmodium falcipuram*. [41]



(10)

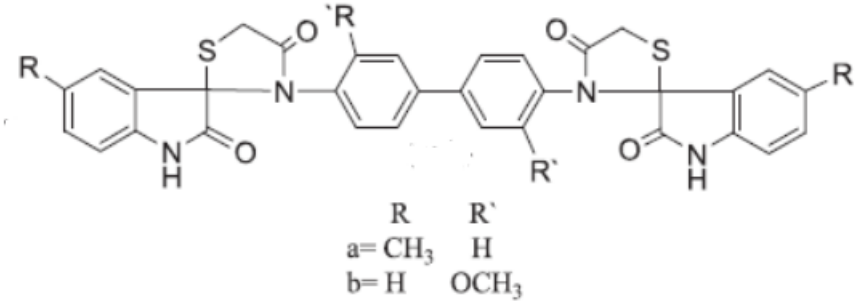
- Sabine et al., synthesis of 4-[5-(4-Phenoxyphenyl)-2H-pyrazol-3-yl]morpholine (13) derivatives. They were screened for antiparasitic activities. [42]



(11)

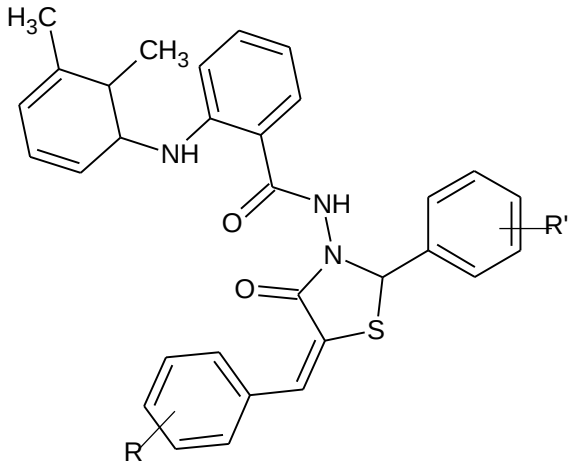
2.2 THIAZOLIDINONE

- Nadia G.Kandile et al., synthesized of new schiff's base bearing thiazolidine derivatives (14). They screened for antibacterial and cytotoxicity evaluation.[43]



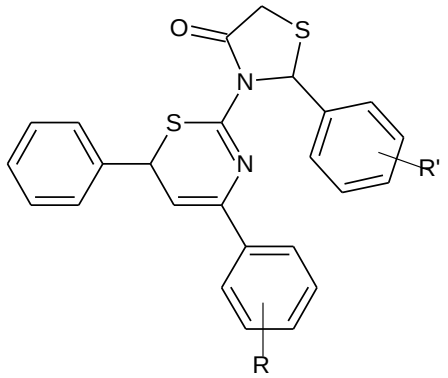
(12)

- Zeki A.Naser AI- Shamkhani et al., synthesized some novel schiff's base thiazolidinone derived from mefenamic acid(15) possessing antibacterial activity against anti*Escherichia coli*.[44]



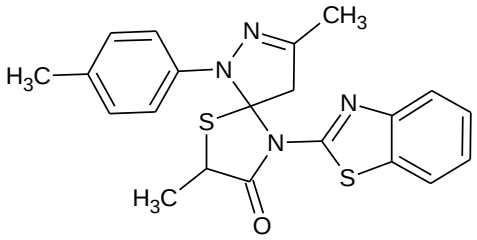
(13)

- Sayaji S.Didwagh et al., synthesized of novel thiazolidin-4-one (16) derivatives possessing antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus substilis*. [45]



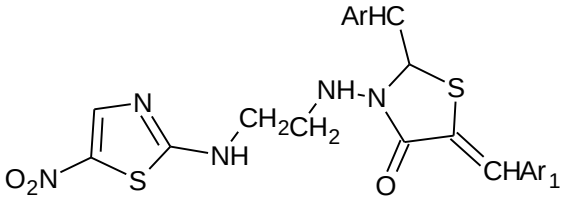
(14)

- Desai et al., synthesized novel Heterocyclic 4-thiazolidinone derivatives (17) and screened for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. [46]



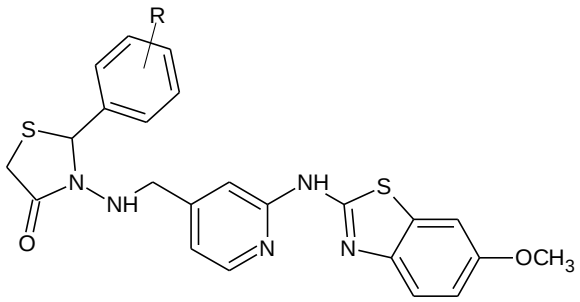
(15)

- Pushkal samadhiya et al., synthesized 4-thiazolidinone derivatives (18) and screened for antitubercular and antimicrobial activity. [47]



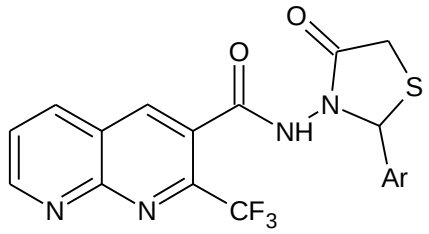
(16)

- Navin B.Patel et al., synthesized new 4-thiazolidinone derivatives (19) possessing antimicrobial activity against *Escherichia coli* and antifungal activity against *candida albicans*. [48]



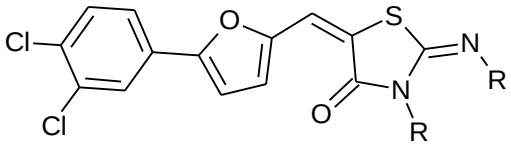
(17)

- Mogilaiah et al., synthesized 4-thiazolidinone (20) and screened for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus substilis*. [49]



(18)

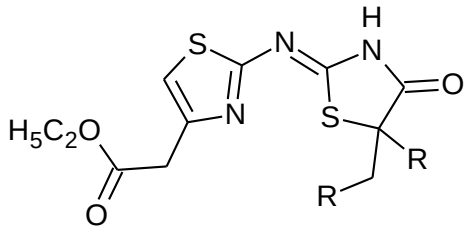
- Bhoot et al., synthesized of 2-(p-tolylimino)-3-(4-tolyl)-5-(5'-(3,4-dichlorophenyl))-thiazolidinone(21) possessing antimicrobial activity against *Escherichia coli*, *Pseudomonas Vulgaris* and antifungal agent against *Aspergillus niger*. [50]



(19)

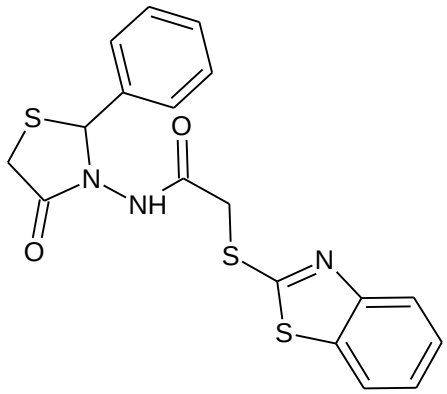
- Altintas et al., synthesized 5-substituted 5-(N,N-disubstituted aminomethyl)-2-[(4-carbethoxymethylthiazol-2-yl)imino]-4-thiazolidinones (22) were screened for their in vitro antibacterial activity

against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi* using disc diffusion method. [51]



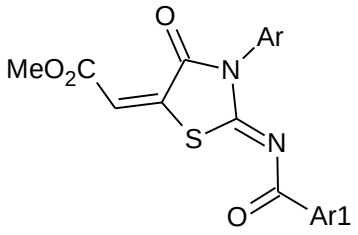
(20)

- Desai KG and Desai KR have synthesized 2-(aryl)-3-[2-(benzothiazolylthio)-acetamidyl]-4-oxo-thiazolidines (23) the compounds have been screened for their antibacterial activity against *Escherchia coli, Staphylococcus aureus* and *Bacillus substilis.* [52]



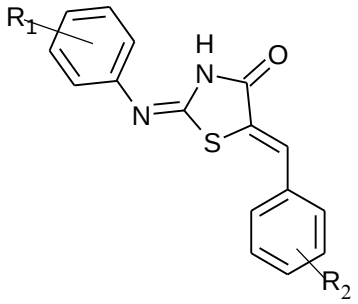
(21)

- Saeed A etal., synthesized derivatives of 2-aroylimino-3-arylthiazolidin-4-ones (24) and were screened for antimicrobial activity. [53]



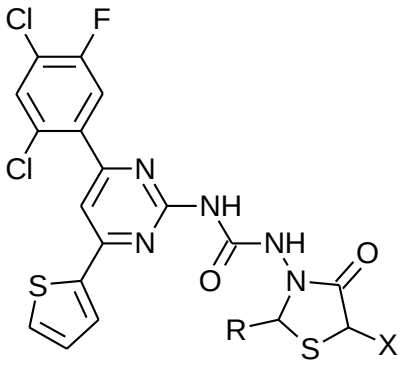
(22)

- Chawla et al., synthesized 2,5-disubstituted 4-thiazolidinone (25) analogues possessing antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal against *Candida albicans.* [54]



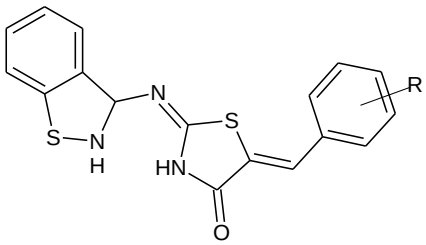
(23)

- Tejaskumar et al., synthesized fluorinated 4-thiazolidinones (26) having antibacterial activity against *Escherichia coli, Pseudomonas aureginosa, Bacillus substilis.* They screened for their antifungal activity against *Candida albican.*[55]



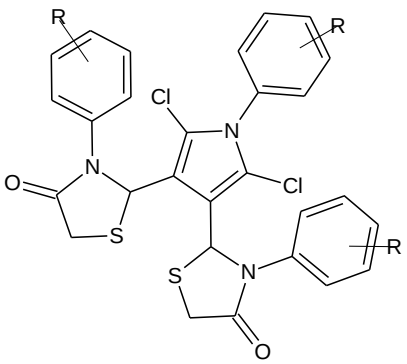
(24)

- Vicini et al., synthesized 2-thiazolylimino-5-benzylidene-4-thiazolidinones (27) were screened for their antibacterial and antifungal activity. [56]



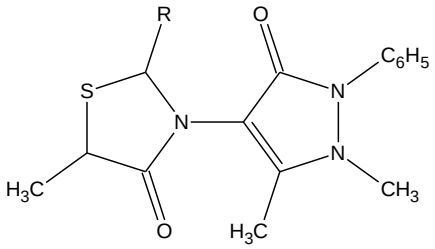
(25)

- A.P. Rajput et al., synthesized 2,5-dichloro-3,4-diformyl (N-substituted phenyl pyrroles thiazolidinones derivatives (28) were screened for their antibacterial activity. [57]



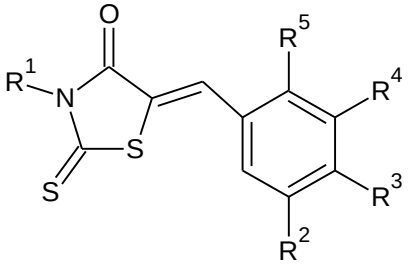
(26)

- A.N. Solankee et al., synthesized 4-thiazolidinones (29) and were screened for their antibacterial and antifungal activity. [58]



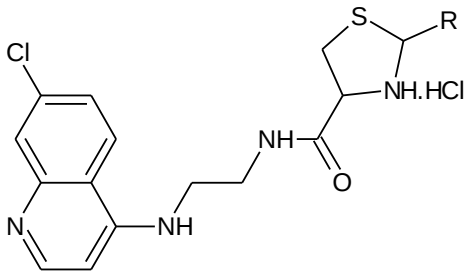
(27)

- Gyanendra et al., synthesis of 5-Benzylidene-2-thioxothiazolidin-4-one (30) Rhodanine class of compounds and were screened for *Pf*ENR enzyme inhibitors. [59]



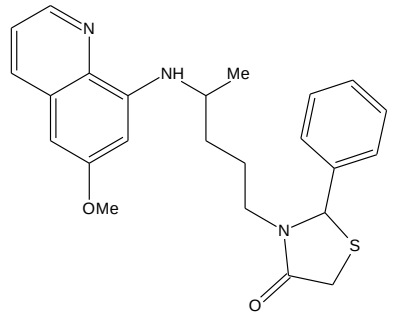
(28)

- Raja et al., designed and synthesized some 4-aminoquinline-derived thiazolidines (31) and were screened for their antimalarial activity and heme polymerization inhibition studies. [60]



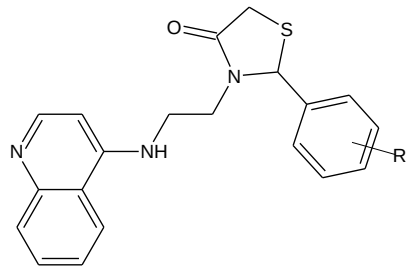
(29)

- Agular et al., synthesized primaquine-thaizolidinones derivatives (32) which possessing antimalarial activity. [61]



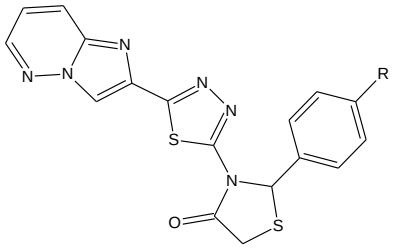
(30)

- F.A. Rojas Ruiz et al., synthesized and screened for antimalarial activity evaluation of new heterocyclic hybrids (33) based on chloroquine and thiazolidinone.[62]



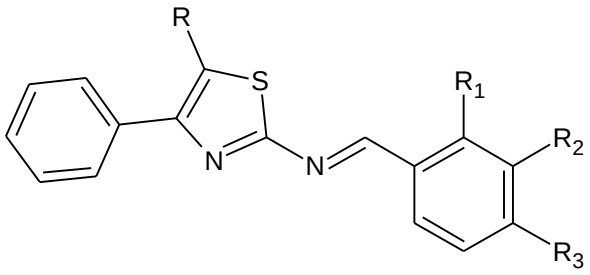
(31)

- Ashish bhatt et al., synthesized novel thiadiazole derivatives incorporating imidazo[1,2-b] pyridazine and thiazolidinone moieties (34) were possessing antimalarial and antibacterial activity. [63]



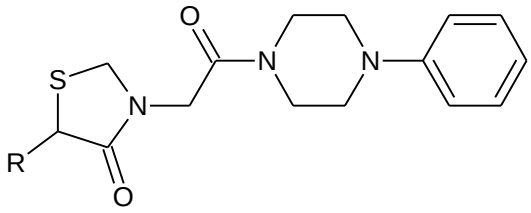
(32)

- Varun A. Morde et al., synthesised of *N*-benzylidene-4-phenyl-1,3-thiazol-2-amine (35) biological evaluation of *Plasmodium falciparum* enoyl-acyl carrier protein reductase (*Pf*ENR) inhibitor.[64]



(33)

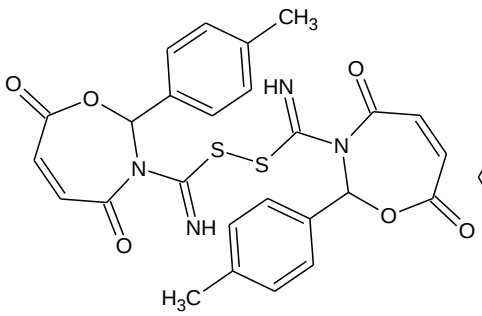
- Rajni Kant Sharma et al., Synthesized and studied and studied SAR of thiazolidinone(36) as antiplasmodial inhibitor of the *Plasmodium falciparum* cysteine protease falcipain-2.[65]



(34)

2.3 OXAZEPINE AND BENZOXAZEPINE

- Naruka al., synthesized formamidine disulfuide Schiff's base and their corresponding 1,3–oxazepine (37)and 2,4-benzoxazepine (38) possessing antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and antifungal activity against *Aspergillus niger*.[66]



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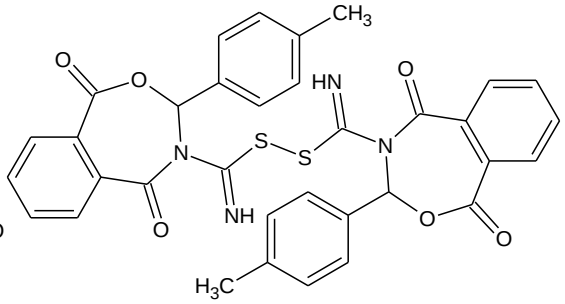
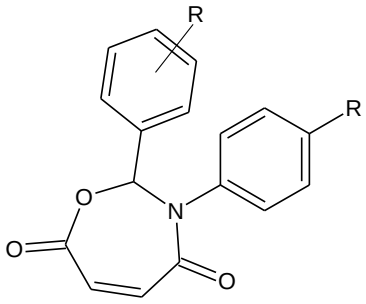


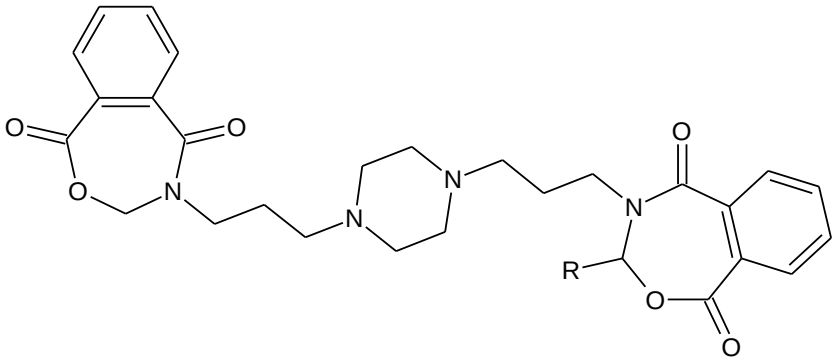
(38)

- Khitam T.A .Alsultani., synthesized oxazepine derived from schiff's base (39) and screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus substilis*.[67]



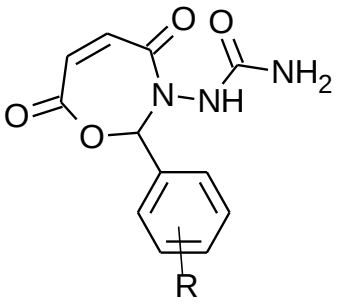
(359)

- Hamak KF et al., synthesized benzoxazepine derivatives (40) from Schiff's base and screened for their antibacterial activity against *Escherichia coli*, *Bacillus substilis*, *Staphylococcus aureus* and anticorrosion activity. [68]



(40)

- Ibrahim et al., synthesized schiff's base derivatives of 1,3-oxazepine(41) and benzoxazepine (42) screened for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. [69]



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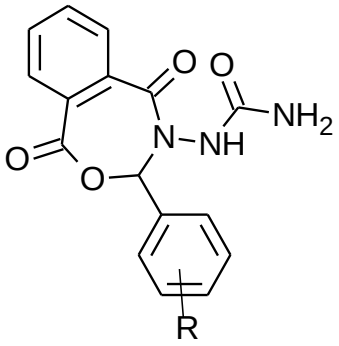
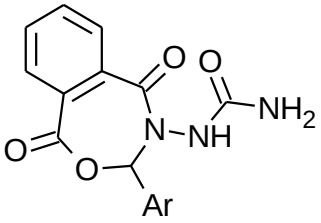


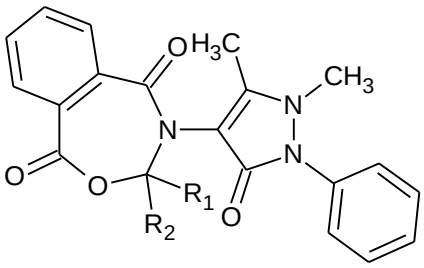
(42)

- Ahmed et al., synthesized some new oxazepine derivatives (43) possessing antibacterial activity against *Escherichia coli*, *Bacillus substilis* and *Staphylococcus aureus.* [70]

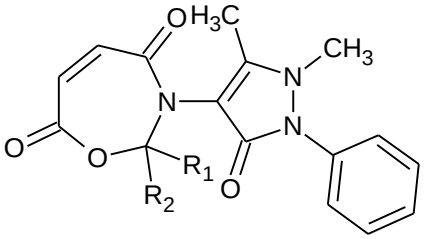


(43)

- Anila et al., synthesized novel 5,6–Benz-1,3-oxazepine-4,7-dione(44) and 1,3-oxazepine (45) derivatives and were screened for their antibacterial , antioxidant and anti-inflammatory activity. [71]

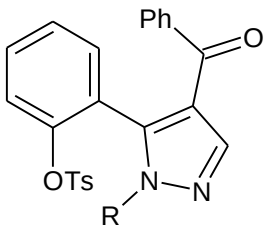


(44)

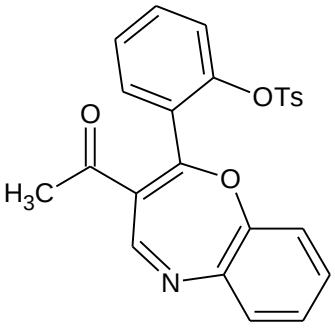


(45)

- Kendre et al., synthesized some novel pyrazole(46), benzoxazepine derivatives(47) and were screened for their antibacterial, antifungal and anti-inflammatory activity.[72]

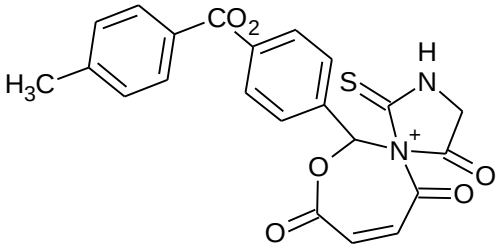


(46)



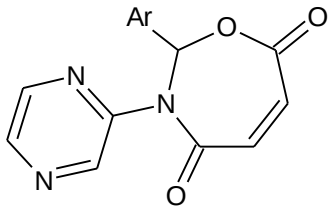
(47)

- Muhsen et al., synthesized and characterized some novel compounds containing oxazepine rings (48) were screened for their antibacterial activity against *E.coli* , *S.aureus and Bacillus cereus.* [73]



(48)

- Hawraa Mohammed sadiq., synthesized and characterized some Novel 1,3-oxazepine (49) derivatives from aminopyrazine. [74]



(49)

PURPOSE AND PLAN OF WORK

The prime motivation of the present work is to design a drug in such a way that it can be used clinically to treat the disease. Drug discovery tools have been utilized now in designing new molecular entities which are safe and effective without consuming much of the research hours. Recent literatures shows that search of new drugs are now focussed on design of drugs as inhibitors of enzyme targets.

Enoyl ACP reductase is such a potential drug target in the development of new antimicrobial agents. From the literature and virtual screening technique Triclosan analogues, pyrazole, thiazolidinone, oxazepine, etc., possess promising enoyl ACP reductase inhibiting action on both *Plasmodium falciparum* and *Escherichia coli.*

Based on these reports an attempt was made here to design and develop new antiplasmodial and antibacterial agents by utilizing computational tools. The primary objective of the present work is to identify and synthesize pyrazole linked thiazolidinone, oxazepine and benzoxazepine as promising antiplasmodial and antibacterial agents by the inhibition of enoyl ACP reductase enzyme.

PLAN OF WORK

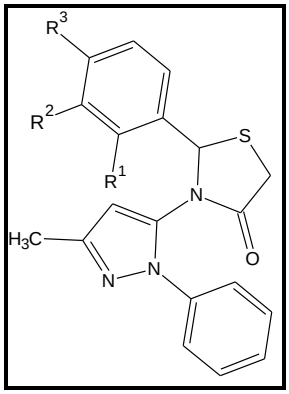
The present work has been carried out under the following sections.

Phase I - *In-silico* studies

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

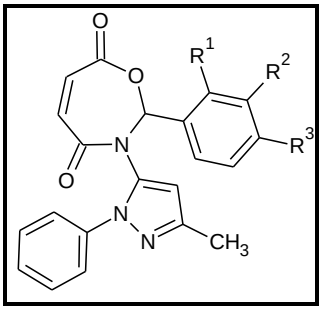
Phase II - Synthesis

- 3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2-phenyl-1,3-thiazolidin-4-one **(2a-j)**



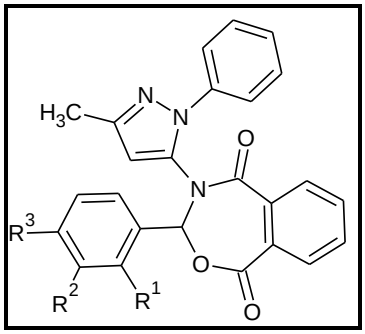
Pyrazolyl Thiazolidinone series **(2a-j)**

- 3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2-phenyl-2,3-dihydro-1,3-oxazepine-4,7-dione **(3a-j)**



Pyrazolyl Oxazepine series **(3a-j)**

- 4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-3-phenyl-3,4-dihydro-2,4-benzoxazepine-1,5-dione **(4a-j)**



Pyrazolyl Benzoxazepine series **(4a-j)**

- **Spectral characterization**

PHASE III - Biological studies

- Antimalarial Studies (*Plasmodium falciparum*)
- Antibacterial Studies (*Escherichia coli*)

CHEMISTRY

4.1 PYRAZOLES[75,76]

Pyrazole was first described by Buchner who discovered it during the decomposition of pyrazole 3,4,5-tricarboxylic acid. Pyrazole is a colourless solid with a melting point of 70°C and is soluble in water. It possesses a penetrating pleasant smell. Pyrazole has a high boiling point of about 187°C.

Synthetic methods

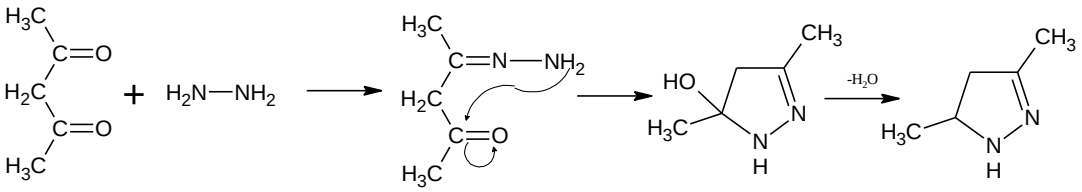
The pyrazoles can be synthesized by the following general methods

1. From (3+2) Cyclization Reaction

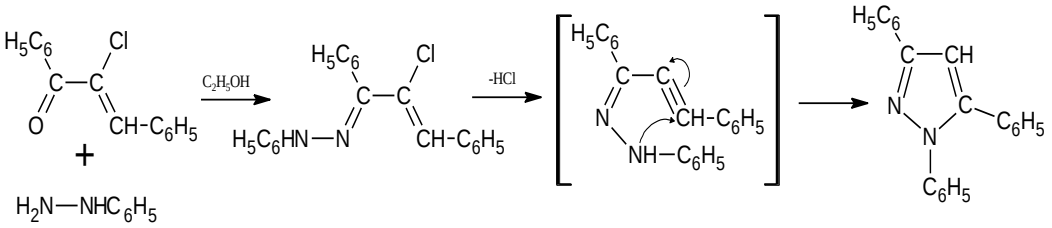
Pyrazoles are synthesized by the condensation of 1,3-difunctionalized compounds with hydrazine or its derivatives involving (3+3) cyclization.

- **Reaction of β-diketones with hydrazine**

The most widely used method and involves the reaction of β-diketones with hydrazine or monosubstituted hydrazine in the presence of an acid.

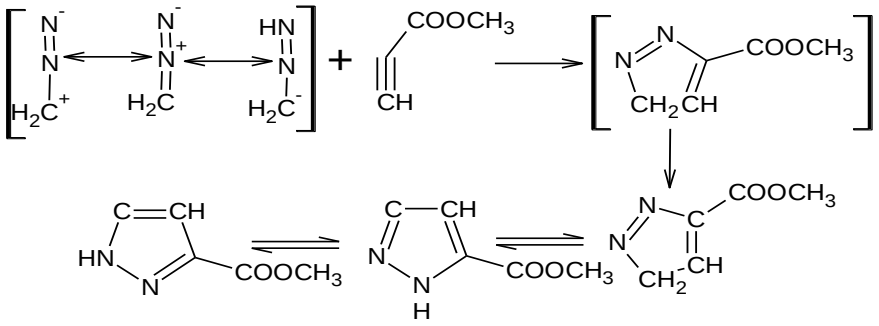


- Reaction of α,β-ethylenic carbonyl compounds, substituted with a readily replaceable substituent of α- or β- position, with hydrazines results in the formation of expected pyrazoles.



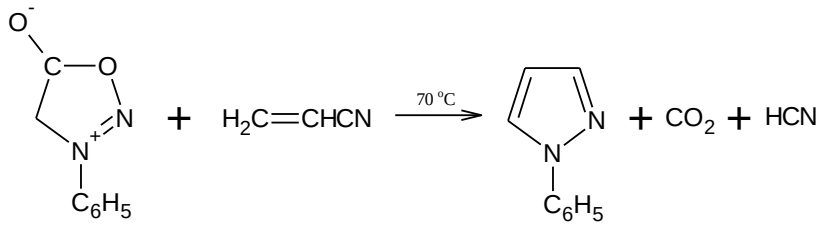
2. 1,3-dipolar cycloadditions

1,3-Dipolar cycloaddition of diazoalkanes with functionalized alkynes results in the formation of pyrazole. The reaction proceeds through a transition state involving energetically most favourable interaction of 4π-electron of 1,3-dipole [diazoalkene-with electrophilic and nucleophillic centres-ambivalent] with 2π-electron of dipolarophile (methylpropiolate)(HOMO of diazoalkene-LUMO of dipolarophile).



3. From other ring system

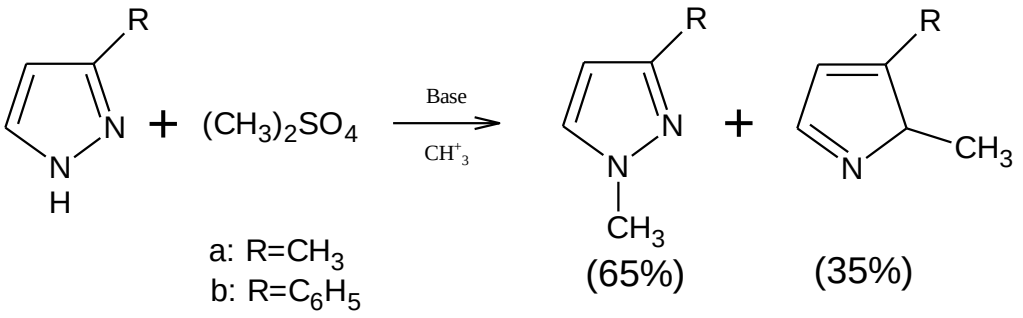
Various heterocyclic compounds transform to pyrazole under appropriate condition. Syndone, for instance, and acrylonitrile result in pyrazole formation. The cyanopyrazole formed as intermediate is immediately converted to pyrazole.



Electrophilic Reaction

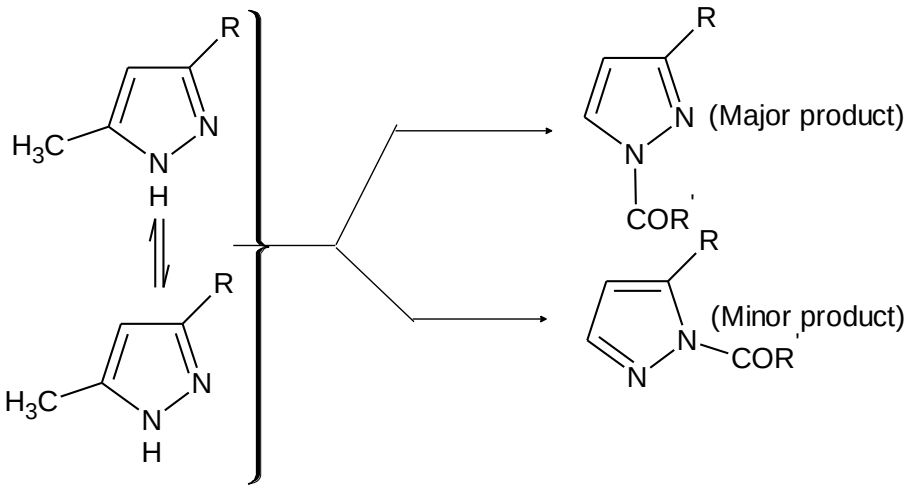
- **N-Alkylation**

The alkylation of the free NH group of pyrazole proceeds with alkylating agents such as alkyl halides, diazomethane or dimethylsulphate, Substituted pyrazoles undergo alkylation to give a mixture of two isomeric products. Excess of alkylating agent causes quarterisation.



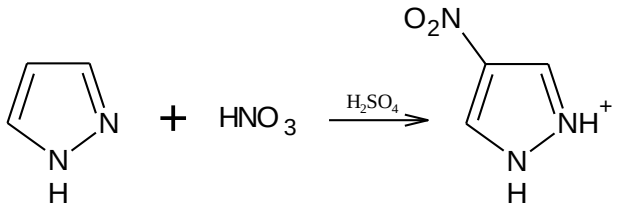
- **N-Acylation**

Pyrazole with free N-H group undergo acylation when treated with acetylchloride (alone in the presence of pyridine) or acetic anhydride.

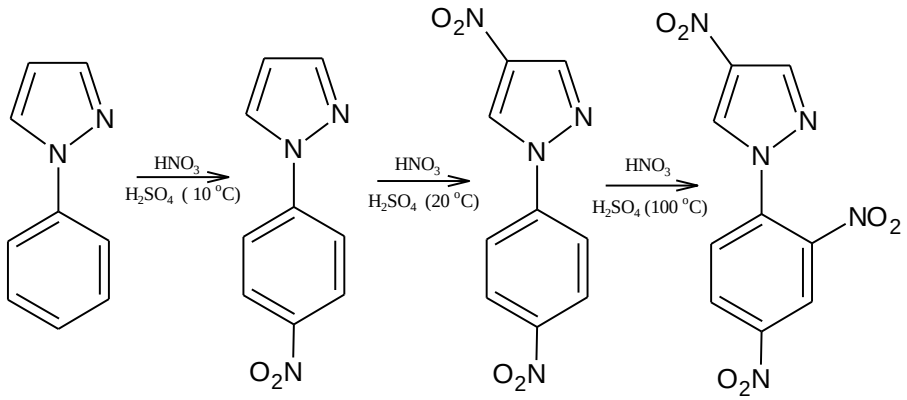


➢ **Nitration**

Nitration of pyrazole with nitrating mixture of concentrated nitric and sulphuric acids occured at the position-4.

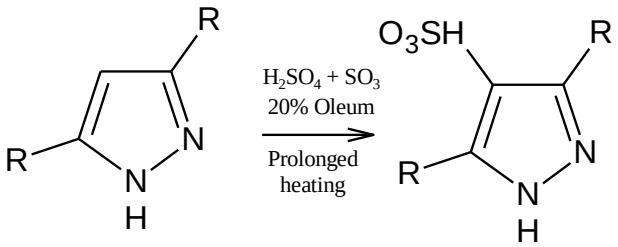


If the pyrazole is substituted with phenyl group at the position-1, it competes with pyrazole ring and nitration occurs at para-position.



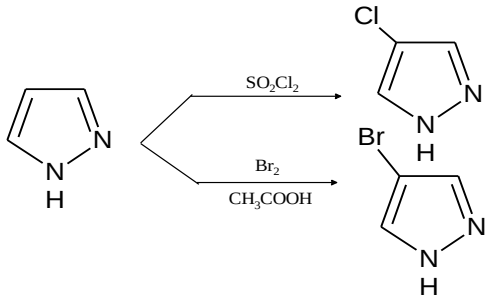
➢ **Sulphonation**

Pyrazole undergo sulfonation only under vigorous reaction conditions with the introduction of sulfonic acid group at the position-4.



