Chapter-1

A: Introduction & review of literature
B: Plan of work
A: Introduction & Review of Literature

Synthetic medicinal chemistry is a specialized science that has evolved to encompass a broad range of disciplines concerned with the identification, synthesis and development of drug-like compounds for therapeutic use. In early days of drug discovery, medicinal chemists often optimized and developed compounds without much knowledge of the drug target or pathway in mind. It was a largely subjective process where chemistry-driven elaboration of chemical structures was undertaken and these compounds were often tested directly in vivo and in vitro.

The heterocyclic skeleton containing nitrogen and sulfur atom is the basis of many essential pharmaceuticals and of many physiologically active natural products [1-2]. Molecules containing heterocyclic substructures continue to be attractive targets for synthesis since they often exhibit diverse and important biological properties. Although there are many antibiotics and chemotherapeutics available since last two decades, the treatment of infectious diseases remains a challenging therapeutic problem due to the inexorable increase and spread of multidrug-resistant [3-5]. Throughout history, there has been a continual battle between humans and the multitude of life threatening diseases like pneumonia, typhoid, diarrhea, skin infection, nosocomial infection, food poisoning, urinary tract infection, ear infection, lung disease, tuberculosis, cancer, AIDS [6].

Thus, studies for the identification of novel targets and drugs for the treatment of infectious diseases are at the forefront. A major challenge of modern drug discovery is the design of highly efficient chemical reaction sequences which provide a maximum of the structural complexity and biological diversity with just a minimum number of synthetic steps to assemble compounds with interesting properties. Among the attractive ways of solving multi-drug resistance is the synthesis of novel molecules which should preferably consist of chemical characteristics that clearly differ from those of existing agents and are biologically active by the virtue of the presence of critical structural features. The development of new synthetic methods leading to structures, which incorporates various biologically active moieties in a single molecule, has attracted much attention in
organic chemistry. This approach to merge such pharmacophores may offer important medical accomplishments to minimize the probability of resistance formation [7].

At the present time, an alleviation of large number of diseases is conducted by drug therapy. The fight against bacterial and fungal infections has been largely succeeded, and significant development has been made in treating drug resistant dilemma. To boast, it can be claimed that certain form of cancers can be cured by chemotherapy.

**Medicinal Chemistry**

The primary objective of medicinal chemistry is the design and discovery of new compounds that are suitable for use as drugs. The discovery or design of a new drug not only requires a discovery or design process but also the synthesis of the drug, a method of administration, the development of tests and procedures to establish how it operates in the body and a safety assessment. Drug discovery may also require fundamental research into the biological and chemical nature of the diseased state [8-10]. These and other aspects of drug design and discovery require input from specialists in many other fields and so medicinal chemists need to have outline knowledge of the relevant aspects of these fields. Medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed to new natural or synthetic organic compounds. Paralleling the development of medicinal agents has come a better understanding to the chemistry of the receptor. The latter has been greatly facilitated by low-cost computers running software that calculates molecular properties, structure and pictures by using high-resolution graphics. Development of organic compounds has grown beyond traditional synthetic methods.

Current trend in drug design for developing new clinically effective agents through the structural modification of the lead nucleus strikes immense importance in medicinal world [11]. The examples of drug discovery without a lead are quite few in number. The lead is a prototype compound that has desired biological activity but may have many undesirable characteristics like high toxicity, insolubility or
metabolism problems. Inspite of the great success of the classical methods of drug
design, their unpredictability and the tremendous amount of wasted effort
expended have necessitated the development of more rational methods with a
much higher predictive capability. New drugs are constantly required to combat drug
resistance even though it can be minimized by the correct use of medicines by
patients. They are also required for improving the treatment of existing diseases, the
treatment of newly identified diseases and the production of safer drugs by the
reduction or removal of adverse side effects.

Medicinal chemistry covers the following stages:

1. In the first stage, new active substances or drugs are identified and prepared
   from natural sources, organic chemical reactions or biotechnological
   processes. They are known as lead molecules.

2. The second stage is the optimization of lead structure to improve potency,
   selectivity and less toxicity.

3. Third stage is the development stage which involves the optimization of the
   synthetic route for bulk production and modification of pharmacokinetic and
   pharmaceutical properties of active substance to render its chemical
   applicability.

Drugs

Drugs are strictly defined as chemical substances that are used to prevent or cure
diseases in humans, animals and plants. The activity of a drug is its pharmaceutical
effect on the subject, for example, analgesic or b-blocker, whereas its potency is the
quantitative nature of that effect. Drugs may be mere chemicals but they are
entering a world of chemical reactions with which they can interact. In order to
invent or prepare a biologically active drug, it must not show toxicity, must show
good pharmacokinetic properties, and must not be rapidly metabolized and most
optimally, must be absorbed after oral administration \[12\]. Hence, ADMET
(Absorption, Distribution, Metabolism, Excretion and Toxicity) i.e. properties of
compounds must be investigated early in the drug discovery path to clarify clinical
utility of new genes as targets. Very few drugs satisfy all the above conditions.
However, in search of novel molecule based on potent therapeutic application still remains the interesting area of research.

**Classification of drugs**

Drugs are classified in different ways depending on where and how the drugs are being used. The methods of interest to medicinal chemists are chemical structure and pharmacological action, which includes the site of action and target system [13]. However, it is emphasized that other classifications, such as the nature of the illness, are used both in medicinal chemistry and other fields depending on what use is to be made of the information. In all cases, it is important to bear in mind that most drugs have more than one effect on the body and so a drug may be listed in several different categories within a classification scheme:

- **Chemical structure**
  
  Drugs are grouped according to the structure of their carbon skeletons or chemical classifications, for example steroids, penicillin and peptides. Unfortunately in medicinal chemistry this classification has the disadvantage that members of the same group often exhibit different types of pharmaceutical activity. Steroids, for example, have widely differing activities: testosterone is a sex hormone, spironolactone is a diuretic and fusidic acid is an antibacterial agent. Classification by means of chemical structure is useful to medicinal chemists who are concerned with synthesis and structure–activity relationships.

- **Pharmacological action**
  
  This classification lists drugs according to the nature of their pharmacodynamic behavior, for example diuretics, hypnotics, respiratory stimulants and vasodilators. This classification is particularly useful for doctors looking for an alternative drug treatment for a patient.

- **Physiological classification**
  
  The World Health Organization (WHO) has developed a classification based on the body system on which the drug acts. This classification specifies seventeen sites of drug action. However, a more practical method but less detailed system often used by medicinal chemists is based on four classifications, namely:
1. *Agents acting on the central nervous system (CNS)*
   The central nervous system consists of the brain and spinal cord. Drugs acting on the CNS are the psychotropic drugs that effect mood and the neurological drugs required for physiological nervous disorders such as epilepsy and pain.

2. *Pharmacodynamic agents*
   These are drugs that act on the body, interfering with the normal bodily functions. They include drugs such as vasodilators, respiratory stimulants and anti-allergy agents.

3. *Chemotherapeutic agents*
   Originally these were drugs such as antibiotics and fungicides that destroyed the microorganisms that were the cause of a disease in an unwitting host. However, the classification has also now become synonymous with the drugs used to control cancer.

4. *Miscellaneous agents*
   This class contains drugs that do not fit into the other three categories, for example hormones and drugs acting on endocrine functions.

- **Prodrugs**
  Prodrugs are compounds that are pharmacologically inert but converted by enzyme or chemical action to the active form of the drug at or near their target site. For example, levodopa, used to treat Parkinson’s syndrome, is the prodrug for the neurotransmitter dopamine. Dopamine is too polar to cross the blood–brain barrier but there is a transport system for amino acids such as levodopa.

