

**SYNTHESIS AND STUDY OF THIENOPYRIMIDINE AND ITS
DERIVATIVES AS POSSIBLE ANTIBACTERIAL AND
ANTIFUNGAL AGENTS**

**THESIS SUBMITTED
UNIVERSITY OF MUMBAI**

**FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
FACULTY OF TECHNOLOGY**

**SUBMITTED BY
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**UNDER THE GUIDANCE OF
DR. B.G. KHADSE**

**HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
MUMBAI – 400 012**

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**SYNTHESIS AND STUDY OF THIENOPYRIMIDINE AND
ITS DERIVATIVES AS POSSIBLE ANTIBACTERIAL
AND ANTIFUNGAL AGENTS**

**A
Thesis Submitted
To The
University Of Mumbai
For The Degree of
Doctor Of Philosophy
In
The Faculty Of Technology
(Pharmaceutical Chemistry)**

**By
Ravindra V. Chambhare
M.Pharm.**

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***DEDICATED
TO MY PARENTS,
NEPHEW PRATEEK***

&

***TO THE MEMORY OF
SISTER-IN-LAW LATE
MRS. SEEMA
CHAMBHARE***

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Ravindra V. Chambhare

STATEMENT

Statement submitted under rule 0.770

For the Thesis, which I intend to submit, no degree or diploma or distinction has been conferred on me before, either in this or any other University.

Statement submitted under rule 0.771

Statement I :

The work embodied in the Thesis to be submitted is original, based on the discovery of new facts, viz. the synthesis and characterization of new and unknown compounds of possible chemotherapeutic value. Also included in the Thesis, are new relations of facts, observed on the basis of in vitro testing.

The assessment of the compounds has been made at the Department of Microbiology and Department of Chemotherapy, Haffkine Institute, Mumbai 400 – 012.

Statement II :

During the study, all available information of related nature published in scientific journals by previous workers in allied fields, has been used as a source of information, as indicated in the “**References**”.

The entire work has been planned and carried out by the candidate.



Dr. B. G. Khadse
Research Guide



Ravindra V. Chambhare
Research Student

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ABBREVIATIONS

α	-	alpha
β	-	beta
b.p.	-	boiling point
$^{\circ}\text{C}$	-	degree celsius (centigrade)
δ	-	delta
cm	-	centimeter(s)
ed.	-	editor(s)
e.g.	-	exempli gratia
et al	-	et alii, 'and others'
$^{\circ}\text{F}$	-	degree fahrenheit
γ	-	gamma
ibid	-	ibidem, 'in the same place'
i.e.	-	id est, 'that is'
IR	-	infra-red
M	-	mole
Mg	-	milligrams(s)
min	-	minute
mL	-	milliliter(s)
m.p.	-	melting point
μg	-	microgram(s)
μl	-	microlitre(s)
vol.	-	volume(s)
wt.	-	weight
PMR	-	Proton Magnetic Resonance



Part I-A

Introduction
and
Review of Literature

INTRODUCTION AND REVIEW OF LITERATURE

Medicinal chemistry remains a challenging science, which provides profound satisfaction to its practitioners. It intrigues those of us who like to solve problems posed by nature. It verges increasingly on biochemist by and on all the physical, genetic and chemical riddles in animal physiology, which bears on medicine. Medicinal chemists have a chance to participate in the fundamentals of prevention, therapy and understanding of diseases and thereby to contribute to a healthier and happier life.

A. Burger

Taken in the prospective sense, medicinal chemistry relates to the design and production of compounds that can be used in medicine for the prevention, treatment or care of human and animal diseases. Medicinal chemistry includes the study of existing drugs for their biological properties and structure-activity relationships.

An early definition of medicinal chemistry was given by an IUPAC specialised commission. 'Medicinal chemistry concerns the discovery, the development, the identification, and the interpretation of the mode of action of biologically active compounds at the molecular level. It may involve synthesis of new compounds, investigations of the relationships between the structure of natural and / or synthetic compounds and their biological activities, elucidations of their interactions with receptors of various kinds, including enzymes and DNA, the determination of their absorption, transport and distribution properties, and studies of the metabolic transformations of these compounds into other chemicals.

Medicinal chemistry covers three critical steps¹

- A discovery step, consisting of identification and production of new active substances, usually called 'lead' compounds. The lead compounds can originate from synthetic organic chemistry, from natural sources or from biotechnological processes.
- An optimization step, dealing mainly with the synthetic modification of the lead structure in order to improve potency, selectivity and to lessen toxicity.
- A development step, consisting of optimization of the synthetic route for bulk products and the modification of the pharmacokinetics and the pharmaceutical

properties of the active substance, to render it suitable for clinical use. This process can involve the preparation of chemical formulations that are better absorbed, or are more water-soluble, or have sustained release properties.

Drug Discovery

The lead is a prototype compound that has the desired biological or pharmacological activity, but may have many other undesirable characteristics, for example, high toxicity, other biological activities, insolubility or metabolism problems. The structure of the lead compound is then modified by synthesis to amplify the desired activity and to minimize or eliminate the unwanted properties. Thus, the paramount duty of medicinal chemist is to prepare compounds which possess significant biological activity by modifying the structure and to see whether the modification results in the enhancement of biological activity. Since the biological properties of a compound are determined by its chemical structure, one ought to be able to change them by varying it².

Choice of lead structure

1. From biological concepts

Three such sources are –

- a. Endogenous compounds governing physiochemical functions. e.g. β -blocker propranolol is a molecular modification of noradrenaline.
- b. Compounds, with activity proven clinically
- c. Compounds, active in pharmacological or biochemical models, but have not been tested clinically.

2. From Biomolecular structures or processes

- a. From resolution of 3-D molecular structures of binding sites
3-D structure of renin and its substrate was proposed from the study of related aspartyl protease³, Inhibition of renin thus developed as a new concept in the therapy of hypertension.
- b. From binding models derived from QSAR
Hansch provided evidence for the feasibility of this approach, whereby it was concluded that the phenyl ring partly enters the hydrophobic part and partly remains exposed to the surrounding aqueous medium, thus,

its lipophilicity can be tailored to the desired result by suitable substitution(s).

- c. From biochemical mechanisms related to drug action

3. From chemical concepts

Search is made on the basis of chemical structure that has been discovered but not pharmacologically investigated, e.g. discovery of benzodiazepine⁴.

4. From natural source

A number of antibiotics, peptides, steroids and several bioactive compounds have been developed from naturally occurring 'lead' compounds.

5. From targeted screening

Various materials like plant extracts, fermentation broth, chemicals, prepared test compounds etc. are subjected to targeted screening.

Lead Discovery

In general, the difficulty arises in the discovery of the lead compound. There are several approaches that can be taken to identify a lead. The first requirement for all of the approaches is to have a means to assay compounds for a particular biological activity.

1) Random Screening

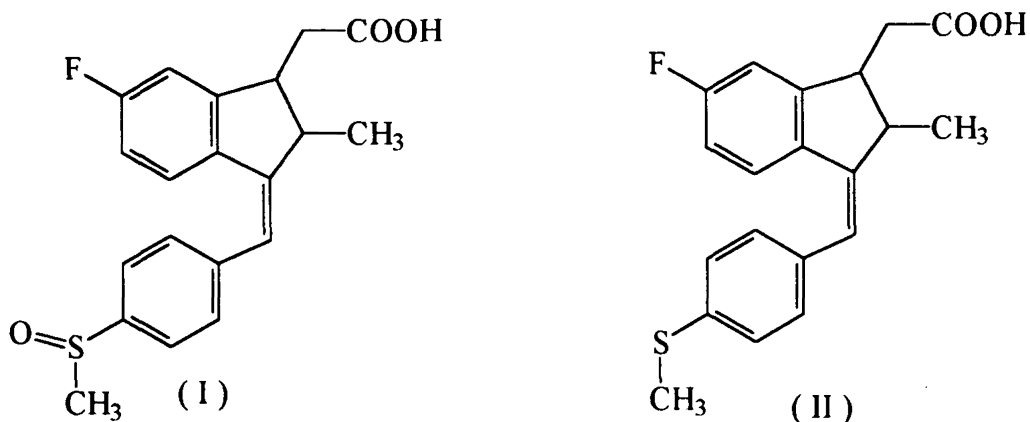
Random screening hardly involves intellectualization; all compounds are tested in the bioassay without regard to their structures. Prior to 1935, random screening was essentially the only approach; today, this method is used to a lesser degree. However, random screening programs are still very important in order to discover drugs for various targets. The two major classes of materials screened for biological activities are synthetic chemicals and natural products.

2) Non-random Screening

It is a slightly narrow approach when compared with random screening. In this case, compounds having a vague resemblance to weakly active compounds, uncovered in a random screen, or compounds containing different functional groups than leads may be tested selectively.

3) Drug Metabolism Studies

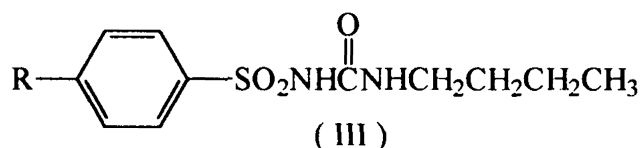
During metabolic studies, drug metabolites that are isolated are screened in order to determine if the activity observed is derived from the drug candidate or from a metabolite. For example, the anti inflammatory drug Sulindac (I) is not the active agent; the metabolic reduction product (II) is responsible for the activity⁵.



4) Clinical Observations

Often a drug candidate, during animal testing or clinical trials, will exhibit more than one pharmacological activity, that is, it may produce a side effect. This compound, then can be used as a 'lead' for the secondary activity.

An antibacterial agent, Carbutamide (III, R = NH₂) was found to have an antidiabetic side effect. However, it could not be used as an antidiabetic drug because of its antibacterial activity. Carbutamide, then, was a lead for the discovery of Tolbutamide (III, R = CH₃), an antidiabetic drug without antibacterial activity.

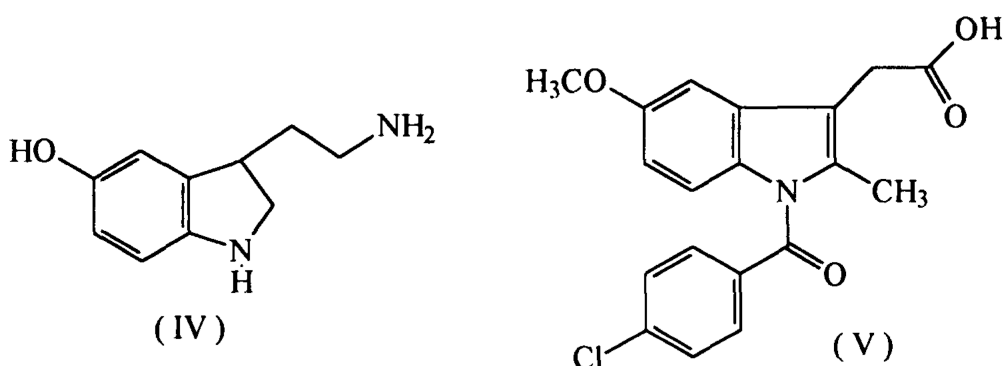


5) Rational Approaches to Lead Discovery

Rational approaches to drug design have now become the major routes to lead discovery. The first step is to identify the cause for the disease state. Most diseases, or at least the symptoms of diseases, arise from an imbalance of particular

chemicals in the body, from the invasion of a foreign organism or from aberrant cell growth.

Once the relevant biochemical system is identified, lead compounds then become the natural receptor agonists or enzyme substrates. For example, it was believed that Serotonin (IV) was a possible mediator of inflammation and consequently, Serotonin was used as a lead for anti-inflammatory agents. From this lead, the anti-inflammatory drug Indomethacin (V) was developed⁶.



Drug Development : Lead Modification

Once your lead compound is in hand, how do you know what to modify in order to improve the desired pharmacological properties?

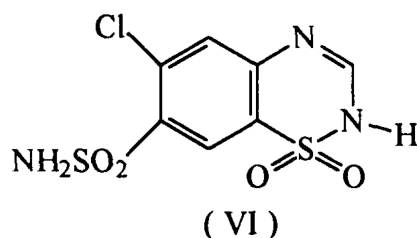
A) Identification of the active part : The Pharmacophore

Interactions of drugs with receptors have been very specific. Therefore, only a small part of the lead compound may be involved in the appropriate interactions. The relevant groups on a molecule that interact with a receptor and are responsible for the activity are collectively known as the pharmacophore. If the lead compound has additional groups, they may interfere with the appropriate interactions. One approach to lead modification is to cut away sections of the molecules in order to determine which parts are essential and which are superfluous.

B) Functional Group Modification

The amino group of Carbutamide (III, R = NH₂) was replaced by a methyl group to give Tolbutamide (III, R = CH₃), and in doing so the antibacterial activity was separated away from the diabetic activity. In some cases, an experienced medicinal chemist knows what functional group will elicit a particular effect. Chlorothiazole (VI)

is an anti-hypertensive agent that has a strong diuretic effect as well. This was predictable because it was known from the work on sulfonamide that the sulfonamides side chain can give diuretic activity.



C) Structure Activity Relationships (SAR)

Drugs can be classified as being structurally specific or structurally-non-specific. Structurally specific drugs, which most drugs are, act at specific sites, such as a receptor or an enzyme. Their activity and potency is very susceptible to small changes in chemical structure. Structurally-nonspecific drugs have no specific site of action and usually have lower potency. Similar biological activities may occur with a variety of structures. e.g. gaseous anesthetics, sedatives and hypnotics.

Even though only a part of the molecule may be associated with the activity, there are a multitude of molecular modifications that could be made for drug development.

Early SAR studies (Prior to the 1960s) simply involved the syntheses of as many analogs as possible of the lead compound and their testing to determine the effect of structure on activity (or potency). Once enough analogues were prepared and sufficient data accumulated, conclusions could be made regarding structure- activity relationships.

D) Structure modification to increase potency and therapeutic index

After years of structure activity relationship studies, various molecular modification approaches have been developed for the systemic improvement of the therapeutic index, which is a measure of the ratio of undesirable to desirable drug effects. The larger the therapeutic index, the greater the margin of safety of the compound. A number of these structural modification methodologies follow

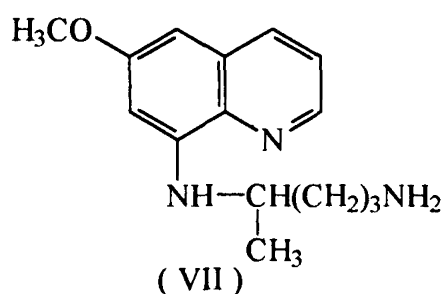
1. Homologation

A homologous series is a group of compounds that differ by a constant unit, generally a CH₂ group for many series of compounds. The lengthening of a saturated

carbon side chain from one methyl to five to nine atoms (pentyl to nonyl) produces an increase in pharmacological activity. Further lengthening results in a sudden decrease in potency. A study by Dohme *et al*⁷ on a 4-alkyl-substituted resorcinol derivative showed that the peak antibacterial activity occurred with 4-n-hexylresorcinol, a compound now used as a topical anesthetic in a variety of throat lozenges.

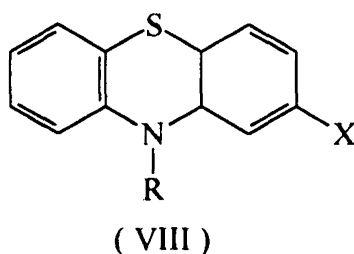
2) Chain Branching

When a simple lipophilic relationship is important then chain branching lowers the potency of a compound. Chain branching can also interfere with receptor binding. Major pharmacological changes can occur with chain branching and homologation. For example, the antimalarial drug Primaquine (VII) is more potent than any of its secondary or tertiary amine homologue.



3) Ring Chain Transformations

Another modification that can be made is the transformation of alkyl substituents into cyclic analogs. Consider Promazine (VIII, X = H, R = (CH₂)₃N(CH₃)₂) and Chlorpromazine [VIII, X = Cl, R = (CH₂)₃N(CH₃)₂] which differ in the substituent X, but are equivalent as tranquilizers in animal tests.



4) Bioisosterism

Bioisosters are substituents or groups that have chemical or physical similarities, and which broadly produce similar biological properties⁸. Bioisosterism is a lead

modification approach that has been shown to be useful to attenuate toxicity or to modify the activity of a lead, and it may have a significant role in the alteration of the metabolism of a lead.

There are classical isosters^{9,10} and nonclassical isosters¹¹. Erlenmeyer¹² later redefined isosters as atoms, ions or molecules in which the peripheral layers of electrons can be considered to be identical. It is, actually, quite surprising that bioisosterism should be such a successful approach to lead modification. On making a bioisosteric replacement, one or more of the following parameters will change-size shape electronic distribution, lipid solubility, water solubility, pka, chemical reactivity and hydrogen bonding. The modifications made to a molecule may affect one or more of the following -

- 1) Receptor interaction
- 2) Pharmacokinetic
- 3) Metabolism

E) Quantitative Structure – Activity Relationships (QSAR)

The concept of quantitative drug design is based on the fact that the biological properties of a compound are a function of its physicochemical parameters, that is physical properties, such as solubility, lipophilicity, electronic effects, ionization and stereochemistry, which have a profound influence on the chemistry of the compound. The first attempt to relate a physicochemical parameter to pharmacological effect was reported in 1893 by Ricket¹³. He observed that the narcotic, action of a group of organic compounds was inversely related to their water solubility. Overton¹⁴ and Meyer¹⁵ related tadpole narcosis induced by a series of non-ionized compounds added to the water in which the tadpoles were swimming, to the ability of the compounds to partition between oil and water. In 1951 Hansch *et al*¹⁶ noted a correlation between the plant growth activity of phenoxyacetic acid derivatives and the electron density at the ortho position.

A number of reviews have documented the history, strategy and successes of quantitative drug design, i.e. design using QSAR method¹⁷⁻²³. The impact of QSAR methods on drug discovery and lead optimization may be manifold (figure 1)²⁴.

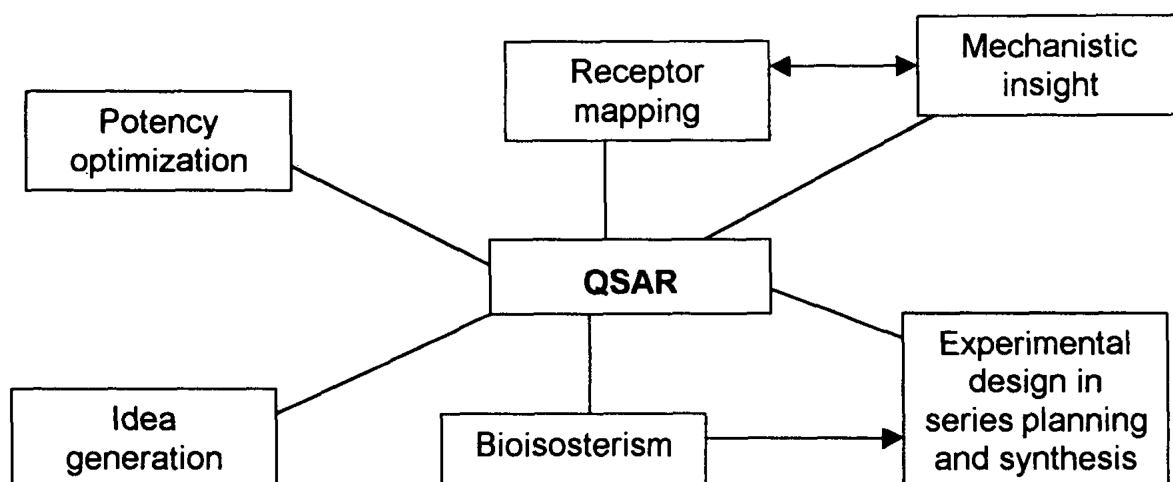


Figure - 1

Physicochemical Parameters

I) Electronic Effects : The Hammett Equation.

The electronic effects of various substituents clearly have an effect on a drug's ionization or polarity. This in turn may have an effect on how easily a drug can pass through cell membranes or how strongly it can bind to a receptor. It is, therefore, useful to have some measure for the electronic effect a substituent can have on a molecule. As far as substituents on an aromatic ring are concerned, the measure used is known as the Hammett substitution constant, denoted by the symbol σ .

The Hammett substitution constant (σ) is a measure of the electron withdrawing or electron-donating ability of a substituent and has been determined by measuring the dissociation of a series of substituted benzoic acid derivatives and comparing them with the dissociation of benzoic acid itself.

The Hammett substituent constant (σ_x) for a particular substituent (x) is defined by the following equation.

$$\sigma_x = \log K_x / K_H = \log K_x - \log K_H$$

II) Lipophilicity effects : The basis for the Hansch Equation.

The crucial breakthrough in QSAR came when Hansch and co-workers^{25,26} conceptualized the action of a drug as depending on two processes. The first process is the journey of the drug from its point of entry into the body to the site of

action (Pharmacokinetics), and the second process is the interaction of the drug with the specific site (Pharmacodynamics).

However, the biological activity of most drugs is related to a combination of physicochemical properties. In such cases, simple equations involving only one parameter are relevant, only if the other parameters are kept constant. In reality, this is not easy to achieve and equations which relate biological activity to more than one parameter are more common. These equations are known as Hansch equations and they usually relate biological activity to the most commonly used physicochemical properties. (p and / or π , σ , and a steric factor).

The Hansch group^{25,27} suggested that a reasonable model for the first step in drug action (transport to the site of action) would be the ability of a compound to partition between 1-octanol, which would simulate a lipid membrane, and water (the aqueous phase). Hansch believed that there should be a linear free energy relationship between lipophilicity and biological activity. As a suitable measure of lipophilicity, the partition coefficient, P , between 1-octanol and water was proposed^{25,27} and P was determined by following equations, where α is the degree of dissociation of the compound in water, calculated from ionization constants.

$$P = [\text{Compound}]_{\text{Oct}} / [\text{Compound}]_{\text{aq}} (1 - \alpha)]$$

If the range of hydrophobicity values is limited to a small range then the equation will be linear as follows -

$$\text{Log } (1/c) = K_1 \log P + K_2 \sigma + K_3 E_s + K_4$$

If P values are spread over a range, then the equation will be parabolic for the same reasons.

$$\text{Log } (1/c) = -K_1 (\log P)^2 + K_2 \log P + K_3 \sigma + K_4 E_s + K_5$$

The constants $K_1 - K_5$ are determined by computer in order to get the best fitting line.

III) Steric Effects : The Taft Equation

Since interaction of a drug with a receptor involves the mutual approach of two molecules, another important parameter for QSAR is the steric effect. Es constant, defined by the following equation (acid-catalyzed hydrolysis of RCOOR' Vs CH₃COOR')²⁸, was the first parameter, which was used to describe steric effects in QSAR studies

$$E_s = \log (K/K_0)_A$$

Two other steric parameters worth mentioning are molar refractivity (MR) and Verloop parameter. Molar refractivity²⁹ is defined by the Lorenz-Lorenz equation given below, where n is the index of refraction at the sodium D line, MW is the molecular weight of the compound and d is the density of the compound. The greater the positive MR value of a substituent, the larger is its steric or bulk effect. This parameter also measures the electronic effects and, therefore, may reflect dipole-dipole interactions at the receptor site.

$$MR = \left[\frac{(n^2 - 1)}{(n^2 + 2)} \right] \left[\frac{MW}{d} \right]$$

The Verloop Steric Parameters³⁰ are used in a program called STERIMOL to calculate the steric substituent values from standard bond angles, van der Waals radii, bond lengths and user-determined reasonable conformations. Five parameters are involved. The length(L) of the substituent along the axis of the bond between the substituent and the parent molecule and four width parameters (B-B₄) are measured perpendicular to the bond axis, are used.

Method used to correlate physicochemical parameters with biological activities

- a) Hansch analysis: A linear multiple regression analysis
- b) Free and Wilson or de novo method
- c) Enhancement factor
- d) Manual stepwise methods. Topliss operational schemes and others.
- e) Batch selection methods. Batchwise Topliss operational scheme, cluster analysis

a) Hansch Analysis: A Linear Multiple Regression Analysis

With the realization that there are (at least) two considerations for biological activity namely, lipophilicity (required for the journey of the drug to the site of action) and

electronic factors (required for drug interaction with the site of action), Hansch and Fujita gave an equation known as the Hansch equation. Here C is the molar concentration (or dose) that elicits a standard biological response, the terms K, K', ρ , and K'' are the regression coefficients derived from the statistical curve and π and σ are the lipophilicity and electronic substituent constants, respectively.

$$\text{Log } (1/c) = -K\pi^2 + K'\pi + \rho\sigma + K''$$

$$\text{Log } (1/c) = -K(\log P)^2 + K'(\log P) + \rho\sigma + K''$$

Because of the importance of steric effects and other shape factors of molecules for receptor interactions, an Es term and a variety of other shape, size, or topography terms (S) have been added to the Hansch equation.

$$\text{Log } (1/c) = -a\pi^2 + b\pi + \rho\sigma + cE_s + dS + e$$

Hansch analysis, is also called the extrathermodynamic method

The advantages of this method are

- 1) The use of descriptors (π , σ , Es, MR) permits data collected from simple organic chemical model systems to be utilized for the prediction of biological activity in complex systems.
- 2) The predictions are quantitative with statistical confidence limits.
- 3) The method is easy to use and is inexpensive.
- 4) The conclusions that are reached may have applications beyond the substituents included in the particular analysis.

The disadvantages of this method are

- 1) There must be parameter values available for the substituents in the data set.
- 2) A large number of compounds must be included in the analysis in order to have confidence in the derived equations.
- 3) An expertise in statistics and computer use is essential.
- 4) Small molecule interactions are imperfect models for biological systems.

5) Since QSAR study is empirical, it is a retrospective technique that depends on the pharmacological activity of compounds belonging to the same structural type, and, therefore, new types of active compounds are not discovered.

b) Free and Wilson or de Novo Method

Free and Wilson³¹ reported a general mathematical method for assessing the occurrence of additive substituent effects, and for quantitatively estimating their magnitude. It is a method for the optimization of substituents within a given molecular framework that is based on the assumption that the introduction of a particular substituent(s) at any one position in a molecule always changes the relative potency by the same amount, regardless of what other substituents are present in the molecule. A series of linear equations have been constructed on the form shown in the equation below -

$$BA = \sum a_i x_i + \mu$$

Where

- BA is the magnitude of the biological activity
- x_i is the i^{th} substituent with a value of 1, if present, and 0, if not
- a_i is the contribution of the i^{th} substituent to the BA
- μ is the overall average activity of the parent skeleton. These equations are solved by the method of least squares for a_i and μ .

c) Enhancement Factor

One of the earliest QSAR observations resulted from a retrospective analysis of a large number of synthetic corticosteroids³². The examination of the biological properties of steroids prepared by the introduction of halogen, hydroxyl, alkyl, or double bond groups revealed that each substituent affects the activity of the molecule in a quantitative sense, and almost independently of other groups.

The effect (whether positive or negative) of each substituent was assigned a numerical value termed as the enhancement factor.

d) Manual stepwise methods : Topliss operational schemes and others

Topliss³³ developed a non-mathematical, non-statistical, and non-computerized

guide to the use of the Hansch principle. This method is most useful when the synthesis of a large number of compounds is difficult and when biological testing of the compounds is readily available. It is an approach for the efficient optimization of the potency of a lead compound with minimization of the number of compounds needed to be synthesized. The only prerequisite for the technique is that the lead compound must contain an unfused benzene ring. This approach relies heavily on π and ρ values and to a much lesser degree on E_s values.

The Topliss scheme for aromatic substituents assumes that the lead compound has been screened for biological activity and contains a mono-substituted aromatic ring. The first analogue in the scheme is a 4-chloro derivative, since this derivative is usually synthesized. The chloro substituent is more hydrophobic and electron-withdrawing than hydrogen, and therefore, π and ρ are positive. Once the chloro analogue has been synthesized, the biological activity is measured. There are three possibilities. The analogue will have less activity (L), equal activity (E), or more activity (M). The type of activity observed will determine which branch of the Topliss scheme is followed next.

If the biological activity increases, then the (M) branch is followed and the next analogue to be synthesized is the 3,4 dichloro- substituted analogue. If on the other hand, the activity stays the same then the (E) branch is followed and the 4- methyl analogue is synthesized. Finally, if activity drops, the (L) branch is followed and the next analogue is the 4-methoxy analogue.

e) Batch selection methods : Batchwise Topliss operational scheme, cluster analysis and others.

The inherent problem with the Topliss operational scheme is its stepwise nature. Topliss³⁴ proposed an alternative scheme that uses batch-wise analysis of small groups of compounds. The substituents were grouped by Topliss according to π , ρ , π^2 and a variety of $x\pi$ and $y\rho$ weighted combinations. The approach starts with the synthesis of five derivatives-the unsubstituted (4-H), 4-chloro, 3,4 dichloro, 4-methyl and 4-methoxy compounds.

The major weakness of this approach is that it is difficult to extend the method to additional parameters, unless computers are utilized.

A computer-based batch selection method, known as cluster analysis, was introduced by Hansch *et al*³⁵. The substituents were grouped into clusters with similar properties according to their σ , π , π^2 , Es, F (field constant), R (resonance constant) MR (Molar refractivity) and MW (molecular weight) values. One member of each cluster would be selected for substitution into the lead compound, and the compound would be synthesized and tested. If a substituent showed dominant potency, then other substituents from that cluster would be selected for further investigations. The important advantage of the batch selection method is that the initial batch of analogues prepared is derived from the widest range of parameters possible so that the dominant physicochemical property can be revealed early in the lead modification process.

Computer Based methods of QSAR related to Receptor Binding

There are a variety of computer based methods that have been used to correlate molecular structure with receptor binding and therefore, activity. Crippen and co-workers^{36a-c} devised a linear free energy model, termed the distance geometry approach, for calculating QSAR from receptor binding data.

The distance geometry approach was extended by Sheriden *et al*³⁷ to treat two or more molecules as a single ensemble. The ensemble approach to distance geometry can be used to find a common pharmacophore for a receptor with unknown structure from a small set of a biologically active molecules.

Hopfinger³⁸ has developed a set of computational procedures termed molecular shape analysis for the determination of the active conformation and, thereby, molecular shapes during receptor-binding. The common pairwise overlap steric volumes calculated from low-energy conformations of molecules are used to obtain three dimensional molecular shape descriptors, which can be treated quantitatively and used with other physicochemical parameters.

Two other descriptors for substructure re-presentation, the atom pair³⁹ and the topological torsion⁴⁰ have been described by Venkataraghavan and co-workers. These descriptors characterize molecules in fundamental ways that are useful for the selection of potentially active compounds from hundreds of thousands of structures

in the database.

The atom pair method can select compounds from diverse structural classes that have atoms within the entire molecule similar to those of a particular active structure. The topological torsion descriptor is complementary to the atom pair descriptor and it focuses on a local environment of a molecule for comparison with active structures.

f) Molecular Graphics Based Drug Design

The quantitative structure activity relationship studies have relied heavily on the use of computers from the beginning for statistical calculations involving multiparameter equations. It was soon realized that drug design could be aided significantly if structures of receptors and drugs could be displayed on a terminal and molecular processes could be visualized. Molecular graphics is the use of computer graphics to represent and manipulate molecular structures. The origin of molecular graphics has been traced by Hassall⁴¹ to the project MAC (Multiple Access Computer)⁴², which produced computer graphic models of macromolecules for the first time.

Medicinal chemists saw the potential of this approach in drug design as well. A three dimensional computer graphics representation that can be manipulated in three dimensions allow the operator to visualize the interactions of small molecules with biologically important macromolecules. The superimposition of structures, which is cumbersome at best with manual models, can be performed easily by molecular graphics. Also, some systems have the capability to synthesize graphically new structures by the assembly of appropriate molecular fragments from a fragments file.

There are numerous molecular graphic systems available^{43,44a-b} but the typical system consists of a mainframe or supermini computer linked to a high resolution graphics terminal with local intelligence. The basic premise in the utilization of molecular graphics is that the better the complementary fit of the drug to the receptor, the more potent the drug will be. In order to apply this concept it would appear that the structure of the receptor would have to be known, so that different drug analogs could be docked into the receptor. Docking is a molecular graphics term for the computer-assisted movement of a terminal displayed molecule into its receptor.

There are relatively few receptors with known X-ray structures. Consequently, it would appear that the approach of receptor-fitting is of little importance to drug design. However, because of the great advances in computer technology and software development, it is possible to use molecular graphics to obtain information about the ligand binding site of an unknown receptor.

The third important molecular graphics technique useful for identification of the pharmacophore geometry is called receptor mapping. This method utilizes data from known ligand binding studies to an unknown receptor. An approach termed steric mapping⁴⁵ uses molecular graphics to combine the volumes of compounds known to bind to the desired receptor. This composite volume generates an enzyme-excluded volume map which defines that region of the binding site available for binding by drug analog and therefore, not occupied by the receptor itself.

A concise review of some of the promising and therapeutically useful antitubercular, antibacterial, antifungal and antimalarial agents is presented in the following pages.

Tuberculosis

Tuberculosis, a disease largely caused by *Mycobacterium tuberculosis*, an acid – fast bacilli, is known to mankind since Vedic times. It occurs throughout the world and is a significant contributor to the morbidity and mortality in several developing countries. Approximately one third of the world's population is infected by *M. tuberculosis*. The spread of human immunodeficiency virus (HIV) into populations in which the incidence of tuberculosis is already high because of socioeconomic factors and malnutrition, has resulted in resurgence of tuberculosis all over the world⁴⁶⁻⁴⁸. The World Health Organization suggests that at least 5.6 million persons worldwide, mostly in the developing countries carry, dual infection, and >1.4 million cases of HIV related tuberculosis will occur by the year 2000⁴⁹.

In India, as per reports nearly 50,000 persons die of tuberculosis, annually. The number of patients at any given time has been estimated to be at least 9-10 million of which one fourth (2-2.5 million) are likely to bear the infectious type⁵⁰.

Apart from *M. tuberculosis*, which is the principle mycobacteria responsible for causing tuberculosis, other causative mycobacteria include *M. bovis* and *M.*

africanum. While *M. tuberculosis* is generally transmitted in airborne droplets dispersed by patients with infectious pulmonary or laryngeal disease, *M. bovis* is transmitted in milk from diseased cows.

Mycobacterial infection such as tuberculosis are chronic diseases which do not elicit a rapid response from the host. After initial infection and multiplication, the bacilli may lie dormant for a long time without giving rise to any of the clinical symptoms. Often, however, the bacilli may be reactivated due to malnutrition, immunosuppressive drugs and diseases including HIV infection. As the human system is endowed with a relatively high resistance through years of natural selection, particularly in our urban communities, the effect of this invasion by a slow growing but persistent organism is to prolong the struggle, thus making the disease chronic.

Tuberculosis is characterized by the formation of nodular bodies or tubercles in the tissues, from which the disease derives its name. In most cases the primary area of infection is the lungs, but other vulnerable areas include lymph nodes, kidneys, bones, the genitourinary tract, the brain and the meninges. In case of infections of the lungs, the lesions often liquify and drain into the bronchial tree giving rise to a persistent cough accompanied by sputum. Tuberculosis causes extensive tissue destruction and virulent bacilli reside in the cavities and debris of the tissue, this being relatively immune to any drug action. The tubercle formation, even though serving a useful purpose of walling off the infection, thus preventing them from spreading, also protects walled off colonies from chemotherapeutic attack.

Also, tubercle bacilli are vulnerable to bactericidal drugs only when they are metabolically effective and replicating. Here, the problem lies in the fact that small subpopulations of bacilli can remain dormant indefinitely, except for short periods when they become transiently active, thus evading chemotherapeutic agents.

Another difficulty is in the existence of drug resistant mutants in populations of bacilli hitherto unexposed to antibiotics.

A truly effective drug should therefore be capable of penetrating the tubercle and the debris, enter the phagocytes within which the bacilli are growing and finally destroy

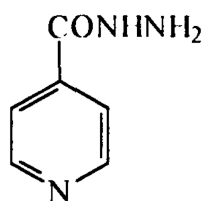
them. It should possess potent bactericidal / sterilizing activity against metabolically active and semidormant bacilli, while also preventing the emergence of resistant strains throughout the normally prolonged period of time. There is no known drug individually self sufficient, possessing all the listed properties. Hence a combination of two or more drugs is used for the treatment which may stretch to several months before a reasonable cure is effected.

Drugs used for the treatment of tuberculosis

Drugs used in the treatment of tuberculosis can be divided into two major classes. "First line" agents combine the greatest level of efficacy with an acceptable degree of toxicity; these include Isoniazid, Rifampin, Ethambutol, Streptomycin and Pyrazinamide. A large majority of patients with tuberculosis can be treated successfully with these drugs. Excellent results can be obtained with a 6 month course of the treatment. The schedule for the first 2 months consists of Isoniazid, Rifampin, and Pyrazinamide, followed by Isoniazid and Rifampin for the remaining 4 months. The administration of Rifampin in combination with Isoniazid for 9 months is also effective therapy for all forms of the disease caused by strains of *Mycobacterium tuberculosis* susceptible to both agents. In areas where primary resistance to Isoniazid occurs, therapy usually is initiated with four drugs- Rifampin, Isoniazid, Pyrazinamide and Ethambutol (or Streptomycin)-until sensitivity tests are completed. Occasionally, however, because of microbial resistance or patient-related factors such as HIV infection or AIDS, it may be necessary to resort to "second line" drugs, in addition, so that the treatment may be initiated with 5 to 6 drugs. This category of agents includes Ofloxacin, Ciprofloxacin, Ethionamide, para aminosalicylic acid, Cycloserine, Amikacin, Kanomycin and Capreomycin

Isoniazid (INH)

Isoniazid (IX), the hydrazide of isonicotinic acid, was first described in 1912, but its action against the tubercle bacillus was only discovered in 1952. It is unrivalled in its bactericidal action and ensures the destruction of the bacilli within few days of starting treatment. It is highly effective against replicating tubercle bacilli but does not affect the semidormant species⁵¹, which resumes multiplication after removal of contact with the drug.



(IX)

Isoniazid is bacteriostatic for 'resting' bacilli, but is bactericidal for rapidly dividing microorganisms. The minimal tuberculostatic concentration is 0.025 to 0.05 µg/mL. The bacteria undergoes one or two divisions before multiplication is arrested.

Although the mechanism of action of Isoniazid is unknown, several hypotheses have been proposed. These include effect on lipids, nucleic acid biosynthesis and glycolysis. Takayama *et al*⁶² have suggested a primary action of Isoniazid to inhibit the biosynthesis of mycolic acid, an important constituent of the mycobacterial cell wall. Because mycolic acid is unique to mycobacteria, this action would explain the high degree of selectivity of the antimicrobial activity of Isoniazid.

Isoniazid is readily absorbed when administered either orally or parenterally. Aluminium containing antacids may interfere with absorption of the drug. The peak plasma concentrations of 3 to 5 µg/mL are developed 1 to 2 hours after oral ingestion of usual doses. Isoniazid diffuses readily into all body fluids and cells. From 75% to a 95% dose of Isoniazid is excreted in the urine within 24 hrs, mostly as the metabolites.

Isoniazid is available for oral and parenteral administration. The commonly used total daily doses of Isoniazid is 5 mg/kg, with a maximum of 300 mg.

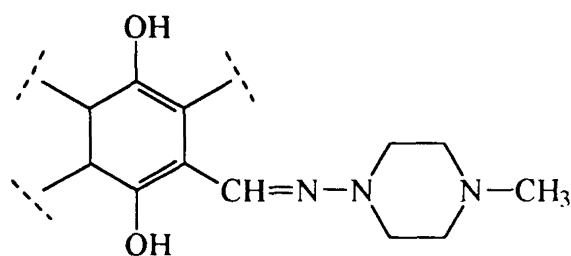
Pyridoxine (15 to 50 mg per day) should be administered with Isoniazid to minimize adverse reactions in malnourished patients, and those predisposed to neuropathy.

The incidence of adverse reactions to Isoniazid was estimated to be 5.4 % among more than 2000 patients treated with the drug. The most prominent of these reactions were rash (2%), fever (1.2 %), jaundice (0.6%) and peripheral neuritis(0.2 %). Among miscellaneous reactions associated with Isoniazid therapy are dryness of the mouth, epigastric distress, methemoglobinemia and urinary retention.

Cross-resistance between Isoniazid and other agents used to treat tuberculosis does not occur. Current evidence suggests that one mechanism of resistance is related to a missense mutation within the mycobacterial *inhA* gene involved in mycolic acid biosynthesis⁵³.

Rifampin

The rifamycins are a group of structurally similar, complex macrocyclic antibiotics produced by *Streptomyces mediterranei*. Rifampin is a semisynthetic derivative of one of these rifamycin B antibiotics.



(X)

Rifampin inhibits the growth of most gram positive bacteria as well as gram negative microorganisms. Rifampin is very active against *Staphylococcus aureus*. Rifampin in concentrations of 0.05 to 0.2 µg/mL inhibits the growth of *M. tuberculosis in vitro*. It increases the *in vitro* activity of Streptomycin and Isoniazid, but not that of Ethambutol, against *M. tuberculosis*⁵⁴.

Rifampin inhibits DNA-dependent RNA polymerase of the mycobacteria and other microorganisms by forming a stable drug-enzyme complex leading to the suppression of initiation of chain formation in RNA synthesis. More specifically, the B subunit of this complex enzyme is the site of action of the drug, although Rifampin binds only to the holoenzymes. Rifampin is bactericidal for both intracellular and extracellular microorganisms.

The effectiveness of Rifampin in the treatment of patients with drug resistant tuberculosis was considered 'extraordinary' when it was first introduced⁵⁵.

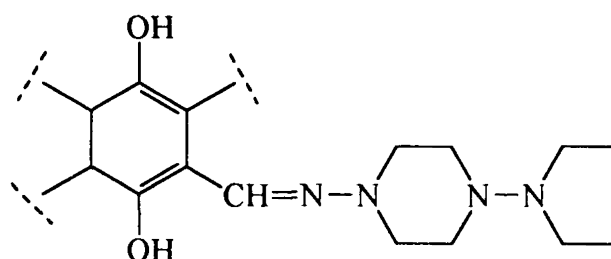
The oral administration of Rifampin produces peak plasma concentrations of about 7µg/mL in 2 to 4 hours, after ingestion of 600 mg, but there is a considerable variability. Following absorption from the GI tract, Rifampin is eliminated rapidly in

the bile, and enterohepatic circulation ensues. The $t_{1/2}$ of Rifampin varies from 1.5 to 5 hours and it increases in the presence of hepatic dysfunction.

Rifampin is available alone and as a fixed dose combination with Isoniazid. Rifampin and Isoniazid are the most effective drugs available for the treatment of tuberculosis in adults (600 mg, given once daily, either 1 hour before or 2 hours after a meal). Rifampin (like Isoniazid) should never be used alone for this disease because of the rapidity with which resistance may develop.

The most common adverse reactions are rash (0.8 %), fever (0.5%), nausea and vomiting (1.5 %) and hepatitis⁵⁶.

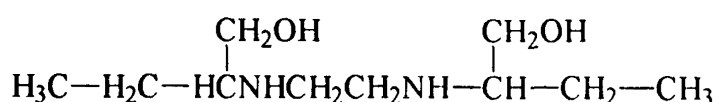
Mycobacteria may develop resistance to Rifampin rapidly *in vitro* as a one step process, and one of every 10^7 to 10^8 tubercle may be resistant to the drug. Among the many other rifampicin derivatives prepared and tested as antimycobacterial agents, Rifapentine (XI) shows interesting characteristics that have been recently exploited for therapeutic use.



(XI)

Ethambutol

Ethambutol(XII) has been classified as a bacteriostatic drug⁵⁷. It has been developed as a synthetic congener of related ethane diamines and is bactericidal against *M. bovis* and some non-specific mycobacteria. It is used in combination with other antitubercular drugs to prevent or delay the emergence of resistant strains.



(XII)

Ethambutol has no appreciable effects on other bacteria. It suppresses the growth of most Isoniazid and Streptomycin resistant tubercle bacilli. Resistance to Ethambutol develops very slowly *in vitro*. The drug has been shown to inhibit the incorporation of mycolic acid into the mycobacterial cell wall⁵⁸.

About 75 % to 80% of an orally administered dose of Ethambutol is absorbed from the gastrointestinal tract. A single dose of 25 mg/kg produces a plasma concentration of 2 to 5 µg/mL at 2 to 4 hours. The drug has a $t_{1/2}$ of 3 to 4 hours.

Within 24 hours, three-fourth of an ingested dose of Ethambutol is excreted unchanged in the urine. Ethambutol has been used with notable success in the therapy of tuberculosis of various forms when given concurrently with Isoniazid.

The oral dose of Ethambutol is 15 mg /kg, given once a day. Ethambutol accumulates in patients with impaired renal function, and adjustment of the dosage is necessary. The side effects that have been observed are decrease in visual acuity, rash, drug fever, etc.. The most important side effect is optic neuritis, resulting in decrease of visual acuity and loss of ability to differentiate red from green.

Streptomycin

Streptomycin was the first clinically effective drug to become available for the treatment of tuberculosis. The antitubercular action of Streptomycin (isolated from *Streptomyces griseus*) was first demonstrated by Schatz and Waksman in 1944. Streptomycin is bactericidal in an alkaline environment. Because the drug is not absorbed from the gut, it must be given parenterally. A peak serum concentration occurs approximately within 1 hour, after an intramuscular dose. Most strains of *M. tuberculosis* are inhibited *in vitro* at a concentration of 8 µg / mL. The half-life in blood is approximately 5 hours and the excretion is entirely renal.

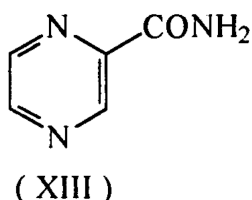
Since other effective agents have become available, the use of Streptomycin for the treatment of pulmonary tuberculosis has been sharply reduced. Many clinicians prefer to give a three drug combination, of which Streptomycin may be one, for the most serious forms of tuberculosis, such as disseminated disease or meningitis. For tuberculosis, adults should be given 15 mg/kg per day in divided doses given every 12 hours, not to exceed 2 g per day.

The therapy is usually discontinued after 2 to 3 months, or sooner if the culture becomes negative.

One of the most common serious adverse effects of Streptomycin is ototoxicity, which usually results in vertigo, but hearing loss may also occur.

Pyrazinamide

Pyrazinamide(XIII) is the synthetic pyrazine analog of nicotinamide. It has following structural formula



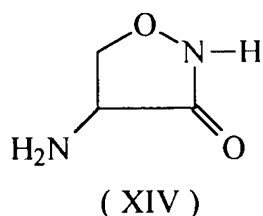
Pyrazinamide was successfully used against human tuberculosis in 1952⁵⁹. Pyrazinamide exhibits bactericidal activity *in vitro* at a slightly acidic pH. The tubercle bacilli within monocytes *in vitro* are killed by the drug at a concentration of 12.5 µg/mL. Resistance develops rapidly if Pyrazinamide is used alone. Pyrazinamide is well absorbed from the gastrointestinal tract, and it is widely distributed throughout the body. The oral administration of 1 gm produces a plasma concentration of about 45 µg/mL at 2 hours and 10 µg/mL at 15 hours.

Pyrazinamide has become an important component of short term (6 month) multi drug therapy for tuberculosis. The daily dose for adults is 15 to 30 mg/kg given orally.

Injury to the liver is the most common and serious side effect of Pyrazinamide. High rates of serious hepatotoxicity were seen when the drug was first introduced in the United States in the 1950s and given in doses of 50 mg/kg/ day.

Cycloserine

Cycloserine(XIV), a broad-spectrum antibiotic produced by strain of *Streptomyces orchidaceus*, is used almost exclusively for the treatment of drug resistant tuberculosis and susceptible strains of non Tuberculus mycobacteria. Cycloserine is D-4-amino-3-isoxazolidone.



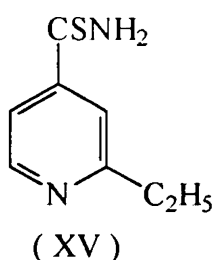
Cycloserine is inhibitory for *M. tuberculosis* in concentrations of 5 to 20 µg/mL *in vitro*. There is no cross resistance between Cycloserine and other tuberculostatic agents. Cycloserine inhibits reactions in which D-alanine is involved in bacterial cell wall synthesis.

When given orally, 70% to 90% of Cycloserine is rapidly absorbed. Peak concentrations are reached 3 to 4 hours after a single dose. Cycloserine should be used only when retreatment is necessary or when microorganisms are resistant to other drugs. When Cycloserine is employed to treat tuberculosis, it must be given together with other effective agents. The usual doses for adults are 250 to 500 mg given twice daily.

Reactions to Cycloserine most commonly involve the central nervous system. Among the central manifestations are somnolence, headache, tremor, dysarthria, vertigo, confusion, nervousness, irritability. Cycloserine is contraindicated in individuals with history of epilepsy.

Ethionamide

The synthesis and study of a variety of congeners of thioisonicotinamide revealed that an alpha-ethyl derivative is considerably more effective than the parent compound.



Ethionamide(XV) is bacteriostatic against *M. tuberculosis* and is used for the treatment of the drug resistant disease. The multiplication of *M. tuberculosis* is suppressed by concentrations of Ethionamide ranging from 0.6 to 2.5 µg/mL. Resistance can develop rapidly *in vitro*. The oral administration of 1 g of Ethionamide

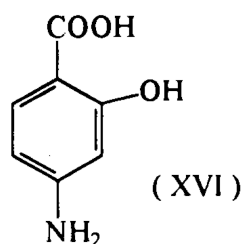
yields peak plasma concentration of about 20 µg/mL in 3 hours and the concentration at 9 hours is 3 µg /mL. The half-life of the drug is about 2 hours.

Ethionamide is a secondary agent, to be used concurrently with other drugs only when therapy with primary agents is ineffective or contraindicated. The initial dose of Ethionamide for adults is 250 mg twice daily. It is increased by 125 mg per day every 5 days until a dose of 15 to 20 mg / kg per day is achieved. The most common reactions to ethionamide are anorexia, nausea and vomiting. A metallic taste also may be noted.

Hepatitis has been associated with the use of the drug in about 5 % of the cases⁶⁰. The concomitant use of Pyridoxine is recommended for patients being treated with Ethionamide.

P-Amino Salicylic Acid (PAS)

PAS(XVI) was the first drug used orally for the treatment of tuberculosis. It is bacteriostatic. *In vitro*, most strains of *M. tuberculosis* are sensitive to a concentration of 1 µg/mL.



PAS is readily absorbed from the GI tract. A single oral dose of 4 g of the free acid produces maximal concentrations in plasma of about 75 µg/mL within 1.5 to 2 hours. The drug has a half-life of about 1 hour. Probenecid decreases the renal excretion of this agent.

PAS is a 'second-line' agent. It is important in the management of pulmonary and other forms of tuberculosis. However, the use of this drug has markedly decreased since more active and better-tolerated drugs have been developed.

Gastrointestinal problems, including anorexia, nausea, epigastric pain, abdominal distress and diarrhoea are the side effects.

Kanamycin

Kanamycin is an aminoglycoside antibiotic produced by *Streptomyces kanamyceticus*. It has quite a broad spectrum of activity, including positive cocci and negative bacteria, as well as *M. tuberculosis*. Its activity against *M. tuberculosis* is weaker than that of Streptomycin⁶¹. The mechanism of action of Kanamycin is similar to that of Streptomycin, since it produces a misreading of genetic code. It interacts with the 30s ribosomal subunit in more than one site (whereas Streptomycin is bound only to one site) and inhibits protein synthesis⁶²⁻⁶⁴. Resistance to Kanamycin can be acquired by conjugation through the transfer of an extra chromosomal DNA, the so-called R-factors or plasmids, coding aminoglycoside – inactivating enzymes. It is not absorbed when given by the oral route, but it is rapidly absorbed after intramuscular administration, reaching high peak serum levels 2-3 h after administration⁶⁵. It has been used in the therapy of tuberculosis in combination with other antituberculous drugs. The common dose is 15 mg/kg per day. Drawbacks of Kanamycin use are its ototoxicity and nephrotoxicity⁶⁶.

Amikacin

It is a semisynthetic analog of Kanamycin. Amikacin is not used for the initial treatment of susceptible tuberculosis, mainly due to its cost, but appears to have merit as an alternative drug for the treatment of resistant *M. tuberculosis* infections.

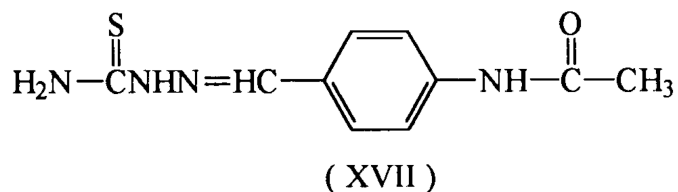
Capreomycin

It is a polypeptide complex isolated from *Streptomyces capreolus*⁶⁷. It is active only against *M. tuberculosis*. The bacterial resistance to Capreomycin develops when it is given alone. Capreomycin is used in conjunction with other appropriate antitubercular drugs in the treatment of pulmonary tuberculosis. It must be given intramuscularly. The recommended daily dose is 15 to 30 mg/kg/day. It should be administered together with other effective agents. It has proven to be of value in the therapy of “resistant” or treatment failure tuberculosis, when given with Ethambutol or Isoniazid⁶⁸.

The reactions associated with the use of Capreomycin are hearing loss and renal functions damage.

Thioacetazone

The synthesis of a series of thiosemicarbazones as intermediates in the preparation of analogs of sulfathiazole lead to the discovery of Thioacetazone (XVII)

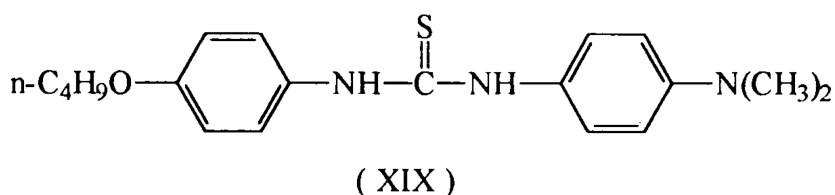
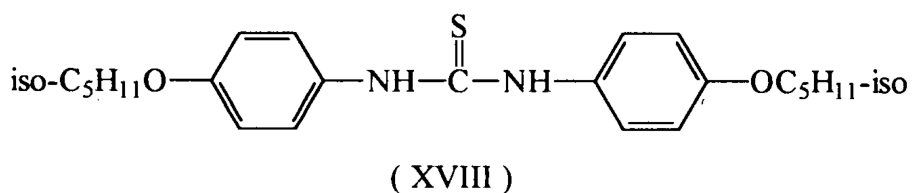


Its good activity *in vitro* and *in vivo*, and the lack of cross-resistance to Isoniazid and Streptomycin, indicate the use of Thioacetazone as a drug in combination with other drugs to delay bacterial resistance. The mechanism of action of Thioacetazone is not known.

Thioacetazone has limitations in clinical use because of serious side effects (such as GI tract disorders, liver damage, and anemia) when administered to humans at the initially proposed daily dose of 300 mg.

Thiocarlide and Thiambutosine

Several thioureas that are structurally related to thiosemicarbazones have been found to be active *in vitro* and *in vivo* against *M. tuberculosis*. Of these, Thiocarlide (XVIII) and Thiambutosine (XIX) have been introduced in clinical use.

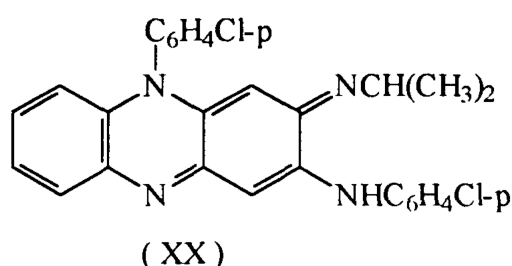


Thiocarlide shows some efficacy against experimental tuberculosis in mice⁶⁹ and is active against tubercle bacilli resistant to PAS, Streptomycin, and Isoniazid^{70,71}. But several clinical trials have indicated little or no usefulness of the drug in the treatment of human tuberculosis, even in combination with other drugs⁷². Thiocarlide acts through inhibition of mycolic acid synthesis of *M. tuberculosis*⁷³.

Thiambutosine has given disappointing results in clinical use for tuberculosis, but it was found to be active in the treatment of leprosy⁷⁴.

Clofazimine and its analogs

Clofazimine (XX) belongs to a peculiar class of phenazines. Its *in vitro* inhibitory activity against *M. tuberculosis* is at concentrations of 0.1-0.5 µg/mL. The strain of microorganisms resistant to Isoniazid and/or Streptomycin, PAS and Thioacetazone are susceptible to the drug.



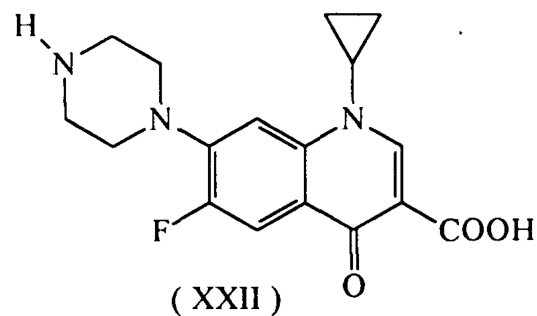
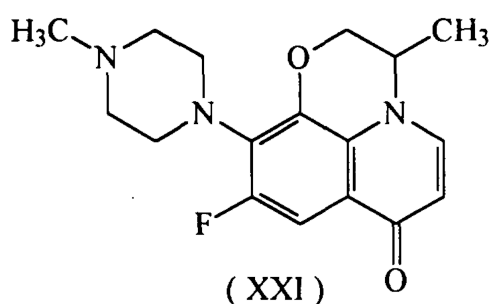
Clofazimine is not only bacteriostatic but also bactericidal and acts only on multiplying mycobacteria.

Other anti-tubercular agents under investigation

In recent years, the need for new drugs to meet the problems connected with the emergence of multidrug resistant tuberculosis and mycobacteriosis, particularly in AIDS patients, in the absence of any really new antituberculous compound, has led to the careful evaluation of the antituberculous activity of antimicrobial agents developed for their activity against common Gram positive and Gram negative bacteria. Some promising drugs have been found among the fluoro quinolones, macrolides, and β-lactams (in combination with β-lactamase inhibitors).

Fluoroquinolones

Fluoroquinolones represent an improvement of the earlier analogs (Nalidixic acid, oxolinic acid, pipemidic and cinoxacin) in being more potent *in vitro* and in having a broader antibacterial spectrum. The derivatives so far recognized with activity against Mycobacteria are Ofloxacin (XXI) and Ciprofloxacin (XXII), which have been studied broadly⁷⁵⁻⁷⁷.



MIC of Ofloxacin and Ciprofloxacin for *M. tuberculosis* range from 0.12 to 2 µg/mL. Both the drugs are bactericidal. Levofloxacin, the levoisomer of Ofloxacin, is twice as active as the parent drug. The main effects of Fluoroquinolones are the inhibition of DNA supercoiling and damage to DNA, whose synthesis is rapidly interrupted⁷⁸. At high quinolone concentrations, RNA and protein synthesis are also inhibited and cell filamentation occurs. *In vitro* combinations of fluoroquinolones with other antitubercular drugs have given variable results. The combination of Ciprofloxacin with major antitubercular drugs did not show any synergistic effects but only difference against *M. tuberculosis*. A triple combination of Sparfloxacin with Ethambutol and Rifampin resulted in synergism⁷⁹⁻⁸¹. The triple combination comprising Rifabutine and either Ciprofloxacin, Ofloxacin or Norfloxacin and Isoniazid, Refapentine and either Pefloxacin or Ciprofloxacin were the most active ones against resistant strains of *M. tuberculosis*.

The daily dosage varies according to the different derivatives as follows⁸²⁻⁸⁵.

- Ofloxacin - 400 mg twice daily
- Ciprofloxacin - 200-400 mg once daily
- Sparfloxacin - 200-400 mg once daily

Fluoroquinolones are usually well tolerated. The most common side effects are gastrointestinal reactions, central nervous system disturbances and skin and hypersensitivity reactions.

Macrolides

Clarithromycin, as well as its hydroxy metabolite (4 hydroxy – clarithromycin), are therapeutically active. *In vitro* combination of Clarithromycin with Amikacin, Ethambutol or Rifampicin did not result in activity superior to that of Clarithromycin

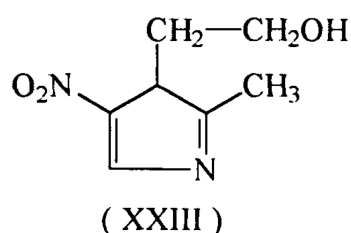
alone, while a combination of Clofazimine and Rifabutin was more active than Clarithromycin alone.

The triple combinations of Clarithromycin-Rifampicin- Clofazimine and Clarithromycin -Clofazimine-Ethambutol were significantly more active than the macrolide alone⁸⁶.

Among the new macrolide derivatives, Azithromycin has a peculiar place due to its chemical structure and its pharmacokinetics.

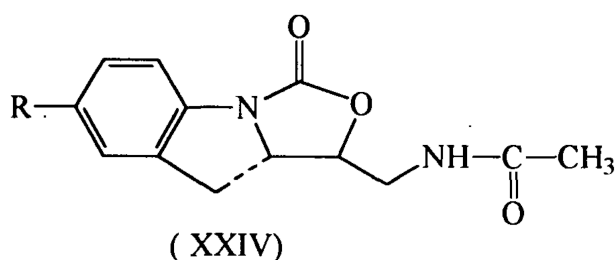
Nitroimidazoles

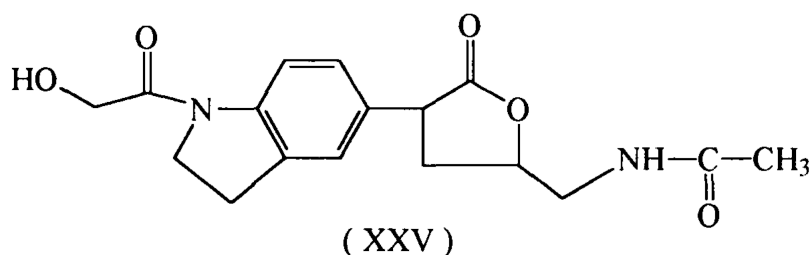
Metronidazole (XXIII), a nitroimidazole, was initially shown to be active against *Trichomonas vaginalis*, *Giardia lamblia* and *Entamoeba histolytica*. An interesting finding reported recently⁸⁷ was that it is highly active *in vitro*, and more importantly, on dormant and slow-growing tubercle bacilli, in conditions simulating those existing in host lesions. After a dose of 250-500 mg/kg, its bactericidal activity against dormant bacilli is seen with 8 µg/mL. Since no antagonism was seen with other antitubercular drugs, it would be of great value to see its action in combination with other drugs.



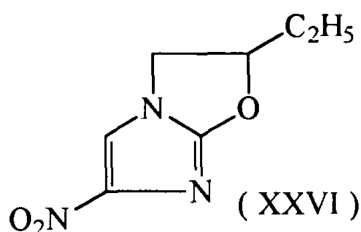
Oxazolidinones

Oxazolidinones (XXIV, XXV) are a relatively new class of drugs unrelated to other agents, with activities against gram-positive and anaerobic bacteria and *M. tuberculosis*⁸⁸. They have similar MIC against MDR(Multi Drug Resistant) tubercle bacilli as with susceptible strains.





One compound, DUP 721(CGP17341)(XXVI), has a MIC of 0.1-0.3 $\mu\text{g/mL}$, with no cross resistance with antituberculosis drugs, and more importantly, has been shown to be highly protective against experimental tuberculosis infection in mice⁸⁹.



Poloxamer surfactants

These compounds were earlier referred to as "defense stimulators" several years ago. Conforth *et al*⁹⁰ attempted to modify the susceptibility of mice to experimental tuberculosis using some surface active agents (now called poloxamers) by condensing p-tetra-octylphenol with formaldehyde to give polynuclear compounds, which have either a linear or macrocyclic structure.

Subsequently, it was shown that tyloxpil, an important member in this series, was one-sixth as active *in vitro* as dihydrostreptomycin, and it acted synergistically with that drug⁹¹. Another surface active agent, alpha- methyl-alpha-n- dodecyl succinic acid (called B-53) was also found to be active in guinea pig tuberculosis. However, it was soon found that its activity was antagonized by serum and other body fluids and was toxic on long-term usage. Chinnaswamy and associates⁹² have evaluated the *in vitro* intracellular and *in vivo* activity of CRL 8131. It was shown to have dose related *in vitro* activity with a MIC of 6.25 $\mu\text{g/mL}$, and on intracellular bacilli the MIC was 1.25 $\mu\text{g/mL}$.

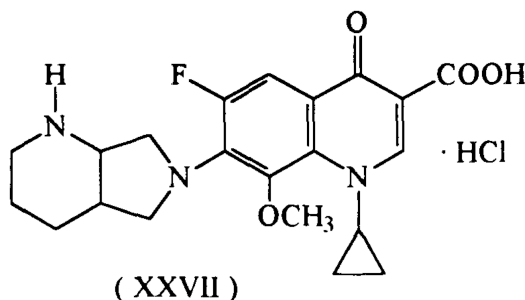
Plant Sources

Considering the seriousness of the disease, several attempts were made to discover antituberculosis drugs from plant sources. In many developing countries, the indigenous system and traditional folk medicines are flourishing very well in close

competition with Western medicine. Allicin, a compound isolated from *Allium sativum* (common name : Garlic), which was shown a long time ago to possess powerful antituberculosis activity by Rao *et al*⁹³ is being reinvestigated recently by *in vitro* studies⁹⁴.

Moxifloxacin HCl (BAY-12-8039)⁹⁵

Bay-12-8039 (XXVII) efficiently reduced *M. tuberculosis* infection in mice, with an activity comparable to that of Isoniazid. A combination of the two compounds shows superior antibacterial efficacy than either compound alone.



Drug resistance in Tuberculosis

Correct application of several powerful drugs in the various types of regimens that have evolved from well-planned clinical trials, could offer a potentially 100 % successful treatment of the disease. Several reasons have been cited for the failure of tuberculosis treatment, chief among them being drug resistance of the tubercle bacilli. From a microbiological standpoint, resistance and susceptibility are relative terms, since practically all populations of drug susceptible bacilli will contain a certain proportion of resistant mutants. According to Mitchison⁹⁶ clinically meaningful resistance is indicated by a decrease of susceptibility to a drug of a sufficient degree to be reasonably certain that the strain concerned is different from a sample of wild strains of tubercle bacilli that had never come into contact with the drug.

Epidemiologically, drug resistance in tuberculosis is classified into three types.

- 1) Primary drug resistance, where previously untreated patients are found to have drug resistant organisms, presumably because they have been infected from an outside source of resistant bacilli.
- 2) Acquired drug resistance, where the patients who initially have drug susceptible tubercle bacilli later become drug resistant due to noncompliance in drug taking.

- 3) Initial drug resistance denotes drug resistance in patients who deny history of previous chemotherapy.

Theoretical Aspects Of Drug Resistance

A) Origin of Drug Resistance

All available evidence shows that drug resistance in *M. tuberculosis* occurs only by mutation, the drug acting merely as a selective agent.

B) Mechanism of drug resistance.

Among the several theories postulated to explain the mechanisms by which a drug resistance develops in microorganisms, only three are not relevant to mycobacteria⁹⁷.

- 1) Development of insusceptible metabolic pathways
- 2) Interference in uptake or penetration of the drug into the bacterial cell, and
- 3) Destruction of drugs.

C) Emergence of Drug resistance

The emergence of drug resistance is due to the selection of preexisting resistant mutant in the original bacterial population.

Special aspects of Drug Resistance

A) Natural Resistance

Natural resistance of the tubercle bacilli was found to exist only against two bacteriostatic drugs, PAS and Thiacetazone. Contrary to the experience with PAS, it was found that the natural resistance to Thiacetazone was associated with the likelihood of an unfavourable response of treatment with Isoniazid plus Thiacetazone.

B) Cross resistance

Cross-resistance normally occurs between drugs that have some chemical relationships, and has been observed among several antituberculous drugs with the available knowledge.

Other Bacterial Infections

There is a continuing need for new antibacterial agents as infectious bacteria have proved to be incredibly adaptable in their fight for survival. Bacteria are mutating and

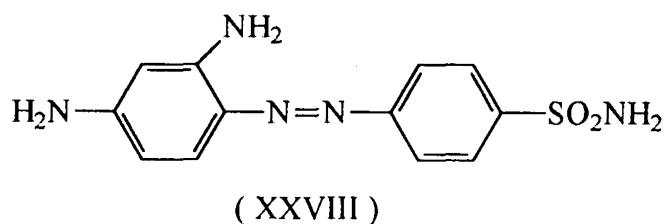
evolving in ways that make them resistant in commonly occurring bacterial infections and hence, better drugs are needed for emerging diseases⁹⁸, such as those caused by invasive *Streptococci*, as well as drug resistant *Enterococci* and *Mycobacteria*. To meet this challenge, research has focused on -

- 1) New analogs of existing classes of antibacterial agents,
- 2) Novel classes lacking cross-resistance, and
- 3) Novel antibacterial screening targets.

The history of antibacterials has thus been dynamic, characterized by the constant emergence of new challenges, followed by investigations, discovery and the production of new drugs. A complete review of the various agents employed as antibacterials would be beyond the scope of this work and hence, is not attempted. A brief summary of the important classes of compounds concerned with this subject matter is presented below.

Sulfonamides

The development of Sulfonamides is one of the most fascinating and informative chapter in the field of medicinal chemistry. Although the word chemotherapy was coined by Ehrlich to articulate the concept of the selective inhibition of microbes by chemicals in the early years of the century, the discovery of the antibacterial activity of Prontosil in the early 1930s was the beginning of the present era of chemotherapy. The story of sulfonamides goes back to the early years of this century when Horlein, Dressel and Kethe found that introduction of a sulfamyl group imparted fastness to acid wool dyes, thus indicating affinity for protein molecules⁹⁹. Mietzch and Klarer synthesized a variety of azo dyes as antibacterials. They synthesized a group of such dyes containing sulfonamide radical, which included Prontosil(XXVIII).



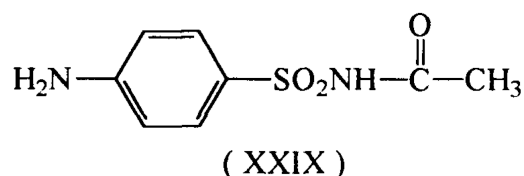
Domagk observed in 1932 that Prontosil protected mice against *Streptococcal* infections and rabbits against *Staphylococcal* infections.

These discoveries have a tremendous impact not only on the development of sulfonamides as antimicrobials, but also as developments in chemotherapy, in general.

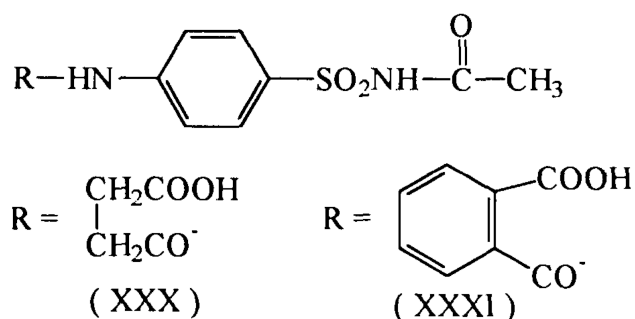
In an effort to improve the efficacy of sulfanilamide, more than 5000 derivatives and analogues were investigated in the first decade of research in this field. By variation of N'-substituent, a variety of N'-substituted sulfonamides having antimicrobial activity have been obtained, all acting by PABA antagonism, and include most of the sulfonamides used in clinical practice today. These developments are discussed below briefly according to the type of the N'-substitution introduced.

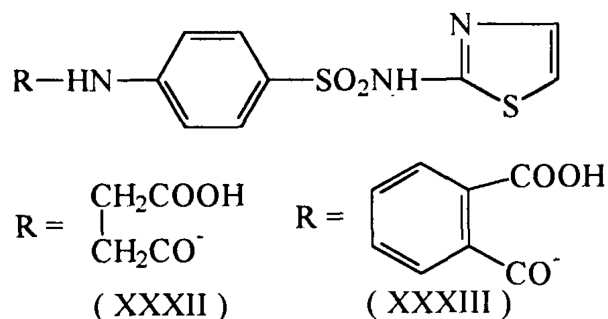
A) N-acyl derivatives

N'-acyl substitution leads to compounds with enhanced biological activity and useful physicochemical properties which are required for special pharmaceutical formulations. The most important member of this group, widely used in ophthalmic practice, is Sulfacetamide (XXIX). It is quickly absorbed and rapidly excreted without the danger of crystallization in the kidney.