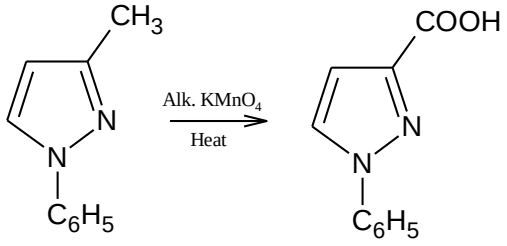
➢ **Halogenation**

Halogenation of pyrazole usually occurs at the position-4. Pyrazoles can be chlorinated by chlorinating reagents such as chlorine water, chlorine in carbon tetrachloride, chlorine in acetic acid. Pyrazole are brominated by bromine in chloroform and bromine in acetic acid.

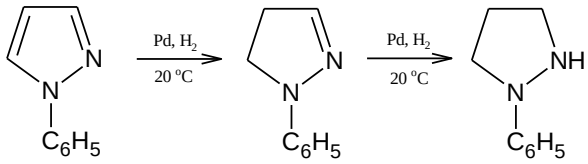


- **Reaction with oxidation and reducing agents**

The pyrazole ring is remarkably stable to the action of oxidizing agents but the side chain may be oxidized to the carboxylic function. The oxidation proceeds well in the presence of alkaline potassium permanganate. Pyrazole and its derivatives have been reduced under a variety of condition. Thus with Na/C_2H_5OH, 2-Pyrazoline in obtained



The catalytic reduction of 1-phenylpyrazole yields both phenylpyrazoline and 1- phenylpyrazolidine.

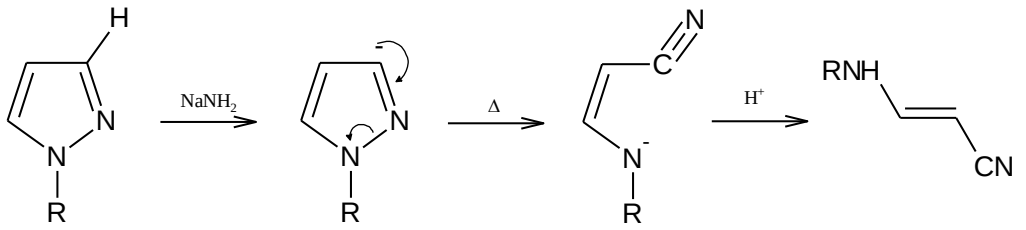


Nucleophilic reactions

Pyrazole are reactive, but the electron-withdrawing substituent attached at α- to the halogen atom makes reactive towards nucleophillic substitutions. Pyrazoles exist partly as anions and thus react with electrophiles as phenols and undergo diazocoupling, nitrosation and Mannich reaction.

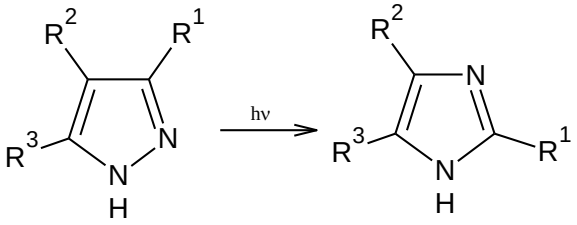
❖ **Ring cleavage via deprotonation**

Pyrazole ring unsubstituted at the position-3 is cleaved by a strong base ($NaNH_2$) via deprotonation at C-3.



❖ **Photochemical reaction**

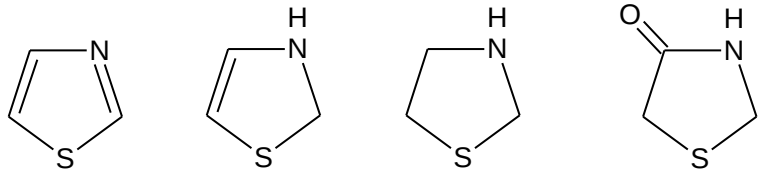
Pyrazole photochemically transformed into imidazoles involving the exchange of positions N-2 and C-3 of pyrazole with the position C-2 and N-3 of imidazole.



4.2 THIAZOLIDIN-4-ONE[77]

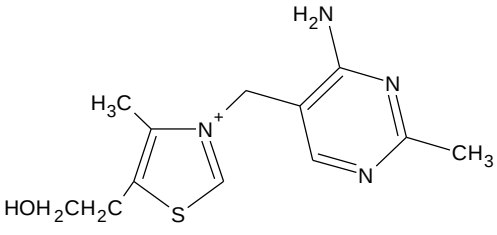
Compounds containing thiazolidi-4-one nucleus find unique place in medicinal chemistry and play significant role as, they are associated with immense biological activity.

Thiazoles are five membered heterocyclic ring system with sulphur and nitrogen in first and third positions respectively. Thiazolidine is nothing but the saturated ring system of thiazole. 4-thiazolidinone are derivatives of Thiazolidines with the carbonyl group in the 4^{th} position

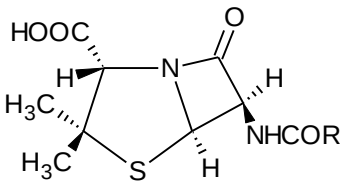


Thiazoles Thiazoline Thiazolidine 4- Thiazolidinone

Thiazolidi-4-ones are important compounds due to their broad range of biological activities. Thiazolidine-4-one have a broad spectrum of pharmacological properties i.e., antifungal, antitubercular, antimicrobial, antioxidant, antibacterial, anti-inflammatory, analgesic, Anti-YFV activity. The most important naturally occurring thiazole derivatives is thiamine (vit B_1) which contain pyrimidine and thiazole ring system. Penicillins are also important naturally occuring products and contain reduced thiazole ring system (thiazolidine)



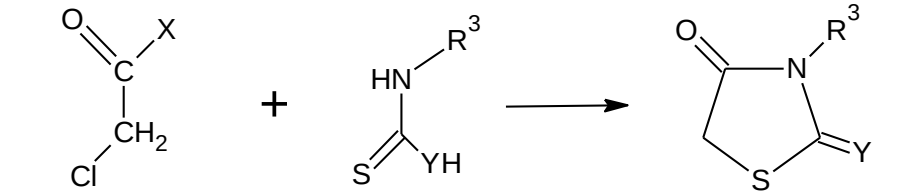
Thiamine (Vitamin B $_1$)



Penicillin G (R = $CH_2C_6H_5$)

SYNTHESIS

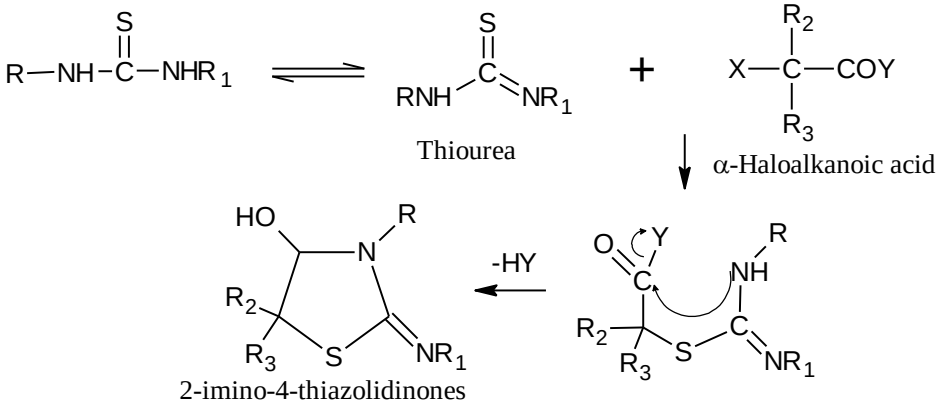
- 2-Substituted thiazolidin-4-ones are easily prepared by the reaction between chloroacetic acid, or its derivatives, and a thiourea, a thiosemicarbazide, or a mono- or di-thiocarbamate.



Chloroacetic acid Di-thiocarbamate 2-sub thiazolidin-4-one

- **Reaction of α-Haloalkanoic acid with primary amine (Hantzsch synthesis)**

Substituted **2-imino-4-thiazolidinones** are obtained in good yields by the reaction of symmetrical and unsymmetrical thioureas with various substituted and unsubstituted α-haloalkanoic acids, their esters, acid chlorides, amides, or carbamates. The proceeds via the intermediate isothiourea which cyclizes while refluxing with acetic acid, ethanol, or benzene in the presence of sodium acetate or pyridine.

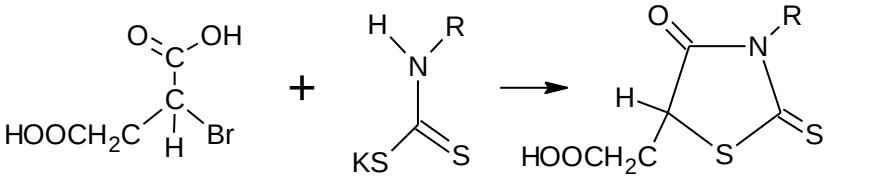


2-imino-4-thiazolidinones

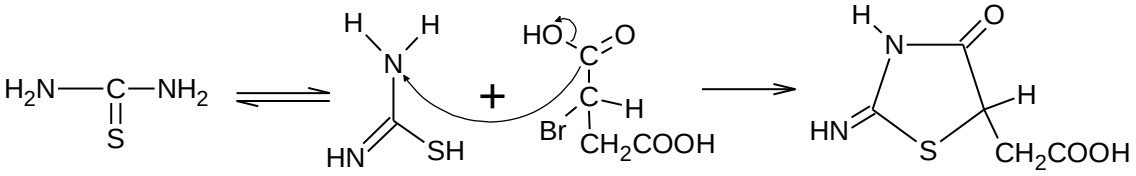
R = Alkyl, aryl or heterocyclic, R_I = Alkyl, aryl or heterocyclic,
R_2 = H or alkyl, R_3= H or alkyl, R_2R_3= Alkylidene or arylidene.

- **From α-Bromosuccinic acid**

α-Bromosuccinic acid mixed with dithiocarbaminates on neutralisation with $NaHC0_3$ followed by acidification and boiling yields **2-thiono-4-oxo-5-thiazolidineaceti**c acids. However, α-bromosuccinic acid on treatment with thioureas in the presence of sodium acetate in methanol yields **2-imino-4-oxo-5-thiazolidineaceti**c acids.

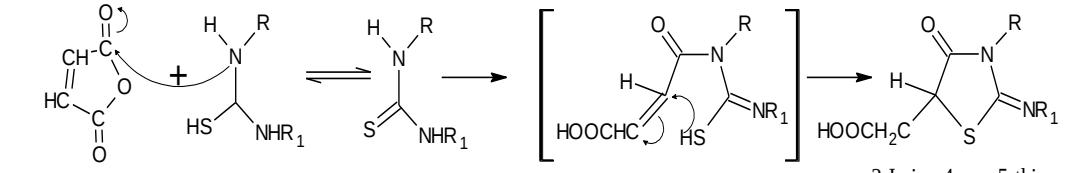


α-Bromosuccinic acid Dithiocarbamates 2-thiono-4-oxo-5-thiaz-lidine acetic acids



Thiourea α-Bromosuccinic acid 2-imino-4-oxo-5-thiaz-lidine acetic acid

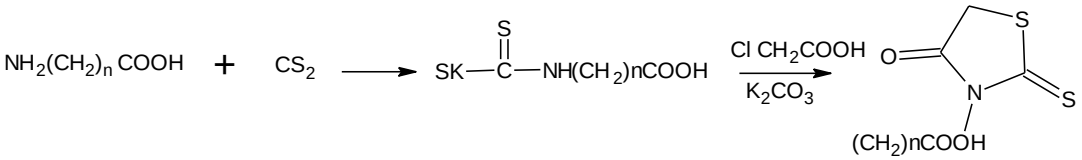
2-Imino-4-oxo-5-thiazolidineacetic **acids** can also be synthesized in good yields by refluxing equimolar amounts of substituted and unsubstituted thioureas and maleic anhydride in acetone.



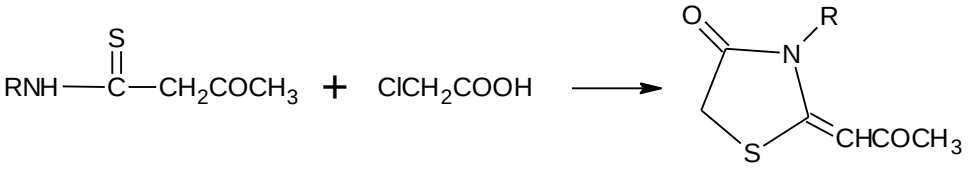
Maleic anhydride Thiourea 2-Imino-4-oxo-5-thiaz -olidineacetic acids

- **3-Carboxyalkyl-2-thiono-4-thiazolidinones** have been synthesized from long-chain amino acids, NH_2-$(CH_2)_n$COOH. The amino acids react with CS_2 and chloroacetic acid in the presence of bases to give the

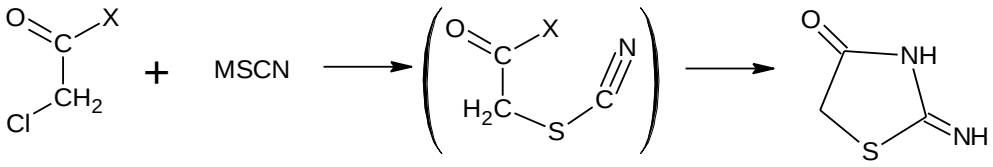
corresponding **N-substituted-4-thiazolidinones** through the intermediate formation of dithionate.



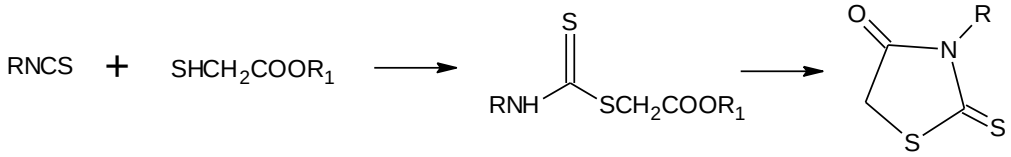
- Various N-substituted acetylthioacetamides on treatment with monochloroacetic acid in the presence of sodium acetate in refluxing acetic acid yield 2-**acetylmethylene-3-substituted-4-thiazolidinones**.



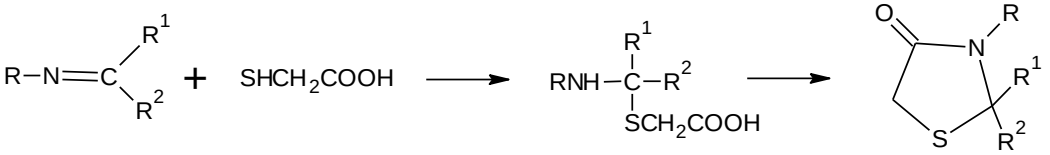
- The α-chloro acid derivatives react with metallic thiocyanates yielding an intermediates, the cyclization of which gives a 2-iminothiazolidin-4-one.



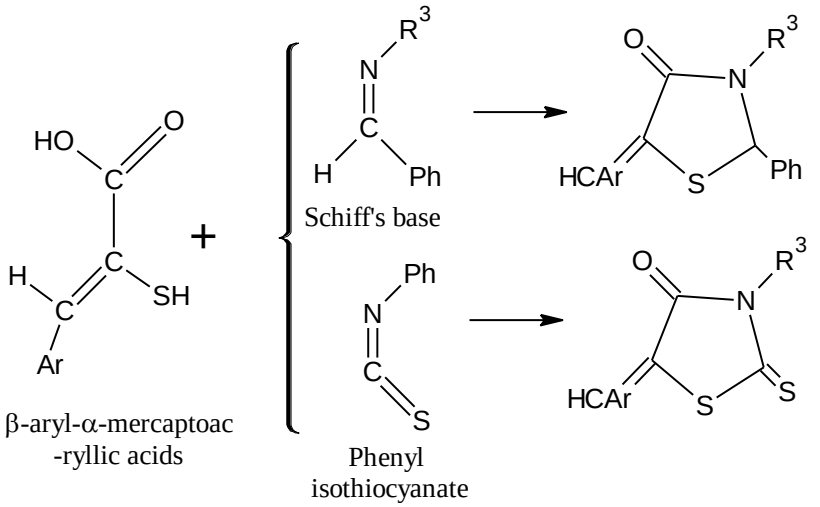
- Various **3-substituted-2-thiono-4-thiazolidinones** can be conveniently prepared by the reaction of substituted isothiocyanates with α-mercaptoacetic acid or its ester followed by acid cyclization of the resulting (thiocarbamoyl) mercaptoacetic acids and acetates.



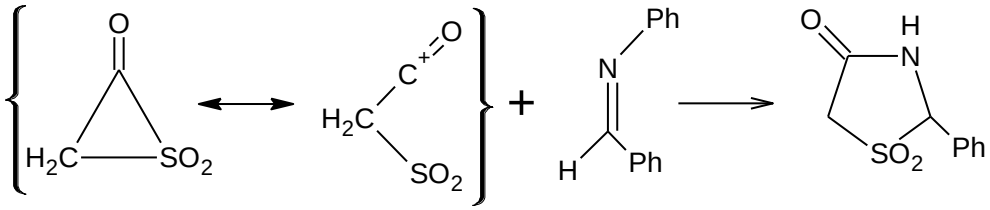
- Schiff bases obtained by the condensation of ketones and amines also react with α-mercaptoacetic acid to give **2,2-disubstituted-4-thiazolidinones.**



- β-aryl-α-mercaptoacryllic acids add to the C=N bond of schiff's base affording thiazolidin-4-ones. The addition to the same acid to phenyl isothiocyanate yields 5-arylidene-3-phenylrhodanines.



- An interesting synthesis of substituted thiazolidin-4-one 1,1- dioxides results from 1,3-dipolar addition of the adduct of ketene and sulphur dioxide(18a) with benzylidene aniline. The ketene-sulfur dioxide adduct may be represented by a mesomeric structure which corresponds to a linear 1,3-dipolar species(18b).



CHEMICAL REACTION

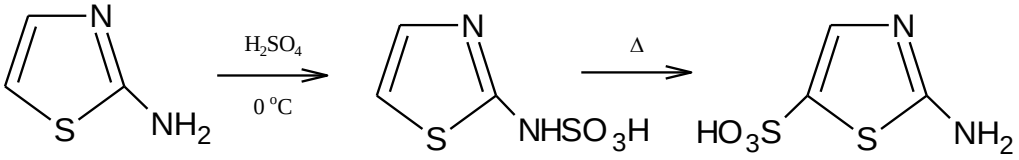
Thiazolidin-4-one presents three nucleophilic centres (C-5, oxygen and nitrogen) and two electrophilic centers (C-4 and C-2)

❖ **Electrophilic attack at Carbon**

The reactivity of thiazole ring is further lowered in electrophilic substitutions proceeding under acidic conditions (nitration, sulfonation and Friedel-Crafts reaction) because of the protonated thiazole. However, thiazoles undergoes electrophilic substitutions readily if the thiazole ring is substituted with electron-releasing substituents (-OH and $–NH_2$). The attack of electrophiles occurs preferentially at the position-5 of the thiazole ring

➢ **Sulfonation**

Thiazole sulfonation occurs only under forcing conditions by the reaction with oleum at 250°C for 3 hours in the presence of Mercury(II)sulphate leads to 65% formation of 4-thiazole sulfonic acid. However, 2-aminothiazole is sulfonated at low temperature 0°C affording at first the formation of 2-sulfamic acid which on heating rearranges to 2-aminothiazole-5-sulfonic acid

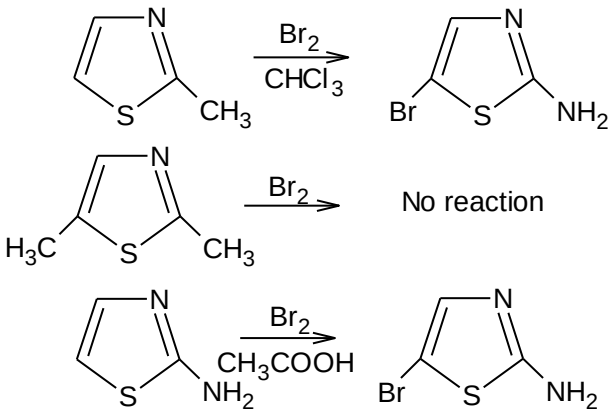


➢ **Nitration**

No nitration of thiazole occurs with the classical nitration reagents, even under forcing conditions.

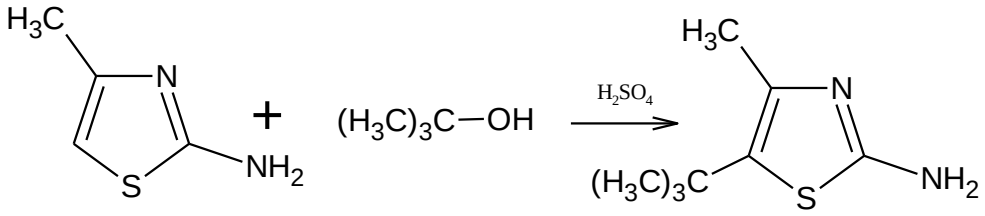
➢ **Halogenation**

Unsubstituted thiazole does not react with Cl or Br in an inert solvent, but 2-methylthiazole undergoes bromination at the position-5 (if the position-5 is occupied, the bromination does not occur. However, the presence of electron releasing substituent at the position-2 facilitates bromination at position-5 even under the mild condition.



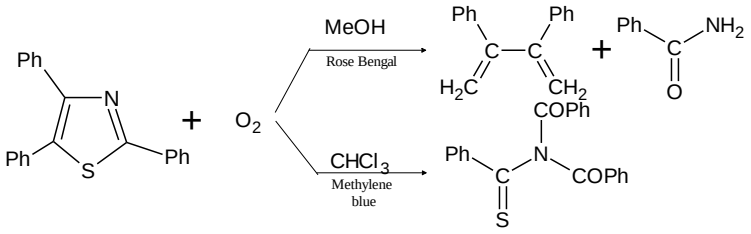
➢ **Alkylation**

Thaizoles activated by the substituents substituted at the position-2 and -4 undergo alkylation at the position -5 when treated with tert-butyl alcohol in the presence of sulphuric acid.



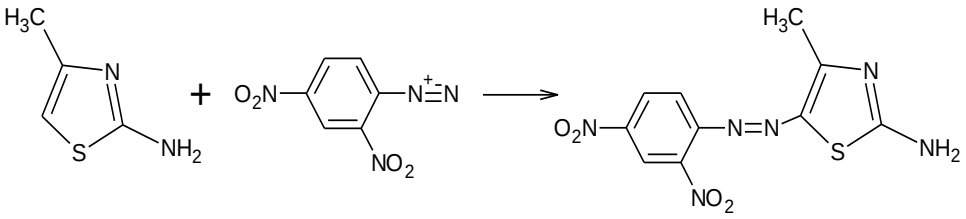
➢ **Oxidation**

Thiazole is relatively resistant to oxidation. Under photosensitized oxygenation, triphenylthiazole affords various products of ring cleavage, depending on the sensitizer and solvent.



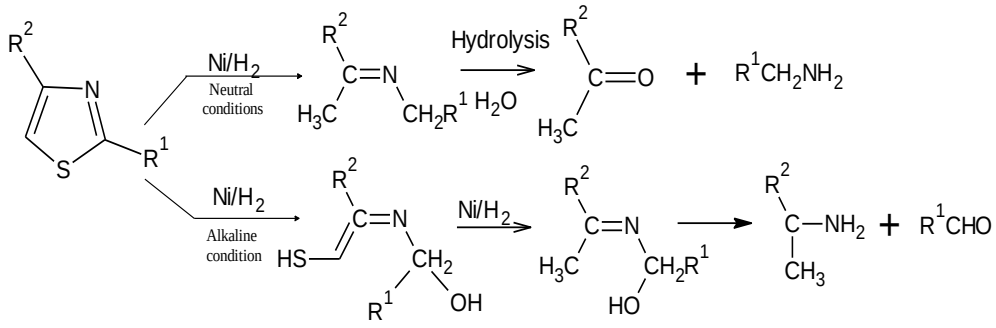
➢ **Diazo coupling**

Only 2-aminothiazole derivatives are reductive enough toward diazonium salts undergo the diazo coupling reaction. The azo group substitutes exclusively at the position-5 when the later is unsubstituted.



➢ **Desulfurization**

Thiazoles undergo desulfurization when treated with Raney nickel, but the products formation depends upon the reaction condition.



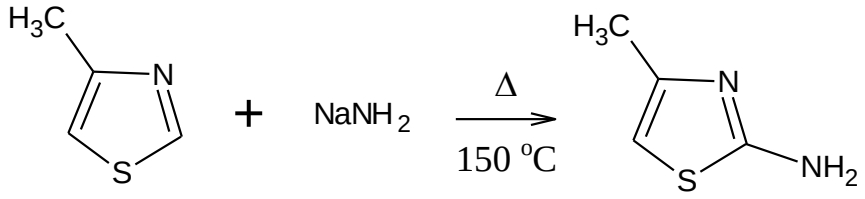
Reactions with nucleophiles

❖ **Nucleophilic attack at carbon**

In the thiazole ring, the position-2 is with lowest π-electron density (position-4 almost neutral and position-5 slightly electron-rich) and is, therefore, most susceptible for the nucleophillic attack (requires activation or strong ncleophiles).

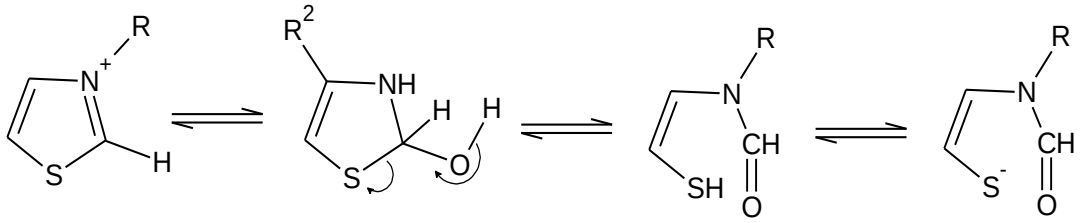
➢ **Amination**

Thiazoles can be aminated at the position-2 by treating with sodium amide at 150°C with the transfer of hydride ion.



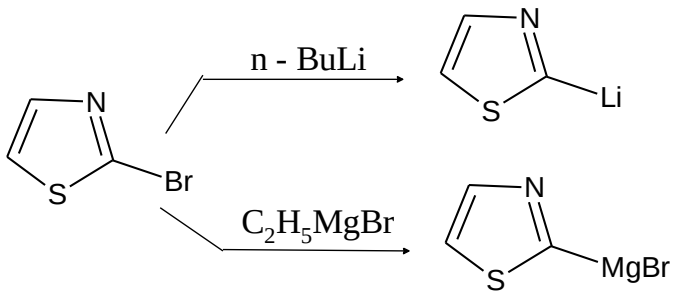
➢ **Ring cleavage**

Thiazole are resistant to attack by hydroxide ion, but thiazolium cation (salts) are susceptible towards attack of hydroxide ion under mild conditions and results in the cleavage of the ring.



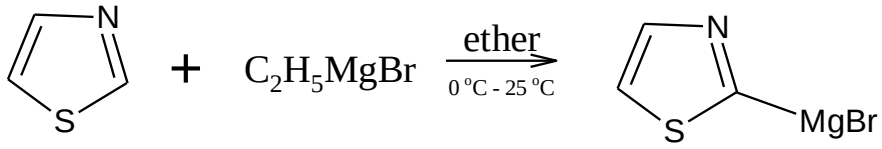
➢ **Metal –Halogen Exchange**

2-Halothiazoles undergo halogen metal exchange when treated with n-butyllithium and Grignard reagents.



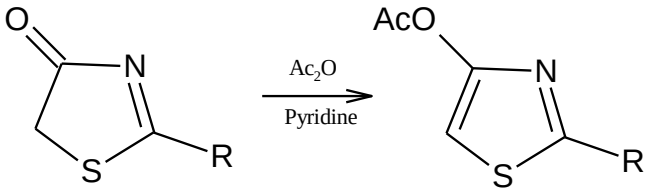
➢ **Metallation**

Thiazoles are deprotonated preferentially at C-2 by a strong base. Thus thiazole is metallated with the abstraction of acidic hydrogen at C-2 when treated with n-butyllithium or ethyl magnesium bromide.



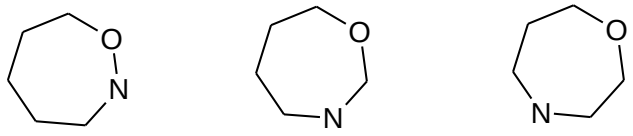
➢ **Reaction at oxygen atom**

The nucleophilic reactivity of the oxygen atom has been observed in the acetylation by acetic anhydride of 2-aryl- and 2-heteryl-thiazolin-4-one.



4.3 OXAZEPINE[78-80]

Oxazepine chemistry includes some interesting rearrangement rections both in synthesis and reactivity, many of which have parallels in the chemistry of the analogous diazepine system.



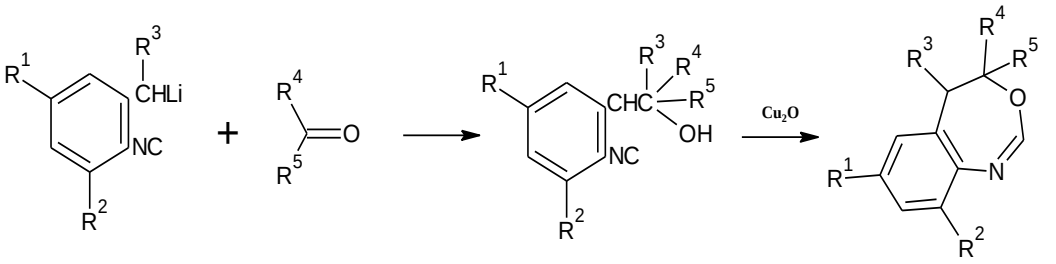
1,2-Oxazepine 1,3-Oxazepine 1,4-Oxazepine

1,3-Oxazepine is unsaturated seven-membered heterocycle consist of oxygen atom in location (1), nitrogen atom in location (3) besides of five carbons. They have been studied the molecular properties of this pharmaceutically important nucleus belong to its entity in some natural products and compounds biological active as antithrombotic, antiepileptic, anticonvulsant, anti-inflammatory, antifungal, progesterone agonist, antipsychotic, antagonist and analgesic, antihistaminic, anxiolytics, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitory and antiaggregating activities

Synthesis

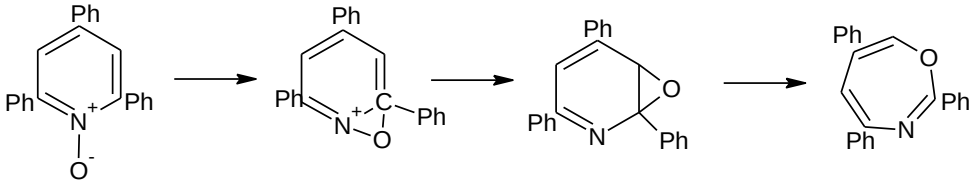
- **Type a (C-N-C-C-C-C-O)**

Formation of this type of bond usually involves ring closure via nucleophilic attack by oxygen on an electrophilic carbon, e.g., a carbonyl group or carbon-halogen bond. Thus 2-methyl-4,5,6,7-tetrahydro-1,3-oxzepine can be prepared by the distillation of 4-acetyl-aminobutanol. The copper-catalysed insertion of isocyanide into the O-H bond of alcohols gives a high yielding route to 4,5 -dihydro-3,1-benzoxazepines.

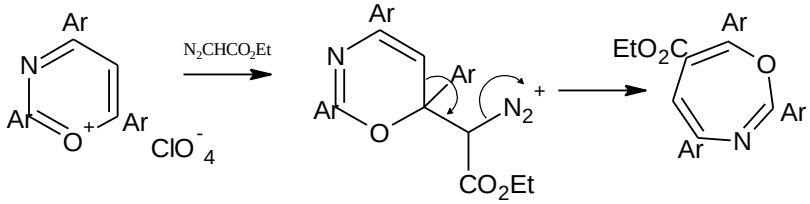


Synthesis from other heterocyclic systems

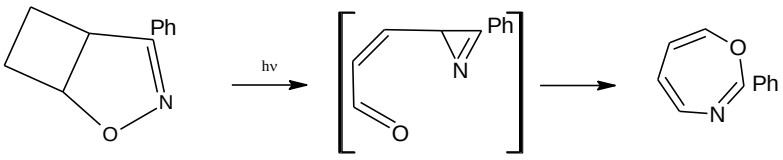
- The photochemical rearrangement of aromatic N-oxides, provides one of the major route to the fully unsaturated 1,3-Oxazepine system. The reaction is thought to proceed by the primary formation of an oxaziridine intermediate which rearranges by a 1,5-sigmatropic shift to give which is then converted to the product by a disrotatory ring opening.



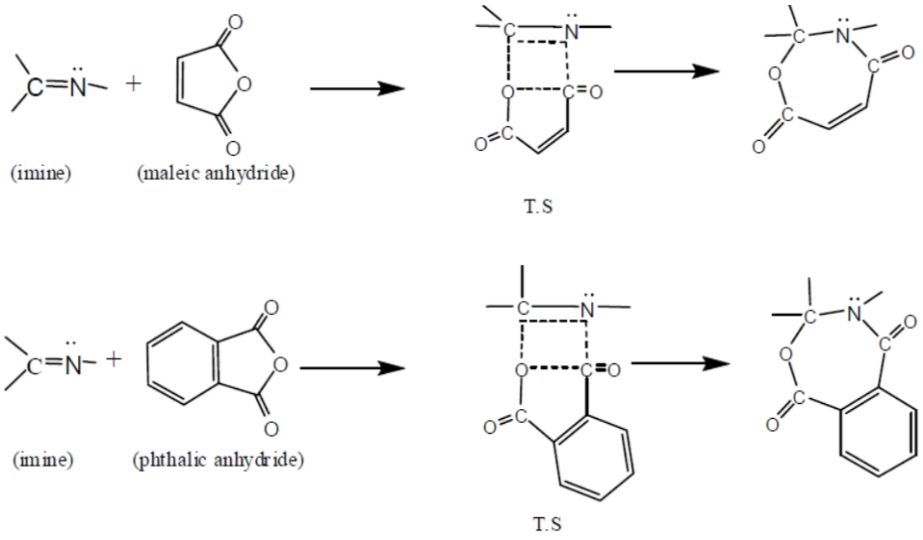
- Monocyclic 1,3-oxazepine with aryl substituents at the 2-, 4- and 7-positions can be prepared in moderate yield (20%-40%) by the reaction of aliphatic diazo compounds with 1,3-oxazinium perchlorates.



- The preparation of 2-phenyl-1,3-oxazepine by the UV irradiation of is mechanistically interesting in that it apparently involves an intermediates.

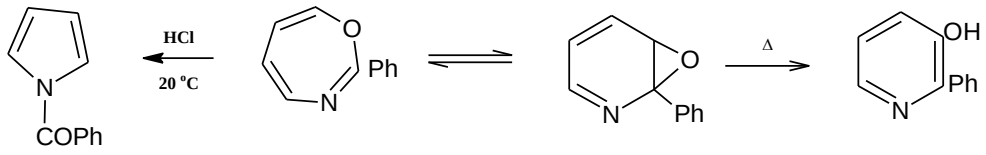


- 1,3-oxazepine ring synthesis of utilized a pericyclic reaction, was classified as (2+5) cycloaddition reaction in which two atoms of the first component (azomethine) react with five-membered component such as phathalic or maleic anhydride to give seven-membered heterocycle. Mechanism of the pericyclic reaction for the synthesis 1,3-oxazepine ring is shown below



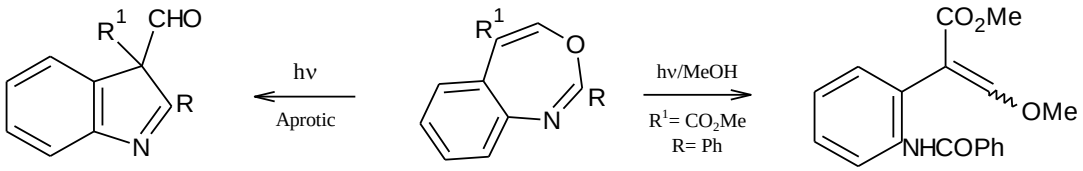
❖ **Reactivity**

- 1,3-Oxazepine are not very stable compounds and are easily decomposed by heat, light, acids and bases to a variety of products. In some of their reaction paths it seems likely that decomposition takes place via hydrolytic opening of the oxazepine but in others via conversion to the oxazanorcaradiene valence tautomer, e.g.