**Drug resistance**
Antimicrobial resistance within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. Increasingly, governments around the world are beginning to pay attention to a problem so serious that it threatens the achievements of modern medicine. A post-antibiotic era—in which common infections and minor injuries can kill—far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century. Antimicrobial resistance is not a new problem, but it has worsened dramatically in
the last decade [14-20]. Antibiotics were discovered in the middle of the nineteenth century and brought down the threat of infectious diseases which had devastated the human race. However, soon after the discovery of penicillin in 1940, a number of treatment failures and occurrence of some bacteria such as staphylococci which were no longer sensitive to penicillin started being noticed. This marked the beginning of the error of antimicrobial resistance. Scientific antibiotic discovery started in the early 1900s by Alexander Fleming [21], who observed inhibition of growth on his agar plate on which he was growing Staphylococcus spp. It was later found that a microorganism that was later to be called Penicillium notatum was the cause of the inhibition of the Staphylococcus around it as a result of excreting some chemical into the media. That marked the beginning of the discovery of penicillin which together with several other different antimicrobial agents was later to save millions of humans and animals from infectious disease-causing organisms.

Increasing prevalence of resistance has been reported in many pathogens over the years in different regions of the world including developing countries. This has been attributed to changing microbial characteristics, selective pressures of antimicrobial use, and societal and technological changes that enhance the development and transmission of drug-resistant organisms. Although antimicrobial resistance is a natural biological phenomenon, it often enhanced as a consequence of infectious agents’ adaptation to exposure to antimicrobials used in humans or agriculture and the widespread use of disinfectants at the farm and the household levels.

In general, the reasons for increasing resistance levels include the following:

- Suboptimal use of antimicrobials for prophylaxis and treatment of infection
- Noncompliance with infection-control practices
- Prolonged hospitalization, increased number and duration of intensive care-unit stays
- Multiple comorbidities in hospitalized patients
- Increased use of invasive devices and catheters
- Ineffective infection-control practices, transfer of colonized patients from hospital to hospital
- Grouping of colonized patients in long-term-care facilities
Antibiotic use in agriculture and household chores

Increasing national and international travel.

Moreover, the level of antibiotic resistance is dependent on the following:

- The population of organisms that spontaneously acquire resistance mechanisms as a result of selective pressure either from antibiotic use or otherwise
- The rate of introduction from the community of those resistant organisms into health care settings
- The proportion that is spread from person to person.

All of these factors must be addressed in order to control the spread of antimicrobial-resistant organisms within health care settings. Community acquired antimicrobial resistance is increasing in large part because of the widespread suboptimal use of antibiotics in the outpatient settings and the use of antibiotics in animal husbandry and agriculture.

**Multidrug Resistance**

We are currently witnessing a dramatic and alarming increase in the incidence of bacterial infections resistant to most common antibiotics. Multidrug resistance among many organisms has become a big challenge to infectious disease management [22-24]. It is increasingly being reported in bacteria and is often mediated by genetic mobile elements such as plasmids, transposons, and integrons.

**Mechanism of Antimicrobial Resistance**

Prior to the 1990s, the problem of antimicrobial resistance was never taken to be such a threat to the management of infectious diseases. But gradually treatment failures were increasingly being seen in health care settings against first-line drugs and second-line drugs or more [25]. Microorganisms were increasingly becoming resistant to ensure their survival against the arsenal of antimicrobial agents to which they were being bombarded. They achieved this through different means but primarily based on the chemical structure of the antimicrobial agent and the mechanisms through which the agents acted [26]. The resistance mechanisms therefore depend on which specific pathways are inhibited by the drugs and the
alternative ways available for those pathways that the organisms can modify to get a way around in order to survive [27-29].

Resistance can be described in two ways:
A) Intrinsic or natural whereby microorganisms naturally do not posses target sites for the drugs and therefore the drug does not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures especially for those that require entry into the microbial cell in order to effect their action or
B) Acquired resistance whereby a naturally susceptible microorganism acquires ways of not being affected by the drug.

Acquired resistance mechanisms can occur through various ways as described as follow (Figure 1.1):

- The presence of an enzyme that inactivates the antimicrobial agent
- The presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent
- Mutation in the antimicrobial agent’s target, which reduces the binding of the antimicrobial agent
- Post-transcriptional or post-translational modification of the antimicrobial agent’s target, which reduces binding of the antimicrobial agent
- Reduced uptake of the antimicrobial agent
- Active efflux of the antimicrobial agent
- Overproduction of the target of the antimicrobial agent
- Expression or suppression of a gene in vivo in contrast to the situation in vitro
- Previously unrecognized mechanisms
Figure 1.1 Illustration of how some antimicrobial agents are rendered ineffective

**Antibacterial Chemotherapy**

The rapid emergence of multidrug resistant bacterial strains and the potential threat pose to future generations means that the development of novel antibacterials is likely neither to be easy nor to offer a permanent solution to the problem [30-31].

Currently, there are two basic strategies for finding novel antibacterials. The first, which has been responsible for the discovery of most novel classes to date, is to screen natural product or corporate compound collections. This strategy provides a compound with demonstrable antibacterial activity, although it may take some time to discover the mode of action of the class, and working with a complex biological system such as a bacterial cell can make the generation of meaningful structure–activity relationships quite problematic, even before taking into account the interspecies diversity encountered. The second strategy, which has become increasingly popular in recent years, is to identify the essential enzymes of the bacterial cell and to screen compounds as inhibitors of these systems. The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. Bacteria are commonly responsible for many diseases, which were
considered until recently to be resistant to chemotherapy. In addition, there are numbers of regimen agents are available to treat such kind of diseases caused by the bacteria described herewith.

**Bacteria**

Bacteria were in existence as long as 3.5 billion years ago, making them one of the oldest living organisms on the earth. Bacterial cells are much smaller than plant or animal cells. They were first seen under a microscope by Anton van Leeuwenhoek in 1676 [32-33]. As microscopes have improved, scientists have come to understand bacterial cell structure better. This appreciation followed the elegant experiments carried out by the French scientist Pasteur, who demonstrated that specific bacterial strains were crucial to fermentation and that these and other microorganisms were far more widespread than was previously thought. The possibility that these microorganisms might be responsible for the disease began to take hold [34][34][34][34][34][34]. Below Figure 1.2 shows the bacterial cell wall.

![Figure 1.2 Bacterial Cell](image)

Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment. Cell wall composition varies
widely amongst bacteria and is one of the most important factors in bacterial species analysis and differentiation.

A technique devised by Danish physician Hans Christian Gram in 1884, uses a staining and washing technique to differentiate between the two forms [35]. When exposed to a gram stain, gram-positive bacteria retain the purple color of the stain because the structure of their cell walls traps the dye. In gram-negative bacteria, the cell wall is thin and releases the dye readily when washed with an alcohol or acetone solution.

<table>
<thead>
<tr>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
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<tbody>
<tr>
<td><em>Diphtheria bacillus</em></td>
<td><em>Coli and Typhoid bacillus</em></td>
</tr>
<tr>
<td><em>Leprosy bacillus</em></td>
<td><em>Gonococcus</em></td>
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<tr>
<td><em>Pneumococcus</em></td>
<td><em>Meningococcus</em></td>
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<tr>
<td><em>Staphylococcus</em></td>
<td><em>Plague bacillus</em></td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td><em>Spirochaetes</em></td>
</tr>
<tr>
<td><em>Tubercle bacillus</em></td>
<td><em>Vibrios (V. Cholerae)</em></td>
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**Antibacterial agents**

The history of antibacterial has been dynamic, characterized by the constant emergence of new challenges followed by investigation, discovery and the production of new drugs. A complete review of the various agents employed as antibacterial would be beyond the scope of this work and hence is not attempted. A brief summary of the important classes of antibacterial compounds is given below.

**Synthetic antibacterial agents**

The synthetic antibacterial agents [36] are comprised of two major classes of compounds; those effective systematically and those used topically.

**Topical antibacterial agents**

Antibacterial agents that are employed topically are commonly termed antiseptics, disinfectants or preservatives depending on how they are employed [37].
Since there is a considerable degree of overlap in usage among these three groups, the more convenient method of classifying them, i.e., according to structural types.

The antiseptics and disinfectants are a large, diverse group of chemical compounds that play an important role in the maintenance of human and animal health. Although they are often improperly utilized and overrated in their effectiveness by both lay and medical personnel, they are invaluable when properly employed. The hexachlorophene tragedy has shown that extensive toxicological studies are just as important for topical agents as for systematic, and older agents should be employed with due care.