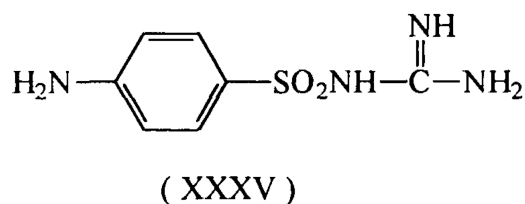
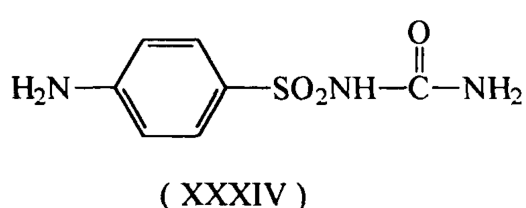
N⁴-carboxyacyl derivatives of Sulfacetamide such as N⁴-phthalyl and N⁴-succinyl Sulfacetamides (XXX) and (XXXI)¹⁰⁰ and of certain N¹-heterocyclic sulfonamides such as N⁴-phthalyl and N⁴-succinyl-sulfathiazole (XXXII), and (XXXIII)¹⁰¹, were developed for parenteral administration.





These agents have been extensively used for presurgical sterilization of the gut and for intestinal infections.

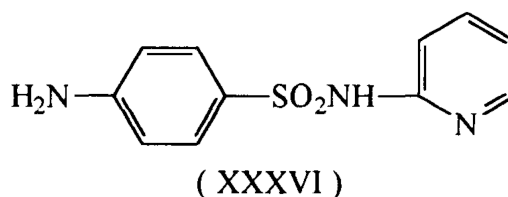
Among the N¹-carbamoyl derivatives, sulfacarbamide (XXXIV) and sulfaguanidine (XXXV) have been clinically used.



B) N¹-heteroaromatic sulfanilamides

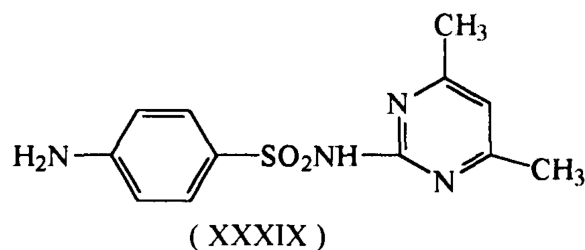
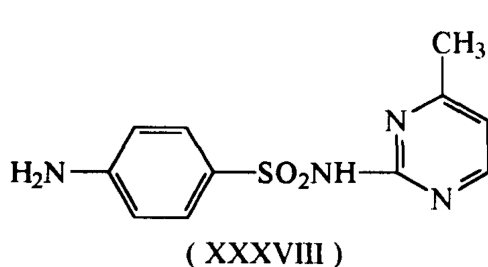
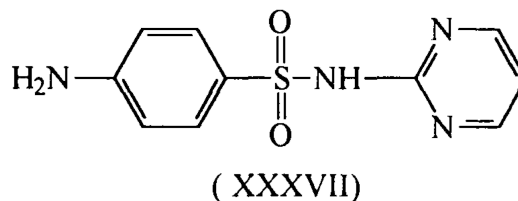
Most of the therapeutically used sulfonamides belong to this class and are obtained by varying the N¹-heterocyclic residue. It has been possible to greatly increase the usefulness of sulfonamides by widening their antimicrobial spectrum, increasing the inhibition index, and modifying their pharmacokinetic properties.

Sulfapyridine(XXXVI) was one of the earliest of the new sulfonamides to be used in clinical practice for the treatment of pneumonia, and remained the drug of choice until it was replaced by Sulfathiazole.



The derivatives of pyrimidine have received the maximum attention and have provided a large number of clinically useful drugs. A sulfonamide linkage at either the 2- or the 4- position gives active compounds. Sulfadiazine (XXXVII) was the first of these derivatives of pyrimidine to be introduced in clinical practice. Two methylated

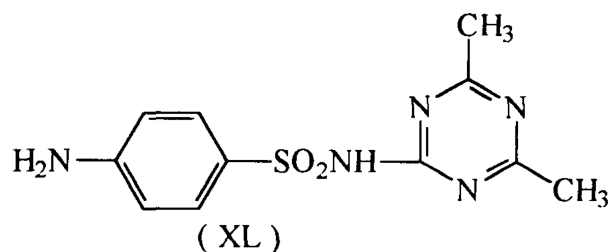
derivatives of Sulfadiazine, Sulfamerazine (XXXVIII) and Sulfamethazine(XXXIX), were soon introduced in therapeutics.



Since the quantitative differences in the properties of three pyrimidine derivatives seem to complement one another, these have been used in combination as “triple sulfa”. This made it possible to -

- 1) Lower the dose of each individual component, thus reducing the toxicity of each and significantly reducing the tendency of crystalluria.
- 2) Maintain higher concentration of the sulfa in plasma and tissue, over a long period.

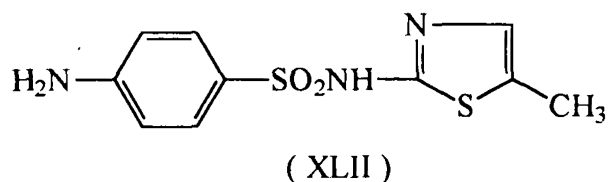
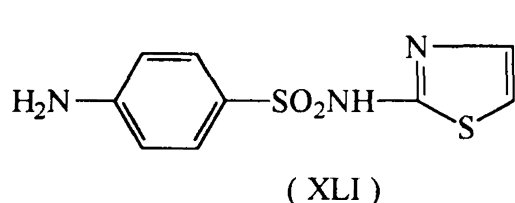
Among the 4-sulfapyrimidine derivatives, sulfasomidine(XL) was shown to possess useful activity¹⁰². Its main advantage was high solubility and low tendency to crystalluria. A useful “disulfa” used was a combination of Sulfasomidine and Sulfadiazine.



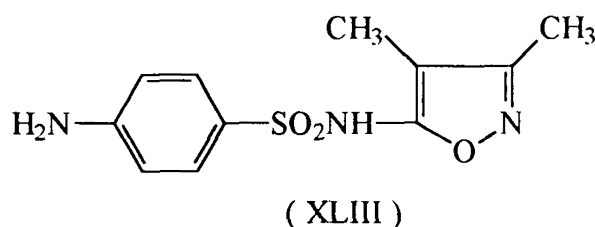
Clinically used sulfas containing five membered heterocycles and have two or more hetero atoms in the rings. The more active compounds belong to the thiazole, oxazole, isoxazole, 1,3,4-thiadiazole and pyrazole groups. Most of the clinically used five- membered heterocyclic compounds possess one or two CH₃ groups, which

must increase their hydrophobicity and impart favourable properties for binding to proteins, and perhaps to the folate enzymes.

Sulfathiazole(XLI) replaced Sulfapyridine because of its wider antibacterial spectrum and higher therapeutic index. Methyl substituent in the 5-position gave sulfamethizole(XLII), which exhibited an increased antibacterial activity.

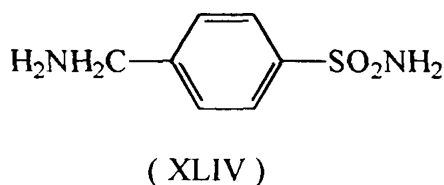


Another sulfonamide introduced during this period was Sulfisoxazole(XLIII). Its significant activity against *Proteus vulgaris* and *Escherichia coli* makes it useful in urinary tract infections¹⁰³.

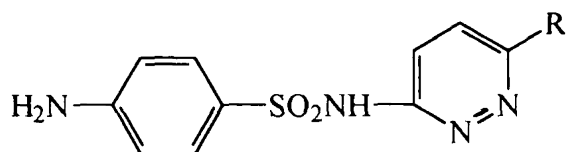


C) 4- substituted derivatives

Mefenide (XLIV) is active against anaerobic bacteria and, therefore, finds use for topical application in burn infected wounds and gas gangrene. It does not act through the PABA mechanism and can be used against organisms resistant to other sulfonamides¹⁰⁴.



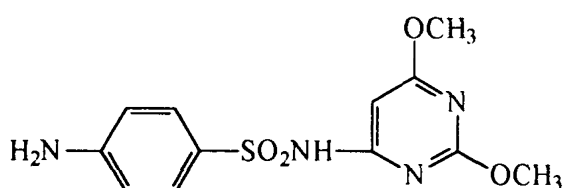
The era of newer long acting sulfonamides started in 1956 with the introduction of sulfamethoxy pyridiazine¹⁰⁵ (XLV) having half-life of around 37 hours. Sulfachlorpyridiazine (XLVI) has been found to have a half-life of about 7 hours and is useful in urinary tract infections.



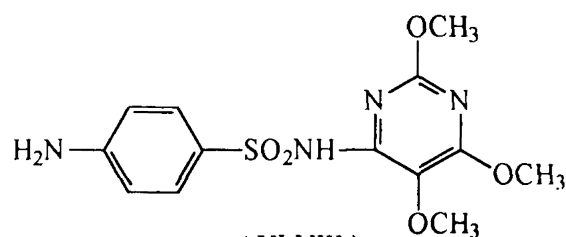
(XLV) R = OCH₃

(XLVI) R = Cl

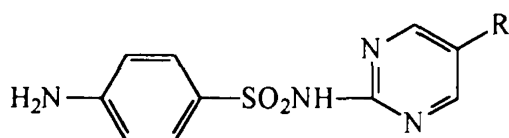
In 1959 Sulfadimethoxine(XLVII) was introduced, with a half-life of approximately 40 hours. The related Sulfamethoxine(XLVIII) having the two methoxyl radicals in 5,6-positions, has by far the longest half life of about 150 hours and needs administration only once a week. Sulfamethyldiazine(XLIX) and Sulfamethoxy-diazine(L)¹⁰⁶ each having a half-life of 36 hours, and Sulfamethoxy-pyrazine(LI)¹⁰⁷ with a half life of 65 hours, were the other long acting sulfonamides introduced during this period.



(XLVII)

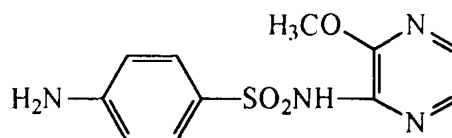


(XLVIII)



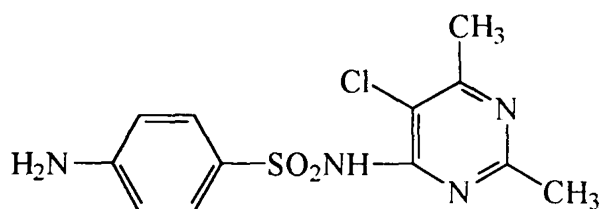
(XLIX) R = CH₃

(L) R = OCH₃

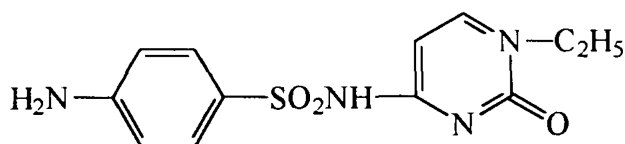


(LI)

A new broad-spectrum sulfonamide, Sulfacloamide (LII), was reported in 1970. It is relatively non-toxic and gives higher serum levels than other sulfonamides. Sulfacytine, N Sulfanilyl-1-ethylcytosine(LIII), is 3-10 times more potent *in vivo* than Sulfisoxazole, Sulfachlorpyridazine and Sulfasomidine, and it was suited for urinary tract infections.



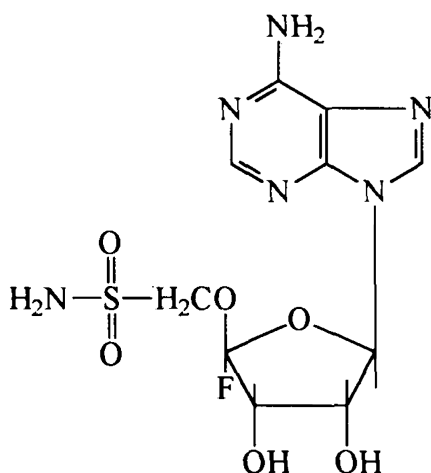
(LII)



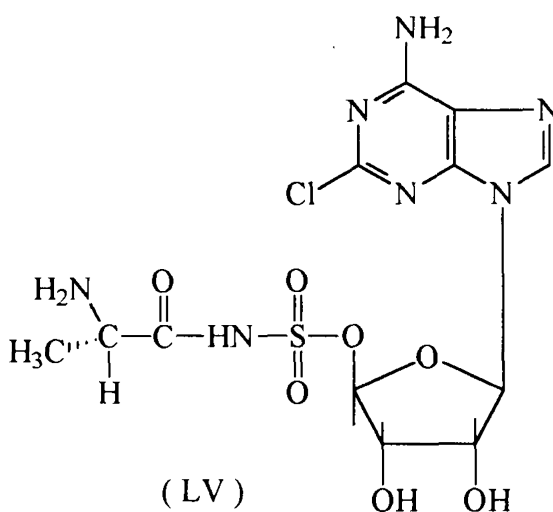
(LIII)

Naturally occurring sulfonamides

Nucleocidine (LIV) and Ascamycin (LV) were obtained from *Streptomyces* species and were isolated from soil samples^{108,109}. Nucleocidine was shown to exhibit a rather broad antibacterial spectrum and a high order of activity against Trypanosomes. Ascamycin showed a very selective action as compared to desalanyl ascamycin.



(LIV)



(LV)

Mechanism of action

Sulfonamides are structural analogs and competitive antagonists of PABA and thus prevent normal bacterial utilization of PABA for the synthesis of folic acid. More specifically, sulfonamides are competitive inhibitors of dihydropteroate synthetase, the bacterial enzyme responsible for the incorporation of PABA into dihydropteroic acid, the immediate precursor of folic acid.

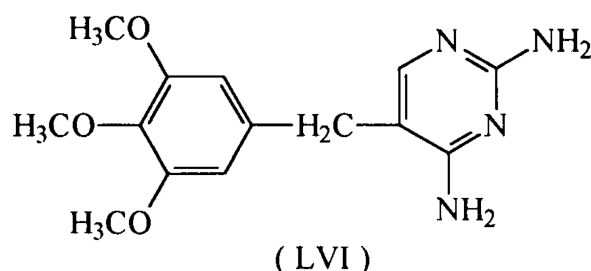
Acquired Bacterial Resistance to Sulfonamides

Bacterial resistances to sulfonamides are presumed to originate by random mutation and selection or by transfer of resistance to plasmids. The resistance to sulfonamides is probably the consequence of an altered enzymatic constitution of the bacterial cell and the alteration may be characterized by -

- i. An alteration in the enzyme that utilizes PABA dihydropteroate synthetase.
- ii. An alternative metabolic pathway for synthesis of the essential metabolite
- iii. An increased capacity to destroy or inactivate the drugs.
- iv. An increased production of an essential metabolite or drug antagonist.

Trimethoprim- Sulfamethoxazole

The introduction of Trimethoprim(LVI) in combination with Sulfamethoxazole (Combination known as co-trimoxazole) constitutes an important progress in the development of clinically effective antimicrobial agents. These two drugs act on sequential steps in the pathway of an obligate enzymatic reaction in the bacteria; the result of their combination is synergistic¹¹⁰.



The antimicrobial activity of the combination of Trimethoprim and Sulfamethoxazole results from its action on two steps of the enzymatic pathways for the synthesis of tetrahydrofolic acid. Sulfonamide inhibits the incorporation of PABA into folic acid and Trimethoprim prevents the reduction of dihydrofolate to tetrahydrofolate

Therapeutic uses

- 1) Urinary tract infections
- 2) Bacterial respiratory tract infections caused by bacteria
- 3) Gastrointestinal infections
- 4) Infection by *Pneumocystis carinii*, a high dose therapy is effective for this severe infection in patients with AIDS.

ANTIBIOTICS

In 1942, Waksman¹¹¹ proposed the widely cited definition that an antibiotic or antibiotic substance is a substance produced by microorganisms, which has the capacity of inhibiting the growth and even of destroying other micro organisms. With the advances made by medicinal chemists to modify naturally occurring antibiotics and to prepare synthetic analogues, it has become necessary to permit the inclusion

of semi-synthetic and synthetic derivatives in the definition. Therefore, a substance is classified as an antibiotic if -

- 1) It is a product of metabolism.
- 2) It is a synthetic product produced as a structural analogue of a naturally occurring antibiotic.
- 3) It antagonizes the growth or the survival of one or more species of microorganisms
- 4) It is effective at low concentrations

β -Lactam Antibiotics

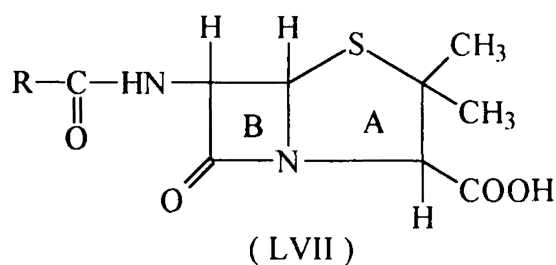
Antibiotics that contain β -lactam (a four membered cyclic amide) ring structure constitute the dominant class of agents currently employed for the chemotherapy of bacterial infections. The β -lactam antibiotics are broadly divided into two classes

- 1) Penicillins
- 2) Cephalosporins

1) The Penicillins

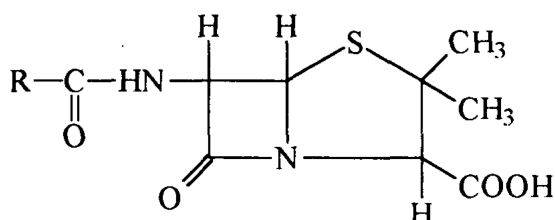
Since Alexander Fleming's serendipitous discovery of Penicillin in 1928, it has remained one of the most important group of antibiotics. Despite the discovery of several other antibacterial agents, the Penicillins are widely used with new derivatives being produced every year. Due to their unique advantages, members of this group of antibiotics are presently the drugs of choice for the treatment of a large number of infectious diseases.

The basic structure of Penicillins(LVII) consists of a thiazolidine ring (A) connected to a β -lactam ring (B) to which is attached a side chain(R). The Penicillin nucleus itself is the chief structural requirement for biological activity; metabolic transformation or chemical alteration of this portion of the molecule causes loss of all significant anti-bacterial activity.



Many of the antibacterial and pharmacological characteristics of Penicillins are determined by the side chain, which can also alter the susceptibility of the resultant compound to inactivating enzymes (β -lactamases). The discovery that 6-amino penicillanic acid could be obtained from a culture of *P. chrysogenum* that was depleted of the side chain precursors led to the development of the semi-synthetic Penicillin.

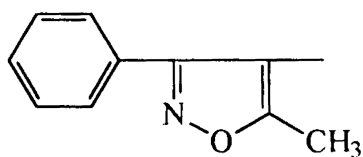
The examples of semi-synthetic Penicillins used in the treatment of bacterial infections are listed below¹¹².



Structure of Penicillins

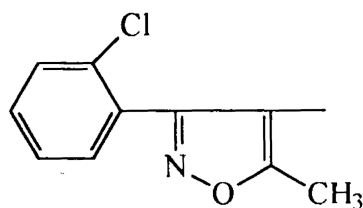
Penicillins	R	No.
Penicillin G		(LVIII)
Penicillin V		(LIX)
Methicillin		(LX)
Nofcillin		(LXI)

Oxacillin



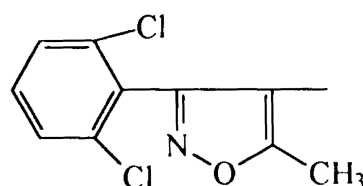
(LXII)

Cloxacillin



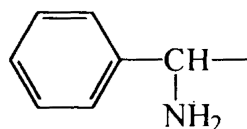
(LXIII)

Dicloxacillin



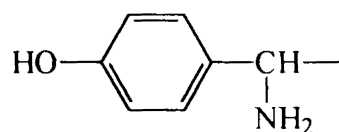
(LXIV)

Ampicillin



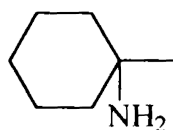
(LXV)

Amoxicillin



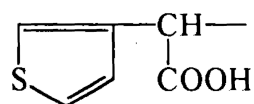
(LXVI)

Cyclacillin



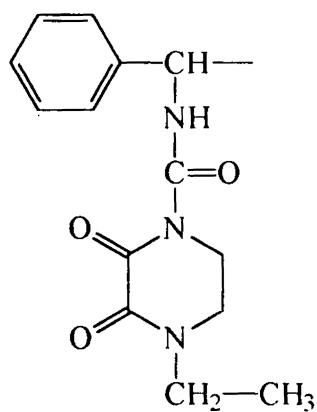
(LXVII)

Ticarcillin



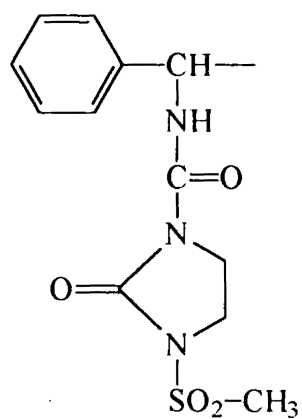
(LXVIII)

Pipercicillin



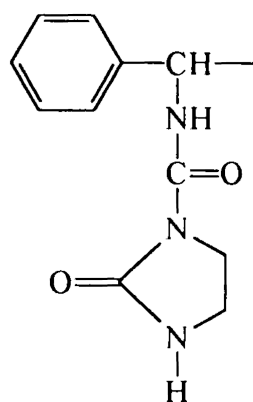
(LXIX)

Mezlocillin



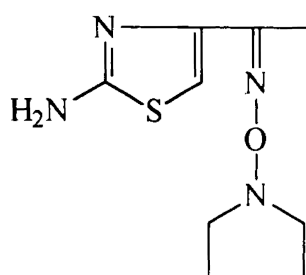
(LXX)

Azolacillin



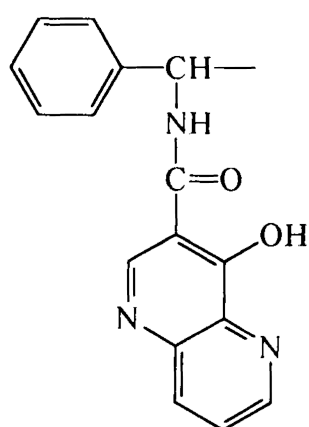
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BRL-44154



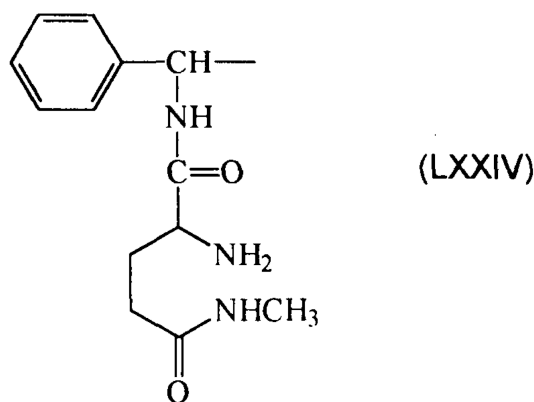
(LXXII)

Apacillin



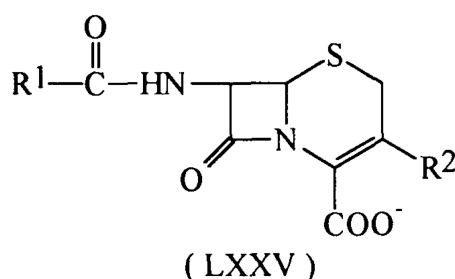
(LXXIII)

Aspoxicillin



2) Cephalosporins

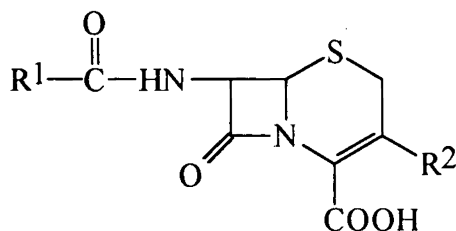
The second major group of β -lactam antibiotics to be discovered was the Cephalosporins(LXXV). The Cephalosporins have shown tremendous growth over the past decade and are usually classified by the "generations" system, which is based on the general features of antimicrobial activity.



Cephalosporium acremonium, the first source of the Cephalosporins, was isolated in 1948 by Brotzu from the sea. Most of the antibiotics introduced since 1965 have been semi-synthetic Cephalosporins. Cephalosporin C contains a side chain derived from D- α -aminoadipic acid, which is condensed with a dihydrothiazine β -lactam ring system. It appears that modifications with alteration in antibacterial activity and substitutions at position 3 of the dihydrothiazine ring, are associated with change in metabolism and pharmacokinetic properties of the drugs¹¹³.

Cephalosporins are traditionally divided into first, second, third and forth generation agents depending on their stability to β -lactamases and spectrum of activity. Generally, the first, and some second generation agents, maintain excellent activity against *Streptococci* and *Staphylococci*, while third generation compounds such as Ceftazidime and Cefoperazone have extended Gram-negative coverage. The Cephameycins are similar to the Cephalosporins but have a methoxy group at 7-

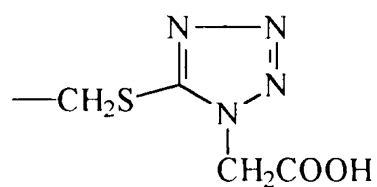
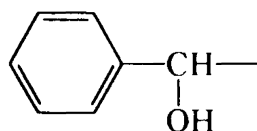
position of the β -lactam ring. A list of different Cephalosporins which are in clinical use¹¹⁴⁻¹¹⁵ is mentioned below -



Structure of Cephalosporins

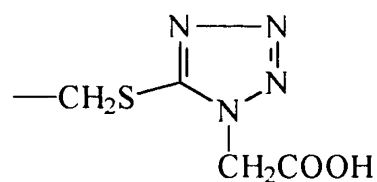
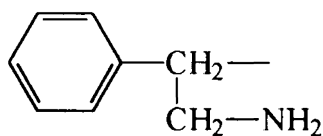
Name	R ¹	R ²	No.
Cephalexin		-CH ₃	(LXXVI)
Cephardine		-CH ₃	(LXXVII)
Cefadroxil		-CH ₃	(LXXVIII)
Cefaclor		-Cl	(LXXIX)
Cephalothin		-CH ₂ OCOOCH ₃	(LXXX)
Cephapirin		CH ₂ OCOCH ₃	(LXXXI)
Cefamandole			(LXXXII)

Cefanacid



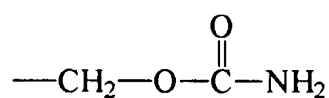
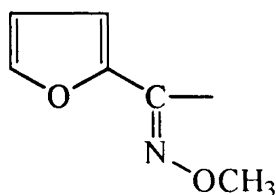
(LXXXIII)

Cefanarid



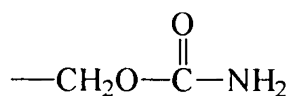
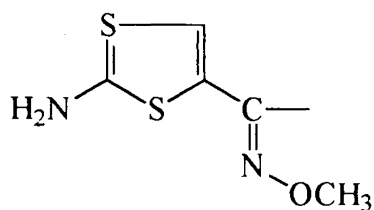
(LXXXIV)

Cefuroxime



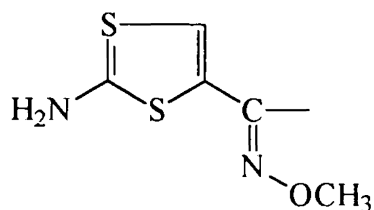
(LXXXV)

Cefotaxime



(LXXXVI)

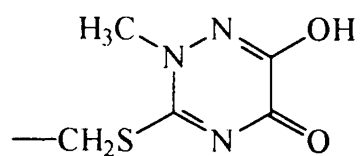
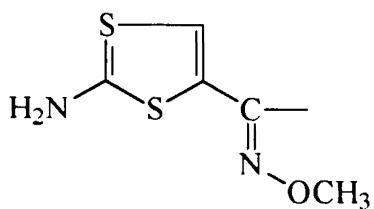
Ceftizoxime



H

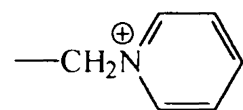
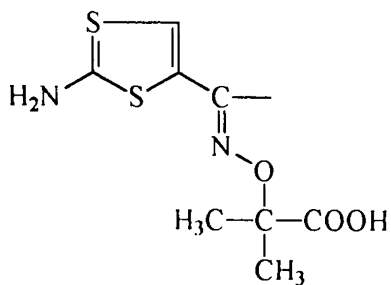
(LXXXVII)

Ceftriaxone



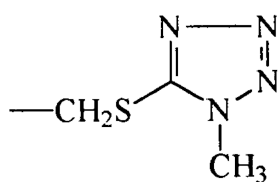
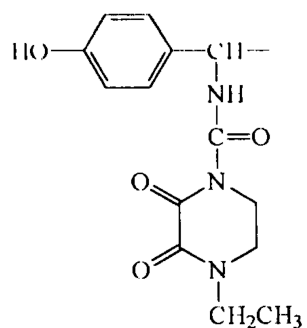
(LXXXVIII)

Ceftazidime



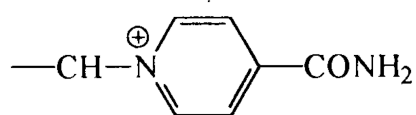
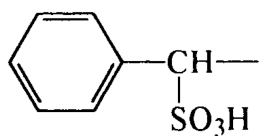
(LXXXIX)

Cefoperazone



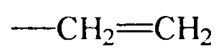
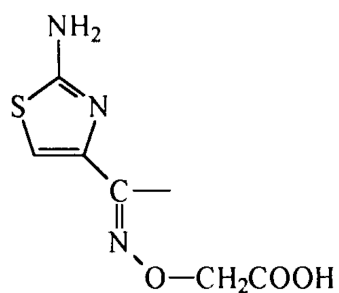
(LXXXXX)

Cefsulodin



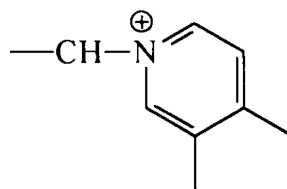
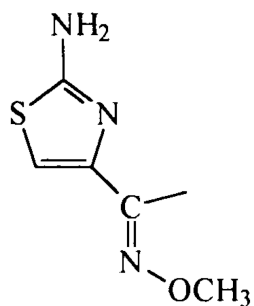
(LXXXXXI)

Cefixime



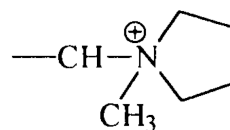
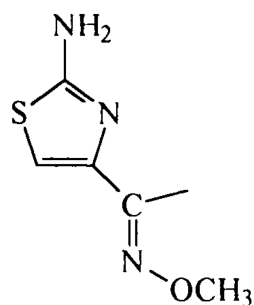
(LXXXXXII)

Cefpirame



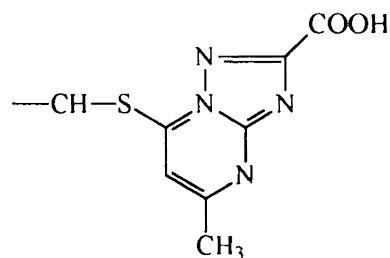
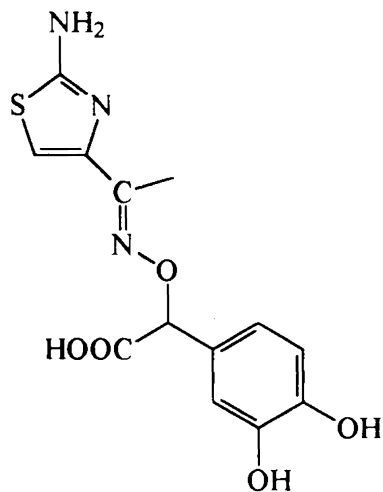
(LXXXXXIII)

Cefepime



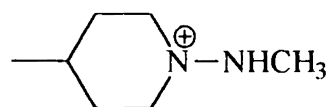
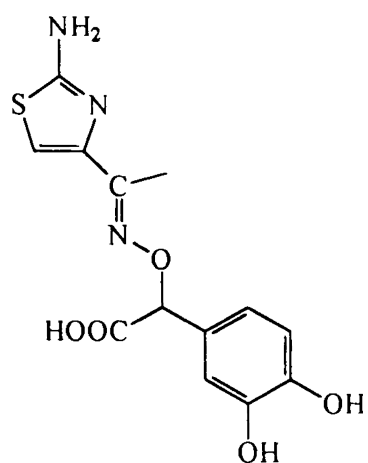
(LXXXXXIV)

M-14659



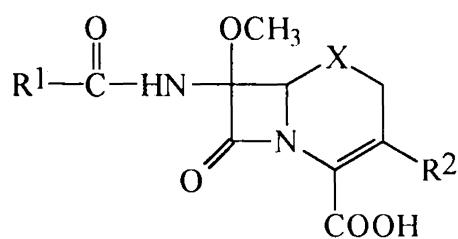
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BRL 57342

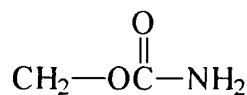
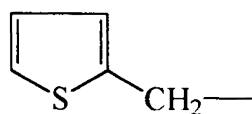


(LXXXXVI)

Parenteral Cephamycin

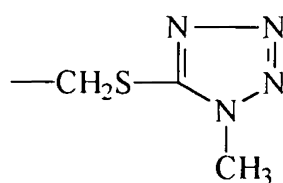
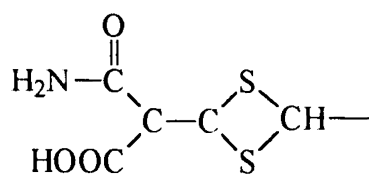


Name
Cefexitin



X
S (LXXXXVII)

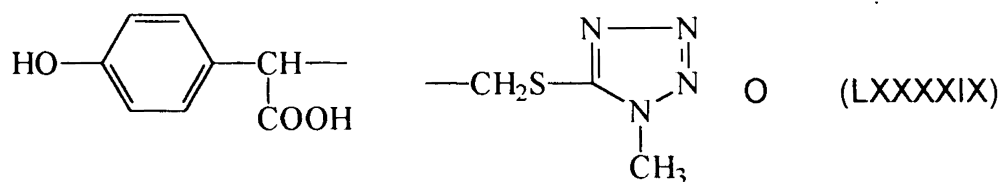
Cefatetan



S (LXXXXVIII)

441

Moxalactam



The most recent cephalosporin derivatives, which are under clinical trials are listed below

Name	R ₁	R ₂	R ₃	No.
Cefdinir ¹¹⁶		—HC=CH_2	H	(LL)
S-1090 ¹¹⁷			H	(LLI)
Cefcapene ¹¹⁸		—C—C(=O)—NH_2	$\text{—CH—O—C(=O)—C(CH}_3)_3$	(LLII)
E-1101 ¹¹⁹		$\text{—CC(=O)N(CH}_3)_2$	$\text{—CHCH}_3\text{OCOCH(CH}_3)_2$	(LLIII)

Cephalosporins - Generations and Biological Spectrum

Generation	Examples	Useful spectrum
First	Cefazolin Cephalothin Cephalexin	<i>Streptococci</i> , <i>S. aureus</i> , No activity against Enterococci
Second	Cefuroxime cofactor Cefoxitin Cefotetan	<i>E.coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>H. Influenza</i> . Not active against gram-positive organisms
Third	Cefotaxime Ceftriazone Cefazidine	<i>Enterobacteriaceae</i> , <i>Pseudomonas aeruginosa</i> , <i>Neisseria</i> , <i>Gonorrhoeae</i> , <i>S. aureus</i>
Fourth	Cetepime	Comparable to third generation, but more resistant to β -lactamases.

Mechanisms of Bacterial Resistance to Penicillins and Cephalosporins

The microorganisms may be intrinsically resistant because of the structural differences in the PBPS which that are the targets of these drugs. Furthermore, it is possible for a sensitive strain to acquire resistance of this type by development of high molecular weight PBPs, which have decreased affinity for the antibiotics.

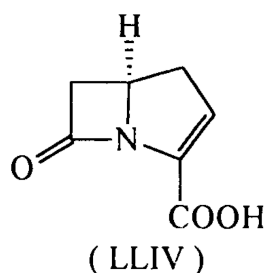
Other instances of bacterial resistance to the β -lactam antibiotics are caused by the inability of the agent to penetrate to its site of action¹²⁰.

Most of the Gram-positive bacteria produce a large amount of β -lactamase, which is secreted extracellularly. The enzymes may hydrolyze Penicillins, Cephalosporins or both.

Other promising Antibiotics

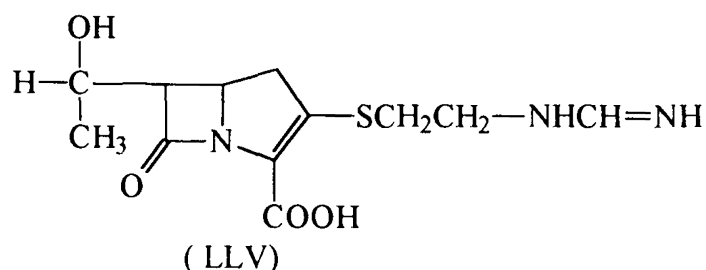
Carbapenems

The general structure of carbapenems is referred to as 1-carbapen-2-em-3-carboxylic acid (LLIV) and it has been isolated as a natural product from certain species of *Serratia* or *Erwinia*.

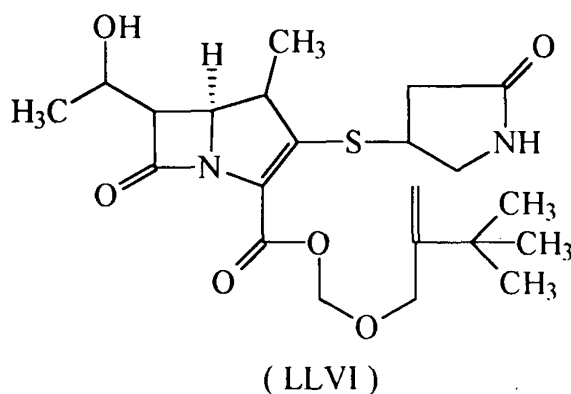


The discovery of this novel series of highly active bicyclic compounds, so markedly different from Penicillins and Cephalosporins, revitalized ideas concerning the structural and stereochemical features required for biological activity and stability amongst the β -lactams.

Imipenem (LLV) is the most active agent available against a wide variety of bacteria.

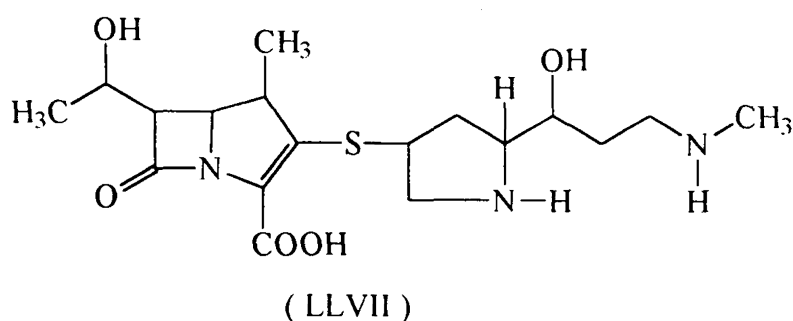


Based on the high stability of its metabolites to penicillinases and its low susceptibility to β -lactamases, it appears that CS 834(LLVVI) would be more effective than other penem antibiotics in treating infections caused by β -lactamases-producing organisms¹²¹.

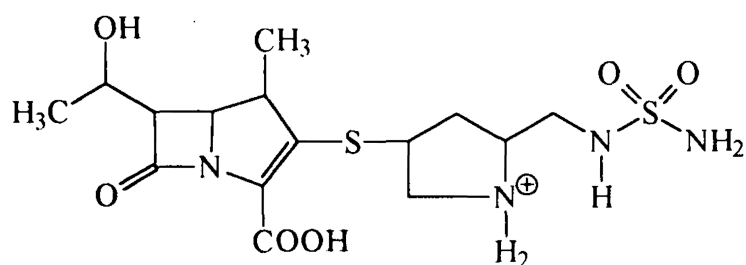


Lenapenem HCl · H₂O (LLVII)

In vitro studies showed that Lenapenem was 4 to 8 fold more active than Meropenem, Imipenem and Blapenem against Methicillin- resistant *Staphylococcus aureus*¹²².

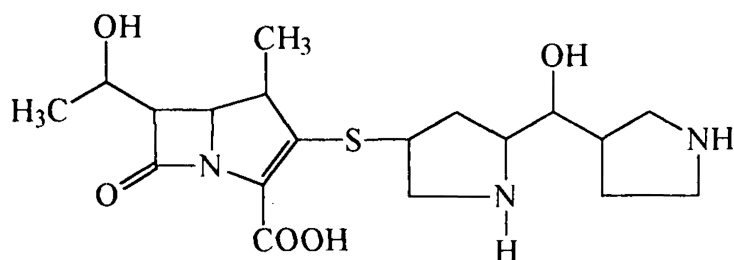


S-4661¹²³



(LLVIII)

ER-35786¹²⁴

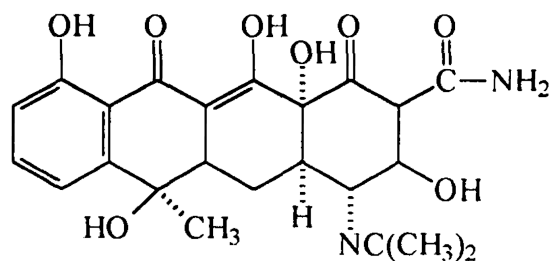


(LLIX)

These agents displayed higher *in vitro* activity against MRSA and *Streptococci* (MIC $\leq 0.39\mu\text{g/mL}$)

Tetracyclines

The Tetracyclines (LLX) exhibit a wide range of activity against Gram-positive and Gram-negative bacteria. In general, Gram-positive bacteria are affected by lower concentrations of tetracycline than the Gram-negative organisms. However, they are recommended for infections caused by Gram-positive bacteria due to problems of resistance and non-availability of superior antibacterial agents.



(LLX)

Microorganisms that have become resistant to one type of Tetracyclines frequently become resistant to the other members of the family. All the Tetracyclines produce gastrointestinal irritation to varying degrees in some individuals, and this is more common after oral administration.

Mechanism of action

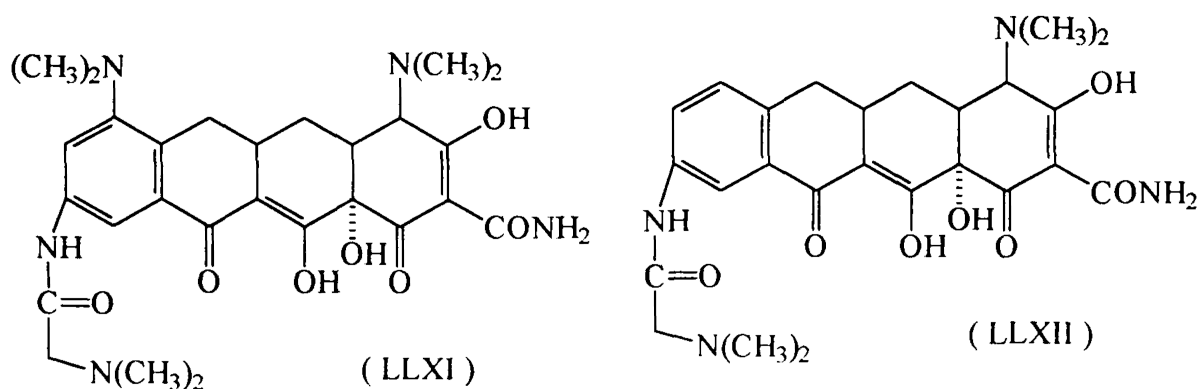
Tetracyclines are thought to inhibit bacterial protein synthesis by binding to the 30s subunit of the bacterial ribosome and preventing access of aminoacyl t-RNA to the acceptor (A) site on the m RNA ribosome complex.

There are two major mechanisms associated with bacterial resistance to Tetracyclines.

- 1) The presence of membrane bound efflux proteins (tet A-tet D-tet L) that constantly remove the antibiotic from the cytoplasm in an energy-dependent manner, and
- 2) The presence of proteins (tet M – tet O) that interact with the ribosome and allow bacterial protein synthesis even in the presence of Tetracyclines¹²⁵.

Tetracycline with an improved antibacterial spectrum

N,N-Dimethylglycylamidomycin (DMG-MINO)(LLXI) and 6-dimethyl-6-deoxytetracycline (DMG-DMDOT) (LLXII) proved to be very active against strains carrying efflux and ribosomal protection resistant determinants. Moreover, the recent identification of the first effective inhibitors of Tet protein efflux in everted vesicles, such as Tetracycline(LLXIII)¹²⁶ opened new avenues for future application of this approach to overcome bacterial resistance.





The macrolide antibiotics have three common chemical characteristics

- The spectrum of antibacterial activity of the more potent macrolides resembles that of Penicillins. They are frequently active against bacterial strains that are resistant to the Penicillins. The macrolides are generally effective against most species of Gram-positive bacteria and also exhibit useful effectiveness against Gram-negative cocci.

Erythromycin has become the most widely used macrolide antibiotic isolated from *Streptomyces erythreus*. Erythromycin has a moderately broad spectrum of activity against Gram-positive bacteria and Gram-negative cocci, and is orally absorbed and safe.

- 1) Roxithromycin
- 2) Dirithromycin
- 3) Flurithromycin
- 4) Azithromycin
- 5) A-69334
- 6) Clarithromycin

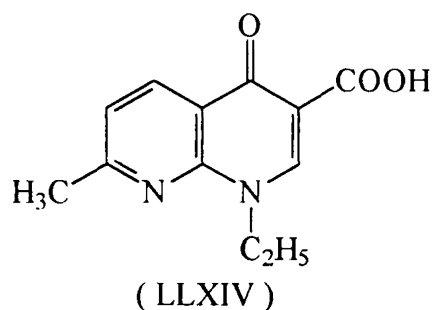
They exhibit a wide diversity of structural variations and are generally subdivided into

Leucomycin and Tylosin.

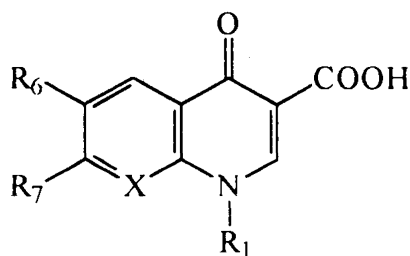
QUINOLONES

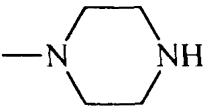
The 4-quinolone antibacterial compounds have a number of advantages over other classes of antibacterials. They are effective against many organisms, well absorbed orally, well distributed in tissues and they have relatively long serum half-lives and minimal toxicity.

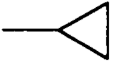
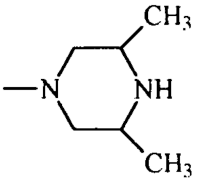
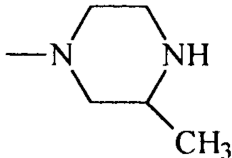
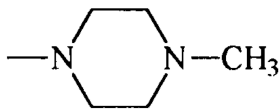
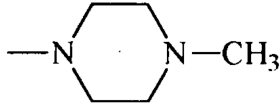
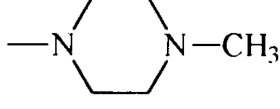
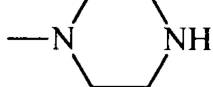
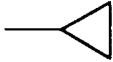
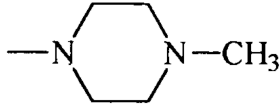
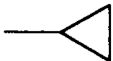
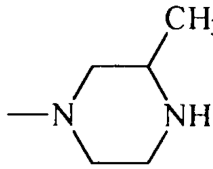
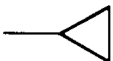
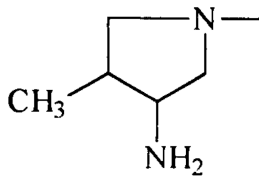
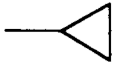
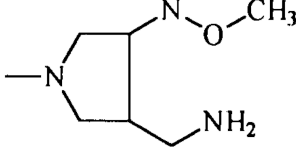
The quinolone carboxylic acid, or 4-quinolones, are a group of synthetic antibacterial agents structurally related to Nalidixic acid (LLXIV). Nalidixic acid is active against Gram-negative bacteria but has little activity against *Pseudomonas* and Gram-positive organisms, because of which its bactericidal use has generally been limited to the treatment of urinary tract infections.

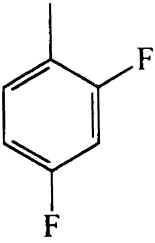
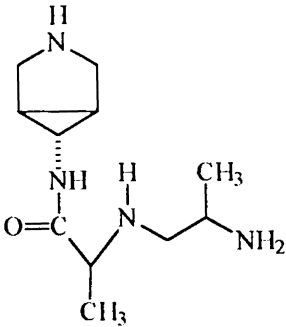
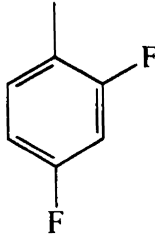
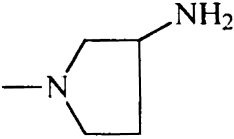
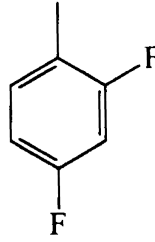
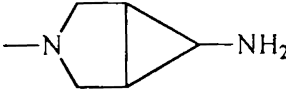
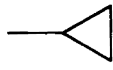
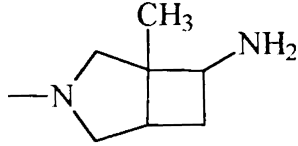
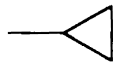
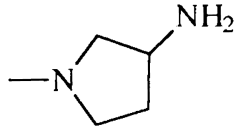
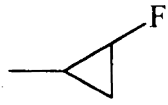
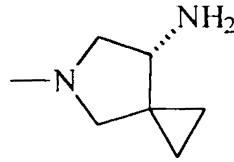
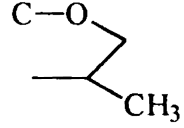
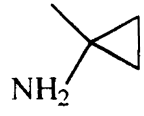


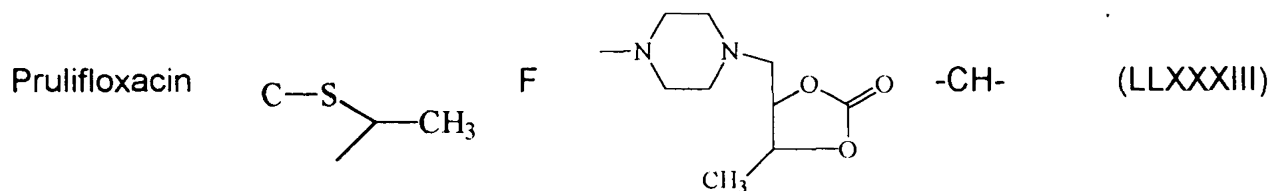
The addition of a 7-piperazinyl group and a fluorine atom at position 6 has produced a group of fluoroquinolones with a broad-spectrum of activity as compared to Nalidixic acid and are more useful for the treatment of systemic infections. They include -



Congener	R ₁	R ₆	R ₇	X	No.
Norfloxacin	—C ₂ H ₅	F		-CH-	(LLXV)

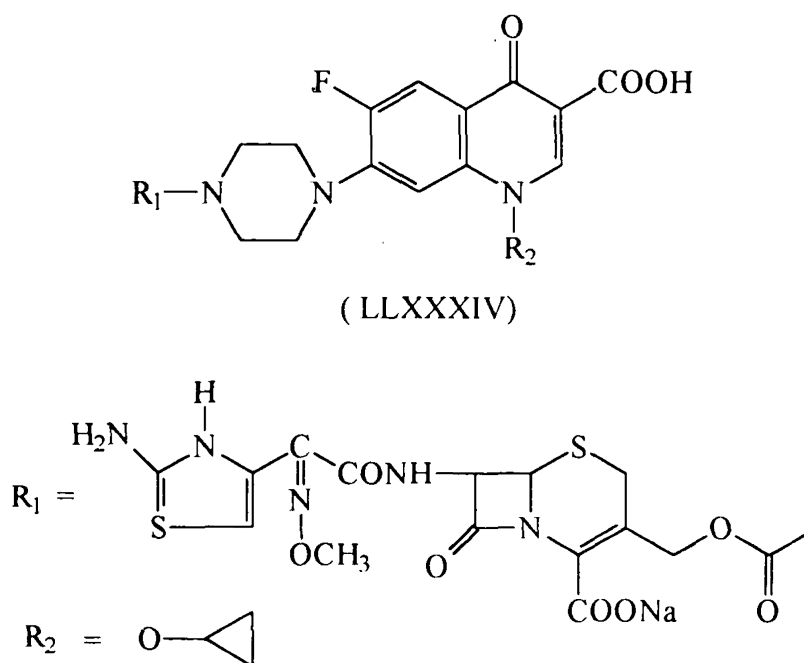
Sparfloxacin		F		-CF-	(LLXVI)
Lomefloxacin	$\text{—C}_2\text{H}_5$	F		-CF-	(LLXVII)
Fleroxacin	$\text{—CH}_2\text{CH}_2\text{F}$	F		-CF-	(LLXVIII)
Pefloxacin	$\text{—C}_2\text{H}_5$	F		-CH-	(LLXIX)
Amifloxacin	—NHCH_3	F		-CH-	(LLXX)
Enoxacin	$\text{—C}_2\text{H}_5$	F		-N-	(LLXXI)
Tefloxacin		F		-CH-	(LLXXII)
Gatifloxacin (AM-1155)		F		-COCH ₃ -	(LLXXIII)
AM -1174		F		-COCH ₃ -	(LLXXIV)
SB-265805		F		-N-	(LLXXV)

Alafloxacin		F		-N-	(LLXXVI)
Tosulfloxacin		F		-N-	(LLXXVII)
Trovafloxacin		F		-N-	(LLXXVIII)
Ecenofloxacin		F		-N-	(LLXXIX)
Clinafloxacin		F		-CCl-	(LLXXX)
Sitafoxacin		F		-CCl-	(LLXXXI)
Pazufloxacin		F		-CH-	(LLXXXII)



Tefloxacin(LLXXII) was demonstrated by Chen *et al*¹²⁷ to exhibit activity against Penicillin- resistant *Staphylococcus*, *Streptococcus* and *Haemophilus*. It is itself a β -lactamase inhibitor and displayed synergy with Amipicillin. AM – 1155 and AM 1174, new quinolones bearing an 8 methoxy group, outperformed Ciprofloxacin against anaerobes and a range of Gram-positive bacteria, with potency upto 16-62 fold greater than Ciprofloxacin¹²⁸.

Recently new β -lactam quinolone hybrids have been reported. The carbamate-linked Ro 24-4383 (LLXXXIV) showed a broad-spectrum of activity and exhibited aqueous solubility much greater than that of the quinolone component alone¹²⁹.



Fluoronaphthyridones and Naphthyridines (LLXXIV-LLXXIX)¹³⁰ are newer analogs currently in clinical trials. These are important for the oral treatment of serious infections specially for Gram-positive cocci. Clinafloxacin¹³¹ was found to be active against *Pseudomonas aeruginosa*, while Sitaefloxacin¹³² was active against *Klebsiella pneumoniae* and *Enterobacter coacae*. It has 16 to 256 fold greater activity than

other quinolones currently in clinical use. Pazufloxacin¹³³ and Prulifloxacin¹³⁴ showed broad bactericidal activity against Gram-positive and Gram-negative bacteria.

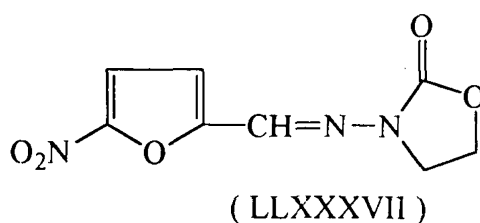
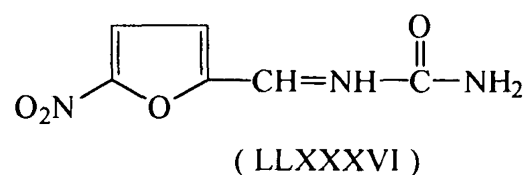
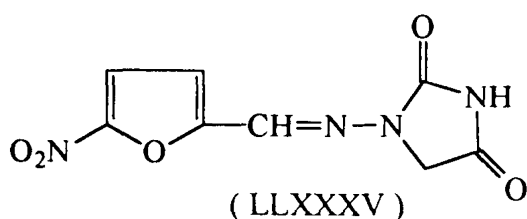
Therapeutic uses

- 1) Urinary Tract infections.
- 2) Prostatitis
- 3) Sexually transmitted diseases
- 4) Gastrointestinal and abdominal infections
- 5) Respiratory tract infections
- 6) Bone, joint and self tissue infections

Arthralgias and joint swelling have been reported in children receiving Fluoroquinolones. Therefore, these drugs are not generally recommended for use in pre-pubertal children or pregnant woman.

Nitrofurans

Nitrofurans are used for the prevention and treatment of infections of the urinary tract. Nitrofurantoin (LLXXXV) is active against many Gram-positive and Gram-negative bacteria at a concentration ranging between of 5-10 µg/mL. Nitrofurazone (LLXXXVI) is a semicarbazone of 5 nitro-2-furaldehyde. It is used topically in the treatment of burns and prevention of bacterial infections. Furazolidone (LLXXXVII) has a broad spectrum of bactericidal activity against intestinal pathogens including *Salmonella*, *Shigella* and *Proteus*.



FUNGAL INFECTIONS

Fungi, like the human host, are eukaryotic organisms, and thus the number of suitable targets for therapeutic attack are limited. Nonetheless considerable progress in treating fungal infections has been achieved through better application of established antifungal agents and the development of promising new investigational agents. This ever expanding battery of antifungal agents exhibits a wide variety of pharmacokinetic profile mechanisms of action and toxicities.

The incidence of systemic mycoses continues to rise steadily. This increase is due in part to improved recognition and diagnosis of fungal infections, and also due to the prolonged survival of patients with global defects in their host defense mechanism, including patients with neoplastic diseases, organ-transplant recipients, diabetics and patients with AIDS. These patients are susceptible to an ever growing list of opportunistic fungi. From the data collected by the Center for Disease Control, it is known that among the 1 and 2 million healthy Americans who have already been infected with AIDS, a significant number of these cases are associated with various fungal infections¹³⁶. One yeast-like fungus, *Cryptococcus neoformans*, is reported to be the most common reason of debilitating and life-threatening infections in AIDS patients¹³⁶.

The agents which are currently in use as antifungals are classified and listed below.

A) Alaylamines

Naftifine
Terbinafine

B) Triazoles

Fluconazole
Itraconazole
Terconazole

C) Imidazoles

Butaconazole
Chlormidazole
Clofrimazole
Econazole
Enilconazole
Fenticonazole
Isoconazole
Ketoconazole
Niconazole
Oxiconazole
Sulconazole
Tioconazole

D) Polyene macrolides

Amphotericin
Candicidin
Hachimycin
Klatamycin
Nystatin
Pentamycin

Most of the antifungals given above are applied in the treatment of infections of hair, mucous membranes, nails and skin caused by *Candida dermatophytes* and other fungi including *Epidermophyton*, *Microsporum*, *Trichophyton* and *Malassezia furfur*. The main agents, which are used in treating these infections, are listed below.

<u>Superficial fungal Infection</u>	<u>Main topical antifungal agents</u>
Candida infections	Azoles, Polyene antifungals
Tinea	Olamine, Haloprogin, Tolnaftate, Azoles, Ciclopirox,
<i>Pityriasis Versicolor</i>	Azoles, Ciclopirox, Olamine, Haloprogin, Tolnaftate

Systemic fungal infections tend to occur in immuno-compromised patients and include infections due to *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*. The principle drugs, which are used in such infections, are listed below:

<u>Systemic fungal infections</u>	<u>Main antifungal agents</u>
Aspergillosis	Amphotericin
Blastomycosis	Amphotericin, Ketoconazole

Candidal infections:

Oesophageal	Fluconazole, Ketoconazole
Disseminated	Amphotericin with or without Fucytosine
Coccidioidomycosis	Amphotericin, Ketoconazole
Cryptococcosis	Amphotericin with or without Elucytosine, Fluconazole
Histoplasmosis	Amphotericin, Ketoconazole
Paracoccidio mycosis	Amphofericin, Ketoconacole

A brief description about major principal drugs, which are used in treating fungal infections, is presented in the following pages.

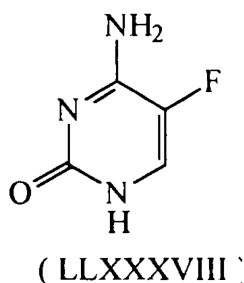
Amphotericin B

It was the first commercially available systemic antifungal drug. It was first isolated in the 1950's from *Streptomyces nodosus*. It is a polyene macrolide that consists of seven conjugated double bonds, an internal ester, a free carboxylic group and a glycoside chain with a primary amino group. It is amphoteric in nature forming

soluble salts in both basic and acidic environments. The primary mechanism of action of the polyenes in general, and Amphotericin B in particular, is due to binding to ergosterol, the principle sterol present in the cell membrane of sensitive fungi¹³⁷. The most significant adverse effect is nephrotoxicity. In spite of the adverse effects, it is still the drug of choice for treating most deeply invasive mycoses, including both endemic mycoses and opportunistic mycoses.

Flucytosine

It is a fluorine analogue of Cytosine (LLXXXVIII). It was found to have *in vitro* and *in vivo* antifungal activity¹³⁸. The drug is being used as an adjunct to Amphotericin B therapy



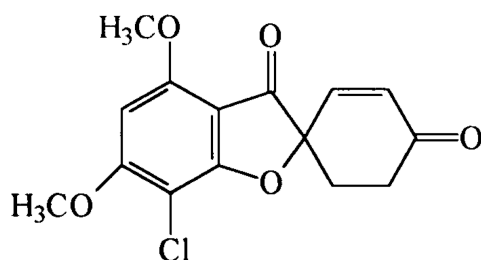
The mechanism of action of Flucytosine is the disruption of protein synthesis by inhibition of DNA synthesis, and alteration of the amino acid pool by inhibition of RNA synthesis. It is used for treating the infections caused by *Candida albicans*, *Cryptococcus neoformans* and *Torulopsis glabrata*.

Nystatin

Nystatin is produced by *Streptomyces noursei*. It was discovered by Hazen and Brown¹³⁹ and possesses good antifungal activity. The toxicity after parenteral administration to animals has led to its use only topically or orally. The MIC of this drug for *Candida* species *Cryptococcus neoformans* is in the range of 1.56-1.26 µg/mL.

Griseofulvin

Griseofulvin (LLXXXIX) was discovered by Oxford *et al* in 1939 as a metabolic product of *Penicillium griseofulvum*. Grove predicted its chemical structure in 1950¹⁴⁰.

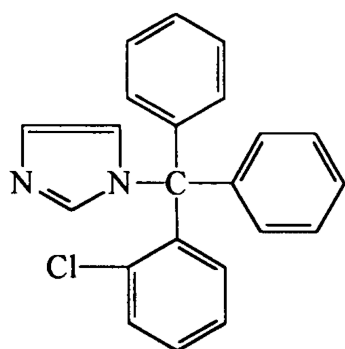


(LLXXXIX)

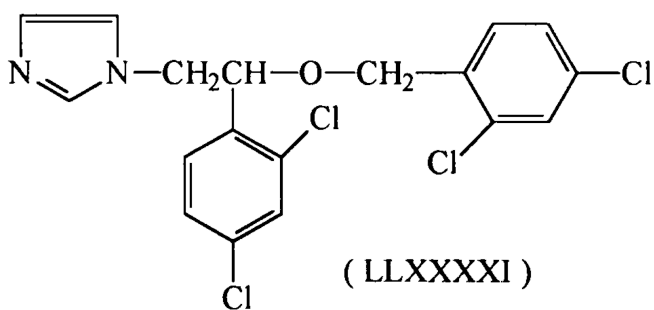
As a “cell-wall antibiotic” particularly active on dermatophytes, Griseofulvin interferes with the biosynthesis of the chitinous elements of the hyphal wall. No resistant strains are observed among dermatophytes during the treatment with this drug.

Synthetic Antifungal Agents

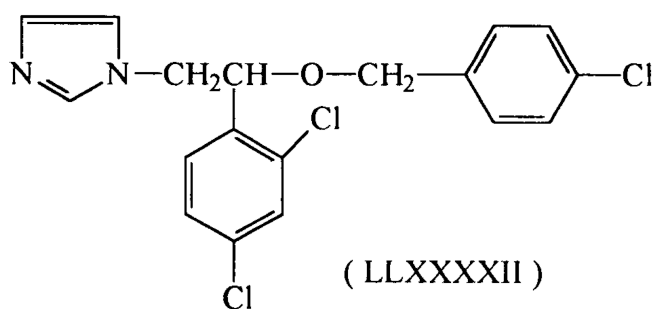
Among all the imidazole compounds, three distinct compounds are actually therapeutic antifungals with very wide spectra against yeast and filamentous fungi. They are Clotrimazole(LLXXXX), Miconazole(LLXXXXI) and Econazole (LLXXXXII)



(LLXXXX)



(LLXXXXI)



(LLXXXXII)

Clotrimazole was developed by investigators at the Bayer Laboratories in 1969, and is utilized topically in superficial mycoses, or orally in clinical trials in deep mycoses¹⁴¹.

Miconazole¹⁴² is a well known antifungal drug developed by the investigators at Janssen Pharmaceutica in 1969. It is utilized in superficial mycoses and by oral or intravenous routes in deep mycoses, in clinical experimental trials.

Econazole is a phenethyl imidazole derivative. Like Miconazole, it is utilized as a topical agent in superficial mycoses and as a systemic antifungal, by oral or intravenous routes, in deep mycoses¹⁴³.

Triazoles

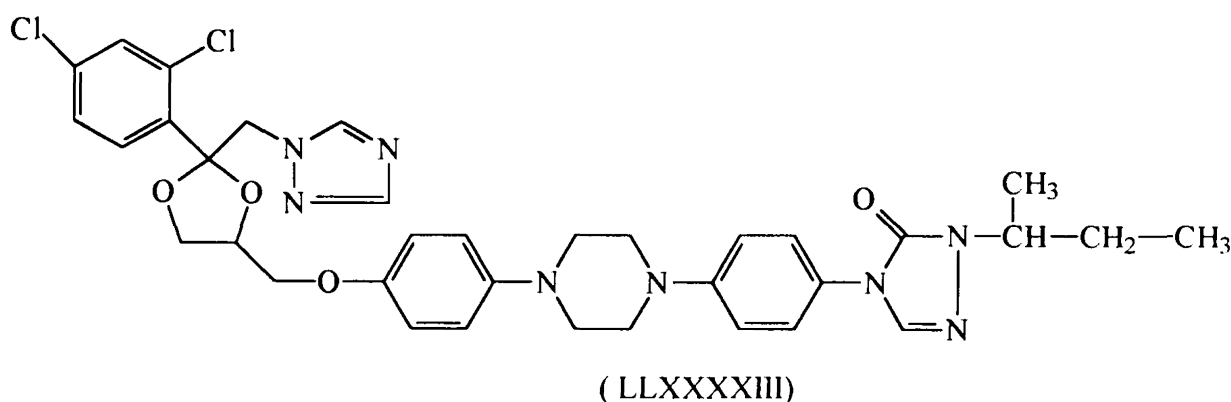
The replacement of imidazole ring with the triazole ring offers many advantages to the newer azoles¹⁴⁴.

- 1) It allows for greater polarity, which may improve solubility and reduce protein binding.
- 2) The triazole ring is less nucleophilic and more resistant to metabolic attack.

There are a number of such compounds that have recently been licensed, or are currently being developed and tested.

Itraconazole

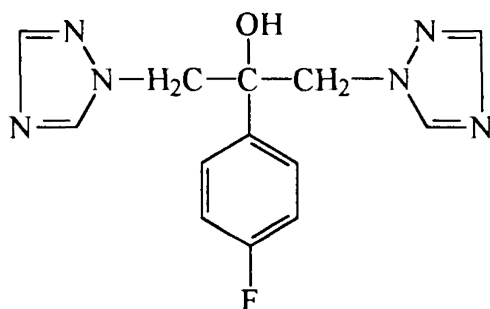
Itraconazole (LLXXXIII) is water-soluble, a lipophilic triazole analogue that exhibits excellent activity against most fungal pathogens. When compared with Ketoconazole, its potential advantages include a broader spectrum of activity (including *Aspergillus* species) a more desirable pharmacokinetic profile and lower toxicity¹⁴⁵.



Fluconazole

Fluconazole (LLXXXIV) is a water-soluble fluorine substituted bis-triazole that has been shown to be effective against a variety of fungal infections in immuno competent and immuno compromised hosts. A major point of confusion during the

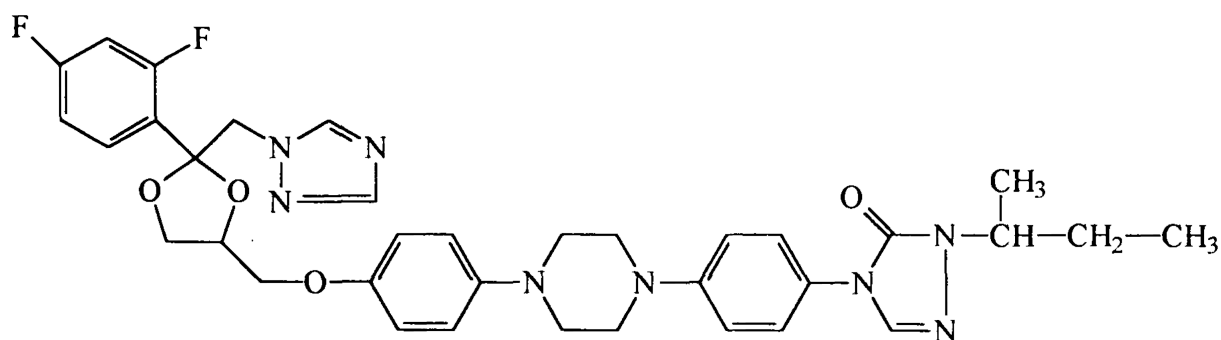
development of this drug, however, has been its relatively low activity *in vitro* compared with its high *in vivo* activity. Fluconazole is a potential candidate for prevention and early treatment of disseminated candidiasis in granulocytopenic patients¹⁴⁶.



(LLXXXIV)

Saperconazole

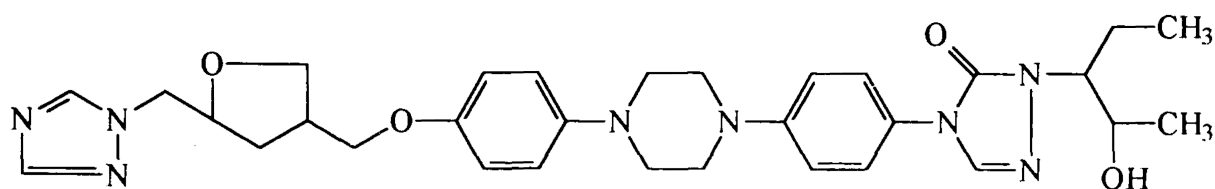
Sperconazole (LLXXXV) is a water-soluble, lipophilic fluorinated triazole with chemical structure similar to Itraconazole, with two chlorine atoms replaced by fluorine.