➢ **Photochemical reaction**

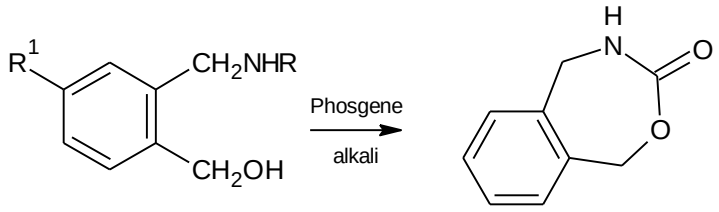
The photolysis of in aprotic solvents gives the labile 3H-indole intermediate as the primary photoproduct but in methanol, the addition reaction which gives is faster.



4.4 SYNTHESIS OF 2,4-BENZOXAZEPINE

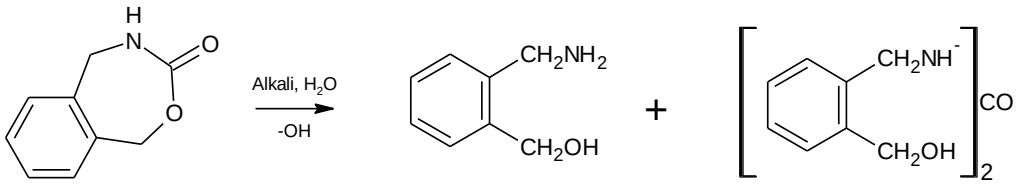
- **From 2-(Amino-methyl)benzyl acohol**

  2-(Amino-methyl)benzyl acohol cyclises with phosgene in alkali to give 1,,3,4,5-thetrahydro-2,4-benzoxazepin-3-one.

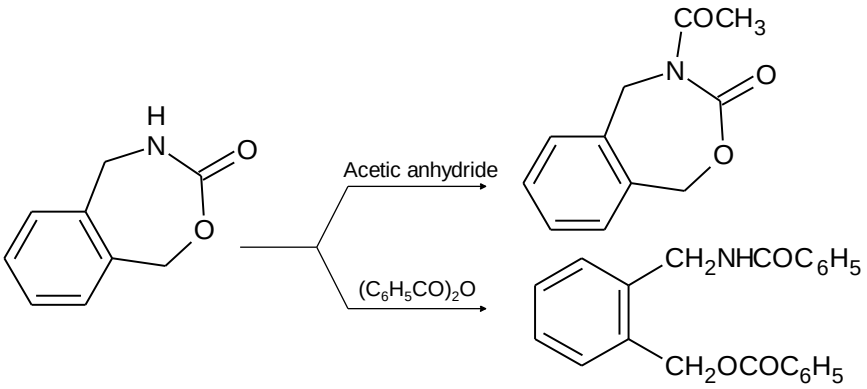


Reactivity

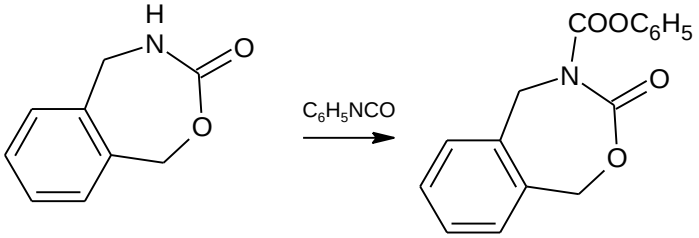
- The reactivity and stability in acidic or basic media of 2,4-benzoxazepin-3-one was found to be the heterocyclic nucleus readily opens in warm dilute alkali to give o-Aminomethyl benzyl alcohol and N,N'-bis-o-hydroxy-methylbenzyl urea.



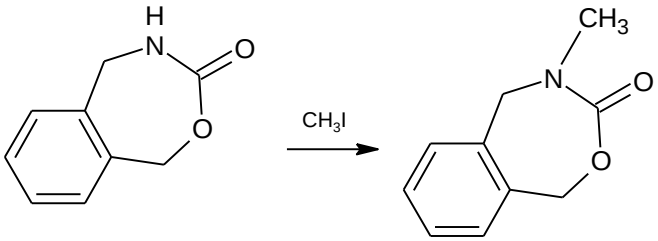
- The acylation of 2,4-benzoxazepin-3-one the compound decomposes on reaction with both aliphatic and aromatic acid chlorides. So, the corresponding N-acyl derivatives (N-Acetyl-4,5-dihydro-2,4-benzoxazepine-3(1H)one) were isolated by heating 2,4-benzoxazepin-3-one with an aliphatic anhydride; with an aromatic anhydride such as benzoic anhydride the ring-opened compounds o-Benzamidomethylbenzyl Benzoate was obtained.



- Benzoxazepine reacted normally with phenyl isocyanate to yield the N-phenylcarbamoyl derivative. Reaction with ethylene oxide in methanol gives methyl o-hydroxymethylbenzylcarbamate.



- Methylation of Benzoxazepine with methyl iodide to give the methylated product.



MATERIALS AND METHODS

5.1. PHASE 1: *INSILICO* STUDIES

Software and databases used

- iGEMDOCK v.2
- Zinc database
- Cygwin
- Mgltools 1.5.6
- Autodock tools 1.5.6
- Python 3.4.3
- Discovery studio visualizer
- Molinspiration server
- RCSB Protein data bank
- Online simile translator

All the *in-silico* experiments are carried out in Department of Pharmaceutical Chemistry, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore.

5.1.1 Virtual Screening

- Identification of drug target
- Selection of the lead

Procedure:

Virtual screening is done by using iGEMDOCKv.2. [81-83]

1. Using ZINC, the free data base of around thirteen million commercially available compounds, a small molecule library consisting of 48 compounds were constructed. The protein with the accession code 1VRW & 1C14 corresponding to enoyl ACP reductase of *P. falciparum* and *E.coli* was selected from the RCSB protein data bank.

2. The protein was uploaded in the iGEMDOCKv.2 & the binding sites were chosen.

3. Similarly ligands were uploaded in the iGEMDOCKv.2

4. Start virtual screening module was clicked & the fitness value was saved.

RESULTS AND DISCUSSION

Among the 48 *Pf*ENR & E.*coli* FabI inhibitors screened, pyrazole, thiazolidinone, oxazepine were identified as the lead. The results are tabulated in **Table1** and the snap shots of ligands binding with 1VRW.pdb and 1C14.pdb are given in **fig.9, 10.**

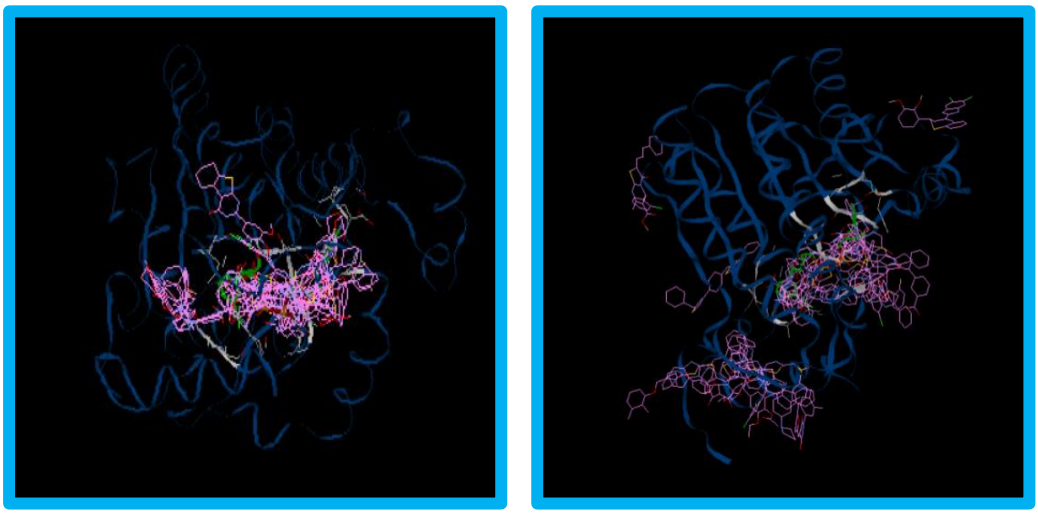


Fig.9: Binding of ligands with 1VRW.pdb

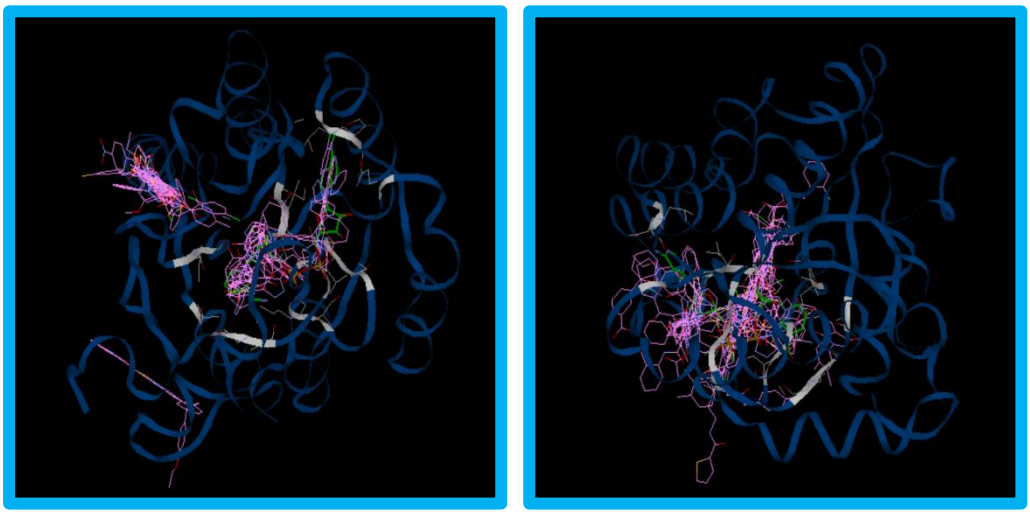
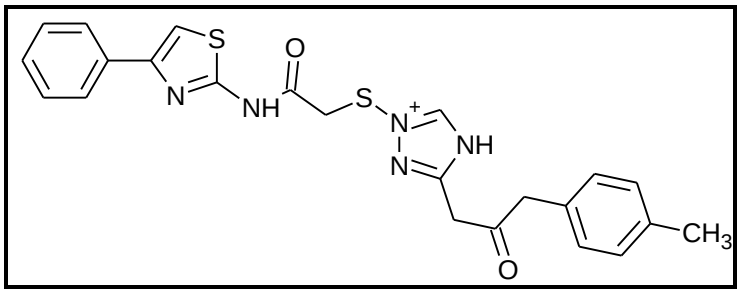


Fig.10: Binding of ligands with 1C14.pdb

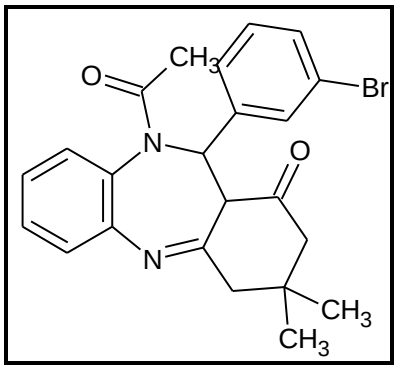
Table.1: Fitness values of 48 compounds

S.No	Compound code	Fitness value	
		1VRW	1C14
1	ZINC-6316009	-106.7	-90.36
2	ZINC-633953	-88.85	-107.17
3	ZINC-664768	**-119.81**	-107.28
4	ZINC-666178	-104.39	-106.31
5	ZINC-667115	-96	-103.47
6	ZINC-856315	-112.56	-118.35
7	ZINC-857211	-99.68	-98.28
8	ZINC-878056	-116.23	-110.95
9	ZINC-1240782	-91.06	-104.91
10	ZINC-2063825	-84.76	-106.61
11	ZINC-2064406	-112.11	-104.84
12	ZINC-2124704	-106.37	-99.53
13	ZINC-2258599	-81.35	-75.67
14	ZINC-3770286	**-115.98**	-103.04
15	ZINC-4180918	**-123.71**	-94.4
16	ZINC-8441250	-85.94	-103.42
17	ZINC-8441387	-99.23	-108.69
18	ZINC-8441604	**-121.98**	-102.08
19	ZINC-8441700	-97.79	-99.24

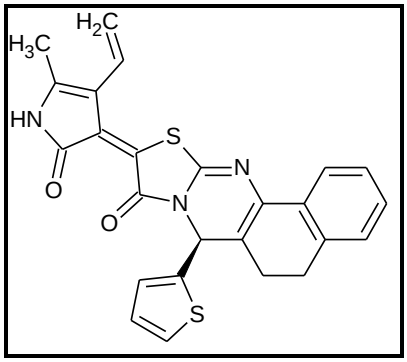
S.No	Compound code	Fitness value	
		1VRW	1C14
20	ZINC-8441816	-105.18	**-116.45**
21	ZINC-8441888	-101.68	-112.39
22	ZINC-8441912	-103.26	-88.53
23	ZINC-8441938	-95.72	-87.42
24	ZINC-844194	-101.85	-102.28
25	ZINC-8441955	-96.54	-103.2
26	ZINC-8441959	**-91.78**	-101.43
27	ZINC-8441975	-91.72	-107.13
28	ZINC-8441999	-105.68	-97.98
29	ZINC-8442087	**-112.52**	**-116.35**
30	ZINC-8442127	-109.34	-103.02
31	ZINC-8442156	-84.77	-111.38
32	ZINC-8442161	-103.86	-110.47
33	ZINC-8442164	-98.51	-109.49
34	ZINC-8442165	-94.44	-94.75
35	ZINC-8442166	-91.38	-103.21
36	ZINC-8442180	**-103.51**	**-129.53**
37	ZINC-8442267	-99.99	**-124.28**
38	ZINC-8442295	-101.52	-113.19
39	ZINC-19794473	-97.82	-104.73
41	ZINC-19794496	-100.18	-95.71
42	ZINC-19795380	-82.84	-98.43
43	ZINC-19795548	-95.87	-93.67
44	ZINC-3770286	**-108.44**	-98.01
45	ZINC-3788342	-102.95	-91.21
46	ZINC-2258574	-100.63	-93.12
47	ZINC-2258650	-107.13	-111.45
48	ZINC-2254930	-113.29	-111.82
49	ZINC-2255236	-94.82	-101.07



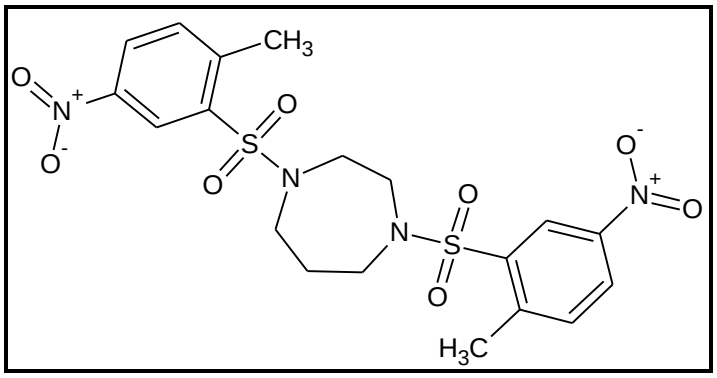
ZINC-8442180



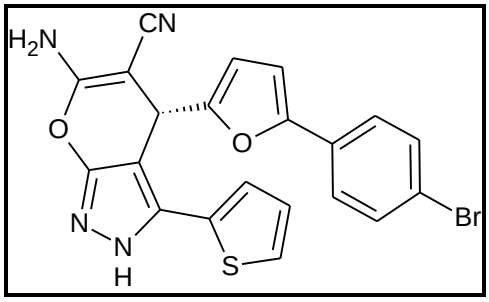
ZINC-4180918



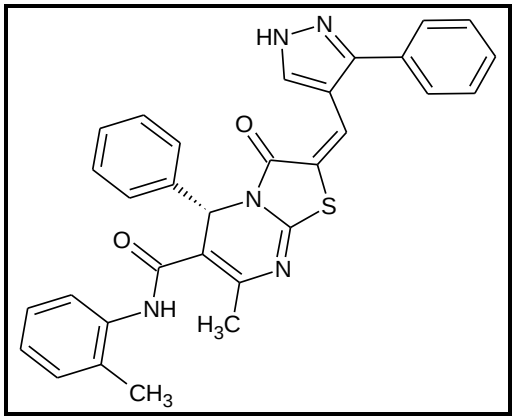
ZINC-8441959



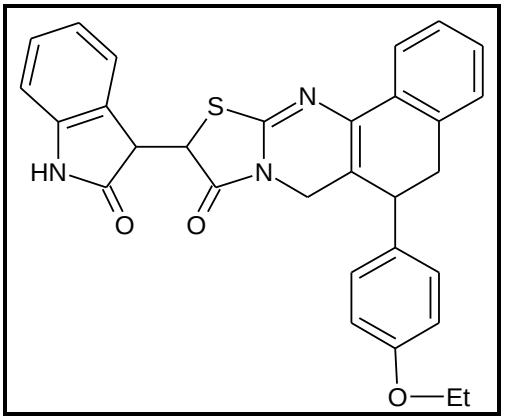
ZINC-8441604



ZINC-664768



ZINC-8441816



ZINC-3770286

By analysing the results, the compounds containing **PYRAZOLE, THIAZOLIDINONE** and **OXAZEPINE** nucleus were found to have good fitness value. Therefore on the basis of the virtual screening performed in iGEMDOCK v2.1 and by the literature, pyrazole linked with thiazolidinone and oxazepine were taken as the lead for *Pf*ENR and *E.coli* FabI inhibitors in the present study.

5.1.2 LEAD OPTIMIZATION

Lead optimization was done by evaluating the drug likeness properties

Computation of drug like properties

Lipinski's rule of five help to find the drug likeness score. For the better oral absorption of the ligands, the drug likeness scores are constructed by getting information about the solubility, diffusion, Log P, molecular weight etc. Lipinski's rule of five can be found out by utilizing Molinspiration server.

Calculation of Lipinski's rule of five [84]

1. Open the Molinspiration home page.
2. Click calculation of molecular properties and prediction of bioactivity.
3. Draw the structure of 2a in JME window or paste the smile notation of the compound.
4. Then click calculate properties.
5. save the properties.
6. JAVA program is required in the computer for the calculation of the properties.

Calculation of properties of the rest of the compounds is done in the same manner. Results are tabulated in **Table 2.**

RESULTS AND DISCUSSION

Table.2: Drug likeliness scores of 2a-j and 3a-j ligands

S.No	Compound code	Log P	Molecular Weight	Hydrogen acceptors	Hydrogen donor	n Violations
1	**2a**	3.32	380.43	7	0	0
2	**2b**	4.06	369.88	4	0	0
3	**2c**	3.44	365.46	5	0	0
4	**2d**	2.73	381.46	6	1	0
5	**2e**	3.49	378.50	5	0	0
6	**2f**	4.01	369.88	4	0	0
7	**2g**	3.10	395.48	6	1	0
8	**2h**	2.60	353.40	6	2	0
9	**2i**	2.81	381.46	6	0	0
10	**2j**	3.33	339.39	4	0	0
11	**3a**	2.96	390.36	9	0	0
12	**3b**	3.70	379.80	6	0	0
13	**3c**	3.08	375.38	7	0	0
14	**3d**	2.36	391.38	8	1	0
15	**3e**	3.12	388.43	7	0	0
16	**3f**	3.65	379.80	6	0	0
17	**3g**	2.73	405.41	8	1	0
18	**3h**	2.46	377.36	8	2	0
19	**3i**	2.67	405.41	8	0	0
20	**3j**	3.18	363.35	6	0	0
21	**4a**	4.29	454.47	9	0	0
22	**4b**	4.81	429.86	6	0	0
23	**4c**	4.19	425.44	7	0	0
24	**4d**	3.47	441.44	8	1	0
25	**4e**	4.24	438.49	7	0	0
26	**4f**	4.76	429.86	6	0	0
27	**4g**	3.85	455.47	8	1	0
28	**4h**	3.57	427.42	8	2	0
29	**4i**	3.78	455.47	8	0	0
30	**4j**	4.52	427.44	6	0	0

The *in-vivo* absorption capabilities of the designed molecules were assessed by means of Lipinski's rule of five using molinspiration server. All the lead compounds satisfied the rule indicating that ligand 2a-j, 3a-j and 4a-j have good oral absorption.

5.1.3 DOCKING STUDIES FOR THE LEAD MOLECULES [85-89]

From the virtual screening and literature review we have selected **Enoyl Acyl Carrier Protein Reductase *Pf*ENR & FabI** as a target for the present study. The enzyme have been selected from **RCSB Protein Data Bank** where the x-ray crystallographic structures were obtained and the docking studies were performed with the AutoDock 4.2 version.

Various steps involved in docking are

Step 1: SELECTION FROM PDB

- *Plasmodium falciparum* : PDB accession code:1VRW.pdb **(*Pf*ENR)**
- *Escherichia coli :* PDB accession code: 1C14.pdb **(*E.coli* FabI)**

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

Step 2: PROTEIN STRUCTURE REFINEMENT

Protein (1VRW & 1C14) downloaded from protein data bank as such cannot be used for docking process. It has to be refined before docking. Refinement of downloaded protein involves the removal of water and bound ligand if any.

The steps involved are

- Open Discovery studio viewer.
- File ⟶ open ⟶ Protein (downloaded from PDB).
- View ⟶ Hierarchy.
- Click water molecule.
- Ctrl + shift and click the last water molecule (select all the water molecule)
- Give right click and cut.
- Select ligand, which is unnecessary. Give right click and cut.

- Save the molecule in our desired area.

The 1VRW.pdb and 1C14.pdb were refined by the above method.

Step 3: LIGAND FILE FORMAT CONVERSION

The ligands 2a-j, 3a-j and 4a-j were drawn in Chemsketch.

- Tools ⟶ Generate ⟶ SMILES notation [Simplified Molecular Input Line Entry System, which is a file format]
- Save the SMILES in a word document.
- Open the online smile translator – catus. nci.nih.gov/services/translate/
- Upload the SMILES
- By choosing the required file format we can save the file. Here, we are saving it as pdb format in Cygwin/usr/local/bin.

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

Step 4: DOCKING

Docking was performed using AutoDock and requires a refined protein and the ligand in PDB format and files like autogrid4 and autodock4.

Docking process is done with AutoDock 4.2

Steps involved are

- Conversion of refined enzyme into .pdb format.
- Conversion of pdb format of ligand into .pdbqt format.
- Preparation of grid box by setting grid parameters at 60, 60 & 60.
- Docking process by setting docking parameters.
- Saving the docked result as .dlg file.

- Viewing the docked conformation.
- Taking snapshots of the interactions.

Docking studies for all the ligands 2a-j, 3a-j and 4a-j were carried out in the same manner.

RESULTS AND DISCUSSION

The docking results of *Pf*-ENR (1VRW.pdb) & *E.coli* FabI (1C14.pdb) with the ligands **2a-j, 3a-j and 4a-j** and standard are reported below. The best docked structures should have the binding energy lower to the standard. The binding sites and the active sites are represented in the snap shots and the binding energy was compared with the standard ligand, triclosan.

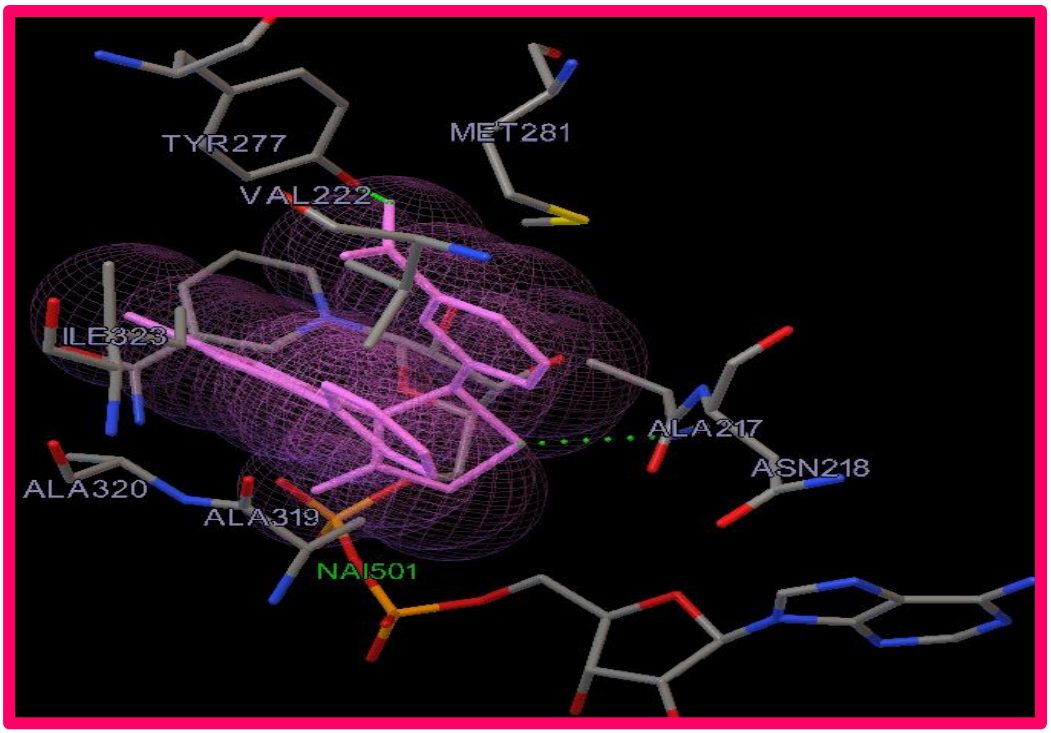
The results have been tabulated in the **Table 3 followed** by the snapshots.

Table 3: Binding energies of 2a-j with *Pf*-ENR (1VRW.pdb) & *E.coli*-FabI 1C14.pdb)

S.No	Compound code	Binding energies (kcal/mol)	
		1VRW.pdb	**1C14.pdb**
1	**2a**	-5.68	-4.54
2	**2b**	-4.78	-5.71
3	**2c**	-6.59	-3.54
4	**2d**	-6.43	-4.41
5	**2e**	-2.89	-3.88
6	**2f**	-2.93	-1.66
7	**2g**	-7.26	-4.66
8	**2h**	-3.89	-5.69
9	**2i**	-4.36	-2.85
10	**2j**	-6.10	-4.01
11	**3a**	-9.33	-1.24
12	**3b**	-9.51	-4.91
13	**3c**	-5.39	-7.58
14	**3d**	-5.88	-6.65
15	**3e**	-7.83	-3.41
16	**3f**	-9.11	-5.24
17	**3g**	-6.63	-3.67
18	**3h**	-9.45	-3.68
19	**3i**	-7.89	-6.49
20	**3j**	-6.78	-3.27
21	**4a**	-7.31	-8.05
22	**4b**	-6.43	-7.55
23	**4c**	-8.76	-8.28
24	**4d**	-8. 43	-8.24
25	**4e**	-4.57	-9.09
26	**4f**	-6.32	-6.91
27	**4g**	-7.58	-5.65
28	**4h**	-5.43	-6.57
29	**4i**	-6.23	-9.19
30	**4j**	-9.19	-6.23
31	**Triclosan**	-5.28	-7.15

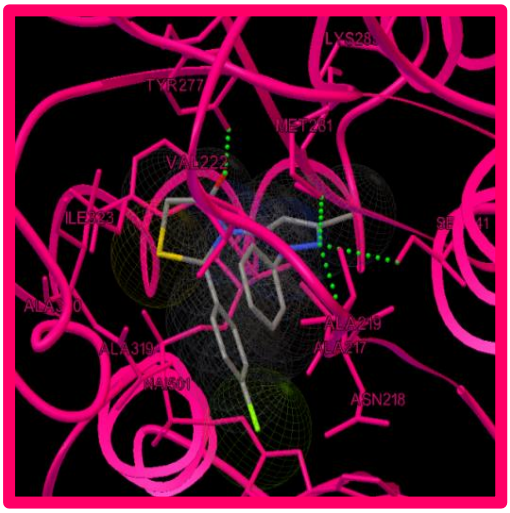
Binding interactions of 2g with *Pf*-ENR (1VRW.pdb)

2g interacts with Enoyl ACP reductase at Tyr 267, Tyr 277, Met 281, Gly313, Pro314 and NAD^+. The binding energy was found to be -7.26 kcal/mol.

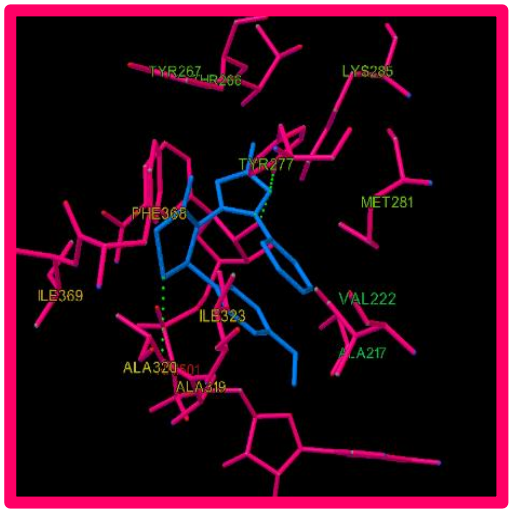


Snap shot of 2g with PfENR (1VRW.pdb)

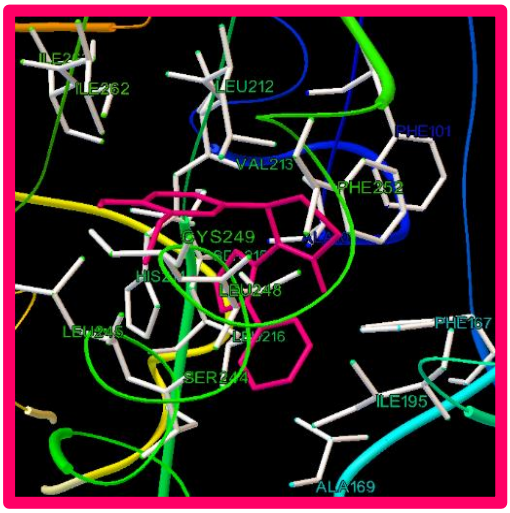
Snapshots of other pyrazolyl thiazolidinone derivatives with *Pf*-ENR (1VRW.pdb)


LXS281
TYR277
MET281
VAL222
ILE323
ALA219
ALA217
ASN218

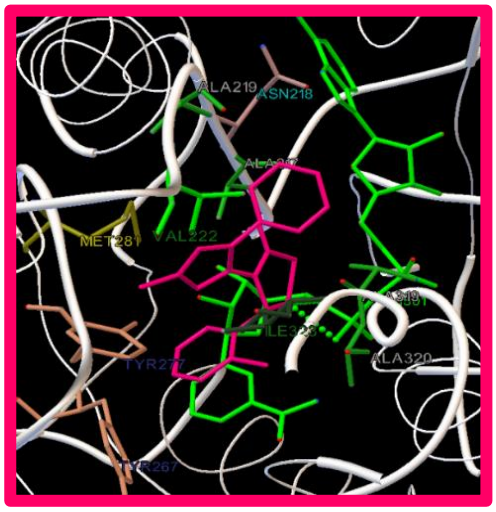
2b


TYR267
LYS285
TYR277
PHE366
MET281
ILE369
VAL222
ILE323
ALA217
ALA320
ALA318

2c


ILE262
LEU212
PHE101
VAL213
PHE252
CYS249
LEU248
LEU216
PHE167
SER214
ILE195
ALA169

2d


ALA219
ASN218
MET281
VAL222
ILE323
ALA320
TYR267

2e


ALA219
ASN218
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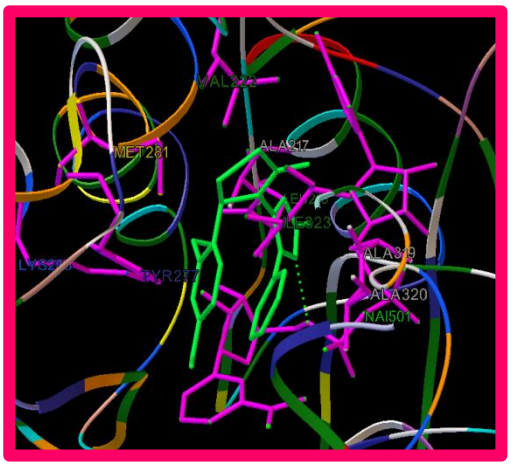
MET281
ALA217
ALA322
ILE323
ALA319
TYR277
NAI501

2f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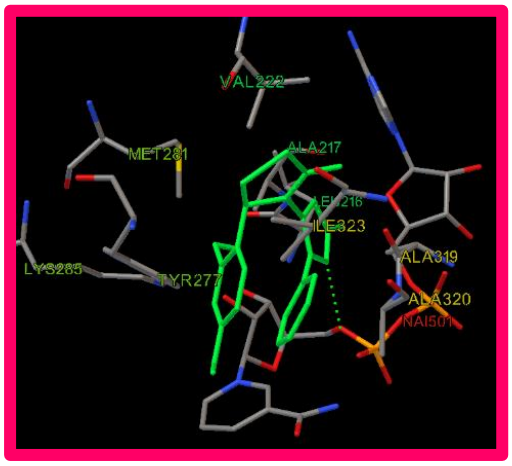
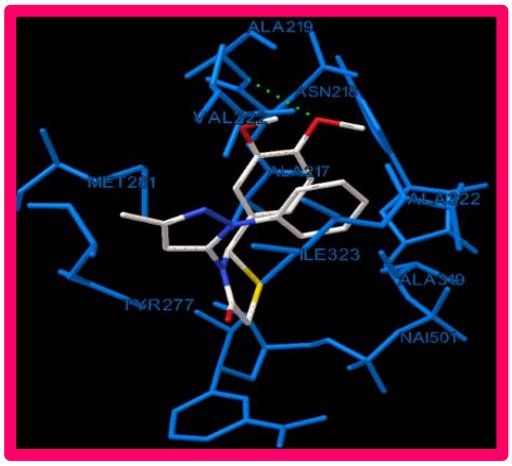
MET281
ALA217
ILE323
ALA319
TYR277
ALA320
NAI501

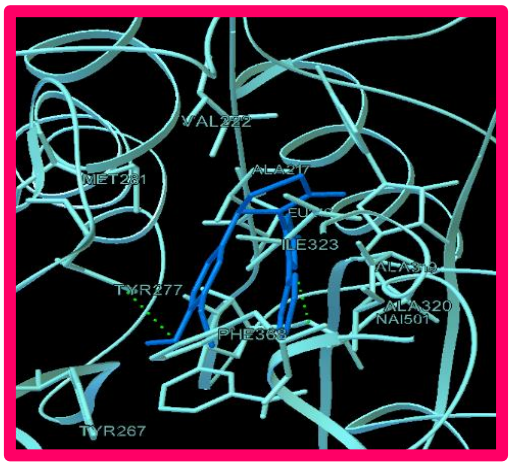
2g


VAL222
MET281
ALA217
ILE323
ALA319
TYR277
ALA320
NAI501

2h


ALA219
ASN218
MET281
ILE323
ALA319
TYR277
NAI501

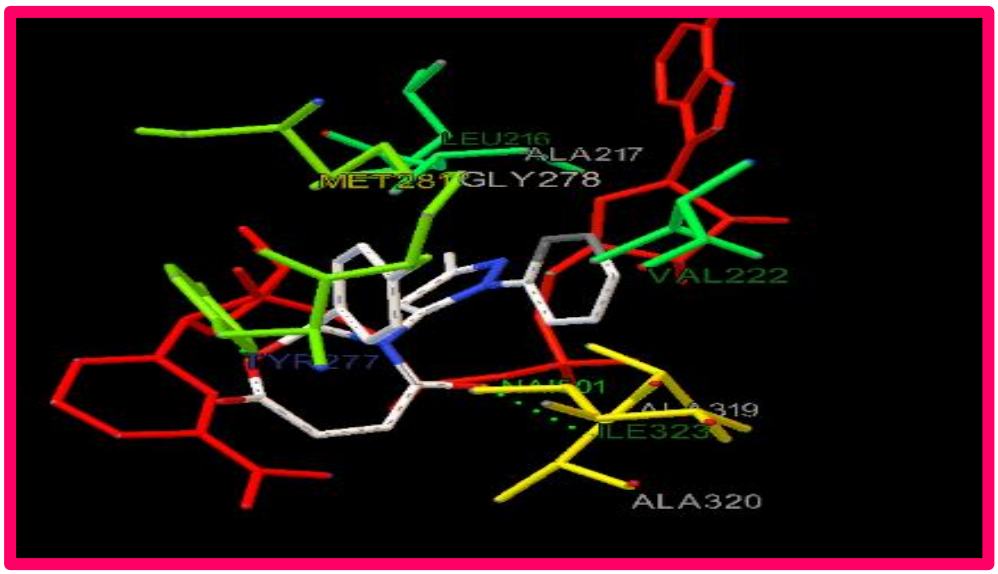
2i


VAL222
MET281
ALA217
ILE323
TYR277
ALA320
NAI501
TYR267

2j

Binding interactions of 3b with *Pf*-ENR (1VRW.pdb)

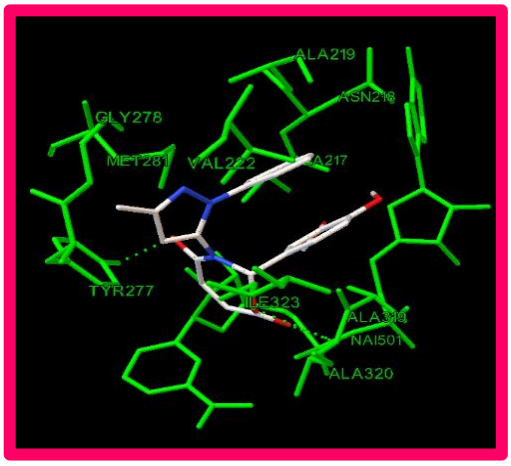
3b interacts with Enoyl ACP reductase at Tyr 267, Tyr 277, Met 281, Gly313, Pro314 and NAD^+. The binding energy was found to -9.51 kcal/mol.



