SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF DIHYDROPYRIMIDINE DERIVATIVES

Thesis submitted in partial fulfillment for the requirement of the
Degree of Master of Pharmacy
(Pharmaceutical Chemistry)
Faculty of Engineering and Technology

By
AJEYA SAMANTA
Exam Roll No.-M4PHA19028
Reg. No.-140852 of 2017-18

Under the Guidance of
PROF. (DR.) TAPAN KUMAR MAITY
Synthetic and Natural Product Research Laboratory
Division of Pharmaceutical Chemistry

Department of Pharmaceutical Technology
Jadavpur University
Kolkata-700032
2019

DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY
JADAVPUR UNIVERSITY
KOLKATA-700032

Certificate of Approval

This is to certify that ***Ajeya Samanta (Examination Roll No: M4PHA19028, Registration No: 140852 of 2017-18)*** *has carried out the project work on the subject entitled* ***"Synthesis, Characterization and Pharmacological Evaluation of Dihydropyrimidine Derivatives"*** *under the supervision of* ***Prof. (Dr.) Tapan Kumar Maity****, Professor, Department of Pharmaceutical Technology, Jadavpur University. This project work is submitted by her in partial fulfillment of the requirements for the degree of* ***Master of Pharmacy*** *(Pharmaceutical Chemistry) of Jadavpur University. She has carried out her work independently and with proper care and attention to our entire satisfaction.*

Forwarded by

_____

Prof. (Dr.) Pulok Kumar Mukherjee
Head
Department of Pharmaceutical Technology,
Jadavpur University
Kolkata-700032

Supervised by

_____

Prof. (Dr.) Tapan Kumar Maity
Synthetic and Natural Product Research Laboratory,
Department of Pharm. Tech.
Jadavpur University
Kolkata- 700032

……..……

Prof. (Dr.) Chiranjib Bhattacharjee
Dean, Faculty of Engineering & Technology
Jadavpur University
Kolkata-700032

ACKNOWLEDGEMENT

First and foremost, I feel immense pleasure in expressing my sincere and deep sense of gratitude to my supervising guide ***Prof. (Dr.) Tapan Kumar Maity****, Professor, Department of Pharmaceutical Technology, Jadavpur University, for giving me such a progressive & unconventional topic for my thesis work and of course for the patience and support in overcoming numerous obstacles I have been facing through my research. This work would have been impossible without his exemplary guidance, sustained interest, creative suggestions & motivation.*

I would like to express my sincere gratitude to ***Prof. (Dr.) Pulok Kumar Mukherjee****, Head, Department of Pharmaceutical Technology, Jadavpur University, for rendering me valuable help and necessary facilities to carry out this work.*

*I would also like to express my thanks to all of my respected teachers, laboratory seniors (****Mr. Tanmoy Guria****,* ***Mrs. Puspita Roy*** *and* ***Mr. Avik Maji****), my friend (****Ms. Suparna Ghosh****) & juniors (****Ms. Sanjukta Saha*** *and* ***Mr. Arnab Sarkar****) for their help and support in each step of work.*

I conevy my heartful gratitude to ***Prof. (Dr.) Amalesh Samanta****, Professor, Department of Pharmaceutical Technology, Jadavpur University, for rendering me valuable help, support and necessary facilities to complete this research work. I would also like to thank* ***Ms. Shilpa Das*** *&* ***Ms. Mousumi Tudu*** *for helping me to perform my research work.*

I also thank my friends and all others who have extended their cooperation and helped me immensely during the entire project work.

Ajeya Samanta
Class Roll No.-001711402028
Exam Roll No.-M4PHA19028

DECLARATION OF THE ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research as part of my work on "***Synthesis, Characterization and Pharmacological Evaluation of Dihydropyrimidine Derivatives."***

All the informations in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that as required by these rules and conduct, I have fully cited and refered all the informations and results that are not original to this work.

NAME: - AJEYA SAMANTA

EXAM ROLL NUMBER: - M4PHA19028

REGISTRATION NUMBER: - 140852 of 2017-18

THESIS TITLE: - "***Synthesis, Characterization and Pharmacological Evaluation of Dihydropyrimidine Derivatives***"

SIGNATURE WITH DATE:

Dedicated to
my guide, family and well wishers

INDEX

CHAPTER -1

INTRODUCTION

1. INTRODUCTION:

Two demons of diseases are knocking at the door: one is‘cancer’ and the other is diseases associated with ‘resistance of antimicrobials’. According to a survey report by World Health Organization, within 30 years there will be more death rate caused by antimicrobial resistance than cancer. Although for now, cancer is the leading cause of unexpected and unnatural death; by 2050, ten million people are set to lose their lives unnecessarily every year as a result of antimicrobial resistance unless drastic action is taken. [1]

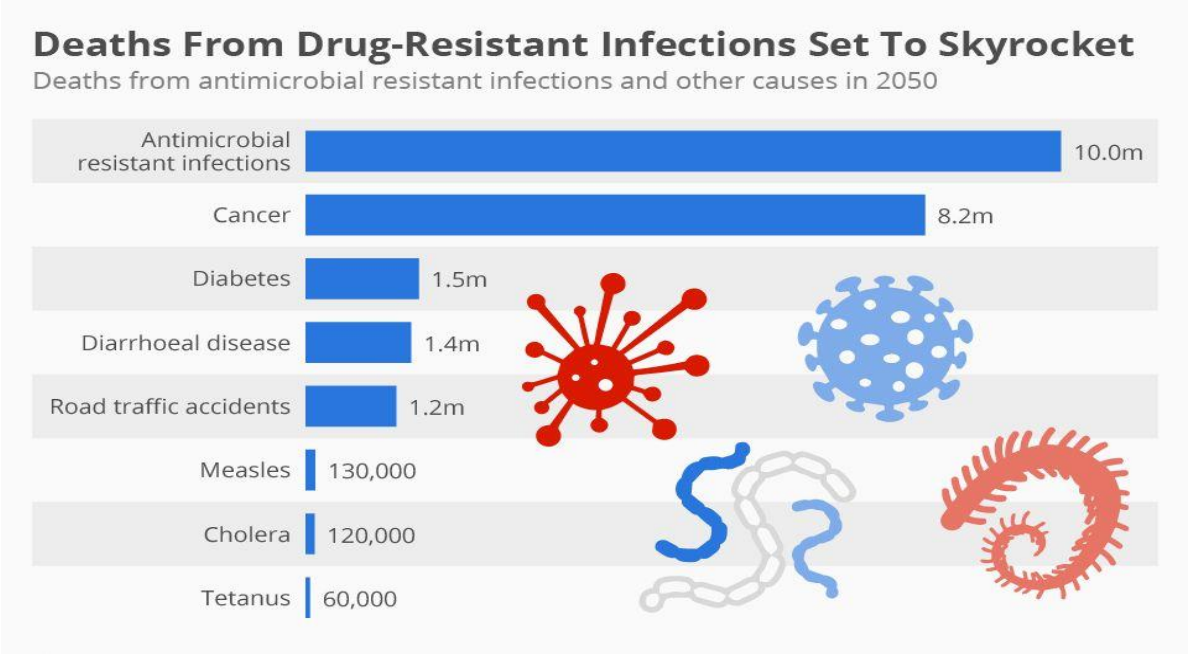


Fig 1.1. Deaths from antimicrobial resistant infections

In 1928, the discovery of penicillin opened the door to the modern era of medicine. Ever since these “magic bullets” have transformed medicine and saved countless lives. In the 1940s, the very first prescription of antibiotics was made. During World War II, penicillin was considered as the drug of choice and used to control bacterial infections among the armed forces.[1][2]

Unfortunately, soon after that resistance against penicillin turned out to be a significant clinical concern. So, most of the advancements made in the 1940s were threatened during the 1950s. However, to maintain the use of antibiotic treatment strategies, scientists soon discovered and developed novel β-lactam antibiotics. However, the first case of methicillin-

resistant *Staphylococcus aureus* (MRSA) was reported in the UK and the US in 1962 and 1968, respectively, the same decade when new antibiotics were applied. [1][2]

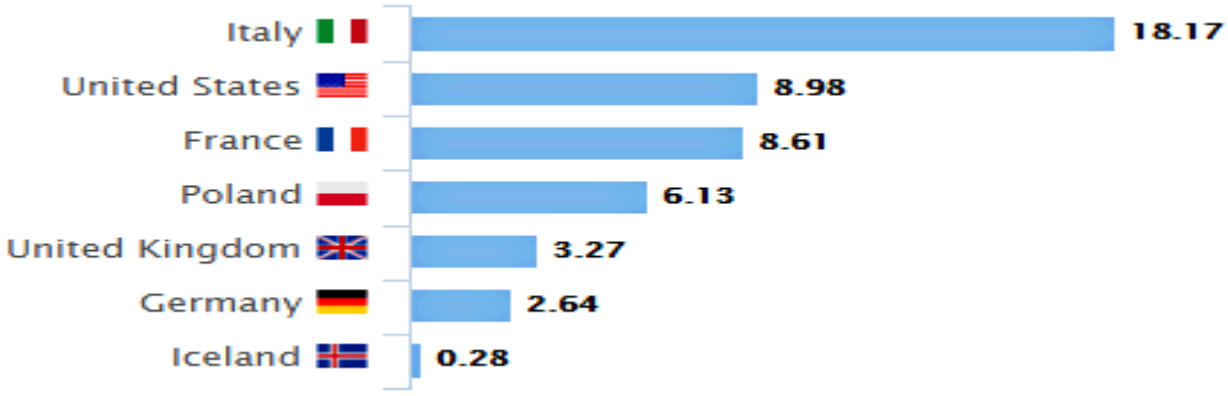


Fig1.2. Predicted death in percentage worldwide due to Antimicrobial resistance within 2015 to 2025