(LLXXXV)

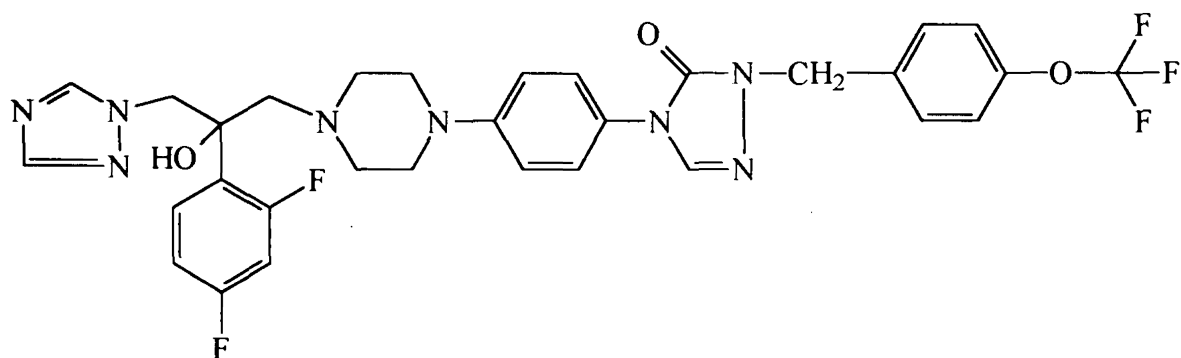
It has a broad spectrum of antifungal activity, including the dimorphic fungi, phacohyphomycetes, *Cryptococcus* species and *Aspergillus* species¹⁴⁷.

In vitro evaluation of the antifungal activity of SCH 56592 (LLXXXVI) against clinical isolates of *Candida albicans* demonstrated good antifungal activity at a concentration of < 0.03 – 0.06 µg/mL¹⁴⁸.



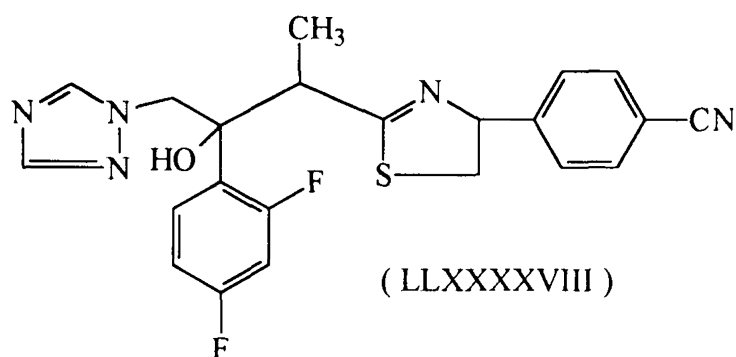
(LLXXXVI)

Syn-2869 (LLXXXXVII) is a new orally active antifungal triazole derivative that has broad spectrum and potent activity against a variety of pathogenic fungi, particularly against *Aspergillus* species. This compound is among the most recent antifungal triazole derivatives being investigated as potential candidates for development¹⁴⁹.



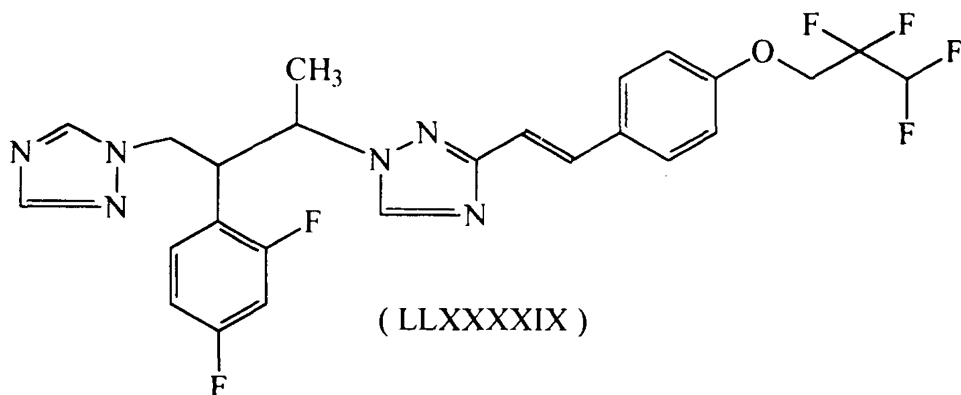
(LLXXXXVII)

ER 30346 (LLXXXXVIII) is found to be more potent than Fluconazole¹⁵⁰.



(LLXXXXVIII)

D-0870 (LLXXXXIX) is more effective than Fluconazole in treating *Candida albicans*, including Fluconazole susceptible and resistant strains¹⁵¹.



(LLXXXXIX)

MALARIA

Malaria is one of the most serious complex and refractory health problems facing humanity this century. It is a serious endemic disease in more than 100 countries in Africa, Asia, Oceania, Latin and South America. Some 300-500 million of the world's people are infected by the disease, presenting over 120 million clinical cases annually. It is estimated that between 1.2 and 1.7 million people die of malaria every year, either directly or in association with acute respiratory infections and anemia, and upto 1 million of those deaths are among children younger than five years. More people have died of malaria than have died from AIDS in the last seventeen years. Malaria is a leading cause of morbidity and mortality in the developing countries, particularly in tropical Africa, and it remains the single outstanding tropical disease priority.

Human malaria is caused by four species of protozoan parasites of the *Plasmodium* genus. These are

- 1) *Plasmodium falciparum*
- 2) *Plasmodium vivax*
- 3) *Plasmodium ovale*
- 4) *Plasmodium malavae*

Plasmodium falciparum is the most widespread of the four, geographically, and most pernicious, causing the majority of malaria related morbidity and mortality.

Malaria is mainly transmitted by the female Anopheline mosquito, and in rare cases by transfusion of infected blood. The life cycle of Plasmodia involves two stages

- 1) A sexual reproductive stage with multiplication (sporogony), which occurs in the gut of the mosquito.
- 2) An asexual reproduction phase with multiplication (schizogony), which takes place in the human host. Sporozoites, the infective stages, are injected during blood meal from the mosquito and circulate to the liver, where there is a period of pre-erythrocytic development into tissue schizonts. Once the tissue schizonts rupture, each rupture releases thousands of merozoites, which enter the circulation and invade the erythrocytes.

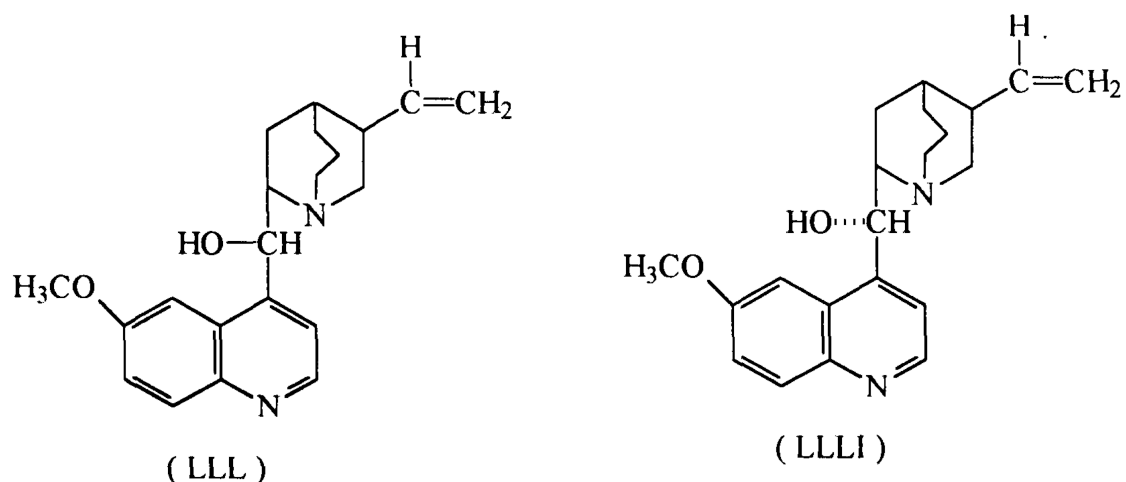
Practical, effective and safe drugs, insecticides and vaccines are needed to combat malaria. Since 1960, transmission of malaria has risen in most regions where the infection is endemic. Chloroquine-resistant and multidrug resistant strains of *P. falciparum* have spread, and the degree of drug resistance has increased.

Most of the antimalarial drugs were developed on the basis of their action against asexual erythrocytic forms of malaria parasites. Highly effective chemotherapeutic agents in this category are Chloroquine, Quinine, Quinidine, Mefloquine and Halofantrine, Pyrimethamine, Sulfonamides, Sulfones and Tetracyclines. These are slower acting, less effective, and nearly always used in combination with other antimalarial compounds. Primaquine is the only antimalarial used clinically to eradicate latent tissue forms responsible for relapses in *P. vivax* and *P. ovale* malarias. Artemisinin derivatives are one of the several novel antimalarial endoperoxides currently being evaluated for use against Chloroquine-resistant and multidrug resistant strains of *P. falciparum*.

Antimalarial Drugs

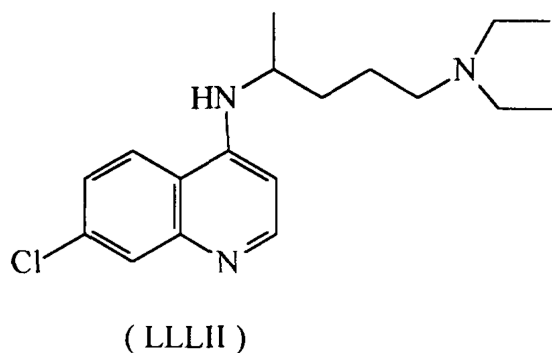
Quinine (LLL) and Quinidine (LLLI) have been used for centuries in treating malarial fevers, and they are still used to control *P. falciparum* malaria. Quinine is the chief alkaloid of Cinchona bark which contains a mixture of more than 20 alkaloids. The most important of these are two pairs of optical isomers, Quinine and Quinidine, and Cinchonidine and Cinchonine. Quinine contains a quinoline group attached through a secondary alcohol linkage to quinuclidine ring. Quinidine has the same structure as quinine except for the steric configuration of the secondary alcohol group. Quinidine is two to three fold more active than Quinine in both Chloroquine-sensitive and Chloroquine-resistant strains of *P. falciparum*¹⁵².

Quinine acts primarily as a blood schizontocide and it has little effect on sporozoites or pre-erythrocytic forms of the malarial parasite.



Chloroquine

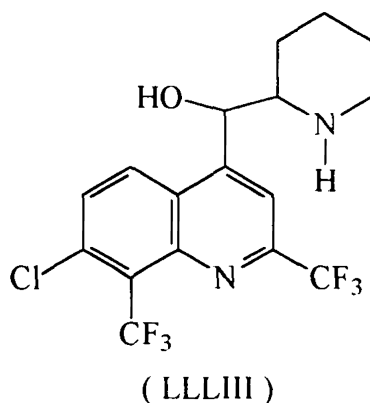
Chloroquine (LLLII) is one of the most successful antimicrobial agents ever produced and it has had an enormous positive impact on human health. Chloroquine is highly effective against erythrocytic forms and chloroquine-sensitive strains of *P. falciparum*. The mechanism of action of Chloroquine consists of (a) DNA intercalation, (b) the weak base hypothesis. Malaria parasites possess a food vacuole in which haemoglobin is degraded into aminoacids, peptides, and free heme. The environment of the vacuole is acidic. The weak base hypothesis suggests that it is the dibasic nature of Chloroquine and its interactions with acidic vacuole of the parasite that results in the antimalarial action.



Mefloquine

Mefloquine (LLLIII), a 4-quinoline methanol structurally related to Quinine, is an orally active agent effective against the intra-erythrocytic stages of the Plasmodium life cycle. It is a relatively slow-acting drug with greatest activity against the more mature blood stages. In order to delay the development of resistance to Mefloquine, it was recommended that Mefloquine be used only in combination with Pyrimethamine and Sulfadoxine. Mefloquine exerts its action by 1) DNA intercalation, 2) weak base hypothesis, or 3). It may interfere with the parasite's

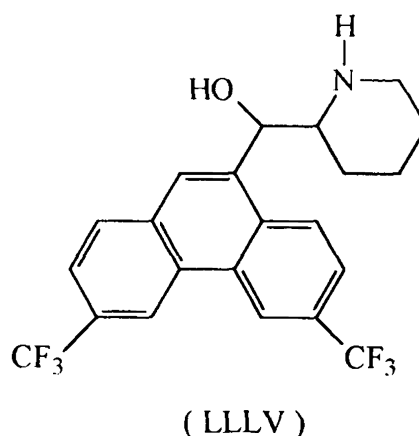
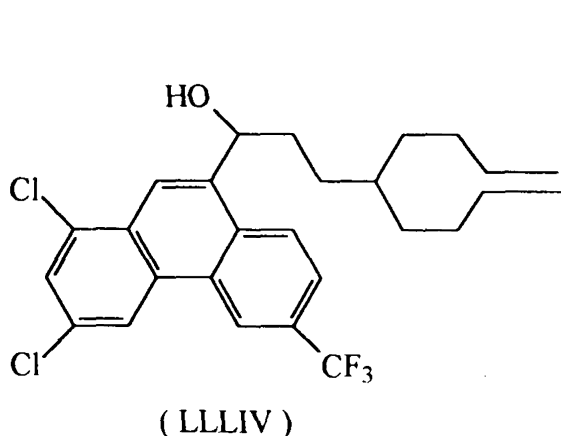
ability to detoxify heme. The drug binds with high affinity to red cell membranes. Mefloquine is given in combination with a variety of Artemisinin-derived agents, such as Artesunate. Mefloquine and Artemether / Mefloquine are the most promising new drugs. Mefloquine is the only newer antimalarial that is recommended for use as a prophylactic agent.



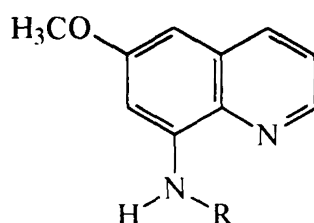
Halofantrine

Halofantrine (LLLIV) a phenanthrene methanol analogue is a recent addition to the anti-malarials to treat MDR *P. falciparum*. *In vitro* it is more active than Mefloquine.

Halofantrine is mainly used or indicated for treating mild to moderate acute malaria in sensitive strains of *P. falciparum* and *P. vivax*. Halofantrine is schizontocidal with selective activity against the erythrocytic stages of Plasmodia. One drawback of Halofantrine is its poor and variable absorption. W.R 33063 (LLLIV) and WR 122455 (LLLVI) analogs of Halofantrine displayed good activity in Chloroquine-resistant strains, but high cure rates required multiple dose over several days.



8-Aminoquinoline



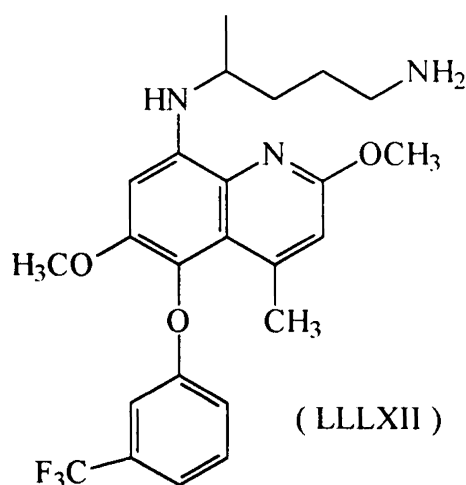
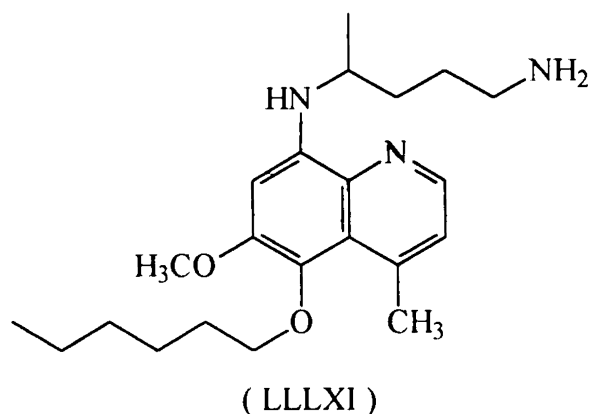
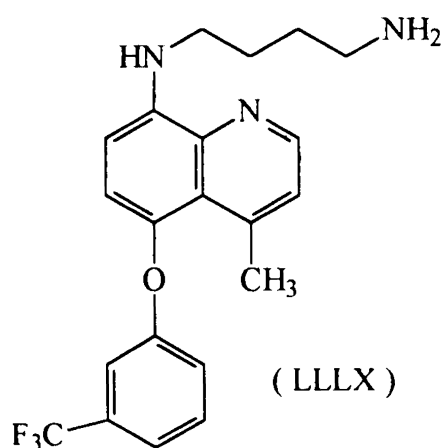
Antimalarial Compound	R	No
Pamaquine	$\begin{array}{c} \text{CH}_3 \\ \\ \text{---CH---}(\text{CH}_2)_3\text{---N} \begin{array}{l} \nearrow \text{C}_2\text{H}_5 \\ \searrow \text{C}_2\text{H}_5 \end{array} \end{array}$	LLLVI
Primaquine	$\begin{array}{c} \text{CH}_3 \\ \\ \text{---CH---}(\text{CH}_2)_3\text{---NH}_2 \end{array}$	LLLVIII
Pentaquine	$\begin{array}{c} \text{H} \\ \\ \text{---}(\text{CH}_2)_5\text{---N---CH}(\text{CH}_3)_2 \end{array}$	LLLIX

Primaquine destroys late hepatic stages and latent tissue forms of *P. vivax* and *P. ovale*. The standard treatment often involves a combined regimen of Chloroquine and Primaquine. The usual dose of Primaquine is 15 mg. Primaquine is used mainly to prevent relapses caused by the exoerythrocytic forms of the parasites. Primaquine is readily absorbed from the gastrointestinal tract after administration. The most serious adverse effect associated with Primaquine is intravascular hemolysis.

The action of pamaquine is clearly different from that of quinine, especially in its ability to prevent relapses. The therapy for vivax malaria involved a combination of

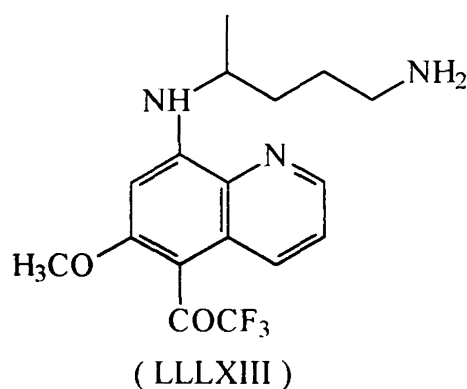
Pamaquine with Quinine or Quinacrine. Due to human toxicity the use of Pamaquine was discontinued.

In order to reduce the toxicity associated with Primaquine several fluorine-containing analogs of Primaquine have been discovered. Among these are WR 225448 (LLLX), WR 242 511 (LLLXI) and WR 238 605 (LLLXII)

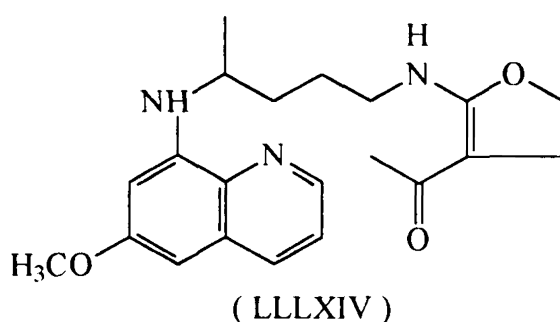


WR 238605 is effective against pre-erythrocytic stages, drug-sensitive and multiresistant erythrocytic stages gametocytes with activity significantly better than Primaquine.

5-trifluoroacetyl Primaquine (M 8506) (LLLXIII) was shown to be a causal prophylactic agent, equivalent in activity to Primaquine.

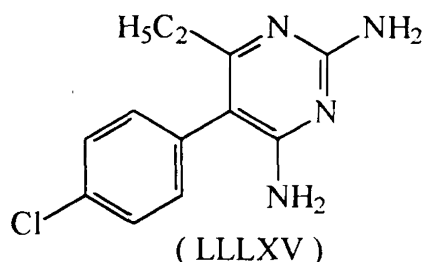


A prodrug of Primaquine, CDRI compound 80/53 (LLLXIV), has been shown to possess radical curative and causal prophylactic action against *P. cynomolgi* in rhesus monkey¹⁵³. This compound caused less methemoglobinemia than did Primaquine.



Diamino Pyrimidines

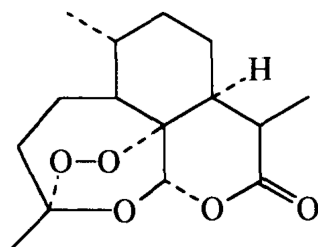
Several 2,4-diaminopyrimidines, including Pyrimethamine (LLLXV) and the antibacterial, Trimethoprim, exhibited significant antimalarial activity in animal models. Pyrimethamine and Sulfadoxine, a long acting sulfonamide, have been used extensively for the prophylaxis and suppression of human malaras, especially those caused by Chloroquine resistant strains of *P. falciparum*.



Pyrimethamine is a slow acting blood schizontocide and it acts by inhibiting dihydrofolate reductase of Plasmodia. However, resistance to Pyrimethamine may develop because of prolonged or extensive drug use.

Artemisinin and its derivatives

The weed *Artemisia annua* has been used for many centuries in Chinese herbal medicine as a treatment for fever and malaria. In 1971, Chinese chemists isolated from the leafy portions of the plant, the substance responsible for its reputed medicinal action¹⁵⁴. Artemisinin (LLLXVI) is a sesquiterpene lactone bearing an endoperoxide



(LLLXVI)

Several thousand malaria patients including those with Chloroquine-resistant strains of *Plasmodium falciparum* were successfully treated with Artemisinin.

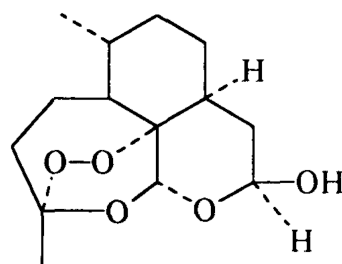
First Generation Artemisinin

Artemisin

Artemisinin (LLLXVI) has rapid action on all erythrocytic stages of the parasite¹⁵⁵. The Gametocytes are the targets for the drug. It is poorly absorbed after oral administration. The combination of Artemisinin with Mefloquine produced a rapid clearance of parasitemia than Artemisinin alone. It has a high rate of recrudescence, poor oral activity with high dose required and a short plasma half-life.

Dihydroartemisinin

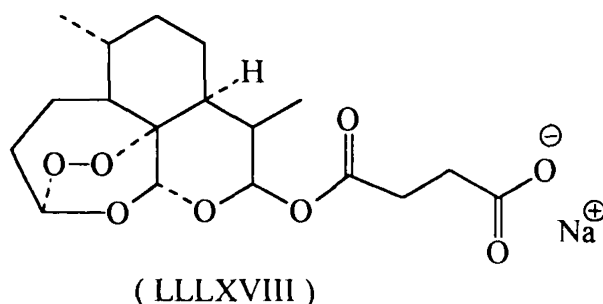
Dihydroartemisinin (LLLXVII) is more active *in vitro* and *in vivo* than Artemisinin and Artesunate. It has low solubility and instability. The treatment is effective and well tolerated in acute uncomplicated *falciparum* malaria¹⁵⁶. It is more bioavailable than Artemisinin.



(LLLXVII)

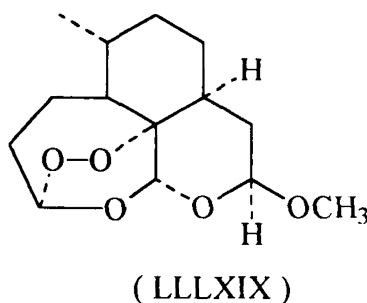
Artesunate

In vivo, Artesunate (LLLXVIII) has higher activity than Artemisinin, and significantly higher parasite clearance and fever clearance times. It is well tolerated. Artesunate was found to inhibit cytoadherence, the pathological process that contributes to microvascular obstruction in severe *falciparum* malaria¹⁵⁷. A combination of Artesunate with Mefloquine appears to be the most active regimen for use against multidrug resistant *falciparum* malaria.



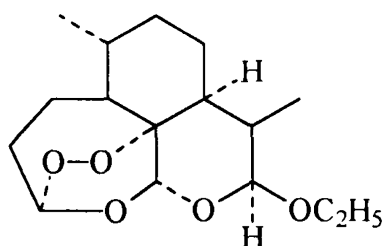
Artemether

Artemether (LLLXIX) is the methyl acetal derivative of dihydroartemisinin. This oil soluble derivative has high blood schizonticidal activity comparable to that of Arteether, *in vitro* and *in vivo*. A single dose of artemether resulted in complete sterilization of the gametocytes 18-20 hours after administration. It inhibits cytoadherence. Artemether was highly effective, especially against Chloroquine and Pyrimethamine resistant infections.



Arteether

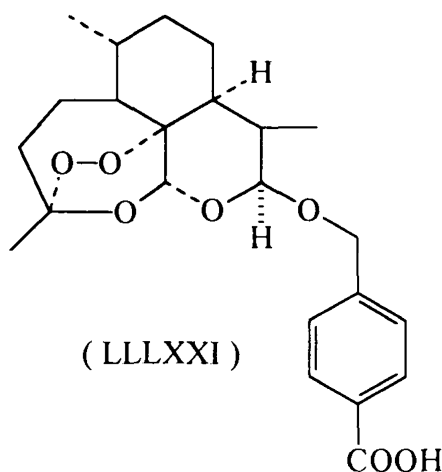
Arteether (LLLXIX) is the ethyl acetal of dihydroartemisinin. It has greater lipophilicity than Artemether, leading to better penetration into the brain. Arteether is comparable in activity to artmethether *in vitro* and *in vivo*. A strain of *P. yoelli* that was highly resistant to Chloroquine, Mefloquine and Quinine was completely susceptible to Arteether¹⁵⁸.



(LLLXX)

Artelinic Acid

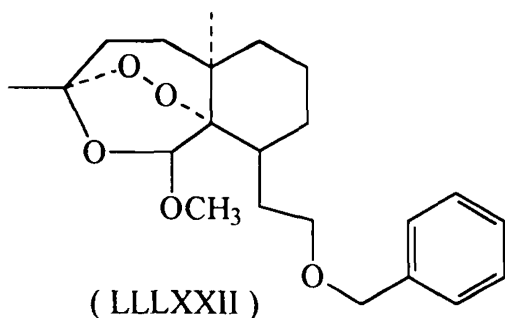
Artelinic acid (LLLXXI) was developed as a water soluble alternative to Artesunate, which was found to have limited stability in aqueous solutions. The drug possesses superior activity against *P. berghei in vitro* when compared with either Artemisinin or Artesunate.



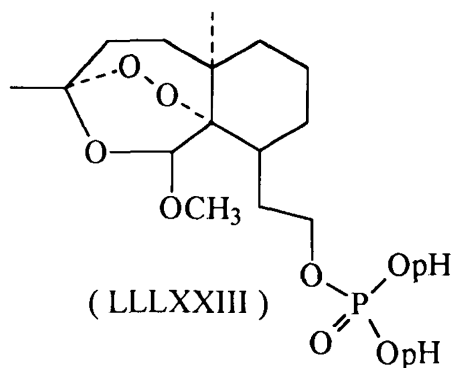
(LLLXXI)

Second Generation Artemisinins

Two synthetic agents (LLLXXII) and LLLXXIII), clearly based on the artemisinin structure, have been evaluated for activity in mice and in monekys¹⁵⁹. The potency of the compounds was comparable to that of Artemether.



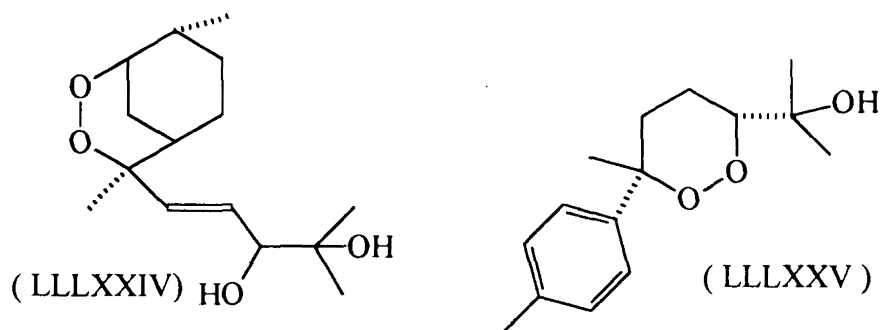
(LLLXXII)



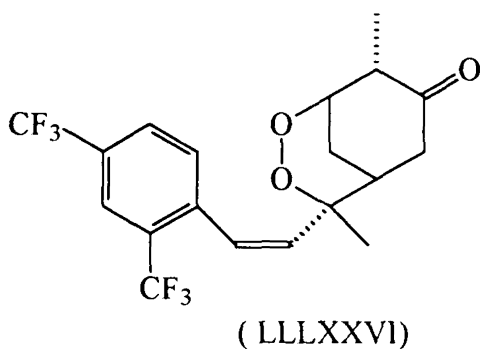
(LLLXXIII)

Yingzhaosu A., Yingzhaosu C., and Arteflene

Yingzhaosu A (LLLXXIV) and Yingzhaosu C (LLLXXV) are cyclic compounds, containing a bisabolene, skeleton have been isolated from the roots of the plant Yingzhao (*Artabotrys uncinatus*), which has been used in the folk treatment of malaria.



Arteflene (LLLXXVI) is a synthetic antimalarial agent developed as an analog of Yingzhaosua¹⁶⁰. The central core ring structure (2,3 dioxabicyclo [3.3.1] nonane) was shown to have inherent antimalarial activity. It is as active as Artemisinin and has better chemical stability. Arteflene may find use both, in treatment and as a prophylactic agent.



Part I-B

Plan of Work

PLAN OF WORK

Bacteria, fungi and viruses are the common causative organisms responsible for the infectious diseases of microbial origin. It was Paul Ehrlich, the pioneer of chemotherapy, who used the term "Chemotherapy" to mean the study and use of agents, which are relatively more toxic to the invading organism than the host. The modern era of chemotherapy of microbial diseases started with clinical use of Sulfonamide in 1936. The introduction of Penicillin brought antimicrobial therapy to golden age. This was followed by discovery of Streptomycin, PAS and INH. Also new kind of drugs will be needed to combat various diseases, widely prevalent in tropical countries like India. Malaria, helminths, tuberculosis, fungal, viral and bacterial infections are some of the major illnesses. Hence, intensive research in development of new agents for combating the communicable diseases is essential.

A considerable progress has been made in the past 30 years in the chemotherapy of microbial diseases. The seemingly uncontrollable phenomenon of drug resistance has become one of the main driving forces behind the search for new therapies and development of new antimicrobials. Although resistance was observed soon after the introduction of the earliest antimicrobials into the market, it was only quite recently that this problem was recognized as a serious threat for the continuing therapeutic utility of antimicrobial in the treatment of infectious diseases. The worldwide emergence of Methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, multiresistant *Mycobacterium tuberculosis*, and Vancomycin-resistant enterocci strains are among the major problems currently plaguing the medical community. The continued use/misuse of antimicrobial agents exerts a strong selective pressure on bacterial populations favouring the survival of those organism that are capable of resisting them. The knowledge of the mechanism of bacterial resistance, the study of induction routes as well as the factors responsible for the changes in antimicrobial resistance patterns is of primary importance for predicting future medical needs and preventing the emergence of resistant pathogens.

In order to prevent or control the emergence of bacterial resistance, the discovery of new broad-spectrum antimicrobials, especially those against which the microorganisms have little chance of developing resistance, is necessary. The newer compounds should have the following properties.

- 1) Potent activity
- 2) Good tolerance
- 3) Lesser side effects
- 4) Efficacy against microbial resistant strains.

The discovery and development of pharmacologically active molecules has been guided not only by classical medicinal chemistry but also by the use of sophisticated mechanistic approaches and new biochemical assay. New concepts of therapy such as the use of enzyme inhibitors to control diseased state has increased the scope of medicinal chemistry research.

The electron-rich properties as well as the slightly smaller steric volume of thiophene as compared to benzene may play an important role in fitting the necessary biological receptor sites. The thiophene moiety has been incorporated into biologically active molecules in two manners. The most chemically interesting of these is the synthesis of molecules containing thiophene as part of the central ring. In such system, synthesis of three possible 2,3- 3,4 and 3,2 isomeric substitution patterns may be possible. Such synthesis targets provide the greatest insight into the effect of thiophene

Many reviews clearly emphasise the importance of heterocycles in naturally occurring as well as synthetic agents, and are an important class itself possessing many pharmacological actions such as antimicrobial, antifungal, antitubercular, antiviral and antimalarial. This points out the need for further investigation in this field. The logic supporting the work presented in this thesis was formulated, bearing in mind the biological activities of known moieties and attempting certain structural modification or adaptations in light of the recent trends in drug research in incorporating newly emerged pharmacophores on existing moieties.

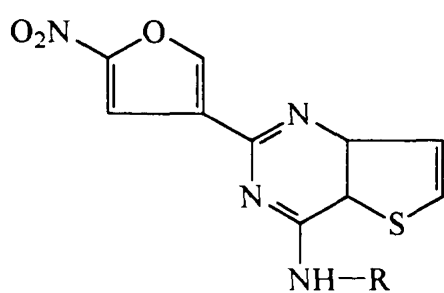
Different biological activities of condensed pyrimidines are well known in the literature. The isosteric properties of benzene and thiophene¹⁶¹ are in general of interest to medicinal chemists. The thienopyrimidine structure (a Purine analogue) has recently received considerable attention. Many of its substituted derivatives including thienotriazolopyrimidines¹⁶², thienotetrazolopyrimidine¹⁶³, thienothiadiazo-

pyrimidine¹⁶⁴ and others have pronounced antibacterial¹⁶⁵⁻¹⁶⁷ antifungal¹⁶⁸, antiviral¹⁶⁹, anti-inflammatory^{170,171}, antitubercular¹⁷², antimalarial¹⁷³ and phosphodiesterase inhibition activities¹⁷⁴.

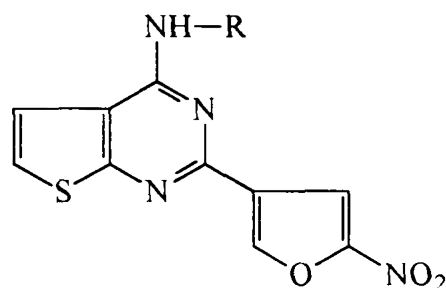
In the present studies, thieno [2,3-d] pyrimidione moiety has been chosen as the basic nucleus. Recently, Hozien, *et al*¹⁷⁵ synthesized and evaluated some derivatives of 4- substituted-5-phenyl-thieno [2,3-d] pyrimidine, as a potential antibacterial and antifungal agents. By introducing different biologically active heterocycles such as 2- furyl, 2-thienyl, 4-pyridinyl, 3-pyridinyl, at 5-position and the halogenated aryl / heteroaryl substituent by different pharamacophoric side chains at 2,3-position of thieno [2,3-d] pyrimidin-4(3H)-one nucleus, it may expected to get potent compounds of desired biological activity as these heterocycles and pharmacophoric side chain play an important role in enhancing the activity spectrum of most of the clinically used drugs.

Part II-A : Synthesis of methyl-3N-[(substituted aryl/heteroaryl)-methylene]-amino-(2-furyl/2-thienyl)-thieno [2,3-d] pyrimidin- 4(3H)-ones

Woitun *et al*¹⁷⁶ have synthesized some derivatives of 4- substituted amino -2(5-nitrofuryl)- thieno [3,2-d] pyrimidine (I) and 4 substituted amino - 2 (5-nitrofuryl) - thieno [2,3-d] pyrimidine (II) and evaluated *in vitro* against *Staphylococcus aureus* and *Trichomonas vaginalis* (I) was found to be active at a concentration of < 3 µg/ml and < 0.1 µg/ml against the above two organism, respectively.

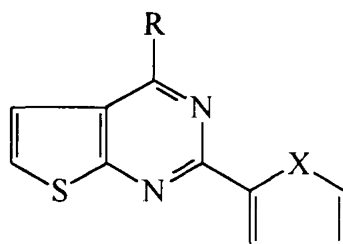


(I)



(II)

Similarly, Bourguignon *et al*¹⁷⁷ also prepared 4-substituted amino 2- furyl-thieno [2,3-d] pyrimidine (III) and 4-substituted amino-2 thienyl-thieno [2,3-d] pyrimidine (IV)

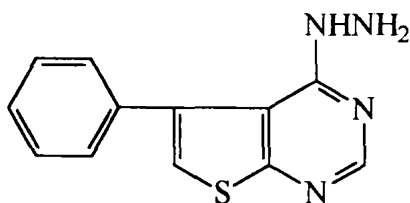


X = O (III)

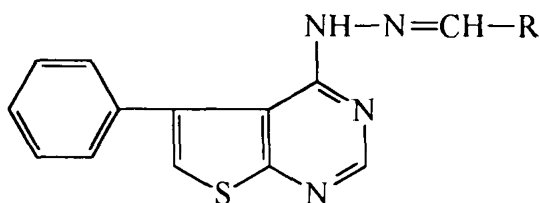
X = S (IV)

R = substituted amine

Hozien *et al*¹⁷⁵ have synthesized various derivatives of 5-phenyl-4-substituted-thieno [2,3-d] pyrimidine, out of which 4-hydrazino-5-phenyl-thieno [2,3-d] pyrimidine (V) and Schiff bases (VI) of 5- phenyl-thieno [2,3-d] pyrimidine have shown high activity against most of fungi and bacteria.

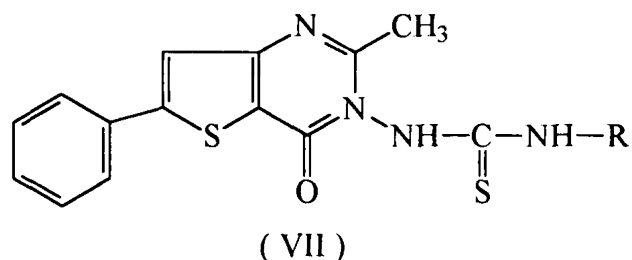


(V)



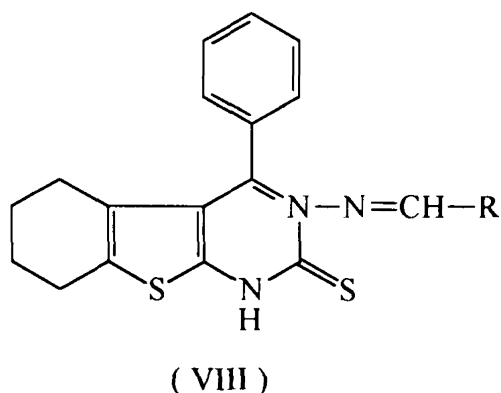
(VI)

Recently, Desai *et al*¹⁷⁸ synthesized 3 N-substituted-thiouredo-2 methyl-6-phenyl thieno [3,2-d] pyrimidin-4-(3H)-one (VII) derivatives which showed good antibacterial activity against various types of bacteria



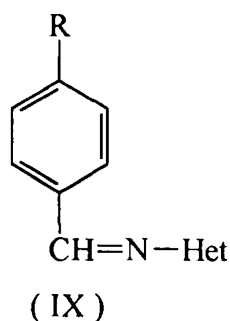
R = substituted aryl

Vega *et al*¹⁷⁰ had prepared the Schiff bases (VIII) of 2-thioxo-4-phenyl-benzothieno [2,3-d] pyrimidine and tested for analgesic and anti-inflammatory activity.



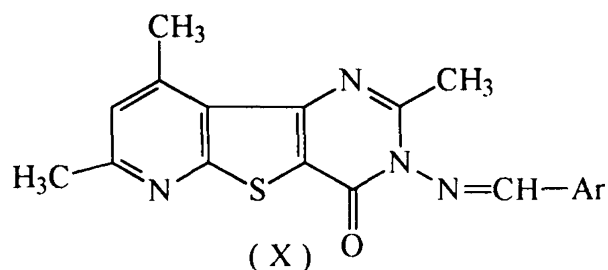
Schiff bases from substituted benzaldehyde have also been reported to have encouraging tuberculostatic action¹⁷⁹. The presence of azomethine linkage in the variety of biologically active compounds such as Nitrofurantoin and also in the field of antibiotics such as Rifampicin has been proved to be very essential.

Fioravanti *et al*¹⁸⁰ have reported antimicrobial activity of some new heteroraryl benzylamines and their Schiff bases (IX)

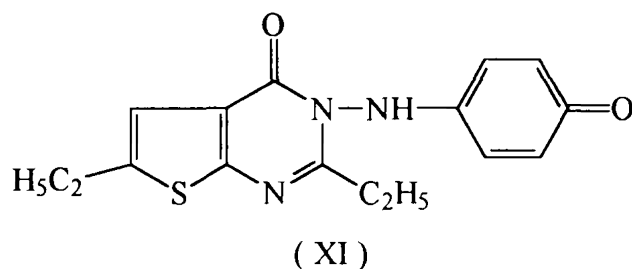


R = Cl, F

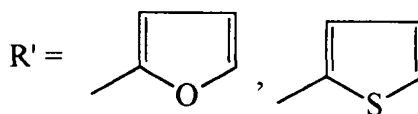
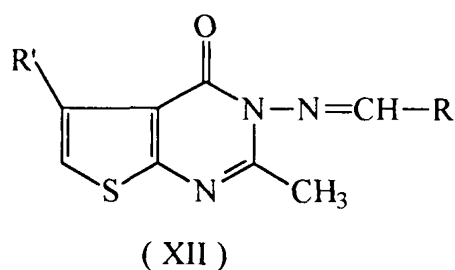
Bousquest *et al*¹⁸¹ prepared a number of Schiff bases of pyrido-thieno [3,2-d] pyrimidione (X). They were found to have interesting analgesic activity without GI complications.



Kharizomenova *et al*¹⁸⁹ synthesized 3-substituted-2,6 diethyl-thieno [2,3-d] pyrimidin-4(3H)-one derivatives and screened for their antiviral activity. Out of these 3-(p-benzoquinonylimino-2,6-diethyl-thieno [2,3-d] pyrimidin-4(3H)-one (XI) was found to be the most active antiviral agent.



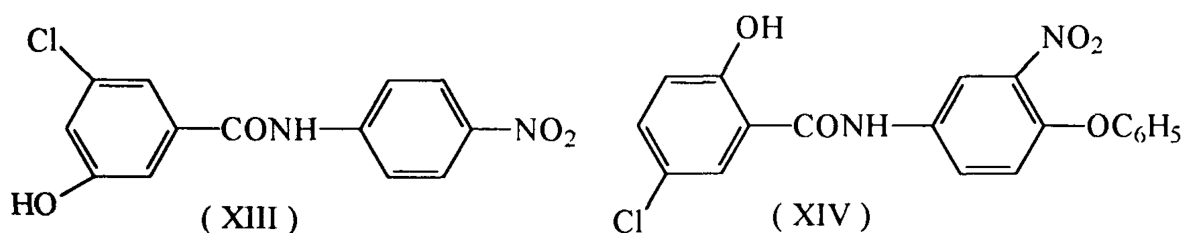
Thus, based on the above data two series of 2-methyl-3N-[(substituted aryl / heteroaryl) methylene]-amino-5-(2-furyl/2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones (XII) have been synthesized and screened for their possible antibacterial, antifungal, antitubercular, and antimalarial activities.



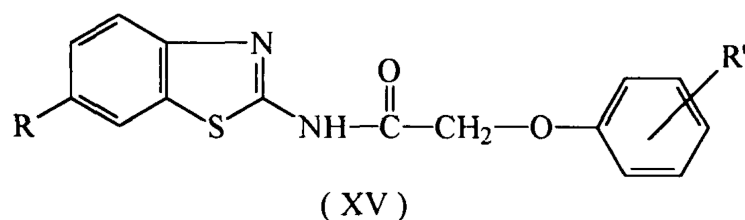
R = substituted aryl/heteroaryl

Part II-B : Synthesis Of 2 – methyl – 3N - [(substituted aryl / heteroaryl) carboxamido] – 5-(2-furyl/2-thienyl) –thieno [2,3-d] pyrimidin-4 (3H)-ones

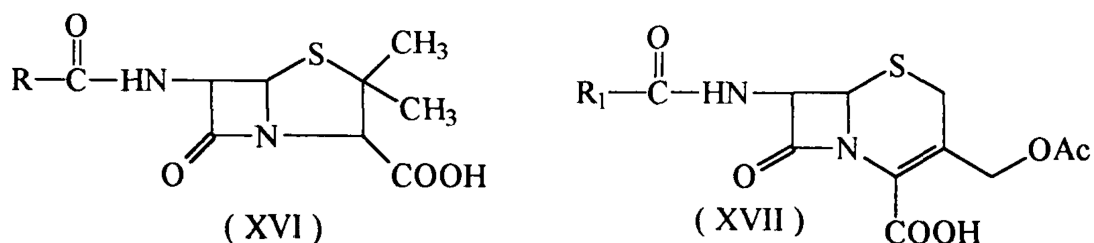
Moderate activity was claimed for 5-chloro salicylanilide derivatives as anthelminthic and antibacterial agent¹⁸². The acid amido linkage was found to be responsible for various kinds of activities as seen from the following examples. Niclosamide (XIII), a 5-chloro salicylanilide derivative, is a drug of choice in the treatment of cestodes. Another salicylanilide derivatives (XIV) was found to be effective against penicillin resistant *S. aureus* at a minimum inhibitory concentration (MIC) of 0.00609 µg/ml¹⁸³.



Lacova *et al*¹⁸⁴ reported compounds of type (XV) having good biological activities.



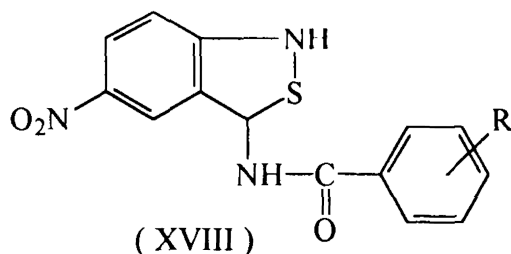
The basic structure of Penicillin (XVI) and Cephalosporin(XVII) antibiotics consist of a thiazolidine and dihydrothiazine ring respectively, connected to a β-lactam ring which is attached to a side chain through amidocarbonyl linkage. Many pharmacological characteristics of Penicillin and Cephalosporine antibiotics are determined by its side chain, which plays an important role in antimicrobial activity



R, R₁ = substituted benzyl

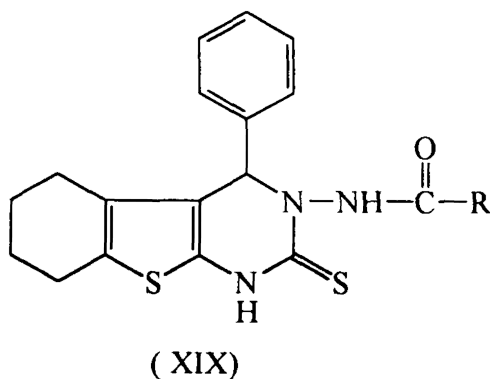
Also, in our laboratory, a number of carbamido linkages of 5-nitro-2,1-benzothiazole (XVIII) have been synthesized and evaluated for their

antitubercular and antifungal activity. Most of the compounds have shown significant antitubercular and antifungal activity¹⁸⁵.



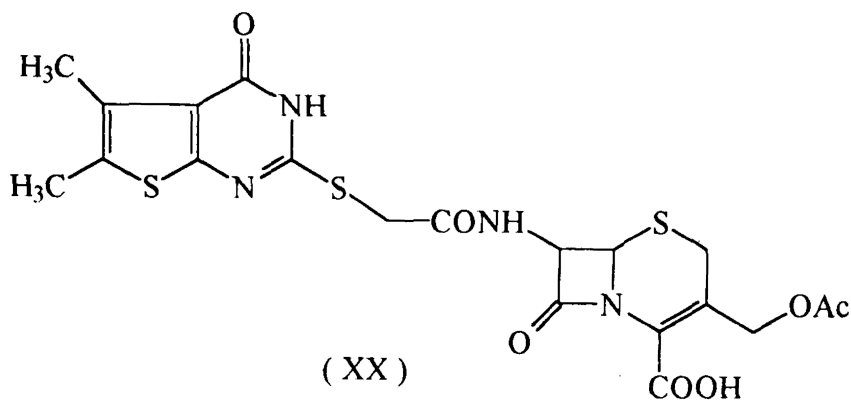
R = Cl, F, Br, CF₃ etc

Vaga¹⁷⁰ prepared 3-acylamino-4-phenyl-2-thioxobenzothieno [2,3-d] pyrimidine (XIX) derivatives and tested for their analgesic and anti-inflammatory activities.



R = substituted aryl

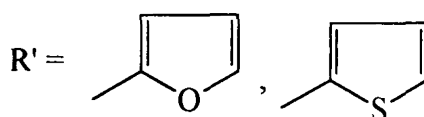
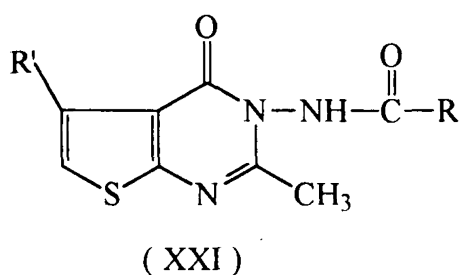
Thieno [2,3-d] pyrimidin-4 (3H)-one ring system, joined to the dihydrothiazine ring through CONH- linkage (XX), was found to be very active against Gram-positive organism¹⁸⁶. The structure of (XX) is given below



Heterocycles such as thienyl and furyl attached at C₂ position of thienopyrimidine ring system (part II-a) are reported to have significant antibacterial and antifungal

activity. Also, a number of β -lactam antibiotics bearing these (furyl/thienyl) heterocycles through acylamino side chain have a broad-spectrum of activity.

All the above examples indicate the importance of the amido linkage in biologically active moieties. Taking into account the above facts and the biological importance of different heteroaryl substituents in molecules, it was thought worthwhile to synthesize molecules where the active thienopyrimidione is substituted at C₅-position with a heterocycles (furyl/thienyl), and at 3-position with various substituted aryl / heteroaryl substituents. The general structure of the compounds(XXI) is given below. The compounds were studied for antibacterial, antifungal, antitubercular, and antimalarial activities.

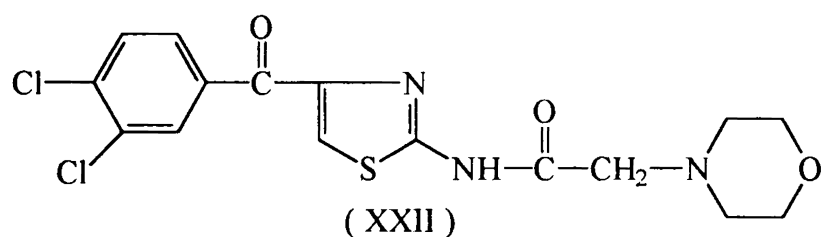


R = substituted aryl/heteroaryl

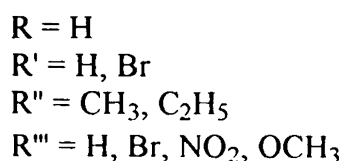
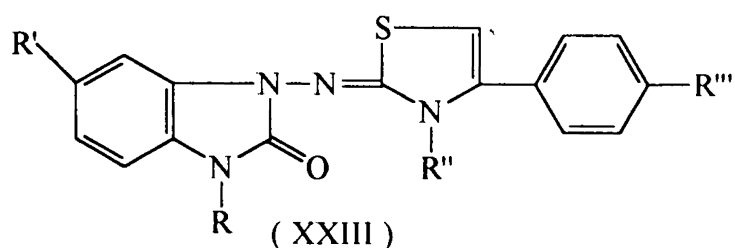
Part II-C : Synthesis of 2-methyl-3-[3-ethyl-4 (substituted aryl/heteroaryl)-2,3 dihydrothiazolidine amino] - 5 - (2-furyl/2-thienyl) - thieno [2,3-d] pyrimidin-4 (3 H) - ones

Thiazole nucleus first described by Arthur Hantzsch in 1887 is an important member of the heterocyclic family. Over the period of years, it has undergone intensive research and finds several applications in the field of medicinal chemistry. In 1965 Romanian research workers¹⁸⁷ showed the bacteriostatic activity of thiazole with amino substituents at 2 position and alkyl or substituted alkyl at 4 position.

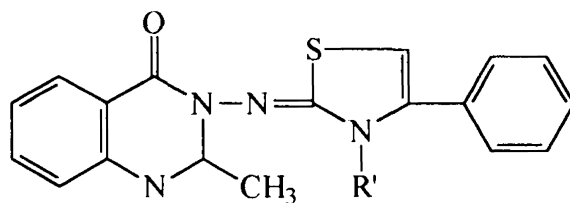
Our laboratory reported a number of 2,4 –disubstituted thiazole derivatives with good antitubercular activity. Of that 2-[(morpholin-1-yl)-Acetamido]-4-(3,4-dichloro benzoyl)-thiazole (XXII) showed antitubercular activity at the concentration of 0.2 µg/ml¹⁸⁸.



Recently, Karali *et al*¹⁸⁹ synthesized derivatives of 3- [3,4-disubstituted-4-thiazolin- 2 ylidine) hydrazono-]-1H-2 indolinones (XXIII) as potent antitubercular agents having MIC <12.5 µg/ml



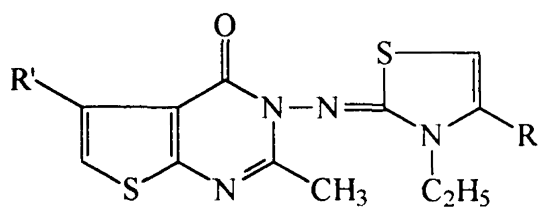
Ahmad and Farghaly¹⁹⁰ synthesized 4(3H)-quinazolinone derivatives having substituted thiazoline moiety at the 3-position (XXIV), which exhibited good antibacterial and antifungal activities.



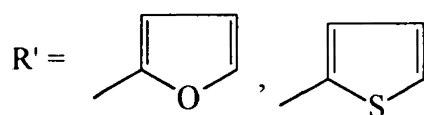
(XXIV)

R = C₄H₉, Allyl, C₆H₁₁, C₆H₅, C₆H₄Br

Hence, on the basis of the above evidence, a series of 5-(2-furyl/2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-one derivatives (XXV) having substituted thiazoline moiety at 3-position have been synthesized and tested for their antibacterial, antifungal, antitubercular, and antimalarial activities.



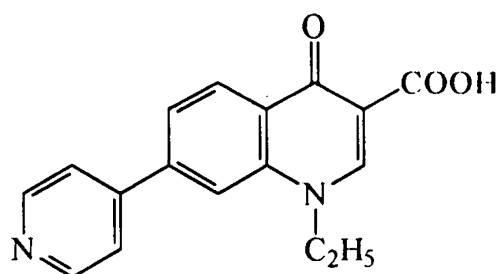
(XXV)



R = substituted aryl/heteroaryl

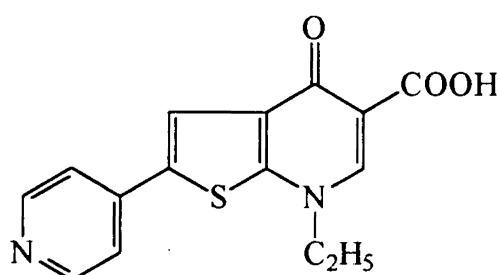
Part II-D : Synthesis of 2-[(substituted aryl / heteroaryl)-thio]ketomethylene]-3- phenyl - 5 - (3-pyridinyl / 4-pyridinyl)-thieno [2,3-d] pyrimidin - 4(3H)-ones

The pyridinyl moiety has played important role in active antitubercular drugs viz. Isoniazid, Ethionamide, and antibacterial Cephapirin. Rosoxacin (XXVI), the first quinolone having 4-pyridinyl moiety at C₇ position proposed for treatment of systemic infection, demonstrated activity against both Gram-positive and Gram-negative bacteria and is marketed for the treatment of gonorrhea¹⁹¹.

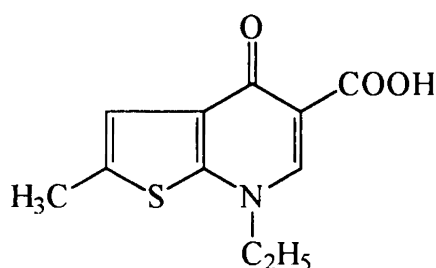


(XXVI)

Edward *et al*¹⁹² synthesized compound (XXVII), which is structurally related to the known antimicrobial (XXVIII), an analogue of Nalidixic acid reported to have a similar antibacterial profile of activity¹⁹³.

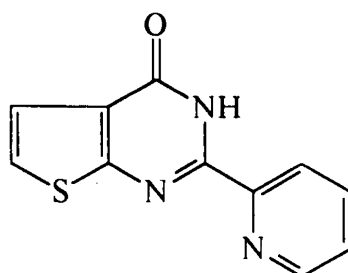


(XXVII)



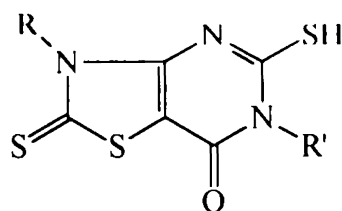
(XXVIII)

Also Bourguignon *et al*¹⁷⁷ prepared 2-(2-pyridinyl)-thieno [2,3-d] pyrimidione (XXIX) for their biological activity.



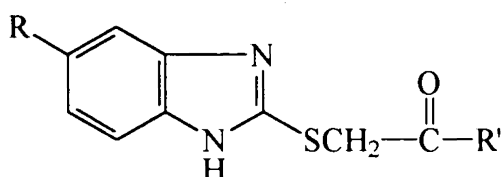
(XXIX)

Badaway *et al*¹⁹⁴ synthesized 3,6-diaryl-2-thioxothiazolo-[4,5-d] pyrimidin-7(6H)-ones (XXX) having mercapto group at C₂ position which showed pronounced antibacterial and antifungal activity.



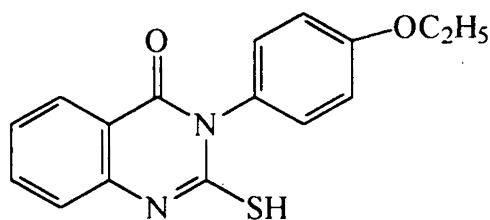
(XXX)
R, R' = phenyl

Lozanosa *et al*¹⁹⁵ synthesized the following types of 2-phenacyl thiobenzimidazoles (XXXI) which are known for their diverse biological activity.



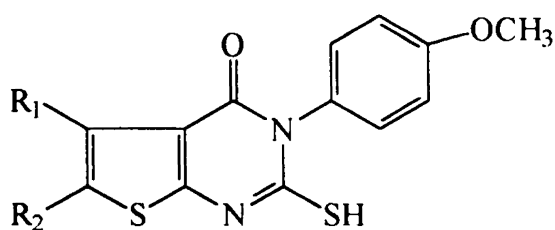
(XXXI)
R = NO₂, Cl
R' = substituted aryl

Muraveva *et al*¹⁹⁶ have reported significant antitubercular activity of 2-thioquinazoles (XXXII).

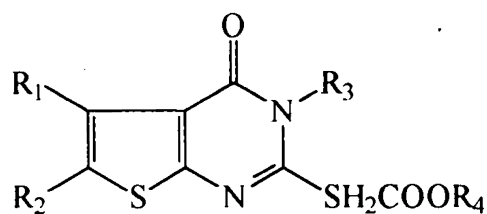


(XXXII)

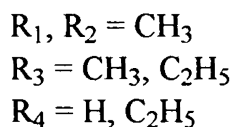
Devani *et al*^{197,198} have reported the antitubercular, antibacterial, and antifungal activity of 2-mercapto-thieno-[2,3-d] pyrimidin-4 (3 H) ones (XXXIII) and thieno [2,3-d] pyrimidin- 4(3 H) – one-2-mercaptoacetic acid and ethyl ester (XXXIV).



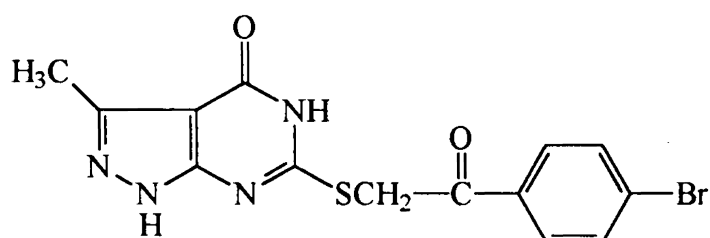
(XXXIII)



(XXXIV)

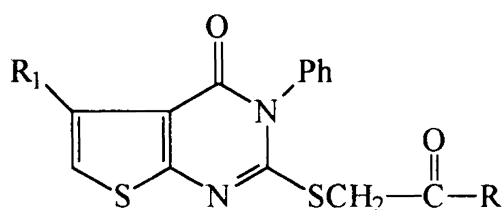


Recently Guccione *et al*¹⁹⁹ have prepared 3-methyl-4-oxo-6-(phenacyl thio)-pyrazolo (3,4-d) pyrimidin (XXXV) by condensation of α -haloketones and 3-methyl-4-oxo-6-mercapto pyrazolo (3,4-d) pyrimidine and screened it for anti-inflammatory and analgesic activity.

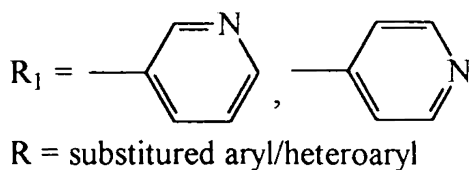


(XXXV)

Careful study of the literature exemplifies the pharmacophoric importance of the pyridine ring and thieno [2,3-d] pyrimidine moiety in various antimicrobial agents. In view of the above findings two series of 2- [(substituted aryl/heteroaryl)-thioketo methylene]-3 phenyl-5-(3-pyridinyl/ 4-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3 H) ones were synthesized having the following general structure (XXXVI), and screened for their antibacterial, antifungal, antitubercular, and antimalarial activity.

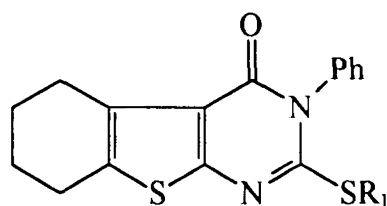


(XXXVI)

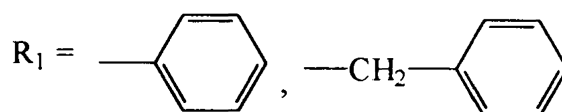


Part II-E : Synthesis of 2-[(substituted benzyl)-thio]-3-phenyl-5-(3-pyridinyl / 4-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3 H)-ones

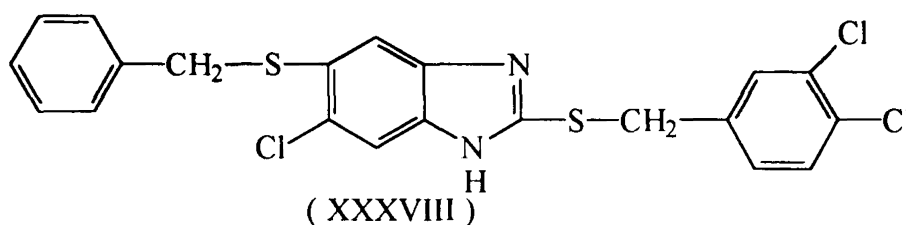
Vishnu *et al*²⁰⁰ have synthesized and studied the biological activity of 2-benzylmercapto-3-phenyl-5, 6 tetramethyleno-thieno [2,3-d] pyrimidin-4 (3H)-one (XXXVII)



(XXXVII)

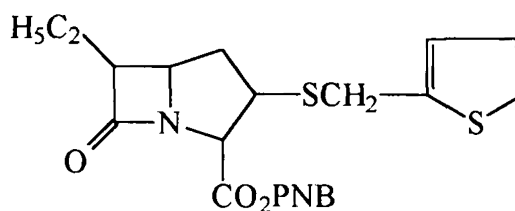


Our laboratory reported a number of sulfide linkage of benzimidazole derivatives having good antitubercular activity, out of which 5-(benzyl thio)-6-chloro-2-(3,4-dichloro benzyl)-thio-benzimidazole (XXXVIII) showed antitubercular activity at a concentration of 0.32 µg/ ml²⁰¹.



(XXXVIII)

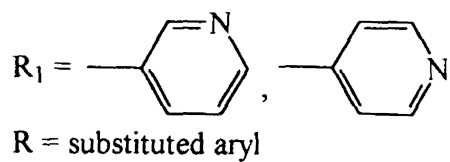
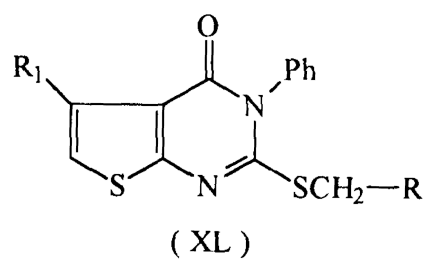
Yamamoto *et al*²⁰² have reported thienamycin analogue (XXXIX) having antimicrobial activity superior or comparable to that of thienamycin.



(XXXIX)

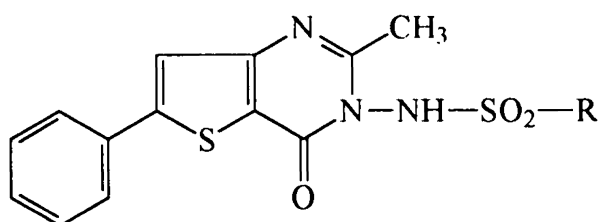
As mentioned in part II-D the importance of pyridinyl moiety attached to thienopyrimidine ring system and based on the above literature finding, we plan to synthesize thieno [2,3-d] pyrimidin- 4(3H)-ones having the following structure (XL)

bearing substituted aryl moiety at C₅ position. All the compounds were subjected for their *in vitro* antibacterial, antitubercular, antifungal and antimalarial activity.



Part II-F : Synthesis of 2-[(substituted aryl / heteroaryl)-sulfonamido]-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H) –ones

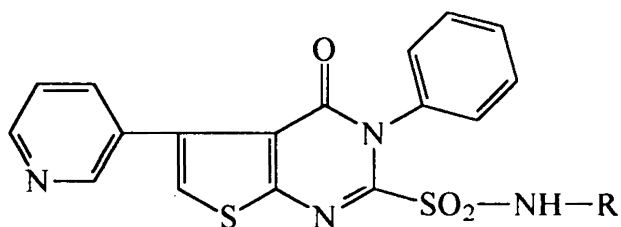
Desai²⁰³ synthesized substituted thieno [3,2-d] pyrimidin- 4(3H)-one derivatives (XLI) by joining the sulfonamido group at 3-position and phenyl group at C₆ position of thienopyrimidione moiety. These derivatives showed significant antimicrobial activity.



(XLI)

R = 4MeC₆H₅, C₆H₅
AcNHC₆H₄, etc.

Thus considering the importance of the sulfonamido linkage as effective sytnhons in preparing compounds of medicinal value, several aryl/heteroryl amines have been attached at C₂ position and 3-pyridinyl group at C₅ position of thieno [2,3-d] pyrimidin-4 (3H)-one moiety in order to get potent compounds of following types (XLII) and screened for their antibacterial, antifungal antitubercular, and antimalarial activity.



(XLII)

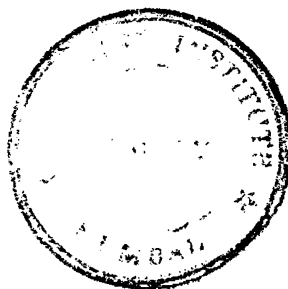
R = substituted aryl

Part I-C

Chemistry

THIS PART DEALS WITH THE CHEMISTRY OF ALL COMPOUNDS / INTERMEDIATES INVOLVED IN THE WORK

- **BUTENOIC ACID-2-CYANO-3-(2-FURYL/2-THIENYL)-ETHYL ESTER**
- **2-AMINO-3-CARBOETHOXY-4-(HETEROARYL)-THIOPHENE**
- **2-METHYL - 3 - AMINO - 5 - (HETEROARYL) - THIENO [2,3-d] PYRIMIDIN -4(3H)-ONES**
- **SCHIFF BASES**
- **AMIDE**
- **HALOGENATION OF KETONES**
- **2- MERCAPTO-3-PHENYL - 5 - (3-PYRIDINYL/4-PYRIDINYL) - THIENO [2,3-d] PYRIMIDIN-4(3H)-ONES**
- **2-[(SUBSTITUTED ARYL/HETEROARYL)-THIOKETOMETHYLENE]- 3 - PHENYL - 5 - (3 - PYRIDINYL / 4-PYRIDINYL)-THIENO [2,3-d] PYRIMIDIN-4(3H)-ONES**
- **2- [(SUBSTITUTED BENZYL) - THIO] - 3 - PHENYL - 5 - (3-PYRIDINYL /4-PYRIDINYL)-THIENO[2,3-d]PYRIMIDIN-4(3H)-ONES**
- **THIOUREA**
- **SULFONYL CHLORIDE**

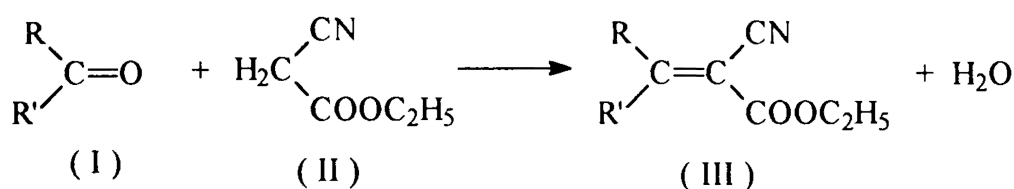


This part deals with the chemistry of all the compounds / intermediates involved in the work

1) Chemistry of butenoic acid-2- cyano -3- (2-furyl/2-thienyl)-ethyl ester

The recent studies of Cope and his associates^{204,205} have introduced a number of alkylidenecyano acetates that may be prepared from ketones by the Knoevenagel reaction. The condensation of cyanoacetic acid and its esters with simple ketones has been reported by a number of earlier investigators. In general, the usual conditions of the Knoevenagel reaction, employing basic catalysts such as piperidine²⁰⁶, diethylamine²⁰⁷ or other amines, were effective. Cope recently found that with simple ketones better yields could be obtained using catalysts such as acetamide or the acetate salts of amines, such as piperidine, diethyl amine or ammonia.

The Knoevenagel reaction is shown below -



The maximum yield was obtained when the reaction was carried out in the presence of acetic acid and in an inert, water-insoluble solvent (benzene), which was added to remove water. Cope and his associates condensed a variety of ketones, including aliphatic, alicyclic or mixed aromatic aliphatic types, with cyano acetic esters.

Many salts catalyze the condensation of 1-ketones with cyanoacetic esters and that the ammonium and amine salts of organic acids are better catalysts than the free bases. Knoevenagel has employed amine salts in several condensation reactions of aldehydes and in the condensation of ketones with acids.

Several different solvents have been reported to be useful in aiding the removal of water by distillation from the reaction mixtures. Among these are toluene²⁰⁸, benzene and chloroform²⁰⁹.

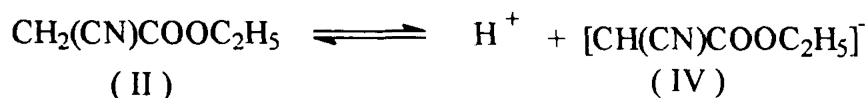
At higher temperatures, ammonium acetate dehydrates very rapidly to acetamide, which is not a catalyst for the reaction. In a reaction, the catalyst must completely

decompose, long before the reaction is completed. Therefore, it is necessary to add the ammonium acetate catalyst in smaller portions at frequent intervals, until further addition gives no more reaction.

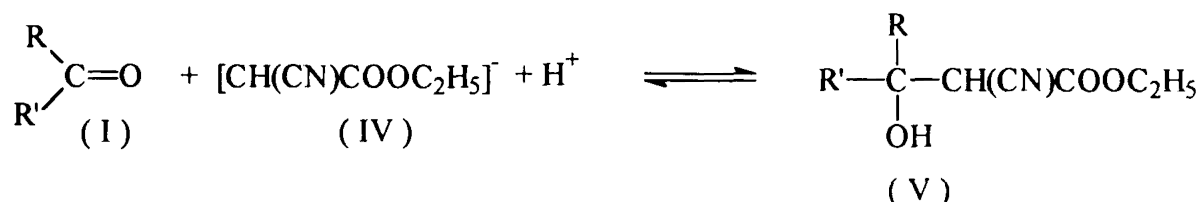
Better yields were obtained by using acetic acid as a solvent and removing the water formed by distillation during the reaction.