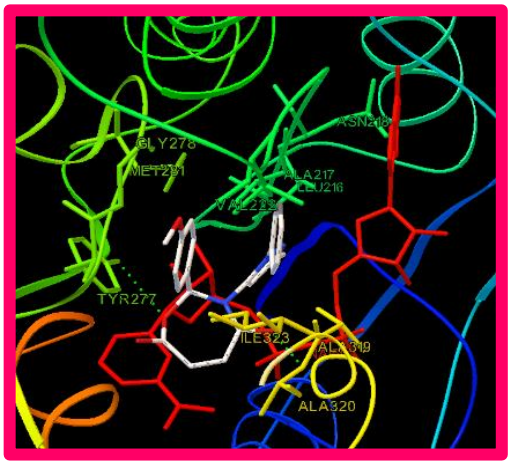
Snap shot of 3b with PfENR (1VRW.pdb)

Snapshots of other pyrazolyl oxazepine derivatives with *Pf*-ENR (1VRW.pdb)

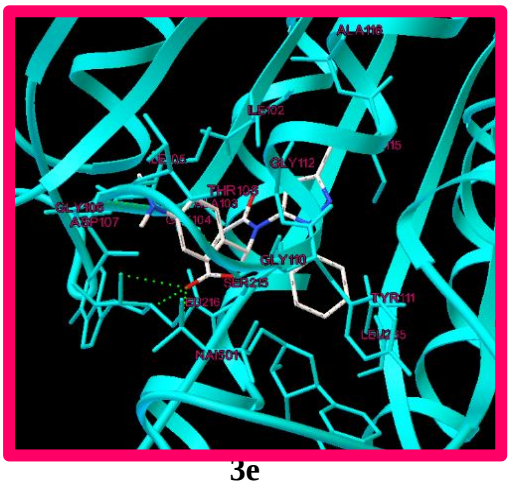
3a



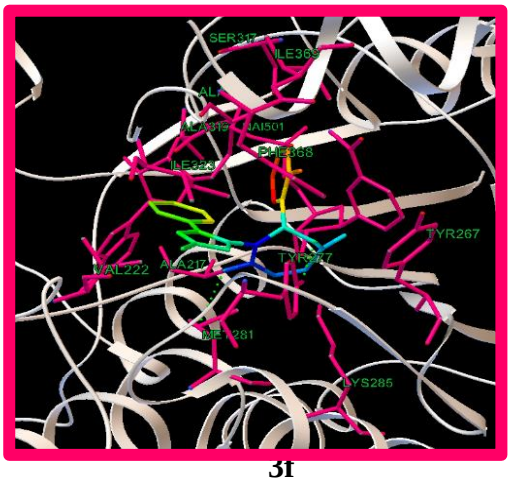
3c



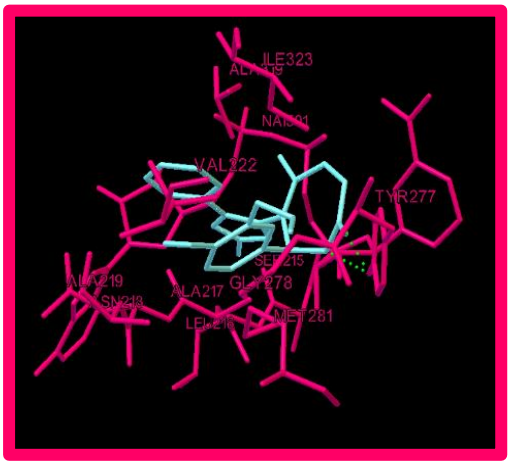
3d



3e

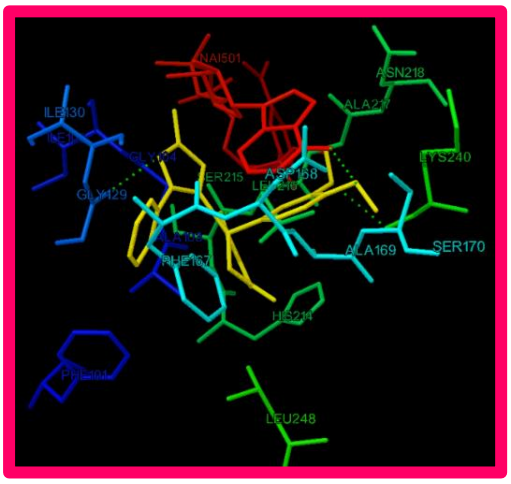


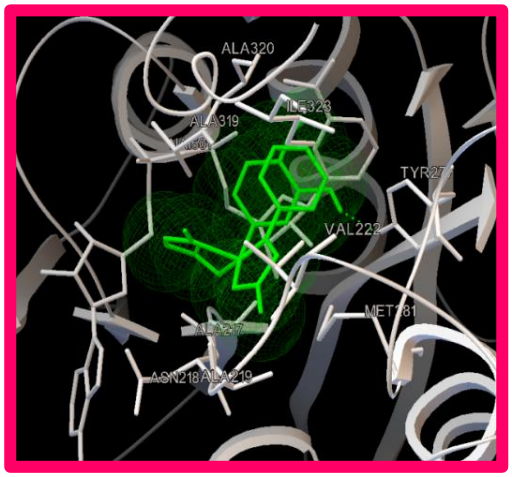
3f



3g

3h

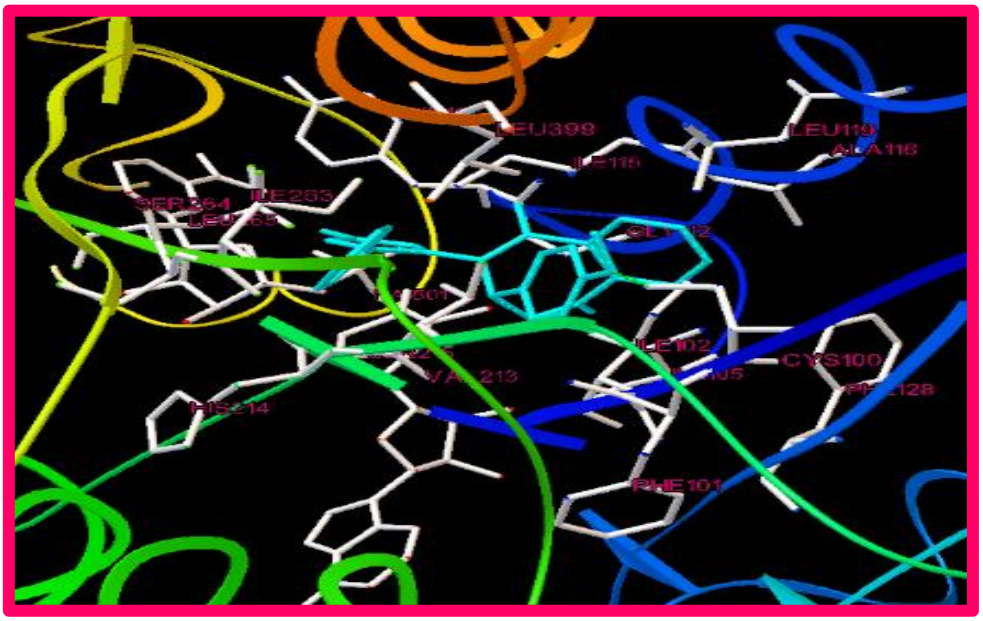




3i **3j**

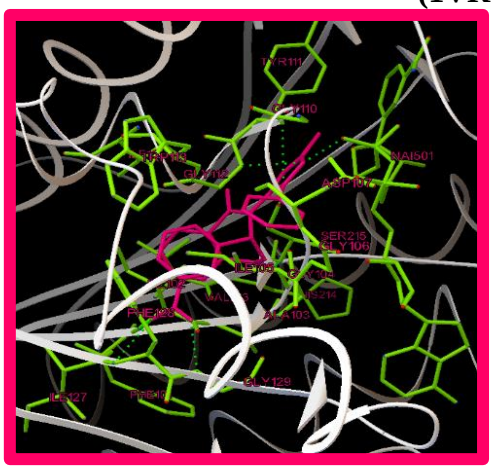
Binding interactions of 4c with *Pf*-ENR (1VRW.pdb)

4c interacts with Enoyl ACP reductase at Tyr 267, Tyr 277, Met 281, Gly313, Pro314 and NAD^+. The binding energy was found to be -8.76kcal/mol.

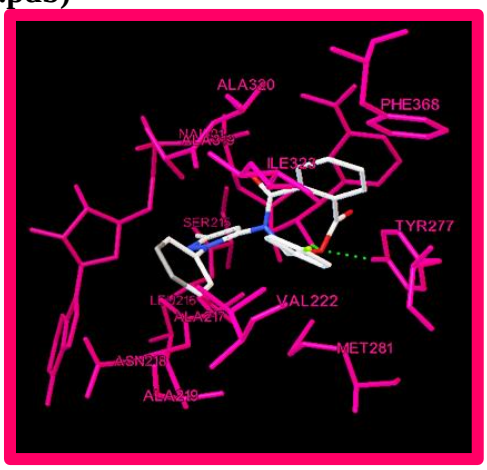


Snap shot of 4c with *Pf*-ENR (1VRW.pdb)

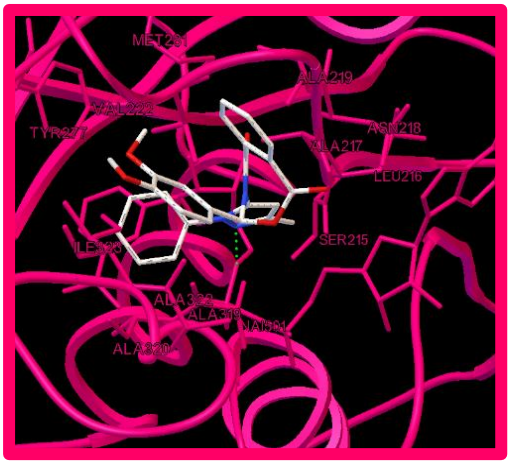
Snapshots of other pyrazolyl benzoxazepine derivatives with *Pf*-ENR (1VRW.pdb)



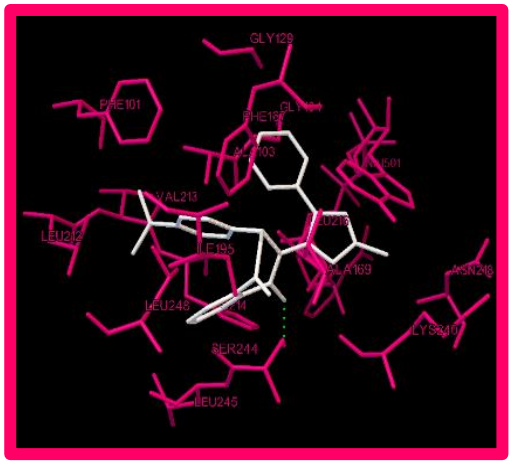
4a



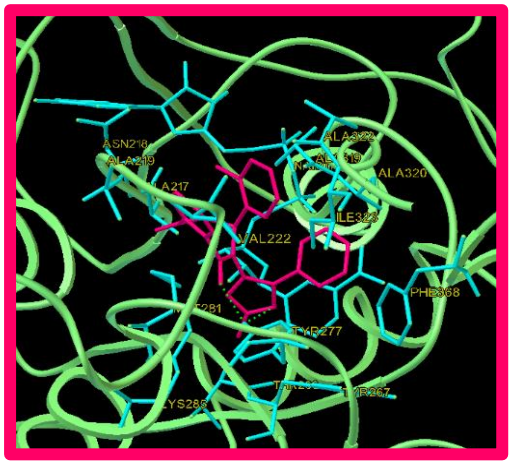
4b



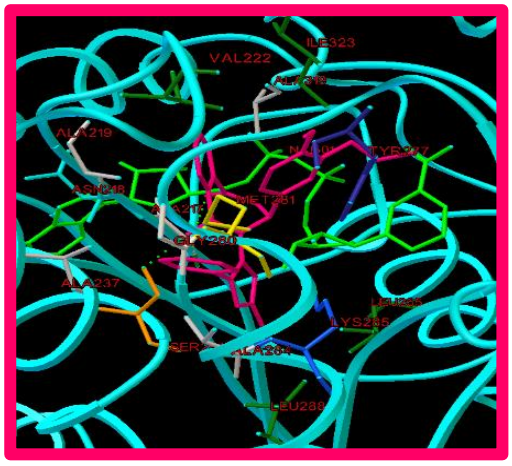
4d



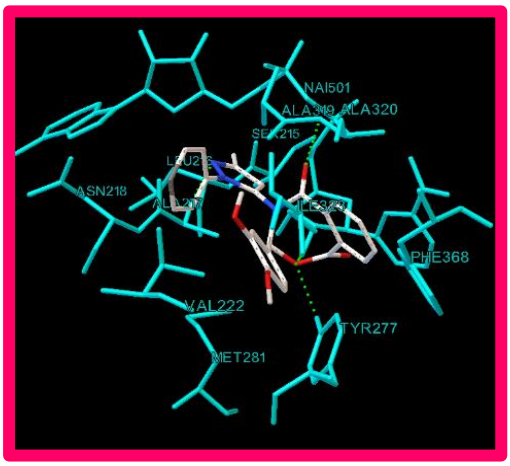
4e



4f



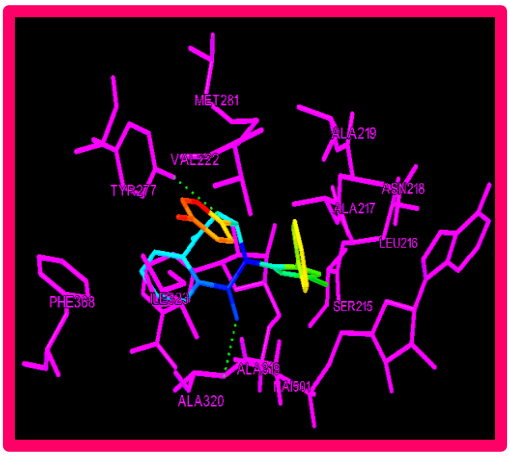
4g


NAI501
ALA320
SER215
LEU216
ASN218
PHE368
VAL222
TYR277
MET281

4h


VAL213
ILE310
ILE102
ALA103
LEU265
ALA312
GLY104
NAI501

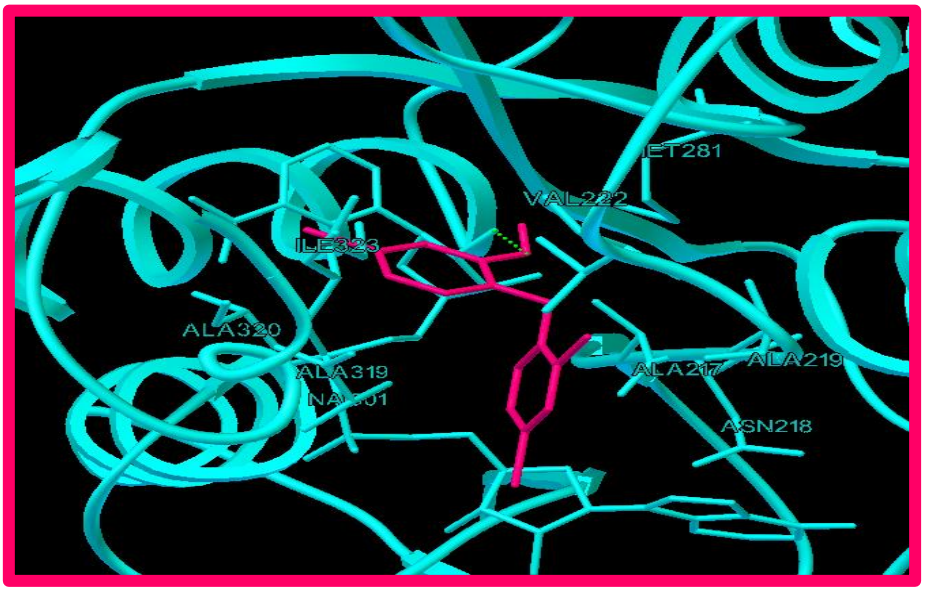
4i


MET281
ALA219
VAL222
TYR277
ASN218
ALA217
LEU216
PHE368
SER215
ALA320

4j

Binding interactions of Triclosan with *Pf*-ENR (1VRW.pdb)

Triclosan interacts with Enoyl ACP reductase at Tyr 267, Tyr 277, Met 281, Gly313, Pro314 and NAD^+. The binding energy was found to be -5.28 kcal/mol.

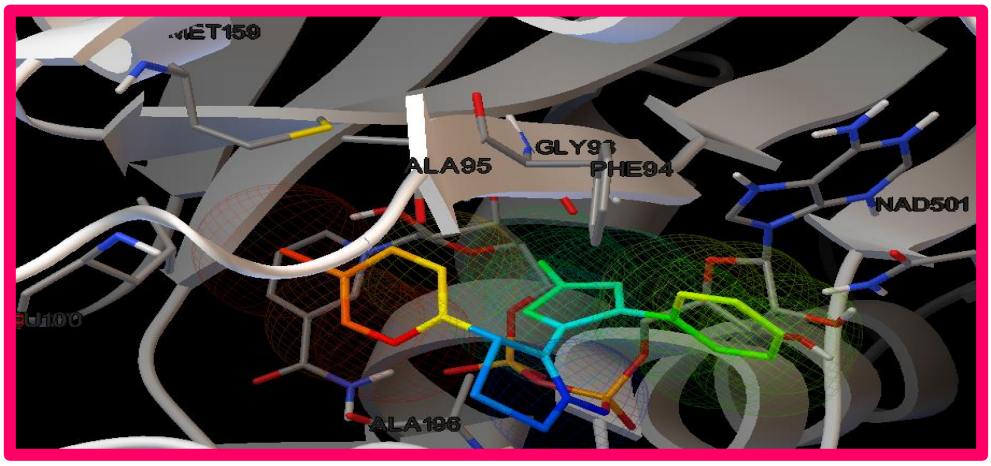


Snapshot of triclosan with PfENR (1VRW.pdb)

In the pyrazolyl thiazolidinone series, all the ligands (**2a-j**) showed excellent binding interactions with the *Pf*ENR (1VRW.pdb). Among the derivatives, **2g** (ethoxy), **2c** (methoxy), **2d** (4-hydroxy-3-methoxy) and **2j** (fluoro) had shown the highest binding energies, -7.26, -6.59, -6.43 and -6.10kcal/mol . Among the pyrazolyl oxazepine (**3a-j**) derivatives, **3b** (p-chloro), **3h** (dihydroxy), **3a** (nitro) and **3f** (o-chloro) had shown the highest binding energies, -9.51, -9.45, -9.33 and -9.11kcal/mol. Among the pyrazolyl benzoxazepine (**4a-j**) derivatives, **4j** (fluoro), **4c** (methoxy), **4d** (4-hydroxy-3-methoxy) and **4g** (ethoxy) had shown the highest binding energies, -9.19, -8.76, -8.43 and -7.58kcal/mol when compared to the standard, triclosan (-5.28kcal/mol).

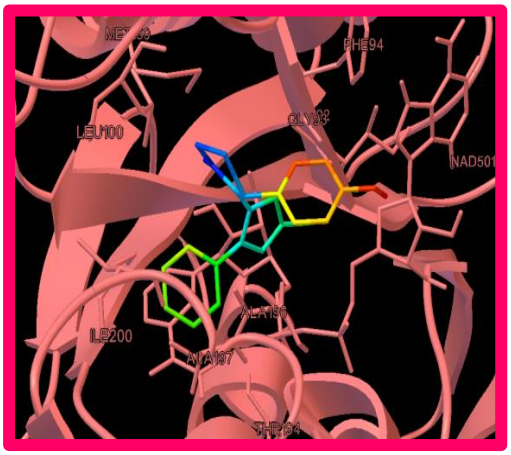
Binding interaction of 2b with *E.coli*-FabI (1C14.pdb)

2b interacts with Enoyl ACP reductase at Gly 93, Leu195, Tyr 158, Ala 196, Lle 200 and alse with NAD. The binding energy was found to be -5.71 Kcal/mol.

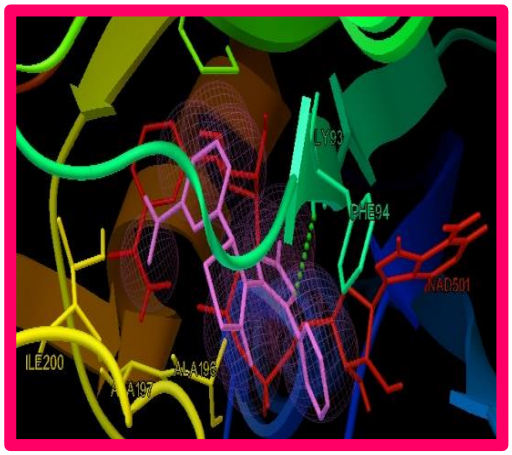


Snapshot of 2b binding with *E.coli*-FabI (1C14.pdb)

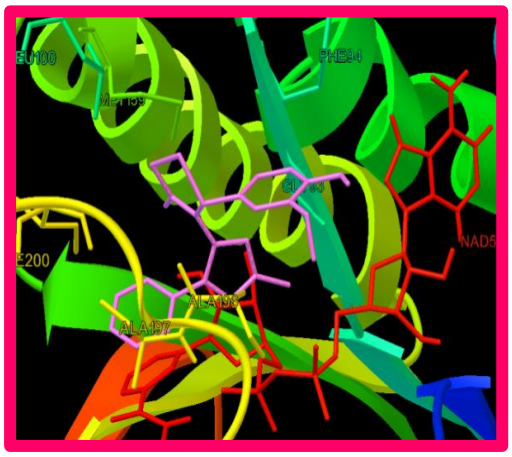
Snapshots of other pyrazolyl thiazolidinone derivatives with *E.coli*-FabI (1C14.pdb)



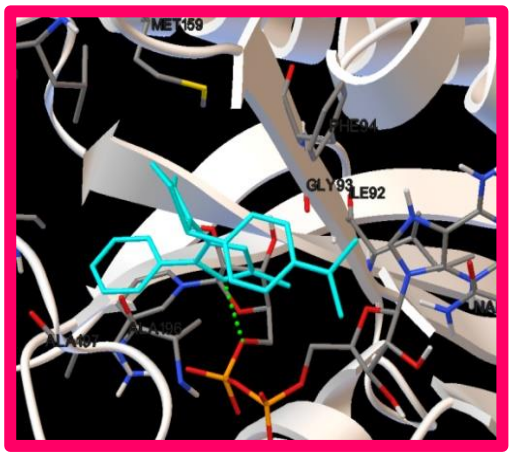
2a



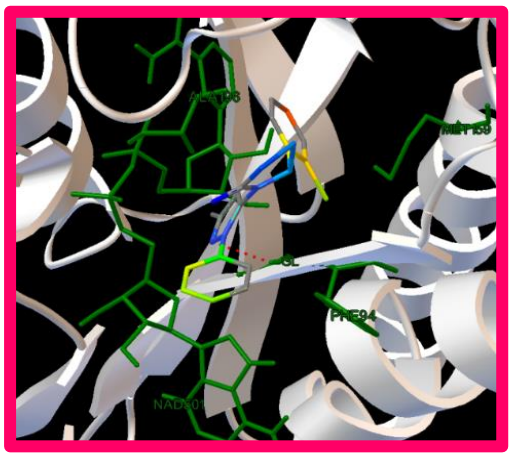
2c



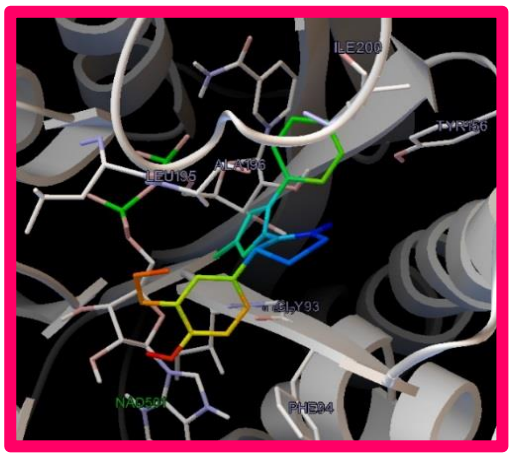
2d



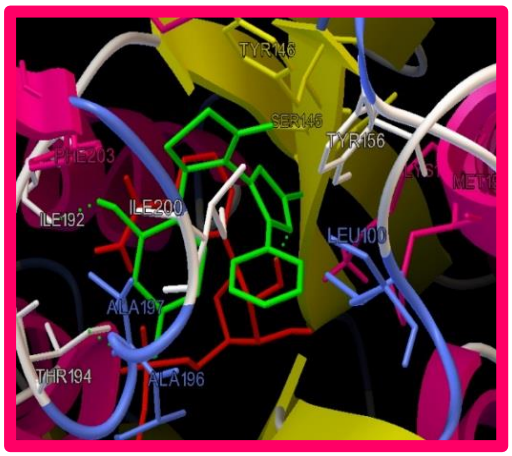
2e



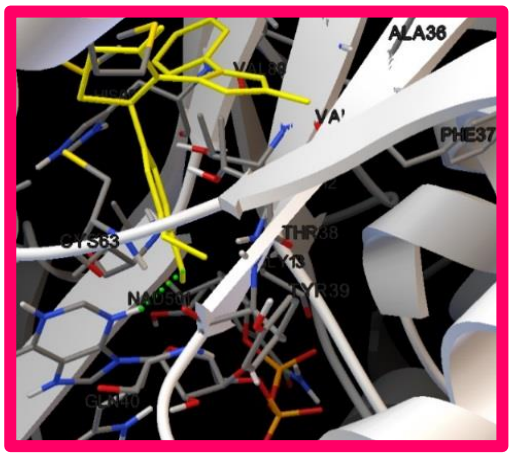
2f



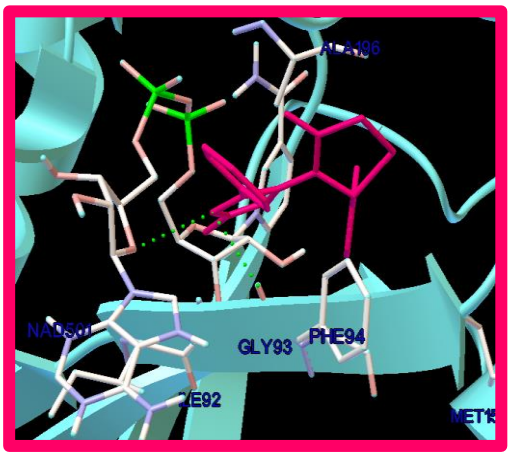
2g



2h



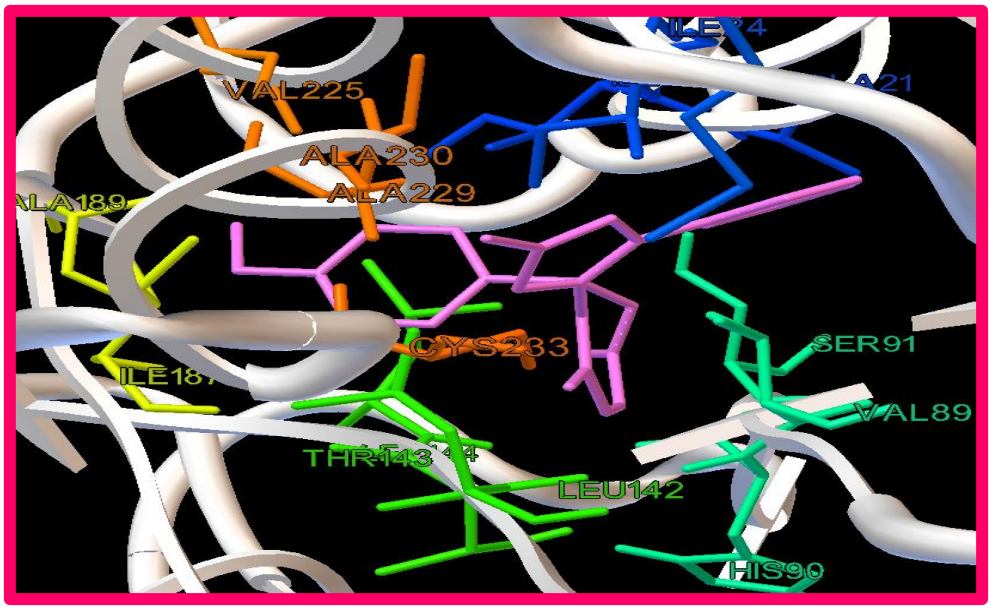
2i



2j

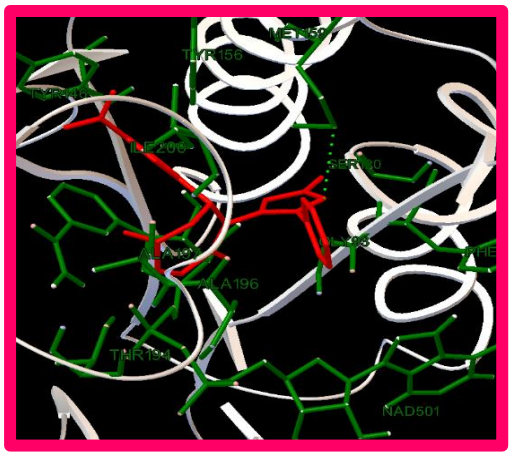
Binding interaction of 3c with *E.coli*-FabI (1C14.pdb)

3c interacts with Enoyl ACP reductase at Gly 93, Leu195, Tyr 158, Ala 196, Lle 200 and alse with NAD. The binding energy was found to be -7.58kcal/mol.

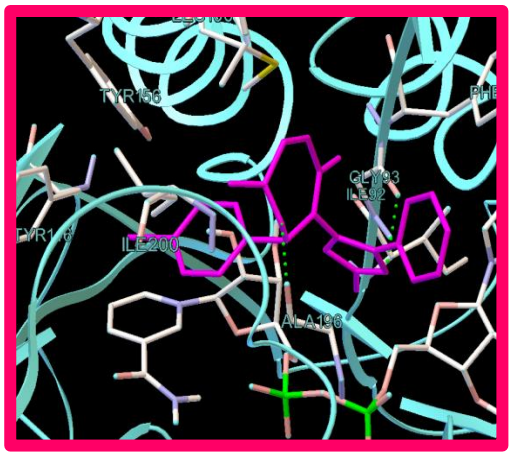


Snapshot of 3c binding with *E.coli*-FabI (1C14.pdb)

Snapshots of other pyrazolyl oxazepine derivatives with *E.coli*-FabI (1C14.pdb)

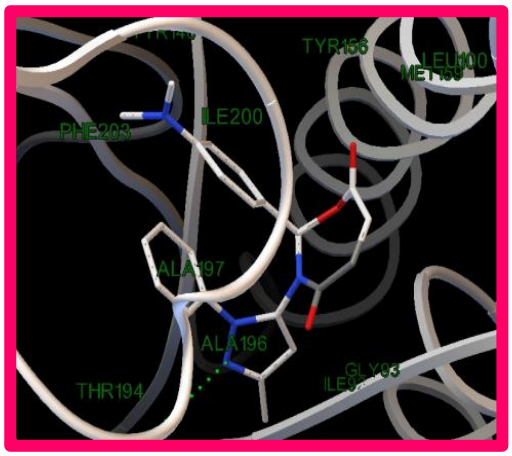


3a

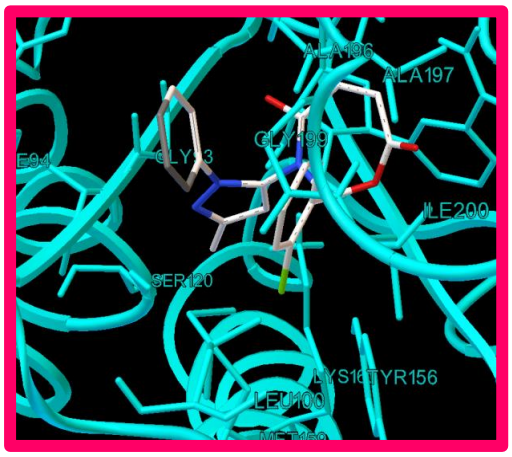


3b

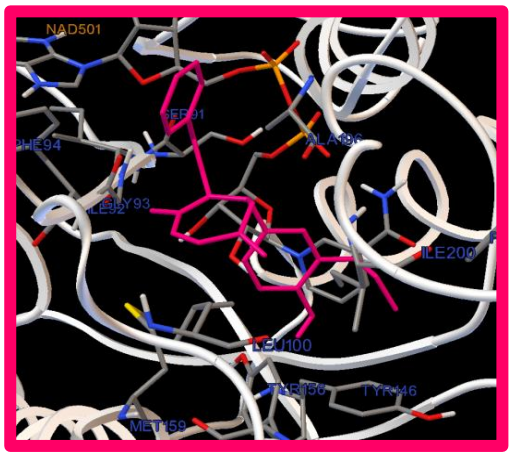
3d



3e


ALA196
ALA197
GLY199
GLY13
ILE200
SER120
LYS162
TYR156
LEU100

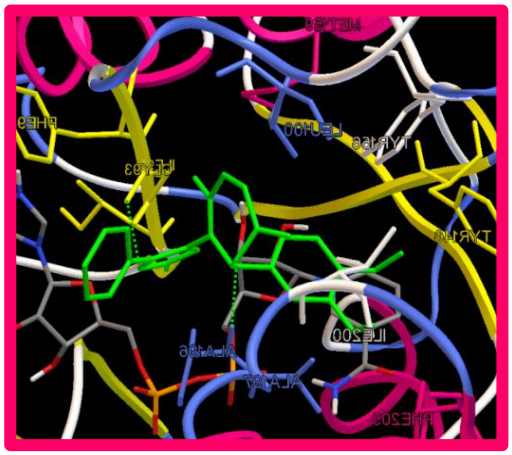
3f


NAD501
ILE200
LEU100
TYR156
TYR146
MET159

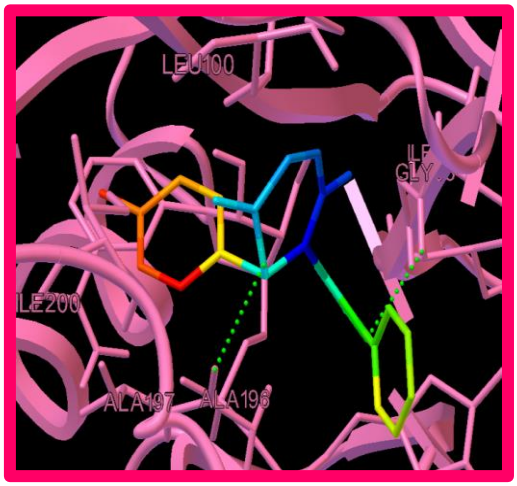
3g


LEU100
TYR146
ALA196
GLY93
ILE92
ILE94

3h


LEU100
ILE200

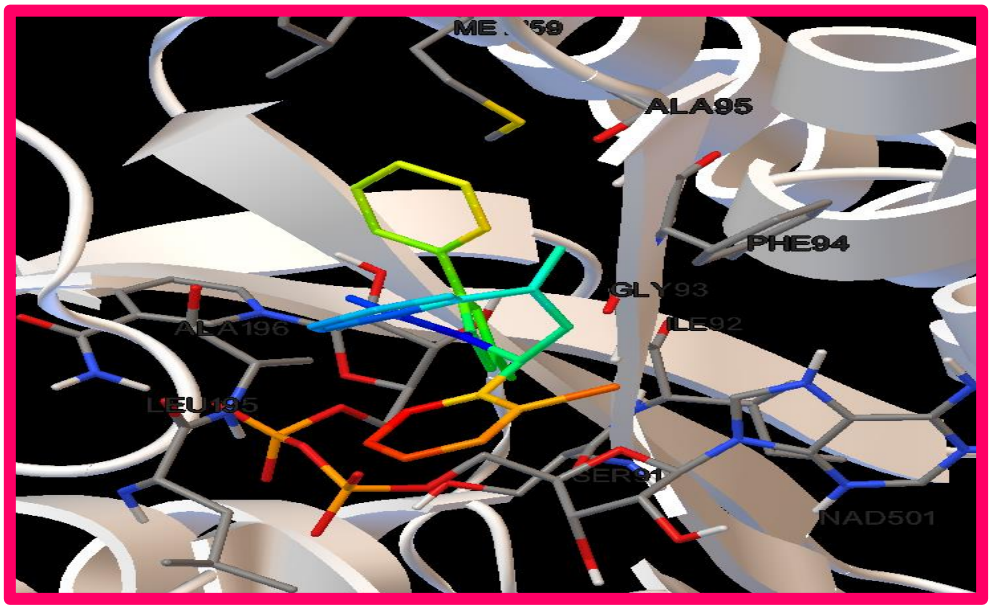
3i


LEU100
ILE200
ALA197
ALA196

3j

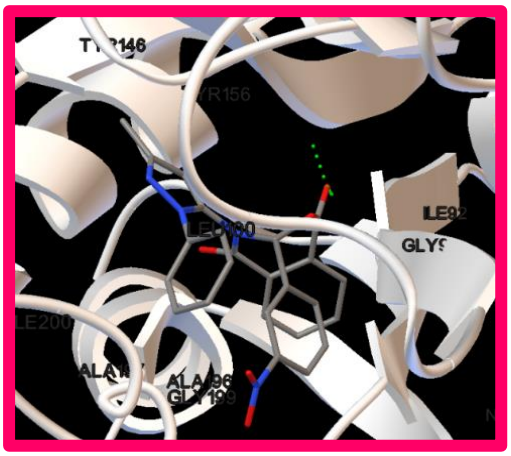
Binding interaction of 4i with *E.coli*-FabI (1C14.pdb)

4i interacts with Enoyl ACP reductase at Gly 93, Leu195, Tyr 158, Ala 196, Lle 200 and alse with NAD^+. The binding energy was found to be -9.19kcal/mol.