Numerous important organizations, like the Centers for Disease Control and Prevention (CDC), Infectious Diseases Society of America, World Economic Forum, and the World Health Organization (WHO) have declared antibiotic resistance to be a 'global public health concern'. As new forms of antibiotic resistance can cross international boundaries and spread between continents with ease, so it is a worldwide problem. Many forms of resistance spread with remarkable speed. World health predictors have described antibiotic-resistant micro-organisms as 'nightmare bacteria' that 'pose a catastrophic threat' to people in every country in the world. Each year globally, at least 2 million people acquire serious infections with bacteria that are resistant to one or more of the antibiotics designed to treat those infections. At least 23,000 people die each year as a direct result of these antibiotic-resistant infections. Many more die from other conditions that were complicated by an antibiotic-resistant infection. [3]

As AMR grows, there is an urgent need for novel compounds to be developed to treat life-threatening infections. Yet there is little incentive for pharmaceutical companies to invest in antimicrobial R&D, not least because of the major technical challenges involved in discovering and developing new antimicrobial classes. There is also little promise of a swift return on investment. New antibiotics are particularly highly sought after, yet must be used conservatively to limit the risk of resistance emerging. This makes high-volume, high-return markets less likely to develop. Nevertheless, a core group of companies remain committed and have dedicated antimicrobial R&D divisions. To stimulate pharmaceutical company investment in R&D for new antimicrobials, the global AMR community has established a

range of both “push” and “pull” incentives; these either lower the cost of developing a new antimicrobial medicine or reward its successful development. [4]

Numerous pharmaceutical companies have publicly committed to tackling AMR, with many signing the “Declaration by the Pharmaceutical, Biotechnology and Diagnostics Industries on Combating Antimicrobial Resistance” (The Davos Declaration), which was made in 2016. This was followed by the publication by a core group of manufacturers of an “Industry Roadmap for Progress on Combating Antimicrobial Resistance” (Industry Roadmap). Both documents assure us that several pharmaceutical and biotechnology companies are poised to play their part in addressing AMR. [5]

Substantial investment and research in the field of anti-infectives are now desperately needed if a public health crisis is to be averted.

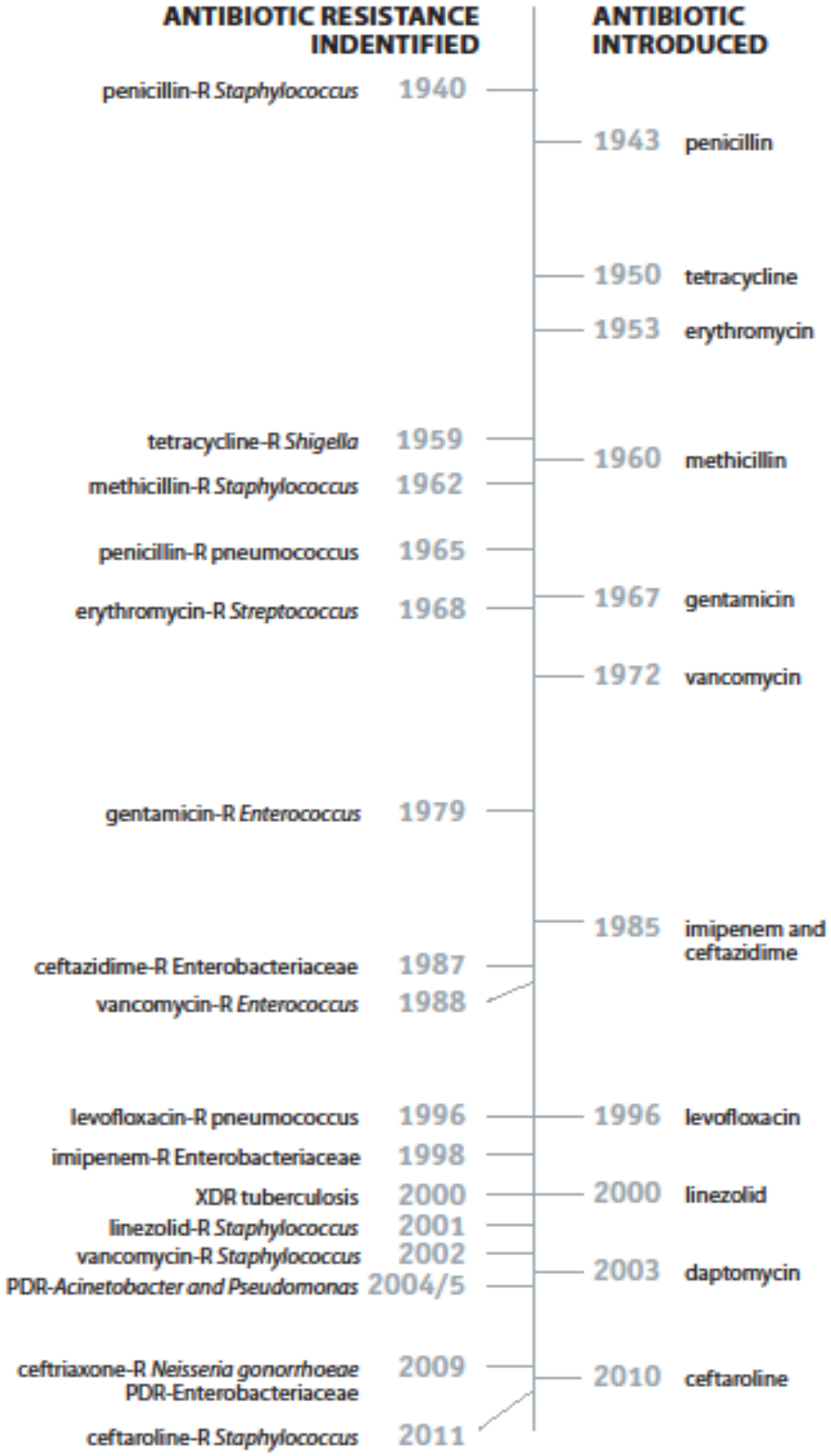


Fig 1.3. Evolution of Antimicrobial Agents [8]

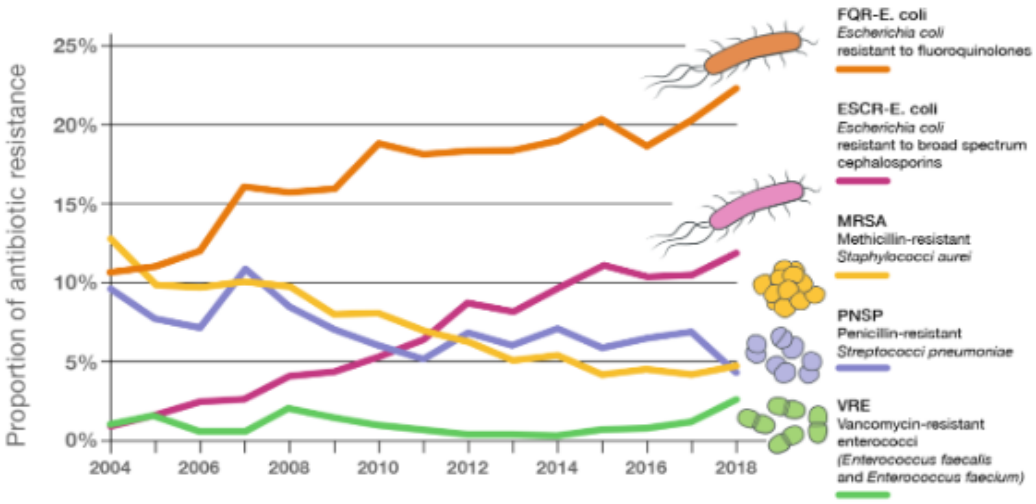


Fig1.4. Antibiotic resistance in some bacterial species from 2004 to 2018 [9]

The data accumulated since 2004 show mixed results: antibiotic resistance has increased strongly in some species of bacteria and has decreased or remained stable in others. For *Escherichia coli* – frequently involved in lower tract urinary infections – resistance to fluoroquinolones (a commonly used class of antimicrobial agent) has increased, as has resistance to another class of broad-spectrum antibiotics (3^{rd} and 4^{th} generation cephalosporin). For *Klebsiella pneumoniae*, potentially responsible for urinary tract and respiratory tract infections, resistance similarly increased until 2014, but has been declining in the last 3 years. The reasons for this change in trend are not clear and are still under investigation.The proportion of vancomycin-resistant enterococci has remained stable but has increased since early 2018. [9] Unfortunately, its presence in a hospital can lead to the cancellation of some surgical operations.Infections due to penicillin-resistant streptococci – which can cause pneumonia, for example – have probably declined. Thanks to vaccination, since the vaccine also protects against antibiotic-resistance streptococci.The proportion of invasive infections caused by methicillin-resistant *Staphylococcus aureuos* (MRSA) has also decreased, thanks to improved detection and rapid treatment of infected patients in hospitals. [10]

1.1. Antimicrobial Agents:

1.1.1. Definition: An antimicrobial substance is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against it. For example, antibiotics are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. [11]

1.1.2. History of the anti-microbial agents:

Bacteria are simple one-celled organisms, which were first identified in the 1670's by Van Leeuwenhoek. Later in the nineteenth century, concepts have been developed that there is the strongest correlation between bacteria and diseases. Such considerations attracted interest of the researchers not only to answer some mysterious questions about infectious diseases, but also to find a substance that could kill, inhibit, or at least slow down the growth of such disease-causing bacteria. These efforts led to the revolutionary discovery of the antibacterial agent "penicillin" in 1928 from *Penicillium notatum* by Sir Alexander Fleming. The discovery unlocked the field of microbial natural products and so new agents were continually added, such as newly introduced daptomycin, tigecycline, linezolid, and so on. Gradually, due to various issues arising during the use of antibacterial agents, such as the resistance phenomenon, an enormous increase in the number and types (e.g. structurally different and agent with a slightly different pattern of activity) of the newly added antibacterial agents has been observed. This made it necessary to review and compile the existing classification and functions of almost all the antibacterial agents. [12]

1.1.3. Classification:

- **According to chemical structures:**
  A. β-Lactams: Penicillin derivatives, cephalosporins, monobactams, and carbepenems, e.g. imipenems, all belong to this class.
  B. Aminoglycoside: Streptomycin, gentamicin, sisiomicin, netilmicin, kanamycin, amikacin, neomycin, tobramycin, toframycin, spectinolycin, and paromonucin.
  C. Macrolides: Erythromycin and roxithromycin [15]

D. Quinolones and flouroquinolones: Nalidixic acid (first generation), ciprofloxacin (second generation), levofloxacin (third generation), and trovafloxacin (fourth generation).
E. Streptogramin antibiotics: Dalfopristin and quinopristin are representative example of the streptogramin A and streptogramin B groups, respectively. Alteration of the group B structural units has been mainly achieved on the 3-hydroxypicolinoyl, the 4-dimethylaminophenylalanine, and the 4-oxo pipecolinic residues.
F. Sulphonamides: these have a sulphonamide functional group (R_1-SO_2-NR_2-R_3) in their structures, sulfadiazine.
G. Tetracyclines: Oxytetracycline and doxycycline
H. Nitroimidazoles: This class of drugs contains a basic imidazole ring. The most commonly used example is metronidazole.