The Knoevenagel reaction is supposed to take place by aldol-like mechanism, which was first postulated by Hann and Lapworth²¹⁰, and later supported by Kohler and Corson²¹¹. According to this mechanism, the reaction involves the following steps.

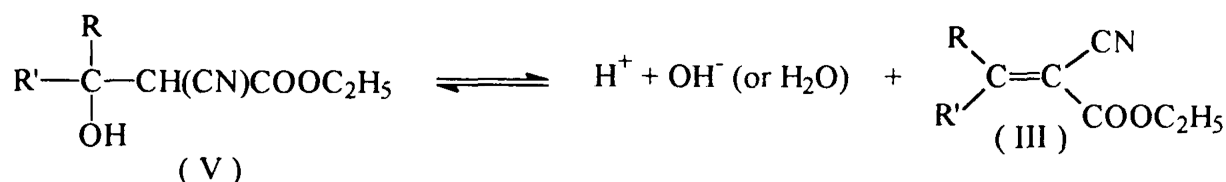
- i) Enolization of the adding methylene compound, probably through dissociation of hydrogen ion.



- ii) Addition of the enol, probably through its ions, to the carbonyl compound.



- iii) Elimination of water from the aldol-like intermediate.



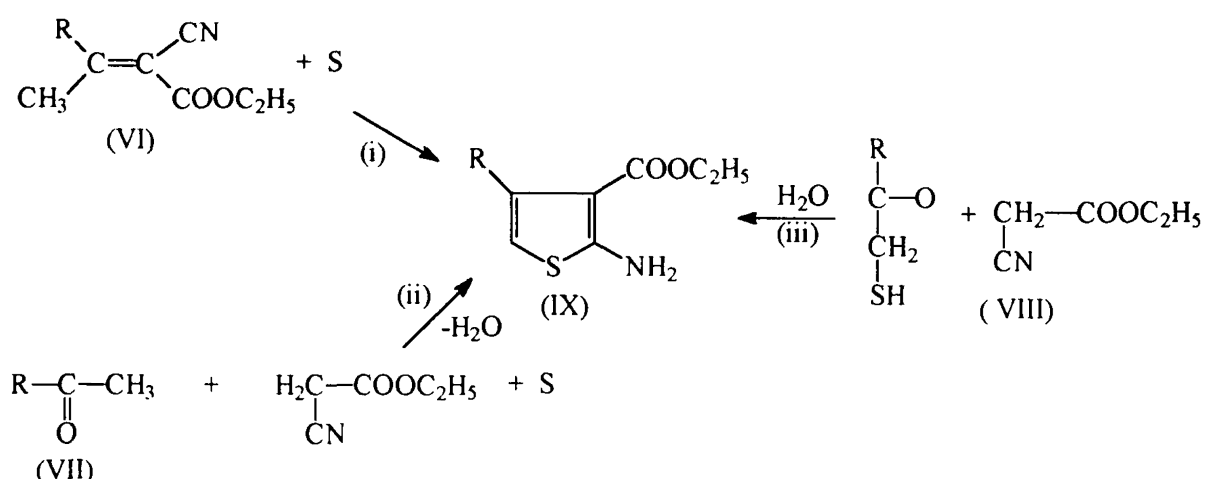
The following facts are known in general about the above types of equilibria. The enolization equilibria is catalyzed by both acids and bases. Bases produce higher concentration of the enolate anion by removing the hydrogen ion. The rate of addition reaction as such depends upon the degree to which the addition to carbonyl group is hindered, or upon the size of the alkyl group. Reactions similar to (iii) in which water is eliminated are catalyzed by both acids and bases, but in general more powerfully by acids.

Acetic acid was chosen as an ionizing solvent which itself catalyzes enolization, and which by distillation allows the continuous removal of water, thus preventing reversal of equilibria. Acetamide acetate, ammonium acetate and ethylene diamine diacetate were the most active catalysts.

2) Chemistry of 2-amino-3-carboethoxy - 4 - heteroaryl-thiophene

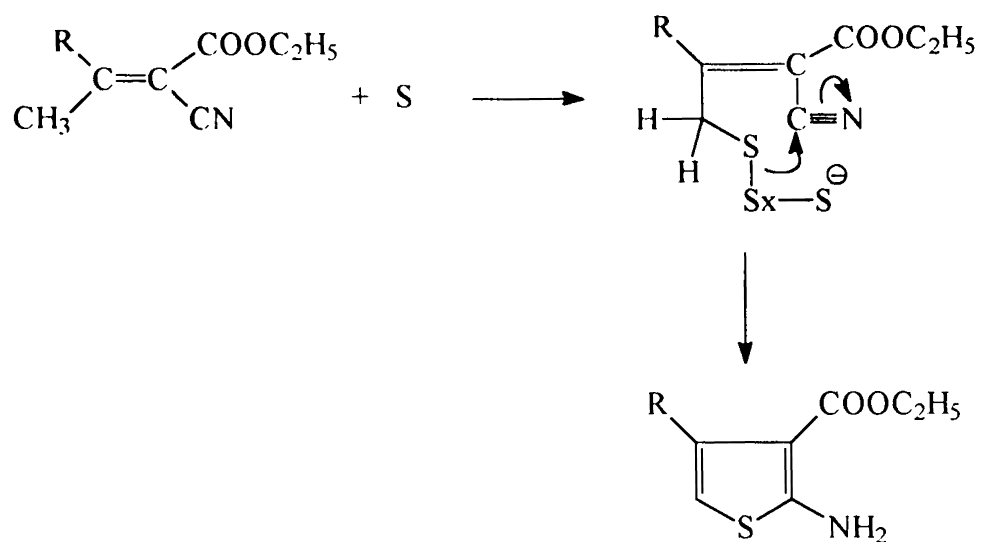
The substituted thiophenes could easily be synthesized by the well known Gewald²¹² procedure which involves-

- The reaction of ethyl heteroarylidene cyano acetate with elemental sulphur in the presence of diethyl amine using absolute alcohol as the solvent.
- The reaction of aryl/heteroarylketone, ethyl cyano acetate, and elemental sulphur in the presence of morpholine using absolute alcohol as the solvent.
- The reaction of α -oxo-mercaptan with ethylcyano acetate.

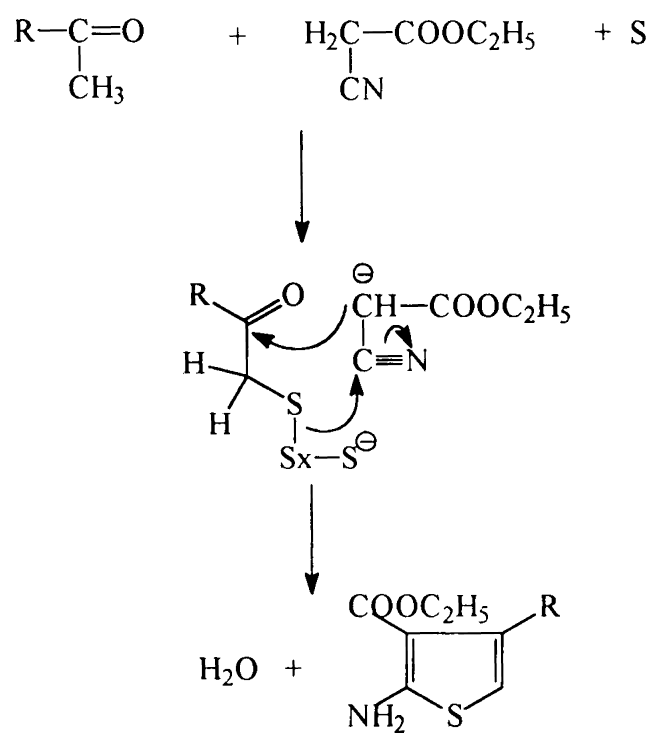


In our synthesis of substituted thiophenes, we chose route (i) and (ii). 2-acetyl furan and 2-acetyl thiophene react only through route (i), while 3-acetyl pyridine and 4-acetyl pyridine react through route (ii) with ethylcyano acetate.

The probable mechanism for route (i)

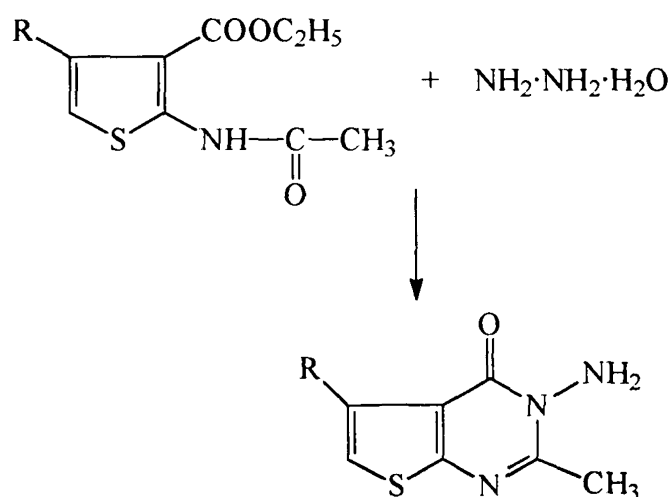
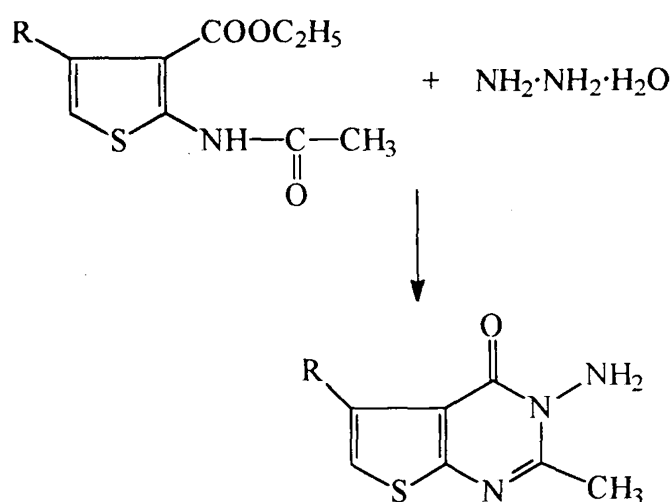


The probable mechanism for route (ii)

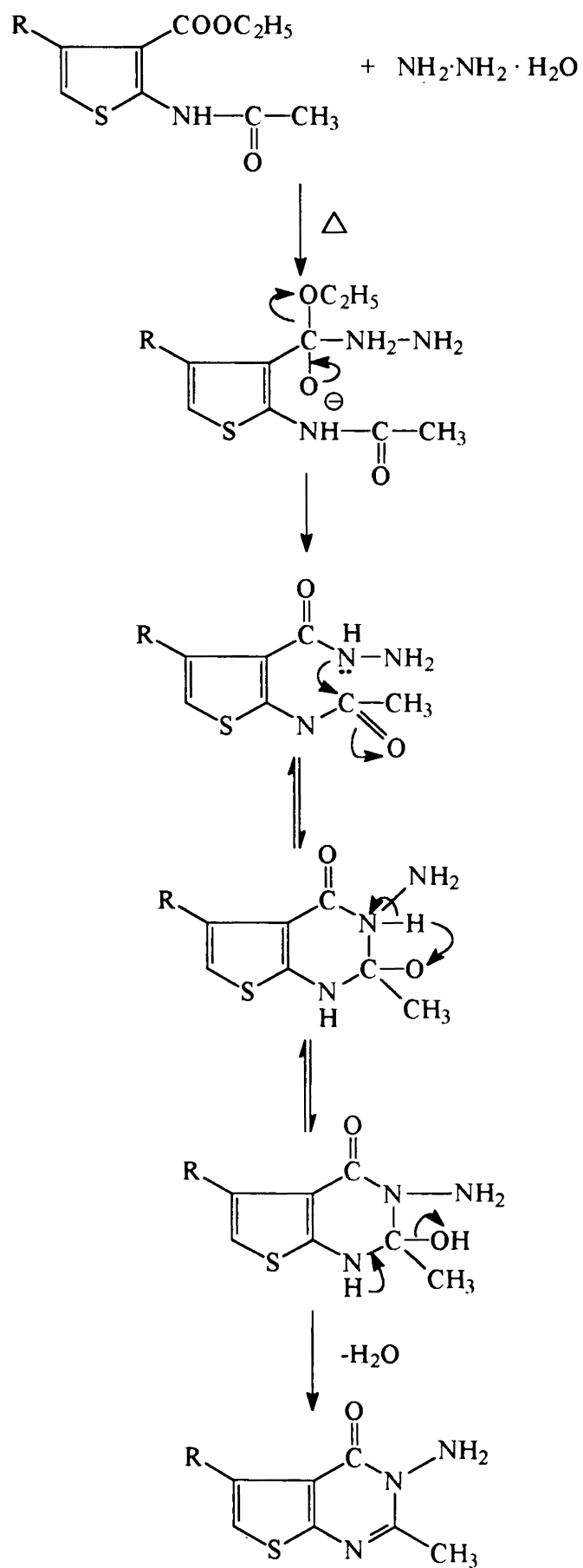


3) Chemistry of 2 - methyl - 3 - amino - 5 - (heteroaryl) - thieno [2,3-d] pyrimidin - 4(3H) - one

In literature various procedures are reported for the preparation of 2-methyl-3-amino-5,6-disubstituted-thieno [2,3-d] pyrimidin-4 (3H)-ones, which involves the reaction of 2- acetamido -3-carbethoxy 4-(heteroaryl) thiophene with hydrazine hydrate (80 %) under reflux²¹³. For preparation of 2 methyl 3 amino 5 (heteroaryl) thieno [2,3-d] pyrimidin-4 (3H)-ones, we reacted 2 acetamido-3-carbethoxy-4 (heteroaryl) thiophene with excess of hydrazine hydrate using alcohol as a solvent under reflux.



Reaction Mechanism

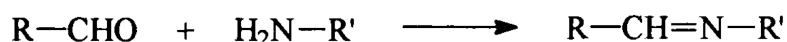


4) Chemistry of Schiff bases

Schiff bases are formed by the condensation of amino group and carbonyl functional group of aldehyde or ketone.

The presence of azomethine linkage in compounds has been shown to decrease their toxicity considerably^{214,215}. In our laboratory, Schiff bases of various aldehydes have shown encouraging antitubercular activity *in vitro*.

Schiff²¹⁶, for the first time, reported the condensation of primary amine with carbonyl compounds and hence, condensation products are referred to as "Schiff bases".

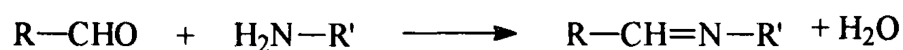


Aromatic, as well as heterocyclic amines and aldehydes can be condensed as above to give Schiff bases. The literature describes many procedures to prepare Schiff bases. The important methods are listed below:

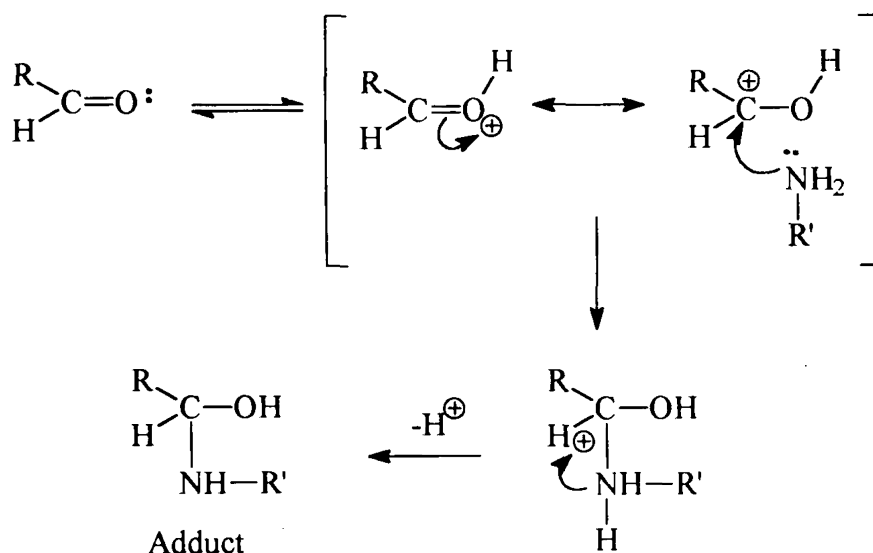
- 1) The simplest method appears to be one in which condensation of equimolar quantities of amine and an aldehyde is effected in boiling methanol.
- 2) In 1946, Tipson and Clapp²¹⁷, obtained Schiff bases by refluxing the amine and aldehyde in toluene, with a few drops of piperidine. The water formed during the course of the reaction was removed by employing a Dean-Stark's trap.
- 3) In 1950, Hayes *et al*²¹⁸ prepared Schiff bases by heating a mixture of an aldehyde and amine in benzene and removing water formed during the reaction using Dean Stark apparatus.
- 4) In 1953, Giovambettista and Rabassa²¹⁹ suggested the use of acetic acid as a catalyst for the synthesis of Schiff bases in good yields.

As the first method was found to give Schiff bases in good yields, it was followed initially during the course of this investigation. Later on, it was found that the use of a few drops of acetic acid improved the yields.

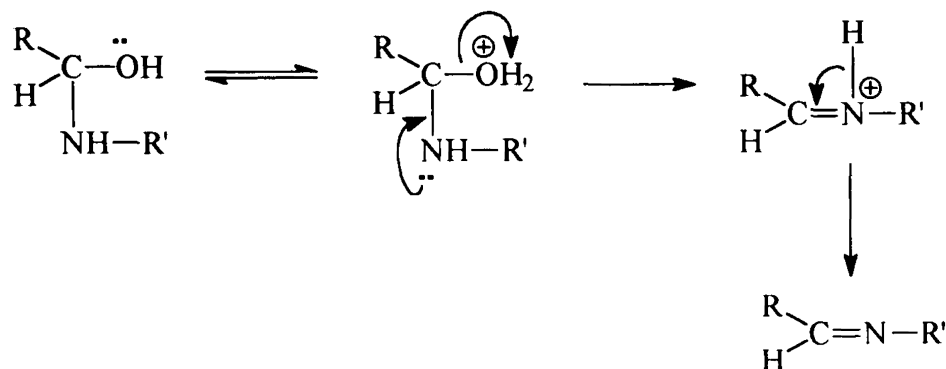
Reaction Mechanism



The addition of amines to aldehydes under mild acid catalysis causes. The acid catalyst to protonate the carbonyl oxygen and promote the attack of nucleophilic amine.



The adduct that is formed in this reaction undergoes a further reaction to eliminate water, and form a carbon nitrogen double bond.

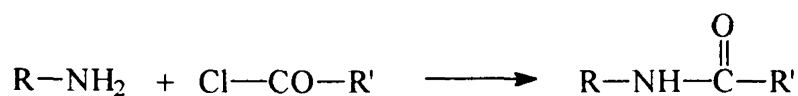


Hence, a combination of modified first and the fourth method was used during the synthesis of the Schiff bases of 2 methyl-3-amino-5 (2 furyl/2 thienyl) –thieno [2,3-d] pyrimidin-4(3H)-ones, which were condensed with various substituted benzaldehydes.

5) Chemistry of amide

Aromatic or hetero amines condense with benzoyl chloride in solvent at elevated temperature to form amide linkage. Various solvents such as benzene, dioxane etc. can be used.

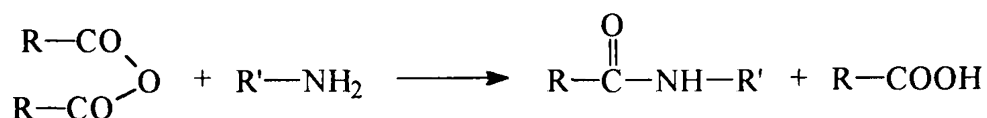
The synthetic route can be represented as below:



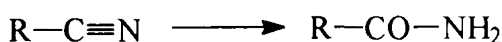
The heterocyclic amines are in general less reactive than the aromatic amines. The refluxing of the amine with substituted acid chloride in dioxane, in the presence of triethylamine, was found to be the best method. Hydrogen chloride liberated in the reaction is trapped by triethylamine, forming its hydrochloride salt. Triethylamine hydrochloride $[(\text{C}_2\text{H}_5)_3\text{N}^+\text{HCl}^-]$ is highly soluble in water, the product being insoluble in water. The product is isolated easily by dumping the reaction mixture in water.

The use of pyridine in place of triethylamine is also very common. Other methods of synthesis of amide include.

- i) action of amine on ester
- ii) action of acetic anhydride on amine

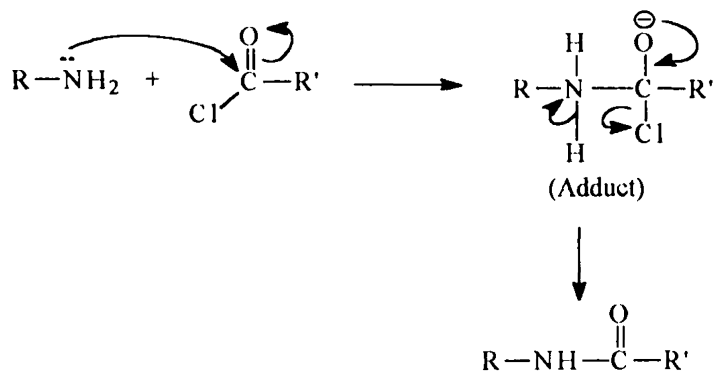


- iii) hydrolysis of alkali nitriles



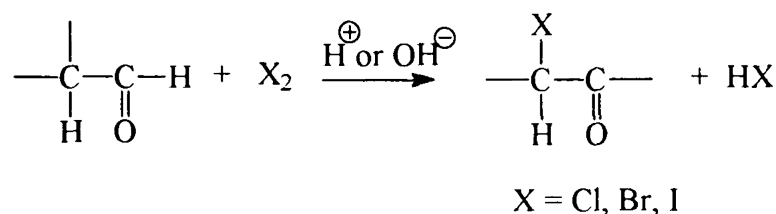
Mechanism of reaction

The acid chlorides are readily attacked by the nucleophile at the carbonyl carbon. The resulting adduct eliminates chloride ion. So although each step of the reaction is theoretically reversible, the reaction by-product is hydrogen chloride gas, which is usually abstracted by base from the reaction mixture, shifting the equilibrium to the right side.



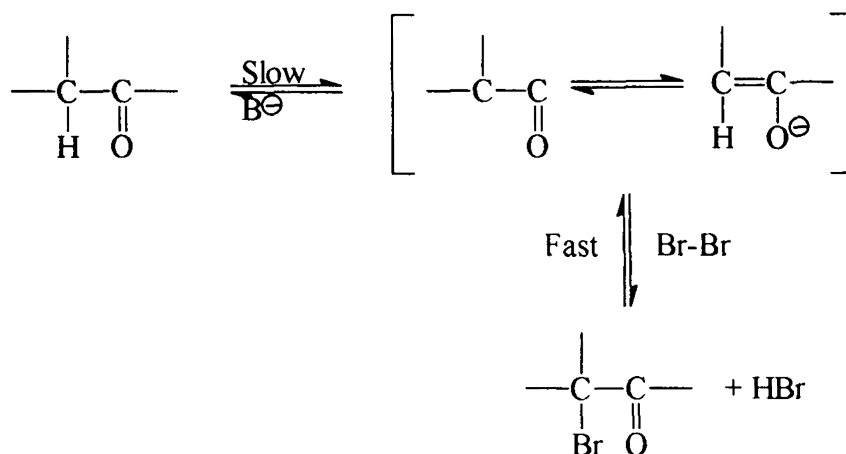
6) Chemistry of halogenation of ketones

Ketones that have an (α)-hydrogen can readily be halogenated with Bromine, Chlorine or Iodine in an acidic, as well as basic medium.



Base-promoted halogenation of ketones:

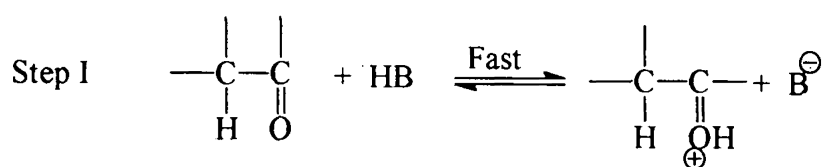
When basic catalysts are used, the base slowly abstracts a proton from the ketone to form the carbanion (I), which then rapidly reacts with Bromine to yield the bromoketone(II).

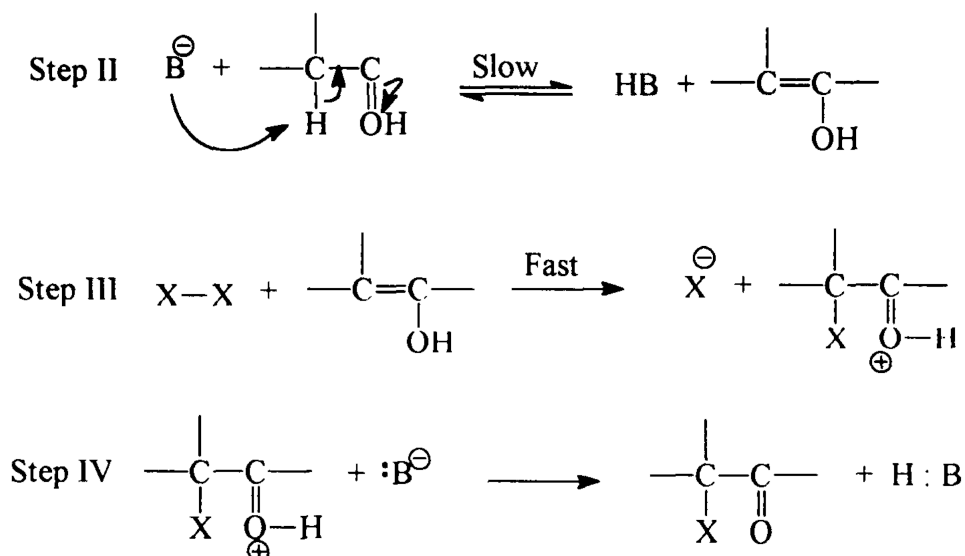


Since the rate of generation of carbanion determines the overall rate of the reaction, it is the rate determining step. As fast as the carbanions are generated, they are snapped by the Bromine molecule.

Acid-catalysed halogenation of ketones

Acids, like bases, speed up the halogenation of ketones. Since the acids are not consumed, the reaction may be called acid-catalysed halogenation of ketones.





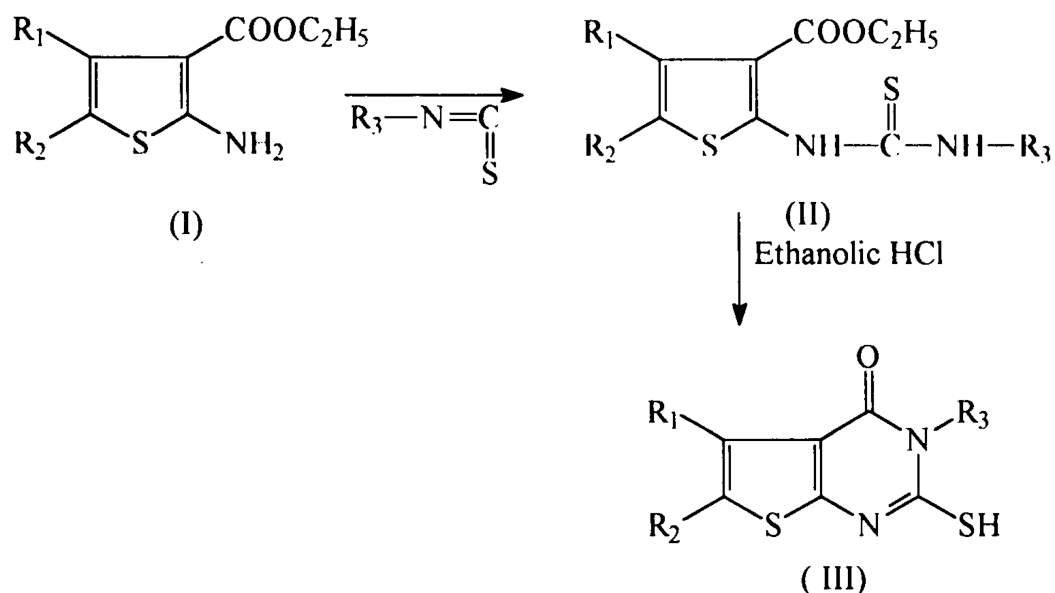
The rate-determining step here is the formation of the enol, which itself involves 2 steps : rapid reversible protonation step (I) of the carbonyl oxygen, followed by the slow loss of an α -hydrogen step (III). Once formed, the enol reacts rapidly with halogen to give the haloketone.

Solvents like chloroform, carbon tetrachloride, diethyl ether can be used conveniently with better results. When diethyl ether is used as a solvent, aluminium chloride is often added for prompt enolization.

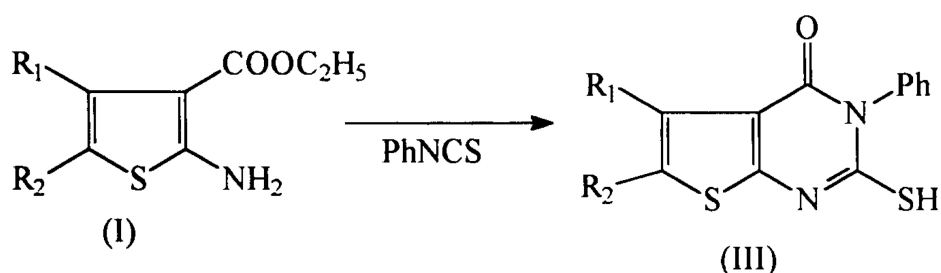
It is observed during bromination that the presence of sunlight or slight heating during the initial stages facilitates the initiation of the reaction. Heat or light may help bromine molecule to get polarized to Br radical, which would favour attack on the enol form of the ketone.

7) Chemistry of 2-mercapto-3-phenyl-5-(3-pyridinyl/4-pyridinyl)-thieno [2,3-d] pyrimidin- 4(3H) -ones

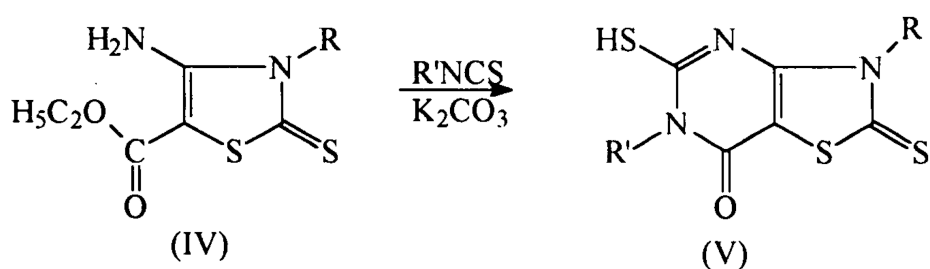
For the preparation of 5,6 disubstituted 2-mercapto-3(aryl/alkyl)-thieno[2,3-d] pyrimidione, various methods are reported. First method involves the reaction of 2-amino-2,3-carbethoxy-4,5-disubstituted-thiophene and substituted aryl / alkyl isothiocyanate in the ethanol saturated with dry hydrochloric acid, under reflux conditions¹⁹⁷.



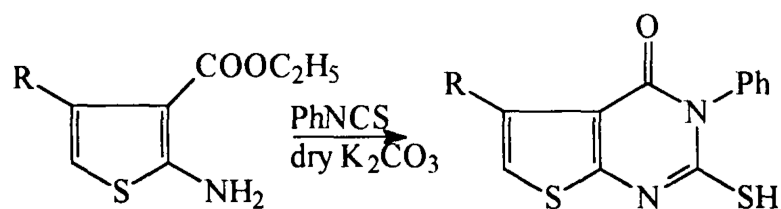
In the second procedure, a mixture of 2-amino-3-carbethoxy-4,5-disubstituted thiophene and phenyl isothiocyanate in ethanol was heated for 15 to 20 hrs¹⁶⁸.



Also, 3-aryl-4-amino-2,3-dihydro-2 thioxothiazole-5-carboxylate was cyclized to 3,6 diaryl-5-mercapto-2-thioxothiazole [4,5-d] pyrimidin-7(6H)-one by using phenyl isothiocyanate, dry K_2CO_3 and acetonitrile as solvent, under reflux conditions¹⁹⁴.

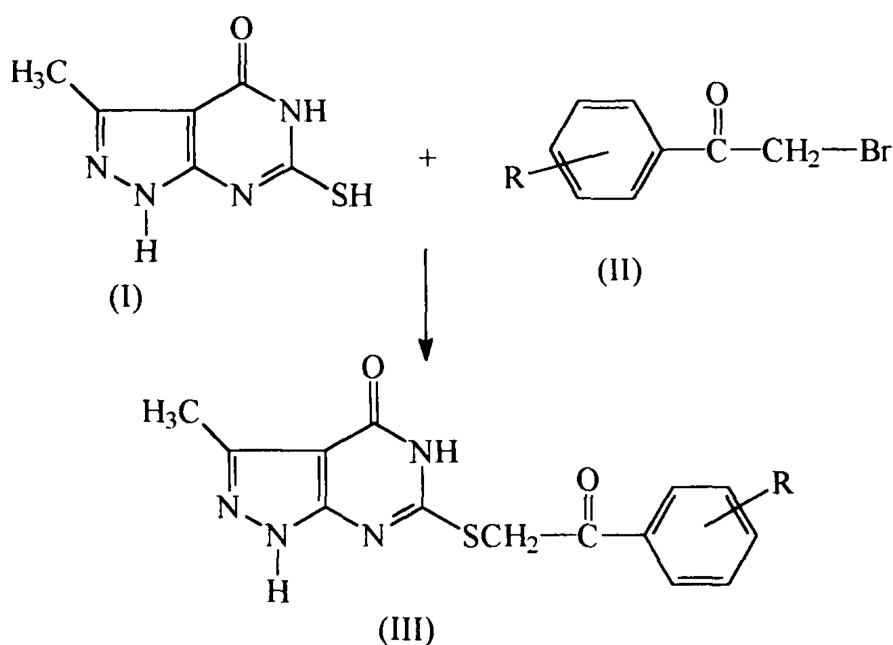


Therefore, we decided to cyclize 2-amino-3-carbethoxy-5-(3-pyridinyl / 4-pyridinyl)-thiophene with phenyl isothiocyanate using K_2CO_3 and acetonitrile as solvent, under reflux conditions, to give 2-mercapto 3-phenyl-5(3-pyridimnyl / 4-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H) ones.



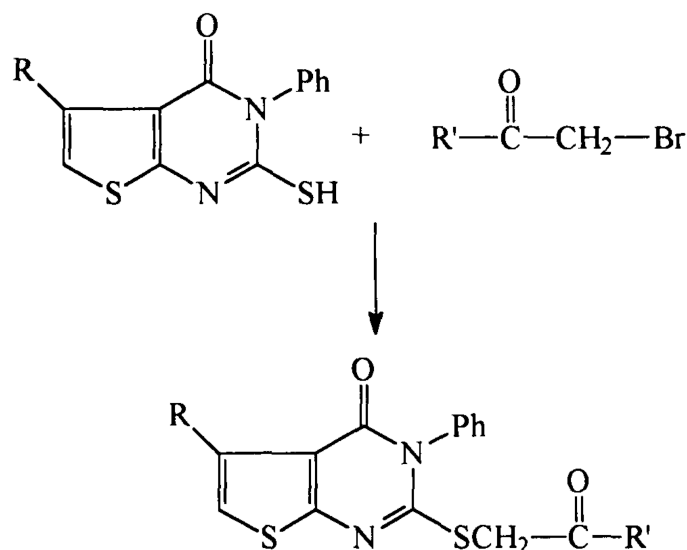
8) Chemistry of 2-[(substituted aryl / heteroaryl) - thioketomethylene]-3-phenyl-5-(3 -pyridinyl /4-pyridinyl)-thieno [2,3-d] pyrimidin- 4 (3H) -ones

In the literature, 3-methyl-6-[(substituted aryl)-thioketomethylene]] pyrazolo [3,4,-d] pyrimidin-4(3H)-ones have been prepared by stirring 3-methyl 6-mercapto pyrazolo [3,4-d] pyrimidin-4(3H)-one with the pertinent α -haloketone in anhydrous acetone and sodium carbonate at room temperature for 24 hours.



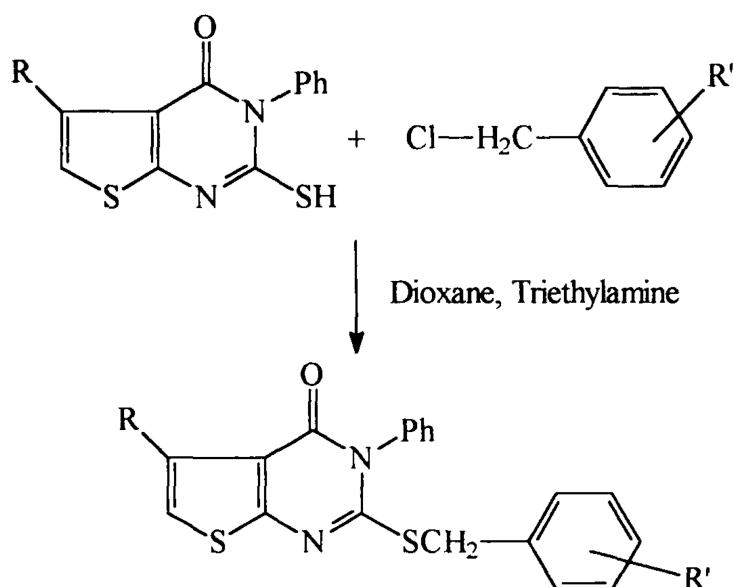
In our course of synthetic work, we used this method for the preparation of 2-[(substituted aryl/heteroaryl)-thioketomethylene]-3-phenyl-5-(3 pyridinyl / 4-pyridinyl) -thieno [2,3-d] pyrimidin- 4 (3 H)-ones.

In this method the α -haloketone was condensed with 2-mercapto-3-phenyl-5-(3-pyridinyl / 4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones.



9) Chemistry of 2-[(substituted benzyl)-thio]- 3-phenyl-5-(3-pyridinyl / 4-pyridinyl) - thieno [2,3-d] pyrimidin-4 (3H)-ones

2-mercapto-3-phenyl-5-(3-pyridinyl/4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one was refluxed with various substituted benzyl chlorides in equimolar quantities in dry 1,4, dioxane and triethylamine, yielding the title compounds.



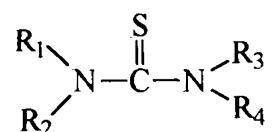
10) Chemistry of thiourea

Since the first synthesis of thiourea and its derivatives was made in about 1870, a vast literature on these compounds has accumulated. In fact, an extensive knowledge of the properties and principles of synthesis and reactivity of thiourea was available at a relatively early stage in the history of thiocarbonyl chemistry. The main

reason for this situation is that thioureas are of high stability and are more easily accessible by relatively simple synthetic procedures.

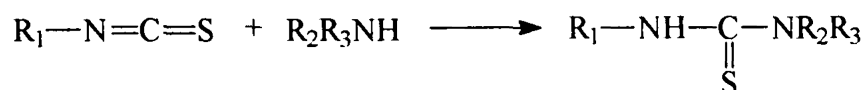
Thioureas have found wide applications in pharmaceutical, insecticides, preservatives, rodenticides and are of commercial use in dyes, photographic films, plastics, textiles etc.

Thioureas in common practice are represented by the following structure:



$\text{R}_n = \text{H}, \text{Alkyl}, \text{Aryl}, \text{etc}$

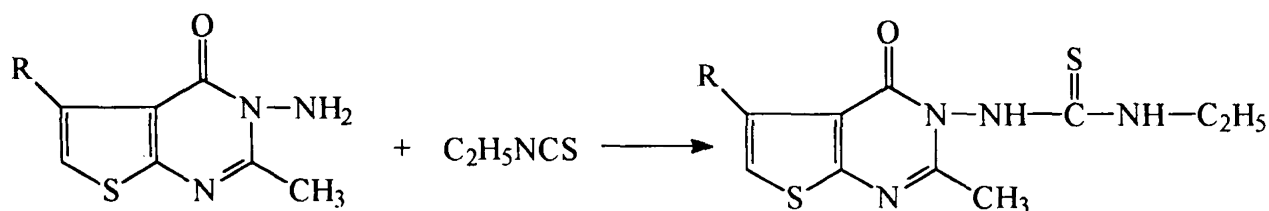
The most important standard method of synthesis is based on the reaction of alkyl or aryl isothiocyanates with ammonia or amine²²⁰. By this reaction, mono substituted, 1,3 disubstituted, and 1,1,3 – trisubstituted thioureas can be obtained.



$\text{R}_1, \text{R}_2, \text{R}_3 = \text{H}, \text{alkyl}, \text{aryl}, \text{etc.}$

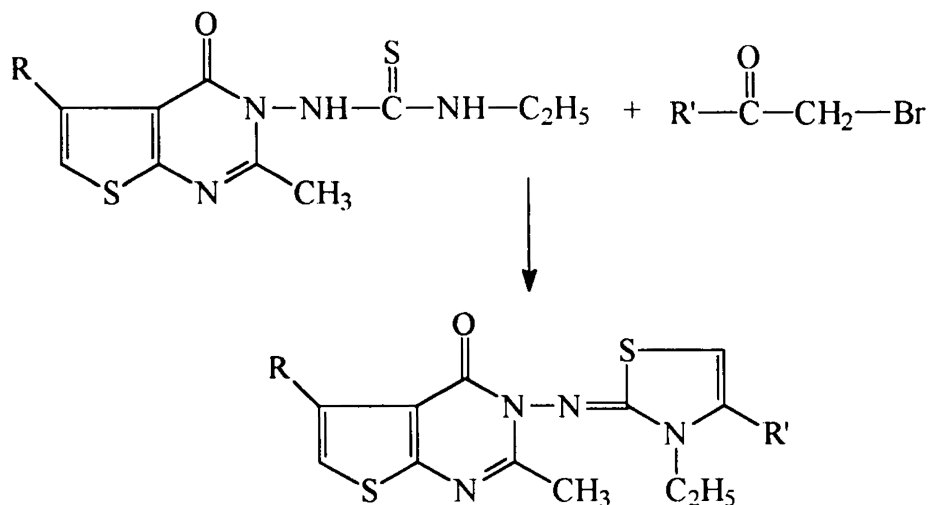
The broad scope of the reaction is most clearly demonstrated by a large variety of thioureas that have been synthesized on the basis of active participation of aliphatic, aromatic, open chain and cyclic amines, as well as of amine derivatives such as ketimines, hydroxylamines, iminoesters and amidines²²¹⁻²²³.

The same reaction is used to synthesize 2-methyl-3N-ethyl thioureido-5-(2-furyl / 2-thienyl)-thieno [2,3-d] pyrimidin-4 (3H)-one.

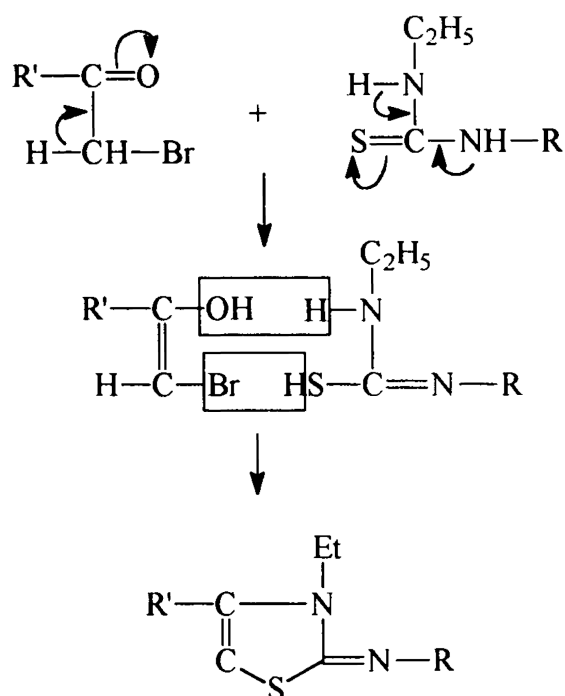


In the course of our synthetic work, for the preparation of 2- methyl-3-[3-ethyl-4 (substituted aryl / heteroaryl)-2,3 dihydrothiazolidene amino)]-5- (2-furyl / 2-thienyl)-

thieno[2,3-d] pyrimidin-4(3H)-ones, α -haloketones was condensed with 2-methyl-3 N-ethyl thioureido-5-(2-furyl / 2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-one.



Reaction Mechanism



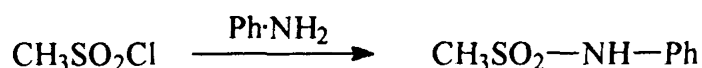
11. Chemistry of Sulfonyl chloride

The preparation of Sulfonamide from sulfonyl chloride is a nucleophilic substitution at RSO₂Cl, in a manner similar to the attack of R-COCl, but the mechanism is not the same because a 'tetrahedral' intermediate(I) in this case would have five groups on the central atom²²⁴.



(1)

This mechanism more closely resembles the SN^2 mechanism, but the reaction of the substrate, which carries a α -hydrogen, is a strong evidence that the reaction takes place by an elimination addition mechanism going through sulfene intermediate, e.g. reaction of methane sulfonyl chloride and aniline gives sulfonamide as follows



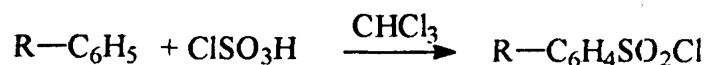
Preparation of sulfonyl chloride :

The sulfonyl chlorides are prepared by various methods –

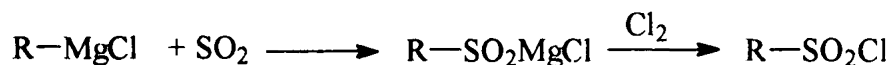
1. By reacting sodium sulfonate with PCl_5 or POCl_3 at 180°C . In this reaction, benzene sulfonate reacts with the PCl_5 or POCl_3 to yield benzene sulfonyl chloride.



2. Chlorosulfonic acid is utilized for the conversion of aromatic hydrocarbon to sulfonyl derivatives²²⁵. In this, chlorosulfonyl chloride is used to provide the sulfonyl chloride ($-\text{SOCl}_2$) group to the aromatic hydrocarbon. Thus, the sulfonyl chloride group is introduced to the aromatic nucleus by treatment with an excess of chlorosulfonic acid in chloroform²²⁶.

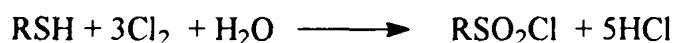


3. By reaction of Grignard reagent first with SO_2 , and then with chlorine gas²²⁷.

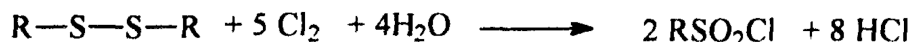


4. Mercaptans or disulfides on reaction with chlorine in aqueous hydrochloric acid can be converted to sulfonyl chloride²²⁸.

Thus, methane mercaptan is converted into methane sulfonyl chloride by simultaneous hydrolysis and chlorination at $40\text{--}70^\circ\text{C}$ in saturated HCl .

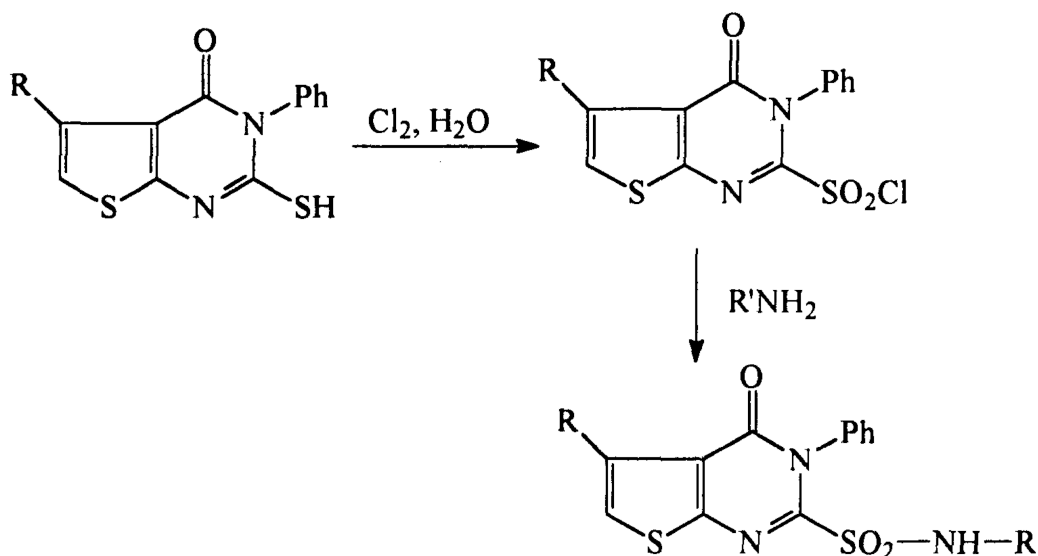


Disulfides can also be converted to sulfonyl chloride by simultaneous hydrolysis and chlorination.



There are relatively few the heterocyclic sulfonamides in which the sulfur atom is joined directly to a carbon of heterocyclic ring. In such a case, sulfonyl chloride (Sulfonamide) are usually prepared by chlorosulfonation or conversion to sulfonic acid chloride, followed by the amidation to get sulfonamide. The oxidative chlorination, at low temperature, of such heterocycles has been found to be quite widely applicable to the preparation of these compounds.

In the course of our synthetic work, we used the oxidative chlorination method (Method 4) for the preparation of 2-(aryl/heteroaryl)-sulfonamido]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one. In this method, mercaptan compound was converted into sulfonyl chloride in the presence of Cl_2 and water at low temperature, followed by the amidation to get sulfonamide.

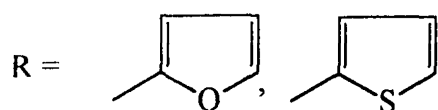
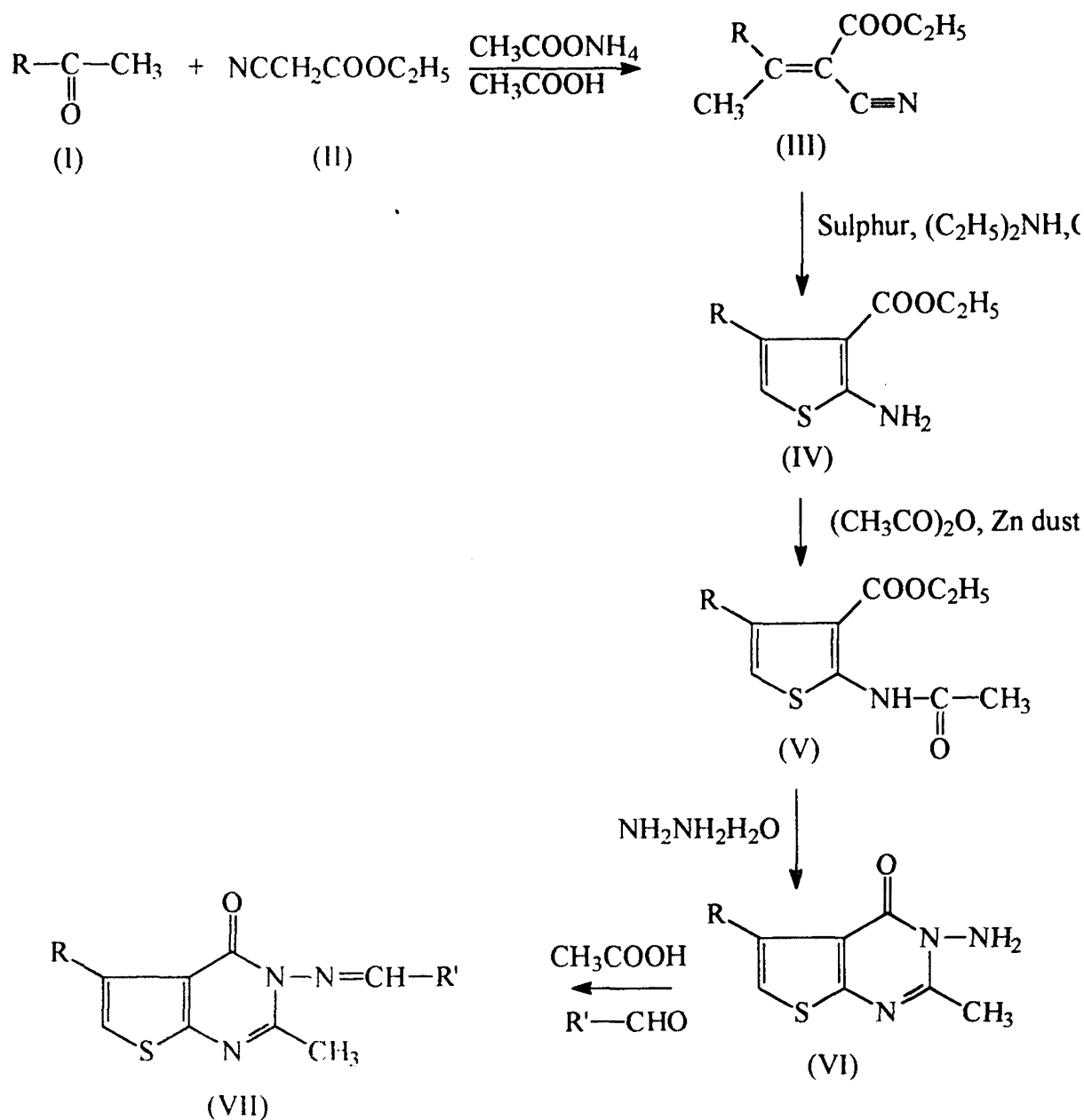


Part I-D

***Schematic
Representation of the
Series of Compounds
Synthesised***

SCHEME I

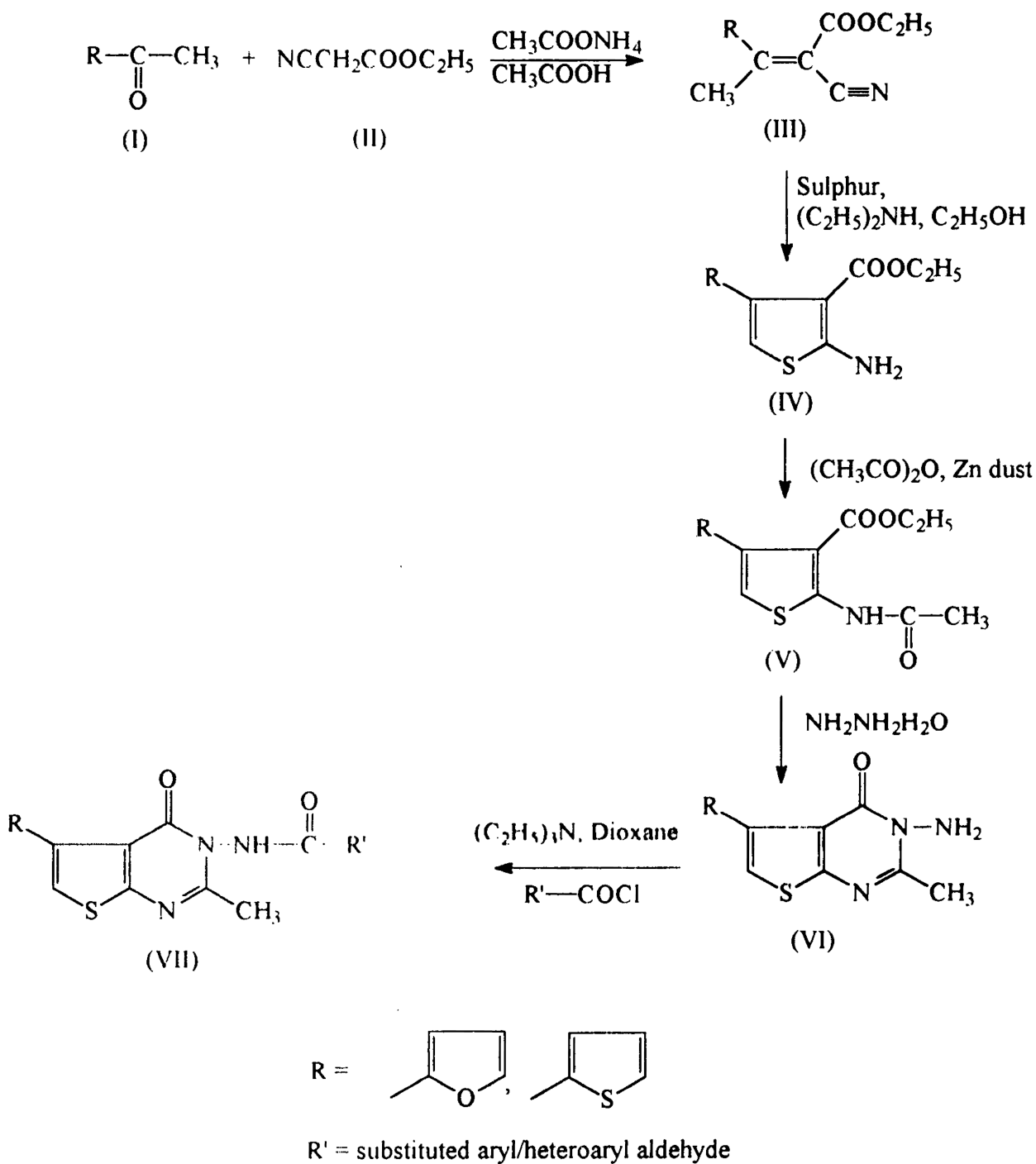
Synthesis of 2-methyl-3N-[(substituted aryl /heteroaryl)- methylene]-amino-5-(2-furyl / 2-thienyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones



R' = substituted aryl/heteroaryl aldehyde

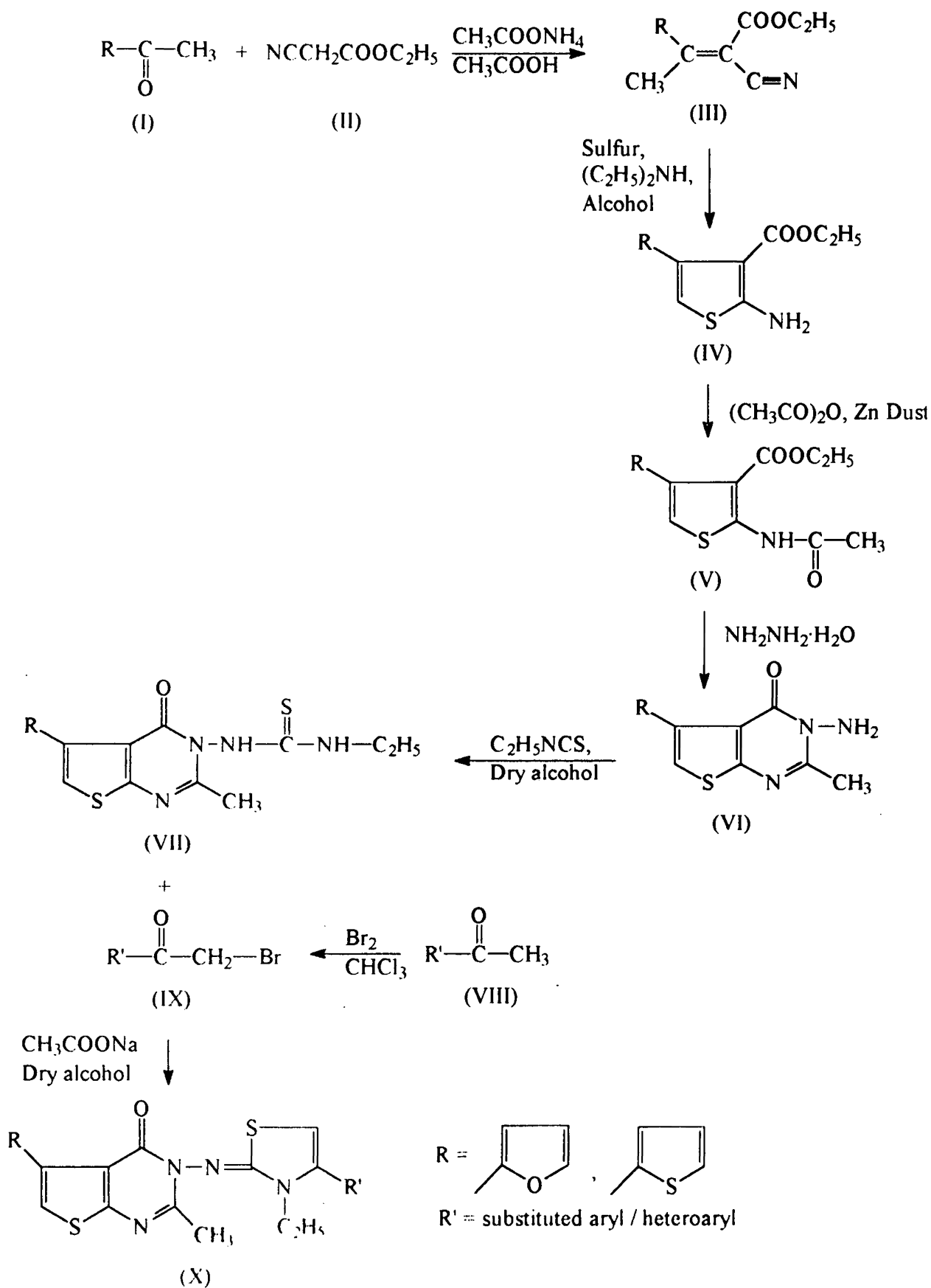
SCHEME II

Synthesis of 2-methyl-3N-[(substituted aryl / heteroaryl)-carboxamido]-5-(2-furyl / 2-thienyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones



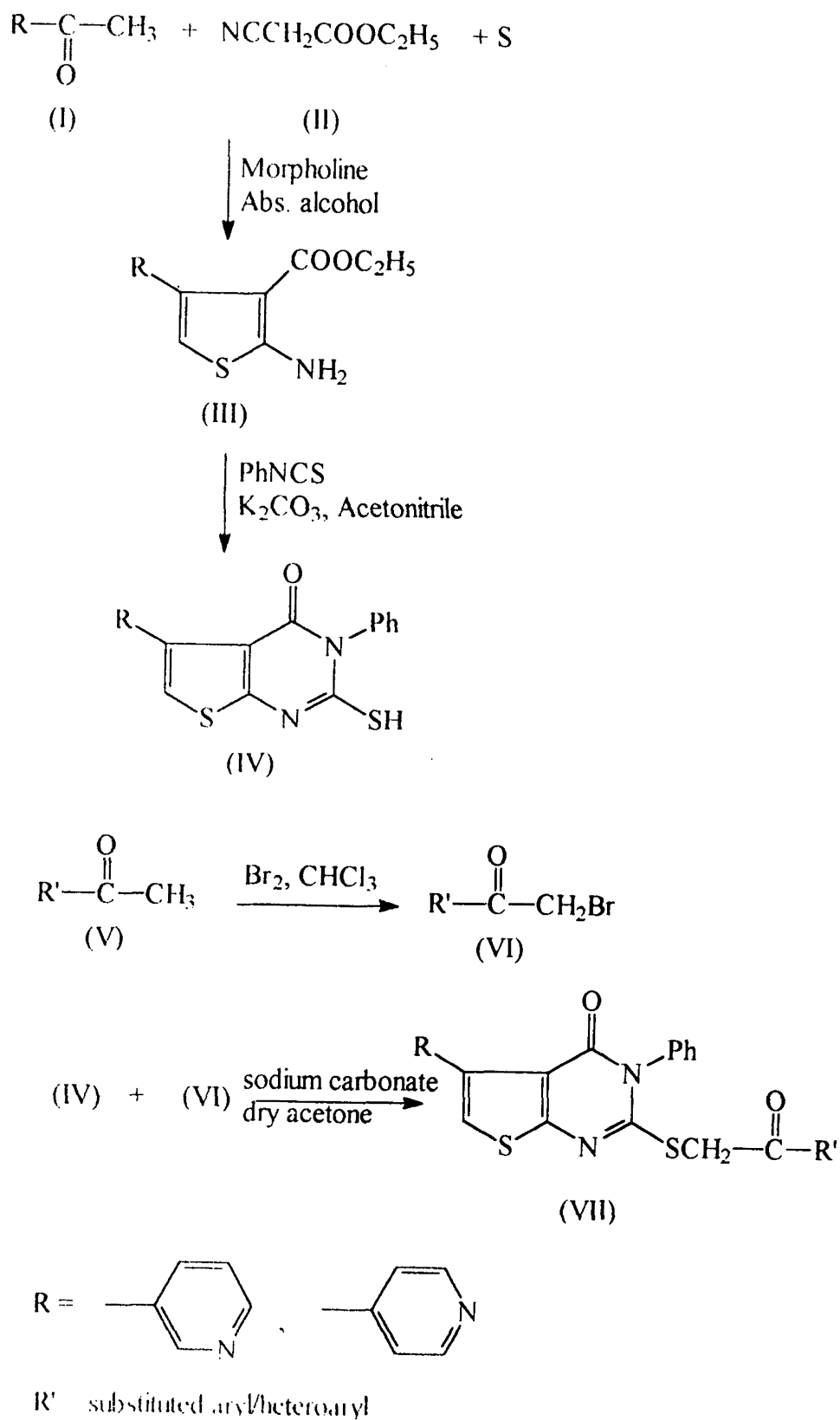
SCHEME III

Synthesis of 2-methyl-3-[3-ethyl-4-(substituted aryl / heteroaryl)-2,3 dihydro thiazolidine amino]-5-(2-furyl / 2-thienyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones



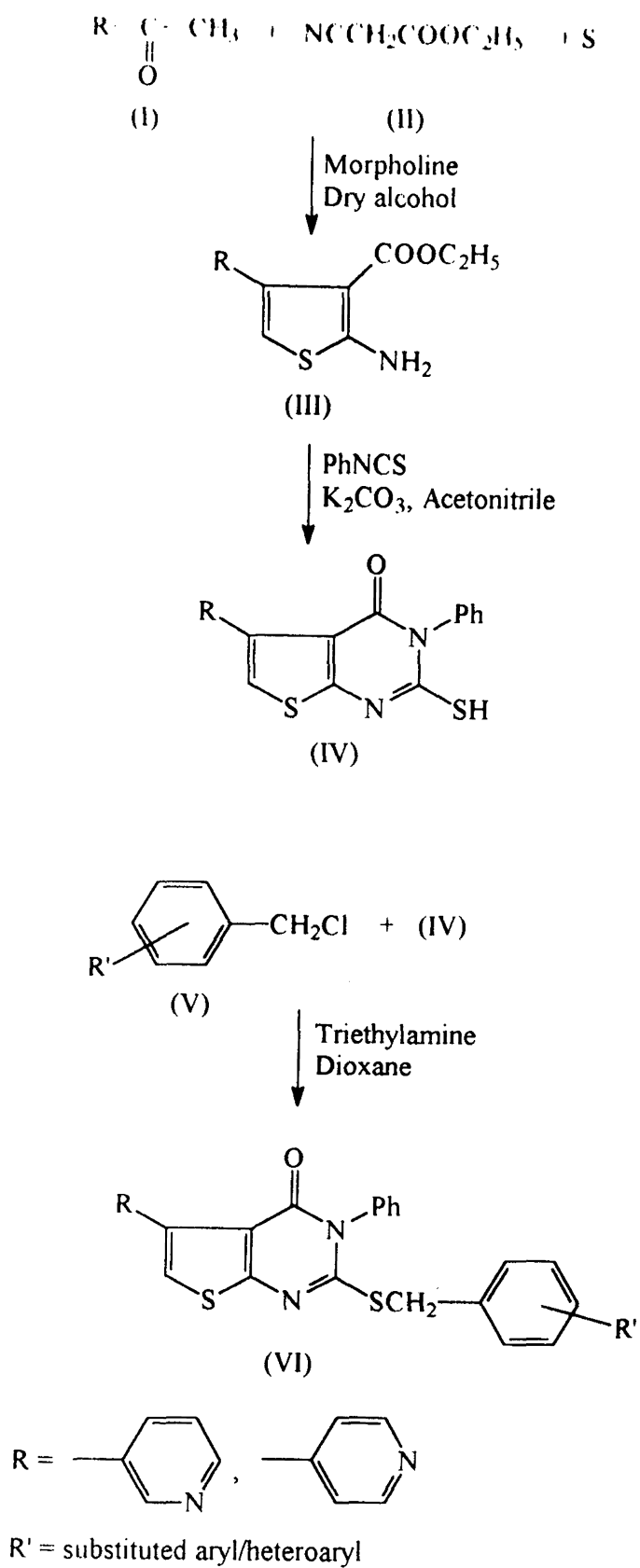
SCHEME IV

Synthesis of 2-[(substituted aryl / heteroaryl)-thio]ketomethylene]-3-phenyl-5-(3-pyridinyl / 4-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones



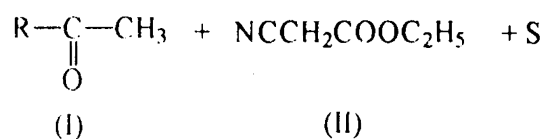
SCHEME V

Synthesis of 2-[(substituted benzyl)-thio]-3-phenyl-5-(3-pyridinyl / 4-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones

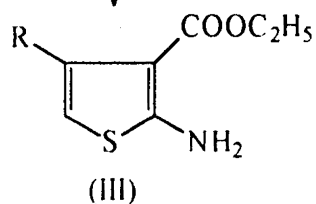


SCHEME VI

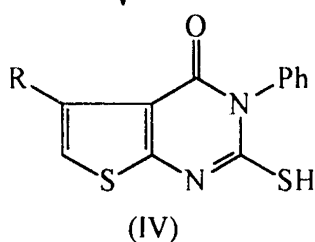
Synthesis of 2-[(substituted aryl / heteroaryl)-sulfonamido]-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones



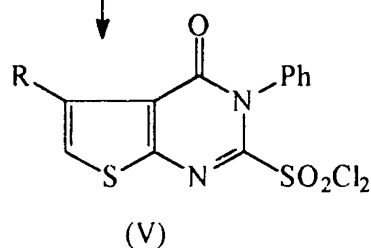
↓
Morpholine
Dry alcohol



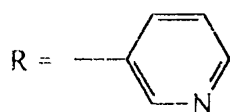
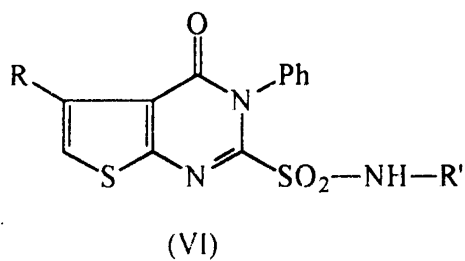
↓
PhNCS
K₂CO₃, Acetonitrile



↓
Cl₂, CH₃COOH, HCl, H₂O



↓
R'NH₂
Dry acetone, (C₂H₅)₃N



R' = substituted aryl/heteroaryl

Part II

Experimental

Part II-A.i

***Synthesis of 2-methyl-3N-
[(substituted aryl / hetero
aryl)-methylene]-amino-5-(2-
furyl)-thieno [2,3-d]
pyrimidin-4 (3H)-ones***

This part of the thesis deals with the synthesis of 2-methyl-3N-[(substituted aryl / heteroaryl)-methylene]-amino-5-(2-furyl)-thieno [2,3-d] pyrimidin-4(3H)-ones which involves the following steps -

- 1) Synthesis of 2-butenic acid-2-cyano-3-(2-furyl)-ethyl ester
- 2) Synthesis of 2-amino-3-carbethoxy-4-(2-furyl) thiophene
- 3) Synthesis of 2-acetamido-3-carbethoxy-4-(2-furyl)- thiophene
- 4) Synthesis of 2-methyl-3-amino-5-(2-furyl)-thieno [2,3-d] pyrimidin-4 (3H)-one
- 5) Synthesis of the title compounds by condensation of 2-methyl-3-amino-5-(2-furyl)- thieno [2,3-d] pyrimidin-4(3H)-ones with various substituted aldehydes

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1) Synthesis of 2-butenic acid- 2-cyano- 3-(2- furyl)- ethyl ester

Ethyl cyanoacetate (10.66 mL, 0.1 M), 2-acetyl furan (10.09 mL, 0.1 M), anhydrous ammonium acetate (6.16 g, 0.08 M), glacial acetic acid (4.61 mL, 0.08 M), and dry benzene (150 mL) were placed in a 250 mL round bottom flask attached to a modified Dean - Stark constant water-separator and double walled water condenser. The reaction mixture was boiled under reflux for 7 to 9 hours with constant stirring. Refluxing continued for one hour after which water ceased to separate. On cooling to room temperature, it was diluted with benzene (150 mL) and washed with water (3 x 100 mL) and dried over sodium sulfate. The solvent was removed in *vacuo*. The solid obtained was recrystallized from methanol to give a yellow coloured crystalline product.