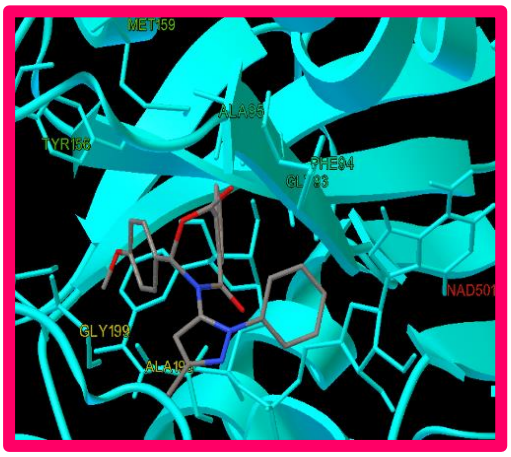
Snapshot of 4i binding with *E.coli*-FabI (1C14.pdb)

Snapshots of other pyrazolyl benzooxazepine derivatives with *E.coli*-FabI (1C14.pdb)



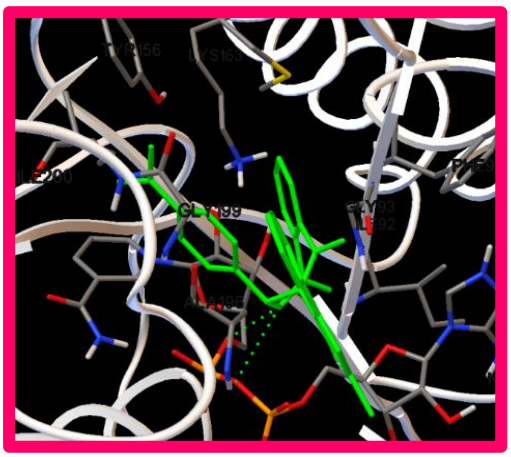
4a

4b



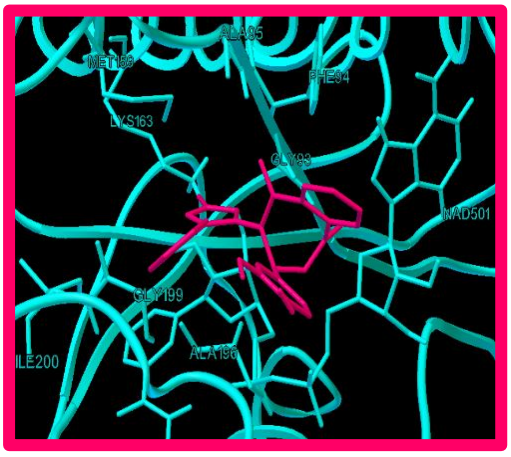
4c

4d

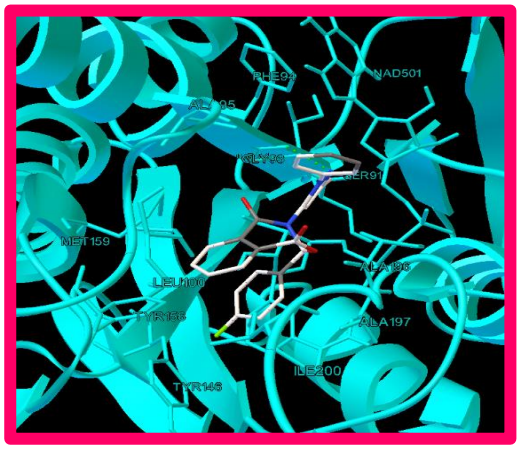


4e

4g



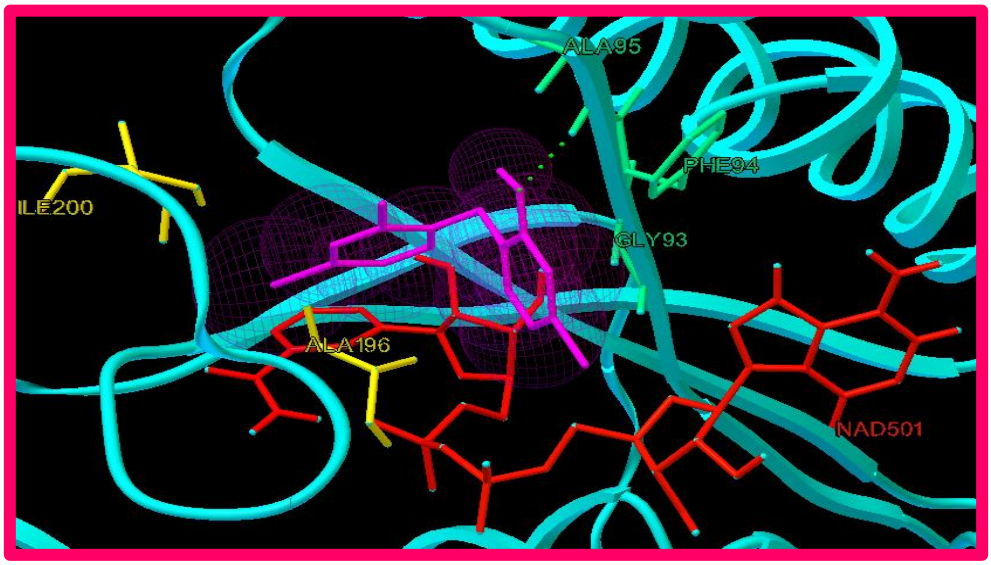
4h



4j

Binding interaction of triclosan with *E.coli*-FabI (1C14.pdb)

Triclosan interacts with Enoyl ACP reductase at Gly 93, Leu195, Tyr 158, Ala 196, Lle 200 and also with NAD. The binding energy was found to be -7.15kcal/mol



Snapshot of triclosan binding with *E.coli* FabI (1C14.pdb)

In the pyrazolyl thiazolidinone series, all the ligands (**2a-j**) showed excellent binding interactions with the *E.coli* FabI (1C14.pdb). Among the derivatives, **2b** (chloro), **2h** (dihydroxy) and **2g** (ethoxy) had shown the maximum binding energies, -5.71, -5.69 and -4.66kcal/mol. Among the pyrazolyl oxazepine (**3a-j**) derivatives, **3c** (methoxy), **3d** (4-hydroxy-3-methoxy), **3i** (dimethoxy) and **3f** (o-chloro) had shown the maximum binding energies, -7.58, -6.65, -6.49 and -5.24kcal/mol. Among the pyrazolyl benzoxazepine (**4a-j**) derivatives, **4i** (dimethoxy), **4e** (p-dimethyl amino), **4c** (methoxy) and **4a** (nitro) had shown the maximum binding energies, -9.19, -9.09, -8.28 and -8.05kcal/mol when compared to the standard, triclosan (-7.15kcal/mol).

5.2 PHASE II – SYNTHESIS

Chemicals and reagents used

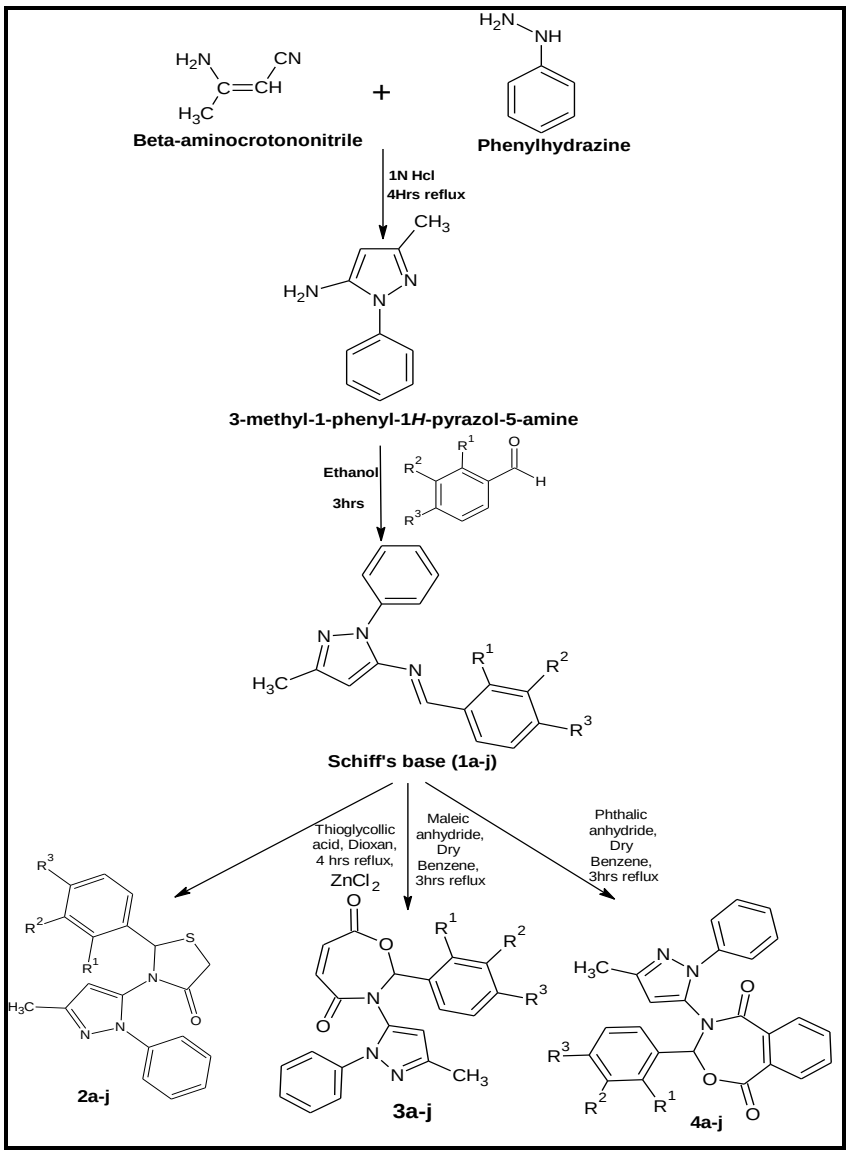
3-nitro benzaldehyde, 4-chloro benzaldehyde, 4-methoxy benzaldehyde, 4-hydroxy 3-methoxy benzaldehyde, 4-dimethylamino benzaldehyde, 2-chloro benzaldehyde, 3-ethoxy 4-hydroxy benzaldehyde, 2,4-dihydroxy benzaldehyde, 3,4-dimethoxy benzaldehyde, 4-fluoro benzaldehyde, β-aminocrotononitrile, phenylhydrazine, 1N HCl, thioglycolic acid, maleic anhydride, phthalic anhydride, dry benzene, dioxan, ethanol, etc.

All the chemicals and reagents were procured from Sigma Aldrich, High Media and LobaChem. All the compounds procured were purified and dried, whenever necessary before use, by following standard methods.

Analytical work

- Melting point were determined by using melting point apparatus MR-VIS, visual melting range apparatus, LABINDIA and corrected.
- Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel-G plates using iodine vapour as visualizing agent.
- UV spectra were recorded on JASCO V-530 UV/VIS Spectrophotometer in the Department of Pharmaceutical Analysis, SRIPMS, College of Pharmacy, Coimbatore.
- IR spectra on JASCO FTIR-420 in the Department of Pharmaceutical Analysis, SRIPMS, College of Pharmacy, Coimbatore.
- NMR were recorded on the Bruker Ultra Shielded NMR-300MHz.
- MASS spectra were recorded on JEOL GC Mate GC-MS Spectroscopy.

SCHEME



Compd code	R^1	R^2	R^3
a	H	NO_2	H
b	H	H	Cl
c	H	H	OCH_3
d	H	OCH_3	OH
e	H	H	$N(CH_3)_2$
f	H	Cl	H
g	H	OC_2H_5	OH
h	OH	H	OH
i	H	OCH_3	OCH_3
j	H	H	F

Procedure

Step 1: Synthesis of 3-methyl-1-phenyl-1*H*-pyrazol-5-amine[90]

An amount of 3.48g (0.042 mol) β-aminocrotononitrile was dissolved in 40ml 0f 1NHCl. To this mixture 5.86g (0.04mol) of phenylhydrazine hydrochloride was dissolved and then refluxed for 4 hours. The resulting reddish liquid was cooled to room temperature and basified to alkaline p^H with aqueous sodium hydroxide. The product obtained was filtered and dried.

Step 2: Synthesis of Schiff base (1a-j) [91]

A mixture of 3-methyl-1-phenyl-1*H*-pyrazol-5-amine (0.005mol) and benzaldehyde (0.005mol) were dissolved in minimum quantity of ethanol and few drops of glacial acetic acid was added and the mixture was refluxed for 3 hours. After cooling, the mixture was added to crushed ice by stirring. The separated compounds was filtered, washed, dried and recrystallised from ethanol.

Step 3:

❖ **Synthesis of Thiazolidinone derivatives (2a-j)**

Schiff's base (0.001 mol) treated with mercaptoacetic acid (Thioglycolic acid) (0.002 mol) in dioxin 25ml in presence of anhydrous $ZnCl_2$ and reaction mixture was refluxed for 4hrs. After cooling the reaction mixture was poured in crushed ice with stirring. Thus the separated solid was then filtered, washed with sodium bicarbonate to remove unreacted thioglycollic acid and recrystallized from methanol.

❖ **Synthesis of Oxazepine derivatives (3a-j)**

To a solution of maleic anhydride (0.01mol, 0.8g) in 10ml of dry benzene was added dropwise to a hot dry benzene solution (20ml) containing 0.01mol of a Schiff's base and the reaction mixture was refluxed for 3hrs. The solvent distilled off and the solid obtained was washed with cold water. And the resulting was recrystallized from Ethanol.

❖ **Synthesis of Benzoxazepine derivatives (4a-j)**

To a solution of phthalic anhydride (0.01mol, 0.8g) in 10ml of dry benzene was added dropwise to a hot dry benzene solution (20ml) containing 0.01mol of a Schiff's base and the reaction mixture was refluxed for 3hrs. The solvent distilled off and the solid obtained was washed with cold water. And the resulting was recrystallized from Ethanol.

RESULTS AND DISCUSSION

The novel 30 docked compounds(2a-j, 3a-j, 4a-j) were synthesized by the schemes as mentioned above and the structure of the synthesized compounds were established on the basis of the physical data (melting point and TLC) and spectral data (IR, UV, NMR and MASS) respectively. The purity of the compounds was established by TLC plates.

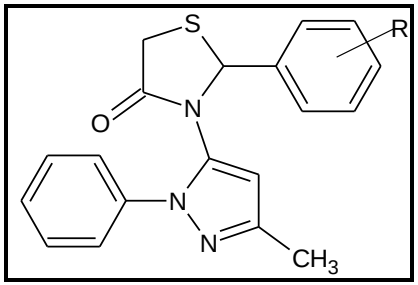
Recrystallisation solvent : Ethanol

Solvent system : Ethyl acetate: n-Hexane (3:7)

Visualizing agent : Iodine vapour

PHYSICAL CHARACTERISATION DATA

1) Pyrazolyl Thiazlidinone series (2a-j)

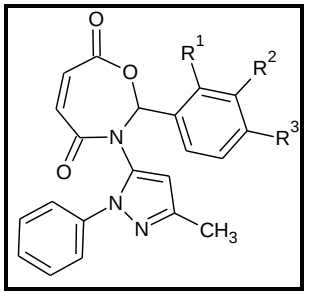


3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2-phenyl-1,3-thiazolidin-4-one

Table 4: Physical characterization of Pyrazolyl Thiazolidinone series (2a-j)

SI no.	Compd code	R^1	R^2	R^3	Molecular formula	Mol.weight	% Yield	Melting point °C	R_f value
1	2a	H	NO_2	H	$C_{19}H_{16}N_4O_3S$	380.42	67	93.50	0.36
2	2b	H	H	Cl	$C_{19}H_{16}ClN_3OS$	369.86	56	105.34	0.50
3	2c	H	H	OCH_3	$C_{20}H_{19}N_3O_2S$	365.44	76	89.61	0.46
4	2d	H	OCH_3	OH	$C_{20}H_{19}N_3O_3S$	381.448	54	93.67	0.83
5	2e	H	H	$N(CH_3)_2$	$C_{21}H_{22}N_4OS$	378.49	58	135.32	0.54
6	2f	H	Cl	H	$C_{19}H_{16}ClN_3OS$	369.86	69	120.43	0.43
7	2g	H	OC_2H_5	OH	$C_{21}H_{21}N_3O_3S$	395.47	45	105.28	0.63
8	2h	OH	H	OH	$C_{18}H_{15}N_3O_3S$	353.395	55	118.10	0.48
9	2i	H	OCH_3	OCH_3	$C_{20}H_{19}N_3O_3S$	381.44	67	85.34	0.73
10	2j	H	H	F	$C_{18}H_{14}FN_3OS$	339.38	45	98.32	0.66

2) Pyrazolyl Oxazepine series (3a-j)

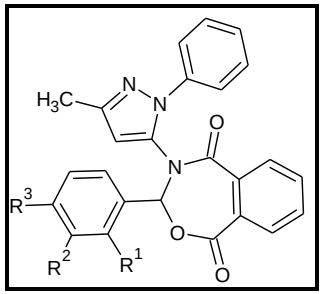


3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2-phenyl-2,3-dihydro-1,3-oxazepine-4,7-dione

Table 5: Physical characterization of Pyrazolyl Oxazepine series (3a-j)

SI no.	Compd code	R^1	R^2	R^3	Molecular formula	Mol. weight	% Yield	Melting point °C	R_f value
1	3a	H	NO_2	H	$C_{20}H_{14}N_4O_5$	390.34	56	120.21	0.47
2	3b	H	H	Cl	$C_{20}H_{14}ClN_3O_3$	379.79	45	93.84	0.42
3	3c	H	H	OCH_3	$C_{21}H_{17}N_3O_4$	375.37	63	128.62	0.34
4	3d	H	OCH_3	OH	$C_{21}H_{17}N_3O_5$	391.37	56	93.42	0.40
5	3e	H	H	$N(CH_3)_2$	$C_{22}H_{20}N_4O_3$	388.41	58	112.65	0.67
6	3f	H	Cl	H	$C_{20}H_{14}ClN_3O_3$	379.79	62	89.24	0.72
7	3g	H	OC_2H_5	OH	$C_{22}H_{19}N_3O_5$	405.40	57	103.70	0.77
8	3h	OH	H	OH	$C_{20}H_{15}N_3O_5$	377.35	43	143.34	0.75
9	3i	H	OCH_3	OCH_3	$C_{22}H_{19}N_3O_5$	405.40	49	132	0.62
10	3j	H	H	F	$C_{20}H_{14}FN_3O_3$	363.34	54	121	0.81

3) Pyrazolyl Benzoxazepine series (4a-j)



4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-3-phenyl-3,4-dihydro-2,4-benzoxazepine-1,5-dione

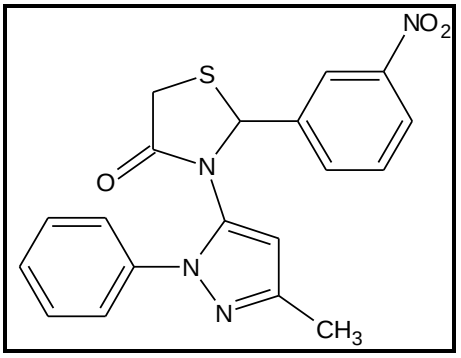
Table 6: Physical characterization of Pyrazolyl Oxazepine series (3a-j)

SI no.	Compd code	R^1	R^2	R^3	Molecular formula	Molecular weight	% Yield	Melting point °C	R_f value
1	4a	H	NO_2	H	$C_{25}H_{18}N_4O_5$	454.43	55	121	0.30
2	4b	H	H	Cl	$C_{24}H_{16}ClN_3O_3$	429.85	43	119.62	0.52
3	4c	H	H	OCH_3	$C_{25}H_{19}N_3O_4$	425.43	64	105	0.48
4	4d	H	OCH_3	OH	$C_{25}H_{19}N_3O_5$	441.43	54	118.20	0.70
5	4e	H	H	$N(CH_3)_2$	$C_{26}H_{22}N_4O_3$	438.47	65	116.72	0.69
6	4f	H	Cl	H	$C_{24}H_{16}ClN_3O_3$	429.85	43	132	0.33
7	4g	H	OC_2H_5	OH	$C_{26}H_{21}N_3O_5$	455.46	45	109.49	0.52
8	4h	OH	H	OH	$C_{24}H_{17}N_3O_5$	427.40	56	117	0.39
9	4i	H	OCH_3	OCH_3	$C_{26}H_{21}N_3O_5$	455.46	43	147	0.53
10	4j	H	H	F	$C_{25}H_{18}FN_3O_3$	427.42	65	123	0.37

SPECTRAL CHARACTERIZATION OF COMPOUNDS[94-96]

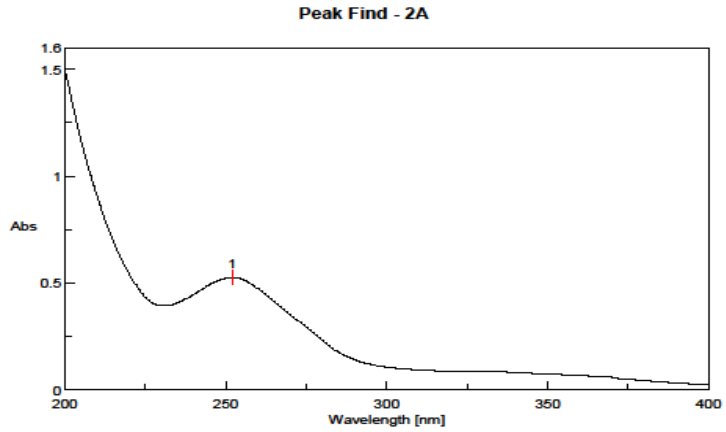
The structures of synthesized compounds were established on the basis of the IR, UV, NMR, and Mass spectral data .

Compound code : 2a



Chemical name	:	3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2-(3-nitrophenyl)-1,3-thiazolidin-4-one
UV Spectrum		
Solvent used	:	Acetonitrile
λ_{max}	:	252.0 nm
IR (KBr, νmax in cm-1)	:	3487.63 (Aromatic, C-H), 1683.55 (C=O,Thiazolidinone), 1596.77 (C=N), 1499.38 (N=N), 1384.64(C-N), 821.527(Aromatic, C-H Bending), 755.95(C-S),
^{1}HMR spectral data	:	2.83 (s, 3H, CH_3), 3.0 (s, 2H, Thiazolidinone), 5.20 (s, 1H, CH-S), 6.8-7.92 (m, 9H, Ar-H), 8.29 (s, 1H, Pyrazole)
^{13}C NMRSPECTRA	:	35.59(CH_3), 39.97 (C-S), 41.93 (C_5 of Thiazolidinone), 42.63(C-N), 100.146(C=C, Pyrazole), 132.75-138.66 (Aromatic carbons), 149.140 (C_3, Pyrazole), 170.23 (C=O).
Mass spectral data	:	381.25 M+1 ion peak

UV spectrum



[Comments]

Sample name	2a
Comment	
User	
Division	
Company	SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
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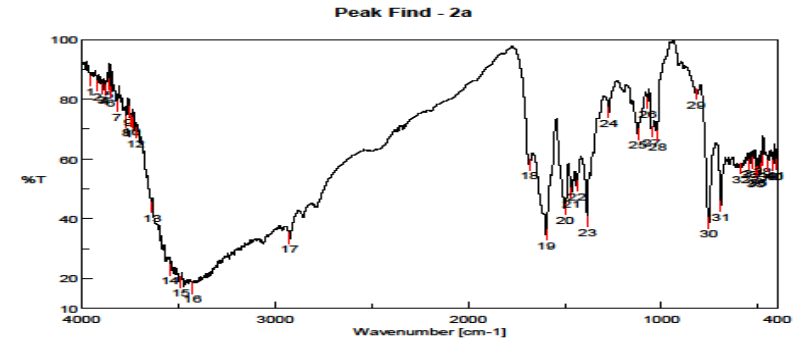
IR Spectrum



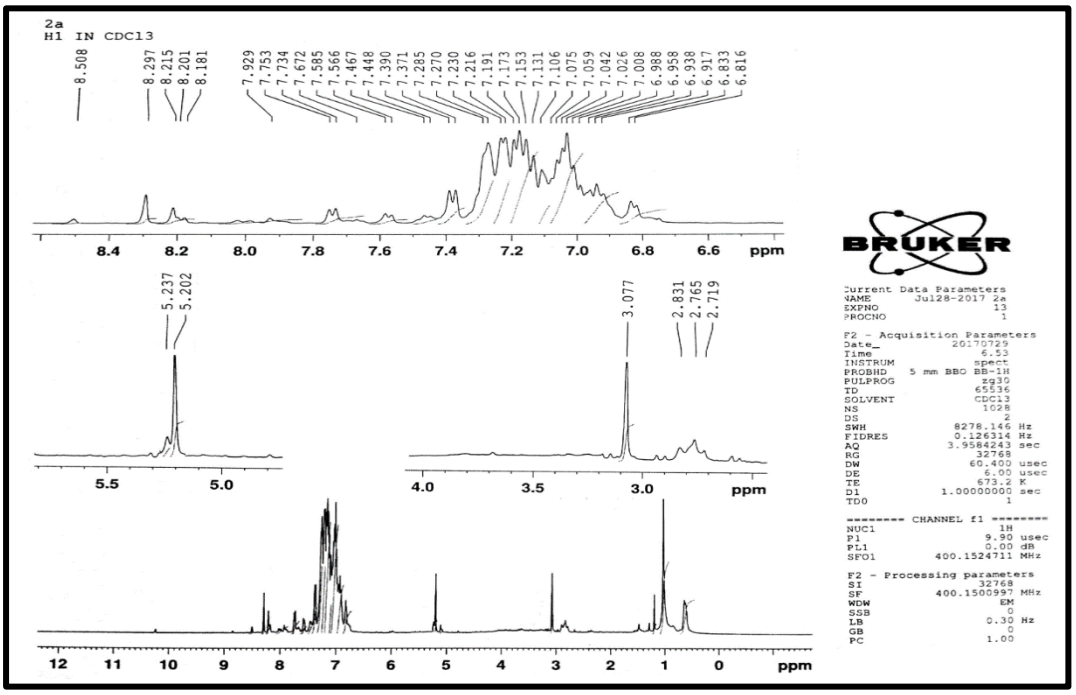
[Comments]

Sample name	2a
Comment	FTIR SPECTRUM
User	
Division	
Company	SRIPMS

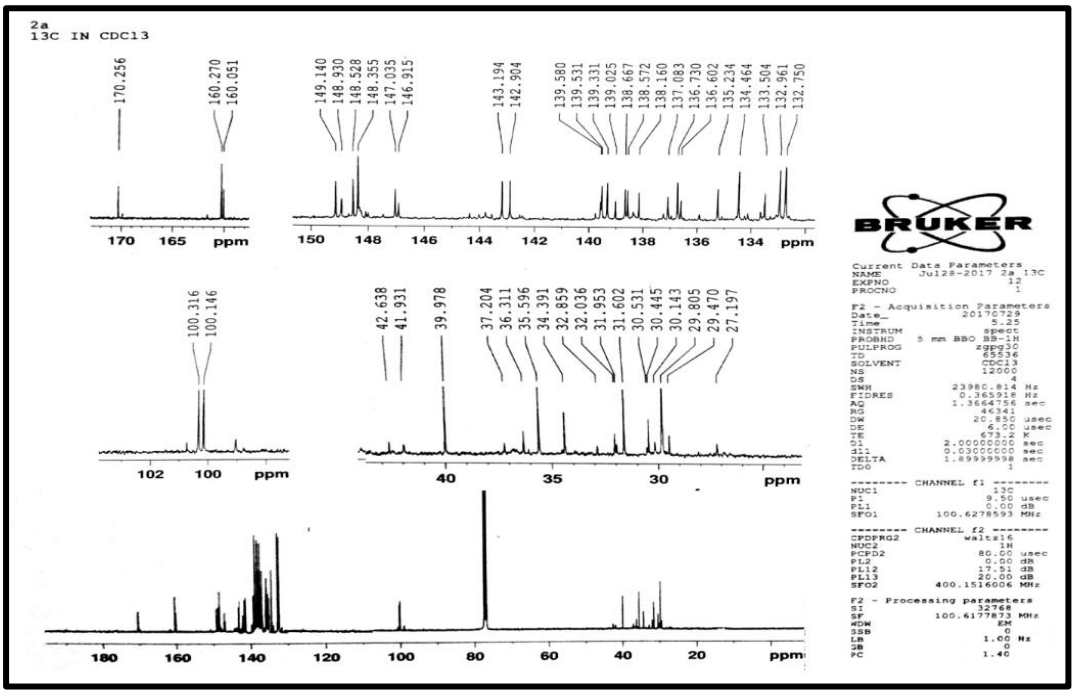
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3954.32	86.135	2	3919.61	84.4719
3	3896.47	83.34	4	3881.04	83.0318
5	3859.83	85.0161	6	3849.22	82.4302
7	3816.44	77.5585	8	3769.19	72.2973
9	3758.58	75.8263	10	3743.15	72.7016
11	3733.51	72.24	12	3722.91	68.7588
13	3635.16	43.8108	14	3542.59	22.8065
15	3487.63	18.6691	16	3427.85	16.6015
17	2924.52	33.356	18	1683.55	58.0687
19	1596.77	34.464	20	1499.38	43.207
21	1466.6	48.6753	22	1438.64	51.0662
23	1384.64	38.9483	24	1271.82	75.584
25	1123.33	68.321	26	1072.23	78.6297
27	1050.05	69.1818	28	1022.09	67.8071
29	821.527	81.6774	30	755.959	38.5301
31	693.284	44.1356	32	588.182	56.6874

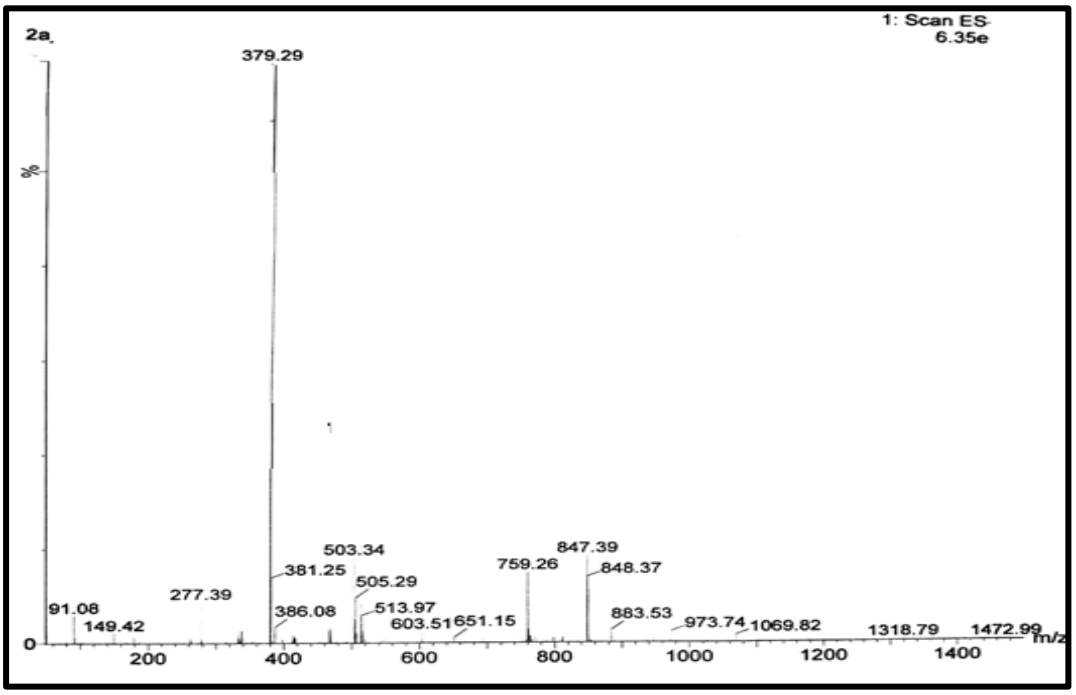
^{1}H NMR



^{13}C NMR



Mass Spectrum


2a
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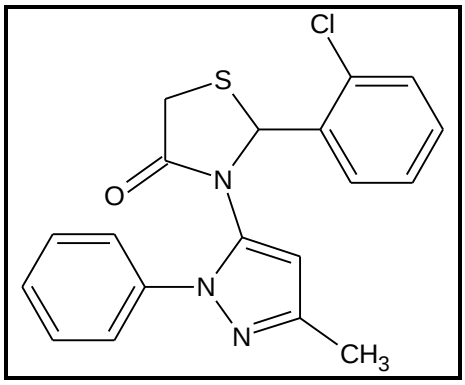
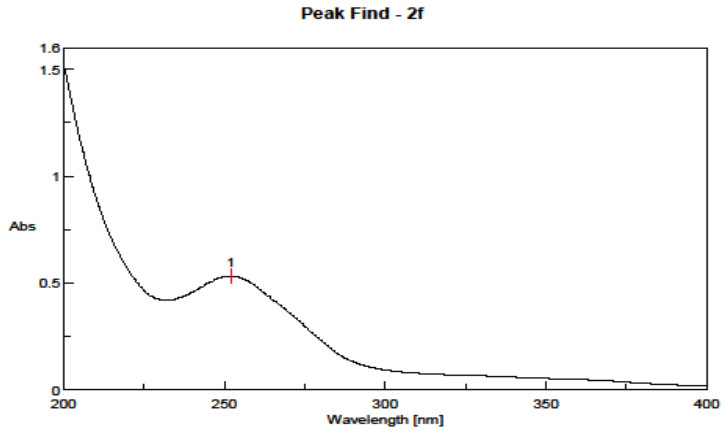
6.35e
379.29
%
503.34
847.39
759.26
381.25
848.37
505.29
277.39
91.08
513.97
883.53
386.08
973.74
1069.82
149.42
603.51
651.15
1318.79
1472.99
m/z
0
200
400
600
800
1000
1200
1400