- **According to type of action:**

A. Bacteriostatic antibacterials:
   a. Sulphonamides
   b. p-nitrobenzene derivatives, e.g. Chloramphenicol
   c. Spectinomycin
   d. Trimethoprim
   e. Tigecycline; it belongs to the glycylcycline class (class of antibiotics derived from tetracycline)
   f. Erythromycin, clarithromycin and azithromycin are macrolides
   g. Linezolid is a member of the oxazolidinone class
   h. Doxycycline, tetracycline, and minocycline belong to tetracycline class
B. Bactericidal antimicrobials:
   a. Penicillins, e.g. penicillin V, penicillin G, procaine penicillin G, benzathine penicillin G, methicillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin. They belong to β-lactams antibiotic class
   b. Carbapenems like imipenem, meropenem, aztreonam, ticaracillinclvulnate and piperaciin-tazobactam; these are β-lactam/β-lactamase inhibitors. Some other β-lactam inhibitors are cephalosporin, e.g. cefotaxime, ceftriaxone, ceftazidime, and cefepime
   c. Gentamicin, tobramycin, and amikacin are aminoglycosides

d. Quinolones and flouroquinolones, such as levofloxacin, ciprofloxacin, and oxifloxacin
e. Vancomycine is a glycopeptide
f. Polymyxin B and colistin are polymyxins [13]

- **According to source of Antimicrobial agents:**

A. Naturally obtained from fungal sources:Cephalosporins, cefamycins, benzylpenicillin, and gentamicin
B. Semi-synthetic members which are chemically altered natural product and/or synthetic:Ampicillin, amikacin, moxifloxacin and norfloxacin [14]

- **According to spectrum of activity:**

A. Narrow spectrum: these are considered to be those which can work on a narrow range of microorganisms, that is, they act against Gram-positive only or Gram-negative bacteria only e.g. penicillin G, benzathine penicillin G, penicillin V, procaine penicillin, propicillin, pheneticillin, azidocillin, clometocillin, and penamecillin, β-Lactamase-resistant, Ist generation include; Cloxacillin (dicloxacillinflucloxacillin), methicillin, nafcillin, oxacillin and temocillin, Cephalosporins (first generation and second generation), Vancomycin, clindamycin, isoniazid, rifampin, ethambutol, pyrazinamide, bacitracin, polymixins, sulfonamides, glycopeptide and nitroimidazole.
B. Broad spectrum: these affect a wide range of pathogenic bacteria, including both Gram-positive and Gram-negative bacteria e.g. Ampicillin, Amoxicillin/clavulanic acid, Quinolones such as lomefloxacin, ofloxacin, norfloxacin, gatifloxacin, ciprofloxacin, Aminoglycosides kanamycin A, amikacin, tobramycin, dibekacin, gentamicin, sisomicin, netilmicin, neomycins B, C and paromomycin, Cephalosporins (third, fourth, and fifth generations), Carbepenems, Macrolides such as erythromycin, roxithromycin, clarithromycin, azithromycin, and dirithromycin, Chloramphenicol,Ticarcillin, Tetracycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, minocycline, and tigecycline, Rifamycins.

- **According to mechanism of action:**

A. Cell wall synthesis inhibitors: β-Lactam drugs, including penicillin derivatives, cephalosporins, monobactams and carbapenems. Gram-positive and Gram-negative bacteria vary in the susceptibility to the β-lactam drugs because of the structural differences in their cell wall, i.e. Gram-negative bacteria usually have less susceptibility because these antibiotics fail to reach the cell wall as they are blocked by the outer membrane of the Gram-negative bacteria. Factors such as the amount of peptidoglycan, receptors, and lipids availability, nature of crosslinking, autolytic enzymes activity greatly influence the activity, permeation, and incorporation of the drugs.

B. Inhibitors of membrane function: Polymyxins are active antibacterial agents, which are cyclic peptides, having a long hydrophobic tail.

C. Protein synthesis inhibitors:
   a) Macrolides (binds with 50s ribosome)
   b) Tetracyclines and glycyclines (tigecycline) (binds with 30s ribosome).
   c) Ketolides: This is a novel class of protein synthesis inhibitors, which exhibit excellent activity against resistant organisms. [16]

C. Inhibitors of nucleic acid synthesis: Quinolones, including nalidixic acid and ciprofloxacin, work as DNA inhibitors. Some other antibacterial drugs, which act upon anaerobic bacteria by creating metabolites that are bound into DNA strands, which then are more likely to rupture. Examples of such drugs include nitrofurantoin and metronidazole.

1.1.4. Anti-microbial activity testing:

Antibacterial activity of the synthetic compounds using disk diffusion method:

- **Preparation of synthetic compounds for microbiological assay:** A stock solution of 10 mg of each synthetic compound dissolved in 1 mL of dimethyl sulfoxide (DMSO) as solvent is prepared. The antimicrobial activity of the synthesized compounds is evaluated by the disk diffusion method and the microbroth dilution method, which determines minimum inhibitory concentration (MIC).
- **Determination of antibacterial activity by disk diffusion method:** Antibacterial activity of the prepared synthetic compounds against the Gram-negative and the Gram-positive bacterial strains are examined by disk diffusion assay. Isolated pure

colonies from fresh grown bacteria are transferred from the plates into sterile normal saline solution and vortexed to form bacterial homogenous suspensions. The turbidity is then adjusted to 0.5 McFarland standard units, and the suspensions are poured over Mueller–Hinton agar (MHA) plates. Sterile filter paper disks with a diameter of 6 mm are placed over these plates. The sterile disks are impregnated with 20 µL of the tested compounds (10 mg/mL dissolved in DMSO). Positive control (Amoxicillin) and negative control (sterile distilled water) are used. Finally, the plates are incubated at 37°C for 24 hours. The inhibition zones are measured in millimeter. [17]

1.1.5. Antifungal disk diffusion method:

- The fungal strains are cultured on Sabouraud dextrose agar and incubated at 35°C for 24 hours and for 5 days on potato dextrose agar slant for the mold fungi. Using a sterile loop, pure colonies of the Candida species are transferred into a tube containing sterile normal saline. For the mold, 1 mL of sterile distilled water supplemented with 0.1% Tween 20 is used to cover and resuspend the colonies. Using a hemocytometer, the suspension is adjusted to $2–5\times10^6$ conidia/mL. The suspension is further diluted 1:10 to obtain final working inoculums $2–5\times10^5$ conidia/mL. The inoculums are poured over MHA supplemented with 2% of glucose. The sterile 6 mm disks that are impregnated with 20 µL test compounds (with a concentration of 10 mg/mL) are placed over the plate.
- Standard antifungal drug Nystatin is used as positive control and sterile distilled water as negative control and incubated at 35°C for 48 hours. The zone of inhibition is measured in millimeter. [18]

1.1.6. MICs by microbroth dilution method:

The MIC is determined by measuring the absorbance of microtiter plates at 570 nm for bacteria and 530 nm for fungi. While for *Aspergillus niger*, MIC is determined visually. The optical density from each well is compared with optical density from the positive control wells, the lowest concentration with optical density <0.1 signifies inhibition and considered as MIC.

1.1.7. Antibacterial susceptibility testing by microbroth dilution method:

The pure bacterial culture of each microorganism is adjusted to 0.5 McFarland

standards in Mueller–Hinton broth (MHB). The bacterial inoculums are vortexed and diluted in MHB. The bacterial suspension is used within 30 minutes after turbidity adjustment to avoid duplication of the bacterial cells. Eight different concentrations are prepared from each stock with the medium (0.125, 0.25, 0.5, 1, 2, 4, 6, and 8 mg/mL). A total of 50 µL of each compound concentration is added to sterile 96-well microtiter plates. After that, 50 µL of diluted bacterial inoculums are added to each well including the negative control lane and 100 µL of broth is added to positive control lane. (Each well reaches the final desired concentration of 5×10^5 CFU/mL.) The plates are then incubated at 37°C for 18–24 hours. Then, they are examined to check where growth had taken place, and the inhibition of growth is determined by measuring the absorbance at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader. [19]