Yield = 80 %

M.P = 75-76°C

Elemental analysis of $C_{11}H_{11}NO_3$

	C%	H%	N%
Calculated	64.39	5.36	6.82
Found	64.70	5.27	6.93

2) Synthesis of 2-amino-3-carbethoxy-4-(2-furyl)- thiophene

In a 100 mL round bottomed flask equipped with water condenser was placed a suspension of 2-butenic acid -2-cyano-3-(2-furyl)-ethyl ester (6 g, 0.029 M) in absolute ethanol (15 mL), diethylamine (1.35 mL, 0.013 M) and sulphur (0.92 g, 0.029 M). The mixture was warmed on a steam bath for 30 minutes when most of the reactants dissolved. The solution was further warmed on the steam bath below 60°C for 2 hours. On cooling to 10 °C, a red coloured crystalline product was formed. This was filtered and recrystallized from methanol.

Yield = 4.85 g (70 %)

M.P. = 86-87°C

Elemental analysis of $C_{11}H_{11}NO_3S$

	C%	H%	N%
Calculated	55.69	4.64	5.90
Found	55.41	4.51	5.66

3) Synthesis of 2-acetamido-3-carbethoxy-4-(2-furyl)- thiophene

In a 100 mL round bottom flask was placed a mixture of 2-amino-3-carbethoxy- 4- (2-furyl)- thiophene (10 g, 0.04 M), acetic anhydride (12.14 mL, 0.12 M) and zinc dust (2.85g) This mixture was stirred and warmed on a water bath at 70-75°C, when the solid dissolved after 10 minutes. The reaction mixture was then cooled and the resulting solid was filtered, washed with water and crystallized from methanol.

Yield = 9.41 g (80 %)

MP =103-104°C

Elemental analysis of $C_{13}H_{13}NO_3S$

	C%	H%	N%
Calculated	59.31	4.94	5.32
Found	59.45	4.87	5.50

4) Synthesis of 2- methyl- 3- amino-5-(2-furyl)-thieno [2,3-d] pyrimidin - 4 (3H)- ones

In a 100 mL round bottom flask equipped with water condenser was placed a solution of 2-acetamido-3-carbethoxy-4-(2-furyl)-thiophene (4 gm 0.014 M) in alcohol (20 mL).To it hydrazine hydrate (14 mL) was added. The reaction mixture was heated under reflux for 1 hour. After cooling, the isolated product was recrystallized from acetone to obtain a white crystalline product.

Yield = 2 g (57%)

M.P.= 174-175°C

Elemental analysis of $C_{11}H_9N_3O_2S$

	C%	H%	N%
Calculated	53.44	3.64	17.00
Found	53.28	3.78	17.08

5) General method for the synthesis of 2- methyl-3N-[(substituted aryl/ heteroaryl)-methylene]-amino-5-(2-furyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones

Synthesis of 2-methyl-3N-[4-fluorophenyl]-methylene]-amino-5-(2-furyl)-thieno [2,3-d] pyrimidin-4 (3H)-one

In a 25 mL round bottomed flask equipped with water condenser was placed a mixture of 2-methyl-3-amino-5-(2-furyl)-thieno [2,3-d] pyrimidin-4 (3H)-one (0.5 g, 0.002 M) and 4 - fluorobenzaldehyde (0.24g, 0.002 M) in 5 mL of acetic acid. It was refluxed on oil bath for 4 hours. The reaction mixture was then allowed to cool to room temperature. The solid separated was filtered and washed with water. It was then recrystallized from dichloromethane.

Yield = 0.45 g (63 %)

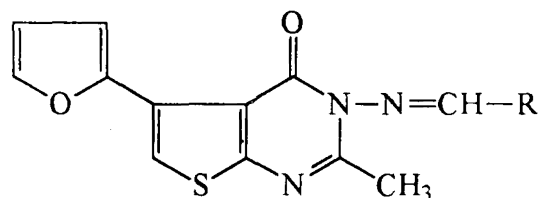
M.P. = 234-36°C

Elemental analysis of $C_{18}H_{12}FN_3O_2S$

	C%	H%	N%
Calculated	61.18	3.39	11.89
Found	61.00	3.49	12.19

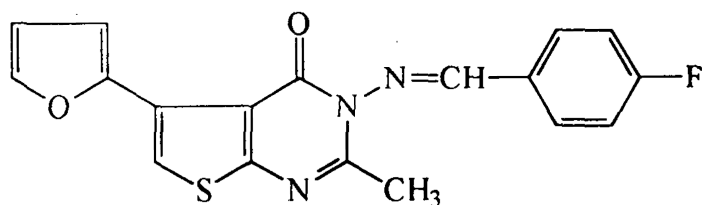
In a similar manner, all the other 2 - methyl - 3N - [(substituted aryl / heteroaryl) methylene] - amino - 5 - (2-furyl) - thieno [2,3-d] pyrimidin - 4 (3H) -ones have been synthesized and their physical data has been recorded in **Table 1**

Table 1 : 2-methyl - 3N - [(substituted aryl / heteroaryl) - methylene]-amino-5-(2- furyl-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	C ₆ H ₅	C ₁₈ H ₁₃ N ₃ O ₂ S	335	160-61	60	64.47	3.86	12.53	64.70	3.97	12.49
2	4-Cl-C ₆ H ₄	C ₁₈ H ₁₂ ClN ₃ O ₂ S	369	218-19	75	58.53	3.25	11.38	58.36	3.40	11.57
3	4-F-C ₆ H ₄	C ₁₈ H ₁₂ FN ₃ O ₂ S	353	234-35	63	61.18	3.39	11.89	61.00	3.49	12.19
4	4-NO ₂ -C ₆ H ₄	C ₁₈ H ₁₂ N ₄ O ₄ S	380	225-26	65	56.84	3.15	14.73	57.20	2.91	15.00
5	4-CF ₃ -C ₆ H ₄	C ₁₉ H ₁₂ F ₃ N ₃ O ₂ S	403	150-53	50	56.57	2.97	10.42	56.68	3.02	10.67
6	2-CF ₃ -C ₆ H ₄	C ₁₉ H ₁₂ F ₃ N ₃ O ₂ S	403	113-15	58	56.57	2.97	10.42	56.63	3.10	10.62
7	2-F, 4-CF ₃ -C ₆ H ₃	C ₁₉ H ₁₁ F ₄ N ₃ O ₂ S	421	155-56	58	54.15	2.61	9.97	54.18	2.87	10.10
8	4-OH-C ₆ H ₄	C ₁₈ H ₁₃ N ₃ O ₂ S	351	248-50	42	61.50	3.70	11.96	61.80	4.00	12.20
9	3-Pyridine	C ₁₇ H ₁₂ N ₄ O ₂ S	336	199-200	41	60.71	3.57	16.66	60.91	3.79	16.51
10	N-Methyl-pyrrole	C ₁₇ H ₁₄ N ₄ O ₂ S	338	209-10	73	60.53	4.14	18.10	60.79	4.05	17.96
11	2-Thienyl	C ₁₆ H ₁₁ N ₃ O ₂ S ₂	341	148-50	57	56.30	3.20	12.31	55.96	3.65	12.60
12	5-Bromo-thienyl	C ₁₆ H ₁₀ BrN ₃ O ₂ S ₂	419	250-51	70	45.82	2.38	10.02	45.88	2.48	9.98

Spectral data of 2- methyl- 3N-[(4-fluoro phenyl)-methylene]-amino-5-(2-furyl)-thieno [2,3-d] pyrimidin- 4 (3H) - one (Compound No. 3, Table 1) has been given below



IR (KBr) cm^{-1} : 1686 (-CO-); 1603 (C=N), 3009, 1383 (-CH₃) ; 1480, 1239, 1139, 1002 (Furyl); 3122, 1156, 1097, 1588, 1449 (Aromatic); 770, 748, 730 (C-H Aromatic) ; 1021 (C-Halo)

PMR (CDCl_3) δ : 2.61 (s, 3H, CH₃); 6.46 (s, 1H, Furyl); 7.20 – 7.9 [m, 8H (4H, Aromatic ; 1H, Thienyl ; 1H, N=CH; 2H, Furyl)]



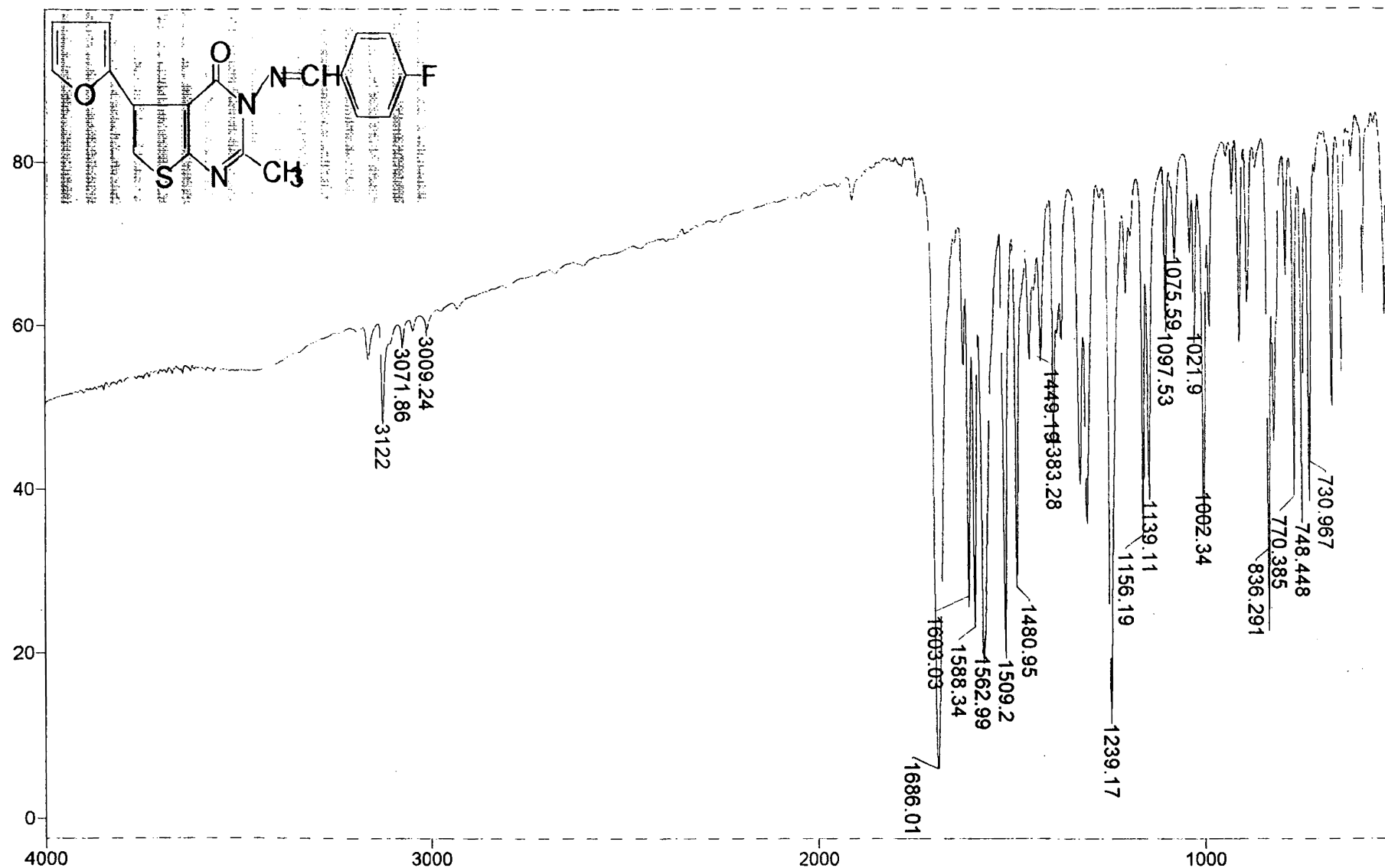
Sample Description: 4nitrobenzylidene-furylthienopyrimidineone

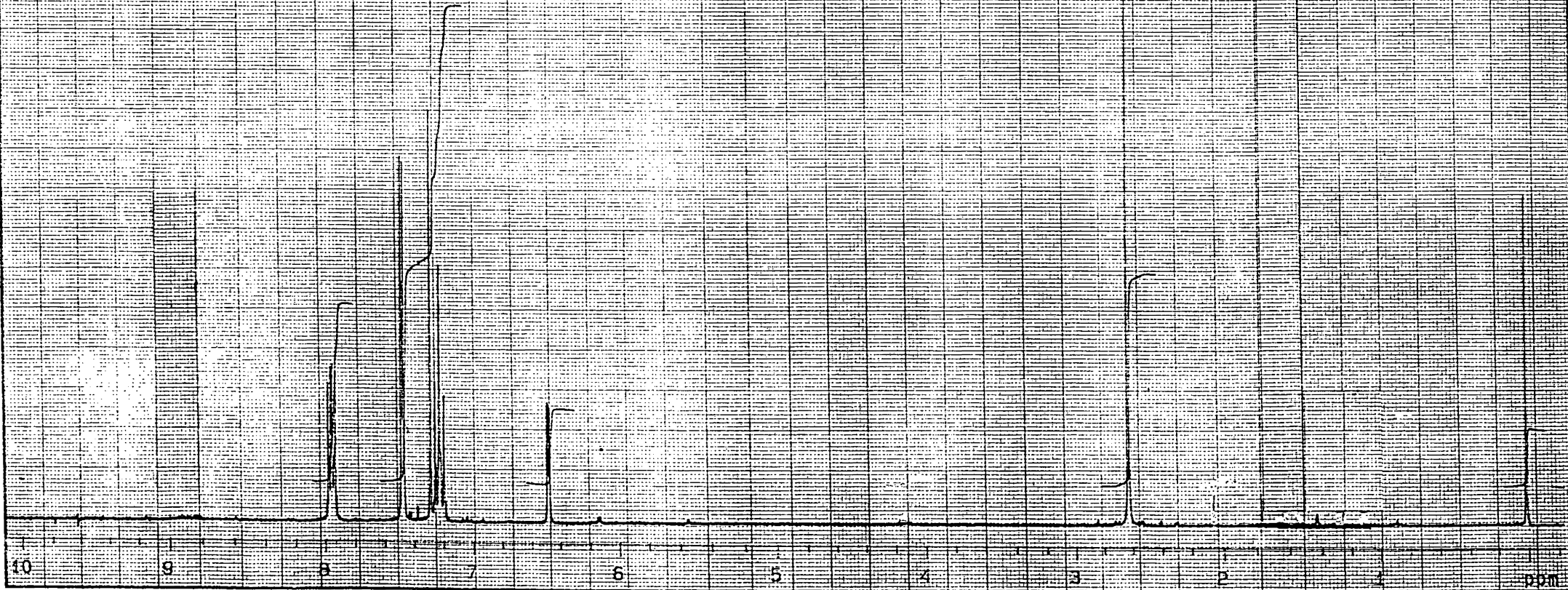
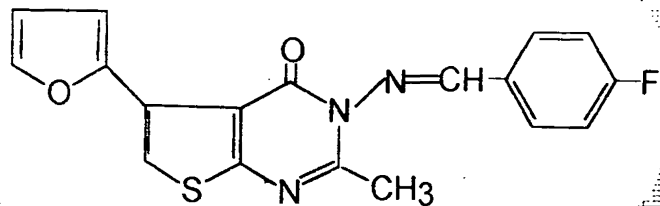
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





Part II-A.ii

***Synthesis of 2-methyl-3N-
[(substituted aryl/heteroaryl)-
methylene]-amino-5-(2-thienyl)-
thieno [2,3-d] pyrimidin-4(3H)-
ones***

This part of the thesis deals with the synthesis of 2-methyl-3N-[(substituted aryl/heteroaryl)-methylene]-amino-5-(2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-ones which involves the following steps

- 1) Synthesis of 2-butenic acid-2-cyano-3-(2-thienyl)-ethyl ester
- 2) Synthesis of 2-amino-3-carbethoxy-4-(2-thienyl) thiophene
- 3) Synthesis of 2-acetamido-3-carbethoxy-4-(2-thienyl)-thiophene
- 4) Synthesis of 2-methyl-3-amino-5-(2-thienyl)-thieno[2,3-d] pyrimidin- 4 (3H) -one
- 5) Synthesis of the title compounds by condensation of 2-methyl-3-amino-5-(2-thienyl)- thieno[2,3-d]pyrimidin- 4(3H) - ones with various substituted aldehydes

1) Synthesis of 2-butenic acid-2-cyano- 3-(2-thienyl)- ethyl ester

In a three necked 250 mL round bottomed flask equipped with a modified Dean - Stark constant water separator and double walled water condenser was placed ethyl cyanoacetate (10.66 mL, 0.1 M), 2 - acetyl thiophene (10.86 mL, 0.1 M), anhydrous ammonium acetate (6.16 g, 0.08m), glacial acetic acid (4.61 mL, 0.08 M), and dry benzene (150 mL). The reaction mixture was boiled under reflux for 15 hours with constant stirring. The refluxing was continued for one hour, after which water ceased to separate. On cooling to room temperature, it was diluted with benzene (150 mL) and washed with water (3 x 100 mL) and dried over anhydrous sodium sulphate. The solvent was removed *in vacuo*. A dark yellow coloured liquid was obtained, which was distilled under reduced pressure.

Yield = 67 %

B.P_{20mm} = 210 °C

Elemental analysis of C₁₁H₁₁NO₂S

	C%	H%	N%
Calculated	59.72	4.97	6.33
Found	59.40	4.86	6.66

2) Synthesis of 2-amino-3-carbethoxy-4-(2-thienyl)-thiophene

In a 100 mL round bottomed flask equipped with water condenser was placed a suspension of 2-butenic acid-2-cyano-3-(2-thienyl)- ethyl ester (6.43 g, 0.029 M) in absolute ethanol(15 mL), diethylamine (1.35 mL 0.013 M) and sulphur (0.92 g, 0.029 M). Mixture was warmed on a steam bath for 30 minutes when most of the reactants dissolved. The solution was further warmed on a steam bath below 60°C for 2 hours. On cooling to 10°C for 4 to 5 hours, a crystalline product was formed. This was filtered and recrystallized from methanol.

Yield = 6.40 g (87 %)

M.P = 83-84 °C

Elemental analysis of C₁₁H₁₁NO₂S

	C%	H%	N%
Calculated	52.17	4.34	5.53
Found	51.89	4.10	5.42

3) Synthesis of 2- acetamido - 3- carbethoxy – 4-(2-thienyl)-thiophene

In a 100 mL round bottomed flask was placed a mixture of 2-amino-3-carbethoxy-4(2-thienyl)-thiophene (10.12 g, 0.04 M), acetic anhydride (12.41 mL, 0.12 M), and Zinc dust (2.85 g). This mixture was stirred and warmed on a water bath at 70-75°C, when the solid dissolved after 10 minutes. The reaction mixture was then cooled and the resulting solid was filtered, washed with water and recrystallized from methanol.

Yield = 8.26 (70 %)

M.P. = 96-97 °C

Elemental analysis of $C_{13}H_{13}NO_3S_2$

	C%	H%	N%
Calculated	52.88	4.40	4.74
Found	52.76	4.35	4.80

4) Synthesis of 2-methyl-3-amino-5-(2-thienyl)-thieno[2,3-d] pyrimidin-4(3H) one

In a 100 mL round bottomed flask equipped with water condenser was placed a solution of 2-acetamido-3-carbethoxy –4-(2-thienyl)- thiophene (4.13 g, 0.014 M) in alcohol (20 mL). To it hydrazine hydrate (14 mL) was added. The reaction mixture was heated under reflux for 1 hour. After cooling, the isolated product was recrystallized from acetone to obtain a white crystalline product.

Yield = 1.47 g (40%)

M.P. = 142-43°C

Elemental analysis of $C_{11}H_9N_3OS_2$

	C%	H%	N%
Calculated	50.19	3.42	15.96
Found	49.95	3.34	15.91

5) General method for the synthesis of 2-methyl-3N-[(substituted aryl / heteroaryl)-methylene]-amino-5-(2-thienyl)-thieno[2,3-d] pyrimidin-4 (3H)-ones

Synthesis of 2-methyl-3N-[(4-nitrophenyl)-methylene]-amino- 5-(2-thienyl)- thieno[2,3-d] pyrimidin-4 (3H)-one

In a 25 mL round bottomed flask equipped with water condenser was placed a mixture of 2-methyl-3-amino-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-one (0.5g, 0.0019 M) and 4 -nitrobenzaldehyde(0.28 g, 0.0019m) in 5 mL of acetic acid. It was refluxed on oil bath for 4 hours. The reaction mixture was then allowed to cool to room temperature. The solid separated was filtered and washed with water. It was then recrystallized from ethyl acetate.

Yield = 0.58 g (78 %)

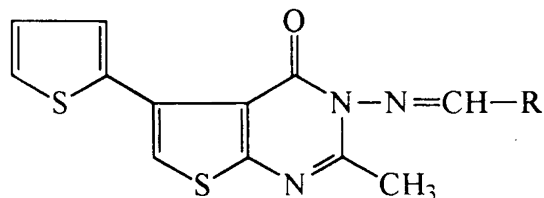
M.P. = 198-99°C

Elemental analysis of $C_{18}H_{12}N_4O_3S_2$

	C%	H%	N%
Calculated	54.50	3.39	14.10
Found	54.74	3.39	14.10

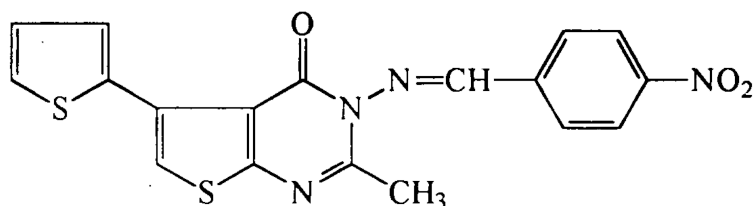
In a similar manner, all the other 2-methyl-3N-[(substituted aryl / heteroaryl)-methylene]-amino-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones have been synthesized and their physical data has been recorded in **Table 2**

Table 2 : 2-methyl - 3N - [(substituted aryl / heteroaryl) - methylene] - amino - 5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	4-Cl-C ₆ H ₄	C ₁₈ H ₁₂ ClN ₃ OS ₂	385	184-85	67	56.10	3.10	10.90	56.40	2.96	11.20
2	4-NO ₂ -C ₆ H ₄	C ₁₈ H ₁₂ N ₄ O ₃ S ₂	396	198-99	78	54.50	3.39	14.10	54.74	3.39	14.10
3	4-F-C ₆ H ₄	C ₁₈ H ₁₂ FN ₃ OS ₂	369	179-80	73	58.88	3.25	11.38	58.96	3.16	11.63
4	4-CF ₃ -C ₆ H ₄	C ₁₉ H ₁₂ F ₃ N ₃ OS ₂	419	173-74	63	54.41	2.86	10.02	54.68	3.10	10.20
5	4-OH-C ₆ H ₄	C ₁₈ H ₁₃ N ₃ O ₂ S ₂	367	240-43	82	58.85	3.54	11.44	59.96	3.22	11.64
6	2-F, 4-CF ₃ -C ₆ H ₃	C ₁₉ H ₁₁ N ₃ OS ₂	437	159-60	57	52.17	2.51	9.61	51.98	2.74	9.90
7	2-OH, 5-Br-C ₆ H ₃	C ₁₈ H ₁₂ BrN ₃ O ₂ S ₂	445	250-53	65	48.53	2.64	9.43	48.82	2.78	9.60
8	3-Pyridine	C ₁₇ H ₁₂ N ₄ OS ₂	352	190-92	52	57.95	3.40	15.90	58.21	3.55	16.20
9	2-Thienyl	C ₁₆ H ₁₁ N ₃ OS ₃	357	200-01	69	53.70	3.08	11.76	53.70	3.08	11.76
10	N-Methyl-pyrrole	C ₁₇ H ₁₄ N ₄ OS ₂	354	194-95	72	57.62	3.95	15.81	57.79	3.88	16.10

Spectral data of 2-methyl-3N-[(4-nitro phenyl) methylene]-amino- 5-(2-thienyl)- thieno [2,3-d] pyrimidin-4 (3H)-one (Compound No. 2, Table 2) is given below..



IR (KBr) cm^{-1} : 1678 (-CO-); 1591 (C=N), 3007, 1367 (-CH₃) ; 1491, 1404, 1367, 1034, 824 (Thienyl); 3104, 1168, 1137, 1445, 1457 (Aromatic); 769, 748 (C-H Aromatic) ; 1571, 1288 (Nitro)

PMR (CDCl₃) δ : 2.67 (s, 3H, CH₃); 7 – 8.35 [m, 9H (4H, Aromatic ; 1H, N=CH; 4H, Thienyl)]

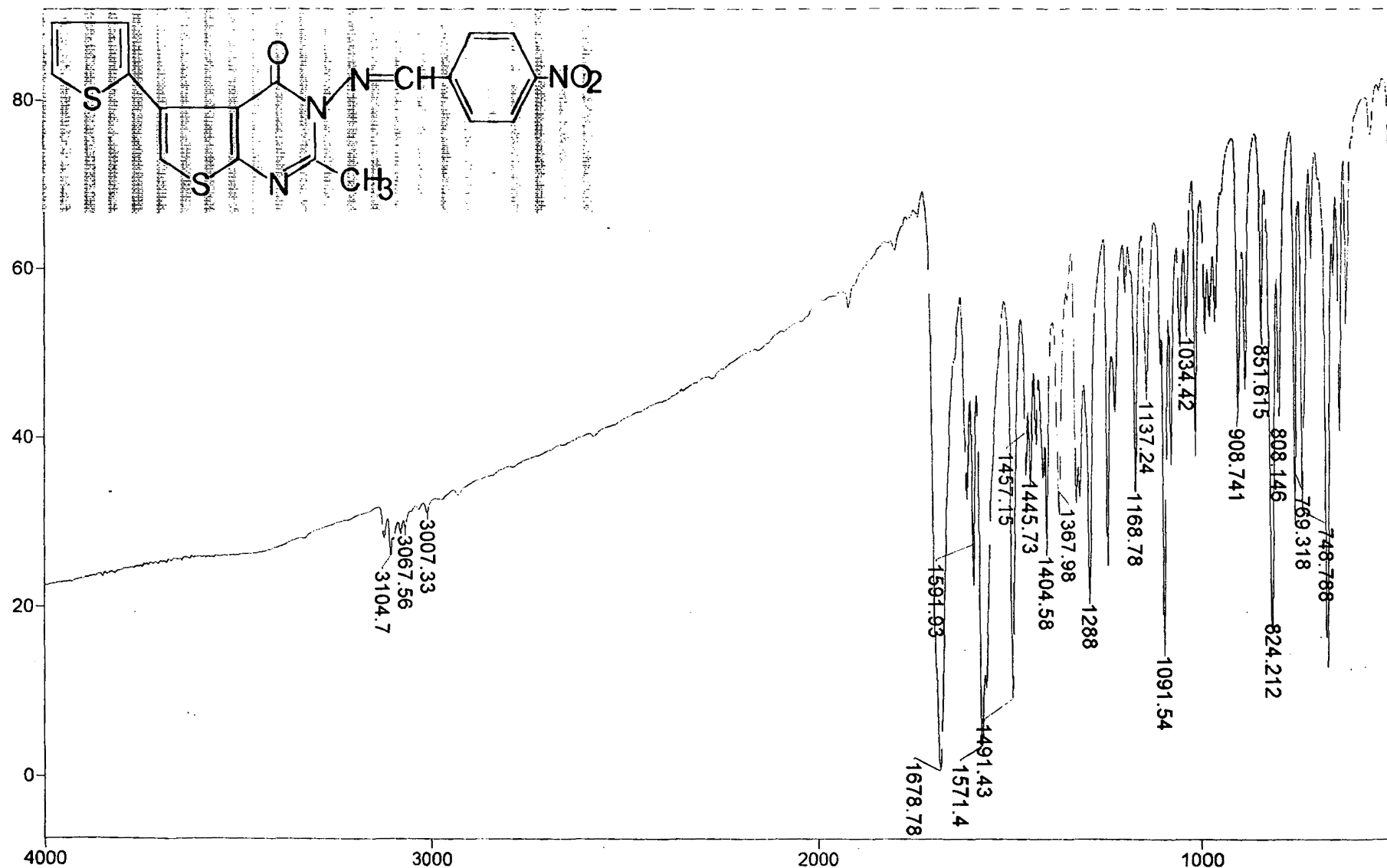
Sample Description: chlorobenzylideneethienylthienopyrimidineone

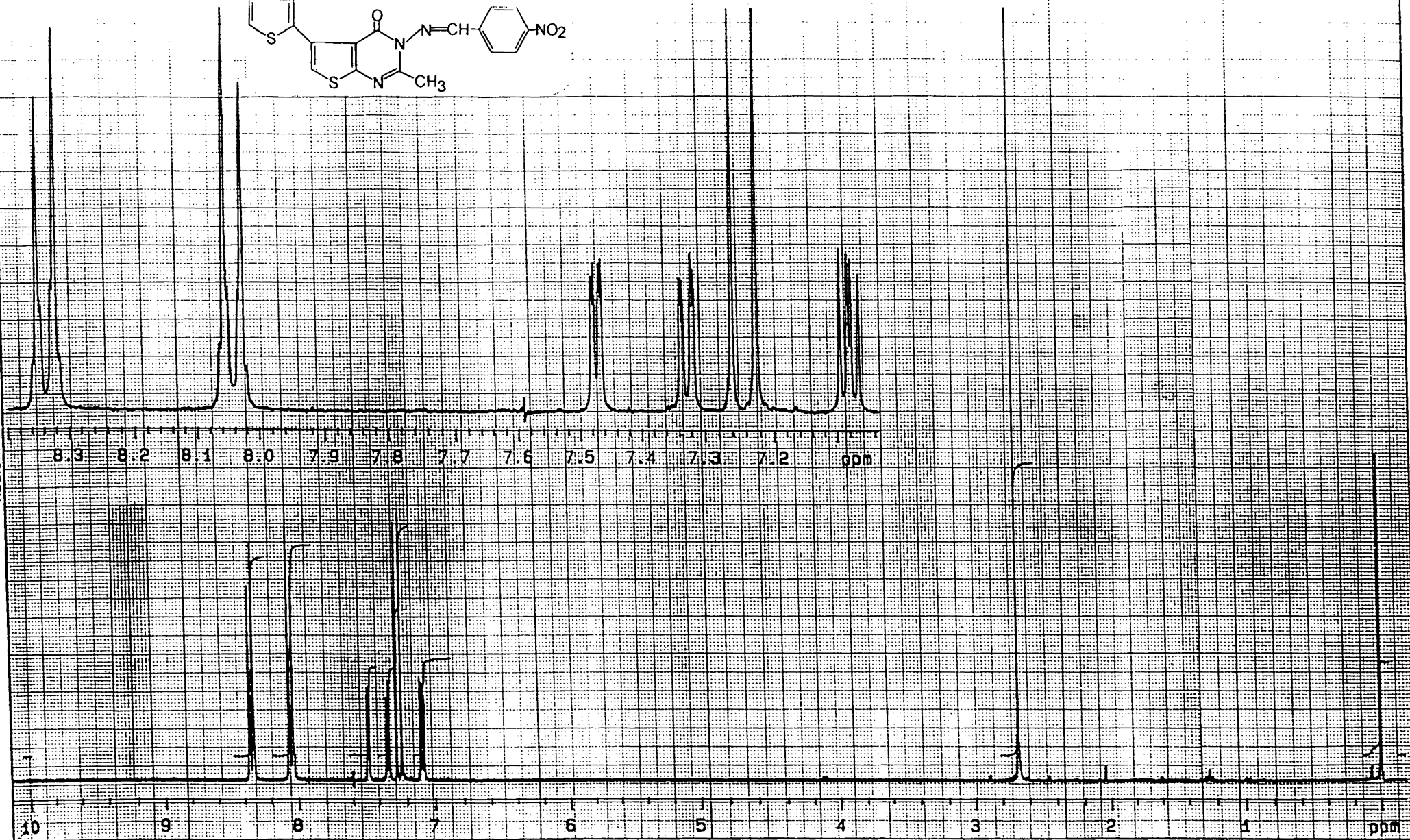
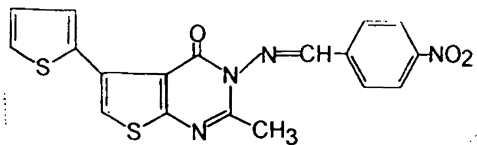
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





Part II-B.i

***Synthesis of 2-methyl-3N-
[(substituted aryl/heteroaryl)-
carboxamido]-5-(2-furyl)-thieno
[2,3-d] pyrimidin-4(3H)-ones***

This part of the thesis deals with the synthesis of 2-methyl-3N-[(substituted aryl / heteroaryl)-carboxamido]-5-(2-furyl)-thieno [2,3-d] pyrimidin- 4 (3H) - ones which involves the following steps

- 1) Synthesis of 2-butenic acid - 2-cyano- 3-(2- furyl)-ethyl ester*
- 2) Synthesis of 2-amino - 3- carbethoxy- 4-(2-furyl)- thiophene*
- 3) Synthesis of 2-acetamido- 3-carbethoxy – 4-(2-furyl)-thiophene*
- 4) Synthesis of 2-methyl- 3-amino-5-(2-furyl)- thieno [2,3 -d] pyrimidin-4 (3H)-one*
- 5) Synthesis of the title compounds by condensation of 2-methyl-3-amino-5-(2-furyl)-thieno [2,3-d] pyrimidin-4(3H)-ones with various substituted benzoyl / heteroaryl acid chlorides.

* Synthesis has been described in **Part II-A.i**

5) General method for the synthesis of 2- methyl- 3N-[(substituted aryl / heteraryl) - carboxamido]-5-(2-furyl) – thieno [2,3-d] pyrimidin-4 (3H)-ones

Synthesis of 2-methyl-3N-[(4-chlorophenyl)-carboxamido]-5-(2-furyl)-thieno [2,3-d] pyrimidin- 4 (3 H)-one

In a three necked round bottomed flask equipped with a dropping funnel and reflux condenser with guard tube was placed a mixture of triethylamine (0.2g, 0.002 M), 2-methyl- 3 – amino-5-(2-furyl)-thieno [2,3-d] pyrimidin-4 (3 H)-one (0.5 g, 0.002 M) and 10 mL dry 1,4-dioxane. The reaction mixture was stirred on a magnetic stirrer at 70 - 80 °C for 30 min. To this was added dropwise a solution of 4 - chlorobenzoyl chloride (0.35 g, 0.002 M) in 10 mL dry 1,4 - dioxane at the same temperature. After the addition, the reaction mass was stirred for 4 hours at 100-110°C. It was then poured on crushed ice. The solid separated out was filtered and washed with 1 % potassium bicarbonate and water. It was then recrystallized from methanol.

Yield = 0.64 g (83 %)

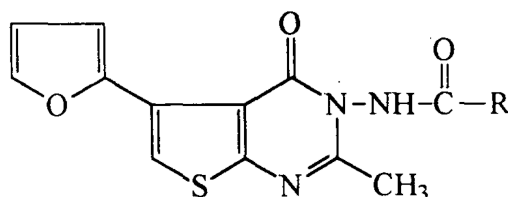
M.P. = 264-66°C

Elemental analysis of $C_{18}H_{12}ClN_3O_3S$

	C%	H%	N%
Calculated	56.10	3.11	10.07
Found	56.29	3.21	10.18

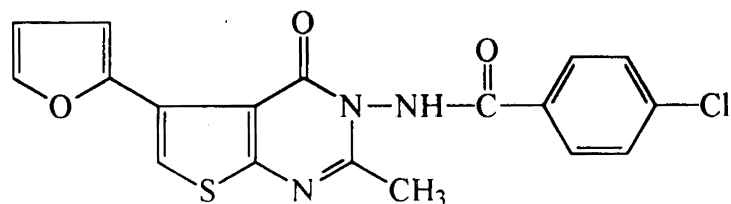
In a similar manner all the other 2-methyl-3N-[(substituted aryl / heteroaryl carboxamido] -5-(2-furyl)-thieno [2,3-d] pyrimidin 4-(3H)-ones have been synthesized and their physical data has been recorded in **Table 3**.

Table 3: 2-methyl - 3N - [(substituted aryl / heteroaryl) - carboxamido] - 5-(2-furyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	4-Cl-C ₆ H ₄	C ₁₈ H ₁₂ ClN ₃ O ₃ S	385	264-66	83	56.10	3.11	10.07	56.29	3.21	10.18
2	2-F, 6-F-C ₆ H ₃	C ₁₈ H ₁₁ F ₂ N ₃ O ₃ S	387	190-92	62	55.81	2.84	10.85	55.86	3.00	11.00
3	3,4 di-Cl-C ₆ H ₃	C ₁₈ H ₁₂ Cl ₂ N ₃ O ₃ S	419	230-33	72	51.42	2.61	10.00	51.26	2.78	9.72
4	2-F-C ₆ H ₄	C ₁₈ H ₁₂ FN ₃ O ₃ S	369	229-30	96	58.53	3.25	11.38	58.63	3.34	11.28
5	4-OCH ₃ -C ₆ H ₄	C ₁₉ H ₁₅ N ₃ O ₄ S	381	235-36	65	58.53	4.0	11.38	58.74	3.88	11.46
6	4-F, C ₆ H ₄	C ₁₈ H ₁₂ FN ₃ O ₃ S	369	223-24	47	58.53	3.25	11.38	58.63	3.57	11.14
7	3-F, C ₆ H ₄	C ₁₈ H ₁₂ FN ₃ O ₃ S	369	167-70	70	58.53	3.25	11.38	58.73	3.35	11.15
8	Cyclohexane	C ₁₈ H ₁₈ N ₃ O ₃ S	357	269-70	65	60.67	5.05	11.79	60.54	5.13	11.65
9	Cyclopropane	C ₁₅ H ₁₃ N ₃ O ₃ S	315	238-40	36	57.14	4.12	13.33	57.36	4.27	13.04
10	2-Furyl	C ₁₆ H ₁₁ N ₃ O ₄ S	341	210-11	63	56.30	3.22	12.31	56.47	3.59	12.28
11	2-Thienyl	C ₁₆ H ₁₁ N ₃ O ₃ S ₂	357	269-70	58	53.78	3.08	11.76	54.02	3.23	12.00

Spectral data of 2- methyl - 3N- [(4-chloro phenyl)-carboxamido] – 5-(2-furyl)-thieno [2,3-d] pyrimidin - 4 (3H)-one (Compound No. 1, Table 3) has been given below



IR (KBr) cm^{-1} : 3284 (-NH-); 1698 (-CO-); 1574 (C=N), 1670 (-NH-CO-); 1296 (C-N) ; 2970, 1382 (-CH₃) ; 1479, 1227, 1012 (Furyl); 3112, 1150, 1093, 1434 (Aromatic); 778, 755 (C-H Aromatic) ; 818, 736 (C-Halo)

PMR (CDCl_3) δ : 2.52 (s, 3H, CH₃); 6.44 (s, 1H, furyl); 7.2 – 7.9 [m, 7H (4H, Aromatic ; 1H, Thienyl, 2H, Furyl)] ; 9.1 (s , 1H, -NH)

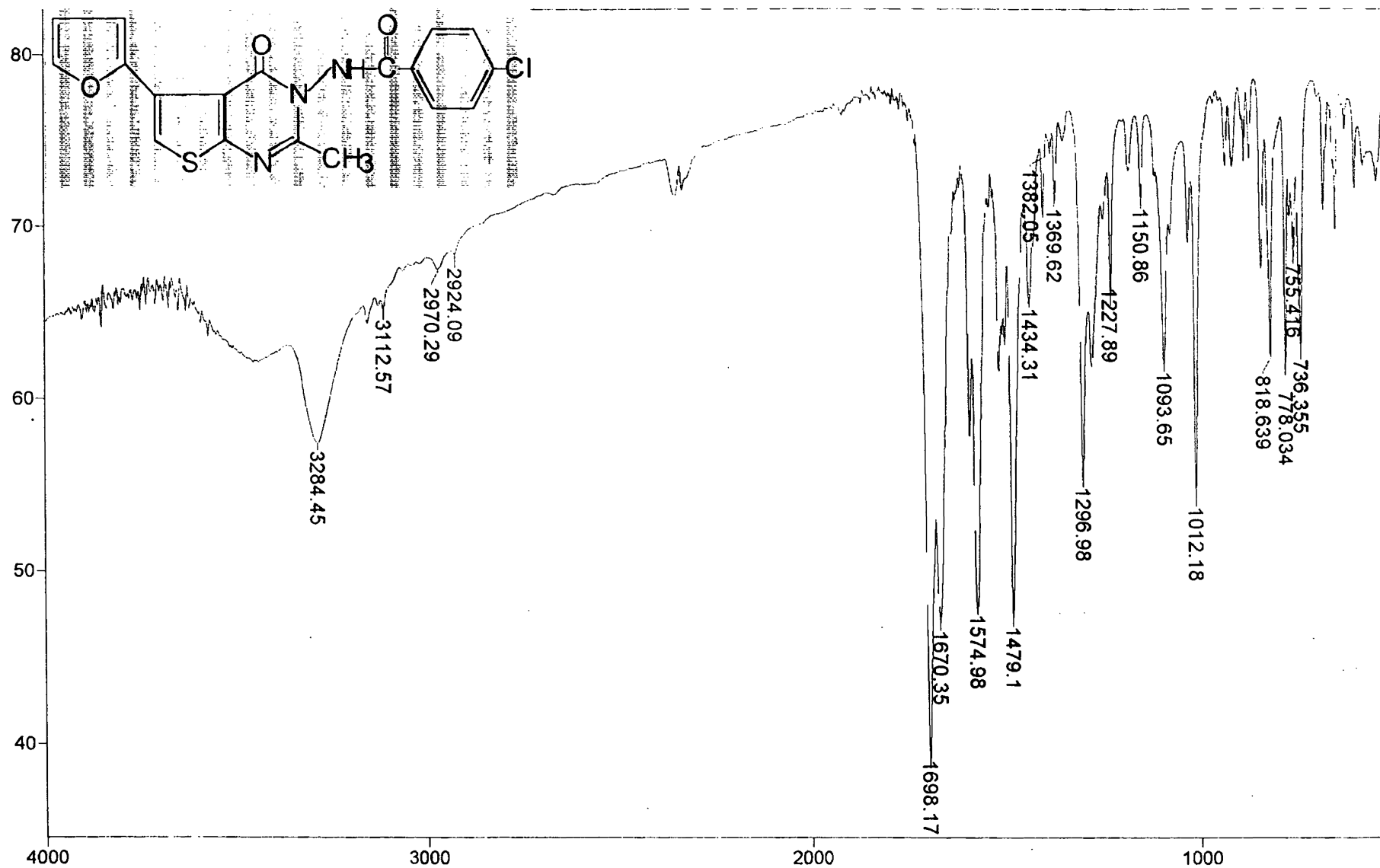
Sample Description: methylamidofurylthienopyrimidineone

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Part II-B.ii

***Synthesis of 2-methyl-3N-
[(substituted aryl/heteroaryl)-
carboxamido]-5-(2-thienyl)-thieno
[2,3-d] pyrimidin-4(3H)-ones***

This part of the thesis deals with the synthesis of 2-methyl-3N-[(substituted aryl / hetero aryl)-carboxamido]-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones which involves the following steps

- 1) Synthesis of 2-butenic acid-2 cyano-3-(2-thienyl)- ethyl ester.*
- 2) Synthesis of 2-amino – 3-carbethoxy-4-(2-thienyl)- thiophene.*
- 3) Synthesis of 2-acetamido-3-carbethoxy-4-(2-thienyl)- thiophene.*
- 4) Synthesis of 2-methyl-3-amino-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3 H)-one.*
- 5) Synthesis of the title compounds by condensation of 2-methyl-3-amino-5-(2-thienyl)- thieno [2,3-d] pyrimidin-4(3H)-ones with various substituted benzoyl/ heteroaryl acid chlorides.

* Synthesis has been described in **Part I – A.ii**

5) General method for the synthesis of 2- methyl-3N-[(substituted aryl / heteroaryl)-carboxamido] - 5- (2-thienyl) - thieno [2,3 -d] pyrimidin-4(3H)-ones

Synthesis of 2 methyl - 3N-[(cyclohexane)-carboxamido] -5-(2-thienyl)-thieno [2,3-d] pyrimidin - 4 (3H)-one

In a three necked round bottomed flask equipped with a dropping funnel and reflux condenser with guard tube was placed a mixture of triethyl amine (0.2 g, 0.002 M), 2-methyl-3-amino-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-one (0.5 g, 0.002 M) and 10 mL of dry 1,4- dioxane. The reaction mixture was stirred on a magnetic stirrer at 70-80°C for 30 min. To this was added dropwise a solution of 4-cyclohexyl carbonyl chloride (0.24 g, 0.002 M) in 10 mL dry 1,4 dioxane at the same temperature. After the addition the reaction mass was stirred for 4 hours at 100-110°C. It was then poured on crushed ice. The solid separated out was filtered and washed with 1 % potassium bicarbonate and water. It was then recrystallized from methanol.

Yield = 0.21 g (50 %)

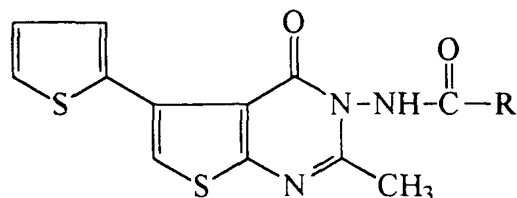
M.P = 218-20°C

Elemental analysis of $C_{18}H_{18}ClN_3O_2S_2$

	C%	H%	N%
Calculated	58.06	4.83	11.29
Found	58.21	4.86	11.48

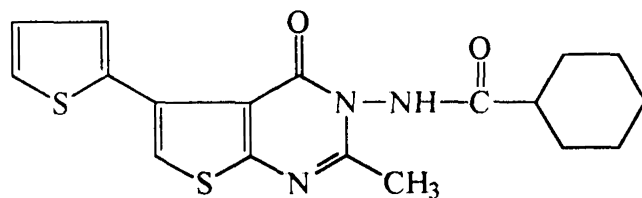
In a similar manner, all the other 2-methyl-3N-[(substituted aryl / hetero aryl) carboxamido]- 5-(2-thienyl)- thieno [2,3-d] pyrimidin – 4 (3H) -ones have been synthesized and their physical data has been recorded in **Table 4**

Table 4 : 2-methyl - 3N - [(substituted aryl / heteroaryl) - carboxamido] - 5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	4-Cl-C ₆ H ₅	C ₁₈ H ₁₂ ClN ₃ O ₂ S ₂	401	240-42	80	53.86	2.99	10.47	54.00	3.09	10.60
2	3-Cl, 4-Cl-C ₆ H ₄	C ₁₈ H ₁₁ Cl ₂ N ₃ O ₂ S ₂	435	256-58	66	49.65	2.52	9.65	49.63	2.47	9.75
3	4-F-C ₆ H ₄	C ₁₈ H ₁₂ FN ₃ O ₂ S ₂	385	190-93	51	56.26	3.12	11.93	55.98	3.42	12.10
4	2-F, 6-F-C ₆ H ₃	C ₁₈ H ₁₂ FN ₃ O ₂ S ₂	402	207-09	51	53.73	2.48	10.44	54.00	2.66	10.60
5	4-OH ₃ -C ₆ H ₄	C ₁₈ H ₁₁ F ₂ N ₃ O ₂ S ₂	397	224-26	52	57.43	3.77	13.57	57.64	3.65	13.75
6	2-F-C ₆ H ₄	C ₁₈ H ₁₂ FN ₃ O ₂ S ₂	385	180-83	49	56.25	3.12	10.93	56.45	3.26	10.87
7	Cyclohexane	C ₁₈ H ₁₈ N ₃ O ₂ S ₂	372	218-20	50	58.06	4.83	11.29	58.21	4.86	11.48
8	Cyclopropane	C ₁₅ H ₁₃ N ₃ O ₂ S ₂	331	157-59	45	54.38	3.92	12.68	54.41	4.03	12.73
9	4-Morphine	C ₁₆ H ₁₆ N ₄ O ₃ S ₂	376	227-28	35	51.06	4.2	14.89	51.02	4.31	14.89
10	2-Furyl	C ₁₆ H ₁₁ N ₃ O ₃ S ₂	357	204-06	65	57.78	3.0	11.76	58.00	3.11	11.45
11	2-Thienyl	C ₁₆ H ₁₁ N ₃ O ₂ S ₃	373	229-31	73	51.47	2.94	11.26	51.88	2.94	11.45

Spectral data of 2- methyl - 3N-[(cyclohexane) carboxamido]-5-(2-thienyl)-thieno [2,3-d] pyrimidin - 4 (3H) one (Compound no. 7, Table 4) has been given below.



IR (KBr) cm^{-1} : 3266 (-NH-); 1690 (-CO-); 1574 (C=N), 1670 (-NH-CO-); 1288 (C-N) ; 3097, 1375 (-CH₃) ; 1500, 1443, 1100, 1042, 833 (Thienyl); 2940, 2885 (Cyclohexane)

PMR (CDCl_3) δ : 1.2 – 2 (m, 11H, Cyclohexane); 2.5 (s, 3H, CH₃); 7 – 7.5 (m, 4H, Thienyl) ; 8.4 (s, 1H, -NH-)

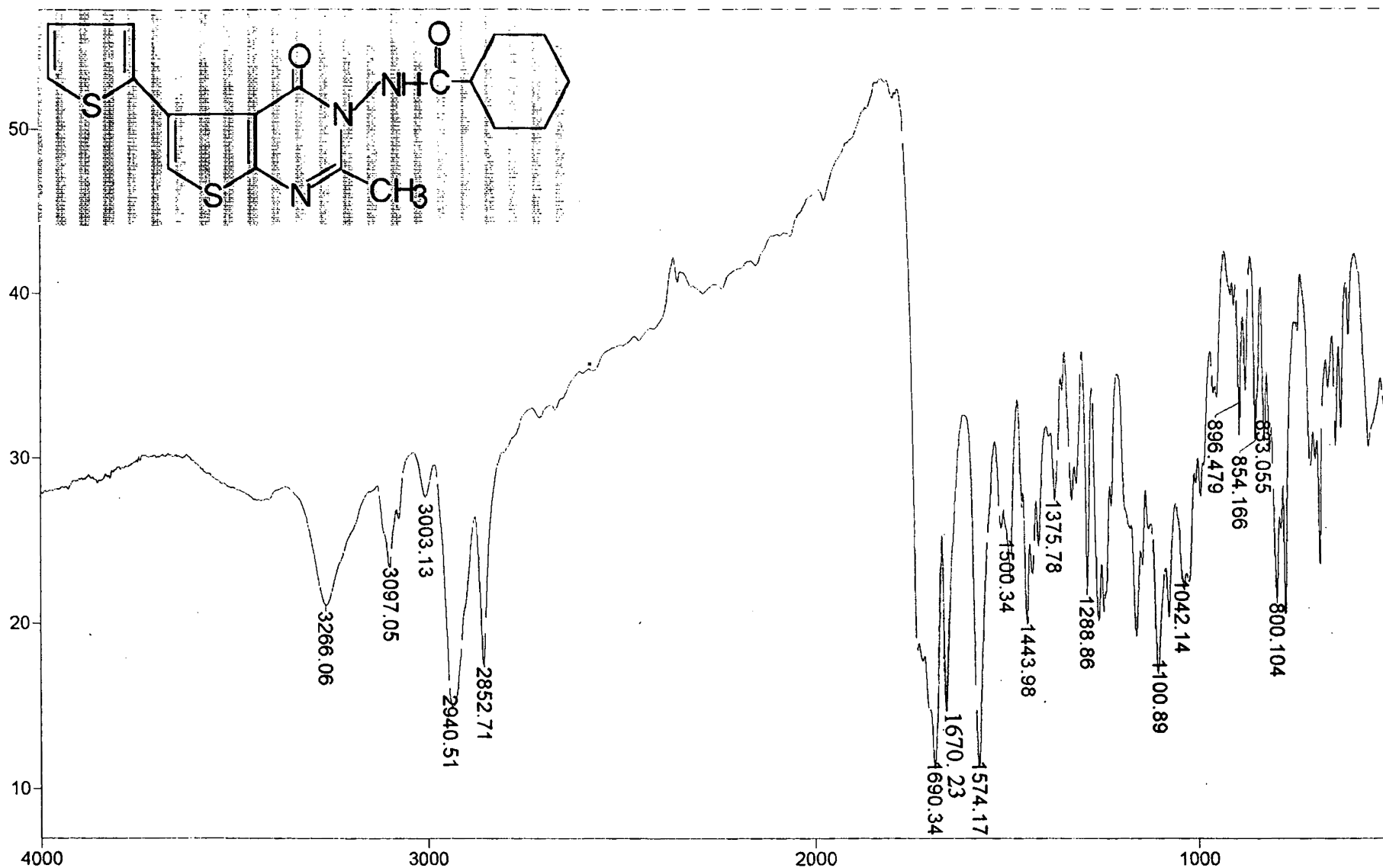
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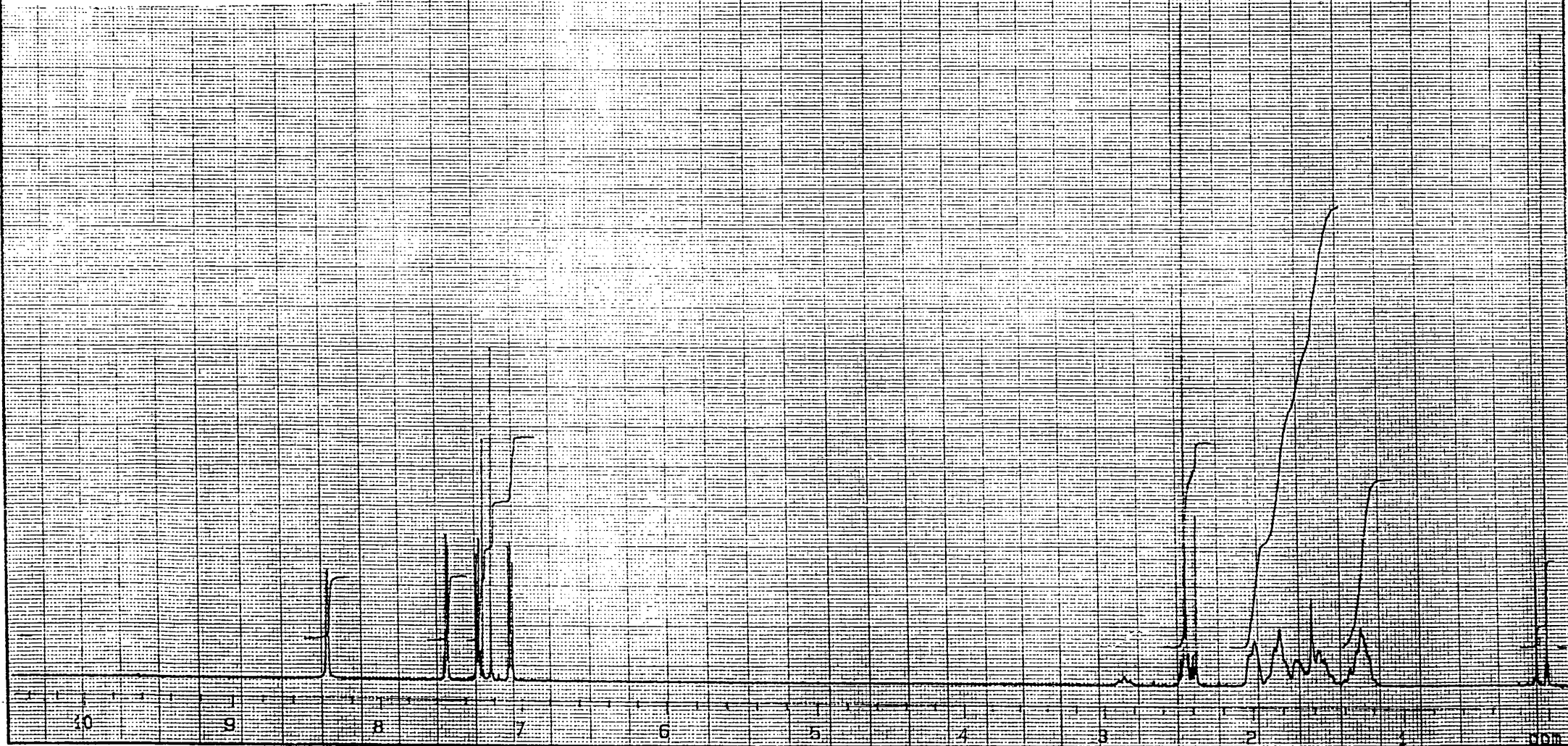
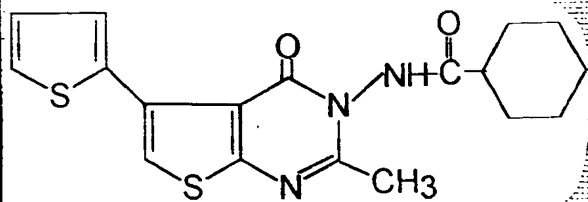
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Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





Part II-C.i

***Synthesis of 2-methyl-3-[3-ethyl-4
-(substituted aryl/heteroaryl)-2,3
dihydrothiazolidine amino]-5-(2-
furyl)-thieno [2,3-d] pyrimidin-
4(3H)-ones***

This part deals with the synthesis of 2-methyl-3-[3-ethyl-4 (substituted aryl / hetero aryl)-2,3 dihydrothiazolidine amino]-5-(2-furyl)-thieno [2,3-d] pyrimidin-4(3H)-ones which involves the following steps

- 1) Synthesis of 2-butenic acid-2 cyano-3-(2-furyl)-ethyl-ester*
- 2) Synthesis of 2-amino-3-carbomethoxy -4-(2-furyl)- thiophene*
- 3) Synthesis of 2-acetamido-3-carbomethoxy-4-(2-furyl)- thiophene*
- 4) Synthesis of 2-methyl-3-amino-5-(2-furyl)-thieno [2,3-d] pyrimidin-4 (3H)-one*
- 5) Synthesis of 2-methyl-3N-ethyl thiourea-5-(2-furyl)-thieno[2,3-d] pyrimidin-4(3H) one
- 6) Synthesis of the title compounds by condensation of 2-methyl-3N-ethyl thiourea-5-(2-furyl)-thieno [2,3-d] pyrimidin-4(3H)-ones with various substituted α -haloketones

* Synthesis has been described in **Part II-A.i**

5) Synthesis of 2-methyl-3N-ethyl thiouredo-5-(2-furyl)-thieno [2,3-d] pyrimidin-4 (3H)-one

In a 100 mL round bottomed flask equipped with water condenser and guard tube was placed a mixture of 2-methyl-3-amino-5-(furyl)-thieno[2,3-d] pyrimidin-4(3H)-one (1g, 0.004 M), ethyl isothiocyanate (0.34 g, 0.004 M), and absolute alcohol (20 mL). The reaction mixture was heated under reflux for 9- 10 hours, partially concentrated and cooled. The separated white coloured solid was filtered and crystallized from methanol.

Yield = 0.82 g (61 %)

M.P. = 219-20°C

Elemental analysis of $C_{14}H_{14}N_4O_2S_2$

	C%	H%	N%
Calculated	50.99	4.19	16.76
Found	50.89	4.41	17.01

6) General method for the synthesis of 2-methyl- 3-[3-ethyl-4-(substituted aryl/heteroaryl)- 2,3 dihydrothiazolidine amino]- 5 - (2-furyl) - thieno [2,3-d] pyrimidin-4(3H)-ones

Synthesis of 2- methyl – 3-[3-ethyl-4-(3-nitro phenyl)-2,3 dihydrothiazolidine amino] – 5-(2-furyl)-thieno [2,3-d] pyrimidin - 4 (3H)-one

In a 50 mL round bottomed flask equipped with water condenser and guard tube was placed a mixture of 2-methyl-3N-ethyl thioureido-5-(2-furyl)-thieno [2,3-d] pyrimidin-4(3H)-one (0.5 g, 0.0014 M), 3-nitro phenacyl bromide (0.34 g, 0.0014 M) and anhydrous sodium acetate (0.45 g, 0.005 M) in absolute alcohol (15 mL). The reaction mixture was heated under reflux for 6-8 hours. It was then partially concentrated and left overnight. The precipitate thus obtained was filtered off and crystallized from methanol.

Yield = 0.48 g (67 %)

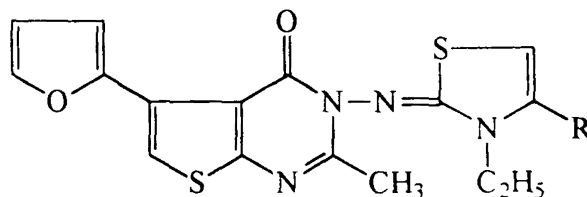
M.P = 243-5°C

Elemental analysis of $C_{22}H_{15}Cl_2FN_4O_2S_2$

	C%	H%	N%
Calculated	55.11	3.50	14.61
Found	55.32	3.31	14.80

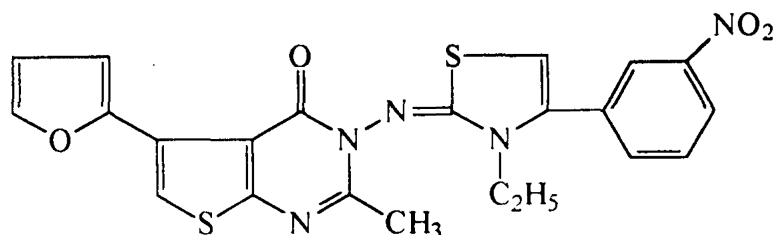
In a similar manner all other 2-methyl-3-[3-ethyl-4-(substituted aryl/ hetero aryl) -2,3 dihydrothiazolidene amino]-5-(2-furyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones have been synthesized and their physical data has been recorded in **Table 5**

**Table 5 : 2-methyl - 3- [3-ethyl-4-(substituted aryl / heteroaryl)-2,3 dihydrothiazolidine amino]-5-(2-furyl)-thieno [2,3-d]-
spyrimidin-4(3H)-ones**



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	2,4-DiCl, 5-FC ₆ H ₂	C ₂₂ H ₁₅ Cl ₂ FN ₄ O ₂ S ₂	521	229-31	55	50.67	2.87	10.74	50.40	2.59	10.84
2	3-NO ₂ -C ₆ H ₄	C ₂₂ H ₁₇ N ₅ O ₄ S ₂	479	243-45	67	55.11	3.50	14.61	55.32	3.11	14.80
3	5-Cl-Thienyl	C ₂₀ H ₁₅ ClN ₄ O ₂ S ₂	442	190-91	45	54.29	3.39	12.66	54.02	3.31	12.48
4	4-OCH ₃ -C ₆ H ₄	C ₂₃ H ₂₀ N ₄ O ₂ S ₂	424	215-17	51	65.09	4.71	13.24	64.83	4.61	13.11
5	3-CF ₃ -C ₆ H ₄	C ₂₃ H ₁₇ F ₃ N ₄ O ₂ S ₂	503	165-66	56	54.87	3.37	11.13	54.63	3.14	10.88
6	4-F-C ₆ H ₃	C ₂₂ H ₁₂ FN ₄ O ₂ S ₂	447	205-06	42	59.06	2.68	12.52	58.93	2.45	12.34

Spectral data of 2-methyl-3-[3-ethyl-4-(3-nitro phenyl)-2,3 dihydrothiazolidine amino]-5-(2-furyl)-thieno [2,3-d] pyrimidin - 4 (3 H)-one (Compound No. 2, Table 5) has been given below.



IR (KBr) cm^{-1} : 1662 (-CO-); 1595 (C=N), 2973, 2935, 1369 (CH_3 and C_2H_5) ; 1202, 1147, 1007 (Furyl) ; 3079, 1479, 1434, 1097 (Aromatic); 775, 760 (C-H Aromatic) ; 1519, 1067, 885, 865 (Thiazolidine) ; 1557, 1349 (NO_2)

PMR (CDCl_3) δ : 1.25 (t, 3H, CH_3); 2.59 (s, 3H, CH_3); 3.61 (s, 2H, CH_2) ; 6.4 (s, 1H, Furyl) ; 7.28 – 8.4 [m, 8H (4H, Aromatic ; 1H, Thiazolidine ; 1H, Thienyl ; 2H, Furyl)]

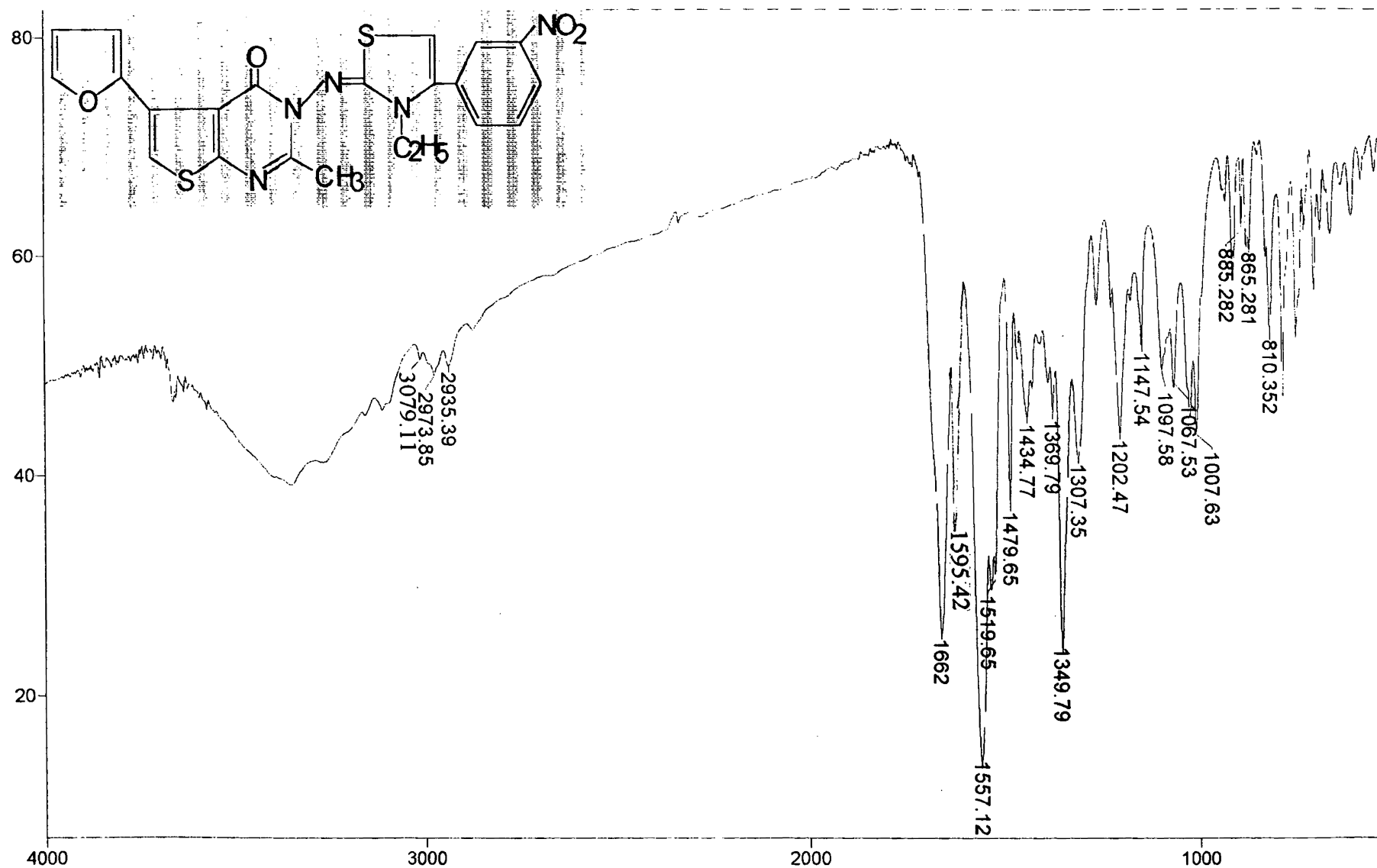
Sample Description: thi-ftp

Scans= 10 Slow

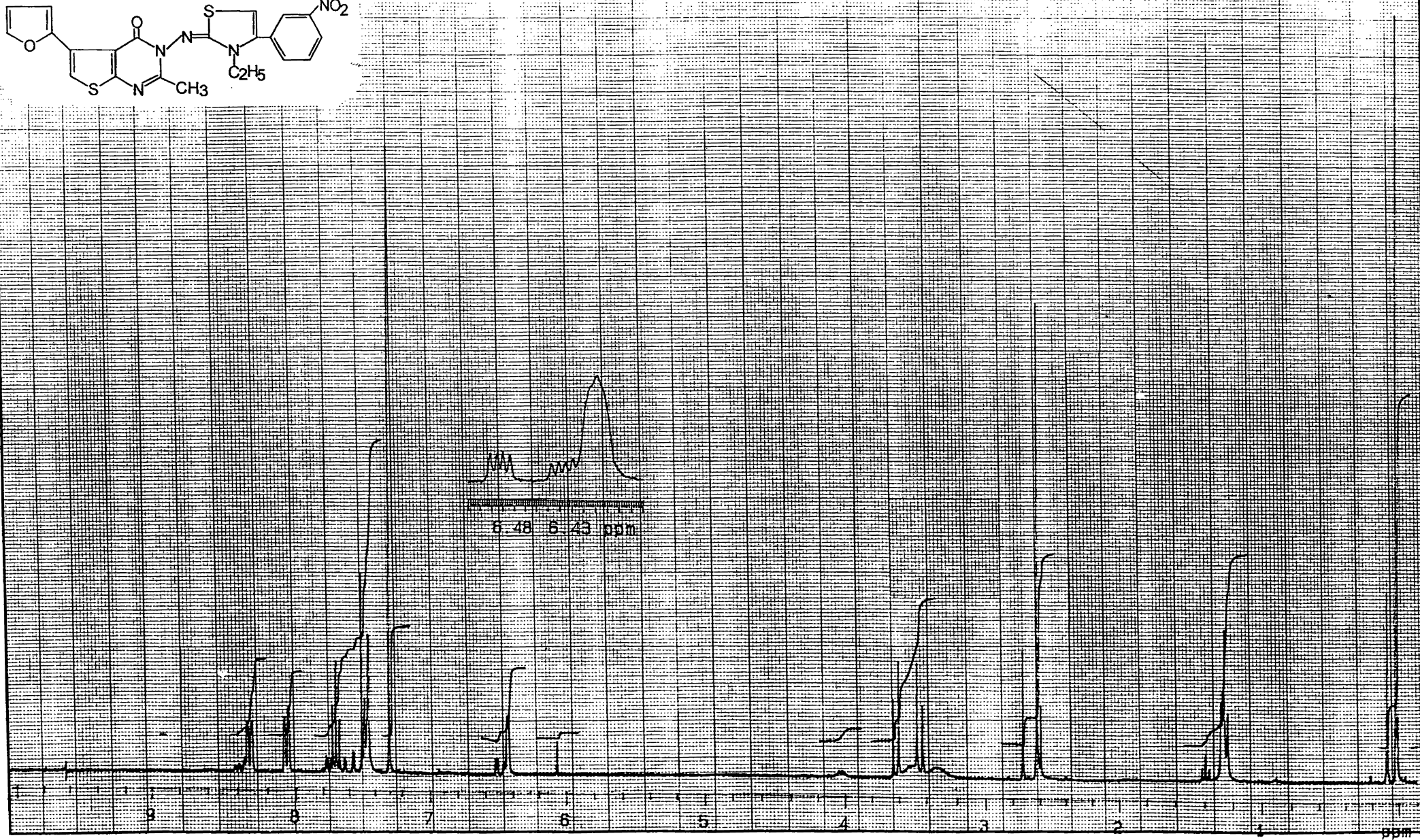
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)



Part II-C.ii

Synthesis of 2-methyl-3-[3-ethyl-4-(substituted aryl/heteroaryl)-2,3 dihydrothiazolidine amino]-5-(2-thienyl)-thieno[2,3-d] pyrimidin-4(3H)-ones

This part of the thesis deals with the synthesis of 2-methyl-3-[3-ethyl-4-(substituted aryl / hetero aryl)-2,3 dihydrothiazolidine amino]-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4 (3H)-one which involves the following steps

- 1) Synthesis of 2- butenoic acid- 2 - cyano- 3 -(2-thienyl)- ethyl ester*
- 2) Synthesis of 2- amino - 3 - carbethoxy - 4-(2-thienyl) thiophene*
- 3) Synthesis of 2- acetamido - 3 – carbethoxy-4-(2-thienyl)-thiophene*
- 4) Synthesis of 2-methyl-3-amino-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4 (3H)-one*
- 5) Synthesis of 2-methyl-3N-ethyl thiouredo-5-(2-thienyl)-thieno[2,3-d] pyrimidin-4 (3H)-one
- 6) Synthesis of the title compounds by condensation of 2- methyl-3N-ethyl thiouredo-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-one with various substituted α - haloketones

* Synthesis has been described in **Part II-A.ii**

5) Synthesis of 2- methyl - 3 N - ethyl thioureido-5-(2-thienyl) - thieno [2,3-d] pyrimidin - 4 (3 H)-one

In a 100 mL round bottomed flask equipped with water condenser and guard tube was placed a mixture of 2-methyl-3-amino-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-one (1.5 g, 0.0055 M), ethyl isothiocyanate (0.47 g, 0.0055m) and absolute alcohol (20 mL). The reaction mixture was heated under reflux for 9-10 hours, partially concentrated and cooled. The separated solid was filtered and crystallized from methanol.