Compound code : 2f



Chemical name	:	2-(2-chlorophenyl)-3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-1,3-thiazolidin-4-one
UV Spectrum		
Solvent used	:	Acetonitrile
λ_{max}	:	252.0 nm
IR (KBr, νmax in cm-1)	:	2921.83 (Aromatic C-H), 1683.55 (C=N), 1596.77(C=O), 1498.42 (N=N), 1383.68 (C-N), 1125.26 (N-N), 758.85(C-Cl), 617.10 (C-S,Thiazolidinone).
^{1}HMR spectral data	:	2.25 (s, 3H, CH_3), 3.31 (s, 2H, Thiazolidinone), 5.64 (s, 1H, CH-S), 7.14-7.53 (m, 9H, Ar-H), 8.51 (s, 1H, Pyrazole)
^{13}C NMRSPECTRA	:	35.10 (C-Cl), 35.52(CH_3), 39.97 (C-S), 41.98 (C_5 of Thiazolidinone), 100.28 (C=C Pyrazole), 132.98-137.080 (Aromatic carbons), 149.065 (C_3, Pyrazole), 161.78 (C=O).
Mass spectral data	:	370.47 M+1 ion peak

UV Spectrum



[Comments]
Sample name 2f
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

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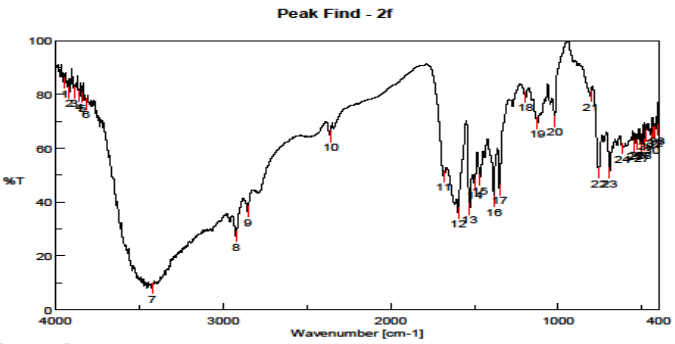
IR specturm

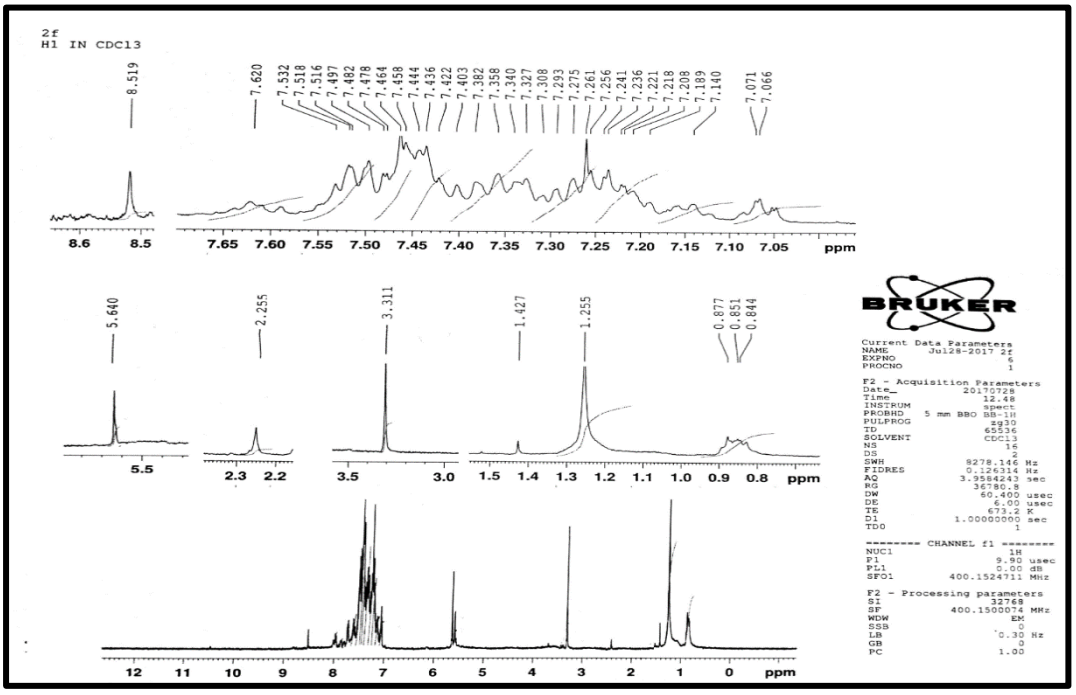


[Comments]
Sample name 2f
Comment FTIR SPECTRUM
User
Division
Company SRIPMS

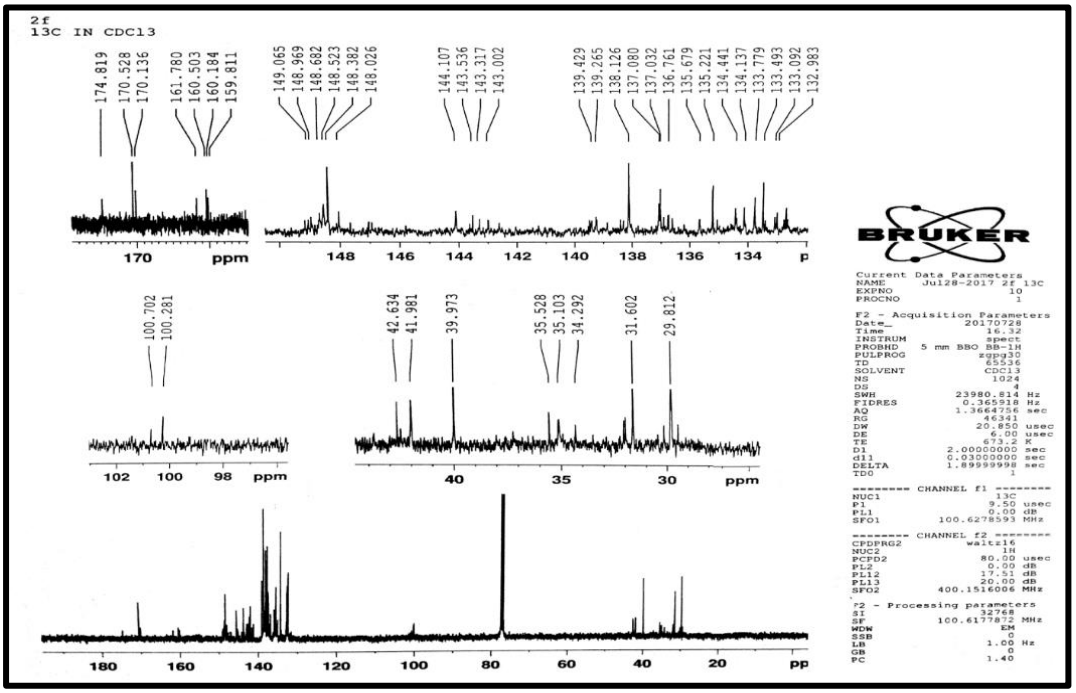
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3942.75	84.208	2	3917.68	80.7014
3	3887.79	80.7117	4	3854.04	79.2362
5	3839.58	78.9691	6	3816.44	76.5338
7	3423.03	8.03005	8	2921.63	27.2075
9	2852.2	36.3529	10	2358.52	64.3588
11	1683.55	49.5579	12	1596.77	35.8424
13	1529.27	37.306	14	1498.42	46.5215
15	1467.56	48.2256	16	1383.68	40.1591
17	1349.93	44.5148	18	1193.72	79.0987
19	1125.26	89.3139	20	1021.12	89.9836
21	806.099	79.4137	22	758.852	50.91
23	694.248	50.8193	24	617.109	60.008
25	545.756	60.9756	26	528.4	61.6064
27	501.401	60.3792	28	489.831	61.3268

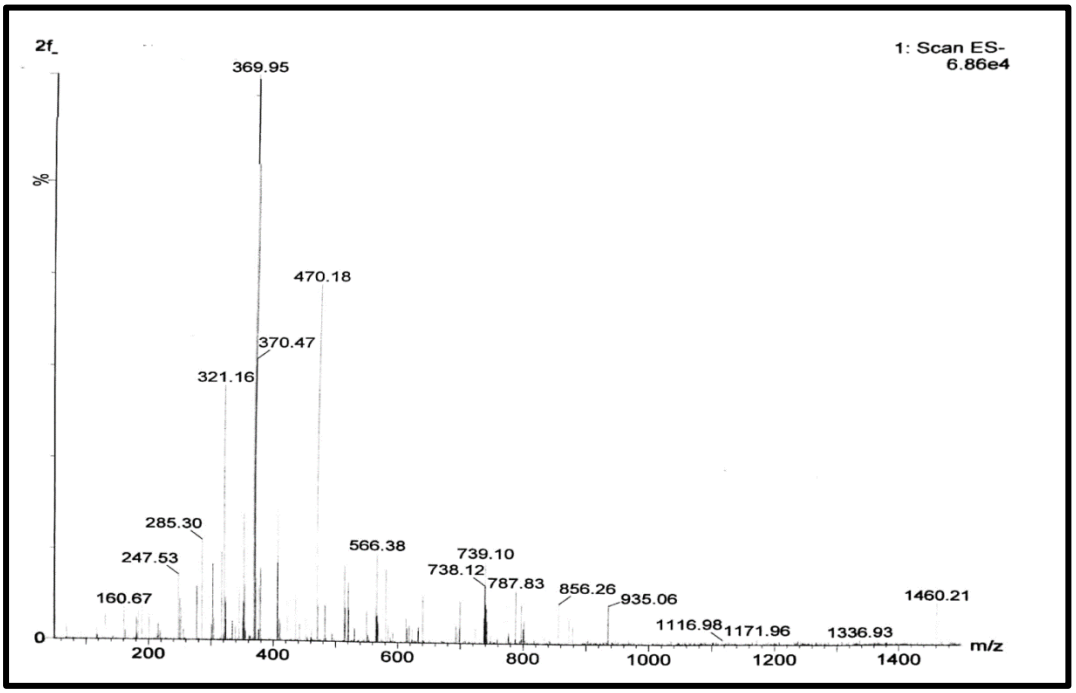
^{1}H NMR



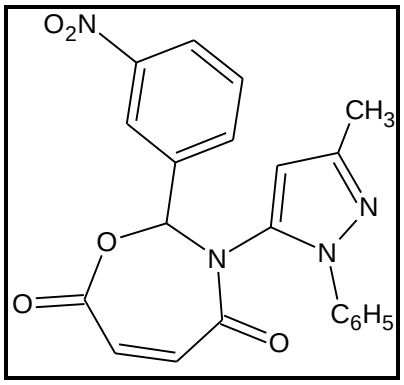
^{13}C NMR



Mass spectrum

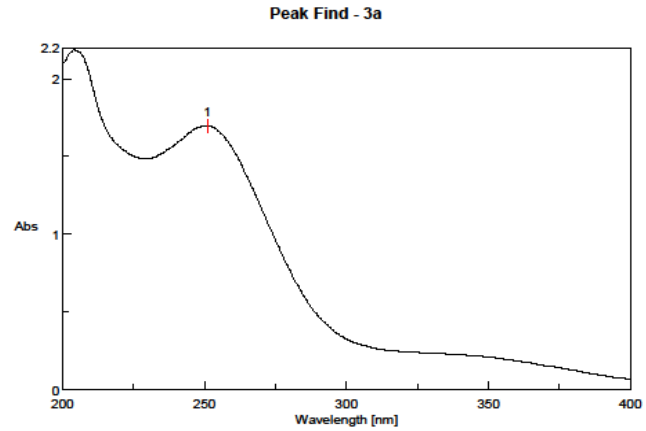


Compound code : 3a



Chemical name	:	3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2-(3-nitrophenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
UV Spectrum		
Solvent used	:	Acetonitrile
λ_{max}	:	251.0 nm
IR (KBr, νmax in cm-1)	:	3087.48(Aromatic,C-H), 1725.55 (C=O, Lactone),1616.06(Amide,C=O), 1596.77(C=N), 1502.28(C=C), 1094,4 (N-N), 732.81 (Aromatic C-H, bending)
^{1}HMR spectral data	:	3.5 (s, 3H, CH_3),6.36-6.95 (d, 2H, Oxazepine), 7.06-8.52 (m, 9H, Ar-H), 8.68 (s, 1H, Pyrazole), 10.14 (s, 1H, Oxazepine)
^{13}C NMRSPECTRA	:	39.29 (CH_3), 60.52 (C_7 of oxazepine), 128.58-135.450 (Aromatic carbons), 147.95 (C_3, Pyrazole), 166.88 & 163.40 (C_8 & C_{11} of oxazepine)
Mass spectral data	:	405.21 M+1 ion peak

UV Spectrum



[Comments]
Sample name 3a
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
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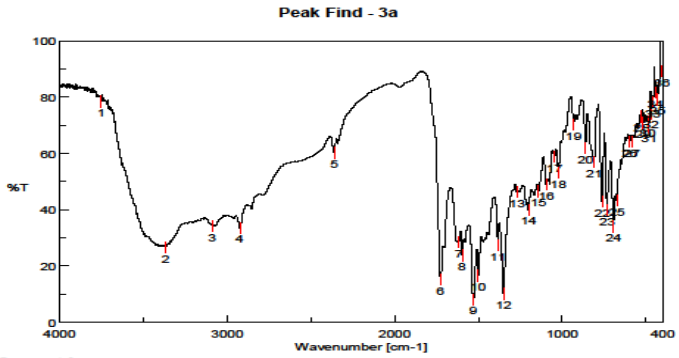
IR Spectrum

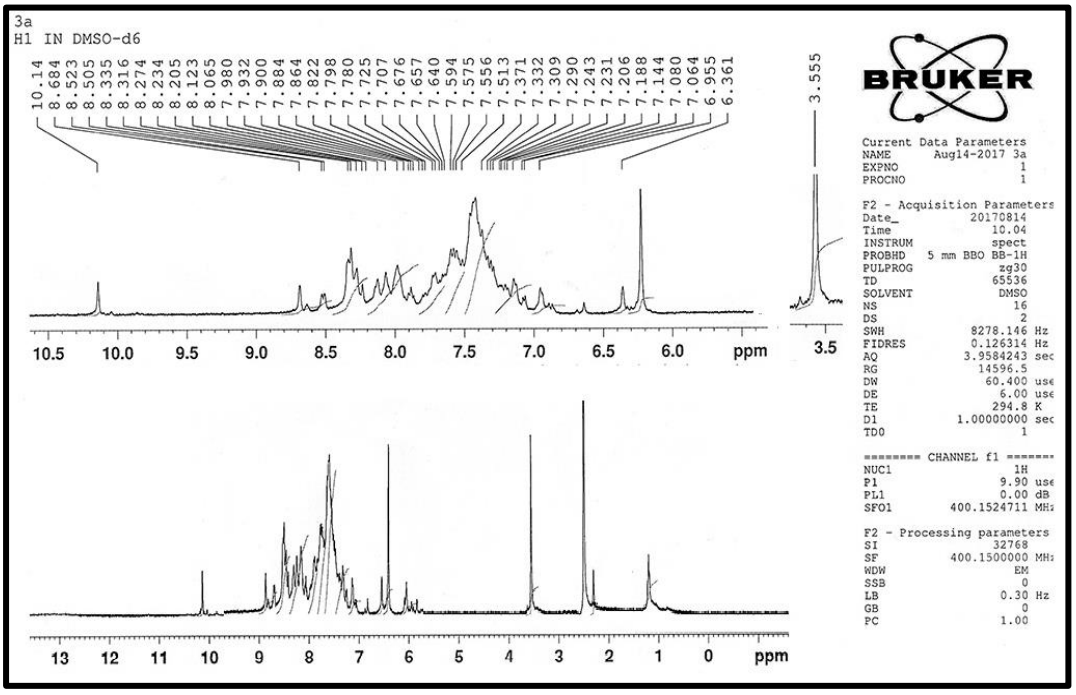


[Comments]
Sample name 3a
Comment FTIR SPECTRUM
User
Division
Company SRIPMS

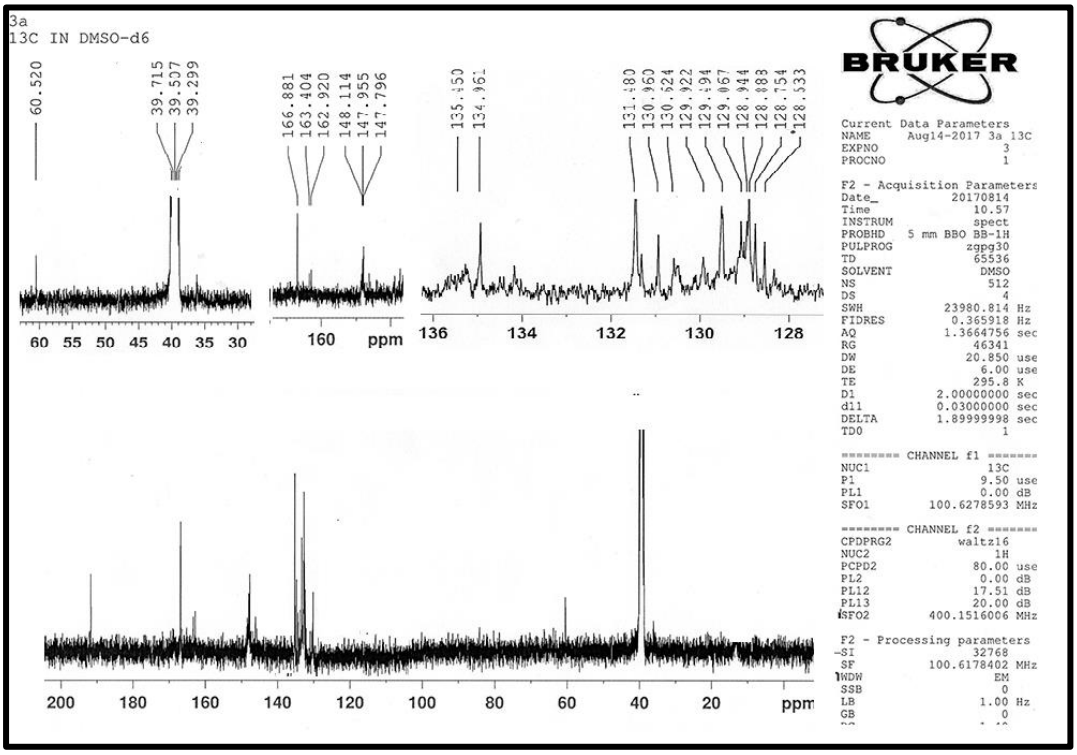
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3749.9	78.4093	2	3365.17	26.5185
3	3087.48	34.0937	4	2923.56	33.4626
5	2359.48	60.2046	6	1725.98	15.1003
7	1616.06	28.4599	8	1596.77	23.7201
9	1530.24	8.07276	10	1502.28	16.4678
11	1383.68	27.3058	12	1350.89	10.0687
13	1269.9	46.285	14	1201.43	39.9532
15	1141.65	46.5468	16	1094.4	48.9088
17	1047.16	58.8725	18	1023.05	53.0996
19	933.378	70.1037	20	862.025	61.7898
21	810.92	56.9406	22	762.709	42.9224
23	732.817	39.862	24	695.212	33.9432

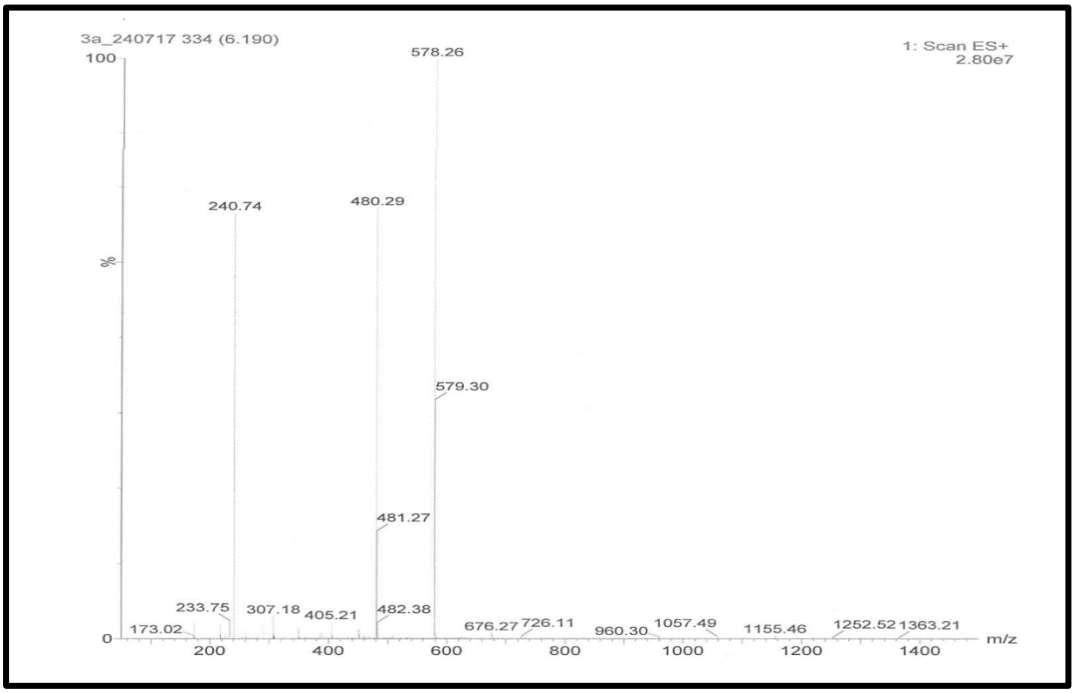
^{1}H NMR



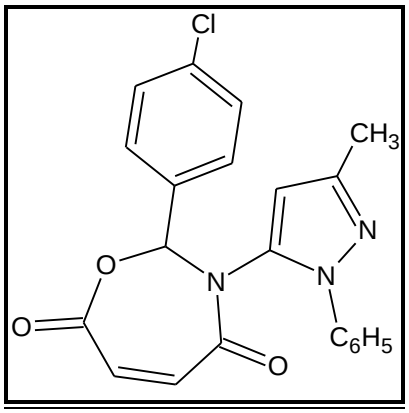
^{13}C NMR



Mass Spectrum

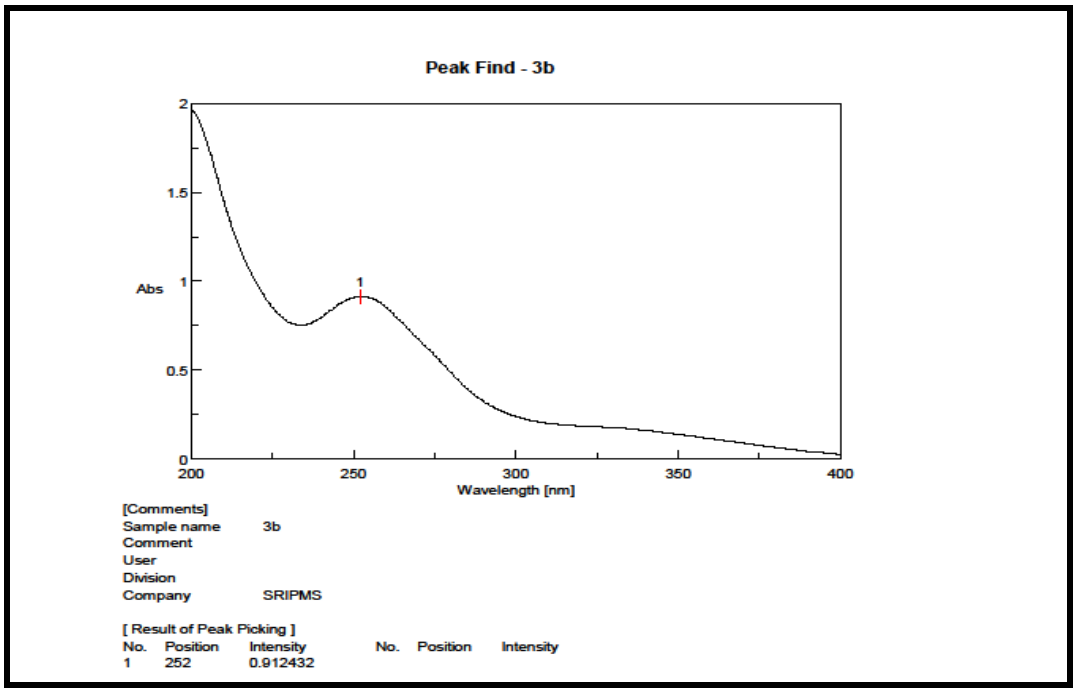


Compound code: 3b



Chemical name	:	2-(4-chlorophenyl)-3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2,3-dihydro-1,3-oxazepine-4,7-dione
UV Spectrum		
Solvent used	:	Acetonitrile
λ_{max}	:	252.0 nm
IR (KBr, νmax in cm-1)	:	2922.59(Aromatic, C-H), 1725.98(C=O, Lactone), 1625.7(C=O, amide, Oxazepine ring), 1595.81 (C=N), 1089.58 (N-N), 695.212 (C-Cl).
^{1}HMR spectral data	:	3.56 (s, 3H, CH_3), 6.23-6.36 (d, 2H, Oxazepine), 6.96-8.33 (m, 9H, Ar-H), 8.35 (s, 1H, Pyrazole), 10.34 (s, 1H, Oxazepine)
^{13}C NMRSPECTRA	:	**39.31 (CH_3), 3 (C-Cl), 66.41 (C_7 of oxazepine), 128.58-135.450 (Aromatic carbons), 147.08 (C_3, Pyrazole), 166.89 & 163.12 (C_8 & C_{11} of oxazepine).**
Mass spectral data	:	394.11 M+1 ion peak

UV Spectrum



[Comments]
Sample name 3b
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

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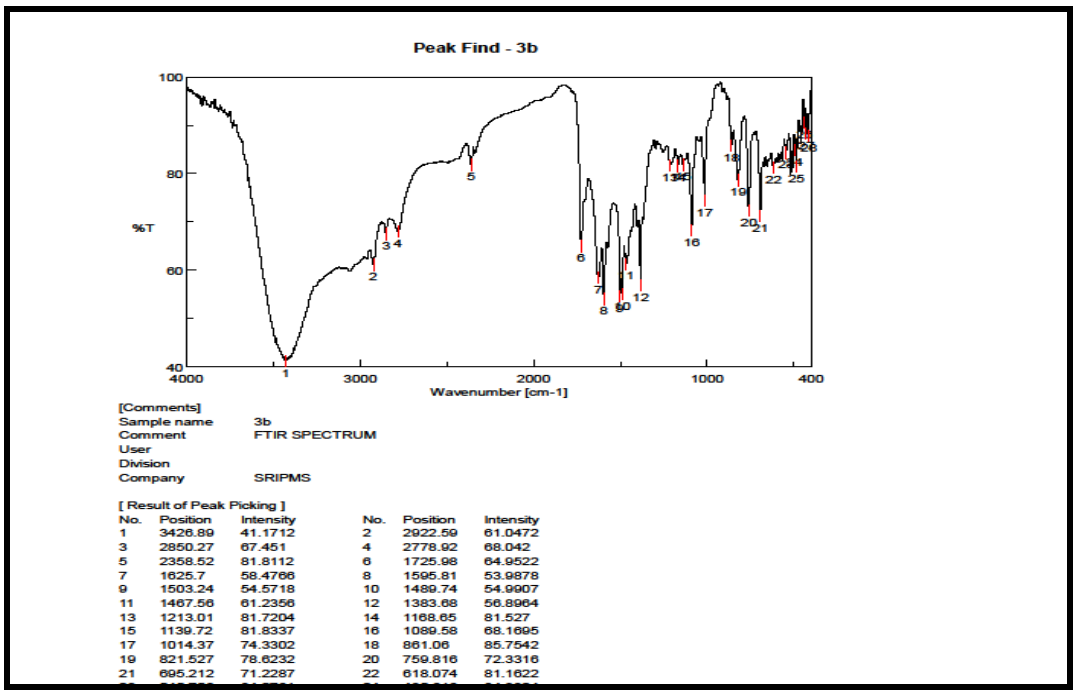
IR Spectrum

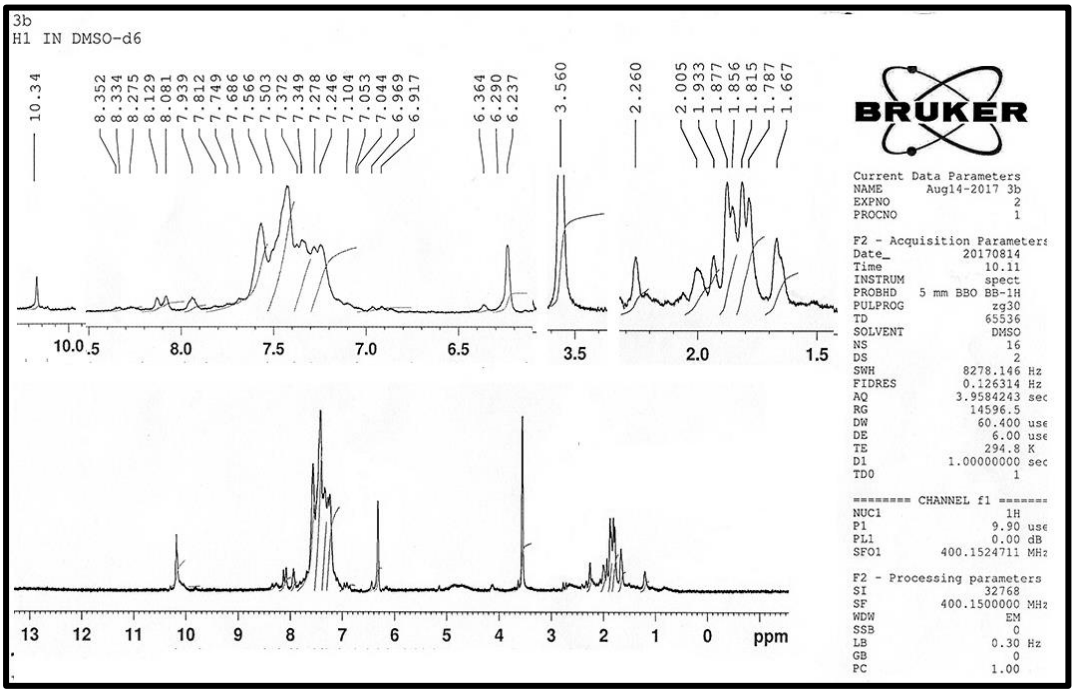


[Comments]
Sample name 3b
Comment FTIR SPECTRUM
User
Division
Company SRIPMS

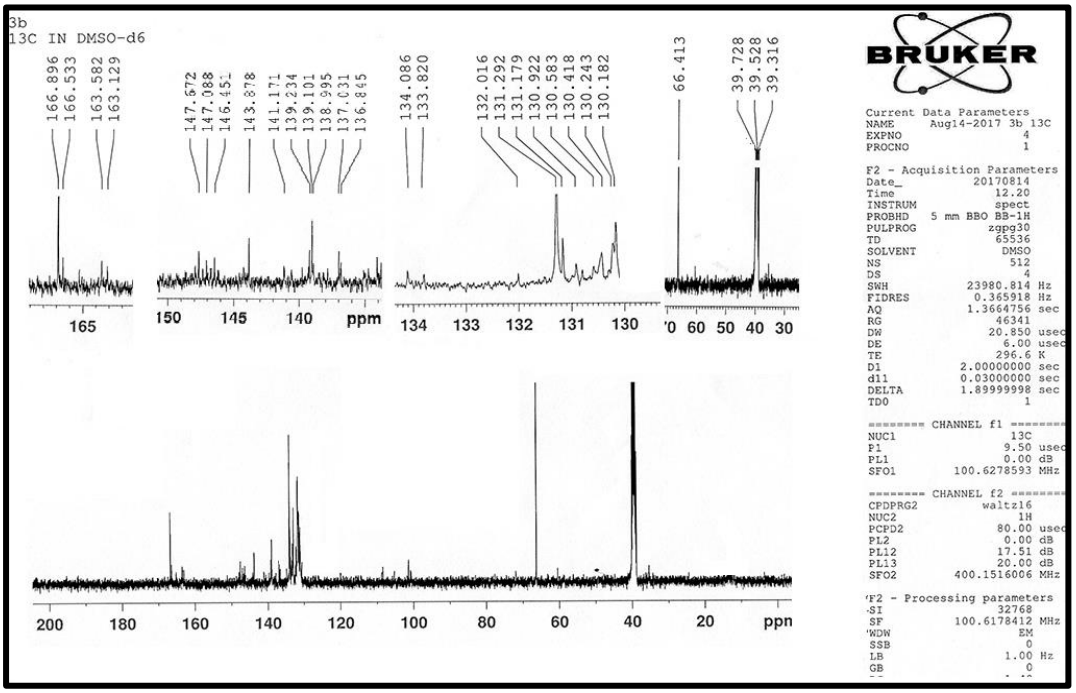
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3426.89	41.1712	2	2922.59	61.0472
3	2850.27	67.451	4	2778.92	68.042
5	2358.52	81.8112	6	1725.98	64.9522
7	1625.7	58.4766	8	1595.81	53.9878
9	1503.24	54.5718	10	1489.74	54.9907
11	1467.56	61.2356	12	1383.68	56.8964
13	1213.01	81.7204	14	1168.65	81.527
15	1139.72	81.8337	16	1089.58	68.1695
17	1014.37	74.3302	18	861.06	85.7542
19	821.527	78.6232	20	759.816	72.3316
21	695.212	71.2287	22	618.074	81.1622

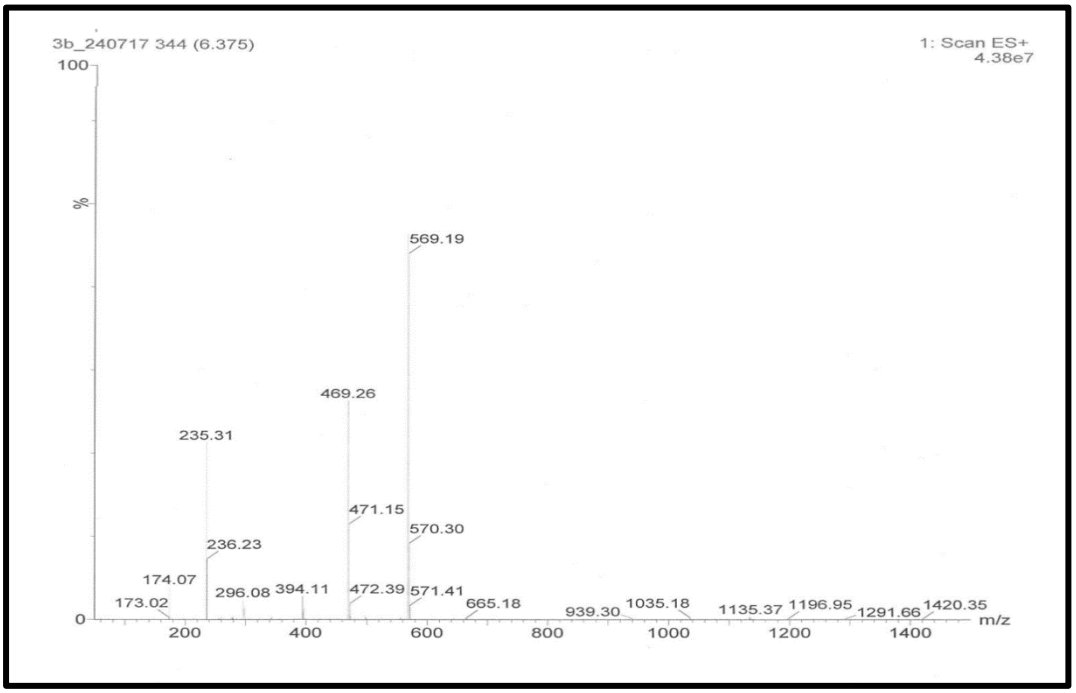
^{1}H NMR



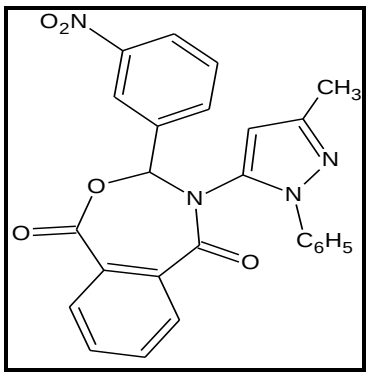
^{13}C NMR



Mass spectrum

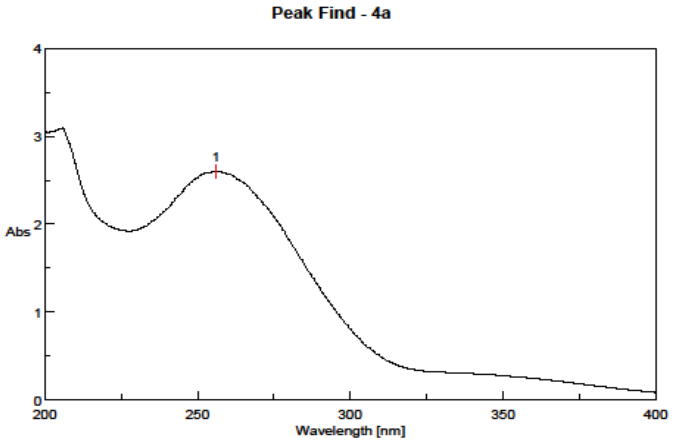


Compound code : 4a



Chemical name	:	3-(4-chlorophenyl)-4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
UV Spectrum		
Solvent used	:	Acetonitrile
λ_{max}	:	256.0 nm
IR (KBr, νmax in cm-1)	:	3068.19 (Aromatic C-H, stretching), 1710.55 (Lactone, C=O), 1614.13 (Amide, C=O), 1596.77(C=N), 1578.45(Aromatic, C=C, Bending), 1500.35 (C=C), 1350.89 (Aromatic Nitro grp), 1093.44 (N-N).
^{1}HMR spectral data	:	2.50 (s, 3H, CH_3), 7.2-8.07 (m, 13H, Ar-H), 8.32 (s, 1H, Pyrazole), 13.26 (s, 1H, Oxazepine)
^{13}C NMRSPECTRA	:	35.55 (CH_3), 40.15(C-N), 68.37(C_7 of oxazepine), 100.186 (C=C, Pyrazole), 132.94-147.79 (Aromatic carbons), 148.35 (C_3, Pyrazole), 168.72 & 163.42 (C_8 & C_{11} of oxazepine).