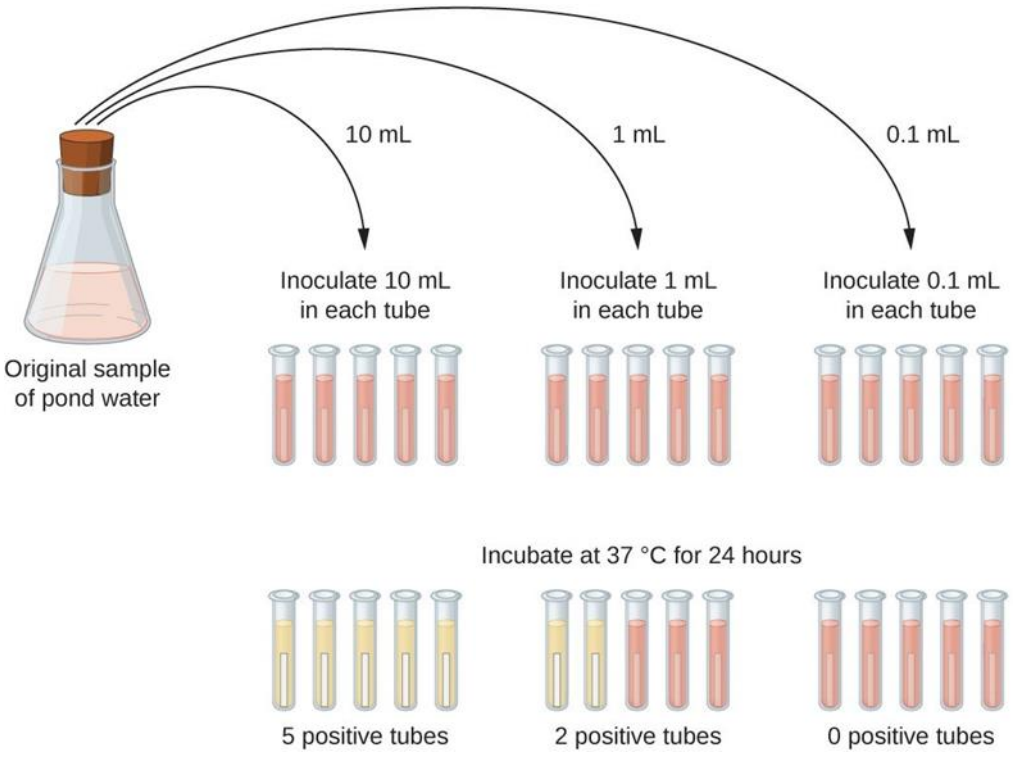


Fig 1.4: Microbroth Dilution Method

1.1.8. Antifungal susceptibility testing by microbroth dilution method:

The MICs for *Candida albicans* and *Candida krusei* are determined using the reference procedure of the Antifungal Susceptibility Testing of CLSI M27-A319 and

EUCAST for the testing of fermentative yeasts. MICs for *Aspergillus niger* (mold) are determined in accordance with EUCAST22 and CLSI M38-A.20 Briefly, testing is performed in sterile 96-well microtiter plates with Roswell Park Memorial Institute (RPMI) 1640 medium with L-glutamine, without sodium bicarbonate ($NaHCO_3$, RPMI 1640; Gibco, Carlsbad, CA, USA) supplemented with 2% glucose, buffered to pH 7.0 with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) medium.

- **Preparation of fungal (yeasts and molds) inoculums:**

  Regarding preparation of yeast inoculums, the fungal strains are subcultured on Sabouraud's dextrose agar slant and incubated for 24–48 hours at 35°C to obtain a freshly grown pure culture. Homogenous suspension is adjusted to 0.5 McFarland standards. Then, the inoculum size is further adjusted to 0.5×105 or 2.5×105. In addition, regarding the preparation of mold inoculums, the mold suspension of conidia is obtained from 5 days culture Sabor and dextrose agar slant is incubated at 35°C. Colonies are covered with 5 mL of sterile distilled water supplemented with Tween 20. The conidia are collected with a sterile cotton swab, transferred to a sterile tube, and vortexed to homogenize the suspension. The suspension is standardized by counting the conidia in a hemocytometer to 2–5×106 conidia/mL. The suspension is diluted 1:10 with RPMI to obtain final inoculums of 2–5×105 conidia/mL. A total of 50 µL of each compound concentration and 50 µL of fungal suspension are added to each well for the negative control lane, but 100 µL of broth is added to the positive control lane (each well reaches the final desired concentration of 2–5×105 CFU/mL). The plate is sealed with aluminum foil and is incubated at 35°C for 2,448 hours in humid atmosphere. The MIC is determined using an ELISA reader at 530 nm for the yeast species and visually for mold species after 48 hours of incubation as the lowest concentration of drug that results in 50% inhibition of growth compared to that drug-free growth control. [20]

1.1.9. Connection between Antimicrobial activity & Pyrimidine:

- Heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, and antibiotics [21, 22] hence, they have attracted considerable attention in

the design of biologically active molecules [22, 23] and advanced organic chemistry [24, 25]. Also in the family of heterocyclic compounds nitrogen containing heterocycles are an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes [26]. A totally unsaturated membered six-ring containing nitrogen is known as azine [27] or pyridine with two nitrogen atoms it is known as diazine [28], and with a nitrogen at 1-position, it is known as pyridine, at 1,3- position as pyrimidine, and at 1,4-position as pyrazine. However it is intended to focus on the significance of pyrimidine class of antimicrobial agents along with clinical and in vitro applications of pyrimidine derivatives to facilitate the development of more potent as well as effective antimicrobial agents. That's why it was decided to mold pyrimidine derivatives into anti-infectives and serve the global clinical crisis.

Here are some marketed drugs which consist of pyrimidine skeleton in their structures.

1.2. Marketed Drugs having Pyrimidine skeleton:

Activity	Drugs	IUPAC Name
Anticancer	1.5-Fluorouracil	5-Fluoropyrimidine-2,4(1H,3H)-di-one
	2. Cytarabine	1-β-d-Arabinofuranosylcytosine
	3. Capecitabine	4-Amino-1-β-d-Arabinofuranosylpyrimidine-2(1H)-one
	4. Uramustine	5-Bis(2-chloroethylamino)uracil
	5. Methotrexate	N-{4-[(2,4-Diamino-6-pteridinylmethyl)methylamino]benzoyl}-l-glutamic acid
	6. Tegafur	5-fluoro-1-(tetrahydro-2-furyl)pyrimidine-2,4(1H,3H)dione
	7. Floxuridine	2'-Deoxy-5-flurouridine
	8. PiritreximIsetionate	2,4-Diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-d}pyrimidinemono(2-hydroxyethanesulphonate)
Antifungal	1. 5-Flucytosine	5-Fluorocytosine;4-Amino-5-fluoro-pyrimidine-2(1H)-one
Antibacterial	1. Sulfadiazine	(4-aminophenyl)sulfonyl](pyrimidin-2-yl)azanide
	2. Sulfadimidine	4-amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide
	3. Metioprim	5-(3,5-Dimethoxy-4-methylthiobenzyl-2,4,diyldiamine
	4. Tetroxoprim	5-{3,5-Dimethoxy-4-(2-methoxyethoxy) benzyl}pyrimidine-4-diyl-diamine
Antimalarial	1.Trimethoprim	5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine

1.3. References:

1) McCarthy N. "Deaths From Drug-Resistant Infections Set To Skyrocket"*Statista.*2015
2) Sengupta S., Chattopadhyay M. K. & Grossart H.-P. "The multifaceted roles of antibiotics and antibiotic resistance in nature". Frontiers in Microbiology. *Resonance* (2013). 4. https://doi.org/10.3389/fmicb.2013.00047
3) Piddock L. J. "The crisis of no new antibiotics—what is the way forward?"*The Lancet Infectious Diseases.* (2012). 12(3), 249–253. https://doi.org/10.1016/s1473-3099(11)70316-4
4) "Antibiotic and Antimicrobial resistance. Biggest threats and data". 2015
5) "First independent framework for assessing pharmaceutical company action". *Access to medicine foundation.* 2017
6) *Centre for disease control and prevention*. 2017
7) "Research project in Vietnam: results indicate very high rates of resistance to most antibiotic"
8) "Evolution of the main resistant bacterial strains in Switzerland"
9) Kim E. S. & Hooper D. C. "Clinical Importance and Epidemiology of Quinolone Resistance". *Infection & Chemotherapy*.(2014). 46(4), 226. https://doi.org/10.3947/ic.2014.46.4.226
10) Abd El-Salam N. M., Mostafa M. S., Ahmed G. A. &Alothman O. Y. "Synthesis and Antimicrobial Activities of Some New Heterocyclic Compounds Based on 6-Chloropyridazine-3(2H)-thione". *Journal of Chemistry*. (2013). 2013, 1–8. https://doi.org/10.1155/2013/890617
11) "Antimicrobial"*Merriam-Webster Online Dictionary*. 2009.
12) Ullah H. and Alidoi S. "Classification of Anti-Bacterial Agents and Their Functions". *Intech Open*. (2017). doi:10.5772/intechopen.68695
13) Aminov R. I. "A brief history of the antibiotic era: Lessons learned and challenges for the future". Frontiers in Microbiology. *Frontiers*. (2010). 1, 1-5. doi: 10.3389/fmicb.2010.00134
14) Oloke J. K. "Activity pattern of natural and synthetic antibacterial agents among hospital isolates". *Microbios*. (2000). 102, 175-181
15) Zhu Z. J., Krasnykh O., Pan D., Petukhova V., Yu G., Liu Y., Liu H., Hong S., Wang Y., Wan B., Liang W., Franzblau S. G. "Structure activity relationships of macrolides

against *Mycobacterium tuberculosis*". *Tuberculosis*. (2008). 88, 49-63, doi: 10.1016/S1472-9792(08)70036-2

16) Kohanski M. A., Dwyer D. J., Collins J. J. "How antibiotics kill bacteria: From targets to networks". *Nature Reviews Microbiology*. (2010). 8, 423-435. doi: 10.1038/nrmicro2333

17) Wiegand I., Hilpert K., Hancock R. E. "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances". *Natural Protocol*. (2008). 3(2), 163–175.

18) Esmadi F. T., Khabour O. F., Albarqawi A. I., Ababneh M., Al-Talib M. "Synthesis and characterization of some transition metal complexes of thiocarbohydrazone Schiff bases". *Jordan Journal of Chemistry*. (2013). 8(1), 31–43.

19) Wiegand I., Hilpert K., Hancock R. E. "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances". *Natural Protocol*. (2008). 3(2), 163–175.