Yield = 0.82 g (61%)

M.P. = 219-20°C

Elemental analysis of $C_{14}H_{14}N_4OS_3$

	C%	H%	N%
Calculated	48.00	4.00	16.00
Found	48.07	4.15	15.81

6) General method for the synthesis of 2- methyl -3-[3-ethyl- 4- (substituted aryl/heteroaryl)-2,3 dihydrothiazolidine amino] - 5- (2-thienyl)-thieno [2,3-d] pyrimidin- 4 (3H)-ones

Synthesis of 2- methyl -3-[3-ethyl-4-(2,4-dichloro-5-fluoro phenyl)-2,3 dihydro thiazolidine amino] - 5-(2-thienyl)-thieno [2,3,-d] pyrimidin - 4 (3H)-one

In 50 mL round bottomed flask equipped with water condenser and guard tube was placed a mixture of 2-methyl-3N-ethyl thioureido-5-(2-thienyl)-thieno[2,3-d] pyrimidin-4-(3H)-one (0.49 g, 0.0019m), 2,4 dichloro - 5-fluoro phenacyl bromide (0.42, 0.0014 M) and anhydrous sodium acetate (0.45 g, 0.005 M) in absolute alcohol (15 mL). The reaction mixture was heated under reflux for 6-8 hours. It was partially concentrated and left overnight. The precipitate thus obtained was filtered off and crystallized from methanol.

Yield = 0.35 g, (47 %)

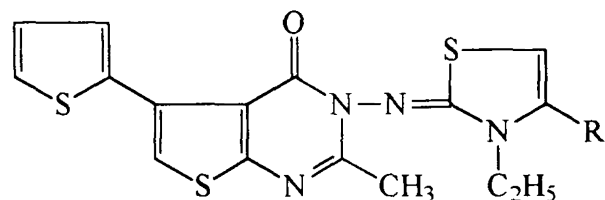
M.P. = 187-90°C

Elemental analysis of $C_{22}H_{15}FCl_2FN_4OS_4$

	C%	H%	N%
Calculated	49.16	2.79	10.42
Found	49.35	3.00	10.39

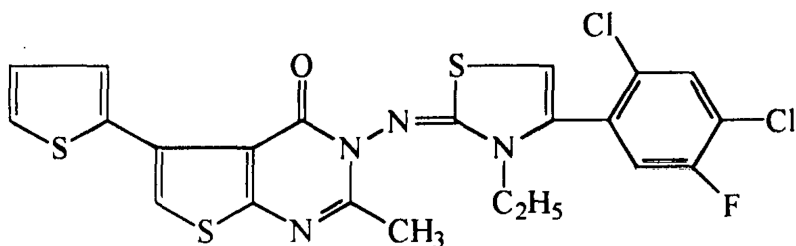
In a similar manner all other 2-methyl-3-[3-ethyl-4-(substituted aryl/ heteroaryl) -2,3 dihydrothiazolidine amino]-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones have been synthesized and their physical data has been recorded in **Table 6**

Table 6 : 2-methyl - 3- [3-ethyl-4-(substituted aryl / heteroaryl)-2,3-dihydrothiazolidine amino]-5- (2-thienyl)-thieno [2,3-d]-pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	2,4-DiCl, 5-FC ₆ H ₂	C ₂₂ H ₁₅ FCl ₂ N ₄ OS ₃	537	187-90	47	49.16	2.79	10.42	49.35	3.00	10.39
2	5Cl-Thienyl	C ₂₀ H ₁₅ ClN ₄ OS ₄	490	147-50	61	48.92	3.05	11.41	49.13	3.10	11.61
3	4-OCH ₃ -C ₆ H ₄	C ₂₃ H ₂₀ N ₄ O ₂ S ₃	480	195-98	53	56.50	4.16	11.66	56.12	4.15	11.70
4	4-NO ₂ -C ₆ H ₄	C ₂₂ H ₁₇ N ₅ O ₃ S ₃	495	243-46	57	53.33	3.43	14.14	53.55	3.22	14.50
5	4-CF ₃ -C ₆ H ₄	C ₂₃ H ₁₇ F ₃ N ₄ OS ₂	518	193-94	49	53.23	3.28	10.81	52.94	3.16	11.09
6	4-F-C ₆ H ₄	C ₂₂ H ₁₇ FN ₄ OS ₃	468	190-91	57	56.41	3.63	11.98	56.30	3.43	11.45

Spectral data of 2- methyl- 3-[3- ethyl- 4- (2,4-dichloro-5-fluoro phenyl) -2,3 dihydro thiazolidine amino]-5-(2-thienyl)-thieno [2,3-d] pyrimidin-4 (3 H)-one (Compound No. 1, Table 6) has been given below.



IR (KBr) cm^{-1} : 1668 (-CO-); 1585 (C=N), 2977, 2937, 1375 (-CH₃ and -C₂H₅); 1496, 1404, 1036 (Thienyl) ; 3084, 1496, 1173, 1098 (Aromatic); 775 (C-H Aromatic) ; 1542, 888, 811 (Thiazolidine), 726 (C-Cl) ; 997 (C-F)

PMR (CDCl₃) δ : 1.28 (t, 3H, CH₃); 2.55 (s, 3H, CH₃); 3.5 (s, 2H, CH₂), 7 – 7.62 [m, 7H (2H, Aromatic; 1H, Thiazolidine; 4H, Thienyl)]

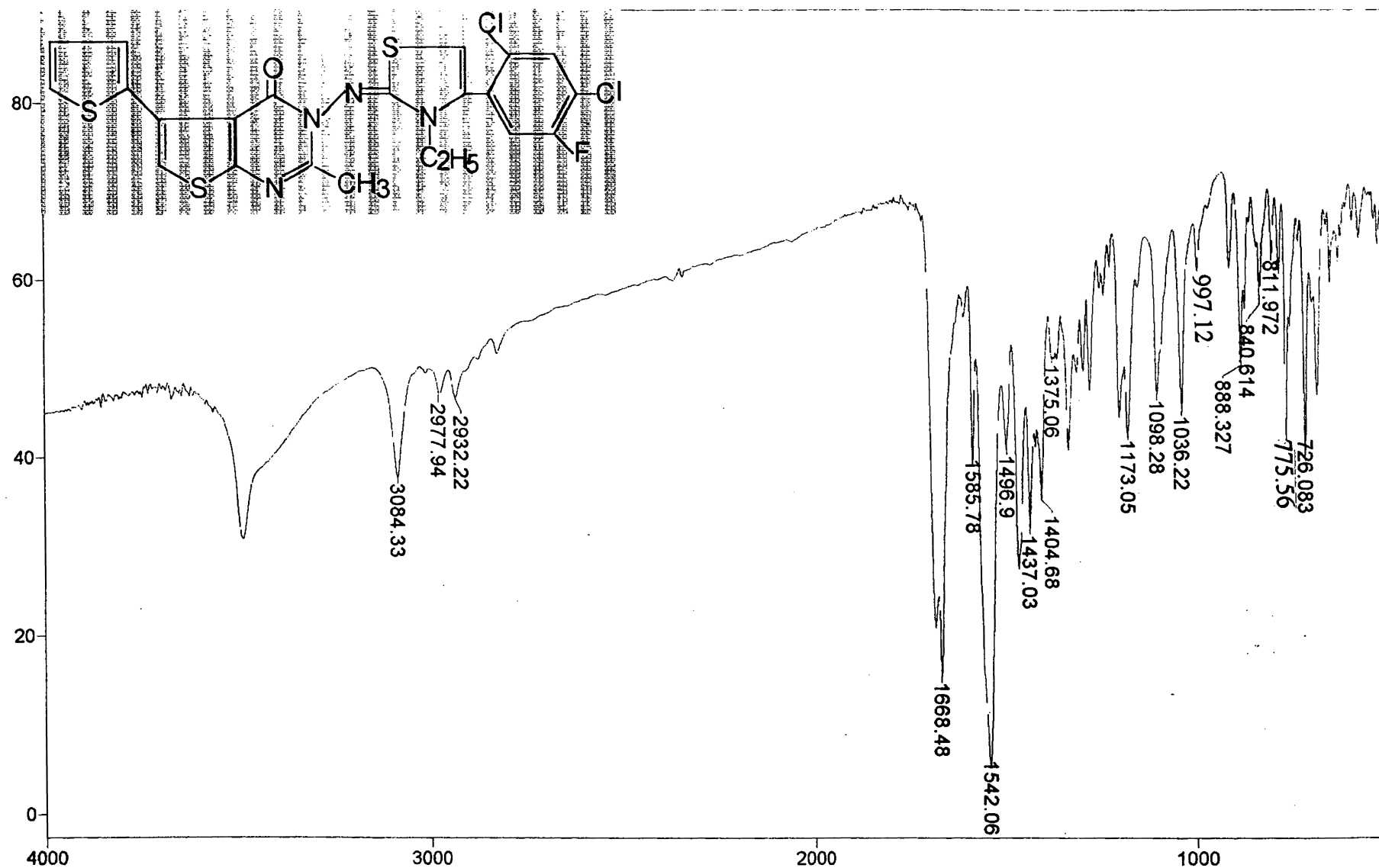
Sample Description: thi-ttpo

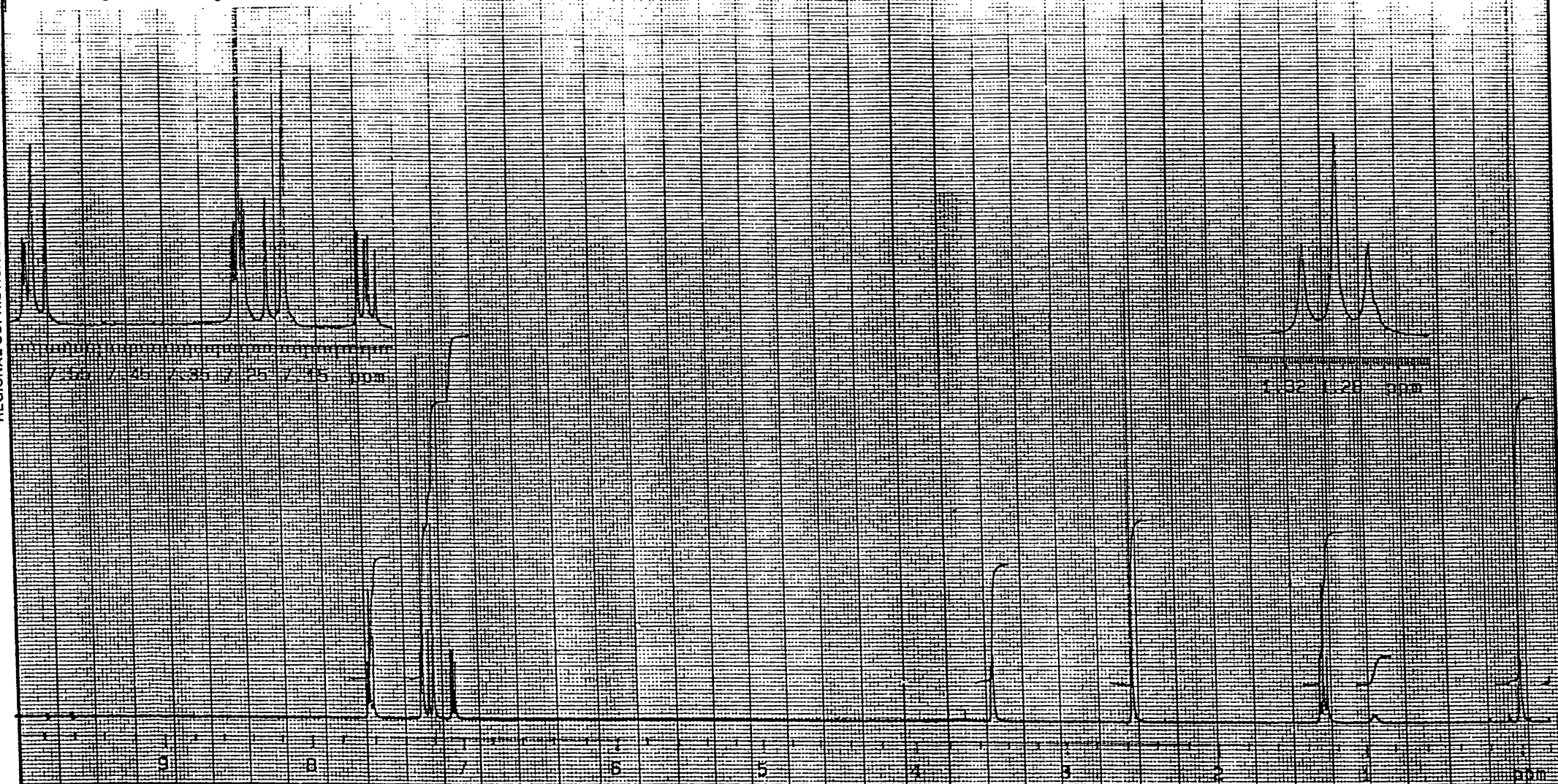
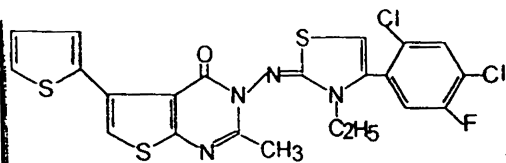
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





Part II-D.i

***Synthesis of 2-[(substituted aryl
/heteroaryl)-thioketomethylene]-
3-phenyl-5-(3-pyridinyl)-thieno
[2,3-d] pyrimidin-4(3H)-ones***

This part of the thesis deals with the synthesis of 2- [(substituted aryl / hetero aryl) thioketomethylene]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-ones, which involves the following steps.

- 1) Synthesis of 2- amino - 3 - carbethoxy - 4- (3-pyridinyl) thiophene
- 2) Synthesis of 2-mercapto-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-one
- 3) Synthesis of substituted α -haloketones
- 4) Synthesis of the title compounds by condensation of 2-mercapto-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-one with various substituted α -haloketones

1) Synthesis of 2 - amino - 3 - carbethoxy - 4-(3-pyridinyl)-thiophene

In a 250 mL round bottomed flask equipped with water condenser and a guard tube was placed a mixture of 3 - acetyl pyridine (12.g, 0.1 M), ethyl cyano acetate (11.3 g, 0.1 M), sulphur (3.2 g 0.1 M) and morpholine (3 mL) in absolute alcohol (150mL). The mixture was heated at 80-85°C for 6 hours and then chilled in ice. The resultant crystals were collected, washed with hexane and recrystallized from ethanol.

Yield = 9.2 g (45%)

M.P. = 134-35°C

Elemental analysis of $C_{14}H_{14}N_4OS_3$

	C%	H%	N%
Calculated	58.04	4.8	11.29
Found	58.28	4.98	11.56

2) Synthesis of 2-mercapto-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin -4(3H)-one

In a 250 mL round bottomed flask equipped with a water condenser and a guard tube was placed a mixture of 2-amino-3-carbethoxy-4-(3-pyridinyl)-thiophene (10 g, 0.048 M) and phenyl isothiocyanate(6. 48 g, 0.048 M) in acetonitrile (150 mL). The reaction mixture was heated under reflux for 15 hours in the presence of anhydrous potassium carbonate (6 g). The reaction mixture was filtered and, water was added tp it, prior to its neutralization with hydrochloric acid (2 N). The solid product thus obtained was filtered, washed with water, dried and recrystallized from acetone.

Yield = 8.14 g (60%)

M.P. = 303-5°C

Elemental analysis of $C_{17}H_{11}N_3OS_2$

	C%	H%	N%
Calculated	60.53	3.26	12.46
Found	60.79	3.20	12.70

3) General method for the synthesis of substituted α -haloketones

Synthesis of 3-nitrophenacyl bromide

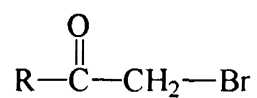
3-nitroacetophenone (3.465 g, 0.021 M) was dissolved in 21 mL of dry chloroform. This solution was transferred to a 3-necked round bottomed flask, fitted with efficient mechanical stirrer, a dropping funnel and a water condenser with a guard tube (anhydrous CaCl_2). The reaction mixture was exposed to light from a the 200 watts bulb while being warmed to 50°C. Bromine (1.075 mL, 0.042m) in 20 mL of dry chloroform, was then added drop wise to the well stirred reaction mixture. This stirring was continued at 50°C for half an hour. A stream of dry air was then passed through the reaction mixture to sweep out the HBr gas evolved in the reaction. The reaction mixture was then diluted with petroleum ether (40 : 60) and refluxed to dissolve the syrupy mass. The clear petroleum ether solution was treated with charcoal and filtered. It was concentrated to obtain crystalline product.

Yield = 3.58 g (70.0%)

M.P. = 94-95°C, Lit. M.P.²²⁹ = 96°C

Similarly, the various other substituted α -haloketones have been prepared by the above method and their physical constants have been recorded in **Table 7**

Table 7 : Substituted α -haloketones



Sr. No.	R	Yield	M.P. °C / B.P. °C		Reference
			Observed	Reported	
1	2,4-DiCl, 5-F C ₆ H ₂	78	64-65	*	-
2	3-NO ₂ -C ₆ H ₄	70	94-95	96	229
3	4-OCH ₃ -C ₆ H ₄	78	74-75	75-76	230
4	Cyclopropane	55	88-89/15mm	90/15mm	231
5	4-F-C ₆ H ₄	62	48-50	47-49	232
6	5-chloro-thienyl	71	79-81	80-81	233
7	3,4-DiCl-C ₆ H ₃	81	54-56	*	-
8	Cyclohexane	53	70-71/15mm	71/15mm	234
9	3-F-C ₆ H ₄	74	44-45	*	-
10	3-CF ₃ -C ₆ H ₄	69	51-52	*	-
11	2,3,4 trimethoxy C ₆ H ₂	40	61-62	*	-

* Confirmed by TLC & Elemental analyses

4) **General method for the synthesis of 2-[(substituted aryl / heteroaryl) -thioketomethylene]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d]pyrimidin-4(3H) ones**

Synthesis of 2-[(2,4-dichloro - 5 - fluoro phenyl)-thioketomethylene]-3-phenyl-5-(3-pyridinyl) - thieno [2,3-d] pyrimidin- 4 (3 H)-one

In a 150 mL three necked round bottomed flask equipped with a condenser, guard tube, and a dropping funnel, was placed a suspension of 2-mercapto-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one (0.5 g, 0.001 M) and sodium carbonate (0.2 g) in anhydrous acetone (40 mL). The suspension was stirred for 45 minutes on a magnetic stirrer. A solution of 2,4 dichloro-5 fluorophenacyl bromide (0.285 g, 0.001 M) in 10 mL dry acetone was slowly added over a period of 2 hours. The mixture was then stirred for an additional 24 hours and the solid obtained was collected by filtration under reduced pressure. It was repeatedly washed with water, dried and crystallized from methanol.

Yield = 0.48 (60 %)

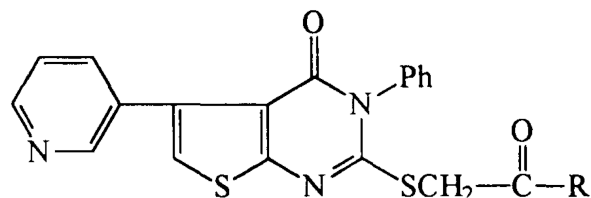
M.P. = 194-96°C

Elemental analysis of $C_{17}H_{11}N_3OS_2$

	C%	H%	N%
Calculated	55.35	2.58	7.74
Found	55.59	2.62	7.85

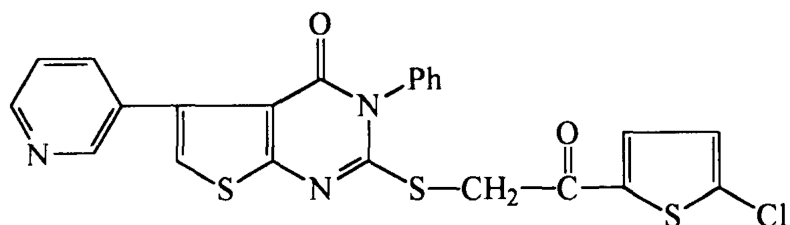
In a similar manner, all other 2-[(substituted aryl / heteroaryl)-thioketomethylene]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones have been synthesized and their physical data has been recorded in **Table 8**

Table 8 : 2-[(substituted aryl / heteroaryl)-thioketomethylene]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	2,4-DiCl, 5-F-C ₆ H ₂	C ₂₅ H ₁₄ Cl ₂ FN ₃ O ₂ S ₂	542	194-96	60	55.35	2.58	7.74	55.59	2.62	7.85
2	C ₆ H ₅	C ₂₅ H ₁₇ N ₃ O ₂ S ₂	455	213-15	85	65.93	3.73	9.23	65.63	3.85	9.40
3	4-F-C ₆ H ₄	C ₂₅ H ₁₆ FN ₃ O ₂ S ₂	473	225-27	57	63.42	3.38	8.87	63.64	3.52	9.13
4	3-CF ₃ -C ₆ H ₄	C ₂₆ H ₁₆ F ₃ N ₃ O ₂ S ₂	523	124-27	40	59.65	3.05	8.03	59.41	3.17	7.96
5	3-F-C ₆ H ₄	C ₂₅ H ₁₆ FN ₃ O ₂ S ₂	473	167-70	60	63.42	3.38	8.87	63.44	3.50	8.69
6	3,4-DiCl-C ₆ H ₃	C ₂₅ H ₁₅ Cl ₂ N ₃ O ₂ S ₂	524	210-11	59	57.25	2.86	8.03	57.25	2.75	7.92
7	5-Cl-Thienyl	C ₂₃ H ₁₄ ClN ₃ O ₂ S ₃	495	239-42	40	55.75	2.82	8.48	56.00	2.71	8.35
8	Cyclopropane	C ₂₂ H ₁₇ N ₃ O ₂ S ₂	419	160-63	60	63.00	4.05	10.02	63.39	4.09	9.78
9	Cyclohexane	C ₂₅ H ₂₃ N ₃ O ₂ S ₂	461	139-42	63	65.00	4.90	9.10	65.30	5.00	8.90
10	3-NO ₂ -C ₆ H ₄	C ₂₅ H ₁₆ N ₄ O ₄ S ₂	500	202-05	55	60.00	3.20	11.39	59.92	2.91	11.70

Spectral data of 2- [(5-chloro-thienyl)-thioketomethylene]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin - 4 (3 H)-one (Compound No. 7, Table 8) has been shown below



IR (KBr) cm^{-1} : 1689 (-CO-); 1591 (C=N), 2915, 1452 (-CH₂); 1513, 1073, 816 (Thienyl) ; 3092, 1569, 1477, 1413, 665 (Aromatic); 1186, 1016 (Pyridine) ; 755 (C-Halo) ; 694 (S-C)

PMR (CDCl₃) δ : 4.4 (s, 2H, -CH₂); 7 – 8.7 [m, 12H (3H, Thienyl ; 5H, Aromatic ; 4H, Pyridinyl)]

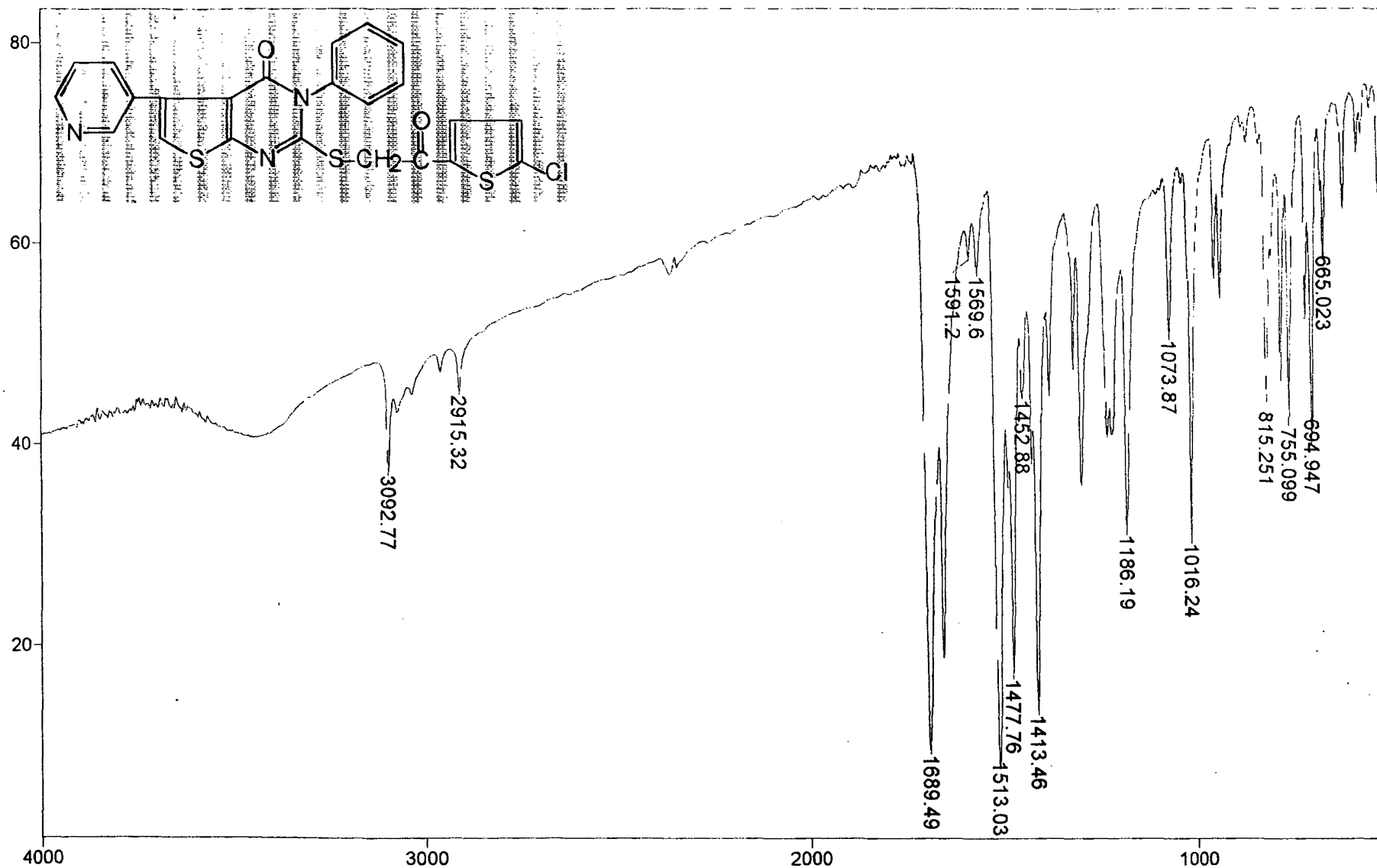
Sample Description: pp4ptp

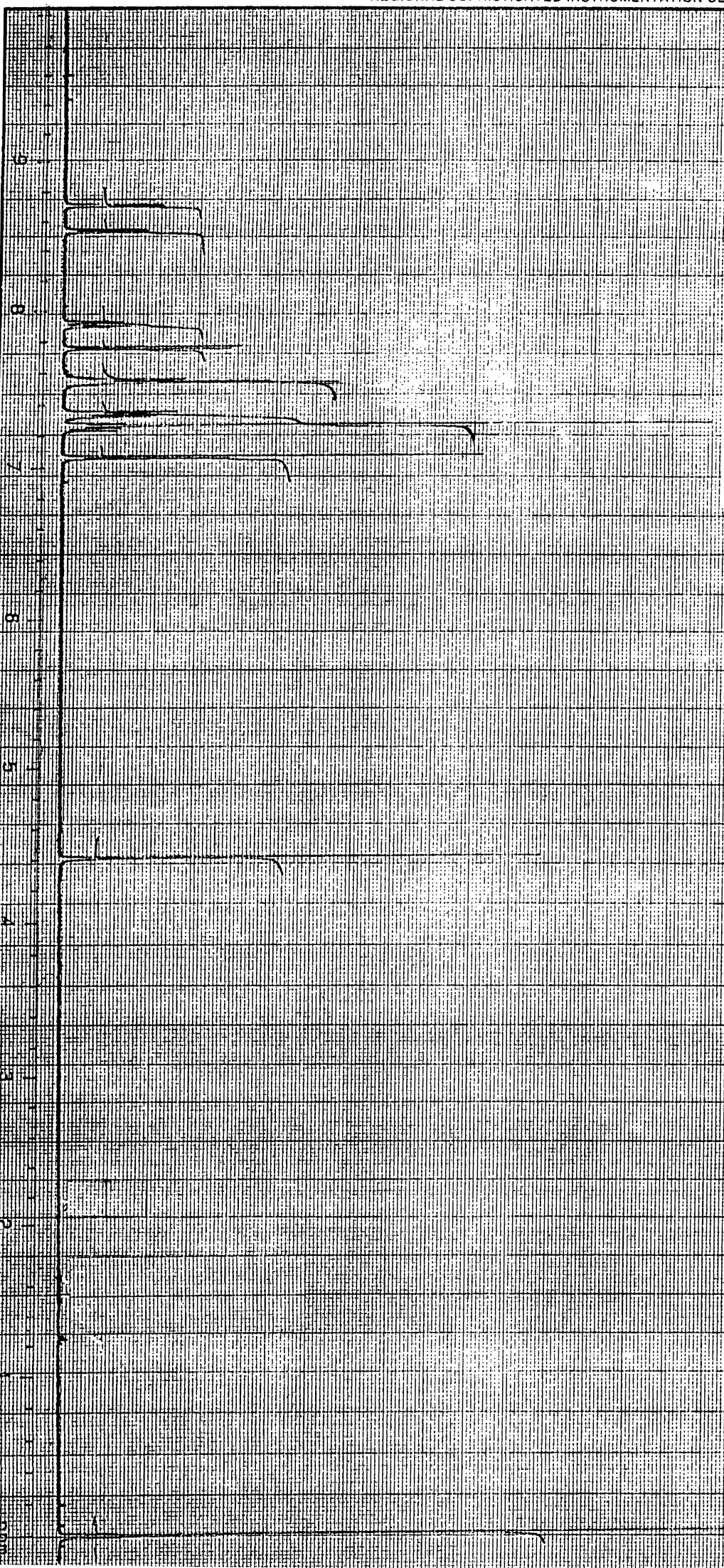
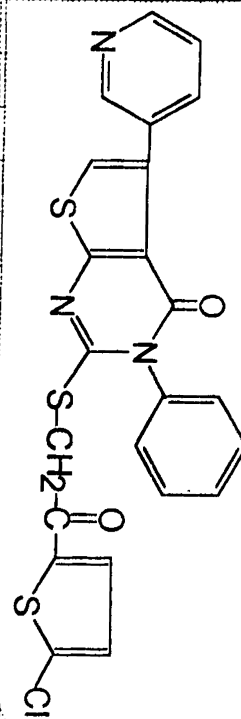
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





Part II-D.ii

***Synthesis of 2-[(substituted aryl/
heteroaryl)-thioketomethylene]-3-
phenyl-5-(4-pyridinyl)-thieno [2,3-
d] pyrimidin-4(3H)-ones***

This part of the thesis deals with the synthesis of 2-[(substituted aryl / heteroaryl) thioketomethylene] -3- phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones which involves the following steps

- 1) Synthesis of 2- amino- 3- carbethoxy - 4-(4-pyridinyl)- thiophene
- 2) Synthesis of 2-mercapto-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one
- 3) Synthesis of the title compounds by condensation of 2-mercapto-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one with various substituted α -haloketones

1) Synthesis of 2-amino-3-carbethoxy-4-(4-pyridinyl)-thiophene

In a 250 mL round bottomed flask equipped with a water condensor and a guard tube was placed a mixture of 4-acetyl pyridine (12.1 g, 0.1m), ethyl cyanoacetate (11.3 g, 0.1m), and morpholine (3 mL) in absolute alcohol (150 mL). The mixture was heated at 80-85°C for 6 hours and then chilled in ice. The resulting yellow coloured crystals were collected, washed with hexane and recrystallized from ethanol.

Yield = 18.6 (75 %)

M.P. = 200-01°C

Elemental analysis of $C_{12}H_{12}N_2O_2S$

	C%	H%	N%
Calculated	58.04	4.83	11.29
Found	58.28	4.66	11.55

2) Synthesis of 2-mercapto-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin - 4(3H)-one

In a 250 mL round bottomed flask equipped with a water condenser and a guard tube was placed a mixture of 2-amino-3-carbethoxy-4-(4-pyridinyl)-thiophene (10 g, 0.048 M) and phenyl isothiocyanate (6.48g,0.048m) in acetonitrile (150 mL). The reaction mixture was heated under reflux for 15 hours in the presence of anhydrous potassium carbonate (6 g). It was then filtered, and after the addition of water and neutralization with hydrochloric acid (2N), a solid product was obtained. It was filtered, washed with water, dried and recrystallized from acetone.

Yield = 9.1 g (67 %)

M.P. = 288-90°C

Elemental analysis of $C_{17}H_{11}N_3OS_2$

	C%	H%	N%
Calculated	60.53	3.26	12.46
Found	60.75	3.26	12.54

3) General method for the synthesis of 2- [(substituted aryl / heteroaryl) -thiketomethylene]-3-phenyl-5-(4-pyridinyl)-thieno[2,3-d] pyrimidin-4(3H)-ones

Synthesis of 2-[(2,4-dichlorophenyl)-thiketomethylene]-3-phenyl-5-(4-pyridinyl) – thieno [2,3-d] pyrimidin-4(3 H)-one

In a 150 mL three necked round bottomed flask equipped with a condenser, a guard tube and a dropping funnel, was placed a suspension of 2-mercapto-3-phenyl-5-(4-pyridinyl) -thieno [2,3-d] pyrimidin-4 (3H)-one (0.5 g, 0.001 M) and sodium carbonate (0.2 g) in anhydrous acetone (40 mL). The suspension was stirred for 45 minutes on a magnetic stirrer. A solution of 3, 4-dichlorophenacyl bromide (0.267 g, 0.001 M) in 10 mL dry acetone was slowly added over a period of 2 hours. The mixture was then stirred for an additional 24 hours and the solid obtained was collected by filtration under reduced pressure. It was repeatedly washed with water, dried and crystallized from methanol.

Yield = 0.41 g (61 %)

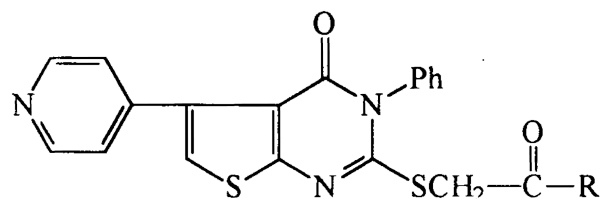
M.P. = 205-6°C

Elemental analysis of $C_{25}H_{15}Cl_2N_3O_2S_2$

	C%	H%	N%
Calculated	57.25	2.86	8.03
Found	57.49	2.90	7.93

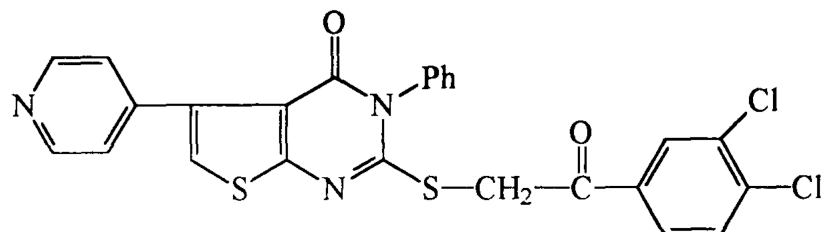
In a similar manner, all other 2- [(substituted aryl / heteroaryl)-thiketomethylene] -3-phenyl-5-(4- pyridinyl)-thieno [2,3-d] pyrimidin- 4(3H)-ones have been synthesized and their physical data has been recorded in **Table 9**

Table 9 : 2-[(substituted aryl / heteroaryl)-thioketomethylene]-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	2,4-DiCl, 5-F-C ₆ H ₂	C ₂₅ H ₁₄ Cl ₂ N ₃ O ₂ S ₂	542	153-55	51	55.35	2.58	7.74	55.27	2.75	8.00
2	C ₆ H ₅	C ₂₅ H ₁₇ N ₃ O ₂ S ₂	455	209-10	77	65.93	3.73	9.23	65.05	3.79	9.40
3	3,4-DiCl-C ₆ H ₃	C ₂₅ H ₁₅ Cl ₂ N ₃ O ₂ S ₂	524	205-06	60	57.25	2.86	8.03	57.49	2.90	7.93
4	4-F-C ₆ H ₄	C ₂₅ H ₁₆ FN ₃ O ₂ S ₂	473	221-22	50	63.42	3.38	8.87	63.42	3.31	8.70
5	3-CF ₃ -C ₆ H ₄	C ₂₆ H ₁₆ F ₃ N ₃ O ₂ S ₂	523	204-06	55	59.65	3.05	8.03	59.43	3.26	8.03
6	3-F-C ₆ H ₄	C ₂₅ H ₁₆ FN ₃ O ₂ S ₂	473	147-50	62	63.42	3.38	8.87	63.30	3.15	9.00
7	2,3,4-Tri OCH ₃ -C ₆ H ₂	C ₂₈ H ₂₃ N ₃ O ₅ S ₂	545	230-33	49	61.65	4.22	7.70	61.81	4.24	7.05
8	5-Cl-Thienyl	C ₂₃ H ₁₄ ClN ₃ O ₂ S ₃	495	199-201	61	55.75	2.82	8.48	55.52	3.20	8.29
9	Cyclopropane	C ₂₂ H ₁₇ N ₃ O ₂ S ₂	419	181-84	54	63.00	4.05	10.02	62.85	4.11	10.19

Spectral data of 2- [(3,4-dichloro phenyl)-thioketo methylene]-3-phenyl-5-(4-pyridinyl) - thieno [2,3-d] pyrimidin -4(3H)-one (Compound No. 3, Table 9) is given below



IR (KBr) cm^{-1} : 1686 (-CO-); 1595 (C=N), 3912, 1429 (-CH₂-); 3096, 1569, 1473, 1096 (Aromatic); 775, 655 (C-H Aromatic) ; 1513 (Thienyl) ; 1225, 1028 (Pyridinyl), 752 (C-Halo) ; 694 (S-C)

PMR (CDCl₃) δ : 4.5 (s, 2H, -CH₂-); 7.1 – 8.6 [m, 13H (8H, Aromatic ; 1H, Thienyl, 4H, Pyridinyl)]

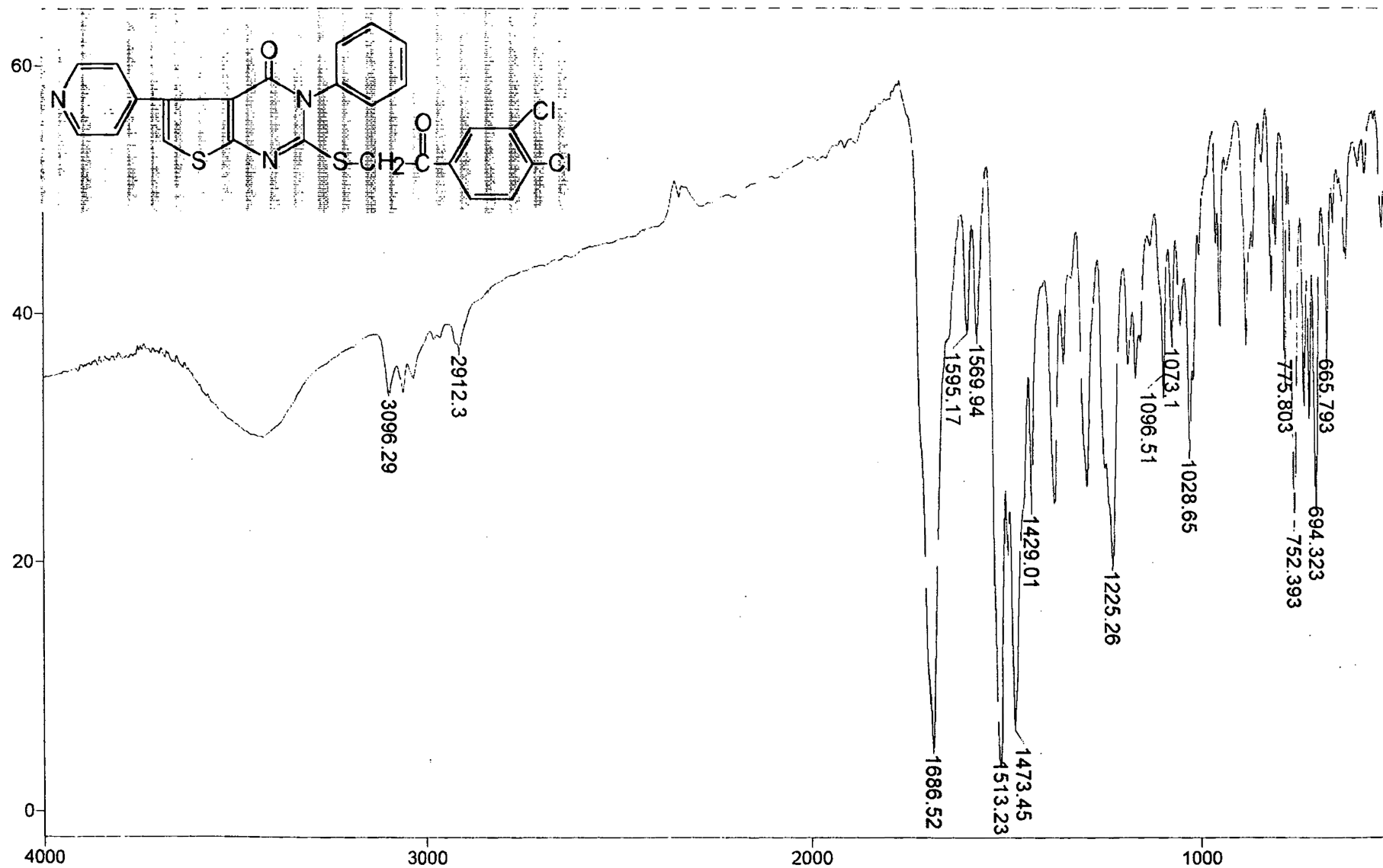
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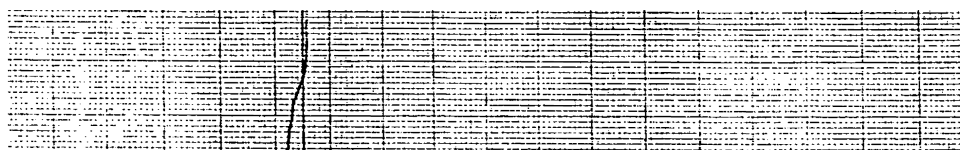
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Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





Part II-E.i

***Synthesis of 2-[(substituted
benzyl)-thio]-3-phenyl-5-(3-
pyridinyl)-thieno [2,3-d]
pyrimidin-4(3H)-ones***

This part of the thesis deals with the synthesis of 2-[(substituted benzyl)-thio]-3-phenyl-5-(3-pyridinyl)-thieno[2,3-d] pyrimidin-4(3H)-ones which involves the following steps-

- 1) Synthesis of 2-amino-3-carbethoxy-4-(3-pyridinyl)-thiophene*
- 2) Synthesis of 2-mercapto-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-one*
- 3) Synthesis of the title compounds by condensation of 2- mercapto - 3- phenyl- 5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-one with various substituted benzyl chlorides

* Synthesis has been described in **Part II-D.i**

3) General method for the synthesis of 2-[(substituted benzyl)-thio]-3-phenyl- 5-(3-pyridinyl)-thieno [2,3-d] pyrimidin -4(3H)-ones

Synthesis of 2-[(2-fluorobenzyl)-thio]-3-phenyl-5-(3-pyridinyl)-thieno- [2,3-d] pyrimidin -4 (3H)-one

In a 50 mL round bottomed flask equipped with a water condenser and guard tube was placed a mixture of 2-mercapto-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones (0.5 g, 0.0014 M), 2-fluoro benzyl chloride (0.20 g, 0.0014 M) and triethylamine (0.25 mL) in 15 mL of dry 1,4-dioxane. The reaction mixture was heated on water bath at 70-75°C for 3 to 4 hours, allowed to cool room temperature and dumped into crushed ice. The solid compound separated out, was filtered, washed with water, dried and recrystallized from methanol.

Yield = 0.39 g (60 %)

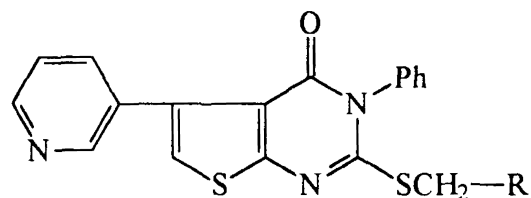
M.P. = 170-72°C

Elemental analysis of $C_{24}H_{16}FN_3OS_2$

	C%	H%	N%
Calculated	64.57	3.81	9.41
Found	64.23	3.99	9.66

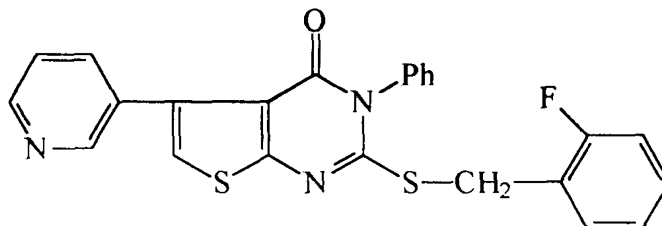
In a similar manner, all other 2-[(substituted benzyl)-thio]- 3- phenyl- 5-(3-pyridinyl) thieno [2,3-d] pyrimidin - 4 (3H)-ones have been synthesized and their physical data has been recorded in **Table 10**

Table 10 : 2-[(substituted benzyl)-thio]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	4-Cl-C ₆ H ₄	C ₂₄ H ₁₆ ClN ₃ OS ₂	461	190-93	85	62.47	3.47	9.11	62.55	3.63	9.40
2	4-OCH ₃ -C ₆ H ₄	C ₂₅ H ₁₉ N ₃ O ₂ S ₂	457	186-87	73	65.93	3.73	9.23	65.81	4.00	8.98
3	3,4-DiCl ₂ -C ₆ H ₃	C ₂₄ H ₁₅ ClN ₃ O ₂ S ₂	496	198-99	65	58.06	3.02	8.46	58.28	3.28	8.43
4	2-F-C ₆ H ₄	C ₂₄ H ₁₆ FN ₃ OS ₂	445	170-72	60	64.57	3.81	9.41	64.23	3.99	9.66
5	4-F-C ₆ H ₄	C ₂₄ H ₁₆ FN ₃ OS ₂	445	176-78	53	64.57	3.81	9.41	64.10	3.86	9.53
6	4-CF ₃ -C ₆ H ₄	C ₂₅ H ₁₆ F ₃ N ₃ OS ₂	495	183-85	71	60.60	3.23	8.48	60.25	3.00	8.66

Spectral data of 2-[(2-fluorobenzyl)-thio]-3-phenyl - 5 - (3-pyridinyl) -thieno [2,3-d] pyrimidin -4 (3H)-one (Compound No. 4, Table 10) is given below



IR (KBr) cm^{-1} : 1686 (-CO-); 1594 (C=N), 2925, 1453 (-CH₂-); 1514 (Thienyl) ; 3090, 1571, 1490, 1427 (Aromatic); 757, 669 (C-H Aromatic) ; 1186, 1018 (Pyridine), 1072 (C-F) ; 700 (S-C)

PMR (CDCl₃) δ : 4.43 (s, 2H, -CH₂-); 7 – 8.7 [m, 14H (9H, Aromatic ; 1H, Thienyl; 4H, Pyridinyl)]

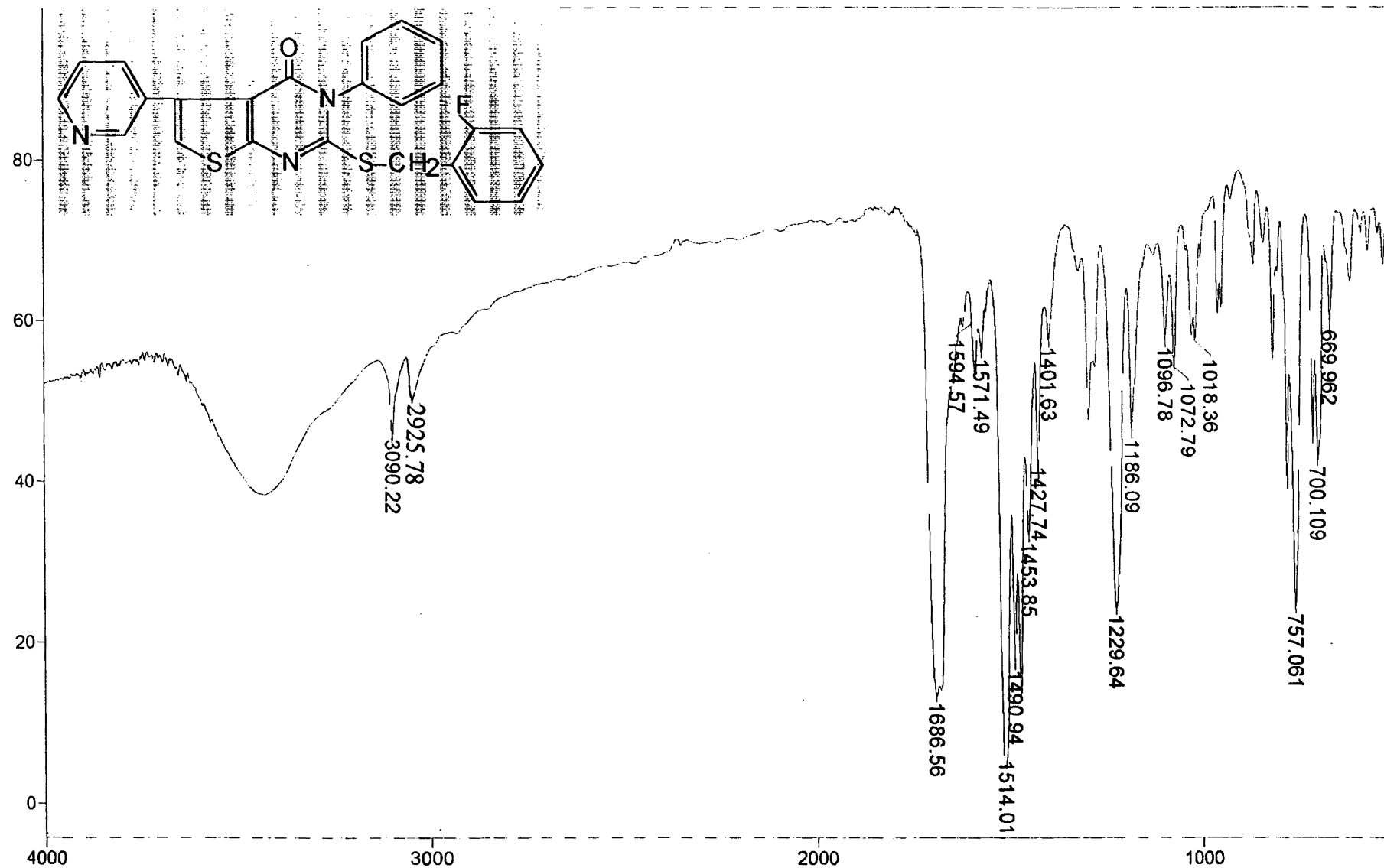
Sample Description: ft3ptp

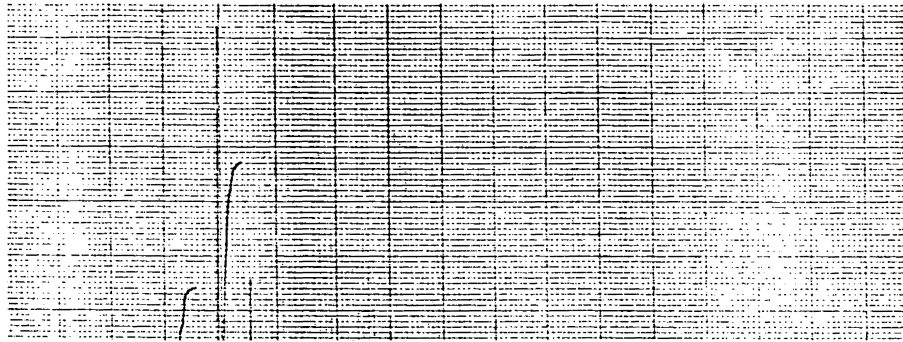
Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x





Part II-E.ii

***Synthesis of 2-(substituted
benzyl)-thio]-3-phenyl-5-(4-
pyridinyl)-thieno [2,3-d]
pyrimidin-4(3H)-ones***

This part of the thesis deals with the synthesis of 2-[(substituted benzyl)-thio]-3-phenyl-5-(4-pyridinyl)-thieno[2,3-d] pyrimidin-4(3H)-ones which involves the following steps

- 1) Synthesis of 2-amino-3-carbethoxy-4-(4-pyridinyl)-thiophene*
- 2) Synthesis of 2-mercapto-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one*
- 3) Synthesis of the title compounds by condensation of 2- mercapto-3-phenyl-5-(4-pyridinyl)-thieno[2,3-d] pyrimidin-4(3H)-one with various substituted benzyl chlorides

* Synthesis has been described in **Part II-D.ii**

3. General method for the synthesis of 2-[(substituted benzyl)-thio]- 3-phenyl- 5-(4-pyridinyl)-thieno [2,3-d] pyrimidin - 4 (3H)-ones

Synthesis of 2-[(4-fluorobenzyl)-thio]-3-phenyl-5-(4-pyridinyl)-thieno-[2,3-d] pyrimidin - 4 (3 H)-one

In a 50 mL round bottomed flask equipped with a water condenser and a guard tube was placed a mixture of 2- mercapto-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one (0.5 g, 0.0014m), 4-fluorobenzyl chloride (0.20 g, 0.0014 M) and triethyl amine (0.25 mL) in 15 mL of dry 1,4 - dioxane. The reaction mixture was heated on a water bath for 3 to 4 hours allowed to cool at room temperature and dumped into crushed ice. The solid separated out was filtered, washed with water, dried and recrystallized from methanol.

Yield = 0.43 g (67 %)

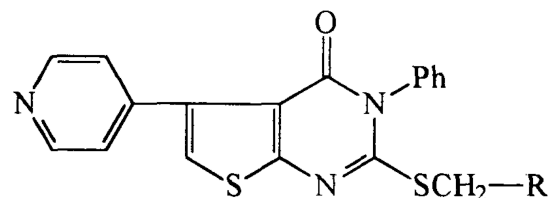
M.P. = 170-71°C

Elemental analysis of $C_{25}H_{16}FN_3OS_2$

	C%	H%	N%
Calculated	64.57	3.81	9.41
Found	64.40	4.10	9.15

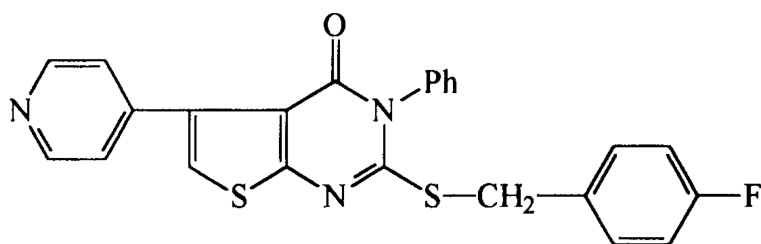
In a similar manner, all other 2-[(substituted benzyl)-thio]-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin- 4 (3H)-one have been synthesized and their physical data has been recorded in **Table 11**

Table 11 : 2-[(substituted benzyl)-thio]-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	4-Cl-C ₆ H ₄	C ₂₄ H ₁₆ ClN ₃ OS ₂	461	185-86	59	62.58	3.47	9.11	62.28	3.56	8.93
2	4-F-C ₆ H ₄	C ₂₄ H ₁₆ FN ₃ OS ₂	445	170-71	67	64.57	3.81	9.41	64.20	4.18	9.25
3	3,4-DiCl-C ₆ H ₃	C ₂₄ H ₁₅ Cl ₂ N ₃ OS ₂	496	208-10	48	58.06	3.02	8.46	57.87	3.10	8.43
4	4-OCH ₃ -C ₆ H ₄	C ₂₅ H ₁₉ N ₃ O ₂ S ₂	457	135-37	56	65.93	3.73	9.23	65.83	4.00	8.98
5	2-F-C ₆ H ₄	C ₂₄ H ₁₆ FN ₃ OS ₂	445	157-59	49	64.57	3.81	9.41	64.21	3.79	9.22

Spectral data of 2-[(4-fluorophenyl)-thio]-3-phenyl-5-(4-pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-one (Compound No. 2, Table 11) is given below.



IR (KBr) cm^{-1} : 1684 (-CO-); 1603 (C=N), 2965, 1451 (-CH₂-); 1510 (Thienyl) ; 3078, 1566, 1469, 1413 (Aromatic); 751, 670 (C-H Aromatic) ; 1160, 1019 (Pyridine), 1071 (C-F) ; 696 (S-C)

PMR (CDCl₃) δ : 4.3 (s, 2H, -CH₂-); 7 – 8.3 [m, 14H (9H, Aromatic; 1H, Thienyl; 4H, Pyridinyl)]

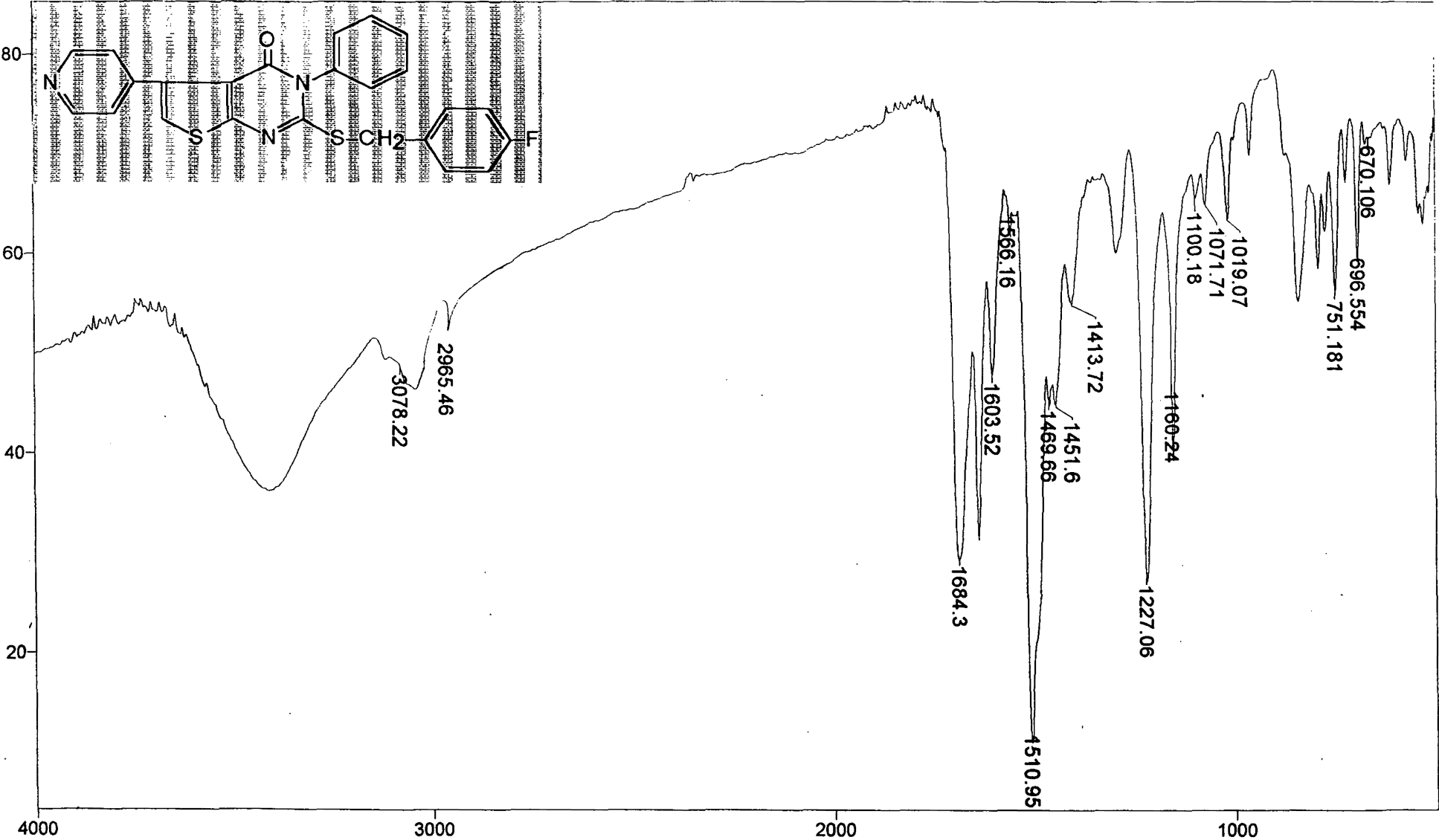
Sample Description: ft4ptp

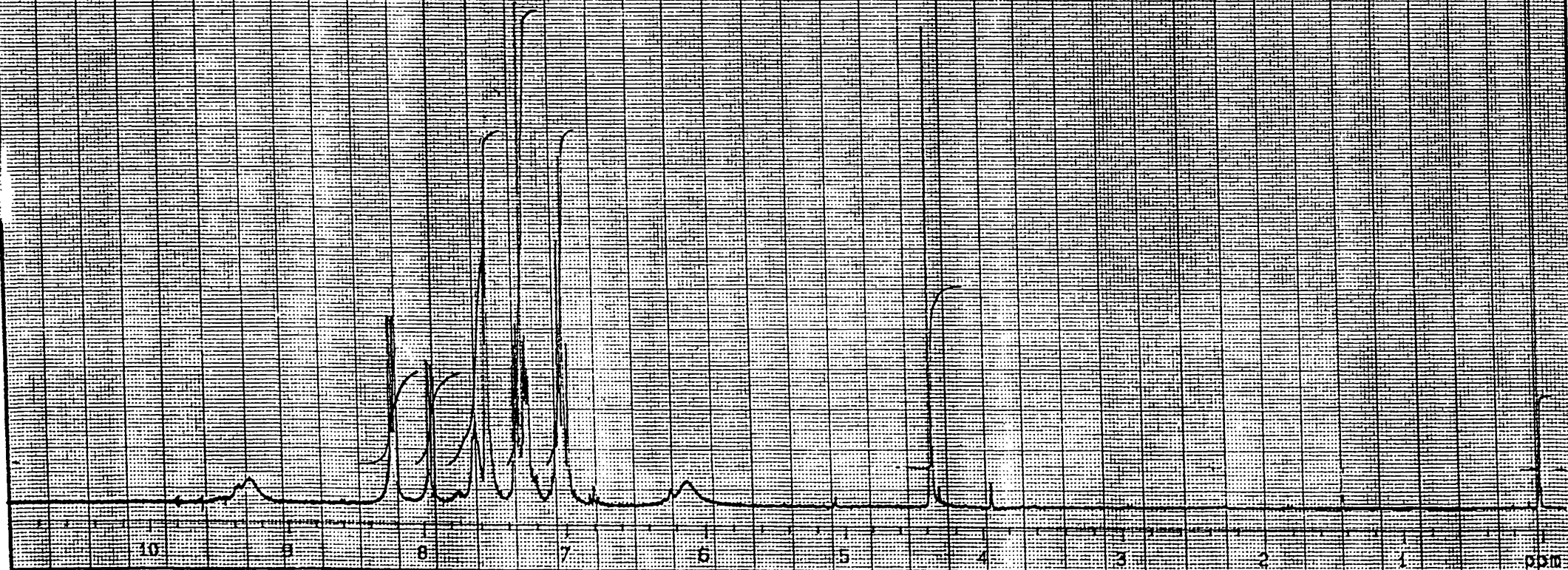
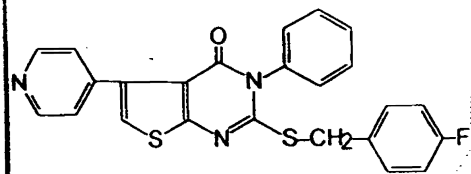
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





Part II-F

***Synthesis of 2-[(substituted
aryl/heteroaryl)-Sulfonamido] -5-
(3-pyridinyl)-thieno [2,3-d]
pyrimidin-4(3H)-ones***

This part of the thesis deals with the synthesis of 2-[(substituted aryl/heteroaryl)-sulfonamido]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones which involves the following steps

- 1) Synthesis of 2- amino-3- carbethoxy - 4-(3-pyridinyl) – thiophene*
- 2) Synthesis of 2- mercapto - 3 - phenyl- 5- (3- pyridinyl)-thieno [2,3-d] pyrimidin-4 (3H)-one*
- 3) Synthesis of the title compounds by condensation of 2 - sulfonyl chloride - 3 - phenyl- 5 (3- pyridinyl) thieno [2,3-d] pyrimidin-4 (3H)-ones with different substituted aryl / heteroaryl amines

- Synthesis has been described in **Part II-D.i**

3) General method for the synthesis of 2-[(substituted aryl/heteroaryl)-sulfonamido]-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones

Synthesis of 2-[(2-pyrimidine)-sulfonamido]-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one

Step I

In a 150 mL three necked round bottomed flask equipped with air condensor, chlorine gas inlet tube and stirrer, was placed a suspension of 2-mercapto-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one (1 g, 0.0028 M), concentrated hydrochloric acid (4ml), acetic acid (1ml) and water 25 mL.

Chlorine gas was bubbled into the suspension by maintaining the temperature between 8 to 10°C, with moderate stirring. Chlorine gas was passed for a period of one and half hours and the reaction mixture was filtered. The solid obtained was washed with ice cold water, dried and used immediately in the next step.

Step II

In a 25 mL conical flask was placed a mixture of 2-sulfonyl chloride-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one (0.5 g, 0.0012 M), 2-amino-pyrimidine (0.1 g, 0.001 M), and Triethylamine (0.1 mL) in dry acetone (25 mL). The mixture was stirred in the flask for 15 to 20 minutes. It was kept overnight and then filtered off to obtain a solid. The excess acetone was removed under reduced pressure to obtain a sticky mass, which was dissolved in water. The water layer was extracted with ethyl acetate, twice. The ethyl acetate layer was discarded and the water layer was evaporated. The white solid obtained was crystallized from methanol.