Topical synthetic antibacterials are classified as follows:

1. Halogens and Halophors [34]
2. Phenols [38-41]
3. Alcohols
4. Aldehydes [42]
5. Quaternary ammonium compounds [43-44]
6. Dyes [45-47]
7. Ureas [48], Amidines [49-50] and Biguanides [51]
8. Heavy metal compounds [52-53]
9. Miscellaneous [54]

**Systematically active antibacterial agents**

The systematically active antibacterial has been divided into three groups, two of which, are sulfonamides and the antimycobacterial agents. The remaining compounds are the principally agents which are used for the treatment of urinary tract infections.

Except for the sulfonamides and antimycobacterial drugs, only a few systemically active synthetic antibacterials are commercially important today. The multitudes of highly effective relatively nontoxic antibiotics available for the treatment of bacterial infections have provided stiff competition for the medicinal chemist attempting to synthesize new antibacterial agents.
Systemic synthetic antibacterials are classified as follows:

1. Antimycobacterial agents [55-56]
2. β-Lactam antibiotics [57-61]
3. Trimethoprim, Cotrimoxazole [62]
4. Methanamine
5. Nitrofurans [63]
6. Quinolones [64]
7. Sulfonamides [65]

According to the effect produced, antibacterial drugs can be bacteriostatic (inhibit growth of bacteria) or bactericidal (kill the bacteria). Commonly used bacteriostatic and bactericidal drugs are given below.

<table>
<thead>
<tr>
<th>Bactericidal Drugs</th>
<th>Bacteriostatic Drugs</th>
</tr>
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<tbody>
<tr>
<td>Penicillin</td>
<td>Sulfonamides</td>
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<tr>
<td>Aminoglycosides</td>
<td>Nitrofurans</td>
</tr>
<tr>
<td>Polymyxin, Colistin</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Tetracyclines</td>
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<tr>
<td>Isoniazid</td>
<td>Chloramphenicol</td>
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<tr>
<td>Cotrimoxazole</td>
<td>Lincomycin</td>
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**Mechanism of action of antimicrobial agents**

In order to appreciate the mechanisms of resistance, it is important to understand how antimicrobial agents act [28, 66-68]. Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. Broadly, antimicrobial agents may be described as either bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of the bacteria giving the immune system of the host time to clear them from the system.
Complete elimination of the bacteria in this case therefore is dependent on the competence of the immune system. Bactericidal agents kill the bacteria and therefore with or without a competent immune system of the host, the bacteria will be dead [69-70]. However, the mechanism of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents [71]. These include generally the following:

- Inhibition of the cell wall synthesis
- Inhibition of ribosome function
- Inhibition of nucleic acid synthesis
- Inhibition of folate metabolism
- Inhibition of cell membrane function

**Figure 1.3** Sites of action and potential mechanisms of bacterial resistance to antimicrobial agents
Antimicrobial drugs can damage pathogens in several ways. The most selective antibiotics are those that interfere with the synthesis of bacterial cell walls (e.g., Penicillins, Cephalosporins, Vancomycin, Bacitracin). These drugs have a high therapeutic index because bacterial cell walls have a unique structure not found in eucaryotic cells. Streptomycin, Gentamycin, Spectinomycin, Clindamycin, Chloramphenicol, Tetracycline, Erythromycin and many other antibiotics inhibit protein synthesis by binding to the prokaryotic ribosome (Figure 1.3). Because of these drugs discriminate between prokaryotic and eukaryotic ribosomes, their therapeutic index is fairly high, but not as favourable as that of cell wall synthesis inhibitors. The antibacterial drugs that inhibit nucleic acid synthesis or damage cell membranes often are not as selective toxic as other antibiotics. This is because procaryotes and eucaryotes do not differ as greatly with respect to nucleic acid synthetic mechanisms or cell membrane structure. Good examples of drugs that affect nucleic acid synthesis or membrane structure are quinolones and polymyxins. Quinolones inhibit the DNA gyrase and thus interfere with DNA replication, repair and transcription. Polymyxins act as detergents or surfactants and disrupt the bacterial plasma membrane.

**HIV virus**

AIDS, caused by the HIV virus, is one of the world’s leading causes of death with a major medical and economic impact on society. The current combination therapy of three or more drugs is increasing the survival of HIV-infected patients by many years and provides an improved quality of life [72-74]. The present therapies are mainly based on the inhibition of two key viral enzymes: HIV reverse transcriptase (RT) and HIV protease (PI) as well as the inhibition of viral fusion. Structure of RT is depicted in Figure 1.4. Based on the chemical structures and the inhibitory mechanism, the RT inhibitors can be divided into nucleoside and nucleotide RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs) which bind to an allosteric site of HIV-1 RT located about 10 Å away from the catalytic site.
Inhibitors of HIV RT

Since reverse transcriptase is virus-specific and essential for virus replication, and exerts its function before the irreversible integration of the viral DNA into the host genome, it is an excellent target enzyme to inhibit retroviral replication in an HIV infected individual. Over the last 15 years a variety of compounds, identified to interact with the RT, can be divided into four distinct groups of RT inhibitors [75-76].

- 2', 3'-Dideoxynucleoside analogues, designated nucleoside RT inhibitors or NRTIs,
- Acyclic nucleoside phosphonate analogues (ANPs), designated as nucleotide RT inhibitors (NtRTIs),
- Pyrophosphate analogous and
- Non-nucleoside RT inhibitors (NNRTIs).

**NNRTIs (Non-nucleoside Reverse Transcriptase Inhibitors)**

A structurally diverse group of compounds, which comprises at least 50 distinct structural classes different from the nucleoside structure, is designated as non-nucleoside reverse transcriptase inhibitors (NNRTIs) [77-79]. All these NNRTIs bind to the NNRTI binding pocket: an allosteric hydrophobic site close to, but distinct from,
the catalytic active site of the RT[80]. As they are inhibitory against RT as such, NNRTIs do not require intracellular metabolic activation. NNRTIs are non-competitive (or mixed-type) inhibitors with respect to the substrates and non-competitive or uncompetitive inhibitors with respect to the template/primer. Whereas NRTIs and NNRTIs do not markedly discriminate between RTs from different lentiviruses, the NNRTIs are highly specific for HIV-1 RT and do not recognize other lentiviral RTs or any other DNA or RNA polymerases from viral or cellular origin. This explains the highly selective anti-HIV-1 activity of the NNRTIs [81]. NNRTIs that are approved for clinical use by the FDA are Nevirapine (I), Efavirenz (II) and Delavirdine (III), whereas 9-Cl-TIBO (IV), Dapivirine (V), Etravirine (VI) and Rilpivirine (VII) are currently subject to clinical phase I/II studies, either for potential microbicide use (Dapivirine) or for systemic (oral) therapy of HIV-1.

The general structure of an NNRTI resembles that of a butterfly [82]. One wing contains a substituted aromatic ring and can form a hydrogen bridge to the peptide main chain of K101. The other wing contains often a π-electron system that interacts with the aromatic residues of Y181, Y188 and W229. The body of the butterfly is polar and close to Y181 and results in a movement of the hydroxyphenyl group of
this amino acid upon binding. Although most NNRTIs adapt such a shape, several NNRTIs do have impaired wings like Delavirdine, Dapivirine, Etravirine and Rilpivirine are Diarylpyrimidine (DAPY) derivatives and resemble a horseshoe rather than a butterfly shape. Structural and molecular modeling studies of these inhibitors with HIV-1 RT revealed a binding to RT in different conformations. The torsional flexibility of the inhibitors can generate numerous conformational variants, and the compactness of the inhibitors permits significant repositioning and reorientation (translation and rotation) within the pocket. Such adaptations appear to be critical for the ability of the DAPY compounds to retain their potency against a wide range of drug-resistant HIV-1 RTs [83-85].

Figure 1.5 NNRTI pocket with inhibitor and relevant amino acid residues

TSAO NNRTI molecules (VIII), which have a totally different structure comparing to other NNRTIs, seem to interact with a binding site at the interface between p66 and p51 of HIV-1 RT, which is distinct from the binding site of the other NNRTIs [86-87]. These unusual properties of the TSAO NNRTIs were found by modeling of the TSAO molecules in the RT, and proved to be in agreement with biochemical, enzyme
kinetic and structural data and are also in agreement with the observation that TSAO molecules enhance urea- and acetonitrile-mediated destabilization of the dimmer\([88-89]\). These findings point to an interaction of TSAO with the earlier described $\beta7-\beta8$-loop of the p51 subunit. Therefore, this area in RT can be potentially used as a novel target site on RT for inhibitor design either by small molecules like TSAO derivatives or by oligopeptides or peptidomimetics. Previous studies have shown that the tryptophan-rich area at the p66/p51 interface can be targeted by oligopeptides resulting in destabilisation of RT and even a pronounced anti-HIV activity in cell culture [90-92].