20) "Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). Method for the determination of Broth dilution MICs of antifungal agents for fermentative yeasts." *Clinical MicrobiologicalInfectitious Diseases*. (2008). 4(4), 398–405.

21) Ju Y. and Varma R. S., "Aqueous N-heterocyclization of primary amines and hydrazines with dihalides: microwave-assisted syntheses of N-azacycloalkanes, isoindole, pyrazole, pyrazolidine, and phthalazine derivatives," *Journal of Organic Chemistry*. (2006). 71(1),135–141.

22) Ju Y., Kumar D., and Varma R. S., "Revisiting nucleophilic substitution reactions: microwave-assisted synthesis of azides, thiocyanates, and sulfones in an aqueous medium," *Journal of Organic Chemistry*. (2006). 71(17), 6697–6700.

23) Lokhande P. D., Waghamare B. Y., and Sakate S. S., "Regioselective one-pot synthesis of 3,5-diarylpyrazoles," *Indian Journal of Chemistry B*. (2005). 44(11), 2338–2342.

24) Reddy G. J., Manjula D., Rao K. S., Khalilullah M., and Latha D., "A Direct single step synthesis of 1,3-diaryl-4-cyanopyrazoles and their conversion to 1,3-diaryl-4-(4,6-diamino 1,3,5-triazin2-yl)pyrazoles," *Indian Journal of Chemistry .(*2005). 44, 2412– 2415.

25) Zificsak C. A. and Hlasta D. J., "Current methods for the synthesis of 2-substituted azoles," *Tetrahedron*. (2004). 60(41), 8991–9016.

26) Haino T., Tanaka M., Ideta K., Kubo K., Mori A., and Fukazawa Y., "Solid-phase synthesis of liquid crystalline isoxazole library," *Tetrahedron Letters*. (2004). 45(11), 2277–2279.

27) Garc´ıa-Valverde M. andTorroba v, "Special issue: sulfurnitrogen heterocycles," *Molecules*. (2005). 10(2), 318–320.

28) Hopper D. W., Crombie A. L., Clemens J. J., and Kwon S., "Six-membered ring systems: pyridine and benzo derivatives," *Progress in Heterocyclic Chemistry*. (2009). 21, 330–374.

29) Manlove A. and Groziak M. P., "Six-membered ring systems:diazines and benzo derivatives". *Progress in Heterocyclic Chemistry*. (2009). 21, 375–414.

CHAPTER-2

CHEMISTRY OF PYRIMIDINE

2. CHEMISTRY OF PYRIMIDINE:

1. Pyrimidine is an aromatic heterocyclic organic compound similar to pyridine. One of the three diazines (six-membered heterocyclics with two nitrogen atoms in the ring), it has the nitrogen atoms at positions 1 and 3 in the ring. The other diazines are pyrazine (nitrogen atoms at the 1 and 4 positions) and pyridazine (nitrogen atoms at the 1 and 2 positions). In nucleic acids, three types of nucleobases are pyrimidine derivatives: cytosine (C), thymine (T), and uracil (U). [1]

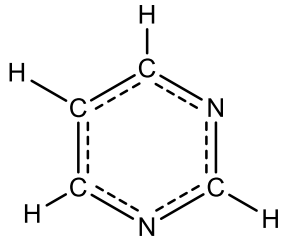


Fig 2.1. Structure of Pyrimidine ring

2. The classification by Albert six-membered heterocycles can be described as π-deficient. Substitution by electronegative groups or additional nitrogen atoms in the ring significantly increases the π-deficiency. These effects also decrease the basicity. [1]

3. Like pyridines, in pyrimidine the π-electron density is decreased to an even greater extent. Therefore, electrophilic aromatic substitution is more difficult while nucleophilic aromatic substitution is facilitated. An example of the last reaction type is the displacement of the amino group in 2-aminopyrimidine by chlorine and its reverse. [2]

4. Electron lone pair availability (basicity) is decreased compared to pyridine. Compared to pyridine, N-alkylation and N-oxidation are more difficult. The pK_a value for protonated pyrimidine is 1.23 compared to 5.30 for pyridine. Protonation and other electrophilic additions will occur at only one nitrogen due to further deactivation by the second nitrogen. The 2-, 4-, and 6- positions on the pyrimidine ring are electron deficient analogous to those in pyridine and nitro- and dinitrobenzene. The 5-position is fewer electrons deficient and substituents there are

quite stable. However, electrophilic substitution is relatively facile at the 5-position, including nitration and halogenations. [2]

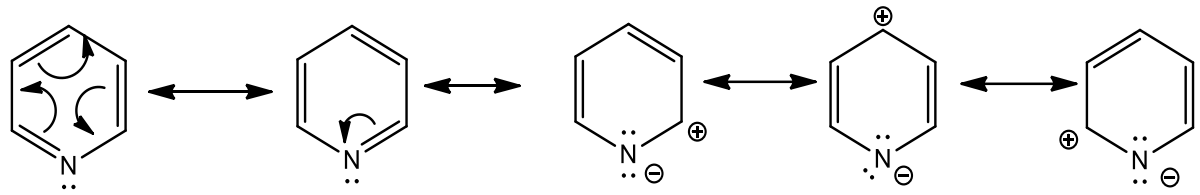


Fig 2.2. Resonance of Pyridine

5. Reduction in resonance stabilization of pyrimidine may lead to addition and ring cleavage reactions rather than substitutions. One such manifestation is observed in the Dimroth rearrangement. [3]

6. The biological significance of the pyrimidine derivatives has led us to the synthesis of substituted pyrimidine. As pyrimidine is a basic nucleus in DNA & RNA, it has been found to be associated with diverse biological activities. The synthesis of substituted pyrimidine and many detailed reviews have been appeared. Synthetic strategies involved four main routes based on the condensation of two fragments, as illustrated by (i) to (iv). Of these strategies, the one illustrated by (i) i.e. the condensation of a three carbon unit with an N - C - N fragment, appears to be the most widely used, offering direct entry in to the pyrimidine nucleus. [4]

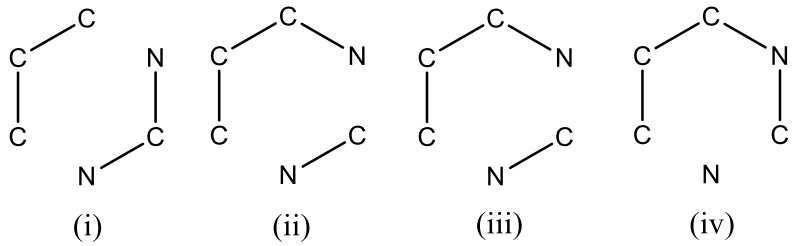


Fig 2.3. Condensation of 3 carbon units

This approach has been called ‘the common syntheses’ because of its general applicability to the synthesis of a whole range of pyrimidine derivatives. [4]

2.1. STRUCTURE OF NITROGENOUS BASES:

The three pyrimidine nitrogenous bases, thymine (T), cytosine (C), and uracil (U), are modified forms of the aromatic compound pyrimidine. They consist of a six-membered ring with two nitrogen atoms and four carbon atoms, but instead of being an aromatic ring with alternating double and single bonds they all have a ketone (carbonyl group) on the 2′ carbon atom (the carbon between the two nitrogen atoms). The addition of this double bond removes a bond from the ring, resulting in two double bonds and four single bonds. [5]

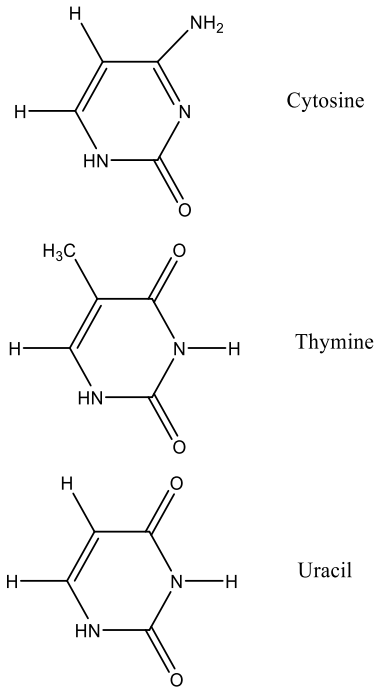


Fig 2.4. Different nitrogenous bases of Pyrimidines

In addition to the carbonyl group, the three nitrogenous bases also have a functional group attached to the 4′ carbon (a ketone for T and U, and an amino group for C), and T has a methyl group attached to the 5′ carbon as well. The addition of another ketone in T and U removes another double bond from the ring, leaving only one double bond in U and T, and two double bonds in C. In all three there are only two bonds to the 1′ nitrogen; this is where the nitrogenous base attaches to the sugar in the nucleic acid to form a nucleoside (or a nucleotide when phosphorus is attached). [2]

2.2. STRUCTURE ACTIVITY RELATIONSHIP (SAR) STUDY:

1. Five membered saturated heterocyclic ring substitution leads to anticancer and antiviral activities.

2. a) 2nd position six or five membered saturated heterocyclic ring substitution leads to anthelmintic, antiparkinsonism expectorant and treatment of GI disturbance, peripheral neuropathies.

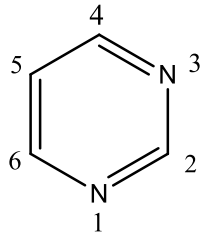


Fig 2.5. Structure activity relationship of Pyrimidine ring

2. b) 2nd and 4th position keto group substitution or amino substitution or mixed keto, amino group's substitution leads to anticancer, antiviral, antibacterial, antifungal, and treatment of respiratory tract infection and liver disorder.

3. 5th position with halogen or substituted amine or saturated distal heterocyclic ring substitution leads to antibacterial and anticancer activities.