Yield = 0.22 g (40 %)

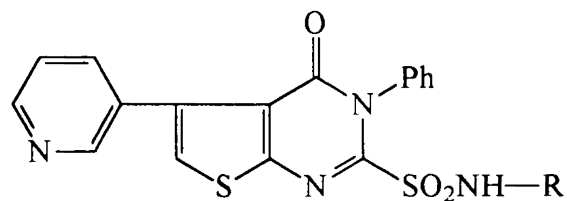
M.P. = 194-96°C

Elemental analysis of $C_{21}H_{14}N_6O_3S_2$

	C%	H%	N%
Calculated	54.54	3.0	18.18
Found	54.21	3.3	18.18

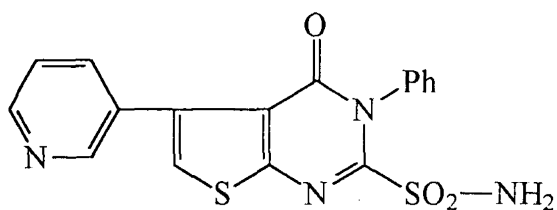
In a similar manner, all other 2-[(substituted aryl/heteroaryl)-sulfonamido]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones have been synthesized and their physical data has been recorded in **Table 12**

Table 12 : 2-[(substituted aryl/heteroaryl)-sulfonamido]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)	Elemental Analysis					
						% Calculated			% Found		
						C	H	N	C	H	N
1	H	C ₁₇ H ₁₂ N ₄ O ₃ S ₂	384	249-50	45	53.12	3.10	14.58	53.04	3.13	14.60
2	2-Pyrimidine	C ₂₁ H ₁₄ N ₆ O ₃ S ₂	462	194-96	40	54.54	3.00	18.18	54.21	3.30	18.18
3	3-methyl-2-pyrimidine	C ₂₃ H ₁₇ N ₅ O ₃ S ₂	475	165-68	41	58.10	3.57	14.73	57.85	3.49	14.60
4	4-methyl-2-pyrimidine	C ₂₃ H ₁₇ N ₅ O ₃ S ₂	475	196-99	47	58.10	3.57	14.73	57.90	3.57	14.50
5	2-CF ₃ , 4-NO ₂ -C ₆ H ₃	C ₂₄ H ₁₄ F ₃ N ₅ O ₅ S ₂	373	>300	50	50.20	2.40	12.21	49.96	2.12	12.42
6	3,4-DiCl-C ₆ H ₃	C ₂₃ H ₁₄ Cl ₂ N ₄ O ₃ S ₂	528	290-93	47	52.27	2.65	10.60	52.00	2.61	10.36
7	2,4-DiCl-C ₆ H ₃	C ₂₃ H ₁₄ Cl ₂ N ₄ O ₃ S ₂	528	>300	50	52.27	2.65	10.60	52.24	2.81	10.68
8	3,5-DiCl-C ₆ H ₃	C ₂₃ H ₁₄ Cl ₂ N ₄ O ₃ S ₂	528	>300	43	52.27	2.65	10.60	52.40	2.90	10.48

Spectral data of 2-sulfonamido-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-one (Compound No. 1, Table 12) have been shown below



IR (KBr) cm^{-1} : 3473, 3291, 1640 ($-\text{NH}_2$); 1697 ($-\text{CO}-$) 1592 ($\text{C}=\text{N}$), 1372, 1189, 1312, ($-\text{SO}_2-$); 3053, 1570, 1527, 1415 (Aromatic); 722, 664 ($\text{C}-\text{H}$ Aromatic) ; 1189, 1017 (Pyridine) ; 698 ($\text{S}-\text{C}$) ; 1503 (Thienyl)

PMR (CDCl_3) δ : 4.8 (s, 2H, $-\text{NH}_2$); 7.3 – 8.6 [m, 10H (5H, Aromatic ; 1H, Thienyl; 4H, Pyridinyl)]

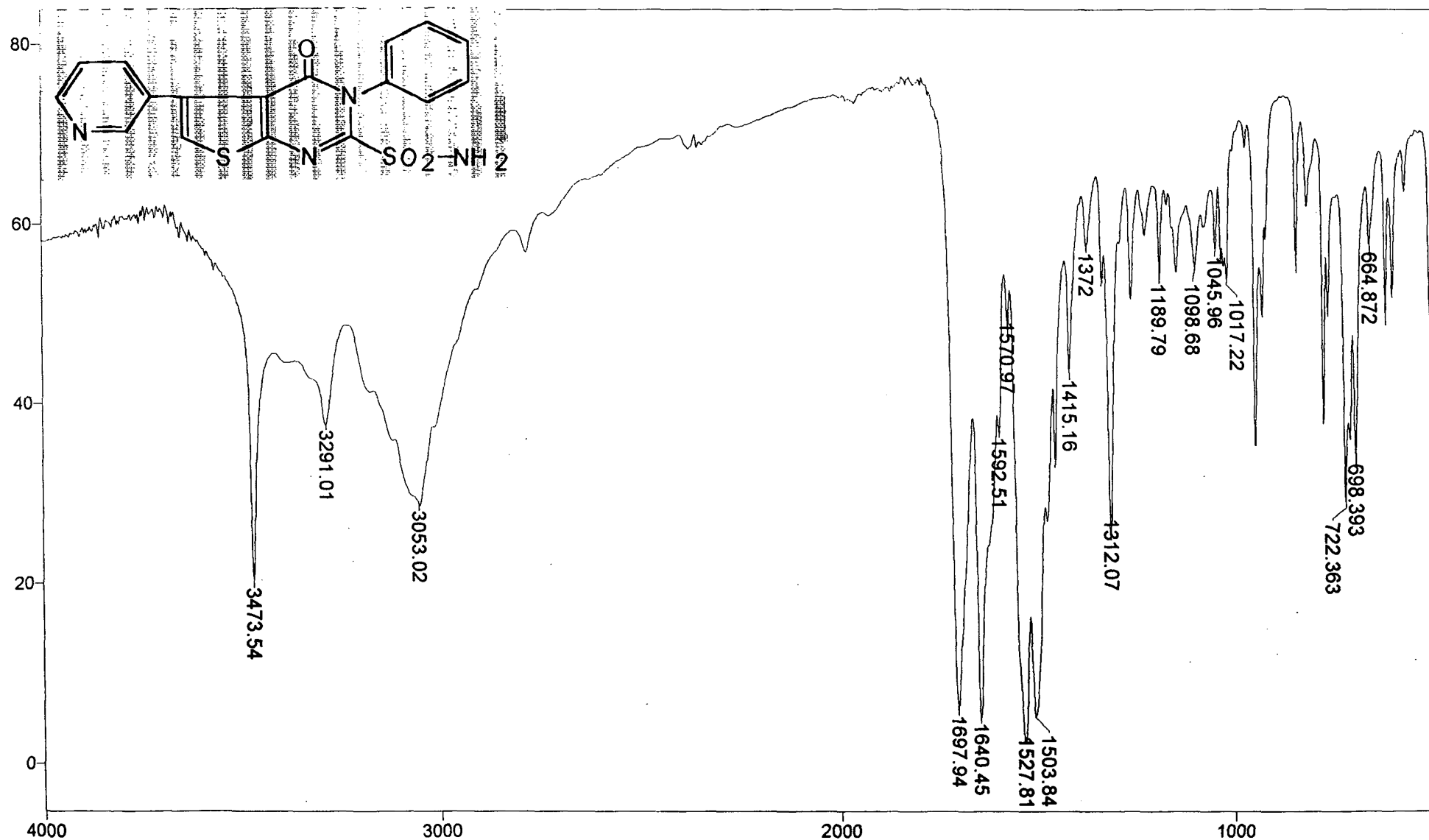
Sample Description: 3pyridinesulfonamide

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2433	2434	2435	2436	2437	2438	2439	2440	2441	2442	2443	2444	2445	2446	2447	2448	2449	2450	2451	2452	2453	2454	2455	2456	2457	2458	2459	2460	2461	2462	2463	2464	2465	2466	2467	2468	2469	2470	2471	2472	2473	2474	2475	2476	2477	2478	2479	2480	2481	2482	2483	2484	2485	2486	2487	2488	2489	2490	2491	2492	2493	2494	2495	2496	2497	2498	2499	2500	2501	2502	2503	2504	2505	2506	2507	2508	2509	2510	2511	2512	2513	2514	2515	2516	2517	2518	2519	2520	2521	2522	2523	2524	2525	2526	2527	2528	2529	2530	2531	2532	2533	2534	2535	2536	2537	2538	2539	2540	2541	2542	2543	2544	2545	2546	2547	2548	2549	2550	2551	2552	2553	2554	2555	2556	2557	2558	2559	2560	2561	2562	2563	2564	2565	2566	2567	2568	2569	2570	2571	2572	2573	2574	2575	2576	2577	2578	2579	2580	2581	2582	2583	2584	2585	2586	2587	2588	2589	2590	2591	2592	2593	2594	2595	2596	2597	2598	2599	2600	2601	2602	2603	2604	2605	2606	2607	2608	2609	2610	2611	2612	2613	2614	2615	2616	2617	2618	2619	2620	2621	2622	2623	2624	2625	2626	2627	2628	2629	2630	2631	2632	2633	2634	2635	2636	2637	2638	2639	2640	2641	2642	2643	2644	2645	2646	2647	2648	2649	2650	2651	2652	2653	2654	2655	2656	2657	2658	2659	2660	2661	2662	2663	2664	2665	2666	2667	2668	2669	2670	2671	2672	2673	2674	2675	2676	2677	2678	2679	2680	2681	2682	2683	2684	2685	2686	2687	2688	2689	2690	2691	2692	2693	2694	2695	2696	2697	2698	2699	2700	2701	2702	2703	2704	2705	2706	2707	2708	2709	2710	2711	2712	2713	2714	2715	2716	2717	2718	2719	2720	2721	2722	2723	2724	2725	2726	2727	2728	2729	2730	2731	2732	2733	2734	2735	2736	2737	2738	2739	2740	2741	2742	2743	2744	2745	2746	2747	2748	2749	2750	2751	2752	2753	2754	2755	2756	2757	2758	2759	2760	2761	2762	2763	2764	2765	2766	2767	2768	2769	2770	2771	2772	2773	2774	2775	2776	2777	2778	2779	2780	2781	2782	2783	2784	2785	2786	2787	2788	2789	2790	2791	2792	2793	2794	2795	2796	2797	2798	2799	2800	2801	2802	2803	2804	2805	2806	2807	2808	2809	2810	2811	2812	2813	2814	2815	2816	2817	2818	2819	2820	2821	2822	2823	2824	2825	2826	2827	2828	2829	2830	2831	2832	2833	2834	2835	2836	2837	2838	2839	2840	2841	2842	2843	2844	2845	2846	2847	2848	2849	2850	2851	2852	2853	2854	2855	2856	2857	2858	2859	2860	2861	2862	2863	2864	2865	2866	2867	2868	2869	2870	2871	2872	2873	2874	2875	2876	2877	2878	2879	2880	2881	2882	2883	2884	2885	2886	2887	2888	2889	2890	2891	2892	2893	2894	2895	2896	2897	2898	2899	2900	2901	2902	2903	2904	2905	2906	2907	2908	2909	2910	2911	2912	2913	2914	2915	2916	2917	2918	2919	2920	2921	2922	2923	2924	2925	2926	2927	2928	2929	2930	2931	2932	2933	2934	2935	2936	2937	2938	2939	2940	2941	2942	2943	2944	2945	2946	2947	2948	2949	2950	2951	2952	2953	2954	2955	2956	2957	2958	2959	2960	2961	2962	2963	2964	2965	2966	2967	2968	2969	2970	2971	2972	2973	2974	2975	2976	2977	2978	2979	2980	2981	2982	2983	2984	2985	2986	2987	2988	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Part III

Biological Results
and
Discussion

BIOLOGICAL RESULTS AND DISCUSSION

The compounds synthesized in the present investigation have been subjected to various biological screening programmes, based on their structural features, so as to ascertain their suitability as potential chemotherapeutic agents.

The various screening programmes carried out include the *in vitro* study against H₃₇R_v strain of *Mycobacterium tuberculosis* (M.T.) for estimating antitubercular activity and the *in vitro* study against various species of Gram-positive and Gram-negative bacteria, viz. *Staphylococcus aureus* ATCC 3750 and *Salmonella typhi* NCTC 786 for estimating antimicrobial activity.

Similarly, other screening programmes conducted have been in the *in vitro* study against various strains of fungi, viz. *Candida albicans*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

In antimalarial screening, the test compounds have been subjected to *in vivo* screening against *P. berghei* in albino mice.

The methodology adopted for the various biological screening programmes undertaken in this study has been described in the following section.

The test compounds (10mg) were dissolved in a suitable solvent such as dimethylformamide, ethanol, dilute HCl, as the case may be, so as to prepare a drug stock solution (1000 µg/mL). From this stock solution, following drug concentrations were made - 200, 100, 40, 20 and 10µg/mL. For lower drug concentrations, the drug stock solution was further diluted to obtain dilutions such as 5, 2.5, 1.25, 0.5, 0.2, 0.1 and 0.05 µg/mL etc.

Controls

- 1) Media control : Sterilised medium was kept as control to assure sterility of the medium. If this control shows growth, medium is not properly sterilised and in that case results are not valid.
- 2) Organism Control : It contains medium and organism. Organism should grow in the medium. If no growth is observed, it can be concluded that the medium is unable to support the growth.

- 3) Solvent control : This includes sterilised medium, solvent (used to dissolve the drug) and the culture of the organism, which should be able to grow in the quantity of solvent used in testing. If no growth is observed, it can be concluded that the solvent used to solubilise the drug inhibits the growth of the organism and in that case results of the test are not valid.

Antitubercular screening

The test compounds have been subjected to *in vitro* screening by the tube dilution technique employing the human virulent H₃₇R_V strain of *M. tuberculosis*. In this method, using Kirchner's medium containing Tween-80 as described by Rake *et al*²³⁶ dispersed growth of *M. tuberculosis* was obtained.

Procedure

Sterile Kirchner's medium was dispensed in each borosilicate test tube (150 x 20 mm) and to this 0.5 mL of sterile horse serum was added. The drug solution was added in each tube so as to attain a final drug concentration as 200, 100, 40, 20, 10, 5, 2, 1, 0.5, 0.25, 0.1, 0.05 µg/mL. Finally, the inoculum of standard suspension (0.1 mL) of *M. tuberculosis* (10⁶ bacilli per mL) was added. The tubes were incubated at 37°C for ten days and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as the end point, i.e. minimum inhibitory concentration (MIC). Isoniazid (INH) was used as a standard drug and was also subjected to similar screening pattern. Minimum inhibitory concentration of Isoniazid is 0.02-0.05 µg/mL.

Antibacterial screening

The test compounds have been subjected to *in vitro* screening by the tube dilution technique²³⁶ against various species of Gram-positive and Gram-negative bacteria viz. *S. aureus* and *S. typhi* using Muller-Hinton broth as the culture medium. Muller Hinton broth (sterilised) was dispensed in each borosilicate test tube (150 x 20 mm). The drug solution was added in order to attain a final drug concentration of 200, 100, 40, 20, 10, 5, 2, 1, 0.5, 0.25, 0.1, 0.05 µg/mL etc. The inoculum of standard suspension (0.1ml) of the desired bacterial strain (*S. aureus* or *S. typhi*) containing 10⁶ bacilli per mL was added to the tube. The tubes were incubated at 37°C for 24 hrs. and then examined for the presence or absence of growth of the test organisms.

The lowest concentration which showed no visible growth was taken as an end point (MIC).

Ampicillin and Trimethoprim were used as standard drugs against Gram -positive and Gram-negative bacteria, respectively, and were subjected to similar screening program.

MIC of Ampicillin is 0.01 µg/mL against *S. aureus* and that of Trimethoprim is 1 µg/mL against *S. typhi*.

Antifungal screening

The organisms employed in the test were 1) *Candida albicans* 2) *Trichophyton rubrum* 3) *Trichophyton mentagrophytes*. These organisms were maintained on Saboraud's agar slants by regular subculturing. *C. albicans* was cultured every week and the rest of the organisms at the interval of 12 days.

Test medium:

The liquid medium described by Stokes *et al*²³⁷ was used for screening the compounds for their antifungal activity. The final pH of the medium was adjusted to 5.2. The medium was dispensed in each borosilicate tube (150 x 20 mm) and to these were added the requisite test solution in graded concentrations varying from 200 to 0.5 µg/mL.

Preparation of inoculum

Candida albicans

A 48 hours old growth on Sabouraud's agar slant was suspended in 4-5 mL of the test medium with the help of platinum loop. The cell count of the suspension (yeast phase) was carried out using Neubaur chamber and then diluted with requisite volume of the medium to give a final concentration of 5×10^6 cells per mL.

T. rubrum and *T. mentagrophytes*

To 12 to 19 days old growth on Sabouroud's slant, 5 mL of sterile test medium and 3-4 sterile glass beads (3 mm diameters) were added and gently shaken for 10 minutes. The supernatant was decanted into another sterile tube and the count of spores in the suspension was carried out using Neubaur chamber. The final strength of the inoculum was adjusted to 5×10^6 spores per mL, using the test medium.

Screening procedure:

To each test tube was then added 0.1 mL of standardised inoculum of the test organism. The tubes were then incubated at room temperature (28-30°C) in a dark place. The presence or absence of growth was noted visually after 7 days in case of *Candida albicans* and after 14 days in case of *T. rubrum* and *T. mentagrophytes*.

Miconazole was used as the standard drug and was subjected to similar screening pattern. MIC of Miconazole is 6.25 µg/mL against *C. albicans* and 1.56 µg/mL against *T. mentagrophytes* and *T. rubrum*.

Antimalarial screening

The first isolation of the rodent malaria was reported by Vincke and Lips²³⁸. Thereafter many species and subspecies of rodent malarias have been isolated and divided into two groups, the Berghei group and the Vincke group. The group used for the majority of chemotherapy research projects is the berghei group.

Some compounds were screened for their antimalarial activity employing rodent malaria strain of *P. berghei* in mice (Haffkine strain, weighing 22-25 gms) *in vivo* by Rane's preliminary rapid screening method²³⁹. Pyrimethamine was used as the standard drug for comparison.

The rodent *Plasmodium* model is considered to be the most important tool in the primary phase of the search for better antimalarial drugs. Its unparalleled simplicity and convenience helps in the screening of a large number of compounds.

To test the blood schizontocidal activity in the rodent malaria system, two tests were mentioned in a report by WHO. Scientific Group²⁴⁰ (WHO 1973)

The tests were

- a) Evaluation of activity by assessment of survival time : Rane test for blood schizontocidal screening²³⁹.
- b) Evaluation of activity by means of parasite counts : The 4-day suppressive test using *P. berghei* (in Swiss mice) modified by Thurston²⁴¹.

The *P. berghei* infection in laboratory mice is the most economically feasible approach, since it acts as a primary and secondary screen before monkey malaria

screens. Hence, the *P. berghei* strain maintained in Haffkine Institute was used for this study.

Materials and Methods

Strain

Plasmodium berghei used for the study was obtained from Hindustan Ciba-Geigy, Mumbai. The strain was maintained by regular passage in mice.

Survival Time (Δ MST) :

This period was determined from the day the mouse was inoculated to the day it was found dead. Mean survival time was calculated at each dose level by subtracting the average mean survival time of control mice (MSTC) from the average mean survival time of treated mice (MSTT) i.e.

$$\text{MSTT} - \text{MSTC} = \Delta \text{MST}$$

Experimental Chemotherapy of Rodent Malarial Infection

Susceptibility of *P. berghei* strain to standard antimalarial agents :

This was confirmed by Rane's single dose test and Peter's 4 days suppressive test. Pyrimethamine was used to study the pathogenicity of the *P. berghei* maintained in the laboratory.

Pyrimethamine at 20 mg/kg (of the body weight) gave curative results in a single subcutaneous dose. The compounds were prepared and screened at the dose levels of 160, 80, 40 and 20 mg/kg.

Methodology

Animal model : Rodent malaria, white Swiss male of Haffkine strain weighing 22.25 g, average.

Primary screen : involved the recording of survival time

Secondary screen : Involved the evaluation of activity by means of parasite count.

Rane's test for blood Schizontocidal activity

Three to four week old male mice weighing 22-25gm were housed in plastic cages with metal lids, and given pelleted food and water *ad libitum*. The animals were infected with 0.1 mL of citrated heart blood, containing 1 million parasitized R.B.C.s.

The title compounds were suspended in distilled water by addition of 6-7 drops of Tween-80. A single dose was given subcutaneously, 72 hours after infection. A control group of 5 mice, which was not administered any drug, was kept as untreated control. The dose range selected was 160, 80, 40 and 20 mg/kg. Pyrimethamine was taken as the standard drug for comparison.

Observations and Results

1. Untreated infected animals died within 6 to 7 days (average). The survival time of these control animals varied with every batch of experiment.
2. The animals administered with the drug and did not recover from infection were observed till death.
3. The drug which enhanced the survival time (twice the period of untreated control mice) was considered to be active.
4. The smallest dose at which the survival time was doubled was considered minimum effective dose (MED)
5. Deaths within 48 hours after administration of compounds were considered toxic(T).

In case of those compounds, which showed activity, the animals were found to be normal and healthy, unlike untreated mice.

RESULTS AND DISCUSSION

PART II-A

2-methyl - 3N - [(substituted aryl / heteroaryl) - methylene] - amino - 5 - (2-furyl / 2-thienyl) - thieno[2,3-d]pyrimidin-4(3H)-ones

In this series a total of 22 compounds have been synthesized. The screening results exhibit the minimum inhibitory concentration (MIC) against Gram-positive bacteria in the range of 0.1-100 µg/mL. Compounds no 6, 7 and 12 showed most significant activity, while the rest of the compounds have shown activity upto 100µg/mL against *S. aureus* (Table 13). Compound no 6 and 10 showed moderate activity against *S. aureus*.

Against Gram-negative bacteria, most of the compounds have shown promising activity in the range of 0.1 – 4 µg/mL. Compounds no. 3, 5, 7, 8, 9, 10 and 11 are the most active agents against *S. typhi* at concentrations of 4, 1, 2, 0.1, 4, 4, and 0.4 µg/mL respectively (Table 13), while compounds no 1, 3, 4, 6 and 10 are active against *S. typhi* at concentrations of 0.1, 1, 1, 0.1 and 1 µg/mL respectively (Table 14). Other compounds from Table 13 and 14 exhibit moderate activity against *S. typhi*.

In anti-tubercular screening, it was observed that compounds (Table 13) are active against H₃₇R_v strain of *Mycobacterium tuberculosis* at the concentration of 4-100 µg/mL. Compound No. 12 is the most active agent (4 µg/mL). Similarly, compound no. 6 and 10 (Table 14) showed anti-tubercular activity at a concentration of 4µg/mL. The remaining compounds from Table 13 and 14 showed moderate activity against *Mycobacterium tuberculosis*.

Twelve compounds from **Part II-A** were screened for antimalarial activity against *P. berghei*. Only compound no. 2 (Table 24) was found to be active at a concentration of 160mg/kg. The rest of the compounds were found to be inactive.

All the compounds listed in **Part II-A** have been evaluated for their *in vitro* antifungal activity against *C. albicans*, *T. rubrum* and *T. mentagrophytes*, but unfortunately none of the compounds have shown activity even at 200µg/mL.

Compound No. 6 (0.1 µg/mL) was the most active compound in this series for antibacterial activity against Gram-positive bacteria (Table 13), where trifluoromethyl group is present at 2-position of the phenyl ring. A drastic reduction in antibacterial activity against *S. aureus* has been observed when trifluoromethyl group is present at 4-position of the phenyl ring (Table 13 compound no. 7).

Against gram-negative bacteria, Compound no. 8 (Table 13), where electron releasing –OH group is present at 4-position of the phenyl ring, was found to be active at a concentration of 0.1µg/mL. Compound no. 11 (Table 13), having 2-thienyl group directly attached to the azomethine linkage, was found to be active at 0.4µg/mL, but introduction of Br atom on thienyl ring (compound no 12) reduced antibacterial activity against *S. typhi*. Compound no. 5 (Table 13) having trifluoromethyl group at 4-position and compound no. 7 (Table 13) having fluoro group at 2-position and trifluoromethyl group at 4-position of phenyl ring exhibited inhibiting activity at a concentration of 1µg/mL and 2µg/mL.

The antibacterial screening results of compounds listed in Table 14, against Gram-negative bacteria, show an interesting trend based on the various substitutions carried out on the phenyl ring. The 4-Cl substituted Compound no. 1 and 2-fluoro-4-trifluoromethyl substituted Compound no. 6, have exhibited highest activity at 0.1µg/mL, which is because of the electron withdrawing effect of the halogen. Also, compound no. 3 and 4 (Table 14) having fluoro and trifluoromethyl group at 4-position, respectively exhibited highest activity at 1µg/mL. On this basis, an inference may be drawn that an electron-withdrawing group, such as halogen at the 4-position, favours activity.

Compound no. 10 (Table 14), in which electron releasing group is at 1-position of the pyrrole ring, exhibits good antibacterial activity against gram-negative bacteria. Also, it has significant antitubercular activity at 4µg/mL.

Part II-B

2-methyl-3N- [(substituted aryl / heteroaryl)- carboxamido]-5-(2-furyl / 2-thienyl) - thieno [2,3-d] pyrimidin -4(3H)-ones

In this series, a total of 22 compounds have been synthesized and screened against *S. aureus* and *S. typhi*. The results once again were very interesting. Compounds no. 1, 2, 5, 7 were found to be active at a concentration of 0.1 µg/mL against *S. aureus* (Table 15), whereas Compounds no. 4, 8 and 10 were found to be active at 4 µg/mL. The rest of the compounds are active upto 100 µg/mL against the same organism. In Table 15, 4-Cl substituted compound (Compound no. 1) exhibited the highest activity at 0.1 µg/mL, while 3,4-dichloro-substituted compound (Compound no. 3) showed moderate activity at 100 µg/mL, which suggested that for activity against *S. aureus* Cl-atom should be present at 4-position of the phenyl ring. In Table 15, Compounds no. 2 and 7, have exhibited activity at a concentration of 0.1 µg/mL against *S. aureus*, while compound no. 4 and 6 show less activity at 4 µg/mL and 100 µg/mL, respectively. This indicated that F-atom when present at 2,6 and 3 position of the phenyl ring enhances the activity of parent moiety, but when it is introduced at 4-position, the compound shows drastic reduction in activity.

Also compound no. 5 (Table 15) having electron releasing group -OCH₃ at 4-position of the phenyl ring, shows highest activity against *S. aureus* at 0.1 µg/mL. Other heterocyclic substituents did not show any significant activity.

On this basis, an inference may be drawn that not only electron donating group, but also electron releasing group favours the biological activity of the compound.

In Table 15, Compounds no. 3, 4 and 11, which are less active against *S. aureus* are found to be most active against *S. typhi* at a concentration of 0.1, 0.1 and 0.4 µg/mL, respectively. Other compounds no. 1, 2, 5, 6, 7, 9 and 10 are active at a concentration of 4-10 µg/mL against *S. typhi*.

Out of 11 compounds, three compounds, viz. 6,7 and 11 (Table 15), have exhibited MIC at 0.05 µg/mL against H₃₇R_v strain of *Mycobacterium tuberculosis*, in which compounds 6 and 7 have an electron-withdrawing F-substituent at their 4- and 3-position, respectively. Compound no 11 contains an electron-rich thienyl moiety

directly attached to the amide linkage. Other compounds such as compound no. 5, in which electron releasing $-OCH_3$ substituent is present at 4-position of the phenyl ring was found to be active at $1\mu\text{g/mL}$, and compound no 9 bearing a cyclopropane moiety directly attached to the amide linkage was found to be active at $0.1\mu\text{g/mL}$ against *M. tuberculosis*. Compound no. 2 & 3 show activity at a concentration of $4\mu\text{g/mL}$ against *M. tuberculosis*. Hence, the antitubercular activity of the compounds is mostly governed by the electron-withdrawing and releasing groups and heterocycles such as thienyl and cyclopropane.

Out of 11 compounds (Table 15), 6 compounds were screened for their antimalarial activity against *P. berghei*. Compounds no. 8, 9 and 10 (Table 25) were found to be active at 160 mg/kg and had an electron withdrawing or electron releasing group substituted on the phenyl ring. The rest of the compounds were inactive against *P. berghei*.

In Table 16, Compounds no. 2, 3 and 8 were found to be active at a concentration of 0.1 , 0.2 and $0.1\mu\text{g/mL}$, respectively, while compound no 1, 7 and 9 were found to be active at a concentration of $4\mu\text{g/mL}$ against *S. aureus*. The remaining compounds were active at concentrations between 100 - $200\mu\text{g/mL}$ against *S. aureus*. Compounds having electron-withdrawing group at 3- and 4- position of the phenyl ring and cyclohexane, cyclopropane, morpholine moiety attached to the amide linkage were the active members against *S. aureus*. Against *S. typhi*, compound no. 4, 7 and 10 have highest activity at concentration of 0.1 , 0.4 and $0.1\mu\text{g/mL}$, respectively. Compounds no. 1, 2, 3, 6, 8, 9 and 11 have moderate activity in the range of 1 - $10\mu\text{g/mL}$, but compound no. 5 which has an electron releasing group on the phenyl ring, shows drastic reduction in the activity against *S. typhi*. The above results indicate that electron-withdrawing group and heterocycles such as cyclohexane, cyclopropane, morpholine, furyl and thienyl favour the antibacterial effect, while electron-releasing groups reduce activity against *S. typhi*.

All the compounds listed in Table 16 were screened for their antitubercular activity against H₃₇R_v strain of *M. tuberculosis*. Compound no. 9 and 10 having a morpholine and furyl moiety attached to the amide linkage, respectively, were found to be the most active compounds at a concentration of $0.05\mu\text{g/mL}$. Compound no. 7, having cyclohexane moiety attached to the amide linkage showed activity at $4\mu\text{g/mL}$. All

other compounds from Table 16 did not show any significant antitubercular activity. It seems that antitubercular activity in this series appears to be governed by the cyclopropane, morpholino and furyl heterocycles, which are attached through the amide linkage to the parent moiety.

All the compounds listed in Table 16 were found to be inactive at a concentration of 200µg/mL against *C. albicans*, *T. mentagrophytes* and *T. rubrum*.

Out of 11 compounds (Table 16), 7 compounds were screened for their antimalarial activity against *P. berghei*. In Table 25, compounds no 3,4 and 5 were found to be active up to 80mg/kg. Compound no 3 has an electron-withdrawing group at 4-position of the phenyl ring, while compounds no. 4 & 5 have cyclopropane and furyl moiety attached through the amide linkage to the parent moiety.

In general, the activity exhibited by these compounds (**Part II-B**) against *S. aureus*, *S. typhi* and *M. tuberculosis* and against the malarial parasite, *P. berghei*, is of most significant nature.

Part II-C

2-methyl-3-[3-ethyl-4(substituted aryl/hetroaryl)-2,3 dihydrothiazolidine-amino] -5-(2-furyl/2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones

In this series, a total of 12 compounds have been synthesized and screened against *S. aureus* and *S. typhi*. The screening results (Table 17) exhibit that the minimum inhibitory concentration (MIC) against *S. aureus* is in the range of 10-100 µg/mL. Only compound no. 5 (Table 17) showed the MIC upto 10 µg/mL, since it has a trifluoromethyl group substituted on the phenyl ring. Other compounds showed moderate activity against *S. typhi*, in the range of 2-40µg/mL. Compounds no. 1,2 and 5 (Table 17), which have electron-withdrawing group attached to the phenyl ring, are the most active against *S. typhi*.

All the compounds listed in Table 17 were screened for their antitubercular activity against H₃₇R_v strain of *M. tuberculosis*. None of the compounds showed promising activity. Similarly, these compounds were screened for their antifungal activity against *C. albicans*, *T. mentagrophytes* and *T. rubrum*, but they were found to be inactive at a concentration of 200µg/mL.

Compounds no. 5,6,7 and 8 (Table 26) were screened for their antimalarial activity against *P. berghei*. None of these compounds were found to be active.

The antibacterial screening of compounds listed in Table 18 against *S. aureus* and *S. typhi* showed that Compound no 2 is the only active member of the series against *S. aureus* with MIC of 4µg/mL. Compound no. 1,2 and 4 (Table 18) showed activity at concentration of 1µg/mL against *S. typhi*. All these compounds have an electron-withdrawing group present on the phenyl ring. All the compounds were also screened for their antitubercular activity against H37Rv strain of *M. tuberculosis*. Only compound no. 1 (Table 18) was found to be active at a concentration of 10µg/mL.

Four compounds (Table 26) were screened for their antimalarial activity but all were found to be inactive against *P. berghei*.

In general, it can be inferred from the biological screening results that these compounds do not possess any significant biological activity.

Part II-D

2-[(substituted aryl / heteroaryl)-thioketomethylene]-3-phenyl-5-(3-pyridinyl/4-pyridinyl) -thieno [2,3-d] pyrimidin-4(3H)-ones

In this series a total of 19 compounds have been synthesized and screened for their biological activity. Among all the series, maximum compounds from this series showed significant antitubercular activity against *M. tuberculosis*. Compounds no.1 and 5 (Table 19) showed activity at a concentration of 0.1 and 4 µg/mL, respectively. Other compounds from Table 19 are active only at 100 µg/mL against *S. aureus*. However, against *S. typhi*, compounds from Table 19 show variable results. Compounds no. 3,5,7 and 9 were found to be active at 0.1µg/mL, while compound no. 8 was active at 1µg/mL. The remaining compounds from Table 19 showed moderate activity. Against *S. typhi*, compounds bearing a halogen substituent on phenyl ring and heterocycle exhibits enhanced activity. Compounds no. 4 and 10 (Table 19) having a trifluoromethyl group and nitro group at 3-position of the phenyl ring, respectively, were found to be inactive against Gram-positive and Gram-negative bacteria.

All the ten compounds from Table 19 have been screened for *in vitro* antitubercular activity against H₃₇R_v strain of *M. tuberculosis*. Compounds no. 2, 3, 5, 6, 7 and 9 were found to be the most active compounds at the concentration of 0.05µg/mL against *M. tuberculosis*. These compounds bear a phenyl ring substituted by electron-withdrawing group, unsubstituted phenyl ring, substituted thienyl ring, and cyclohexane ring which is attached through thioketomethylene linkage to the parent moiety. Other compounds showed moderate activity against *M. tuberculosis*.

Four compounds from Table 19 have been screened for antimalarial activity. Only compound no 4 (Table 27) was found to be active at 160mg/kg against *P. berghei*. The rest of the compounds were inactive.

Compounds from Table 20 have been screened for their antibacterial activity against *S. aureus* and *S. typhi*. Against *S. aureus*, none of the compounds, except compound no. 9 (MIC 4µg/mL), have shown any significant activity. Against *S. typhi*, compounds no. 4, 5 and 7 showed significant activity at a concentration of 1µg/mL, while others were active at 10µg/mL. Here, compounds containing electron-withdrawing as well as electron releasing group on the phenyl ring, appear to be active against *S. typhi*.

All these compounds have been screened for their antitubercular activity against H₃₇R_v strain of *M. tuberculosis*. Compounds no. 1, 2, 3 and 8 (Table 20) showed most significant activity at a concentration of 0.05µg/mL. These compounds contain a substituted or unsubstituted phenyl ring and substituted thienyl ring attached through thioketomethylene linkage to the parent moiety. The substituents are electron-withdrawing halogen groups. Compounds no. 6 and 9 (Table 20) were found to be active at 0.4µg/mL and 1µg/mL, respectively.

Antitubercular activity in this series (**Part II-D**) appears to be governed by the halogen groups, which are attached to the phenyl ring and /or thienyl ring and play a vital role as regards antitubercular activity.

All the compounds from the series (**Part II-D**) were screened for their antifungal activity against *C. albicans*, *T. mentagrophytes* and *T. rubrum*, but unfortunately all

the compounds were found to be active at the concentration of 200µg/mL, which is not really significant.

Eight compounds from Table 20 have been screened for their antimalarial activity. Only compound no. 12 (Table 27) was found to be active at 160mg/kg, while the rest of the compounds were inactive against *P. berghei*.

Part II-E

2-[(substituted benzyl)-thio] – 3 - phenyl-5-(3-pyridinyl/4-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones

In this series, a total of 11 compounds were synthesized (Table 21 & 22) and evaluated for their antibacterial activity against Gram-positive and Gram-negative bacteria. Compounds no. 1 and 3 (Table 21) have exhibited good activity against *S. aureus* at a concentration of 2µg/mL and 0.1µg/mL, respectively. Both of these compounds bear an electron-withdrawing group on the phenyl ring which is attached to the parent moiety through a thio linkage. Compound No. 3 having two chlorine atoms at 3- and 4- position of the phenyl ring, was found to be more potent than compound no. 1 which has a single chlorine atom at 4-position of the phenyl ring. This indicates that when two chlorine atoms are present on the phenyl ring instead of one, there is an increase in activity against *S. aureus*. The rest of the compounds from Table 21 have not shown significant antibacterial activity against *S. aureus*.

Against *S. typhi*, compounds from Table 21 have not shown any promising activity. Here, compounds bearing an electron-withdrawing as well as electron-releasing substituent on the phenyl ring were found to be active up to 10µg/mL (Compound no. 1, 2, 5 and 6).

All six compounds from Table 21 have been screened for their antitubercular activity against H₃₇R_v strain of *M. tuberculosis*. Here, Compound no. 6 was found to be the most active at a concentration of 1µg/mL. Compounds no. 3 and 4 were active at 4µg/mL, while Compounds no. 4 and 5 were found to be active at 100 and 10 µg/mL, respectively. From the above results, it is clear that not only electron-withdrawing but also electron-releasing substituents, present on the phenyl ring are important for antitubercular activity.

None of the compounds from Table 21 were found to be active at 200µg/mL against *C. albicans*, *T. mentagrophytes* and *T. rubrum*.

Five compounds from Table 21 were screened for their antimalarial activity. Only Compound no. 5 (Table 28) was found to be active at 160 mg/kg, while remaining four compounds were found to be inactive against *P. berghei*. Compound no. 5 bears an electron-withdrawing trifluoromethyl group at 4-position of the phenyl ring.

Similarly, all the compounds from Table 22 have been screened against *S. aureus*. Compounds no. 3 and 5 have shown activity at 0.1µg/mL, while compounds no. 1 and 4 have shown activity at 4 and 2 µg/mL and compound no. 2 has shown activity at 100µg/mL.

Against *S. typhi*, four compounds from Table 22 have shown significant activity in the range of 0.1 – 0.2 µg/mL, while only compound no. 1 was active at 10µg/mL. All these four compounds (compound nos. 2 to 5) bear electron-withdrawing and electron-releasing substituents on the phenyl ring.

All the compounds from Table 22 have been screened against H₃₇R_v strain of *M. tuberculosis*. Compounds no. 1, 2, 3 and 5 have shown good activity at concentrations of 10, 5, 2 and 2 µg/mL, respectively. All these four compounds bear an electron-withdrawing substituents on the phenyl ring, which is attached to the parent moiety through thio linkage. Only Compound no. 4, bearing an electron-releasing substituent on the phenyl ring, has shown activity up to 20µg/mL, which is ten times less as compared to compound nos. 3 and 5. It indicates that only electron-withdrawing substituents on the phenyl ring favour the antitubercular activity against *M. tuberculosis*.

All the five compounds from Table 22 were found to be inactive when screened for their antifungal activity against *C. albicans*, *T. mentagrophytes* and *T. rubrum*.

Three compounds from Table 22 were screened for their antimalarial activity. Only compound no. 7 (Table 28) was found to be active at 160mg/kg against *P. berghei*.

Part II-F

2-[(substituted aryl / heteroaryl)-sulfonamido]-3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones

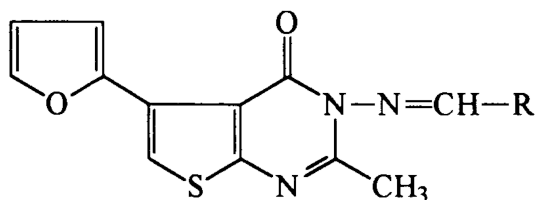
In this series a total of eight compounds have been synthesized (Table 23) and screened for their antibacterial activity against *S. aureus* and *S. typhi*. Compound no. 1 (Table 23) was found to be active against *S. aureus* at a concentration of 4 µg/mL. The rest of the compounds from Table 23 have not shown significant antibacterial activity against *S. aureus*.

Against *S. typhi*, Compound no.2 (Table 23) has shown activity up to 0.1µg/mL, while Compound no. 1 and 5 has shown activity at 10 and 4µg/mL, respectively. The remaining five compounds were found to be active at 100µg/mL, which is not very significant.

All the eight compounds (Table 23) have been screened for their antitubercular activity against H₃₇R_v strain of *M. tuberculosis*. Amongst all, Compound no. 1 was found to be the most active with MIC of 0.05 µg/mL. The remaining compounds from Table 23 have not shown any significant activity against *M. tuberculosis*. This indicates that a sulfonamido group at 2-position of the parent moiety is essential for antitubercular activity; any modification of this group results in a drastic reduction in antitubercular activity.

Two compounds from Table 29 were screened for antimalarial activity, but unfortunately both were found to be inactive against *P. berghei*.

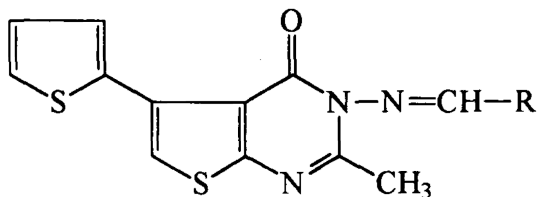
Table 13 : 2-methyl - 3N - [(substituted aryl / heteroaryl) - methylene] - amino – 5-(2- furyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	C ₆ H ₅	100	10	-	-	-	100
2	4-Cl-C ₆ H ₄	100	40	-	-	-	10
3	4-F-C ₆ H ₄	100	4	-	-	-	10
4	4-NO ₂ -C ₆ H ₄	100	10	-	-	-	10
5	4-CF ₃ -C ₆ H ₄	100	1	-	-	-	40
6	2-CF ₃ -C ₆ H ₄	0.1	10	-	-	-	40
7	2-F, 4-CF ₃ -C ₆ H ₃	10	2	-	-	-	10
8	4-OH-C ₆ H ₄	100	0.1	-	-	-	10
9	3-Pyridine	100	4	-	-	-	40
10	N-methyl-pyrrole	100	4	-	-	-	40
11	2-Thienyl	100	0.4	-	-	-	40
12	5-bromo-thienyl	4	10	-	-	-	4

- → inactive at 200 µg/mL

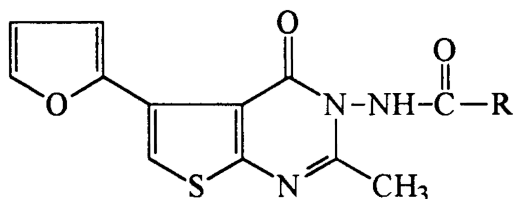
Table 14 : 2-methyl - 3N - [(substituted aryl / heteroaryl) - methylene] - amino - 5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	4-Cl-C ₆ H ₄	100	0.1	-	-	-	20
2	4-NO ₂ -C ₆ H ₄	100	40	-	-	-	40
3	4-F-C ₆ H ₄	100	1	-	-	-	40
4	4-CF ₃ -C ₆ H ₄	100	1	-	-	-	40
5	4-OH-C ₆ H ₄	100	10	-	-	-	20
6	2-F, 4-CF ₃ -C ₆ H ₃	10	0.1	-	-	-	4
7	2-OH, 5-Br-C ₆ H ₃	100	100	-	-	-	40
8	3-pyridine	100	10	-	-	-	10
9	2-thienyl	100	40	-	-	-	20
10	N-methyl-pyrrole	20	1	-	-	-	4

- → inactive at 200 µg/mL

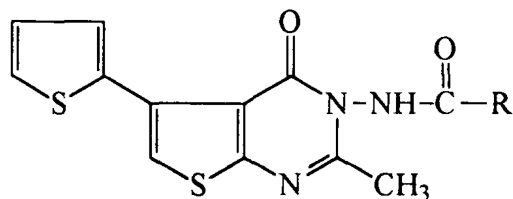
Table 15 : 2-methyl - 3N - [(substituted aryl / heteroaryl) - carboxamido] - 5-(2-furyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	4-Cl-C ₆ H ₄	0.1	10	-	-	-	40
2	2-F, 6-F-C ₆ H ₃	0.1	10	-	-	-	100
3	3-Cl, 4-Cl-C ₆ H ₃	100	0.1	-	-	-	4
4	2-F-C ₆ H ₄	4	0.1	-	-	-	4
5	4-OCH ₃ -C ₆ H ₄	0.1	10	-	-	-	1
6	4-F, C ₆ H ₄	100	10	-	-	-	0.05
7	3-F, C ₆ H ₄	0.1	10	-	-	-	0.05
8	Cyclohexane	4	100	-	-	-	100
9	Cyclopropane	100	4	-	-	-	0.1
10	2-furyl	4	4	-	-	-	100
11	2-thienyl	100	0.4	-	-	-	0.05

- → inactive at 200 µg/mL

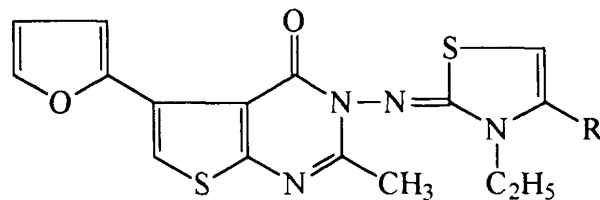
Table 16 : 2-methyl - 3N - [(substituted aryl / heteroaryl) - carboxamido] - 5-(2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	4-Cl-C ₆ H ₄	4	10	-	-	-	20
2	3-Cl, 4-Cl-C ₆ H ₃	0.1	4	-	-	-	40
3	4-F-C ₆ H ₄	0.2	10	-	-	-	100
4	2-F, 6-F-C ₆ H ₃	100	0.1	-	-	-	100
5	4-OCH ₃ -C ₆ H ₄	100	40	-	-	-	100
6	2-F-C ₆ H ₄	200	4	-	-	-	200
7	Cyclohexane	4	0.4	-	-	-	4
8	Cyclopropane	0.1	1	-	-	-	10
9	4-morpholino	4	4	-	-	-	0.05
10	2-furyl	100	0.1	-	-	-	0.05
11	2-thienyl	100	4	-	-	-	200

- → inactive at 200 µg/mL

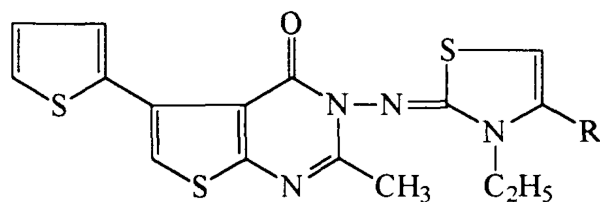
Table 17 : 2-methyl - 3- [3-ethyl-4-(substituted aryl / heteroaryl)- 2,3-dihydrothiazolidene amino]- 5- (2-furyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	2,4-DiCl, 5-F C ₆ H ₂	100	4	-	-	-	100
2	3-NO ₂ -C ₆ H ₄	40	4	-	-	-	100
3	5-Cl-thienyl	100	10	-	-	-	100
4	4-OCH ₃ -C ₆ H ₄	100	40	-	-	-	200
5	3-CF ₃ -C ₆ H ₄	10	2	-	-	-	100
6	4-F-C ₆ H ₃	100	10	-	-	-	100

- → inactive at 200 µg/mL

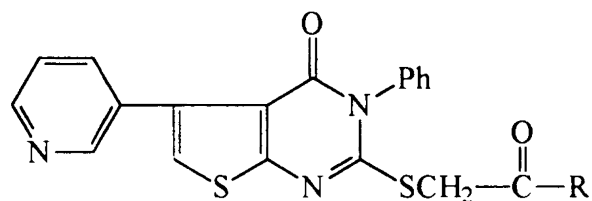
Table 18 : 2-methyl - 3- [3-ethyl-4-(substituted aryl / heteroaryl)- 2,3-dihydro thiazolidene amino] 5- (2-thienyl)-thieno [2,3-d] - pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	2,4-DiCl, 5-FC ₆ H ₂	100	1	-	-	-	10
2	5-Cl-Thienyl	4	1	-	-	-	200
3	4-OCH ₃ -C ₆ H ₄	100	100	-	-	-	100
4	4-NO ₂ -C ₆ H ₄	100	1	-	-	-	100
5	3-CF ₃ -C ₆ H ₄	100	100	-	-	-	200
6	4-F-C ₆ H ₄	100	100	-	-	-	200

- → inactive at 200 µg/mL

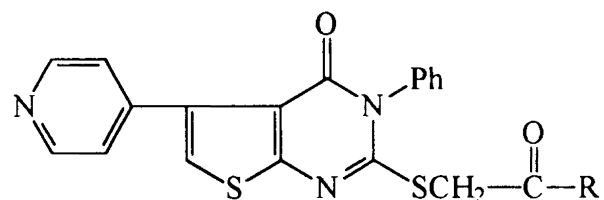
Table 19 : 2-[(substituted aryl / heteroaryl)-thioketomethylene]- 3-phenyl-5-(3-pyridinyl)-thieno-[2,3-d]-pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	2,4-DiCl, 5-F-C ₆ H ₂	0.1	10	-	-	-	40
2	C ₆ H ₅	100	10	-	-	-	0.05
3	4-F-C ₆ H ₄	100	0.1	-	-	-	0.05
4	3-CF ₃ -C ₆ H ₄	100	100	-	-	-	100
5	3-F-C ₆ H ₄	4	0.1	-	-	-	0.05
6	3,4-DiCl-C ₆ H ₃	100	10	-	-	-	0.05
7	5-Cl-thienyl	100	0.1	-	-	-	0.05
8	Cyclopropane	100	1	-	-	-	100
9	Cyclohexane	100	0.1	-	-	-	0.05
10	3-NO ₂ -C ₆ H ₄	100	100	-	-	-	100

- → inactive at 200 µg/mL

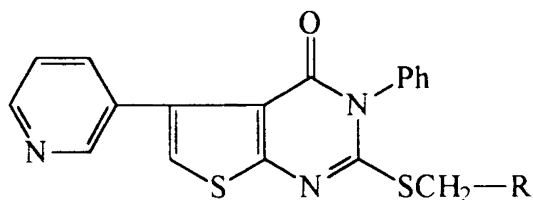
Table 20 : 2-[(substituted aryl / heteroaryl)-thioketomethylene]-3-phenyl-5-(4-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	2,4-DiCl, 5-F-C ₆ H ₂	100	10	-	-	-	0.05
2	C ₆ H ₅	100	10	-	-	-	0.05
3	3,4-DiCl-C ₆ H ₃	100	10	-	-	-	0.05
4	4-F-C ₆ H ₄	100	1	-	-	-	100
5	3-CF ₃ -C ₆ H ₄	100	1	-	-	-	100
6	3-F-C ₆ H ₄	100	10	-	-	-	0.4
7	2,3,4-Tri OCH ₃ -C ₆ H ₂	100	1	-	-	-	100
8	5-Cl-thienyl	100	10	-	-	-	0.05
9	Cyclopropane	40	10	-	-	-	1

- → inactive at 200 µg/mL

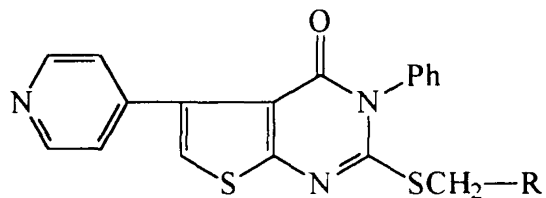
Table 21 : 2-[(substituted benzyl)-thio]-3-phenyl- 5- (3-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	4-Cl-C ₆ H ₄	2	10	-	-	-	10
2	4-OCH ₃ -C ₆ H ₄	100	10	-	-	-	4
3	3,4-DiCl-C ₆ H ₃	0.1	40	-	-	-	4
4	2-F-C ₆ H ₄	100	100	-	-	-	100
5	4-F-C ₆ H ₄	100	10	-	-	-	10
6	4-CF ₃ -C ₆ H ₄	100	10	-	-	-	1

- → inactive at 200 µg/mL

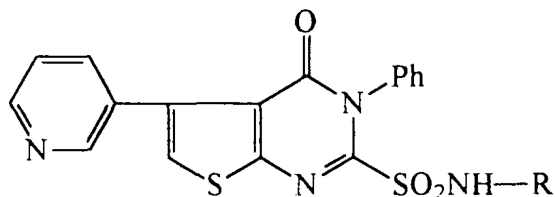
Table 22 : 2-[(substituted benzyl)-thio]-3-phenyl-5-(4-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	4-Cl-C ₆ H ₄	4	10	-	-	-	10
2	4-F-C ₆ H ₄	100	0.1	-	-	-	5
3	3,4-DiCl-C ₆ H ₃	0.1	0.2	-	-	-	2
4	4-OCH ₃ -C ₆ H ₄	2	0.1	-	-	-	20
5	2-F-C ₆ H ₄	0.1	0.1	-	-	-	2

- → inactive at 200 µg/mL

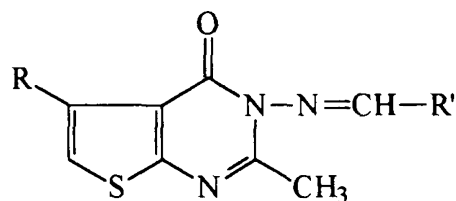
Table 23 : 2-[(substituted aryl/heteroaryl)-sulfonamido]-3-phenyl-5-(3-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones



Sr. No.	R	Antibacterial activity (µg/mL)		Antifungal activity (µg/mL)			Antitubercular activity (µg/mL)
		<i>S. aureus</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	
1	H	4	10	-	-	-	0.05
2	2-Pyrimidine	100	0.1	-	-	-	100
3	3-Methyl-2-pyrimidine	100	100	-	-	-	100
4	4-Methyl-2-pyrimidine	100	100	-	-	-	200
5	2-CF ₃ , 4-NO ₂ -C ₆ H ₃	100	40	-	-	-	100
6	3,4-DiCl-C ₆ H ₃	100	100	-	-	-	100
7	2,4-DiCl-C ₆ H ₃	100	100	-	-	-	200
8	3,5-DiCl-C ₆ H ₃	200	100	-	-	-	200

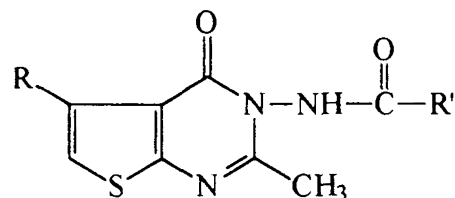
- → inactive at 200 µg/mL

Table 24 : 2-methyl-3N-[(substituted aryl/heteroaryl)-methylene]-amino-5-(2-thienyl / 2-furyl)-thieno[2,3-d]pyrimidin-4(3H)-ones



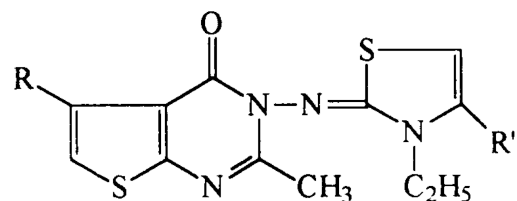
No.	R	R'	Antimalarial activity					Remarks
			Mean Survival Time (Days)			Survival out of 5		
			80 mg	160 mg	Control	80 mg	160 mg	
1	2-Thienyl	4-F-C ₆ H ₄	13.4	15.8	8.8	0/5	0/5	Inactive
2		4-CF ₃ -C ₆ H ₄	14.2	17.8	8.8	0/5	1/5	Active at 160 mg/kg
3		2-F, 4CF ₃ -C ₅ H ₃	14.6	18.0	8.8	0/5	0/5	Inactive
4		2-OH, 6-Br-C ₆ H ₃	11.4	15.8	8.8	0/5	0/5	Inactive
5		2-Thienyl	13.4	16.4	8.8	0/5	0/5	Inactive
6	2-Furyl	4-Cl-C ₆ H ₄	9.6	13.2	8.8	0/5	0/5	Inactive
7		4-F-C ₆ H ₄	10.1	12.2	8.8	0/5	0/5	Inactive
8		4-CF ₃ -C ₆ H ₄	9.9	12.8	8.8	0/5	0/5	Inactive
9		2-CF ₃ -C ₆ H ₄	11.9	14.6	8.8	0/5	0/5	Inactive
10		2-Thienyl	10.8	11.9	8.8	0/5	0/5	Inactive
11		2-Pyridinyl	12.2	14.8	8.8	0/5	0/5	Inactive
12		5-Bromo-2-thienyl	12.2	14.8	8.8	0/5	0/5	Inactive

Table 25 : 2-methyl-3N-[(substituted aryl/heteroaryl)-carboxamido]-5-(2-furyl / 2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



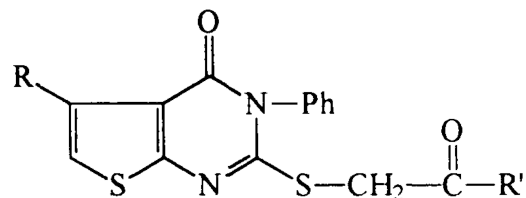
No.	R	R'	Antimalarial activity					Remarks
			Mean Survival Time (Days)			Survival out of 5		
			80 mg	160 mg	Control	80 mg	160 mg	
1	2-Thienyl	4-Cl-C ₆ H ₄	10.8	11.8	7.6	0/5	0/5	Inactive
2		3,4-DiCl-C ₆ H ₃	11.4	13.6	7.6	0/5	0/5	Inactive
3		4-F-C ₆ H ₄	19.4	20.6	7.6	1/5	2/5	Active at 80 & 160 mg/kg
4		C ₃ H ₅	15.8	16.2	7.6	1/5	2/5	Active at 80 & 160 mg/kg
5		2-furyl	15.2	18.2	7.6	1/5	2/5	Active at 80 & 160 mg/kg
6		2,6-DiF-C ₆ H ₃	13.2	16.8	9.6	0/5	0/5	Inactive
7		2-F-C ₆ H ₄	13.2	16.4	9.6	0/5	0/5	Inactive
8	2-Furyl	3,4-DiCl-C ₆ H ₃	11.5	16.0	7.6	0/5	1/5	Active at 160 mg/kg
9		2-F-C ₆ H ₄	12.6	16.2	7.6	0/5	1/5	Active at 160 mg/kg
10		4-OCH ₃ -C ₆ H ₄	12.8	17.2	7.6	0/5	1/5	Active at 160 mg/kg
11		3-F-C ₆ H ₄	11.0	12.8	9.6	0/5	0/5	Inactive
12		2,6-DiF-C ₆ H ₃	12.2	15.8	9.6	0/5	0/5	Inactive
13		4-Cl-C ₆ H ₄	12.4	14.6	8.8	0/5	0/5	Inactive

Table 26 : 2-methyl-3-[3-ethyl-4-(substituted aryl/heteroaryl)-2,3 dihydrothiazolidine amino]- 5-(2-furyl / 2-thienyl)thieno-[2,3-d]pyrimidin-4(3H)-ones



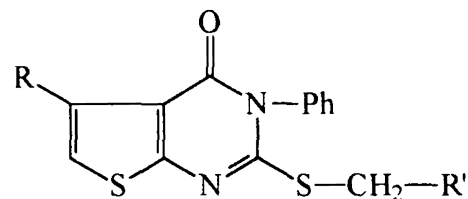
No	R	R'	Antimalarial activity					Remarks
			Mean Survival Time (Days)			Survival out of 5		
			80 mg	160 mg	Control	80 mg	160 mg	
1	2-Thienyl	2,4-DiCl, 5-F-C ₆ H ₂	10.2	13.8	8.8	0/5	0/5	Inactive
2		3-CF ₃ -C ₆ H ₄	10.6	13.8	8.8	0/5	0/5	Inactive
3		4-F-C ₆ H ₄	12.2	14.6	8.8	0/5	0/5	Inactive
4		5-Chlorothieryl	11.9	14.2	8.8	0/5	0/5	Inactive
5	2-furyl	2,4-DiCl, 5F-C ₆ H ₂	14.2	17.4	9.6	0/5	0/5	Inactive
6		4-CF ₃ -C ₆ H ₄	11.6	16.9	9.6	0/5	0/5	Inactive
7		4-F-C ₆ H ₄	11.9	17.1	9.6	0/5	0/5	Inactive
8		5-Chloro thienyl	13.8	16.8	9.6	0/5	0/5	Inactive

Table 27 : 2-[(substituted aryl/heteroaryl)-thioketomethylen]- 3-phenyl-5-(3-pyridinyl / 4-pyridinyl) –thieno [2,3-d] pyrimidin-4(3H)-ones



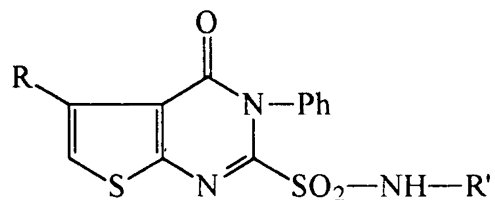
No.	R	R'	Antimalarial activity					Remarks
			Mean Survival Time (Days)			Survival out of 5		
			80 mg	160 mg	Control	80 mg	160 mg	
1	3-Pyridinyl	2,4-DiCl, 5-F-C ₆ H ₂	10.2	16.1	9.6	0/5	0/5	Inactive
2		3-CF ₃ -C ₆ H ₄	10.0	15.6	9.6	0/5	0/5	Inactive
3		C ₃ H ₅	9.8	14.8	9.6	0/5	0/5	Inactive
4		4-F-C ₆ H ₄	16.2	20.0	9.4	1/5	1/5	Active at 160 mg/kg
5	4-Pyridinyl	2,4-DiCl, 5F-C ₆ H ₂	13.4	15.2	9.6	0/5	0/5	Inactive
6		3,4-DiCl-C ₆ H ₃	9.6	12.4	9.6	0/5	0/5	Inactive
7		3-F-C ₆ H ₄	10.4	13.4	9.6	0/5	0/5	Inactive
8		C ₃ H ₅	10.8	13.4	9.6	0/5	0/5	Inactive
9		4-F-C ₆ H ₄	11.4	13.4	8.2	0/5	0/5	Inactive
10		3-CF ₃ -C ₆ H ₄	10.1	13.4	8.2	0/5	0/5	Inactive
11		5-Chloro thienyl	10.2	12.6	9.6	0/5	0/5	Inactive
12		2,3,4 TriOCH ₃ -C ₆ H ₂	12.9	17.4	8.2	0/5	0/5	Active at 160 mg/kg

Table 28 : 2-[(substituted benzyl)-thio]- 3-phenyl-5-(3-pyridinyl / 4-pyridinyl) –thieno [2,3-d] pyrimidin-4(3H)-ones



No.	R	R'	Antimalarial activity					Remarks
			Mean Survival Time (Days)			Survival out of 5		
			80 mg	160 mg	Control	80 mg	160 mg	
1	3-Pyridinyl	4-Cl-C ₆ H ₄	14.2	16.4	9.4	0/5	0/5	Inactive
2		3,4DiCl-C ₆ H ₃	13.6	16.4	9.4	0/5	0/5	Inactive
3		2F-C ₆ H ₄	14.2	17.8	9.4	0/5	0/5	Inactive
4		4F-C ₆ H ₄	10.4	12.8	9.4	0/5	0/5	Inactive
5		4CF ₃ -C ₆ H ₄	14.9	19.0	9.4	0/5	1/5	Active at 160 mg/kg
6	4-Pyridinyl	4F-C ₆ H ₄	13.6	16.0	8.2	0/5	0/5	Inactive
7		3,4-DiCl-C ₆ H ₃	14.2	17.2	8.2	0/5	0/5	Active at 160 mg/kg
8		2F-C ₆ H ₄	12.4	13.8	8.2	0/5	0/5	Inactive

Table 29 : 2-[(substituted aryl/heteroaryl)-sulfonamido]- 3-phenyl-5-(3-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones



No.	R	R'	Antimalarial activity					Remarks
			Mean Survival Time (Days)			Survival out of 5		
			80 mg	160 mg	Control	80 mg	160 mg	
1	3-pyridinyl	H	12.8	15.8	9.4	0/5	0/5	Inactive
2		2-pyrimidine	13.8	16.6	9.4	0/5	0/5	Inactive

Part IV

Conclusion

CONCLUSION

The present investigation was focused on the synthesis of novel organic compounds of possible therapeutic value. To evaluate their biological efficacy, they were screened for *in vitro* antibacterial, antifungal and antitubercular activity and also *in vivo* antimalarial activity.

In **Part II-A**, the synthesis of 2-methyl –3N-[(substituted aryl/heteroaryl)-methylene]-amino-5-(2-furyl / 2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-ones has been described. The antimicrobial and antimalarial activity of the series (Table 13) has revealed the following points –

- 1) The presence of azomethine linkage at 3-position and 2-furyl group at 5-position of the thienopyrimidione moiety has shown good antimicrobial property to thienopyrimidione nucleus.
- 2) All compounds seem to be active against *S. aureus* and *S. typhi*. The presence of halogen substituent in aryl / hetero azomethine linkage is found to be very essential for activity. Trifluoromethyl substituent imparts most significant activity against *S. aureus* at concentration of 0.1 µg/mL.
- 3) Against *S. typhi*, all compounds have shown significant activity ranging between 0.1–40 µg/mL. The presence of electron-withdrawing and electron-donating groups on aryl / hetero aryl ring attached to a 5-furyl-thienopyrimidione ring system through azomethine linkage, is found to be responsible for antibacterial activity against *S. aureus*.
- 4) All the compounds have moderate activity against *M. tuberculosis*.
- 5) All the compounds are inactive against *P. berghei* (Table 24, compound no. 6-12)

The antimicrobial and antimalarial activity of the series (Table 14) has revealed the following points -

- 1) Introduction of 2-thienyl moiety at 5-position and substituted aryl / heterocyclic azomethine linkage at 3-position of the thienopyrimidione nucleus does not impart any significant activity against *S. aureus*, except compound no. 6, which has a fluorine and trifluoromethyl substituent in phenyl azomethine linkage.

- 2) Compounds containing electron-withdrawing substituents and heterocycles such as N-methyl pyrrole attached to the basic thienopyrimidione nucleus, exhibit good activity against *S. typhi*, ranging between 0.1 – 1 µg/mL, as compared to Chloramphenicol (MIC 8µg/mL).
- 3) The antitubercular activity is imparted to Compound no. 6,8 and 10 as an effect of
 - a) substitution of fluorine and trifluoromethyl group on the phenyl azomethine linkage and substitution of 3-pyridinyl and N-methyl pyrrole heterocycles in azomethine linkage.
 - b) Introduction of 2-thienyl moiety at 5-position of thienopyrimidione nucleus.

Compound no. 6,8 and 10 were found to be more potent than Pyrazinamide (MIC 12 µg/mL) and Ethionamide (MIC 10 µg/mL) against *M. tuberculosis in vitro*.
- 4) Compound containing a trifluoromethyl group in phenyl azomethine linkage imparts a moderate antimalarial activity against *P. berghei* and was found to be active at 160 mg/kg.

All the compounds from **Part II-A** are inactive as antifungal agents, which indicates that the introduction of 2-furyl / 2-thienyl group at 5-position and substituted phenyl / heterocyclic azomethine linkage at 3-position of thienopyrimidone nucleus results in the loss of antifungal activity.

In **Part II-B** the synthesis of 2-methyl-3N-[(substituted aryl/heteroaryl)-carboxamido]-5-(2-furyl/ 2-thienyl)-thieno [2,3-d] pyrimidin-4(3H)-ones has been described. The antimicrobial and antimalarial activity of the series (Table 15) reveals the following points –

- 1) The presence of a 2-furyl group at 5-position and substituted aryl / heteroaryl carboxamido linkage at 3-position of the thienopyrimidone nucleus seems to be very essential for antibacterial, antitubercular and antimalarial activity.
- 2) The presence of electron-withdrawing or electron-releasing substituents in phenyl carboxamido linkage favours high activity against *S. aureus* and *S. typhi*. Compound no 1,2,5 and 7 have shown antibacterial activity upto

0.1 µg/mL, which is equivalent to the activity of Ciprofloxacin (0.1 – 1 µg/mL) and greater than the MIC of aminoglycoside antibiotics (2 - 0.25 µg/mL). Also, compound nos. 3,4,9,10 and 11 have been shown higher activity against *S. typhi* than Chloramphenicol.

- 3) The antitubercular screening has given encouraging results. The presence of halogens such as Cl, F, phenyl ring and heterocycles such as propane, thienyl in the carboxamido side chains imparts good antitubercular activity to the compounds and was found to be between 0.05 – 4 µg/mL against H₃₇R_v strain of *M. tuberculosis*.

Compounds no. 6, 7 and 11 were found to be as active as INH and Rifampin against *M. tuberculosis*. Compounds no. 3 and 4 have higher activity than Pyrazinamide and Ethionamide, while compound no. 5 has shown activity equal to PAS against *M. tuberculosis in vitro*.

- 4) Substituents such as Cl, F and OCH₃ substituted in phenyl carboxamido linkage attached at 3-position, and 2-furyl group at 5-position of thienopyrimidone nucleus, impart moderate antimalarial activity against *P. berghei*.

The antimicrobial and antimalarial activity of the series (Table 16) has revealed the following points –

- 1) The antimicrobial and antimalarial activity results of all the compounds indicate that the presence of electron-rich 2-thienyl moiety at 5-position and substituted aryl / heterocyclic carboxamido linkage at 3-position of thienopyrimidone nucleus favours the biological activity.
- 2) It is also observed that the presence of cyclohexane, cyclopropane and 4-morpholine moieties, and electron withdrawing groups at 3- and 4- position of the phenyl ring of carboxamido linkage favour good activity (0.1 – 4 µg/mL) against *S. aureus*.
- 3) From antibacterial results against *S. typhi*, it was concluded that high activity is exhibited by compound bearing an electron-withdrawing groups and cyclohexane, cyclopropane, 4-morpholine, 2-furyl and 2-thienyl moieties, while electron-releasing groups do not contribute significantly towards activity.

- 4) The presence of cyclohexane, cyclopropane, 4-morpholine and 2-furyl moieties in carboxamido linkage and 2-thienyl moiety at 5-position of thienopyrimidione nucleus enhances antitubercular activity against *M. tuberculosis*.

Two compounds have shown antitubercular activity equivalent to INH against *M. tuberculosis*.

- 5) The presence of fluorine atom at 4-position on the phenyl ring, and propane and 2-furyl moieties in the carboxamido linkage, imparts good antimalarial activity.

All the compounds from **Part II-B** were found to be inactive against *C. albicans*, *T. mentagrophytes* and *T. rubrum*. From these results, it was concluded that the presence of 2-furyl / 2-thienyl moiety at 5-position and substituted aryl / heterocyclic carboxamide linkage at 3-position of thienopyrimidione nucleus is not very important as regards antifungal activity.

In **Part II-C** the synthesis of 2-methyl-3-[3-ethyl-4-(substituted aryl / heteroaryl)-2,3 dihydrothiazolidine amino]-5-(2-furyl/2-thienyl) – thieno [2,3-d] pyrimidin- 4(3H) -ones has been described.

The antimicrobial and antimalarial activities of this series (Table 17) has revealed the following points :

- 1) From antimicrobial results of compounds, it is concluded that the introduction of 2-furyl group at 5-position and substituted thiazolidine ring at 3-position of thienopyrimidione nucleus has not shown encouraging activity against *S. aureus* and *M. tuberculosis*.
- 2) It is also observed that against *S. typhi*, compounds containing only electron-withdrawing substituents have shown activity ranging between 2-10 µg/mL, while electron releasing substituents show a drastic reduction in activity.

The antimicrobial and antimalarial activity of the series (Table 18) has revealed the following points -

- 1) The Introduction of 5-chlorothieryl group at 4-position of the thiazolidine side chain is found to be essential for antibacterial activity against *S. aureus* and *S.*

typhi, while introduction of trisubstituted phenyl and 3-nitro phenyl group at 4-position of thiazolidine side chain is found to be essential for antibacterial activity against *S. typhi*.

- 2) It is observed that 2-thienyl moiety at 5-position and 2,4-dichloro-5-fluoro phenyl moiety at the 4-position of the thiazolidine side chain, which is attached at 3-position of thienopyrimidione nucleus are responsible for antitubercular activity.

All compounds from **Part II-C** are inactive as antifungal agents against *C. albicans*, *T. mentagrophytes* and *T. rubrum* and in antimalarial screening against *P. berghei*.

In **Part II-D** the synthesis of 2-[(substituted aryl / heteroaryl)-thioketomethylene]-3-phenyl-5-(3-pyridinyl / 4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones has been described. From the antimicrobial and antimalarial screening results (Table no. 19) following observations are made -

- 1) The presence of 3-pyridinyl moiety at 5-position and substituted aryl / heteroaryl thioketomethylene linkage at 2-position of thienopyrimidione nucleus are essential for potent activity against *M. tuberculosis* and *S. typhi*. Six compounds have antitubercular activity equivalent to that of INH and higher than Ethambutol, Pyrazinamide, Ethionamide and PAS against *M. tuberculosis*, while four compounds have shown activity higher than Chloramphenicol against *S. typhi*.
- 3) The compounds containing Thioketomethylene linkage substituted only with 2,4 dichloro-5-fluoro-phenyl and 3-fluorophenyl substituent has shown good activity against *S. aureus*.
- 3) It is also observed that the compound, bearing a 3-pyridinyl group at 5-position and thioketomethylene linkage substituted by 3-fluorophenyl group at 2-position of the thienopyrimidione nucleus has shown broad activity against *S. aureus*, *S. typhi*, *M. tuberculosis* and *P. berghei*.

From the antimicrobial and antimalarial screening results (Table 20) following observations are made -

- 1) The presence of 4-pyridinyl moiety at 5-position and substituted aryl / heteroaryl thioketomethylene linkage at 2-position of thienopyrimidione

nucleus enhances the activity against *M. tuberculosis* and *S. typhi*, but not against *S. aureus*.

Four compounds have been shown to exhibit antitubercular activity equivalent to that of INH against *M. tuberculosis*. All the compounds are active in the range of 1-10ug/mL against *S. typhi*.

- 2) The substituted and/or unsubstituted phenyl ring with electron withdrawing substituent as well as heterocycles attached to the thioketomethylene linkage mainly contributes towards antitubercular activity, while electron withdrawing as well as electron releasing substituents, and heterocycles attached to the thioketomethylene linkage favour activity against *S. typhi*.
- 3) Only electron releasing substituents on the phenyl thioketomethylene linkage has been shown to exhibit moderate antimalarial activity against *P. berghei*.

All the compounds from **Part II-D** were found to be inactive as antifungal agents against *C. albicans*, *T. mentagrophytes* and *T. rubrum*. It can be concluded that the presence of 3-pyridinyl group at 5-position and substituted thioketomethylene linkage at 2-position of the thienopyrimidione nucleus is not significant as regards antifungal activity.

In **Part II-E** synthesis of 2-[(substituted benzyl)-thio]-3-phenyl-5-(3-pyridinyl / 4-pyridinyl)-thieno [2,3-d] pyrimidin-4(3H)-ones has been described.

The antimicrobial and antimalarial activities of the compounds in this series (Table 21) reveal the following points-

- 1) The antibacterial activity against *S. aureus* is because of the presence of the chloro substituent on the benzyl thio linkage which is attached at 2-position and 3-pyridinyl moiety present at 5-position of the thienopyrimidione nucleus. Compounds with substituents such as fluoro and trifluoromethyl have not shown significant activity against *S. aureus*.
- 2) Compounds have shown moderate activity against *S. typhi*.
- 3) The presence of trifluoromethyl substituent in benzyl thio linkage seems to be responsible for the antitubercular activity against *M. tuberculosis* at a concentration of 1 µg/mL and for the antimalarial activity against *P. berghei* at a concentration of 160 mg/kg.