**Figure 1.5** represents the structure of HIV-1 RT in which the fingers, palm, thumb, connection, and RNase H domains in p66 are colored blue, red, green, yellow, and orange, respectively. The p51 subunit is colored gray. Important sites, including the dNTP-binding site (in gold), RNase H active site (in orange), and NNRTI-binding site (in cyan), are highlighted. The nucleic acid (brown and purple ribbons) binding cleft extends from the polymerase active site to RNase H active site.

**Heterocyclic compounds in drug design and discovery**

Heterocyclic chemistry deals exclusively with the synthesis, properties and applications of active drug design and discovery from many years [93-97]. The Chemistry of heterocyclic compounds continues to be an active field in the medicinal chemistry [98-100]. Finding the treatment for various diseases has stared since several decades and it is integral part of mankind. The common procedures for preparation of novel class of therapeutic agents involve exploitation of recognized lead compounds. Heterocycles are common fragments of the vast majority of marketed drugs. This is a reflection of the central role that heterocycles play in modern drug design [101-104]. They can serve as useful tools to manipulate lipophilicity, polarity, and hydrogen bonding capacity of molecules, which may lead to improve pharmacological, pharmacokinetic, toxicological, and physicochemical properties of drug candidates and ultimately drugs. Heterocycles are routinely used as bioisosteres for a variety of functional groups in drug candidates. Medicinal chemistry is the science that deals with the discovery and design of new therapeutic
chemicals and their development into useful medicines. Heterocyclic compounds containing nitrogen and sulfur atoms are of great importance in the field of pharmaceutical chemistry due to their variety of biological activities and considerable diversity in the ring system. Heterocycles play a central role in the design of therapeutic molecules. They are utilized to optimize potency and selectivity through bioisosterism, pharmacokinetic and toxicological properties by offering wide opportunities to adjust lipophilicity, polarity, and solubility of the target molecules. It should also be noted that sometimes the desirable result of the heterocycle incorporation into the molecule comes at the expense of negative changes of other parameters of the drug. Recognition of the most important properties for the particular target and their careful manipulation to achieve optimal compromises between potency, selectivity, pharmacokinetic properties and toxicity is the hardest part of the medicinal chemists' job [100, 102, 105-106].

Heterocyclic compounds have so far been synthesized mainly due to their wide range of biological activities [107-109]. Hence, much attention has been paid in the past several years to the synthesis of heterocyclic compounds bearing nitrogen and sulfur containing ring systems [110].

The work presented in this thesis has been humble effort in the field of medicinal chemistry though very minute even to be regarded as a small step in offering a practical solution to the innumerable problems, nevertheless, a devoted effort to sincerely contribute to a healthier and happier human life. The work deals with some of the commonest ailments viz. bacterial and fungal infections. Even today these irritate physicians at times, when coupled with other chronic conditions and also due to the resistance offered by infecting organisms to various forms of therapy [41]. The work described in this thesis is yet a modest attempt to synthesize various heterocycles via s-triazine, quinazoline, quinoline, etc. which with the aim of obtaining a bioactive molecule, their characterization and evaluation of their biological potential.
References


Chapter 1


B: Plan of work

- **s-Triazine**

1,3,5-triazine or s-triazine and its derivatives are widely used within the pharmaceutical, textile, plastic, rubber industries, and as pesticides, dyestuffs, optical bleaches, explosives, and surface active agents. Currently, to fight against drug resistance problems and to alleviate opportunistic microbial infections, many researchers have reported 1,3,5-triazine core molecules exhibiting excellent biological activity including anti-microbial, anti-HIV, anti-malarial etc [1].

Synthesis, characterization and anti-microbial activity of 2,4,6-tri-substituted-[1,3,5]-triazine derivatives (I) were carried out by Gavade et al. [2] Some of the compounds appeared with 6.25–12.5 µg/ml MIC range.

![Chemical structure of compound I](image)

Where, R= Substituted amines

Patel et al [3] synthesized 3-(5-sulfanyl-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (II) and evaluated their antimicrobial activity. *In vitro* antibacterial activity result shows that the biological potential of some of the compounds found between 12.5-100 µg/ml MIC value.

![Chemical structure of compound II](image)

Where, X= N, CH₂ or
R= Aliphatic/aromatic or heterocyclic groups

Modh et al [4] reported novel hybrid quinazoline–triazine as potential anti-HIV and anti-microbial agents (III). Anti-HIV activity was measured against HIV-1 (IIIB) and HIV-2 (ROD) viral strains and *in vitro* anti-microbial activity against various bacteria.
Besides this study, they [5] recently executed 3-substituted coumarinyl-triazine derivatives (IV) linked to oxadiazole as promising anti-microbial agents.

Using 1,3 dipolar cycloaddition reaction of C<sub>60</sub> and azomethine ylides, Kumar and Menon [6] prepared novel fullerene derivatives bearing s-triazine nucleus (V) and reported as efficient anti-microbial agents.

Novel s-triazine hybrid analogues (VI) have been synthesized and screened for their anti-fungal activity against different strains. Among the synthesized compounds, mono substituted triazine compounds appeared with better activity than the corresponding di and tri-substituted triazine hybrids [7].
Bhat et al [8] synthesized hybrid 4-aminoquinoline-1,3,5-triazine derivatives (VII) which were emerged as good to potent anti-bacterial agents than standard Ofloxacin.

2-Fluorophenyl-4,6-di-substituted-s-triazines (VIII) were synthesized by Saleh et al [9] and tested for their anti-microbial activity.

Singh et al [10] reported hybrid thiazole-1,3,5-triazines (IX) bearing diphenyl amine moiety that showed excellent anti-fungal activity (0.32 µg/ml). Potency and non-toxicity of synthesized compounds drew a lead in drug-likeness evaluation.
By anticipating various 2,4,6-tri substituted \(s\)-triazine derivatives (X), Srinivas et al [11] reported their \textit{in vitro} anti-microbial assay. Some of the compounds showed the best activity towards the tested strains.

![Diagram of compound X]

Where, X= -NH or -O-; 
R= H, Cl, CH\(_3\), OCH\(_3\)

Bhat and co-workers [12] conjugated 4-aminoquinoline with s-triazine core (XI) to prepare potential biologically active congeners and showed excellent inhibition growth with MIC of 3.125-12.5 \(\mu\)g/ml.

![Diagram of compound XI]

Where, R= Substituted amine or Morpholine

\(N\)-chlorinated alkoxy s-triazine derivatives (XII) were synthesized and assessed for their anti-microbial efficacy [13].

![Diagram of compound XII]

Where, X= H, or Cl; n= 2, 6 or 10

\(N^1\)-Benzyl and \(N^1\)-benzyloxy-1,6-dihydro-1,3,5-triazine-2,4-diamines (XIII) were evaluated for anti-microbial activity by Ma et al [14]. Some compounds were appeared with potent activity against \textit{M. smegmatis} strain.
Venkatraj et al [15] studied novel triazine dimers as anti-viral agents (XIV). Several compounds showed submicromolar activity against wild-type HIV-1 in TZM-b1 cells and moderate activity against single mutant strains.

A novel series of 6-naphthyloxy substituted diaryl triazine (DATA) analogous (XV) were implemented to evaluate anti-HIV activity in MT-4 cells by Xiong et al [16]. Compounds with –N methyl and amine substituent found more potent with IC\textsubscript{50} = 9.3 nM, selectivity index (SI) = 15,385 and IC\textsubscript{50} = 9.4 nM, SI = 14,094 respectively, which was found 15 times more active than standard Nevirapine.