UV spectrum



[Comments]
Sample name 4a
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
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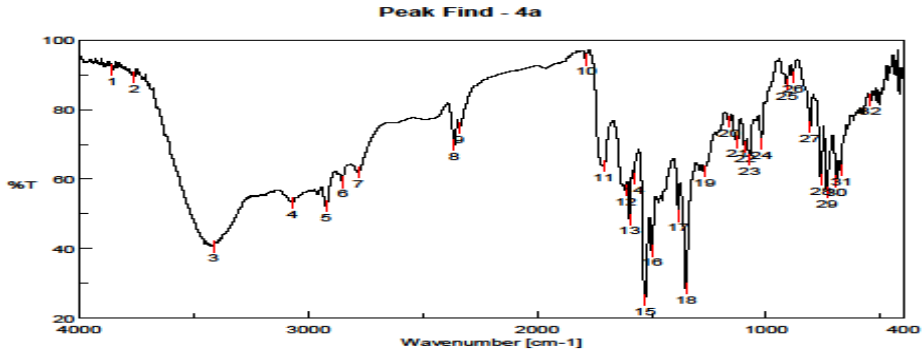
IR Spectrum

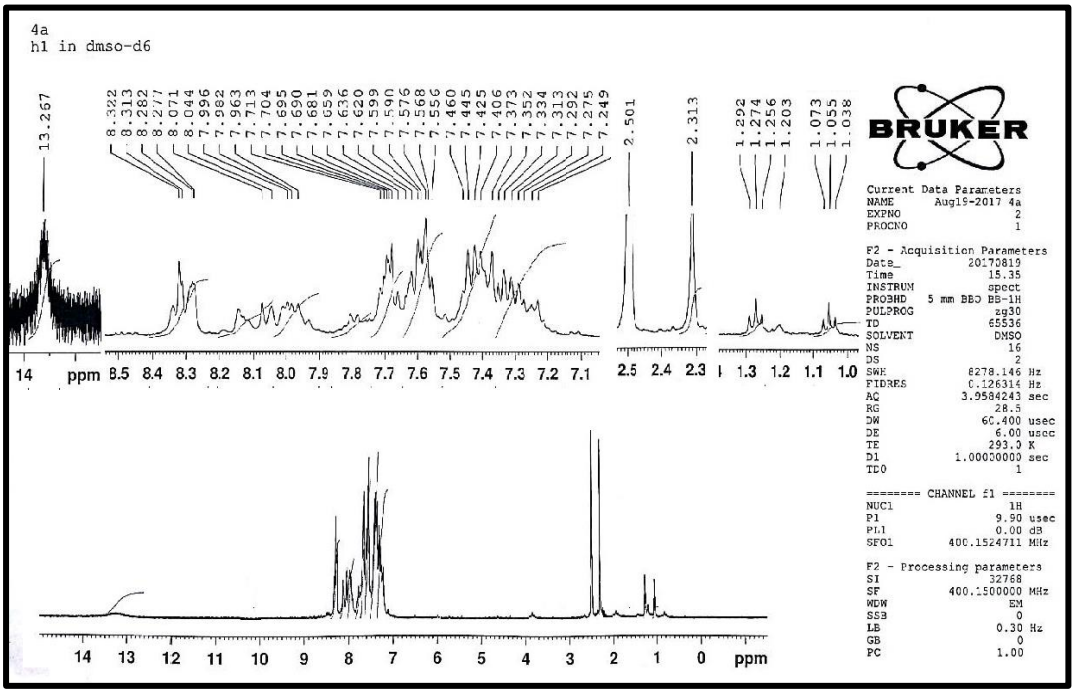


[Comments]
Sample name 4a
Comment FTIR SPECTRUM
User
Division
Company SRIPMS

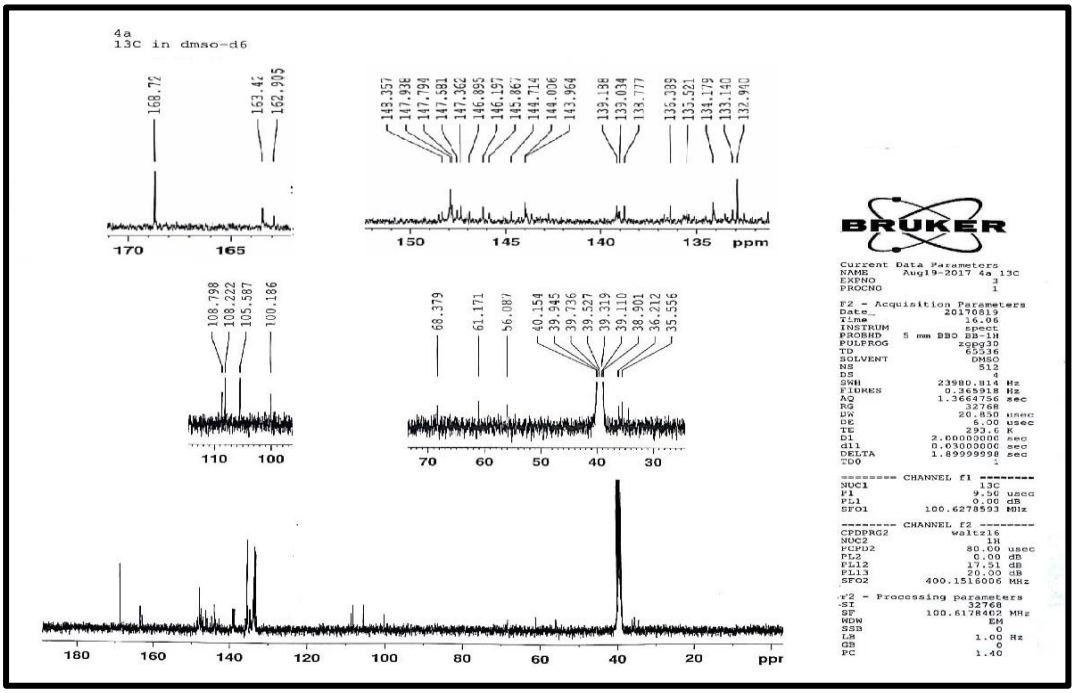
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3854.04	91.4123	2	3760.51	89.2559
3	3414.35	40.5705	4	3068.19	53.144
5	2920.66	52.0971	6	2850.27	58.9884
7	2781.81	62.0001	8	2361.41	69.7811
9	2342.12	74.7376	10	1787.69	94.2385
11	1710.55	63.6272	12	1614.13	56.6719
13	1596.77	48.3174	14	1578.45	60.0842
15	1529.27	25.2333	16	1500.35	39.2885
17	1383.68	49.2901	18	1350.89	28.4085
19	1270.86	62.2644	20	1160.94	76.4939
21	1127.19	70.546	22	1093.44	69.2882
23	1074.16	65.4663	24	1021.12	70.1314
25	910.236	87.1785	26	883.238	89.255
27	808.028	74.9689	28	760.78	59.774

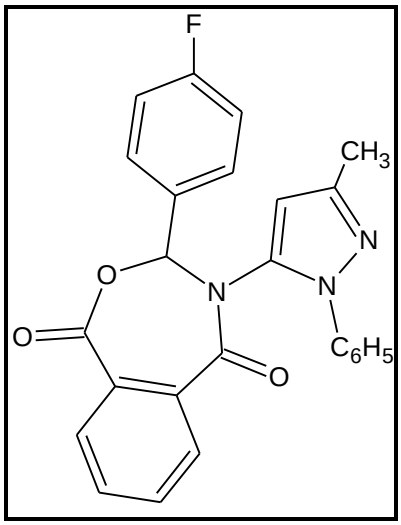
^{1}H NMR



^{13}C NMR

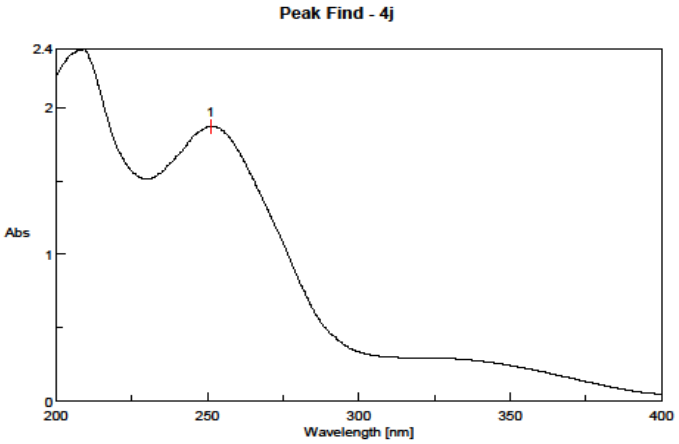


Compound code : 4j



Chemical name	:	3-(4-fluorophenyl)-4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
UV Spectrum		
Solvent used	:	Acetonitrile
λ_{max}	:	251.0 nm
IR (KBr, νmax in cm-1)	:	3060.48(Aromatic C-H), 1719.23 (C=O, Lactone), 1598.7 (C=O, Amide), 1505.17(Aromatic, C=C), 1228.43 (C-F), 1024.98 (N-N).
^{1}HMR spectral data	:	2.50 (s, 3H, CH_3), 7.10-7.79 (m, 13H, Ar-H), 8.15 (s, 1H, Pyrazole), 13.47(s, 1H, Oxazepine)
^{13}C NMR SPECTRA	:	34.32 (C-F), 39.31 (CH_3), 61.17(C_7 of oxazepine), 101.24 (C=C Pyrazole), 130.04-139.335 (Aromatic carbons), 147.91 (C_3, Pyrazole), 168.69 & 163.17 (C_8 & C_{11} of oxazepine).
Mass spectral data	:	425.32 M-2 ion peak

UV Spectrum

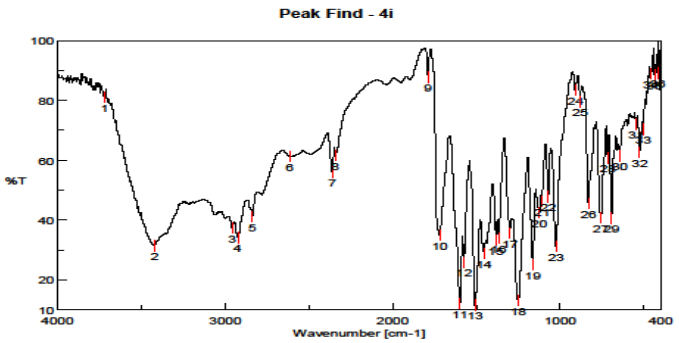


[Comments]
Sample name 4j
Comment
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	251	1.86844			

IR Spectrum

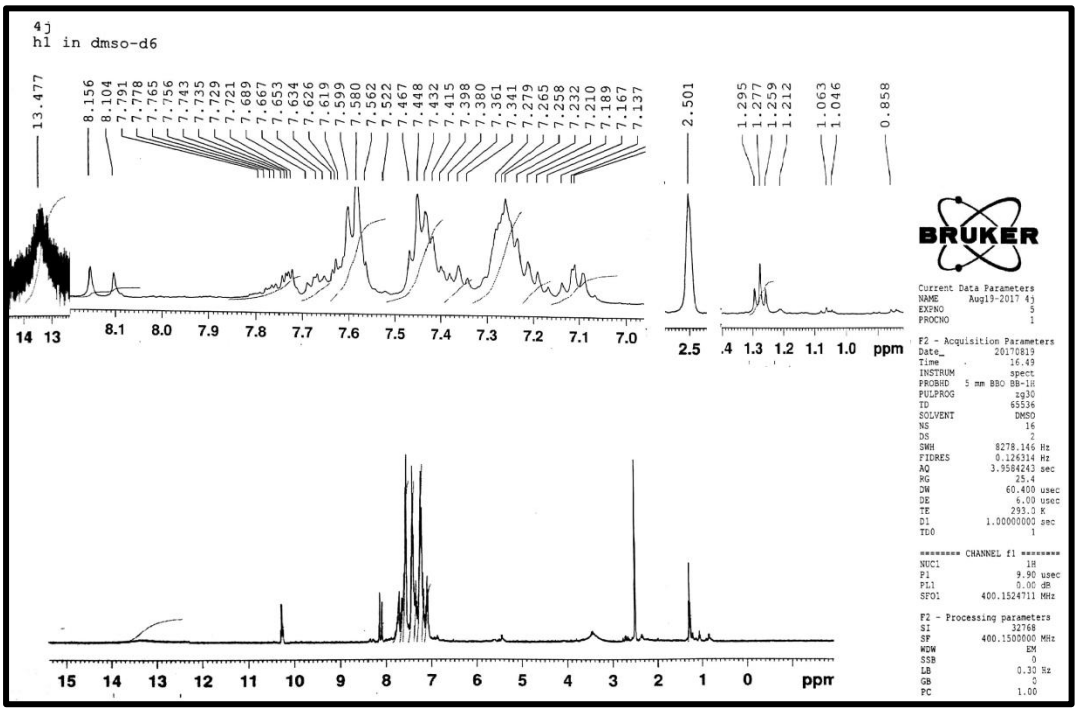


[Comments]
Sample name 4i
Comment FTIR SPECTRUM
User
Division
Company SRIPMS

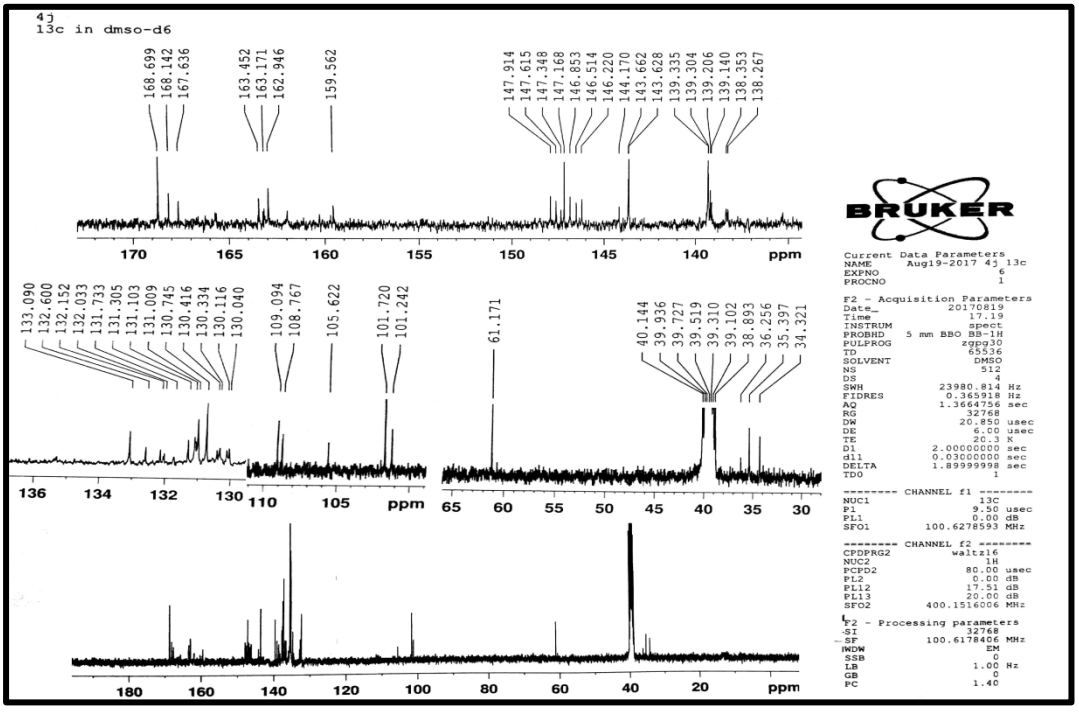
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3717.12	81.1096	2	3421.1	31.3043
3	2956.34	37.2827	4	2922.59	34.128
5	2836.77	41.1567	6	2614.04	61.1824
7	2359.48	55.9197	8	2341.16	61.6034
9	1788.65	87.7028	10	1720.19	34.9261
11	1599.66	11.9436	12	1573.63	25.8334
13	1509.03	11.5101	14	1455.03	28.8307
15	1383.68	33.2644	16	1366.32	33.9581
17	1300.75	35.5476	18	1250.61	12.9752
19	1163.83	24.9622	20	1126.22	42.3991
21	1108.87	46.2012	22	1072.23	47.6961
23	1024.98	31.1283	24	910.236	83.522
25	882.274	79.4809	26	831.169	45.5381
27	757.888	40.6815	28	716.425	60.7543

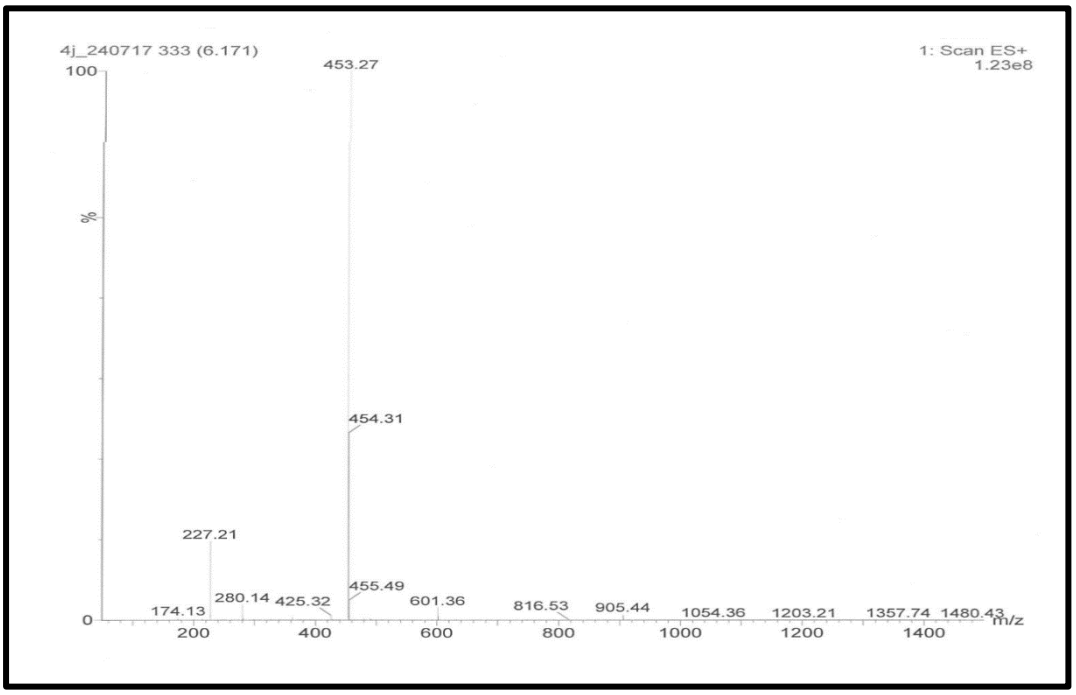
^{1}H NMR



^{13}C NMR



Mass spectrum


4j_240717 333 (6.171)
1: Scan ES+
1.23e8
100
%
0
453.27
454.31
227.21
455.49
280.14
425.32
174.13
601.36
816.53
905.44
1054.36
1203.21
1357.74
1480.43
200
400
600
800
1000
1200
1400
m/z

5.3 PHASE III – BIOLOGICAL SCREENING

5.3.1 *In Vitro* Antimalarial Screening[97-102]

All the synthesized compounds were screened for antimalarial activity in the Microcare laboratory & TRC, Surat, Gujarat

The *in vitro* antimalarial assay was carried out in 96 well microtitre plates according to the microassay protocol of Riekmann and co-workers with minor modification. The culture of *P.falciparum* strain were maintained in medium RPMI 1640 supplemented with 25mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P.falcipuram* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200µl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent of parasitaemia (rings) and uniformly maintained with 50% RBCs (O^+). A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20µl volume were added to the test wells so as to obtain final concentration (at fivefold dilutions) ranging between 0.4µg/ml to 100µg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37°C in a candle jar. After 36 to 40 hours incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observer to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentrations which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentration (MIC). Chloroquine was used as the reference drug.

RESULTS AND DISCUSSION

The *in-vitro* antimalarial assay results showed that the representative compounds exhibited antimalarial activity and the results are shown in **Table 7.**

Table 7: *In-vitro* antimalarial assay

Minimum Inhibitory Concentration (MIC)		
S.No	**Compound Name**	**IC_{50} Mean Values[a]**
01	2g	0.95µg/ml
02	3b	0.32µg/ml
03	3h	1.41µg/ml
04	4c	0.28µg/ml
05	4j	1.07µg/ml
06	Chloroquine	0.020µg/ml
07	Quinine	0.268µg/ml

a: means values in representative assay

In the **pyrazolyl thiazolidinone series**, **2g** (ethoxy) shows minimum inhibitory concentration at **0.95**µg/ml. Among the **pyrazolyl oxazepine series, 3b** (chloro) had shown promising activity with the MIC of **0.32**µg/ml and **3h** (dihydroxy) showed MIC of **1.41**µg/ml. Among the **pyrazolyl benzoxazepine series**, **4c** (methoxy) had shown promising activity with the MIC of **0.28**µg/ml and **4j** (fluoro) showed MIC of **1.07**µg/ml compared to standard chloroquine and quinine with the MIC of 0.02 and 0.26µg/ml. So, in the antimalarial screening for representative compounds, the chloro, methoxy and fluoro substituted derivatives of the three series showed significant activity.

5.3.2 Antibacterial Screening[103,104]

Apparatus and chemicals required

Sterile swab	:	Hi-Media
Non-adsorbent cotton	:	Rama Raju Surgical Cotton Ltd.
Conical flask	:	Borosil
Test tubes	:	Borosil
Petri plates	:	SD Fine-Chem Ltd
Micropipettes	:	VARI Pipettes (Hi-Tab Lab)
Autoclave	:	Universal Autoclave
Laminar Air Flow unit	:	CLEAN Air Instrument Inc.
Micro tips	:	Tarsons
Agar powder	:	Hi Media

The antibacterial screening was carried out in the Pharmaceutical Biotechnology Laboratory, College of Pharmacy, SRIPMS, Coimbatore.

Media used for antibacterial screening

Muller Hinton Agar	:	Hi-Media Laboratories Pvt. Ltd

Ingredients

Casein enzymatic hydrosylate	:	17.5 gm/L
Beef infusion	:	300gm/L
Soluble starch	:	1.5gm/L
Final pH at 25°C	:	7.4 ±0.2

Screening For antibacterial activity

All the newly synthesized compounds were screened for antibacterial activity by agar well diffusion technique.

Preparation

The ingredients were dissolved in distilled water with the aid of heat and pH was adjusted to 7.2-7.6 using dilute alkali or acid. Sterilization 15 ml lots of Mueller Hinton agar were transferred to test tubes and the tubes were plugged with non-absorbent cotton. The test tubes were then autoclaved at a pressure of 15 psi (120^oC) for not less than 15 minutes.

Culture used

Escherichia coli NCIM 2911 was procured from National Collection of Industrial microorganisms, Pune, Maharashtra and stored in the Pharmaceutical Biotechnology Laboratory, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore – 641 044.

Maintenance of culture

The selected strains were confirmed for their purity and identity by Grams staining method and by their characteristic biochemical reactions. The selected strains were preserved by sub culturing them periodically on Nutrient agar slants and storing them under frozen condition. For the study, fresh 24 hrs broth cultures were used after standardization of the culture.

Standardization of inoculum

All the organisms were grown overnight (24 hours) at 37^oC on Nutrient agar and harvested during the stationary growth phase. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes containing Mueller-Hinton broth, incubated for 24 hrs at 37^oC. Inoculum was standardized by matching the turbidity of the culture to 0.5 McFarland standards. The standard was produced by mixing 0.5 ml of 0.048 M $BaCl_2$ (1.175% w/v Barium Chloride dehydrate) with 99.5 ml of 0.36 N H_2SO_4. If the turbidity of the culture matches that of the McFarland standard, then the culture inoculating suspension has approximately 2.0 x 106 CFU/ml of bacteria.

Preparation of inoculums

The inoculum for the experiment was prepared fresh in Mueller Hinton Broth from preserved frozen slant culture. It was kept incubated at 37°C for 24 hours.

Drugs used	:	Newly Synthesized drugs (250µg/ml)
Standard drugs	:	ofloxacin (5µg/ml)
Solvent	:	Dimethyl sulfoxide

Agar well diffusion assay

Mueller Hinton agar plates were prepared aseptically to get a thickness of 5-6mm. The plates were allowed to solidify and inverted to prevent condensate falling on the agar surface. The plates were dried at 37°C before inoculation. The organisms were inoculated as per the following method in the plates prepared earlier. The sterile swab was dipped in the previously standardized inoculums and excess of inoculums was removed by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid. The swab was then streaked all over the surface of the medium three times, rotating the plates through an angle of 60^{o} after each application. Finally, the swab was pressed round the edge of the agar surface. The inoculated medium was allowed to dry at room temperature, with the lid closed. Cork borer was sterilized by using flame and well was made by using cork borer. By using micropipette, the test sample and standard were added into the well and were refrigerated for one hour to facilitate uniform diffusion of the drug. This was then incubated for 18-24 hrs at 37°C. The diameter of the zones of inhibition around the drugs were measured and compared with that of the standard. All the synthesized compounds were tested for antibacterial activity against *Escherichia coli* bacteria.

Table 8: Antibacterial data for 2a-j

Sl no.	Compound code	Diameter of zone of inhibition in mm
1	2a	11
2	2b	12
3	2c	13
4	2d	12
5	2f	13
6	2e	-
7	2g	-
8	2h	-
9	2i	-
10	2j	-
11	3a	13
12	3b	12
13	3c	12
14	3d	11
15	3e	10
16	3f	13
17	3g	13
18	3h	12
19	3i	15
20	3j	13
21	4a	14
22	4b	13
23	4c	16
24	4d	14
25	4e	13
26	4f	12
27	4g	12
28	4h	10
29	4i	12
30	4j	12
31	Standard	32

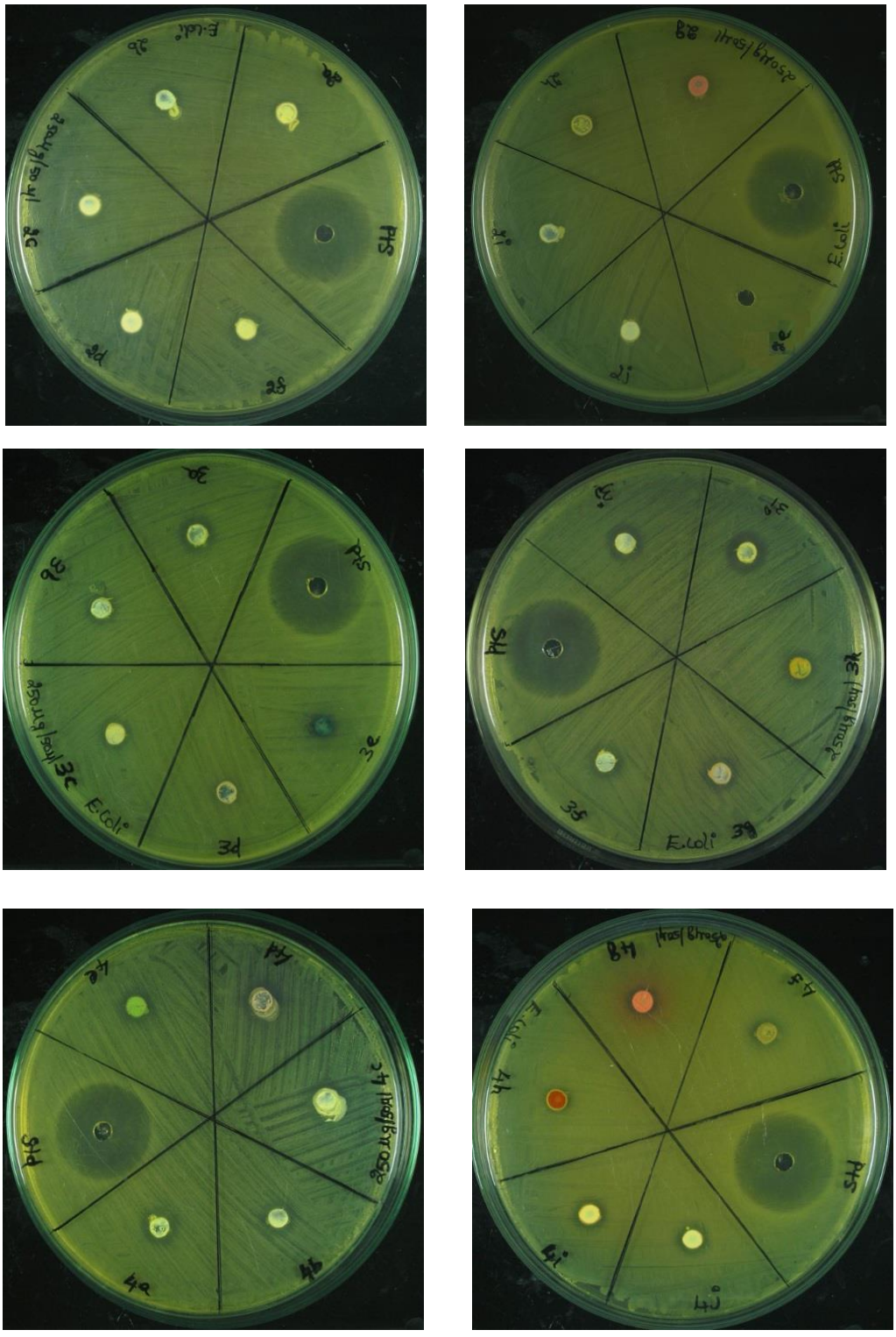


Figure 8: Diameter of zone of inhibition

RESULTS AND DISCUSSION

All the newly synthesized compounds were tested for their antibacterial activity against *Escherichia coli*. Almost all the newly synthesized analogs were active against the tested microorganism at 250 µg/disc concentration by Kirby Bauer agar well diffusion method. It was observed that in the pyrazolyl thiazolidinone series derivatives, **2c** (methoxy substitution) and **2f** (chloro substitution) showed moderate sensitivity with a zone of 13 mm. Activity profile were compared to the standard ofloxacin (32mm). In the pyrazolyl oxazepine series compounds like **3a** (nitro substituted), **3f** (chloro substituted), **3i** (methoxy substituted) and **3j** (fluoro substituted) showed moderate sensitivity (13, 13, 15 aand 13 mm) against *Escherichia coli* at 250 µg/disc concentration. Also in the pyrazolyl benzoxazepine, compound like **4a** (nitro substituted), **4c** (methoxy substituted) and **4d** (3-methoxy-4-hydroxy substituted) showed moderate sensitivity with the zone of 14, 16 and 14 mm respectively. The data for zone of inhibition and the photographs are given in **Table 8 and Figure10** respectively.

Thus the biological screening reports suggested that the novel pyrazole linked thiazolidinone, oxazepine and benzoxazepine derivatives are promising in their antimalarial and antibacterial activity profile. Among the derivatives nitro, fluoro, chloro and methoxy derivatives were found to be significant.

SUMMARY AND CONCLUSION

SUMMARY

The present work was focused on the design, docking, synthesis and evaluation of antimalarial and antibacterial activity of Pyrazolyl linked thiazolidinone, oxazepine and benzoxazepine series as possible Enoyl ACP reductase inhibitors.

Phase I - *In-silico* studies

- **Selection of the target**

The enzyme involved in the fatty acid elongation of *Plasmodium falciparum* and *Escherichia coli* ie., Enoyl ACP reductase was selected as the drug target of the study. The corresponding enzyme were obtained from the protein data bank and their accession codes were 1VRW (*Pf*ENR) and 1C14 (E.*coli* Fab1).

- **Selection of lead by virtual screening**

Virtual screening was performed by iGEMDOCK v.2. Forty nine hits were obtained from ZINC database, from which pyrazole, thiazolidinone and oxazepine were selected as the lead for inhibiting *Pf*ENR and *E.coli* FabI enzymes.

- **Lead optimization**

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

- **Docking**

The optimized leads were subjected to docking studies using Autodock4.2 and the interactions of the derivatives with active sites of the enzymes were studied. The derivatives were subjected to interactions with *Pf*ENR and *E.coli* FabI. Triclosan was used as the standard ligand.