4. 5th and 6th position fused with heterocyclic ring and *o*, *m*, *p* substituted distal aryl ring substitution leads to anticancer, antiviral, antibacterial, vasodilation and treatment of urinary tract infection. [6]

5. As SAR studies give insights into the molecular properties causing receptor affinity and selectivity. The promising nature of the compounds may be attributed to the substitutions at thehydrophobicdomain. [6]

These compounds had electron withdrawing and donating groups at the *ortho*, *meta* & *para* position of the hydrophobic aryl ring. In general it was observed that thesubstituted derivatives were more active than the other derivatives. This may be because of thefact that the substituted

derivatives are better fitted into the receptor site. In all of thepioneering of experiments important core fragments is defined by presence of hydrogen donor/acceptor unit, hydrophobic domain (aryl ring substituted/unsubstituted) and electron donor atomwhich offer the medicinal chemist a continued interest in the pyrimidine skeleton in medicinal chemistry and drug development. [6]

2.3. References:

1) Nishiwaki N., Tamura M., Hori K., Tohda Y. & Ariga M. "The Ring Transformation of 3-Methyl-5-nitropyrimidin-4(3H)-one". *Molecules.* (2003). 8(6), 500–504. https://doi.org/10.3390/80600500
2) Albert, Adrien, *Heterocyclic Chemistry, An Introduction.* 1968.
3) Overberge C. G., Kogon, Irving C, Minin, Ronald,"2-(dimethylamino) pyrimidine. *Organic Syntheses*. (1955). 35, 58. https://doi.org/10.15227/orgsyn.035.0058
4) Botta M., Cavalieri M., Ceci D., De Angelis F., Finizia G. & Nicoletti R. "6-alkyl and 5, 6-dialkyl-2-methoxy-4(3H)-pyrimidinones in the transformations of pyrimidines". *Tetrahedron*. (1984). 40(17), 3313–3320. https://doi.org/10.1016/0040-4020(84)85016-4
5) Chorlton A. P. "Pyrimidine and Pyrimidine Derivatives". *Ullmann's Encyclopedia of Industrial Chemistry*. (2000). Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/14356007.a22_431
6) Blanchet B. "Automatic verification of cryptographic protocols. In Proceedings of the 5th ACM SIGPLAN international conference on Principles and practice of declaritive programming - PPDP '03". *ACM Press*. (2003). https://doi.org/10.1145/888251.888252
7) Karthick V., Selvam T. P., Kumar P. V. & Ramu P. "Antiepileptic properties of novel 2-(substitutedbenzylidene)-7-(4-chlorophenyl)-5-(furan-2-yl)-2H–thiazolo[3,2-a]pyrimidin-3(7 H)-one derivatives". *Journal of Saudi Chemical Society*. (2016). 20, S1–S6. https://doi.org/10.1016/j.jscs.2012.07.018
8) Mallikarjunaswamy C., Bhadregowda D. G. & Mallesha L. "Synthesis of novel (E) - N ′-(2-chloropyrimidin-4-yl)- N -(5-cyano-2-hydroxy-6-phenylpyrimidin-4-yl) formamidine derivatives and their antimicrobial activity*". Journal of Saudi Chemical Society*. (2016). 20, S606–S614. https://doi.org/10.1016/j.jscs.2013.04.005
9) Wang Y., Rong J., Zhang B., Hu L., Wang X.& Zeng C. "Design and synthesis of N-methylpyrimidone derivatives as HIV-1 integrase inhibitors". *Bioorganic & Medicinal Chemistry*. (2015). 23(4), 735–741. https://doi.org/10.1016/j.bmc.2014.12.059

10) DeRosa T. F. "Pyrimidines. In Advances in Synthetic Organic Chemistry and Methods Reported in US Patents". *Elsevier.* (2006). 545–547 https://doi.org/10.1016/b978-008044474-1/50077-3

11) Wu X.-F. & Wang Z. "Synthesis of Pyrimidine. In Transition Metal Catalyzed Pyrimidine, Pyrazine, Pyridazine and Triazine Synthesis". *Elsevier*. (2017).5–54. https://doi.org/10.1016/b978-0-12-809378-8.00002-x

12) Wu X.-F. & Wang Z. "Synthesis of Triazine. In Transition Metal Catalyzed Pyrimidine, Pyrazine, Pyridazine and Triazine Synthesis". *Elsevier*. (2017). 79–93 https://doi.org/10.1016/b978-0-12-809378-8.00005-5

13) Rewcastle G. W. "Pyrimidines and their Benzo Derivatives. In Comprehensive Heterocyclic Chemistry III". *Elsevier*. (2008). 117–272. https://doi.org/10.1016/b978-008044992-0.00702-1

14) Undheim K. &Benneche T. "Pyrimidines and their Benzo Derivatives. In Comprehensive Heterocyclic Chemistry II". *Elsevier.* (1996). 93–231. https://doi.org/10.1016/b978-008096518-5.00118-0

15) Durgadevi D., Manivarman S. & Subash S. "Synthesis, molecular characterization of pyrimidine derivative: A combined experimental and theoretical investigation". *Karbala International Journal of Modern Science.* (2017). 3(1), 18–28. https://doi.org/10.1016/j.kijoms.2017.01.001

16) Gore R. P. & Rajput A. P. "A review on recent progress in multicomponent reactions of pyrimidine synthesis". *Drug Invention Today*. (2013). 5(2), 148–152. https://doi.org/10.1016/j.dit.2013.05.010

17) Bhat A. R., Dongre R. S., Naikoo G. A., Hassan I. U. &Ara T. "Proficient synthesis of bioactive annulated pyrimidine derivatives: A review". *Journal of Taibah University for Science.* (2017). 11(6), 1047–1069. https://doi.org/10.1016/j.jtusci.2017.05.005

18) "Introduction to Pyrimidine Chemistry. In Chemistry of Heterocyclic Compounds: A Series Of Monographs". *John Wiley & Sons Inc.* (2008). 1–47. https://doi.org/10.1002/97804701857395.ch1

19) Brown D. J., Evans R. F., Cowden W. B. &Fenn M. D. (Eds.). "Chemistry of Heterocyclic Compounds. Chemistry of Heterocyclic Compounds: A Series Of

Monographs". *John Wiley & Sons, Inc.* (1985). https://doi.org/10.1002/9780470186770

20) Wang S. Y. "Chemistry of Pyrimidines. I. The Reaction of Bromine with Uracils1-3. The Journal of Organic Chemistry". (1959). 24(1), 11–13. https://doi.org/10.1021/jo01083a003

21) Sujayev A., Garibov E., Nazarov N. & Farzaliyev V. "Synthesis and Investigation of New Different Pyrimidine-Thiones". *Organic Chemistry: Current Research*. (2017). 6(1). https://doi.org/10.4172/2161-0401.1000179

CHAPTER-3

SYNTHETIC BACKGROUND

3. SYNTHETIC BACKGROUND:

The primary aim was to choose a single step multi-component reaction procedure as it would be productive and at the same time less time consuming. Another advantage of this scheme is less impurities are obtained from the process and the materials used.

In the year 1890, a simple and one pot synthesis of dihydropyrimidinones were first observed by P. Biginelli involving three component condensation of a ketoester with an aldehyde and urea.

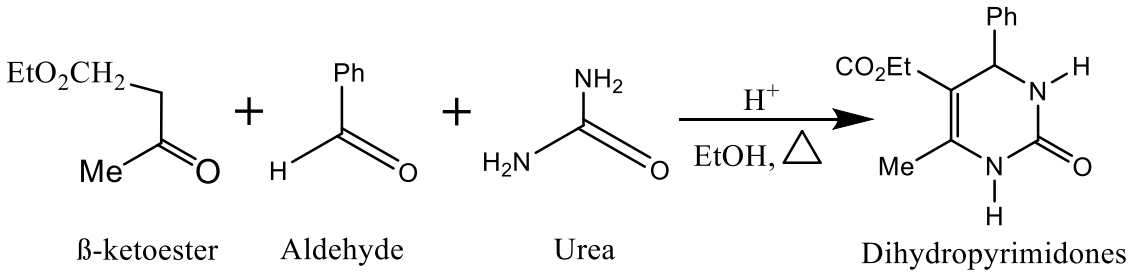


Fig 3.1. Biginelli condensation reaction

The utilization of multi-component reactions (MCRs) to synthesize novel, drug-like scaffold has penetrated in organic transformations from last few decades due to their molecular economy and potential of producing diversed products in single step. More attention has been paid towards developing highly efficient Biginelli reaction owing to the exhibition of a wide range of biological activities in dihydropyrimidinones such as antiviral, antitumor, antibacterial, and anti-inflammatory properties as well as calcium channel modulating activity. [1]

During studying the research papers, it was found that, there were reports on Biginelli type condensation between an aldehyde, malononitrile and urea using P_2O_5 and some other catalysts by both conventional reflux condition and microwave irradiation methods. But in both the cases, it ended up with 2-hydroxy-4-aryl pyrimidine derivatives. [2]

Several researchers reported the synthesis of dihydropyrimidinones including classical conditions with microwave irradiation and by using Lewis acids as well as protic acids as promoters such as Conc. HCl, $BF_3 \cdot OEt_2$, PPE, KSF clay, $InCl_3$, $LaCl_3$, lanthanide triflate, H_2SO_4, ceric ammonium nitrate (CAN), $Mn(OAc)_3$, ion-exchange resin-*n*-butyl-3-methyl

imidazolium tetra fluoroborate ($BMImBF_4$), $BiCl_3$, $LiClO_4$, $InBr_3$, $FeCl_3$, $ZrCl_4$, $Cu(OTf)_2$, $Bi(OTf)_3$, LiBr, ytterbium triflates, NH_4Cl, $MgBr_2$, $SiO_2/NaHSO_4$ and other reagents have been found to be effective.[4]

Although a number of papers were reported concerning the synthesis of pyrimidine derivatives, a few one pot synthesis were published using aromatic aldehydes, malononitrile [5] and thiourea/urea using the reagent concentrated hydrochloric acid.[3][4]

In our present work, conc. HCl is used for the same reaction which resulted in dihydropyrimidinones as a predominant product.