Liu et al [17] synthesized 4,6-diamino-1,3,5-triazin-2-ol compounds (XVI) as novel HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs).
Lozano et al [18] synthesized triazine derivatives (XVII) and functionalized with aromatic amino acids to give monomers, dimers & trimers and concluded that trimers were efficient binders of HIV-1 (gp120) at 1.6-2.8 µM range.

DATA analogues (XVIII) with a fluorinated phenyl group at C-2 position of the s-triazine core were prepared by Xiong et al [19]. From the results, it was concluded that fluorine containing compounds inhibited HIV-1 replication in MT-4 cells with promising SI range.

By molecular modelling, Jorgensen et al [20] proved various s-triazine congeners (XIX) as potential lead toward variant forms of HIV-1. Compounds showed low-nanomolar potency against both wild-type HIV-1 and the Tyr181Cys variant as well.
A novel series of 2-amino-1,3,5-triazines bearing a tricyclic moiety (XX) as heat shock protein 90 (Hsp90) inhibitors is described by Suda and co-workers [21].

By incorporating benzene sulfonamides linked 1,3,5-triazinyl moieties (XXI), Saluja et al [22] attempted to prepare potential human carbonic anhydrase inhibitors. The resultant derivatives appeared with promising biological profile.

Solankee and co-workers [23] designed and synthesized substituted s-triazinyl chalcones (XXII) which was further derivatized to various pyridine, pyrimidine, pyrazoline analogues. Some of the compounds appeared with the best pharmacological profile with lower MIC and MBC values.
Based on these observations, it was thought to consider s-triazine as a principle moiety which is then derivatized to generate active pharmacophore with anticipation of promising pharmacological profile.

➢ **Thiazolidinone**

The diversity in the biological response of 4-thiazolidinones has attracted the attention of many researchers to explore this framework for its potential. It is, therefore, of prime importance that the study of this topic and the development of new synthetic strategies should be based on the most recent knowledge, emerging from the latest research [24]. Moreover, thiazolidin-4-one derivatives are also reported to have important biological activities such as anti-inflammatory [25], anti-tuberculosis [26], anti-cancer [26-27], anti-tumor [28], anti-HIV [29], anti-bacterial [30], anti-fungal [31], anti-oxidant [32], anti-viral [33], anti-convulsant [34], diuretics [35], nematicidal [36], anti-histaminic [37] activity etc.

In this ongoing track to minimize the rapid multidrug-resistance in pathogenic microbes, our group [38] has recently reported s-triazine derivatives (XXIII) containing thiazolidinone and piperazine moieties as potential anti-mycobacterial agents. Some compounds exhibited promising activity towards tested strain.

![Image of chemical structure (XXIII)](image-url)

(XXIII) Where, R= H, F, Cl, Br, OH; X= CH, N

Kumar *et al* [39] synthesized bioactive hybrid thiazolidin-4-one-1,3,5-triazine derivatives (XXIV) and evaluated for their biological profile. Among which majority of compounds displayed promising biological activity.

![Image of chemical structure (XXIV)](image-url)

(XXIV) Where, R₁, R₂, R₃= Cl, F or NO₂
A series of thiazolidin-4-one fused s-triazines (XXV) were synthesized by Patel et al [40] and further subjected to evaluate their anti-microbial and anti-cancer potency. Some of the compounds effectively inhibited the growth and replication of cancer cells.

![Chemical Structure (XXV)](image)

Patel et al [41] synthesized fluoroquinolone-based benzothiazolyl-4-thiazolidinone hybrids (XXVI) as efficient anti-microbial agents which showed least MIC of 1–2 μg/mL. Some of the derivatives were proved as potent biologically active molecules than standard drugs.

![Chemical Structure (XXVI)](image)

A series of novel thiazolidin-4-one analogues (XXVII) were synthesized and evaluated their anti-HIV activity as potential NNRTI agents [42]. Some of the synthesized derivatives demonstrated noticeable binding affinity and interaction towards RT enzyme.

![Chemical Structure (XXVII)](image)

Where, R= NO₂, OH, OCH₃, H, F, Cl, Br, OH; X= CH, N

Where, R= F, Br, CN, CH₃, NO₂

Where, R₁, R₂= F, Cl;
Ar= 4-Cyanophenyl, pyridin-2-yl
Sharath Kumar et al elaborated anti-proliferative effect of 2, 3-disubstituted 4-thiazolidinone analogues (XXVIII) on human leukemic cells [43]. Some of the compounds displayed potent activity in range of 9.71 to 19.29 µM against various cells.

![Chemical Structure of XXVIII](image)

(XXVIII) Where, R= H, Substituted phenyl

Desai et al [44] have published a series of 6-(1H-benzo[d]imidazol-2-yl)-2-(2-(3-nitrophenyl)-4-oxothiazolidin-yl)-4-(aryl)nicotinonitriles (XXIX) with superior antimicrobial activity. Some of the compounds showed impressive results for in vitro cytotoxicity studies against cancer cells.

![Chemical Structure of XXIX](image)

(XXIX) Where, R= H, Cl, OH, CH₃, NO₂

A series of 5-(carbamoymethoxy)benzylidene-2-oxo/thioxo-4-thiazolidinone analogues (XXX) was presented as potential aldose reductase inhibitors and anti-inflammatory agents by Maccari and co-workers [45]. Some of the derivatives endowed with significant inhibitory ability against various species.

![Chemical Structure of XXX](image)

(XXX) Where, X= O, S; R, R₁= H, OCH₃, OCH₂CONH₂
4-[(5-Arylidene-4-oxo-2-phenylimino/oxothiazolidin-3-yl)methyl] benzoic acid conjugates (XXXI) were efficiently prepared [46] and screened their inhibitory activity towards human protein tyrosine phosphatases. Synthesized compounds exhibited moderate to significant selectivity towards C2C12 skeletal muscle cells in mouse.

Bhosle et al [47] approached drug designing of 2-hydrazolyl-4-thiazolidinone-5-carboxylic acid (XXXII) compounds as anti-hyperglycemic agents. Compounds displayed significant anti-hyperglycemic activity in sucrose loaded rat model.

Pardasani and co-workers [48] have condensed 1,2-naphthaquinone and thiazolidin-4-one (XXXIII) which were further subjected for anti-bacterial and anti-fungal screening. Compounds showed remarkable activity as compared to standard drug.

Where, X= O, NPh; 
R, R1= H, OCH3, OCH2CH2Ph
Where, R= H, CH3, F, Br, OH, NO2
Where, X= S, NH
Anticipating computational approach, Devinyak et al [49] showed possible anti-cancer effects of 4-thiazolidinone conjugates (XXXIV) towards various tumor cell lines.

![Chemical Structure of XXXIV]

Where, R= Substituted phenyl;  
R₁= H, COCH₃; R₂= Cl, Br, CH₃

Based on these observations and findings, we have tempted to synthesize s-triazine clubbed thiazolidinone with anticipation of augmented their biological profile.

**Chalcone**

In drug development programme, the chalcone moiety remains a popular scaffold amongst medicinal chemists owing to its structural simplicity. The versatility of chalcone and its wide range of applicability in medicinal chemistry have attracted scientists all over the globe to concentrate their research around it. Chalcones showed diverse biological activities such as anti-inflammatory [50], anti-HIV [51], anti-microbial [52], anti-malarial [53], anti-cancer [54] and anti-leishmanial [55] activities.

Kumar et al [56] prepared pyrimidine based chalcones (XXXV) and evaluated its antimicrobial efficacy against bacterial strains. Some of the compounds appeared with promising activity related to the standard drugs.

![Chemical Structure of XXXV]

Where, R= Phenyl, benzyl, 
4-methyl phenyl, 4-chloro phenyl
A series of 2,5-dimethyl-3-furyl chalcones (XXXVI) has been synthesized and screened for their anti-microbial and insect anti-feedant activities by Subramanian and co-workers [57].

![XXXVI](image)

(XXXVI) Where, X= H, 4-F, 4-Cl, 4-Br, 3-OH, 4-OH

Applying Claisen–Schmidt condensation, Vanangamudi et al [58] synthesized 2,5-dimethyl-3-thienyl chalcones (XXXVII) and examined their anti-microbial, anti-oxidant and insect anti-feedant activities. Majority of compounds exhibited significant anti-fungal activities towards tested strains.