The antimicrobial and antimalarial activities of the series (Table 22) reveal the following points -

- 1) The presence of 4-pyridinyl moiety at C₅-position and substituted benzyl thio linkage at 2-position of the thienopyrimidione nucleus have shown good activity against *S. aureus*, *S. typhi*, and *M. tuberculosis*. Thus, it may be concluded that the electron withdrawing and electron releasing groups favour the antibacterial and antitubercular activity.
2. Only disubstituted benzyl thio-linkage has exhibited antimalarial activity against *P. berghei*.

All the compounds from **Part II-E** were found to be inactive against *C. albicans*, *T. mentagrophytes* and *T. rubrum* which indicates that 3-pyridinyl / 4-pyridinyl moiety at 5-position and substituted benzylthio linkage at 2-position of the thienopyrimidione nucleus not favour antifungal activity at any level.

In **Part II-F** synthesis of 2-[(substituted aryl / heteroaryl)-sulfonamido]-3- phenyl –5-(3-pyridinyl)–thieno [2,3-d] pyrimidin-4(3H)-ones has been described. The antimicrobial and antimalarial results of the series (Table 23) reveal the following points –

- 1) The introduction of SO₂NH₂ linkage at 2-position and 3-pyridinyl group at 5-position of the thienopyrimidione nucleus have contributed significantly towards antitubercular and antibacterial activities.
- 2) Any modification or replacement of hydrogen atom from SO₂NH₂ linkage by another substituted heterocycle or substituted aryl group results in the drastic reduction of antitubercular activity against *M. tuberculosis* and antibacterial activity against *S. aureus*.
- 3) The presence of 2-pyrimidine heterocycles in sulfonamido linkage was found to be essential for antibacterial activity against *S. typhi*.
- 4) All the compounds are inactive as antifungal and antimalarial agents.

In conclusion, it may be stated that the modification of thieno [2,3-d] pyrimidin-4(3H)-one structure by introducing different heterocycles, viz. 2-furyl, 2-thienyl, 3-pyridinyl and 4-pyridinyl at 5-position and pharmacologically active side chains such as azomethine, carboxamide, thioketomethylene, benzyl thio and sulfonamido (bearing

various electron releasing, electron withdrawing, and heterocyclic moieties) at 2 and 3 position resulted in the discovery of the new drug molecules which could be used against *Mycobacterium tuberculosis* infection or infections caused by various Gram-positive and Gram-negative bacteria. Several compounds have exhibited interesting results when subjected to *in vitro* screening against H₃₇R_v strain of *M. tuberculosis*. Fifteen compounds have shown activity equivalent to Isoniazid, two compounds have exhibited activity at a concentration of 0.1 and 0.4 µg/mL (Table 15 and 20), which makes them more active than Ethambutol. Most of the remaining compounds are active at a concentration ranging between 1-200 µg/mL.

Eleven compounds have exhibited antibacterial activity at a concentration of 0.1µg/mL against *S. aureus*, which is equivalent to Ciprofloxacin. Forty-three compounds have shown antibacterial activity against *S. typhi* at concentration ranging between 1-4µg/mL, and are hence more active than Chloramphenicol (MIC 8µg/mL). Three compounds have exhibited antimalarial activity up to 80 mg/kg against *P. berghei*.

Though it can be concluded that the present investigation has been successful in discovering new organic compounds, more active than or comparable to the existing drugs in the class of infections for which these compounds were screened, it may be worthwhile to pursue further investigations bearing in mind certain trends observed in the present investigation.

A better understanding of the disease processes at a molecular level, as well as of the role of proteins and genes in bacterial biology, will be essential in providing a valuable basis for developing better antimicrobials to fight emerging resistant microorganisms.

References

REFERENCES

- 1) Camille G. Wermuth, ***The Practice of Medicinal Chemistry***, Academic Press, London, **1996**, P4
- 2) Austel V., ***Modern Drug Research : Paths To Better and Safer Drugs***, Martin Y. C., Kuffer E., Austel V. (Eds.), Marcel Dekker Inc, New York, **1989**, 243- 73
- 3) Trickle I. J., Sibanda B. L., Pearl C. H., Hemming A. M., Blundell T. L., ***X-Ray Crystallography and Drug Action***, Horn A. S., Derantler C. J. (Eds.), Clarendon Press, Oxford, **1984**, P427-40
- 4) Starbatch L. H., ***The Benzodiazepine Story : Progress In Drug Research***, **1978**, **22**, 2269-29
- 5) Richard B. Silverman, ***The Organic Chemistry of Drug Design and Drug Action***, Academic Press Inc, California, **1992**, P9
- 6) Richard B. Silverman ***The Organic Chemistry of Drug Design and Drug Action***, Academic Press Inc, California, **1992**, P 11
- 7) Dohme A. R. L., Cox E. H. and Miller E., ***J. Am. Chem. Soc.***, **48**, **1926**, 1688
- 8) Thornber C. W., ***Chem. Soc. Rev.***, **1979**, 8563
- 9) Burger A., ***Medicinal Chemistry***, Burger A. (Ed), 3rd Edn., Wiley Interscience, New York, **1970**, P 19
- 10) Korolkovas A., ***Essentials of Molecular Pharmacology : Background For Drug Design***, Wiley Interscience, New York, **1970**, P 19
- 11) Lipinski C. A., ***Annu. Rep. Med. Chem.***, **21**, **1986**, 383
- 12) Erlenmeyer H., ***Bull. Soc. Chin. Biol.***, **30**, **1948**, 792
- 13) Richet M. C., ***Soc. Biol. Ses. Fil.***, **45**, **1893** 775
- 14) Overton E., ***Z. Phys. Chem.***, **22**, **1897**, 189
- 15) Meyer H., ***Arch. Exp. Pathol. Pharmacol.***, **42**, **1899**, 109
- 16) Hansch C., Muir R. M. and Metzenberg R. L. Jr., ***Plant Physiol.***, **26**, **1951**, 812
- 17) Tute M. S., ***Comprehensive Medicinal Chemistry, Vol 4 : Quantitative Drug Design***, Hansch C., Sammes P. G. and Tylor J. B. (Eds.), Pergamon press, New York, **1990**, P1-31
- 18) Hansch C., ***Drug. Dev. Res.***, **1**, **1981**, 267-309

- 19) Hansch C., *Drug Inf. J.*, **18**, 1984, 115-12
- 20) Carraig P. N., *Drug Inf. J.*, **18**, 1984, 123-30
- 21) Fujita T., *Acta Pharm. Jugosl.*, **37**, 1987, 4- 57
- 22) Fujita T., *Comprehensive Medicinal Chemistry, Vol 4: Quantitative Drug Design*, Hansch C., Sammes P. G. and Tylor J. B. (Eds.), Pergomon Press, New York, **1990**, P 497-560
- 23) Martin Y. C., *J. Med. Chem.*, **24**, 1981, 229-37
- 24) Camille G. Wermuth, *The Practice of Medicinal Chemistry*, Academic Press, London, **1996**, P368
- 25) Richard B. Silverman, *The Organic Chemistry of Drug Design and Drug Action*, Academic Press Inc, California, **1992**, P26
- 26) Hanscm K. H., Nikaitani D. and Lien E. J., an A, *Vitam. Horm. (N.Y)*, **16**, **1958**, 303
- 27) Topliss J. G., *J. Med. Chem.*, **15**, 1972, 1006
- 28) Topliss J. G., *J. Med. Chem.*, **20**, 1977, 463
- 29) Hansch C., Unger S. H. and Forsythe A. B., *J. Med. Chem.*, **16**, **1973**, 1217
- 30) a) Crippen G. M. 1979 *J. Med. Chem.*, **22**, **1979**, 988
b) Ghose A. K. and Crippen G. M., *J. Med. Chem.*, **25**, **1982**, 892
c) Crippen G. M., *Distance Geometry and Conformational Calculations*, Research Studies Press, New York, **1981**
- 37) a) Sheridan R. P., Nilakantan R., Dixon J. S. and Venkataraghavan R., *J. Med. Chem.*, **29**, **1986**, 899
b) Sheridan R. P. and Venkataraghavan R., *Acc. Chem. Res.*, **20**, **1987**, 322
- 38) a) Hopfinger A. J., *J. Am. Chem. Soc.*, **102**, **1980**, 7196
b) Hopfinger A. J., *J. Med. Chem.*, **24**, **1981**, 818
c) Hopfinger A. J., *J. Med. Chem.*, **26**, **1983**, 990
- 39) Carhart R. E., Smith D. H. and Venkataraghavan R., *J. Chem. Inf. Caoput. Sci.*, **25**, **1985**, 64
- 40) Nilakantan R., Bauman N., Dixon J. S. and Venkataraghavan R., *J. Chem. Inf. Comput. Sci.*, **27**, **1987**, 82
- 41) Hassall C. H., *Chem. Brit.*, **21**, **1985**, 39

- 42) Levinthal C., **Sci. Am.**, **214(6)**, **1966**, 42
- 43) Tollenaere J. P. and Janssen P. A., **J. Med. Res. Rev.**, **8**, **1988**, 1
- 44) a) Gund P., Halgren T. A. and Smith G. M., **Annu. Rep. Med. Chem.**, **22**, **1987**, 269
b) Gund T. and Gund P., **Molecular Structure and Energetics**, Liebman J. F. and Greenberg A.(Eds.), **Vol. 4**, Chapter 10 - VCH, Weinheim, **1987**
- 45) a) Sufrin J. R., Dunn D. A. and Marshall G. R., **Mol. Pharmacol.**, **19**, **1981**, 307.
b) Humblet C. and Marshall G. R., **Annu Rep Med Chem**, **15**, **1980**, 267
- 46) Harries A. D., **Lancet**, **335**, **1990**, 387
- 47) Raviglione M. C., Narain J. P. and Kochi A., **Bull. World Health Organ.**, **70**, **1992**, 515
- 48) Raviglione M.C., Snider D.E. and Kochi A., **J.Am. Med. Asso.**, **273**, **1995**, 220
- 49) Laal S., Samanich K.M. and Sonnenberg M.G., **J. Infect. Dis.**, **176**, **1997**, 133
- 50) Ranadive S. N. and Banerjee K., **ICMR Bulletin**, **19**, **1989**, 11
- 51) Mackaness G. B. and Smith N., **Am. Rev. Tubercul.**, **67**, **1953**, 322
- 52) Takayama K., Schnoes H. K., Armstrong E. L. and Boyle R. W., **J. Lipid Res.**, **16**, **1975**, 308-17
- 53) Banerjee A., Bubnau E., Quemard A., Balasubramanian V., Willson T., Collins D., Delisle G. and Jacobs W. R., **Science**, **263**, **1994**, 227-30
- 54) Hobby G. L. and Lenert T. F., **Am. Rev. Respir. Dis.**, **105**, **1972**, 292-95
- 55) Davidson P. T., Goble M. and Lester W., **Drugs**, **43(5)**, **1992**
- 56) Grosset J. and Leventis S., **Rev. Infect. Dis.**, **5**, **1983**, 5440- 46
- 57) Kucks M. A., Pects E. A. and Forbes M., **Am. Rev. Resp. Dis.**, **87**, **1963**, 805-06
- 58) Takayama K., Armstrong E. L., Kunugi K. A. and Kilburn J. O., **Antimicrob. Agents Chemotherp.**, **16**, **1979**, 240
- 59) Kushner S., Dalalian H., Sanjurjo J. L., Bach F. L. and Safir S. R., **J. Am. Chem. Soc.**, **74**, **1952**, 3617
- 60) Simon E., Veres E. and Banki G., **Scand. J. Respir. Dis.**, **50**, **1969**, 314-22
- 61) Weyer E. M., **Kanamycin : Appraisal After Eight Years of Clinical Application**, Ann N. Y. Acad. Sci., **132**, **2**, **1966**, 2771

- 62) Apirion D. and Schlessinger D., ***J. Bacteriol.***, **96**, **1968**, 768,
- 63) Davies J., Gorini L. and Davies D. B., ***Mol. Pharmacol.***, **1**, **1965**, 93,
- 64) Davies J., Anderson P. and Davies D. B., ***Science***, **149**, **1965**, 1096
- 65) Doluisio J. T., Dittert L. W. and La Piana J. C., ***J. Pharmacokinet. Biopharmaceut.***, **1**, **1973**, 253
- 66) Kuntz E., ***Klin. Wochensche***, **40**, **1962**, 1107
- 67) Herr Jr. E. B. and Redstone M. O., ***Ann. N. Y. Acad. Sci.***, **135**, **1966**, 940
- 68) Wilson T. M., ***Practitioner***, **199**, **1967**, 817-24.
- 69) Freerksen E. and Rosenfeid M., ***Beite Klin. Tuberk.***, **127**, **1963**, 386; Cited From ***Chem. Abstr.***, **60**, **1969**, 4659c
- 70) Trnka L., Urbanick R. and Polenska H., ***Rozhl. Tuberk.***, **23**, **1963**, 147 ; Cited From ***Chem. Abstr.***, **59**, **1963**, 8016v
- 71) Oka S., Konno K., Kudo M., Oizumi K. and Yamaguchi S., ***Jpn. J. Chest. Dis.***, **23**, **1964**, 326
- 72) Editorial, ***Tubercle***, **46**, **1965**, 298
- 73) Winder G., Collins P. B. and Whelan D., ***J. Gen. Microbiol.***, **66**, **1971**, 379
- 74) Davy T. F., ***Lepr. Rev.***, **29**, **1958**, 25
- 75) Heifets L. B., ***Semin. Respie. Infect.***, **9**, **1994**, 84
- 76) Khardory N., Rolston K., Rosenbaum B., Hayat S., and Bodey G.T., ***J. Antimicrob. Chemother.***, **24**, **1989**, 667
- 77) Garcia-Rodriguez J. A. and Gomez-Garcia A. C., ***J. Antimicrob. Chemother.***, **32**, **1993**, 797
- 78) Karabalut N. and Drusano G., ***Quinolone Antimicrobial Agents***, Hooper D. C. and Wolfson J. S. (Eds.), **2nd** Edn., American Society For Microbiology, Washington, **1993**, P53
- 79) Eliopoulos G. M. and Eliopoulos C. T., ***Quinolone Antimicrobial Agents*** Hooper D. C. and Wolfson J. S. (Eds.), **2nd** Edn., American Society For Microbiology, Washington, **1993**, P161
- 80) Leysen D. C., Haemers A. and Pattyn S. R., ***Antimicrob. Agents Chemother.***, **33**, **1989**, 1
- 81) Casal M., Gutierrez J., Gonzales J. and Ruiz P., ***Chemioterapia***, **6**, **1987**, 437
- 82) Young L. S., ***J. Antimicrob. Chemother.***, **32**, **1993**, 179

- 83) Delalla F., Maserati R., Scarpellini P., Marone P., Nicolin R., Caccamo F. and Rigoli R., ***Antimicrob. Agents. Chemother.***, **36**, 1992,1567
- 84) Hongkong Chest Service and British Medical Research Council, ***Tuberc. Lung Dis.***, **73**, 1992,59
- 85) O'brien R. J., ***Quinolone Antimicrobial Agents***, Hooper D. C. and Wolfson J. S. (Eds.), 2nd Ed, American Society For Microbiology, Washington, 1993, P207.
- 86) Klemens S. P., De Stefano M. S. and Cynamon M. H., ***Antimicrob. Agents Chemother.***, **36**, 1992, 2413
- 87) Wayne L. G. and Sarmek H. A., ***Antimicrob. Agents Chemother.***, **38**, 1994, 2054
- 88) Kilburn J. Glickman, Brickner S., Manninen S. M., Ulanowicz P., Lovtaz D. and Zuenko C., ***Programs and Abstracts of the 33rd Interscience Conference On Antimicrobial Agents and Chemotherapy***, American Society of Microbiology, Washington, 1994
- 89) Ashtekar D. R., Costa-Periera R., Srinivasan T., Iyer R., Vishwanathan N., Rittel W., ***Agent Diagn. Microbiol. Infect. Dis.***, **14**, 465.
- 90) Cornforth J. W., Hart P., Rees R. and Stock J., ***Nature***, **168**, 1951,150.
- 91) Cornforth J. W., Hart P., D'Arcy Hart P., Nicholls G. A., Rees R. J. W. and Stock J. A., ***Br. J. Pharmacol.***, **10**, 1955, 73.
- 92) Chinnaswamy J., Allaudeen H. S. and Hunter R. L., ***Antimicrob. Agents Chemother.***, **39**,1995, 1349.
- 93) Raghunath Rao U., Srinivas Rao S., Natarajan S. and Venkataraman P. R., ***Nature***, **157**,1946, 491
- 94) Deshpande R. G., Khan M. B., Bhatt D. A. and Navalkar R.G., ***J. Antimicrob. Chemother.***, **32**,1993,623
- 95) ***Drugs of Future***, **24 (2)**, 1999, 193
- 96) Mitchison D. A., ***Bull. Int. Union Tuberc.***, **32**, 1961,81
- 97) Davis B. D., ***Drug Resistance in Micro Organism***, Ciba Foundation Symp. Little, Brown, Boston P 165.
- 98) From the AMA' s Council on Scientific Affairs, ***J. Am. Med. Asso.***, **275**, 1996, 181.

- 99) Horlein, Dressel and Kethe, **Chem. Ber.**, **71 A**, **1938**, 15
- 100) Basu U. P., **J. Ind. Chem. Soc.**, **26**, **1949**, 130
- 101) Morre M. L. and Miller C. S., **J. Am. Chem. Soc.**, **64**, **1942**, 1572
- 102) Matsukawa T., Ohta. B. and Shirakawa K., **J. Pharm. Soc. Japan**, **70**, **1950**, 283; Cited From **Chem. Abstr.**, **5**, **1951**, 2894s
- 103) Anderson G. W., Faith H. E. and Marson H. W., **J Am Chem Soc**, **64**, **1942**, 2902
- 104) Wacker A. and Krishfeid S., **Arzneim.- Forsch**, **10**, **1960**, 206
- 105) Nichols R. L., Jones W. F. and Finland M., **Proc. Soc. Exp. Bio. Med.**, **92**, **1956**, 637
- 106) Horstmann H., Knott T. and Scholtan W., **Arzneim.- Forsch.**, **11**, **1961**, 682
- 107) Camerino B. and Palamide S. G., **Gazz. Chimital. Ital.**, **90**, **1960**, 1815
- 108) Jenkins I. D., Verheyden J. P. H. and Moffatt J. G., **J. Med. Chem.**, **13**, **1970**, 242
- 109) Castro-Pichel J. and Garcia-Laopez M. T., **Tetrachedron**, **43**, **1987**, 383
- 110) Hitchings G. H., **Ann. N. Y. Acad. Sci.**, **23**, **1961**, 700-08
- 111) Waksman S. A., **Science**, **110**, **1949**, 27
- 112) Wilson and Gisvold's, **Text Book of Organic Medicinal and Pharmaceutical Chemistry**, 10th Edn., Delgado J. N. and Remers W. A. (Eds.), J. B. Lippincott Company, New York, **1998**, P 231-32
- 113) Humber P. M., Chauvette R. R. and Jackson B. G., **Cephalosporins and Penicillins**, Flymn E. H.(Ed), Academic Press Inc, New York, **1972**, P27
- 114) Wilson and Gisvold's, **Textbook of Organic Medicinal and Pharmaceutical Chemistry**, 10th Edn., Delgado J. N. and Remers W. A. (Eds.), J. B. Lippincott Company, New York, **1998**, P 255-57
- 115) Burger A., **Burger Medicinal Chemistry and Drug Discovery**, 5th Edn., **Vol 4 : Therapeatic Agents**, Manfred E. Wolff (Ed), Wiley-Interscience, New York, **1995**, P 295-302
- 116) **Drugs of Future**, **24(3)**, **1999**, 327
- 117) **Drugs of Future**, **24(3)**, **1999**, 344
- 118) **Drugs of Future**, **23(8)**, **1998**, 905
- 119) **Drugs of Future**, **23(8)**, **1998**, 906

- 120) Jaffe A., Chabbert Y. A. and Semonin O., ***Antimicrob Agents Chemother***, **22**, 1982, 942-48
- 121) ***Drugs of Future***, **24(3)**, 1999, 329
- 122) ***Drugs of Future***, **23(8)**, 1998, 910
- 123) ***Drugs of Future***, **23(4)**, 1998, 469
- 124) ***Drugs of Future***, **22(4)**, 1997, 421
- 125) Schnappiger D., Hilen W., ***Arch Microbiology***, **165**, 1996, 359-69
- 126) Setti E. L., Qualtrocchio and Micetich R. C., ***Drugs of Future***, **23(3)**, 1997, 271-84
- 127) Chen Y. L., ***J. Antibiot.***, **44**, 1991, 970
- 128) Mark J., Suto John M., Domajata and Paul F. Miller, ***Annu. Rep. Med. Chem.***, **27**, 1992, 119
- 129) Biskid G., Dikucht H. A., Fallat V. and Keith D. D., ***30th International Conference On Antimicrobial Agents and Chemotherapy***, 1990, 403
- 130) ***Drugs of Future***, **23 (11)**, 1998, 1204
- 131) ***Drugs of Future***, **23 (10)**, 1998, 1117
- 132) ***Drugs of Future***, **23 (9)**, 1998, 1047
- 133) ***Drugs of Future***, **23(8)**, 1998, 917
- 134) ***Drugs of Future***, **23 (8)**, 1998, 921.
- 135) Layman C. A., Walsh T. J., ***Drugs***, **44**, 1992, 9
- 136) Kavacs J. A., Koracs A. A., Blis M., Wright W. C., Gill V. J. and Tuazan C. V., ***Ann. Intern. Med.***, **103**, 1985, 533
- 137) Kerridge D., ***Advances In Mircobiological Physiology***, **27**, 1986, 1
- 138) Grunberg E., Titsworth E. and Bennet M., ***Antimicrob. Agents Chemother.***, **3**, 1964, 566
- 139) Hazen E. L. and Brown R., ***Science***, **112**, 1950, 423
- 140) Grove J. G., Ismay D., Macmillian J., Malhottan T. P. C. and Rogers M. A., ***Chem. Ind.***, **11**, 1951, 219
- 141) Plempel M., Bartmann K. Buchel K. H. and Regel E., ***Post. Grad. Med. J.***, **50**, 1974,11
- 142) Thienpont D. V., Van C. J., Nveten J. M., Niemegue C. J. E. and Marshboom R., ***Arzneim.- Forsch.***, **25**, 1975, 224

- 143) Godefroi E. F., Heeres J. and Van C. J., ***J. Med. Chem.***, **12**, **1969**, 784
- 144) Richardson K., Cooper K., Marriott M. S., Tarbit M. H. and Troke P. F., ***Review of Infections Diseases***, **12**, **1990**, S-267
- 145) Warnick D. W., ***J. Antimicrobial and Chemotherapy***, **240**, **1989**, 275
- 146) Welch T. J., Mc Cully C., Rinaldi M. G., Wallace J. C. and Babs F. M., ***Antimicrob. Agents Chemother.***, **34**, **1990**, 1281
- 147) Van C. J., Van G. F. and Janssen P. A., ***J. Revista Iberica De Micologia***, **1988**, 5
- 148) ***Drugs of Future***, **24 (2)**, **1999**, 217
- 149) ***Drugs of Future***, **24(1)**, **1999**, 30
- 150) ***Drugs of Future***, **24(1)**, **1999**, 86
- 151) ***Drugs of Future***, **23(5)**, **1998**, 543
- 152) Karle J. M., Karle I. L., Gerena L. and Milhous W. K., ***Antimicrob. Agents Chemother.***, **36**, **1992**, 1538- 44
- 153) Paliwal J. K., Gupta R. C. and Grover P. K., ***J. Chromatogr. Biomed. Appl.***, **616**, **1993**, 155-60
- 154) Klaymn D.L., ***Science***, **228**, **1985**, 1049-55
- 155) Alin M. H., ***Am. J. Trop. Med. Hyg.***, **50**, **1994**, 771-76
- 156) Winstanley P., ***Lancet***, **347**, **1996**, 2-3
- 157) Udomsangpetch R., Pipitaporn B. and White N. J., ***J. Infect. Dis.***, **173**, **1998**, 691-98
- 158) Dutta G. P., Bajpai R. and Vishwakarma R. A., ***Pharmacol. Res.***, **21**, **1989**, 415-19
- 159) Posner G. H., Oh C. H., Webstar H. K. and Ager A. L., ***Am. J. Trop. Med. Hyg.***, **50**, **1994**, 522-26
- 160) Hofheinz W., Gocke E., Jaquet C. and Schmid G., ***Trop. Med. Parasito.***, **45**, **1994**, 261-6, **20**, **21**
- 161) Talukdar P.B., Sengupta S.K. and Datta K.A., ***Ind. J. Chem.***, **20B**, **1981**, 538.
- 162) Shishoo C.J., Devani M.B., Ullas G.V., Ananthan S. and Bhadti V.S., ***J. Heter. Chem.***, **18**, **1981**, 43-46.
- 163) Shishoo C.J., Devani M.B. and Marvekar M.D., ***Ind. J. Chem.***, **21B**, **1982**, 666-68.

- 164) Russo F., Santagati M., Carsuo A., Trombaddokar S. and Amico Roxas M., **Eur. J. Med. Chem.**, **21**, **1986**, 119-22.
- 165) Roth B., **US Patent 3 470 183**, **1969**; cited from **Chem. Abstr.**, **72**, **1970**, 21706m
- 166) El-Kerdawy M.M., Yousif M.Y., El-Emam A.A. and Moustafa M A, **Chin. Pharma. J.**, **45**, **1993**, 457-63
- 167) Devani M. B., Shishoo C. J., Pathak U. S. and Sharma B. G., **Ind. J. Chem.**, **16 B**, **1978**, 575-78
- 168) Ram V. J., Pandey H. N. and Mishra L., **Arch. Pharm.**, **312**, **1979**, 586-90
- 169) Samsonova N. V., Kharizomenova L. A., Givenu A. N. and Kaplina E. K., **Kim. Phar. Zh.**, **15**, **1981**, 40-45
- 170) Vega S., Alonso J. and Diaz J. A., **Eur. J. Med. Chem.**, **26**, **1991**, 323-29
- 171) Santilli A. A. and Kim D. H., **US Patent 3 644 351**; Cited From **Chem. Abstr.**, **76**, **1972**, 140878 e.
- 172) Kaplina N. V., Shvedov V. I. and Filitis, **USSR Patent 1 138 752** ; Cited From **Chem. Abstr.**, **123**, **1995**, 228206r
- 173) Rosowsky A., Chaykovsky M., Chen K. K. N. and Lin M., **J. Med. Chem.**, **16**, **1973**, 185-94,
- 174) Maria I. C., Lluís P., Armando V. and Victor S., **J. Med. Chem.**, **41**, **1998**, 4021-35.
- 175) Hozein Z. A., Atta F. M., Hassan Kh M. and Ahmed S. A., **Synthetic Communication**, **26(20)**, **1996**, 3733-55
- 176) a) Woitun E. and Reuter W., **Ger. Patent 2 050 816**; Cited From **Chem. Abstr.**, **77**, **1972**, 34552m
 b) Woitun E. and Reuter W., **Ger. Patent 2 117 658**; Cited From **Chem. Abstr.**, **78**, **1972**, 16214u
- 177) a) Bourguignon J., Moreau M., Querguiner G. and Pastour P., **Bull. Soc. Chem. Fr.**, **(3-4 Pt 2)**, **1975**, 2483-87; Cited From **Chem. Abstr.**, **84**, **1975**, 150586u.
 b) Bourguignon J., Gougenon E., Querguiner G. and Pastour P., **Bull. Soc. Chem. Fr.**, **(3-4 Pt 2)**, **1975**, 815-19; Cited From **Chem. Abstr.**, **83**, **1975**, 114329 h

- 178) Desai J. R., Parikh A. R., Chauhan N. A., *J. Ind. Chem. Soc.*, **74**, **1997**, 160
179. a) Patil A. M., Chaudhari D. T., Patel M. T. and Sabnis S. S., *Bull. Haff. Instt.*, **5(3)**, **1977**, 107
b) Patil A. M., Chaudhari D. T., Patel M. T. and Sabnis S. S., *Bull. Haff. Instt.*, **6(1)**, **1978**, 36
- 180) Fioravanti R., Biava V., Porretta G. C. and Landolfi C., *Eur. J. Med. Chem.*, **30**, **1995**, 123-32
- 181) Bousquest E., Romeo G., Gurrera F. and Amico-Roxas M., *Farmaco*, **40**, **1985**, 869
- 182) Harbindra Singh, Sharma A. K., Iyer R. N. and Srivastva O. P., *J. Med. Chem.*, **20(6)**, **1977**, 826 .
- 183) Lioyed E. L., *Practitioner*, **181**, **1961**, 679
- 184) Locova E. L., Guozdjakova A., Chovancova J. and Volna F., *Chem. Zvesti.*, **38(5)**, **1984**, 693-98
- 185) Kharkar P., M.Pharm. Thesis *Synthesis of Heterocyclic Compounds As Possible Antibacterial, Antifungal and Antitubercular Agents*, **1998**
- 186) Zekany A. and Makleit S., *Pharmazie*, **42**, **1987**, 160
- 187) Carp E. and Toma A., *All Cuza. Isai. Sect.*, **11(1)**, **1965**, 67; Cited From *Chem. Abstr.*, **63**, **1965**, 14843e
- 188) Manian A., Ph.D. Thesis *Synthesis and Study of 2, 4 - Disubstituted Thiazole As Possible Chemotherapeutic Agents*, **1992**
- 189) Nilgun Karali, Nalan Terzioglu and Aysel Gursoy, *Arzneim.- Forsch / Drug Res.*, **48(11)**, **1998**, 758
- 190) Ahmad M. F., Abdel Mohsen M. E., Mounir A., Khalil, Mohamed A., Gaber and Hamida Abou- Shleib, *Euro. J. Med. Chem.*, **22**, **1987**, 369-71
- 191) Dobson R. A., O'Conner J. R., Poulin S. A., Kundsine R.B., Smith T. F. and Came P. F., *Antimicrob. Agents Chemother.*, **18**, **1980**, 738
- 192) Edward R. B. and Sol J. Daum, *J. Hetro. Chem.*, **28**, **1991**, 1953
- 193) Gillis P. M., Haemers A. and Pattyn S. R., *Antimicrob. Agents Chemother.*, **13**, **1978**, 533
- 194) Badaway Esam, Rida S. M., Hazza A. A., Fahmy H.T.Y. and Gohar Y. M., *Euro. J. Med. Chem.*, **28**, **1998**, 91-98

- 195) Lozanosa Kh., Vasiler G. and Kalcheva V., (Inst. Plant Physiol, 1113 Sofia, Bulg), ***Dolk. Bulg. Akad. Nauk.***, **44(9)**, **1991**, 115-18 ; Cited From ***Chem. Abstr.***, **116 1992**, 235516a
- 196) Murav Eva K. M. and Arkhangel Skaya, ***Chem. Abstr.***, **69**, **1968**, 106658e
- 197) Devani M. B., Shishoo C. J., Pathak U. S., Parikh S. H., Shah G. F. and Padhya A. C., ***J. Pharma. Sci.***, **65(5)**, **1976**, 660-64
- 198) Devani M. B., Shishoo C. J., Pathak U. S. and Sharma B. G., ***Ind. J. Chem.***, **15B**, **1977**, 575-77
- 199) Guccione S., Scolaro L. M. and Russo F., ***J. Hetero. Chem.***, **33**, **1996**, 459,
- 200) Vishnu J. R. and Pandey H. J., ***J. Hetero. Chem.***, **18**, **1998**, 1277
- 201) Patil A. S., M. Pharm. Thesis ***Synthesis and Study of Heterocyclic Compounds As Possible Antitubercular Agents***, **1988**
- 202) Yamamoto K., Yoshioka T. and Kato Y., ***J. Antibiot.***, **36**, **1983**, 407
- 203) Desai J. R., ***J. Inst. Chem. (India)***, **67(5)**, **1995**, 136-37 ; Cited From ***Chem. Abstr.***, **125**, **1996**, 247738y.
- 204) Cope, ***J. Am. Chem. Soc.***, **59**, **1937**, 2327
- 205) Cope, Hofmann Wyckoff and Hardenbergh, ***J. Am. Chem. Soc.***, **63**, **1941**, 3452
- 206) Harding, Haworth and Perkin, ***J. Chem. Soc.***, **93**, **1908**, 1943
- 207) Komppa, ***Ber.***, **33**, **1900**, 3532
- 208) Brich and Robinson, ***J. Chem. Soc.***, **488**, **1942**, 173
- 209) Wideqvist, ***Acta. Chem. Scand.***, **3**, **1949**, 303
- 210) Hann and Lapworth, ***J. Chem. Soc.***, **85**, **1904**, 46
- 211) Kohler and Corson, ***J. Chem. Soc.***, **45**, **1923**, 1975
- 212) Gewald K., Schinke E. and Bottcher H., ***Chem. Ber.***, **99**, **1966**, 94
- 213) Marlene Favre and Cuong Luv Duc, ***Euro. J. Med. Chem.***, **23**, **1988**, 453-56
- 214) Popp F. D., ***J. Med. Chem.***, **7**, **1964**, 210
- 215) Popp F. D., ***J. Org. Chem.***, **26**, **1961**, 1566
- 216) Schiff H., ***Arm. Chem.***, **131**, **1864**, 118
- 217) Tipson, Stuart R. and Clapp M. A., ***J. Org. Chem.***, **11**, **1946**, 292
- 218) Hayes K., Gever G. and Orchutt J., ***J. Am. Chem. Soc.***, **72**, **1950**, 1205

- 219) Giovambattista N. and Rabassa R., *Hev. Assoc. Bioquim., Argentina*, **18**, 1953, 220-2; Cited From *Chem. Abstr.*, **49**, 1955, 205f
- 220) Schroeder D. C., *Chem. Rev.*, **55**, 1955, 181
- 221) Dous F., *Organic Compounds of Sulphur, Selenium and Tellurium*, Vol 2, Chapter 4, Reid D. H. (ed.), The Chemical Society, London, 1973
- 222) Dous F., *Organic Compounds of Sulphur, Selenium and Tellurium*, Vol 3, Chapter 5, Reid D. H. (ed.), The Chemical Society, London, 1975
- 223) Dous F., *Organic Compounds of Sulphur, Selenium and Tellurium*, Vol 1, Chapter 5, Reid D. H. (ed.), The Chemical Society, London, 1970
- 224) Adams R. and Marvel C. S., *Org. Syn., Coll. Vol. 1*, 1941, 84
- 225) Huntress E. H. and Auterrieth J. S., *J. Am. Chem. Soc.*, **63**, 1941, 3446
- 226) Huntress E. H., *J. Am. Chem. Soc.*, **62**, 1940, 511
- 227) Scott R.V., Gayle J.B., Heller M.S. and Lutz R.E., *J. Org. Chem.*, **20**, 1955, 1165
- 228) Griolito S. L. and Hoffman H. O., *US Patent 3 600 136*, 1971; Cited From *Chem. Abstr.*, **75**, 1971, 129353v
- 229) *Dictionary of Organic Compounds*, Chapman and Hall, 6th Edn., Vol 2, , P1063
- 230) *Dictionary of Organic Compounds*, Chapman and Hall, 6th Edn., Vol 2, , P1008
- 231) Hussan N. S., Ravindranathan T. and Tilak B. D., *Ind. J. Chem.*, **16(B)**, 1978, 721-2
- 232) Bebooulene M. and Strurtz G., *Chim. Ther.*, **7(6)**, 1972, 493-502 (Fr); Cited From *Chem. Abstr.*, **78**, 1973, 71783d
- 233) Lecomte J. M. and Cugnon M., *Can. J. Chem.*, **15(17)**, 1975, 2598-607
- 234) Carrie R. and Kiryuskin A. A., *J. Chem. Res. Synop.*, **11**, 1986, 428-9
- 235) Rake G., Jambor W. P., Mckee C. M., Pansy F., Wiselogle F. Y. and Donvovic R., *Am. Rev. Tubercul.*, **60**, 1949, 121
- 236) Koneman E. V., Janda W. M., Allen S. D., Sommers H. M., Dowell V. R. and Winn W. C., *Color Atlas and Taxbook of Diagnostic Microbiology*, J. B. Lippincott Company, 3rd Edn., 1988, 487
- 237) Joan Stokes E., *Clinical Bacteriology*, 4th Edn., 1975, 226

- 238) Vincke I. and Lips M., *Ann. Soc. Belge. Med Trop.*, **28**, **1948**, 97-104 ; Cited
From *Antimalarial Drugs*, **Vol. I**, **1986**, P225
- 239) Osdone T. S., Russell P. B. and Rane L., *J. Med. Chem.*, **10**, **1967**, 431-34
- 240) Crowin H., Bruce A. H., Zong-Ru-Guo and Cynthia D., *J. Med. Chem.*, **27**,
• **1984**, 129-43.
- 241) Thurston J. P., *Trans. Roy. Soc. Trop. Med. Hyg.*, **44**, **1950**, 703-06

APPENDIX

1. All the fine chemicals used in the synthetic work were obtained from
 - a. Fluka Chemie AG
 - b. Aldrich Chemical Company
 - c. Lancaster Ltd.
2. All the commercial solvents used in the synthetic work were redistilled and dried before use.
3. The melting points have been determined in open capillaries in a Veego VMP-1 melting point apparatus and are uncorrected.
4. The TLC of the synthesized compounds has been run on E. Merck pre-coated silica gel plates of type 60 F₂₅₄.
5. The infra-red spectra were recorded on a BOMEM MB-104 Fourier Transform Infrared Spectrometer.
6. Elemental analysis (C,H,N) were performed on Heraeus elemental analyser.
7. The PMR spectra were recorded on Varian 300 MHz spectrophotometer using TMS as internal reference. Chemical shifts were reported as δ (ppm) values.

Synopsis

**A SYNOPSIS TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI
IN FULFILLMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY
IN THE FACULTY OF TECHNOLOGY (PHARMACEUTICAL CHEMISTRY)**

Title of the Thesis : Synthesis and study of Thienopyrimidine and its derivatives as possible antibacterial and antifungal agents.

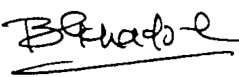
Name of the Candidate : Mr. Ravindra V. Chambhare

Name and Designation of the Guide : Dr.B.G. Khadse,
Director - Haffkine Institute, Parel, Mumbai-400 012.

Place of Research : Department of Chemotherapy,
Haffkine Institute for Training, Research and Testing, Parel, Mumbai - 400 012.

Number and date of Registration : 28 ; Feb. 25, 1997.

Signature of Candidate : 

Signature of the Guide : 

SYNOPSIS

A considerable progress has been made in the past 30 years in the chemotherapy of microbial diseases. The seemingly uncontrollable phenomenon of drug resistance has become one of the main driving forces behind the search for new therapies and development of new antimicrobials. Although resistance was observed soon after the introduction of the earliest antimicrobials into the market, it was only quite recently that this problem was recognised as a serious threat for the continuing therapeutic utility of antimicrobials in the treatment of infectious diseases.

The worldwide emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, multi-resistant *Mycobacterium tuberculosis*, and vancomycin-resistant *Enterococci* strains are among the major problems currently plaguing the medical community. The knowledge of bacterial resistance and the study of induction routes as well as the factors responsible for the changes in antimicrobial resistance patterns are of primary importance for predicting the future medical need and preventing the emergence of resistant pathogens. In order to prevent or control the emergence of bacterial resistance the discovery of new broad spectrum antimicrobials, especially those against which the microorganisms have little chance of developing resistance, is necessary. The newer compounds should have following properties -

1. Potent activity
2. Good tolerance
3. Lesser side effects
4. Efficacy against microbial resistant strain.

Many reviews clearly emphasise the importance of heterocycles in naturally occurring as well as synthetic agents which are an important class itself possessing antimicrobial, antifungal, antitubercular, antiviral and antimalarial activity. This points out the need for further investigations in this field. The logic supporting the work presented in this investigation was formulated, bearing in mind the biological activities of known moieties and attempting certain structural modifications or adaptations in the light of the recent trends in drug research in incorporating newly emerged pharmacophore on existing moieties.

Different biological properties of condensed pyrimidines as sedative, antibacterial and antimalarial are well documented. The isosteric properties of benzene and thiophene in general are of interest to medicinal chemists. The electron-rich properties as well as the slightly smaller steric volume of thiophene as compared to benzene may play an important role in fitting the necessary biological receptor sites. The thienopyrimidine (a purine analogue) has recently received considerable attention. Many of its substituted derivatives including thienotriazolopyrimidine, thienotetrazolopyrimidine, thienothiadiazolopyrimidine, and others have pronounced antibacterial¹, antifungal², antiviral³, anti-inflammatory⁴, antitubercular⁵, antimalarial⁶, and phosphodiesterase-inhibitor activity⁷.

In present studies, thieno(2,3-d)pyrimidin-4(3H)-one moiety has been chosen as basic nucleus. Recently, Hozien *et al*⁸ synthesized and evaluated some derivatives of 4-

substituted-5-phenyl-thieno(2,3-d)pyrimidine as potential antibacterial and antifungal agents. By introducing different biologically active heterocycles such as 2-furyl, 2-thienyl, 3-pyridinyl, and 4-pyridinyl at C₅ position and joining the halogenated aryl/heteroaryl substituents by different pharmacophoric side chain at 2 and 3 - position of thieno(2,3-d)pyrimidin-4(3H)-one nucleus, it may be expected to get potent compounds of desired biological activity as these heterocycles and pharmacophoric side chains play an important role in activity of most of the clinically used drugs. All the compounds have been characterized by elemental analysis and spectral data and screened for their antimicrobial, antifungal and antimalarial activity.

Thesis will be conveniently presented into the four parts as follows :

Part I : Introduction, Review of literature, and Plan of work

Part II-a : Synthesis of 2-methyl-3N-[(substituted aryl/heteroaryl)-methylene]-amino-5(2-furyl/2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-ones.

Part II-b : Synthesis of 2-methyl-3N-[(substituted aryl/heteroaryl)carboxamido]-5(2-furyl/2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-ones.

Part II-c : Synthesis of 2-methyl-3[3-ethyl-4(substituted aryl/heteroaryl)-2,3 dihydro thiazolidene amino]-5(2-furyl/2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-ones.

Part II-d : Synthesis of 2-[(substituted aryl/heteroaryl)-thio keto methylene]-3 phenyl-5-(3-pyridinyl/4-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones.

Part II-e : Synthesis of 2-(substituted benzyl)-thio-3 phenyl-5(3-pyridinyl/4-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones.

Part II-f : Synthesis of 2-(substituted aryl/heteroaryl)-sulfonamido-5(3-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones.

Part III : Biological screening and results.

Part IV : Discussion and Conclusion.

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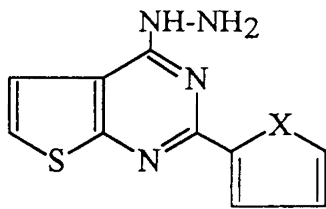
amino-5-(2-furyl/2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-ones

respectively

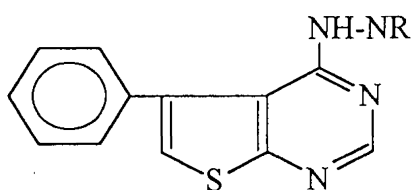


R = Substituted amino

(III) and 4-substituted amino-2-thienyl-thieno[2,3-d]pyrimidine (IV)


$$X = O \quad (\text{III})$$
$$X = S \text{ (IV)}$$

and bacteria

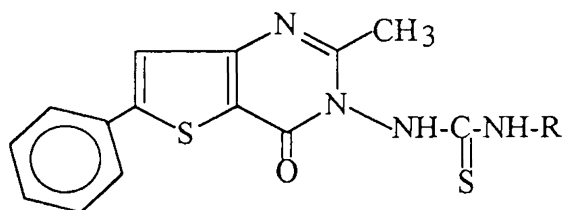


$R = H_2$ (V)

$R = =CH-R'$ (VI)

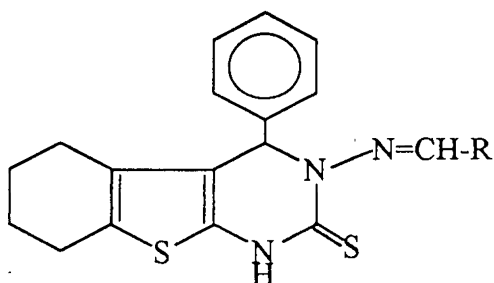
$R' =$ Substituted aryl

Recently, Desai *et al*¹¹ synthesized 3N-substituted-thiouredo-2-methyl-6-phenyl-thieno[3,2-d]pyrimidin-4(3H)-one (VII) derivatives which showed good antibacterial activity against various types of bacteria



(VII) $R =$ Substituted aryl

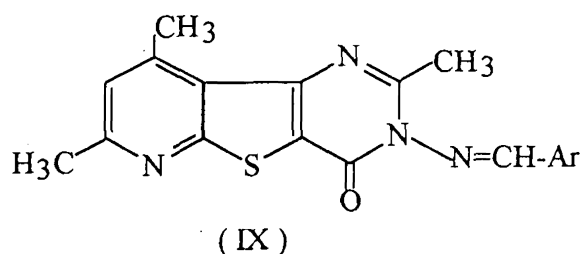
Vega *et al*⁴ had prepared the Schiff bases (VIII) of 2-thioxo-4-phenyl-benzothieno[2,3-d]pyrimidine and tested for analgesic and anti-inflammatory activity



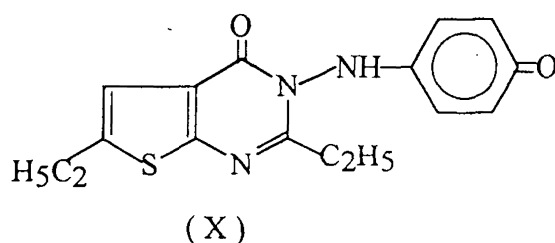
(VIII) $R =$ Substituted aryl

Schiff bases from substituted benzaldehyde have been reported to have encouraging tuberculostatic action . The presence of azomethyl linkage in a variety of biologically active compounds such as Nitrofurantoin and also in the field of antibiotics such as Rifampicin has been proved to be very essential.

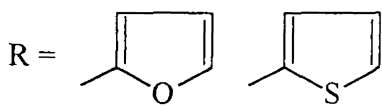
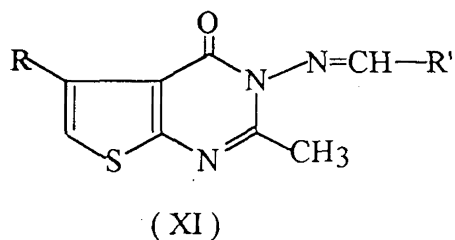
Bousquest *et al*¹² prepared a number of Schiff bases of pyrido-thieno[3,2-d]pyrimidione (IX), they were found to have interesting analgesic activity without GI complications.



Kharizomenova *et al*³ synthesized 3-substituted -2,6-diethyl-thieno[2,3-d]pyrimidin-4(3H)-one derivatives and screened for their antiviral activity. Out of these 3-(p-benzoquinonylimino-2,6-diethyl-thieno[2,3-d]pyrimidin-4(3H)-one (X) was found to be the most active antiviral agent.



Thus, based on the above data two series of 2-methyl-3N-[(substituted aryl/heteroaryl)methylene]amino-5(2-furyl/2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-ones (XI) have been synthesized and screened for their possible antibacterial, antifungal, antitubercular, and antimalarial activities.

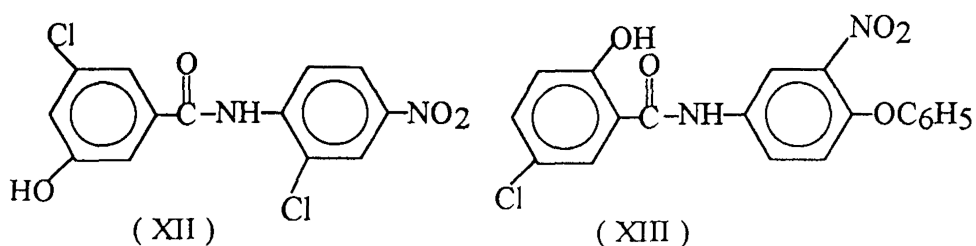


R' = Substituted aryl / heteroaryl

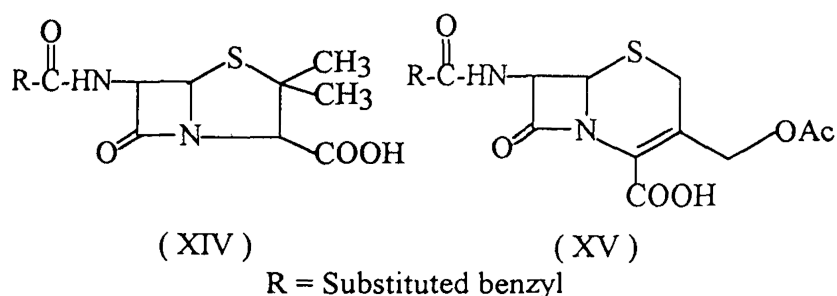
PART II-b : Synthesis of 2-methyl-3N-[(substituted aryl/heteroaryl) carboxamido]-

5(2-furyl/2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-ones

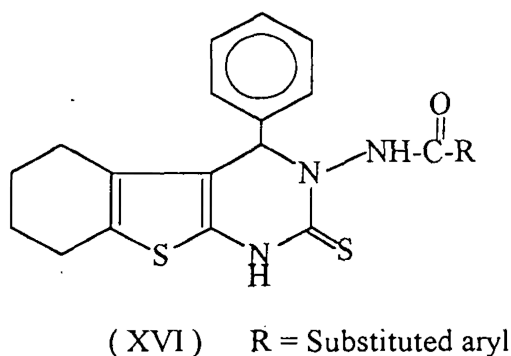
The acid amido linkage was found to be responsible for various kinds of activity as seen from the following examples. Niclosamide (XII), a 5-chloro salicylanilide derivative, is a drug of choice in the treatment of cestodes. Another salicylanilide derivative (XIII) was found to be effective against Penicillin-resistant *S. aureus* at a minimum inhibitory concentration of 0.00609 µg/ml¹³



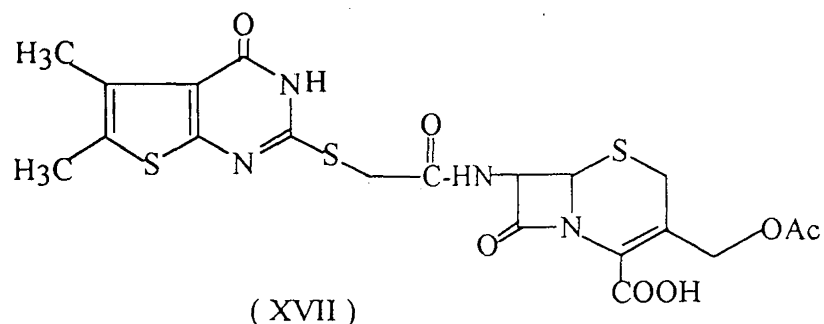
The basic structure of Penicillin (XIV) and Cephalosporin (XV) antibiotics consist of a thiazolidine and dihydrothiazine ring respectively connected to a β -lactam ring which is attached to a side chain through amidocarbonyl. Many pharmacological characteristics of Penicillin and Cephalosporin antibiotics are determined by its side chain which plays an important role in antimicrobial activity.



Vaga⁴ prepared 3-acylamino-4-phenyl-2-thioxobenzothieno[2,3-d]pyrimidine (XVI) derivatives and tested for their analgesic and anti-inflammatory activities.

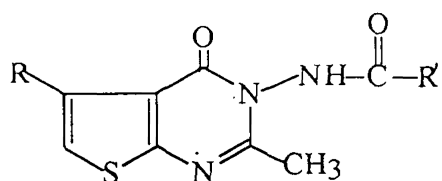


Thieno[2,3-d]pyrimidin-4(3H)-one ring system joined to dihydrothiazine ring through -CONH- linkage was found to be very active against Gram-positive organisms¹⁴. The structure of (XVII) is given below

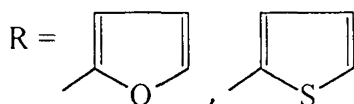


Heterocycles such as thienyl and furyl attached at C₂ position of thienopyrimidine ring system (Part II-a) reported to have significant antibacterial and antifungal activities. Also a number of β -lactam antibiotics bearing these (furyl/thienyl) heterocycles through acylamino side chain are broad-spectrum antibiotics.

All the above examples indicate the importance of the amido linkage in biologically active moieties. Taking into account the above facts and the biological importance of different heteroaryl substituents in molecules, it was thought worthwhile to synthesize molecules where the active thienopyrimidione is substituted at C₅ position with heterocycle (furyl/thienyl) and at 3-position with various substituted aryl/heteroaryl substituents. The general structure of the compounds is given below (XVIII). The compounds were studied for antibacterial, antifungal, antitubercular, and antimalarial activities.



(XVIII)

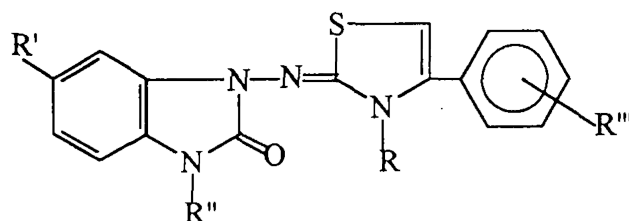


R' = Substituted aryl / heteroaryl

PART II-c : Synthesis of 2-methyl-3[3-ethyl-4(substituted aryl/heteroaryl)-2,3dihydro-thiazolidene-amino]-5(2-furyl/2-thienyl)-thieno[2,3-d]pyrimidin-4 (3H)-ones

The thiazole nucleus first described by Arther Hantzsch in 1887 is an important member of the heterocyclic family. Over the period of years it has underwent intensive research and finds several applications in the field of medicinal chemistry. In 1965, Rumanian research workers showed the bacteriostatic activity of thiazole with amino substituents at 2-position and alkyl or substituted alkyl at 4-position.

Recently, Karali *et al*¹⁵ synthesized derivatives of 3-[(3,4-disubstituted-4-thiazolin-2-ylidene)hydrazono-]-1H-2-indolinones (XIX) as potent antitubercular agents having MIC<12.5µg/ml



(XIX)

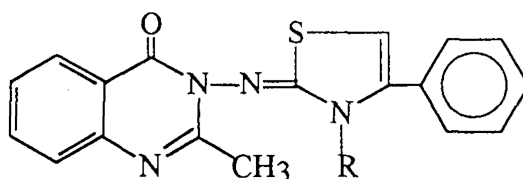
R = Allyl, CH₃, nC₄H₉ etc.

R' = H

R'' = H, Br

R''' = H, Br, NO₂, OCH₃

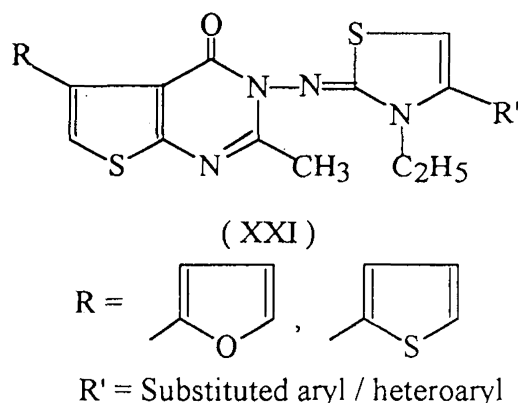
Ahmad and Farghaly¹⁶ synthesized 4(3H)-quinazolinone derivatives having substituted thiazoline moiety at the 3-position (XX), which exhibited good antibacterial and antifungal activities



(XX)

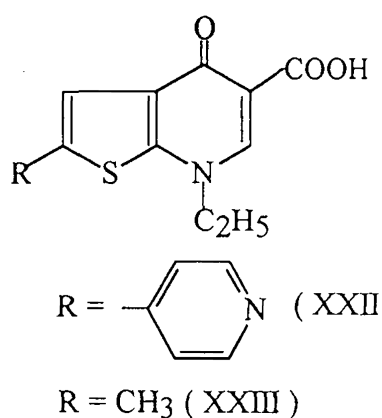
R = Allyl, C₆H₁₁, CH₂C₆H₅, C₆H₅, C₆H₄E

Hence on the basis of above evidence, a series of 5-(2-furyl/2-thienyl)-thieno[2,3-d]pyrimidin-4(3H)-one derivatives (XXI) having substituted thiazoline moiety at 3-position have been synthesized and tested for their antibacterial, antifungal, antitubercular, and antimalarial activities.

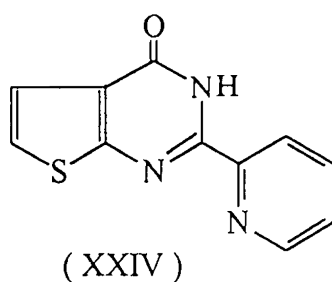


PART II-d : Synthesis of 2-[(substituted aryl/heteroaryl)thio keto methylene]-3-phenyl-5(3-pyridinyl/4-pyridinyl)-thieno[2,3-d]pyridin-4(3H)-ones

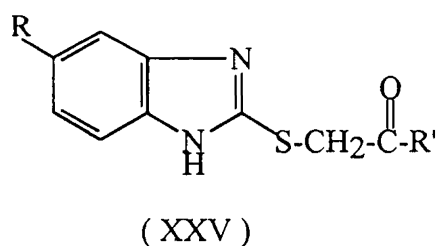
The pyridinyl moiety has played important role in active antitubercular drugs viz. Isoniazid, Ethionamide, and antibacterial Cephapirin. Edward *et al*¹⁷ synthesized compound (XXII) which is structurally related to the known antimicrobial thiophene (XXIII), an analog of nalidixic acid reported to have a similar antibacterial profile of activity



Also Bourguignon *et al*¹⁰ prepared 2-(2-pyridinyl)-thieno[2,3-d]pyrimidione (XXIV) and evaluated its biological activity.



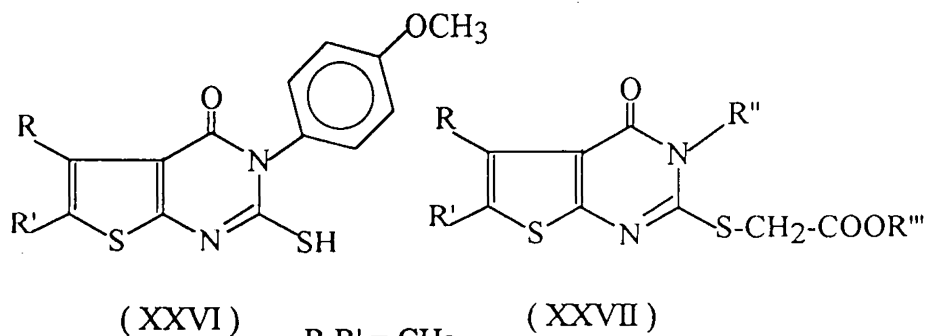
Lozanosa *et al*¹⁸ synthesized the following types of 2-phenacyl thiobenzimidazoles (XXV) which are known for their diverse biological activity



R = NO₂, Cl

R' = Substituted aryl

Devani *et al*¹⁹ have reported the antitubercular, antibacterial, and antifungal activity of 2-mercapto-thieno[2,3-d]pyrimidin-4(3H)-ones (XXVI) and thieno[2,3-d]pyrimidin-4(3H)-one-2-mercaptoacetic acid and ethyl ester (XXVII)

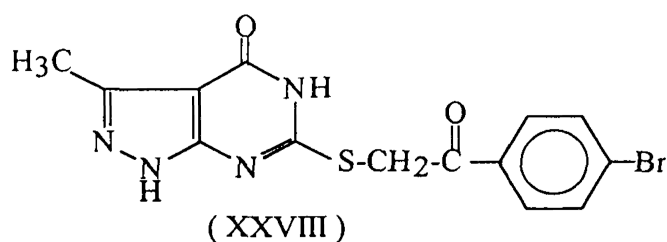


R, R' = CH₃

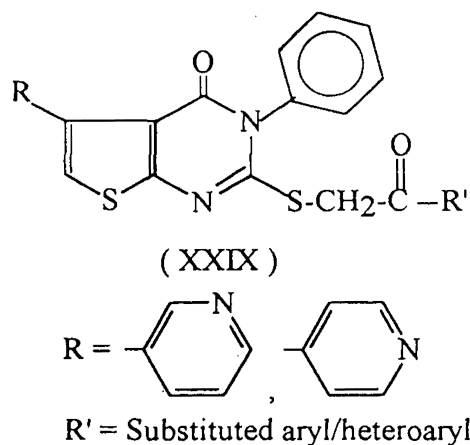
R'' = CH₃, C₂H₅

R''' = H, C₂H₅

Recently Guccione *et al*²⁰ have prepared 3-methyl-4-oxo-6-(phenacyl thio)-pyrazolo[3,4-d]pyrimidin (XXVIII) by condensation of α -haloketones with 3-methyl-4-oxo-6-mercapto pyrazolo[3,4-d]pyrimidine and screened for anti-inflammatory and analgesic activity

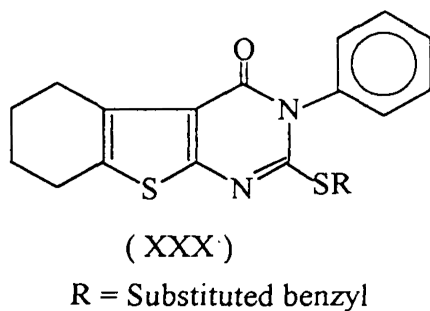


Careful study of the literature exemplifies the pharmacophoric importance of the pyridine ring and thieno[2,3-d]pyrimidine moiety in various antimicrobial agents. In view of the above findings two series of 2-[(substituted aryl/heteroaryl)-thio keto methylene]-3-phenyl-5(3-pyridinyl/4-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones were synthesized having following general structure (XXIX) and screened for their antibacterial, antifungal, antitubercular, and antimalarial activity.

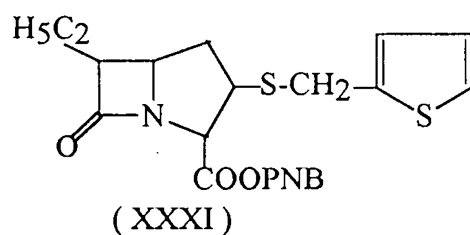


PART II-e: Synthesis of 2-(substituted benzyl)-thio-3-phenyl-5(3-pyridinyl/4-pyridinyl)-thieno[2,3-d]pyrimidin-4(3H)-ones

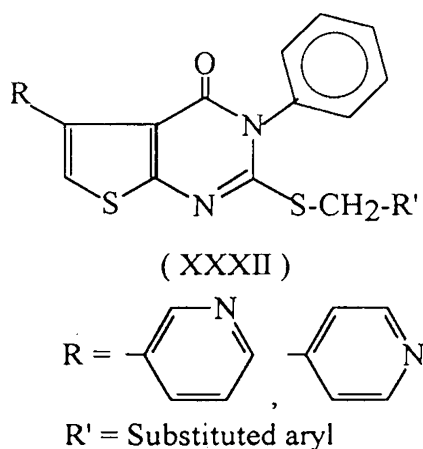
Vishnu *et al*²¹ have synthesized and studied the biological activity of 2-benzylmercapto-3-phenyl-5,6-tetramethylen-thieno[2,3-d]pyrimidin-4(3H)-one (XXX)



Yamamoto *et al*²² have reported Thienamycin analogue (XXXI) having antimicrobial activity superior or comparable to that of Thienamycin



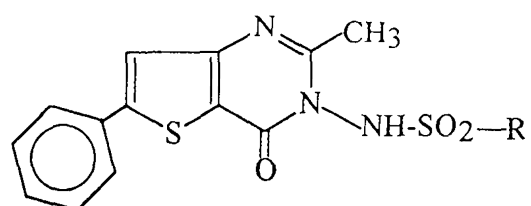
As mentioned in part II-d the importance of pyridinyl moiety attached to thienopyrimidine ring system and based on the above literature finding, we plan to synthesize thieno[2,3-d]pyrimidin-4(3H)-ones of following structure (XXXII) bearing substituted aryl moiety at C₂-position through sulfide linkage and 3-pyridinyl/4-pyridinyl moiety at C₅ position. All the compounds were subjected for their *in vitro* antibacterial, antitubercular, antifungal, and antimalarial activity.



PART II-f : Synthesis of 2-(substituted aryl/heteroaryl)-sulfonamido-5(3-pyridinyl)

-thieno[2,3-d]pyrimidin-4(3H)-ones

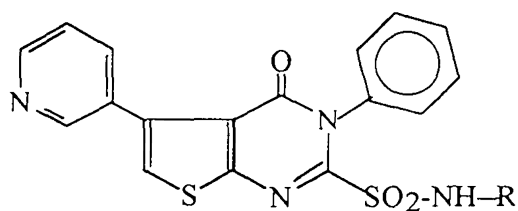
Desai ²³ synthesized substituted thieno[3,2-d]pyrimidin-4(3H)-one derivatives (XXXIII) by joining the sulfonamido group at 3-position and phenyl group at C₆ position of thienopyrimidione moiety. These derivatives showed significant antimicrobial activity



(XXXIII)

R = 4MeC₆H₄, C₆H₅, AcNHC₆H₄
3HO₂CC₆H₄ etc.

Thus considering the importance of sulfonamido linkage as effective synthons in preparing compounds of medicinal value, several aryl/heteroaryl amines have been attached at C₂ position and 3-pyridinyl group at C₅ position of thieno[2,3-d]pyrimidin-4(3H)-one moiety in order to get potent compounds of following types (XXXIV) and screened for their antibacterial, antifungal, antitubercular, and antimalarial activity.



(XXXIV)

R = Substituted aryl/heteroaryl

PART III : Biological Screening and Results

The compounds reported herein have been subjected to elemental and spectral analysis and results have been found satisfactory. The purity of compounds has been checked by TLC. The compounds reported in part II-a to II-f have been screened for their *in vitro* antibacterial, antifungal, antitubercular and *in vivo* antimalarial activity.

Antibacterial activity :

All the compounds have been screened for their *in vitro* antibacterial activity using tube-dilution technique against *Staphylococcus aureus* and *Salmonella typhi*. The culture media used in screening was Muller-Hinton broth²⁴. Ampicillin was used as standard drug.

Antitubercular activity :

The *in vitro* antitubercular screening of compounds was carried out by the tube-dilution technique employing the human virulent H₃₇RV strain of *Mycobacterium tuberculosis*. By this method using Kirchners medium containing Tween 80 as described by Rake *et al*²⁵, dispersed growth of *M.tuberculosis* was obtained. Isoniazid was used as standard drug.

Antifungal activity :

The compounds have also been screened for their antifungal activity *in vitro* by tube dilution technique²⁶ against fungal strains viz. *Candida albicans*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* using Miconazole as standard drug.

Antimalarial activity :

Some compounds have been screened for their antimalarial activity employing rodent malarial strain of *P. Berghei* in mice (Haffkine strain weighing 22-25g) *in vivo* by Rane's preliminary rapid screening method²⁷. Pyrimethamine was used as the standard drug. The drug which enhanced the survival time (twice the period of untreated control mice) was considered to be active. The dose at which the survival time was doubled was considered minimum effective dose(MED). The animals administered with the drug and did not recover from the infection were observed till death.

REFERENCES

1. Devani, M. B., Shishoo, C. J., Pathak, U. S. Sharma, B. G., *Ind. J. Chem.*, 16B, 575-78, (1978)
2. Ram, V. J., Pandey, H. N., Mishra, L., *Arch. Pharma.*, 312, 586-90, (1979)
3. Samsonova, N. V., Kharizomenova, L. A., Givenu, A. N., Kaplina, E. K., *Kim. Pharm. Zh.*, 15, 40-45, (1981)
4. Vega, S., Alonso, J., Diaz, J. A., *Eur. J. Med. Chem.*, 26, 323-29, (1991)
5. Kaplina, N. V., Shvedov, V. I., Filitis, USSR Patent 1 138 752; cited from *Chem. Abstr.*, (1995), 123, 228206r
6. Rosowsky, A., Chaykovsky, M., Chen, K. K. N., Lin, M., *J. Med. Chem.*, 16, 185-94, (1973)
7. Maria, I. C., Lluís, P., Armando, V., Victor, S., *J. Med. Chem.*, 41, 4021-35, (1998)
8. Hozein, Z. A., Atta, F. M., Hassan Kh. M., Ahmed, S. A., *Synth. Commu.*, 26(20), 3733-55, (1996)
9. a. Woitun, E., Reuter, W., Ger. Patent 2 050 816; cited from *Chem. Abstr.*, (1972) 77, 34552m
b. Woitun, E., Reuter, W., Ger. Patent 2 117 658; cited from *Chem. Abstr.*, (1972) 78, 16214u
10. a. Bourguignon, J., Moreau, M., Querguiner, G., and Pastour, P., *Bull. Soc. Chem Fr.* (11-12, pt 2), 2483-7, (1975); cited from *Chem. Abstr.* (1975), 84, 150586u
b. Bourguignon, J., Gougenon, E., Querguiner, G., and Pastour, P., *Bull. Soc. Chem. Fr.* (3-4 pt 2), 815-19, (1975); cited from *Chem. Abstr.* (1975), 83, 114329h
11. Desai, J. R., Parikh, A. R., Chauhan, N. A., *J. Indi. Chem. Soc.*, 74, 160, (1997)
12. Bousquest, E., Romeo, G., Guerrera, F., Amico-Roxas, M., *Farmaco*, 40, 869, (1985)
13. Lioyed, E. L., *Practitioner*, 181, 679, (1961)
14. Zekany, A. and Makleit, S. *Pharmazie*, 42, 160, (1987)
15. Nilgun Karali, Nalan Terzioglu and Aysel Gursoy, *Arzneim-Forsch/Drug Res.*, 48(11), 7, 758, (1998)
16. Ahmad, M. F., Abdel Mohsen, M. E., Mounir, A. Khalil, Mohamed A. Gaber, and Hamida Abou-Shleib, *Euro. J. Med. Chem.*, 22, 369-71, (1987)
17. Edward, R. B. and Sol J. Daum, *J. Hetro. Chem.*, 28, 1953, (1991)
18. Lozanosa, Kh., Vasilier, G., Kalcheva, V., (Inst. Plant Physiol., 1113 sofia, Bulg) *Dolk. Bulg. Akad. Nauk.* 44(9), 115-18(1991); cited from *Chem. Abstr.*, (1992), 116, 235516a
19. a. Devani, M. B., Shishoo, C. J., Pathak, U. S. , Parikh, S. H., Shah, G. F., and Padhya, A.C., *J. Pharm. Sci.*, 65(5), 660-64, (1976)
b. Devani, M. B., Shishoo, C. J., Pathak, U. S. , and Sharma, B. G., *Indian J. Chem.*, 15B, 575-77, (1977)
20. Guccione, S., Scolaro, L. M. and Russo, F., *J. Hetero. Chem.*, 33, 459, (1996)
21. Vishnu, J. R. and Pandey, H. J., *J. Hetero. Chem.*, 18, 1277, (1998)
22. Yamamoto, K., Yoshioka, T., Kato, Y., *J. Antibiot.*, 36, 407, (1983)
23. Desai, J. R., *J. Inst. Chem. (India)*, 67(5), 136-37 (1995); cited from *Chem. Abstr.* (1996), 125, 247738y

24. Koneman, E. V., Janda, W. M., Allen, S. D., Sommers, H. M., Dowell, V. R., Winn, W. C., "*Color Atlas and Text Book of Diagnostic Microbiology*", J. B. Lippincott Co., 3rd edn., 487, (1988)
25. Rake, G., Jambor, W. P., Mckee, C. M., Pancy, F., Wiselogle, F.Y. and Donovic R., *Am. Rev. Tuber.*, 60, 121, (1949)
26. Joan Stokes E., *Clinical Bact.*, Edward Arnold, London, 4th edn., 226, (1975)
27. Osdene, T. S., Russel, P. B. and Rane L., *J. Med. Chem.*, 10, 431, (1967)

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