3.1. SYNTHESIS OF PYRIMIDINE:

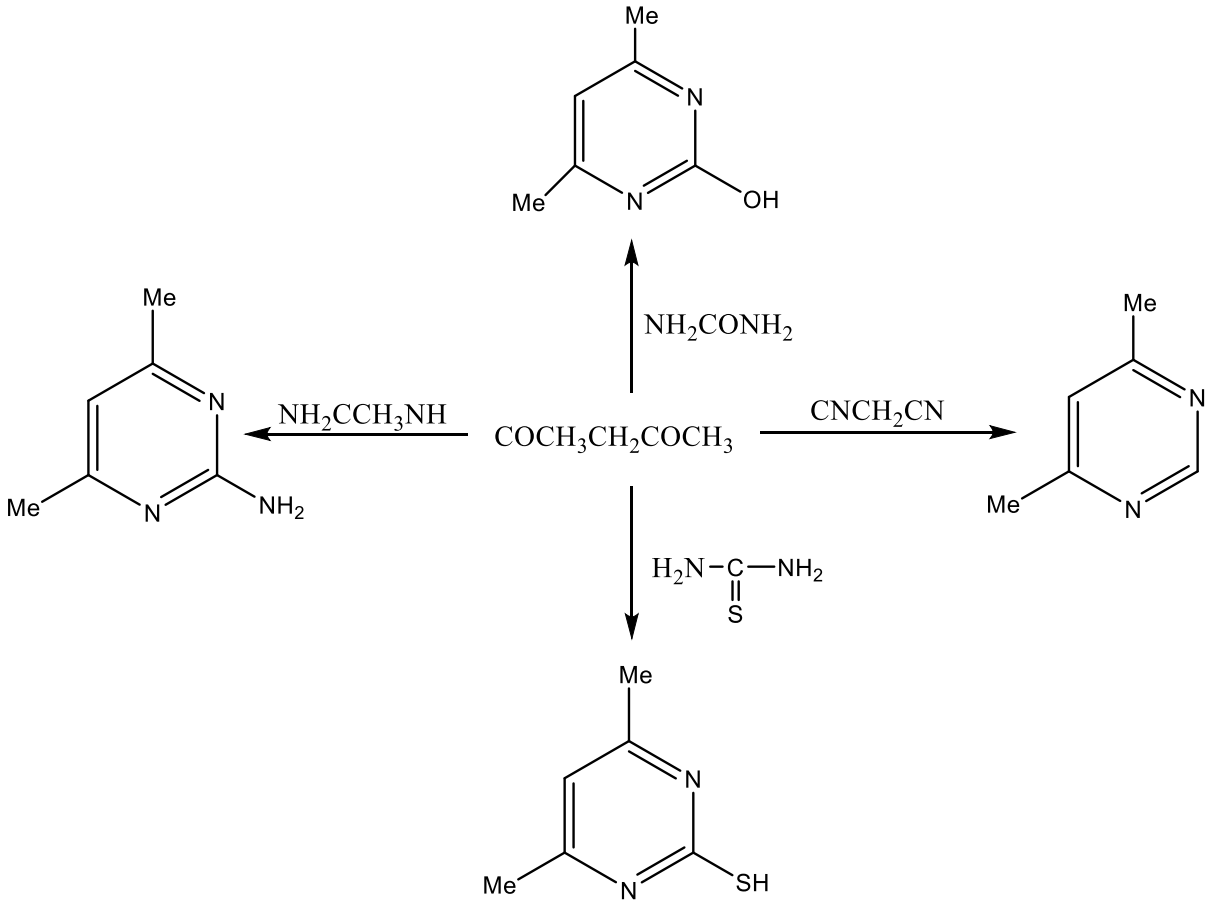
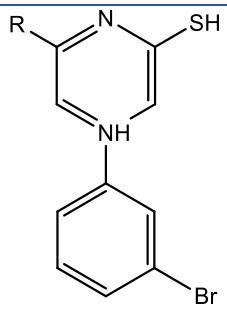
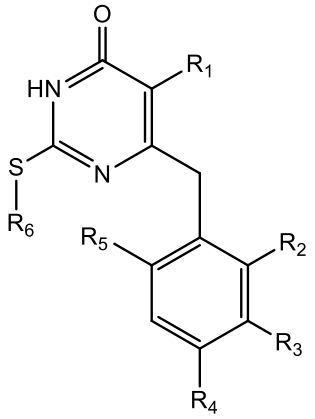
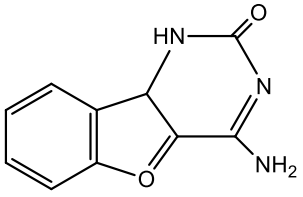
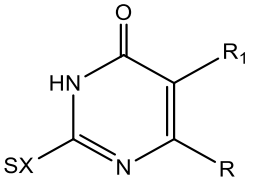
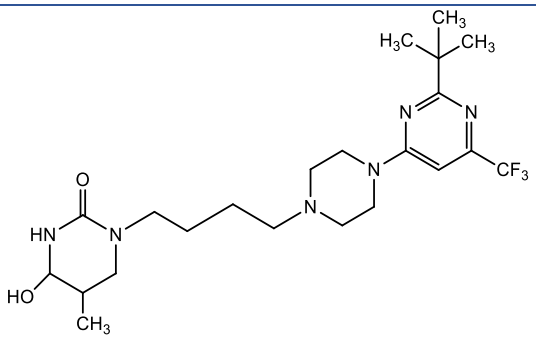
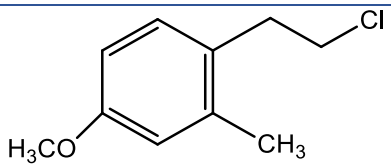
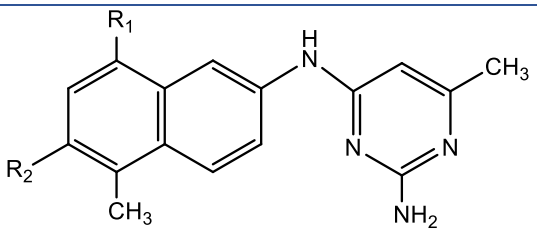


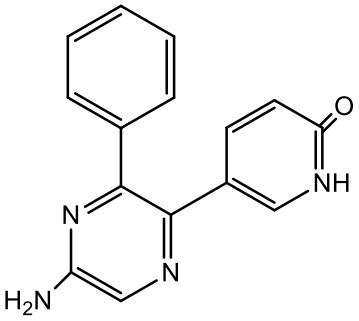
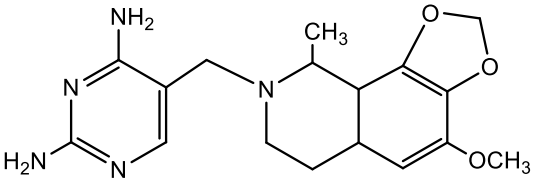
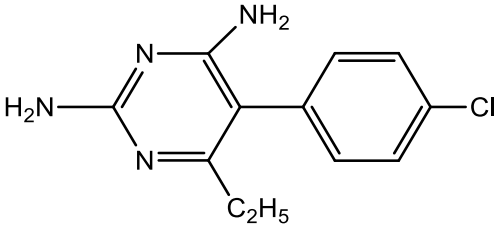
Fig 3.2. Synthesis of various Pyrimidine derivatives from beta-ketoester

An important limitation of this reaction is that, in practice not all possible combinations of reactants produce the expected products, as typified by the failure of beta-ketoesters to undergo condensation with formamidine to produce pyrimidine. It is often possible however; to achieve transformations of this type by modification of the reaction conditions and/or reactants. Thus 1,1,3,3-tetraethoxy propane, a readily available precursor of malondialdehyde, reacts with formamide over an alumina catalyst at 200°C to produce pyrimidine in 70% yield.

3.2. Pyrimidine as different *Avatars* [Various pharmacological activities of pyrimidine] [7]

	Authors	Structure	Activity
1.	K.S.Nimavat, K. H. Popat, S. L vasoya and H. S. Joshi;2003[7]		Antitubercular and Antimicrobial agents
2.	Antonello Mai, Marino Artico,Gianlu caSbardella and Paolo La Colla:1999[8]	 R_1 = H, Me R_{2-4} = Cl, F, NO_2 R_5 = H, Cl, F R_6 = alkyl/cycloalkyl	Anti-HIV-1 agents in both cell-based and enzyme
3.	S. S. Sangopure andA.M.Mul og; 2000[9]		Antimicrobial activity

4.	Viney Lather and A. K. Madan;2005 [10]		Anti-HIV activity
5.	HerveGenest e, Gisela Backfisch, Wilfried Braje.2006[11]		DopamineD3-recepterantagonist's activity.
6.	HerveGenest e, Gisela Backfisch, Wilfried Braje.2006 [12]		Anticancer and antineoplastic activity
7.	Kaplina N. V., Griner A. N., SherdorV.I.,Fomina A.N.1995 [13]		Herpes inhibiting activity

8.	Tsutsumi, Hideo, Yonishi,S atoshi, 2003[14]		Adenosine receptor antagonists
9.	Pierre C. Wyss, Paul Gerber, PeterG.Hartm an 2003[15]		Dihydrofolat ereductase inhibitors
10.	Rastelli,G, Sirawarapor m,W,Sompor npisut, P.2000[16]		Antimalarial activity