![XXXVII](image)

(XXXVII) Where, X= H, 4-F, 4-Cl, 4-Br, 3-OH, 4-OH

Joshi and co-workers [59] attempted to prepare a series of chalcones-bearing 1,3,4-oxadiazole derivatives (XXXVIII) and subjected for anti-microbial screening against broad panel of bacteria and fungi.

![XXXVIII](image)

(XXXVIII) Where, R= 4-Cl, 4-OH, 4-NO₂, 2-NO₂, 4-Br, 2-OH

HIV-integrase inhibitory activity and relevant computational approach of some 3-methyl chalcones (XXXIX) have been elaborated by Babu et al [60]. Few of the derivatives possessed active potency towards viral enzymes.
Kakati et al [61] carried out synthesis of steroidal chalcones (XL) screened for anti-microbial activity. Compounds exhibited potent anti-microbial activities against some pathogens.

Anti-tubercular activity of chalcone derivatives (XLI) was performed and further directed to QSAR and docking studies by Yadav et al [62]. In silico screening showed high binding affinity of active derivatives.

Two series of chalcone based sulfone and bisulfone derivatives (XLII) were prepared and evaluated for their anti-microbial activities [63]. Some of the derivatives endowed with excellent inhibitory action against various bacterial strains.
Mai and co-workers [64] have synthesized a library of chalcone analogues (XLIII) as anti-proliferative agents. Some of the molecules displayed the most potent and selective anti-cancer activity against TRAIL resistant cancer cells.

\[
\text{\textbf{XLIII}} \quad \text{Where, } R, R_2 = H, OH, OCH_3, NH_2; \\
R_1, R_3, R_4, R_5 = H, OCH_3; \\
R_6 = H, OH, OCH_3, Cl; \\
R_7 = H, phenyl, OH, OCH_3; \\
R_8 = H, Cl, Br, NO_2, F
\]

\[N,N\)-dialkyl substituted chalcones (XLIV) have been synthesized as potential anti-cancer conjugates [65]. Few of the derivatives were appeared with good activity against MCF-7 cell lines at low micro-molar concentrations.

\[
\text{\textbf{XLIV}} \quad \text{Where, } X = \text{CH}_2, \text{O, NCH}_2\text{CH}_2\text{OH}; \\
R, R_1, R_2 = H, Cl, Br, \text{CH}_3, \text{OCH}_3
\]

2,4,5-Trimethoxy chalcones and its analogues (XLV) were prepared by Shenvi et al [66] and evaluated their anti-cancer and anti-oxidant activities. Compounds exhibited remarkable in vitro tumour cell inhibitory activity.

\[
\text{\textbf{XLV}} \quad \text{Where, } R = H, OH, OCH_3, OAc; \\
R_1 = H, NO_2, CF_3, OCH_3; \\
R_2 = H, OH, Cl, CH_3, OCH_3; \\
R_3 = H, Cl, OH, OAc; R_4 = H, OCH_3
\]

Moreover, Dinesha et al [67] have also evaluated potential anti-cancer and anti-oxidant activity of imidazoquinoline carrying biologically active chalcones (XLVI).
A series of novel isoxazolyl chalcones (XLVII) were reported as potent anti-lung cancer agents. Compounds, executed by Wan et al [68], displayed significant cytotoxicity against some cell lines.

A series of 4-aminoquinolinyl-chalcone amides (XLVIII) has been synthesized by Smit et al [69] and checked their anti-malarial and anti-cancer efficacy against various cell lines. Some of the compounds were found as the potent drug-candidate compared to reference compound.

Roussaki and co-workers [70] have synthesized quinolinone–chalcone hybrids (XLIX) and observed their anti-parasitic activity. Based on the cytotoxicity and potency, selected compounds were proved active candidate for future drug-development.
In vitro blood stage anti-plasmodial activity of a series of allylated chalcones (L) based on the licorice chalcone A as lead molecule was investigated by Sharma et al [71]. Some of the chalcone derivatives displayed promising drug-like properties.

\[
\text{(L)}
\]

Where, \( R = H, \text{CH}_3, \text{allyl}; \)
\( R_1 = \text{Cl, Br, OH, OCH}_3 \)

Bano et al [50] performed a series of 2’-hydroxy chalcones and 2’-methoxy chalcones (LI) and evaluated for their anti-inflammatory activity in carrageenan induced rat paw oedema model. Some of the analogues appeared with good inhibitory action with very low gastric ulcerogenicity.

\[
\text{(LI)}
\]

Where, \( R = \text{phenyl, 4-chloro phenyl, 3-hydroxy phenyl, 2-chloro phenyl} \)

A library of ring-A-mono substituted chalcone derivatives (LII) has been prepared and observed to possess remarkable anti-inflammatory and anti-oxidant activities [72].

\[
\text{(LII)}
\]

Where, \( R = \text{NH}_2, \text{N}_3, \text{CF}_3, \text{CN, NHSO}_2\text{CH}_3 \)

Gupta and co-workers [73] have carried out the synthesis of some chalcone derivatives (LIII) and screened their in vitro and in vivo anti-leishmanial activity. Some of the compounds showed impressive 75-83% parasite inhibition with good potency.

\[
\text{(LIII)}
\]

Where, \( R = H, \text{Cl}; \)
\( R_1 = H, \text{CH}_3 \)
As the utility of propenoyl derivatives, it was thought worth to synthesize various chalcones on our principle moiety 2,4,6-trichloro-s-triazine.

➤ Amino benzonitrile/2,4,6-trimethyl aniline
A class of 5,7-disubstituted pyrazolo [1,5-a]pyrimidine (LIV) derivatives was synthesized by Tian et al [74] and subjected for evaluation of anti-HIV activity. Compound bearing 4-amino benzonitrile and 2,4,6-trimethyl aniline moieties was found to be the most promising analogue with an EC\textsubscript{50} of 0.07 µM and SI: 3999.

![Chemical Structure](LIV)

Where, R= 2,4,6-TriCH$_3$, 2,6-diCH$_3$, 3,5-diCH$_3$, 2,6-diCH$_3$-4-CN; $R_1$= CN, F, Cl, Br, NO$_2$

A series of new diarylpyrimidine conjugates (LV) containing 4-amino benzonitrile motif has been discovered by Yan and co-workers [75] and evaluated as wild-type HIV-1 NNRTI inhibitors. Remarkable binding affinity to RT pocket and EC\textsubscript{50} values in range of 0.005-0.009 µM of benzonitrile analogues proved their potential as potent drug-molecules.

![Chemical Structure](LV)

Where, X= Cl, Br; R, $R_1$= H, CH$_3$; $R_2$= phenyl, 4-CH$_3$ phenyl, 4-OCH$_3$ phenyl, 3-F phenyl etc.

Approaching structure-guided core-refining method, Li et al [76] synthesized 4-amino benzonitrile bearing pyrimidine derivatives (LVI) as potent anti-HIV agents. Some of the compounds showed excellent NNRTI activity with 4647-13740 SI range.
Chen et al [77] prepared a series of 4-phenoxy-6-(phenylamino)pyridin-2(1H)-one derivatives (LVII) as potent HIV-1 NNRTIs. Compounds executed superior inhibitory action with better EC$_{50}$ level (0.15–0.84 µM).

A series of substituted nitropyridine derivatives (LVIII) were prepared and evaluated its potential as efficient anti-HIV agents by Wang et al [78]. The H-bonding interaction due to 4-amino benzonitrile moiety and low EC$_{50}$ values in range of 0.034-0.16 µM pointed towards the superior activity of the synthesized molecules.