Most of the derivatives were interacting with the key active sites of the ***Pf*ENR (1VRW.pdb)** with superior dock values or binding energies when compared to the **standard** (triclosan) with the binding energy of **-5.28**kcal/mol. Some of the derivatives though were interacting well with the enzymes, showed inferior binding energies predicting less binding affinity to the enzyme. Among the derivatives of **pyrazolyl thiazolidinone**, **2g, 2c** and **2d** (methoxy, chloro and ethoxy, methoxy and chloro) showed maximum binding energies **-7.26, -6.59** and **-6.43**kcal/mol respectively. Also in **pyrazolyl oxazepine derivatives**, **3b, 3h** and **3a** (chloro, dihydroxy and nitro) showed maximum binding energies of **-9.51, -9.45** and **-9.33**kcal/mol respectively. Among the **pyrazolyl benzoxazepine derivatives**, **4j, 4c, 4d** and **4g** (fluoro, methoxy, chloro and ethoxy) showed maximum binding energies of **-9.19, -8.76, -8.43** and **-7.58**kcal/mol respectively. They were interacting well with key binding sites such as Tyr 267, Tyr 277, Met 281, Gly313, Pro314 and NAD^+.

With the ***E.coli* FabI (1C14.pdb)** most of the derivatives were well interacting with the key active sites with excellent binding energies when compared to the **standard** (triclosan) with the binding energy of **-7.15**kcal/mol. Among the derivatives of **pyrazolyl thiazolidinone derivatives**, **2b, 2h** and **2a** (chloro, dihydroxy, and nitro) showed maximum binding energies, **-5.71, -5.69** and **-4.54** kcal/mol respectively. Also in **pyrazolyl oxazepine derivatives, 3c, 3d** and **3j** (chloro, methoxy and fluoro) showed maximum binding energies of **-7.58, -6.65** and **-6.49**kcal/mol respectively. Among the **pyrazolyl benzoxazepine derivatives 4c, 4d, 4a** and **4b** (methoxy, 4-hydroxy-3-methoxy, nitro and chloro) showed maximum binding energies of **-8.28, -8.24, -8.05** and **-7.55**kcal/mol respectively. They were interacting well with key residue such as Gly 93, Leu195, Tyr 158, Ala 196, Lle 200 and alse with NAD^+.

Phase II - Synthesis

Synthesis of 3-methyl-1-phenyl-1*H*-pyrazol-5-amine

An amount of β-aminocrotononitrile and phenylhydrazine hydrochloride was dissolved in dil.HCl and refluxed. The resulting reddish liquid was basified to alkaline pH with aqueous sodium hydroxide. The product obtained results in good yield.

Synthesis of Schiff base (1a-j)

3-methyl-1-phenyl-1*H*-pyrazol-5-amine and benzaldehyde in presence of ethanol and few drops of glacial acetic acid were refluxed for 3 hours to form Schiff's base. The product resulted in good yield.

Synthesis of pyrazolyl thiazolidinone derivatives (2a-j)

Schiff's base and mercaptoacetic acid (Thioglycolic acid) in presence of dioxan and anhydrous $ZnCl_2$ was refluxed for 4hrs. After cooling, the resulting product was washed with sodium bicarbonate to remove unreacted thioglycollic acid to obtain series of pyrazolyl thiazolidinone in good yield.

Synthesis of pyrazolyl oxazepine derivatives (3a-j)

Schiff's base and maleic anhydride in presence of hot dry benzene were refluxed. The solvent distilled off and the solid product obtained was in good yield.

Synthesis of pyrazolyl benzoxazepine derivatives (4a-j)

Schiff's base and phthalic anhydride in presence of hot dry benzene was refluxed and the solvent was distilled off to form the series of pyrazolyl benzoxazepine derivatives with good yield.

Characterization

Melting point of all newly synthesised compounds were determined. R_f values were determined by fixing various suitable solvent system on pre-coated silicagel-G plates. The solvent system used was ethyl acetate: n-Hexane (3:7). The structure was finally characterized by UV, IR, Mass, ^{1}H and ^{13}C NMR spectra.

Phase-III - Biological activity

- **Antimalarial activity**

The representative compounds from novel derivatives were screened for antimalarial activity was performed by *in-vitro* antimalarial assay technique by using *Plasmodium falcipuram*. In the **pyrazolyl thiazolidinone series**, **2g** (ethoxy) showed minimum inhibitory concentration at **0.95**µg/ml. Among the **pyrazolyl oxazepine series, 3b** (chloro) had shown promising activity with the MIC of **0.32**µg/ml and **3h** (dihydroxy) showed MIC of **1.41**µg/ml. Among the **pyrazolyl benzoxazepine series**, **4c** (methoxy) had shown promising activity with the MIC of **0.28**µg/ml and **4j** (fluoro) showed MIC of **1.07**µg/ml compared to standard chloroquine and quinine with the MIC of 0.02 and 0.26µg/ml respectively. So, in the antimalarial screening for representative compounds, the chloro, methoxy and fluoro substituted derivatives of the three series showed significant activity.

- **Antibacterial activity**

Antibacterial activity was performed by Kirby bauer disc diffusion method by using *Escherichia coli* NCIM2911. All the derivatives of three different series were screened for antibacterial activity. Among **pyrazolyl thiazolidinone series, 2b, 2c, 2d** and **2f** (chloro, methoxy, dimethoxy and o-chloro) had shown moderate sensitivity with a zone of inhibition **12, 13, 12** and **13** mm respectively. In the **pyrazolyl oxazepine series**, **3a, 3f, 3g** and **3i** (nitro, chloro, ethoxy and

dimethoxy) showed zone of inhibition **13, 13, 13** and **15**mm respectively. Among the **pyrazolyl benzoxazepine series, 4a, 4b, 4c, 4d** and **4e** (nitro, chloro, methoxy, dimethoxy and p-dimethyl amino) showed zone of inhibition **14, 13, 16, 14** and **13** mm respectively.

Thus in the **pyrazolyl thiazolidinone series,** ethoxy derivatives showed significant activity against the *P. falcipuram* and chloro, methoxy derivatives showed moderated sensitivity against the *E.coli*. In the **pyrazolyl oxazepine series,** chloro and dihydroxy showed promising activity against the *P. falcipuram* and dimethoxy derivatives showed moderate sensitivity against the E.coli. In the **pyrazolyl benzoxazepine series,** fluoro, methoxy derivatives showed activity against the organisms.

Thus, the series of compounds synthesized can be utilized for anti-malarial and antibacterial activity by the mechanism of action of inhibition of Enoyl ACP Reductase enzyme.

CONCLUSION

- The present study establishes that computational tools help in minimizing the tedious process of drug discovery and delivers new drug candidate more quickly.
- Virtual screening was utilized for filtering the compounds and selecting the lead compounds.
- The drug likeness score established the compounds to be pharmacokinetically active.
- The binding energy obtained from docking study further confirmed the possibility of the affinity of the selected leads towards the enzyme, enoyl ACP reductase from *Plasmodium falciparum* and *Escherichia coli.*
- Using the schemes various pyrazolyl linked thiazolidinone, oxazepine and benzoxazepine were synthesized with good yield.
- Structure of the synthesized compounds were confirmed by Melting point, TLC, UV, NMR and MASS spectra.
- The compounds were screened for antimalarial and antibacterial activity.

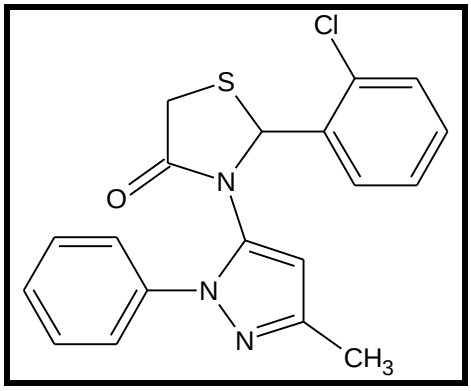
Thus the present study depicts that the utilization of computer aided drug design is an efficient tool in predicting the effectiveness of a series of compounds under study and thus can result in the design of potent antimalarial and antibacterial agents.

FUTURE PERSPECTIVE

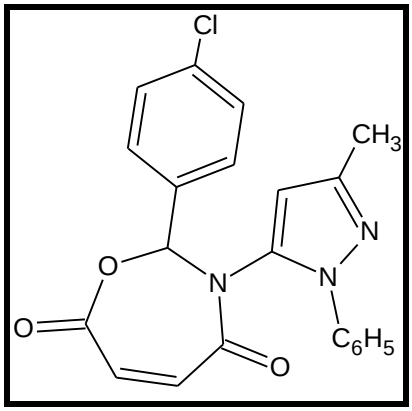
As the extension of the research project the series of pyrazolyl linked thiazolidinone, oxazepine and benzoxazepine can be subjected to *Pf*ENR and *E.coli* FabI enzyme inhibitory studies.

OUT COME OF THE STUDY

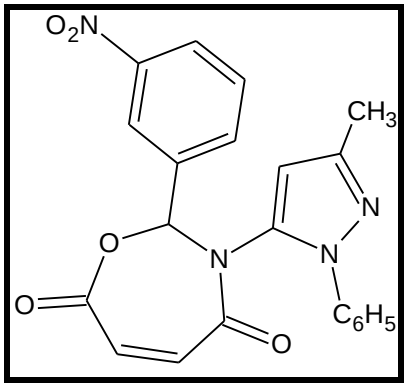
From the present study, the most significant compound were found to be



3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2-(2-chlorophenyl)-1,3-thiazolidin-4-one



2-(4-chlorophenyl)-3-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-2,3-dihydro-1,3-oxazepine-4,7-dione



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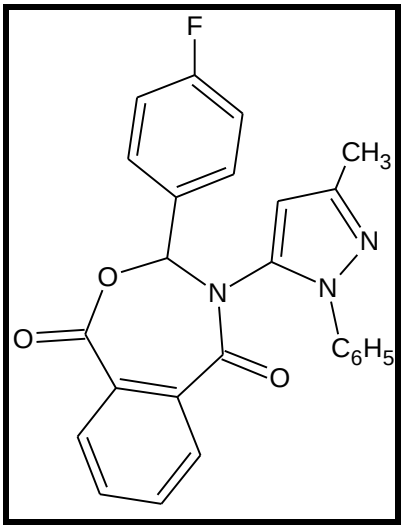
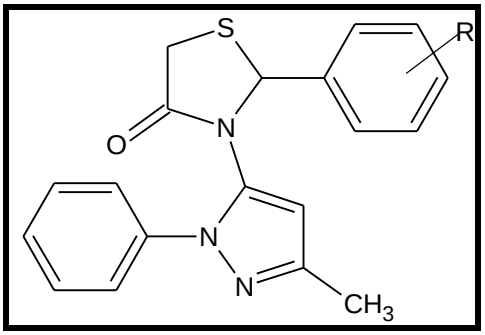


3-(4-fluorophenyl)-4-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione

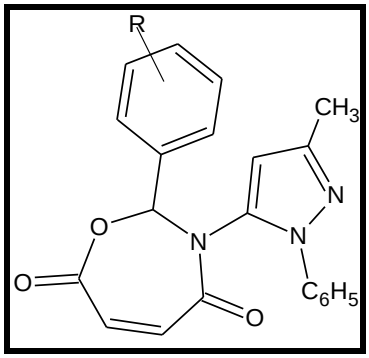
LIST OF NEWLY SYNTHESIZED COMPOUNDS

SERIES-1



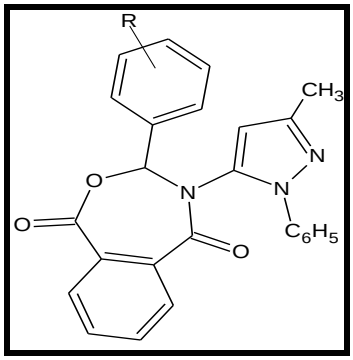
Compd code	R	Compound Name
2a	3-NO_2	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(3-nitrophenyl)-1,3-thiazolidin-4-one
2b	4-Cl	2-(4-chlorophenyl)-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one
2c	4-OCH_3	2-(4-methoxyphenyl)-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one
2d	3-OCH_3,4-OH	2-(4-hydroxy-3-methoxyphenyl)-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one
2e	4-$N(CH_3)_2$	2-[4-(dimethylamino)phenyl]-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one
2f	2-Cl	2-(2-chlorophenyl)-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one
2g	3-OC_2H_5,4-OH	2-(3-ethoxy-4-hydroxyphenyl)-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one
2h	2,4-OH	2-(2,4-dihydroxyphenyl)-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one
2i	3,4-OCH_3	2-(3,4-dimethoxyphenyl)-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one
2j	4-F	2-(4-fluorophenyl)-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1,3-thiazolidin-4-one

SERIES-2



Compd code	R	Compound Name
3a	3-NO_2	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(3-nitrophenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3b	4-Cl	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(4-chlorophenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3c	4-OCH_3	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(4-methoxyphenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3d	3-OCH_3,4-OH	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(4-hydroxy3-methoxy phenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3e	4-$N(CH_3)_2$	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(3-(dimethiamino)phenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3f	2-Cl	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(2-chlorophenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3g	3-OC_2H_5,4-OH	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(3-ethoxy-4-hydroxyphenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3h	2,4-OH	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(2,4-dihydroxyphenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3i	3,4-OCH_3	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(3,4-dimethoxyphenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione
3j	4-F	3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-(4-fluorophenyl)-2,3-dihydro-1,3-oxazepine-4,7-dione

SERIES -3



Compd code	R	Compound Name
4a	3-NO_2	3-(4-chlorophenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4b	4-Cl	3-(4-chlorophenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4c	4-OCH_3	3-(4-methoxyphenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4d	3-OCH_3,4-OH	3-(4-hydroxy3-methoxyphenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4e	4-$N(CH_3)_2$	3-(3-(dimethiamino)phenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4f	2-Cl	3-(2-chlorophenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4g	3-OC_2H_5,4-OH	3-(3-ethoxy-4-hydroxyphenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4h	2,4-OH	3-(2,4-dihydroxyphenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4i	3,4-OCH_3	3-(3,4-dimethoxyphenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione
4j	4-F	3-(4-florophenyl)-4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3,4-dihydro-2,4-benzoxazepine-1,5-dione

REFERENCE

1. http://www.thefullwiki.org/Drug-design
2. Shailza S, Balwant KM and Durlabh KS. Molecular drug targets and structure based drug design: A holistic approach. Bioinformation.2006;1(8):314-320 **Introduction**
3. Regine Sb, Colin MM, Wayne CG. The art and practice of structure based drug design. A molecular modelling perspective. Med Res Rev.1996;16(1):3-50.
4. Donald JA. Burger's Medicinal Chemistry and Drug Discovery. Sixth edition. John Wiley and sons, INC, 1:243-270,715-120.
5. Tudor IO, Hans M. Integrating virtual screening in Lead discovery. Curr Opin Chem Biol.2004;8(4):349-358.
6. Didier R. Development and virtual screening of target libraries. J Physiol.2006:232-244
7. http://en.wikipedia.org/wiki/Virtual-screening
8. Romano T. Kroemer. Structure-Based Drug Design:Docking and Scoring. Current protein and peptide sci.2007;8:312-328.
9. Kitchen DB, Dacornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for Drug Discovery:Methods and applications. Natural Reviews.2004;3: 939-949.
10. Suresh HB , Miwa GT, Liang-Shang Gan, Jing-Tao Wu, Frank W. Lee. Stretegy of utilizing In vitro and in vivo ADME Tool for Lead optimization. Curr Topics in Med Chem.2005;5:1033-1038.
11. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies.2004;1(4):337-341.
12. http:\\en.wikipedia.org/wiki/Druglikeness.
13. http:\\en,Wikipedia.org/wiki/Lipinski's_Rule_of_Five.

14. Terry P Lybrand, Ligand-protein docking and rational drug design. Curr Opin Struc Biol.1995;5(2):224-228.
15. Autodock.scripps.edu/-United States
16. https://en.wikipedia.org/wiki/Malaria
17. https://www.cdc.gov/malaria/
18. KD Thripathi. Essentials of Pharmacology.Sixth edition.2003;735- 737.
19. Tanushree B. Shaliendra kumar S, Neha K, Viashnu D, Namita S, Avadhesha S. Benzothiophene Carboxamide Derivatives as inhibitors of Plasmodium falcipuram Enoyl-ACP Reductase. IUBMB Life.2011;63(12):1101-1110.
20. Tabish Qidwai and Feroz Khan. Antimalarial Drugs and drug targets specific ro Fatty acid metabolic pathway of Plasmodium falcipuram. Chem Biol Drug Des.2012;80:155-172.
21. Manmohan Chhibber, Gyanendra Kumar, Prasanna Parasuraman, T.N.C Ramya, Namita Surolia, Avadhesha Surolia. Novel diphenyl ethers: Design, docing studies, synthesis and inhibition of enoyl ACP reductase of Plasmodium falcipuram and Escherichia coli. Bioorg & Med Chem.2006;14:8086-8089.
22. Avadhesha Surolia, Ramya TNC, Namita Surolia. Review article 'FAS' inhibition of malaria.Biochem J.2004;383:401-412.
23. Lorillee Tallorin, Jacob DD, Auynh GN, McCammon JA, Micheal DB. Celastrol inhibits Plasmodium falcipuram enoyl-acyl carrier protein reductase. Bioorg & Med Chem.2014;25:1-8.
24. Waller RF, Ralph SA, Reed MB, Vanessa Su, Douglas JD, Minnikin DE, Cowman AF, Besra GS, McFadden GI. A typeII Pthway for Fatty acid biosynthesis presents Drug Target in Plasmodium falcipuram.Antimicro agents andChemotheraphy.2003; 47(1):297-301.
25. https://en.wikipedia.org/wiki/Bacteria

26. Health RJ, Rubin R, Holland DR, Erli Zhang, Snoe ME, Rock CO. Mechanism of Triclosan inhibition of Bacterial Fatty Acid synthes. J Bio Chem.1999;274:11110-11114.
27. Jailall R, Mathew DS, Peter BS, Bryan F, Jeff W, Emily F. 2,3,4,5-Tetrahydro-1H-pyrido[2,3-*b* and *e*][1,4] diazepines as inhibitors of the bacterial enoyl ACP reductase FabI. Bioorg Med Chem. 2009; 19: 5359-62.
28. Ashutosh K, Mohammad IS. CoMFA based de novo design of pyrilidonecarboxamides as inhibitors of enoyl acyl carrier protein reductase from Mycobacterium tuberculosis. Biobytes. 2009; 13-17.
29. Sonia G, Ravi TK. Design, synthesis and Antitubercular screening of certain novel thiadiazolyl pyrrolidine carboxamides as enoyl ACP reductase inhibitors. Int J Pharm Pharmaceutical Sci.2011;3:1491.
30. Nisheet C.Desai , Hitesh M. Satodiya , Kiran M. Rajpara, Vivek V. Joshi, Kandarp Bhatt V. Vaghani. Synthesis and Evaluation of N-substituted Thiazolidine-2,4-dione Containing Pyrazole as Potent Antimicrobial Agent. J of Anti-Infective Agent.2014;12:85-94.
31. Pawar RB, Mulwad VV.Synthesis of some Biologically active pyrazole, Thiazolidinone and Azetidinone derivatives.Chem Het Compds.2004; 40(2):219-226.
32. Sah P, Nair S, Garg S P. Synthesis and Evaluation of substituted pyrazoles bearing 2-aryl amino -5- mercepto-1,3,4- thiadiazole as antimicrobial agents.Ind J of Het Chem. 2002;12:9-12.
33. Garkward M S, Mane A S, Shingare B B , and Shingare M S. Synthesis and Evaluation of certain N-1-[2-hydroxy benzoyl]- 5 –substituted phenyl-3- phenyl 4,5- dihydro pyrazoles as antimicrobial agents. Ind J of Het Chem.2004;.13:274-280.
34. Vijaykumar V, Ragavan R V, Suchethakumari N. Synthesis and Evaluation of novel 1,5-diaryl pyrazole derivatives for antibacterial and antifungal activities.Eup J of Med Chem. 2010;45:1173-1180.

35. Garg U, Sareen V, Khatri V, Caugh S. Synthesis and Evaluation of 1-thiocarbonyl 3-trifluoro methyl-5- phenyl-4(2-fluro phenyl azo) pyrazoles as potent Antibacterial agents. Ind J of Het Chem.2002;12:139-142.

36. Mistry.B D, Rna. P B Ptel. J A, Desai K R.Synthesis and Evaluation of a series of pyrazolo (5,4-d) – pyrimidine derivatives for Antimicrobial activity. Ind J of Chem.2009;48:1601-1608.

37. Bhawsar. S B, Chvan. V P, Mane. A S, Singare. M S.Synthesis and Evalyation of some new coumarino pyrazoles as antimicrobial agents. Ind J of Het Chem, 1999;9:135-138.

38. Faidallah. H M, Mohammed S L, Albar H A, Sharashri E M. Design, Synthesis and Evaluation of 3,5-disubstituted pyrazoles (9)from chalcones. Ind J of Het Chem. 2002;12:163-164.

39. Vishnu J R Nath, Mahendra. Indian Journal of Chemistry.1994;33: 1048-1052.

40. Bekhit AA, Hassan MM, Abd EI Razik, Ei-Miligy MM, Ei-Agroudy EJ.New heterocyclic hybrids of Pyrazole and its bioisosteres: Design synthesis and biological evaluation as dual acting antimalarial-antileishmanial agents.E-J Med Chem.2015;1:573-581.

41. Sanjay k, Gynondra k, Mili k, Avadhosa S, Namita S.Synthesis and Evaluation of substituted Pyrazoles : Potential Antimalarial Targetting the Enoyl-ACP Reductase of Plasmodium Falcipuram.Syn Commu.2006;36:215-226.

42. Sabine K, Alfonso Z, Marcal K, Leonardo S, Remo P. Synthesis and Evaluation of 4-[5-(4-Phenoxyphenyl)-2H-pyrazol-3-yl]morpholine derivatives.J. Med. Chem.2007;50:5833-5839.

43. Nadia G. Kandile, Mansoura I. Mohammed, Hind M. Ismaeel . Synthesis of new Schiff Bases bearing 1,2,4-triazole, thiazolidine and chloroazetidine moieties and their pharmacological evalution. J of Enz Inhibition and Med Chem. 2017; 32(1):119-129.

44. Zeki A. Naser Al-Shamkhani , Hanan A.Al-Hazam , Suha K. Al-Mosawi. Microwave Assisted Synthesis, Characterization and Antibacterial Study of Some Novel Schiff's Bases, Thaizolidinone and Chalcone Compounds Derived from Mefenamic Acid. J of Chem and Mat Research. 2015;7(6):50-56.

45. Sayaji S. Didwagh, Pravina B. Piste, Aravind S.Burungale, Avinash M. Nalawade. Synthesis and Antimicrobial evaluation of novel3-(4,6-diphenyl-6h-1,3-thiazin-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one derivatives. J of Applied Pharm Sci.2013;3(11):122-127.

46. K.M. Mistry and K.R.Desai. Synthesis of Novel Heterocyclic Pyrazolyl4-Thiazolidinone Derivatives and their Antibacterial Activity. E-J of Chem. 2004;1(4):189-193.

47. Pushkal Samadhiya, Ritu Sharma, S.K. Srivastava, S.D. Srivastava. Synthesis and Biological evaluation of 4-thiazolidinone derivatives as antitubercular and antimicrobial agents. Arab J of Chem.2011; 1-8.

48. Navin B. Patel, Faiyazalam M. Shaikh. Synthesis and antimicrobial activity of new 4-thiazolidinone derivatives containing 2-amino-6-methoxybenzothiazole.Saudi Pharm J.2010;18:129-136

49. Mogilaiah K, Babu R and K. Narender Reddy. Synthesis of 4-thiazolidinone and 2-azetidinone derivatives of 2-trifluoromethyl-1,8-naphthyridine as antibacterial agents.Ind J of Chem.1999;38B:818-822.

50. Bhoot DP, Khunt RC, Shankhavara VK, Parekh HH. Synthesis of some New Heterocyclic compounds with Potential Biological activity.J Sci Islamic Republic of Iran.2006;17(4):323-325.

51. Altintas H, Ates O, Birteksoz S, Otuk G, Uzun M, Satana D.Synthesis of Mannich bases of some 2,5-Disubstituted 4-Thiazolidinones and evaluation of their Antimicrobial Activities.Turk J Chem.2005; 29:425-435.

52. Krunal GD, Kishor RD.A facile microwave enhance synthesis of sulphur-containing 5-membered heterocycles derived from 2-mercaptobenzothiazole over $ZnCl_2$/DMF and antimicrobial activity evaluation.J Sulfur Chem.2006;27(4):315-328.

53. Aamer S, Naeem A, Ulrich F.Synthesis and Antibacterial activity of some Novel 2-Aroylimino-3-aryl-thiazolidin-4-ones.J Braz Chem Soc.2007;18(3):559-565.

54. Pooja C, Ranjit S, Shailendra KS.Synthesis and evaluation of 2,5-disubstituted 4-thiazolidinone analogues as antimicrobial agents.Med Chem Res.2012;21:2064-2071.

55. Tejaskumar JS, Vikas AD.Synthesis of some novel fluorinated 4-thiazolidinones containing amide linkages and their antimicrobial screening.ARKIVOC.2007;xiv:218-228.

56. Vicini P, Athina G, Matteo I, Franca Z, John Dearden, Mark Hewitt.2-Heteroarylimino-5-benzylidene-4-thiazolidinones analogues of 2-thiazolylimino-5-benzylidene-4-thiazolidinones with antimicrobial activity: Synthesis and structure-activity relationship.Bio & Med Chem. 2008;16:3714-3724.

57. Rajput AP, Kankhare AR.Synthesis, Characterization and microbial screening of dioxolane and thiazolidinones derivatives of 2,5-dichloro-3,4-diformyl(N-substitutedphenyl)Pyrazoles.Phar Chemica.2016;8(13):1-8.

58. Solankee AN, Patel KP, Patel RB.Efficient synthesis and pharmacological evaluation of some new 4-thiazolidinones and 5-arylidenes.Arc Applied Sci Research.2012;4(1):72-77.

59. Gyanendra K, Prasanna P, Shailendra K, Tanushree B, Krishanpal K.Discovery of a 5-Benzylidene-2-thioxothiazolidin-4-one Rhodanine class of compounds as Inhibitors of Plasmodium falcipuram Enoyl-Acyl Carrier Protein Reductase.J Med Chem.2007;50:2665-2675.

60. Raja VS, Haq W, Srivastava K, Puri SK, Katti SB.Design. synthesis of 4-aminoquinoline-derived thiazolidines and their antimalarial activity and heme polymerization inhibition studies.J Enz Inhibition & Med Chem.2013;28(3):619-626.

61. Agular AC, Flguelredo JB, Neuenfeldt PD, Katsuragawa TH, Drawanz BB, Cunico W, Sinnis W.Primaquine-thiazolidinones block malaria transmission and development of liver exoerythrocytic forms.Malar J.2017;16:110:1-11.

62. Rojas Ruiz FA, Garcia-Sanchez RN, Santiago V, Alicia Gomez, Diego FT, Kouznetsov VV.Synthesis and antimalarial activity of new heterocyclic hybrids based on chloroquine and thiazolidinone scaffolds.Bio & Med Chem.2011;19:4562-4573.

63. Ashish Bhatt, Rjesh Kumar, Ravi Kant.Synthesis,antimicrobial and antimalarial study of novel 1,3,4-thiadiazole derivatives incorporating imidazo [1,2-b]pyridazine and thiazolidinone moieties.Chem Bio Lett.2017;4(2):28-34.

64. Varum AM, Shaikh MS, Pissurlenkar RS, Coutinho EC.Molecular modelling studies, synthesis and biological evaluation of Plasmodium falcipuram Enoyl-acyl protein reductase (PfENR) inhibitors.Mol Divers.2009;13:501-517.

65. Rajni KS, Yassir Y, Mugumbate G, Mathew N, Jiri G, Rosenthal PJ, Chibale K.Synthesis and structure-activity-relationship studies of thiazolidinediones as antiplasmodial inhibitors of the Plasmodium falcipuram cysteine protease falcipain-2.E-J Med Chem.2015;90:507-518.

66. Naruka S. Yakubu, Dr. Afolabi, E.O. Synthesis and Antimicrobial Activity of Formamidine Disulfide Schiff Bases and Their Corresponding 1,3-Oxazepines. International Journal of Current Research,2016;8(06):33166-33172.

67. Khitan T.A. Al-Sultani. Preparation Some New heterocyclic Compounds Derived from Schiff Bases and Evaluation its Biological Activity. Int J of Sci And Research. 2017;6(5):1567-1572.

68. Hamak KF, Eissa HH. Synthesis, Characterization, Biological evaluation and Anti Corrosion Activity of Some Heterocyclic Compounds Oxazepine Derivatives from Schiff Bases. Organic chem Curr Res.2013;2(3):1-7.

69. Huda I. Ibrahim. Synthesis and Study the Biological Activity of Schiff Base derivatives of 1,3-Oxazepines.College of Edu. University of Baghdad.

70. Ahmed N G, Al-Hashimi HY.Preparation and Characterization of some new oxazepine compounds.Eur Chem Bull.2016; 5(3):88-91.

71. Anila K. Alexander, Lincy Joseph, Mathew George. Synthesis of Novel 5,6-Benz1,3- Oxazepine 4,7 –Dione Derivatives and Screening for Antbacterial, Antioxidant and Anti-Inflammatory Activities. European Journal of Pharmaceutical and Medical Research, 2016; 3(7): 330-336.

72. Kendre BV, Landge MG, Bhusare SR.Synthesis and biological evaluation of some novel pyrazole, isoxazole, benzoxazepine, benzothiazepine and benzodiazepine derivatives bearing an aryl sulfonate moiety as antimicrobial and anti-inflammatory agents. Arabian J Chem.2015:1-7.

73. Muhsen TR, Tomma JH, Mukhlus A.J.A.Synthesis and characterization of novel compounds containing of imidazolidinone and oxazepine rings.Ibn Al-Haitham. J for Pure and Applied Sci.2012;25(3):276-283.

74. Hawraa Mohammed Sadiq.Synthesis and chacterization of novel 1.3-oxazepine derivatives from aminopyrazine.J Pharmacy and Pharmaceutical Sci.2017;6(5):186-198.

75. R.R. Gupta, M. Kumar, V.Gupta., Heterocyclic chemistry, 1st edition, Springer- Verlag Berli Heidelberg New York:1990:416-455.

76. Katritzky. A.R and Rees,C.W., Comprehensive Heterocyclic Chemistry, 1st edition, Pergamom Press.1984;Vol-6:235-387,239-252.

77. Shiva P. singh, Surendra S. Parmar, Krishna Raman, Virgil I. Stenberg. Chemistry and Biological Activity of Thiazolidinones. Chem Rev.1981: 81:175-203.

78. Zainab Amer Sallal, Hasn Thamer Ghanem, Synthesis of new 1,3-Oxazepine derivatives containing Azo Group. J of Kufa for chem Sci.2011:1-23.

79. S.Coffey.Rodd's Chemistry of Carbon Compounds. 2nd edition.Elsevier Scientific Publishing Company.New york.1986;IV(K):284-286.

80. Giorgio Pifferi, Luigi Fontanella, Emilio Occelli, Riccardo Monguzzi. Synthesis of 4,5-Dihydro-2,4-Benzoxazepine-3(1H)ones and 1,3,4,5-Tetrahydro-2,4-benzoxazepines. J Het Chem.1972;9:1209-1214.

81. Research Collaboratory for Structural Bioinformatics. Protein Data Bank. [Internet].2011 [updated 2011 Mar 22; cited 2011 Mar 26]. Available from: http://www. pdb.org

82. Yang J, BioXGEM Lab, Institute of Bioinformatics, National Chiago-Tung University. Igemdock. [Internet]. 2006 [cited2011 Mar 26]. Available from: http://gemdock.life.nctu.edu.tw/dock/

83. Bioinfomatics and Chemical Informatics Research Center, University of California.ZINC [Internet]. 2009 [updated 2009 Aug 6; cited 2011 Mar 26]. Available from: http://zinc.docking.org/choose.shtm.

84. Molinspiration Cheminformatics. Molinspiration. [Internet]. 2010 [cited 2011 Mar 26]. Available from: http://www.molinspiration.com/cgi-bin/properties

85. http://autodock.scripps.edu/downloads

86. http://www.python.org/download

87. http://www.cygwin.com/

88. Perozzo R, Mack K, Amar bir Singh Sidhu, Jacob T.V, Robert Bittman, William R. Jacobs. Structural Elucidation of the specificity of the Antibacterial Agent Triclosan for malarial Enoyl Acyl Carrier Protein Reductase.J Bio Chem.2002;277(15):13106-13114.

89. Richard J. Health, J.Ronald Rubin, Debra R. Holland, Erli Zhang, Mark E. Snow, Charles O. Rock. Mechanism of triclosan Inhibition of Bacterial Fatty Acid Synthesis.J Bio Chem.1999;274(16):11110-11114.

90. Arasu G, Healthcock CH.Synthesis of Unsymmetrical Pyrazines by Reaction of an Oxadiazinone with Enamines.J Org Chem.1993;58:6155-6157.

91. Havaldar HF, Mishra Sk, Synthesis of azetidine-2-one and thiazolidin-4-one as potent antimicrobial agent.Indian J Het Chem.2004;13:197-199.

92. Pradeep kumar MR, Malleshappa NN, Sheeta S, Satyanarayana D, Veeresh SM.Synthesis and antimicrobial activity of 7-(2-substituted phenylthiazolidinyl)-benzopyran-2-one derivatives. E-J Med Chem.2010;45:85-89.

93. Yeap GY, Abdulkarim TM, Hasnah O.Synthesis, spectroscopic and mesomorphic studies of heterocyclic liquids crystal with 1,3-oxazepine-4,7-dione, 1,3-oxazepane-4,7-dione and 1,3-oxazepine-1,5-dione cores.J Mol Str.2010;982:33-44.

94. Fung FW, Li-Ya Wang, Uramaru N, Hui-Hsuan Chiang, Synthes and structural identification of 5-amin-4-hydroxyiminopyrazoles and (E)-N1-aryl-3-aryl-4[(substituted pyrazolyl)diazenyl] pyrazols from 5-aminopyrazoles with ethyl nitrite or sodium nitrite.Tetrahedron.2014;70:7977-7982.

95. Milan C, Molnar M, Bojan S, Elizabeta HS, Valentina R.Synthesis and Antioxidant Activity of some coumarinyl-1,3-Thiazolidine-4-ones.Molecules.2010; 15:6795-6809: doi:10.3390/ molecules 15106795.

96. Media Noori A, Jala Bahagat Z, NaweenYouns M.Synthesis of some Heterocyclic compounds (oxazepine, Diazepine) using Ultrasound irradiation.SU-ICEIT.2016;April12-14.

97. Rieckmann KH, Campbell GH, Sax LJ, Mrema JE.Drug sensitivity of Plasmodium falcipuram, an *invitro* microtechnique. Lancet.1978; 1:221-113.

98. Desjardins RE. *Invitro* techniques for antimalarial development and evaluation. In:Peter W and Richards, W.H.G. editors. Handbook of Experimental Pharmacology. Springer-Verlag, Germany.1984:179-200.

99. Trager W, Jensen JB. Human Malaria Parasites in Continuous Culture science. 1976;193:673-675.

100. Lambros C, Vanderberg JP. Synchronization of Plasmodium falcipuram intraerythrocytic stages in culture. J Parasitol.1979;65:418-420.

101. Singh J, Stain JSB. A review.Indian J Malariology.1956;10:117-129.

102. Panjarathianam R.Text book of Medical Parasitology. 2nd edition.Orient Longman Pvt.Ltd. Chennai.2007:329-331.

103. Atlas RM, Perks LG, Brown AE. Laboratory Manual of Experimental Microbiology, Louisville: Mosby Publishers; 1995: 5-35.

104. Cappuccino JG, Sherman N. Microbiology – a laboratory manual, 3rd Ed. New York: Benjamin Cummings Publishing Company; 1992.51-66.