Hranjec et al [79] have discovered benzonitrile and nicotinonitrile based benzofuran-2-carboxamides (LIX) and tested for their anti-proliferative potency against various
tumor cell lines. The analogs displayed excellent activity against tested cancerous cells.

\[
\text{(LIX)} \quad \text{Where, } X = \text{CH, N}
\]

Diaryl sulfonamide as agonists (LX) of the free fatty acid receptor 4(FFA4/GPR120) has been discovered by Sparks and co-workers [80]. 2,4,6-Trimethyl aniline bearing sulfonamide derivative was found the promising compound as a selective FFA4 agonist.

\[
\text{(LX)} \quad \text{Where, } R = \text{H, 4-F, 4-Cl, 4-Ac, 4-CH}_3, 4\text{-COOH, 2-CH}_3
\]

Based on these assumptions, we designed following molecules keeping hyperconjugation effect of methyl group i.e. 2,4,6-trimethyl aniline, tight binding characteristic, i.e. hydrogen bonding to the respective enzyme, of p-amino cyano benzene with anticipation of interesting anti-HIV/ NNRTI characteristic.

\[
\text{Bioactive moieties}
\]

\[
\text{s-Triazine-Chalcone hybrid} \quad \text{s-Triazine-4-thiazolidinone hybrid}
\]

Rationalization of Work
Quinazoline

The chemistry of quinazoline compounds has been known from centuries; besides this the intense search for biologically active substances in this series began from last several decades. Earlier research in nineteen fifties and sixties revealed effectiveness of quinazolines not only as anti-malarial but also against various diseases caused by bacteria, protozoa and virus. Quinazoline nucleus can be termed as ‘Master key’ for biological therapy as it is an important scaffold of many reported medicinal agents [81].

Zhang et al [82] have synthesized a series of 4-(4-substituted piperazin)-5,6,7-trialkoxy quinazoline derivatives (LXI) and determined its anti-cancer activity. Some of the compounds showed the lowest IC$_{50}$ values in the range of 1.8 to 2.9 µM.

A series of 1,2,4-triazolo[1,5-a]quinazoline compounds (LXII) has been reported [83] and examined for in vivo anti-hypertensive evaluation for rats and mice. Representative compounds were emerged as potential adrenoblockers and cardiac stimulant.

Hybrid regioisomeric molecules, based on quinazoline and benzimidazole moieties (LXIII), have been synthesized [84] and further subjected to anti-tumor evaluation.
against 60 cancer cell line panel. Some of the derivatives exhibited remarkable anticancer activity.

Patel et al [85] have synthesized \( N\)-(benzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2yl)phenyl)amino)acetamides analogs (LXIV) and examined for microbiological activity. Some of the synthesized analogs demonstrated excellent anti-microbial activity with 6.25-25 µg/ml of MIC.

A series of quinazolines, triazoloquinazolines and triazinoquinazoline bearing a sulfonamide moiety (LXV) have been synthesized [86] and tested for their antimicrobial activity. Some of the compounds appeared with promising bacterial growth inhibition.
Akbari et al [87] have prepared 3-[2-Substituted-4-oxo-3,4-dihydroquinazolin-6-yl]sydnones (LXVI) and screened for their anti-microbial activity. Most of the derivatives appeared with good activity against bacterial species.

\[
\text{(LXVI)} \quad \text{Where, } R = \text{H, 4-NO}_{2}, 2-\text{COOCH}_{3}, 4-(\text{NCH}_{3})_{2}, 2-\text{COOCH}_{3}
\]

1-N-substituted-3-(4-(5-(pyridin-3-yl)-1,3,4-oxadiazol-2-ylthio)quinazolin-6-yl)urea/thiourea derivatives (LXVII) have been reported [88] and evaluated for their anti-microbial activity. Some of the compounds appeared with a broad spectrum of activity against tested species.

\[
\text{(LXVII)} \quad \text{Where, } X = \text{O}, \text{S} ; R = \text{H, 2-Cl, 3-Cl, 4-Cl, 2-CH}_{3}, 3-\text{CH}_{3}, 4-\text{CH}_{3}, 2-\text{OCH}_{3}
\]

Spulák et al [89] have introduced bronchodilatory activity of quinazolines and quinoxalines analogues (LXVIII). Some of the compounds appeared with excellent bronchodilatory effect at low toxicity.

\[
\text{(LXVIII)} \quad \text{R} = \text{, , , , ,}
\]

Novel tricyclic oxazine and oxazepine fused quinazolines (LXIX) have been designed, synthesized [90] and evaluated their anti-tumor activity. Some of the compounds appeared with potent activity against cancer cell lines.
Ravez et al [91] have synthesized series of 7-aminoalkoxy-4-aryloxy-quinazoline ureas (LXX) as multi-tyrosine kinase inhibitors. Analogues also appeared with good anti-proliferative activity on cancer cell lines.

Based on these observations, quinazoline is taken as principle moiety.

**Quinoline**

Quinoline is a remarkable, interesting nitrogen heterocyclic scaffold of paramount importance to human race. Its skeleton is often an attractive framework in the design of several synthetic compounds against diverse pharmacological targets leading to the discovery of potent drugs. The utility of quinoline derivatives in the areas of medicine, food, catalyst, dye, materials, refineries and electronics is well established. These compounds are found to possess various bioactivities such as anti-malarial [92], anti-bacterial [93], anti-fungal [94], anti-cancer agents [95].

Tabassum et al [96] have synthesized 2-chloroquinolin-3-yl ester derivatives (LXXI) and evaluated their ABTS radical-scavenging activity and anti-microbial activities. Some of the compounds appeared with excellent anti-bacterial and anti-fungal activity.

Where, \( R= \) substituted piperazine, morpholine, aliphatic amine
Where, \( X= \) H, Cl, CH\(_3\); \( R_1=\) OCH\(_3\), OC\(_2\)H\(_5\);
\( R_2=\) O(CH\(_2\))\(_2\)NEt\(_2\), O(CH\(_2\))\(_2\)N-piperidine
Where, \( R=\) OCH\(_3\), CH\(_3\); \( R_1=\) CH\(_3\), 2,4-diChlorobenzene, 2,6-diChlorobenzene
Quinoline derivatives containing pyrazoline-5-one and pyrazole motif (LXXII) were synthesized [97] and evaluated their anti-microbial activity. Majority of compounds showed promising anti-microbial activity against bacterial strains.

![LXXII](image)

Where, R= 4-Cl, 4-Br, 4-F, 4-OCH₃, 4-CH₃, 4-COOH, 2-OH

Arayne et al [98] have synthesized a 5-amino-1-cyclopropyl-7-[(3R,5S)3,5-dimethylpiperazine-1-yl]-6,8-difluoro-4-oxo-quinoline-3-carboxylic acid derivatives (LXXIII) and screened for their anti-microbial, anti-fungal, and urease inhibitory studies against different panel of microorganisms.

![LXXIII](image)

Where, R= H, OH, NH₂

A series of sulfur attached quinolines and quinoline–coumarin bisheterocycles (LXXIV) have been synthesized [99] and examined their anti-microbial and cytotoxic activity. Majority of compounds demonstrated moderate to good anti-microbial activities.

![LXXIV](image)

Where, Ar= C₆H₅, 4-CIC₆H₄, 3-CIC₆H₄, 3-BrC₆H₄, 4-PhC₆H₅
Mistry et al [100] have reported quinoline-based azetidinone and thiazolidinone analogues (LXXV) as active anti-microbial and anti-tuberculosis agents. Some of the newly synthesized analogues appeared with promising anti-microbial activity.

![Chemical structure of LXXV](image)

Quinoline based 1,2,4-triazol-3-ylthio-acetamides (LXXVI) were synthesized [101] and evaluated for in vitro anti-microbial potency. Some of the compounds displayed good spectrum of anti-fungal activity.

![Chemical structure of LXXVI](image)

Khan et al [102] have reported anti-microbial evaluation of substituted ethyl 2-(quinolin-4-yl)-propanoates (LXXVII) and discussed their SAR studies.

![Chemical structure of LXXVII](image)

A series of 3-((6-(2,6-dichloroquinolin-3-yl)-4-aryl-1,6-dihydro-pyrimidin-2-yl)thio) propanenitriles (LXXVIII) has been elaborated [103] and further tested for their biological potential. Some of the compounds showed promising anti-tubercular activity with 0.20-3.12 µg/ml of MIC.
Peng et al [104] have synthesized anilinoquinoline derivatives (LXXIX) and evaluated for their anti-tumor activities. Some of the compounds were proved as effective agents against some cancer cell lines.

5-(3-Alkylquinolin-2-yl)-3-aryl isoxazole derivatives (LXXX) were synthesized and their cytotoxic activity were determined [105]. Some compounds appeared with good cytotoxicity at low concentration.

Wang et al [106] have prepared a series of 6-amino-11H- indolo[3,2-c]quinoline derivatives (LXXXI) with various substituents on the quinoline ring and screened for cytotoxic activity against cancer cells. Some of the compounds showed selectivity at 0.052-0.112 µM concentration.
Three series of 8-trifluoromethylquinoline based 1,2,3-triazoles derivatives (LXXXII) were synthesized [107] and screened for their in vitro anti-microbial activity. Some of the compounds showed better activity against strains studied. Where, R=CH2CH3, CH2CH2CH3, CH2CH2CH2CH3.

Miniyar et al [108] have synthesized a series of 2-chloroquinoline fused pyrazole ring (LXXXIII) and evaluated their anti-bacterial and anti-fungal activity. The results obtained were promising against both bacterial and fungal strains. Where, R= H, Br, F, NH2, NO2; R1 = H, Br.

2-Thiophenoxyquinoline-based penta-substituted pyridine derivatives (LXXXIV) were synthesized [109] and investigated for their anti-microbial evaluation. Majority of the compounds were found active agents against strains studied. Where, R= H, CH3; R1, R2= H, CH3, Cl.
Wang et al [110] have reported a series of indolo[3,2-c]quinolines (LXXXV) as active anti-malarial agents. Some of the compounds showed excellent anti-malarial activity against different strains.

![Indolo[3,2-c]quinoline](image)

Where, R = H, 2-F, 2-Br, 2-Cl, 2-CH$_3$; R$_1$ = substituted alkyl amine

Based on the above mentioned bioactive profile of quinoline derivatives, we thought to keep quinoline nucleus as a basic pharmacophore and functionalized to a variety of reactions.

**Thiourea**

Abbas et al [111] have reported thiourea derivatives (LXXXVI) incorporating a hippuric acid moiety and tested against bacterial and fungal strains. Most of the compounds showed significant anti-bacterial and anti-fungal activities.

![Thiourea](image)

Where, R = C$_6$H$_5$, COC$_6$H$_5$, isonicotinonyl, SO$_2$C$_6$H$_5$

A series of acyl thiourea derivatives containing pyrazole ring (LXXXVII) were synthesized [112] and their anti-cancer activities were investigated. Pyrazole-acyl thiourea derivatives possess promising anti-cancer activity against cancer cells.

![Acyl thiourea](image)

Where, R = H, Cl, Me, NO$_2$ etc
Shah et al [113] have synthesized and studied anti-microbial and anti-tuberculosis activity of thiourea based quinazolin-4(3H)-one compounds (LXXXVIII). Majority of the compounds appeared with superior activity against bacterial strains.

\[
(LXXXVIII) \quad \text{Where, } R = \text{H, } 2-\text{Cl, } 3-\text{Cl, } 4-\text{Cl, } 2-\text{F, } 3-\text{F, } 4-\text{F, } 2-\text{CH}_3, \text{3-CH}_3, \text{4-CH}_3
\]

\[
p-(2,7-\text{Dimethyl-4-oxo-4H-thiazolo}[4,5-d]\text{pyridazin-5-yl})\text{benzenesulfonylthioureas (LXXXIX)} \text{ have been synthesized [114] as anti-microbial agents. Some of the compounds were found active against tested strains.}
\]

\[
(LXXXIX) \quad \text{Where, } R = \text{CH}_3, \text{C}_6\text{H}_5, \text{4-ClC}_6\text{H}_4
\]

Yao et al [115] prepared a series of sorafenib derivatives containing diaryl thiourea (XC) and examined their anti-proliferative activities. Most of the compounds exhibited potent anti-proliferative activity towards cancer cells.

\[
(XC) \quad \text{Where, } R = \text{CH}_3, \text{ benzyl, cyclohexyl, cyclopropyl; } R_1 = \text{2,4-diCl, 4-Cl, 3,4-diF}
\]

Pyrazoline substituted benzene sulfonyl urea/thiourea derivatives (XCI) were synthesized [116] and subjected for anti-hyperglycemic activity. Majority of the compounds showed significant activity to inhibit rat lens aldose reductase.

\[
(XCI) \quad \text{Where, } R = \text{benzyl, } C_4\text{H}_9; \quad R_1 = \text{H, 4-Cl,}
\]
A series of novel thiourea and urea derivatives containing 1,2,4-triazole moieties (XCII) were synthesized [117] and evaluated for their anti-fungal and larvicidal activity. Some of the compounds displayed good activity.

\[ \text{XCII} \quad \text{Where, } R = 4-\text{NO}_2, 4-\text{CF}_3, 2,6-\text{diCl}, 2,6-\text{triCl} \]

Khan et al [118] have reported \(N,N'\)-disubstituted thioureas (XCIII) and evaluated for their urease inhibition potential. The synthesized compounds showed a varying degree of \textit{in vitro} urease inhibition with IC\textsubscript{50} values 5.53 - 91.50 \(\mu\text{M}\) range.

\[ \text{XCIII} \quad \text{Where, } X = \text{N, H; } R = \text{H, Cl; } R_1 = 2-\text{Cl, 3-Cl, 4-Cl, 4-Br, 2,4-\text{diF, 3,4-\text{diCl, 2,3-\text{diCl}}} } \]

**Piperazine**

Wang et al [119] have synthesized metronidazole derivatives (XCIV) containing piperazine skeleton as potential anti-bacterial agents. These compounds appeared with good anti-bacterial activities against tested strains.

\[ \text{XCIV} \quad \text{Where, } R = \text{H, 4-F, 2,3-\text{diCl, 2-Cl, 2-F, 3-\text{OCH}_3, 3-Cl, 2-\text{OCH}_3, substituted benzhydryl} } \]

A series of 6-(4-substitutedpiperazin-1-yl)phenanthridine derivatives (XCV) were synthesized [120] and evaluated for their anti-tubercular activity. Some of the compounds showed significant activity with MIC ranging from 1.56 to 6.25 \(\mu\text{g/ml}\).
Patel et al [121] have reported $N$-[4-(piperazin-1-yl)phenyl]cinnamamide analogues (XCVI) and examined for anti-tubercular activity. Majority of compounds exhibited good anti-tubercular activity towards mycobacterial strain.

A series of 1,4-disubstituted piperazines (XCVII) were synthesized [122] and studied their binding and functional properties as $\alpha_1$-adrenergic receptor blockers. Some of the compounds showed blocking activity ranging from 46.73% - 94.74%.

Arnatt et al [123] have synthesized piperazine (XCVIII) based chemokine receptor CCR5 antagonists as anti-prostate cancer agents. A number of compounds showed promising anti-prostate cancer activity and reasonable cytotoxicity profiles.
Bhosale et al. [124] have described biphenyl moiety linked with aryl piperazine derivatives (XCIX) along with their anti-psychotic activity. Some of the compounds appeared with remarkable anti-psychotic potential.

Hydroxyethyl substituted piperazines (C) with different substituents at the N-atoms were reported [125] and further examined for their ability to inhibit selectively the growth of human tumor cell lines. Many of the derivatives appeared with good potency against cancer cells.

Ma et al. [126] have synthesized series of [1,2,4]triazolo[3,4-a]phthalazine and tetrazolo[5,1-a]phthalazine derivatives (CI) bearing substituted piperazine moieties and evaluated for their positive inotropic activity. Several compounds showed favorable activities in terms of the chronotropic effects.
A series of piperazine ureas (CII) were synthesized [127], and evaluated for their potential as orally efficacious fatty acid amide hydrolase (FAAH) inhibitors.

Moreover, Guo et al [128] have prepared 1-aryloxyethyl piperazine derivatives (CIII) as Kv1.5 potassium channel inhibitors. Some of the compounds showed acceptable pharmacodynamics profile with very low acute toxicity.

Above literature survey reveals the biological potential of quinazoline, quinoline, thiourea and piperazine. On account of above activity profile, we were tempted to club these moieties and study their biological potential along with individual motif to the basic pharmacophore and study the electronic effect in their biological profile. The designed molecule with their rationally is mentioned herewith.
Rationalization of Work
References


[34] Ragab, F.; Eid, N. M.; El-Tawab, H. *Pharmazie*, **1997**, *52* (12), 926-929.