3.4. References:

1) Shen Z.-L., Xu X.-P. & Ji S.-J. "Bronsted Base-Catalyzed One-Pot Three-Component Biginelli-Type Reaction: An Efficient Synthesis of 4, 5, 6-Triaryl-3, 4-dihydropyrimidin-2(1H)-one and Mechanistic Study". *The Journal of Organic Chemistry*. (2010). 75(4), 1162–1167. https://doi.org/10.1021/jo902394y
2) ChariM. A., Shobha D., Parameswari, RameshS. B. and Pal R. R. "Synthesis of 3, 4-Dihydropyrimidin-2(1*H*)-ones Using Molybdenum (V) Chloride as a Catalyst". *Asian Journal of Chemistry*, (2012). 24(1), 15-17
3) Kambe S., Saito K., Kishi H., Sakurai A., &Midorikawa H. "A One-Step Synthesis of 4-Oxo-2-thioxopyrimidine Derivatives by the Ternary Condensation of Ethyl Cyanoacetate, Aldehydes, and Thiourea".*Synthesis*.(1979). 1979(4), 287–289. https://doi.org/10.1055/s-1979-28650
4) Li J.-T., Lin Z.-P., Han J.-F., & Li T.-S. "One-Pot Synthesis of 4-Oxo-2-thioxohexahydropyrimidines Catalyzed by Potassium Carbonate under Ultrasound". *ChemInform*. (2004). 35(50). https://doi.org/10.1002/chin.200450121
5) Patil D .R. , Salunkhe S. M. , Deshmukh M. B., and Anbhule P. V. "One Step Synthesis of 6-Amino-5-Cyano-4-Phenyl-2-Mercapto Pyrimidine Using Phosphorus Pentoxide".*The Open Catalysis Journal*. (2010).3, 83-86
6) Sheibani H., Saljoogi A. S., Bazgirb A, "Looking for a new antitubercularpharmacophore site: synthesis and bioactivity of spiroheterocycles 2,3',4'-tri-subsituted-1,2-dihydro-4H,4'H-spiro[isoquinoline-3,5'-isoxazol]-4-ones". (2007). *Arkivoc*. 2008(2), 1. https://doi.org/10.3998/ark.5550190.0009.201
7) Patel K. S.,Raval K. N.,Patel S. V.,Patel A. G. ,Patel S. V. "A review on synthesis and biological activities of pyrimidine derivatives" Indian Journal of Pharmacy & Biological Sciences. (2012). 2(3), 170-182
8) Zalavadiya P., Tala S., Akbari J., & Joshi H. "Multi-Component Synthesis of Dihydropyrimidines by Iodine Catalyst at Ambient Temperature andin-vitroAntimycobacterial Activity".Archiv Der Pharmazie, (2009). 342(8), 469–475. https://doi.org/10.1002/ardp.200800224

9) Mai A., Artico M., Rotili D., Tarantino D., Clotet-Codina I., Armand-Ugón M., Esté, J. A. "Synthesis and Biological Properties of Novel 2-Aminopyrimidin-4(3H)-ones Highly Potent against HIV-1 Mutant Strains". Journal of Medicinal Chemistry.(2007). 50(22), 5412–5424. https://doi.org/10.1021/jm070811e
10) Patel K., Raval K. N., Patel S. P., Patel A. G., Patel S. V., "A review on synthesis and biological activities of pyrimidine derivatives". International Journal of Pharmacy and Biological Sciences. 2012. 2(3), 170-182.
11) Lather V. and Madan A. K.; Bioorganic and Medicinal Chemistry Letters.(2005). 13,1599-1604.
12) Geneste H., Backfisch G., Braje W., Wernet W; Bioorganic & Medicinal Chemistry Letters. (2006). 16(3), 490-494.
13) Kaplina N. V., Griner A. N., SherdorV. I., Fomina A. N. et al, Chemical Abstract, (1995).123.
14) Tsutsumi, Hideo, Yonishi, Satoshi; "PCT" Chemical Abstract. (2002). 139.
15) Wyss P. C., Gerber P., Hartman P. G., Hubschwerlen C.,Stahl M.Journal of Medicinal Chemistry.(2003). 46(12), 2304-2311.
16) Rastelli G., Sirawaraporm W., Sompornpisut P. Bioorganic Medicinal Chemistry Letters. (2000). 8, 1117– 1128.

CHAPTER-4

LITERATURE SURVEY

4. LITERATURE SURVEY:

4.1. SYNTHESIS OF PYRIMIDINE DERIVATIVES:

- Newly synthesized pyrimidine derivatives and thioglycoside and thiazolopyrimidine derivatives were reported by **Mahmoud N. M. *et al*** and the derivatives showed anticancer activity against hepatocellular carcinoma HepG-2, human prostate adenocarcinoma, PC-3 and human colorectal carcinoma in HCT-116 cell lines. [1]
- **Ji-seong Y. *et al*** reported a synthetic route and anticancer activity of fluorocyclopentenylpurines and pyrimidines. [2]
- **Peng-Fei G. *et al*** reported a synthetic procedure in their research paper and also an evaluation process for anticancer activityof novel [1,2,3]triazolo[4,5-d]pyrimidine derivatives containing a thiosemicarbazide moiety.[3]
- An influence of the C-5 substitution in polysubstitutedpyrimidines on inhibition of prostaglandin E_2 production was reported by **Viktor K. *et al***. [4]
- **Sharad S. P. *et al*** designed a synthetic route for the synthesis of 5-cyanodihydropyrimidine. They had used Biginelli reaction in presence of β-ketonitrile. [5]
- **Zeid F. I. F. *et al*** synthesized a series of some new pyrimidine derivatives via the reaction of ethyl cyanoacetate with thiourea and the appropriate aldehydes namely 2-methyl-benzaldehyde and 2-methoxy-benzaldehyde. All the structures of the new compounds were elucidated by their correct elemental analysis and spectral data.[6]
- **Bhata. A. R**. ***et al*** summarized an one-pot multicomponent synthesis of annulated nitrogen- and oxygen-containing heterocycles, such as pyrano[2,3-d]pyrimidines, pyrido[2,3-d]pyrimidines and pyrido[2,3-d;5-6-d]dipyrimidines. The synthetic procedure was based on the chemistry of the domino Knoevenagel-Michael addition mechanism. [7]
- **Yunpeng Y. *et al*** stated a synthetic approach in their research paper for the preparation of target pleuromutilin derivatives and their past study reports the design, synthesis, and antibacterial studies of a series of novel pleuromutilin derivatives with substituted 6-amino pyrimidine moieties. [8]
- **Mostafa. M. G. *et al*** reported in their research workthat antimicrobial evaluation was done for Pyrrole, Pyrazole, Pyrimidine and Pyrrolo [2, 3-d]-Pyrimidine derivatives bearing Sulfonamide moiety. [9]

- **Jitendra *et al*** derived a novel series of 2-amino-4-(coumarin-3-yl)-6-substituted phenyl pyrimidines synthesized from 3-acetylcoumarin. The structures of the synthesizedcompounds were elucidated by I.R., ^{1}H NMR, ^{13}C NMR, and Mass spectroscopic techniques. [10]
- A number of N-substituted pyrimidine glycosides were synthesized by coupling reaction of the pyrimidine base with acetobromosugars followed by deprotection **Mahmoud M. M. Ramiz *et al.***[11]
- A new series of 4(4'-bromophenyl)-6-substituted aryl-1-acetyl pyrimidine-2-thiol derivatives were synthesized by **Rita B. *et al*** by heating chalcones with thiourea, in the presence of ethanolic potassium hydroxide, followed by treatment with acetyl chloride. [12]
- **Mohammad M. *et al*** synthesized some substituted uracils and thiouracils under solvent-free conditions by microwave irradiation. [13]
- In the year 2010, **Dipti R. P. *et al*** reported a one step synthesis of 6-amino-5-cyano-4-phenyl-2-mercaptopyrimidine and its hydroxyl derivatives by using phosphorus pentoxide (P_2O_5). [14]
- **Hemant H. *et al*** showed in their research work a three component Biginelli-type condensation of aldehyde, malononitrile, and urea in the presence of catalytic amount of t-BuOK. The synthetic product reported by them was 6-amino-4-aryl-pyrimidinone-5-carbonitrile derivatives.[15]
- **Nishio *et al*** reported the formation of 4,6-diphenyl-1-propylpyrimidin-2(1*H*)-one, where uriedo nitrogen in the propylurido substratewas made nucleophilic by running the cyclization reaction under alkaline conditions.[16]

4.2. EVALUATION OF PHARMACOLOGICAL ACTIVITY:

- **Chandra B. M. *et al*** informed that a novel pyrimidine derivatives (like Donepezil) were synthesized insilico and biological evaluation was done as multi-target-directed ligands for the treatment of Alzheimer's disease.[17]
- **Yunpeng Y. *et al*** reported in their studies that pleuromutilin derivatives with a substituted pyrimidine moiety were synthesized and they predominantly exhibited antibacterial activity. [18]
- **Wael A. *et al*** studied anticancer activities of a new series of substituted pyrimidine and thiazolopyrimidine glycosides. The anticancer activity against human prostatic adenocarcinoma (PC3), human colorectal carcinoma (HCT116) and human breast adenocarcinoman (MCF7) cell lines in addition to their effect on human normal retinal pigmented epithelial cell line (RPE1) was studied. [19]
- **Chao-Rui Y. *et al*** discovered the synthesis of thieno [2, 3-d] pyrimidine derivatives with a dithiocarbamate side chain at C_2 position. In their studies they reported that all the synthesized compounds exhibited cytotoxicity in cancer cells by targeting tubulin to activate the SAC and potentially acted as a therapeutic lead compound for taxol-resistant cancers. [20]
- **Davide I. W. *et al*** synthesized somepyrimidine derivatives bearing carboxamide andsulphonamide moieties and they also investigated*in vitro*anthelmintic properties of the derived compounds. [21]
- Thienopyrimidine urea derivatives were synthesized by **Eman F. A. *et al*** and they also found that the synthesized compounds showed a potent cytotoxic and pro-apoptotic activity against breast cancer cell line MCF-7. [22]
- **Ranganathaa V. L. *et al*** investigated antibacterial screening data;it was observed that all the tested compounds showed antibacterial activity against four pathogenic bacterial strains. Among the series some compounds exhibited an elevated antibacterial activity against Gram-positive (zone of inhibition 28 - 32 mm) and Gram-negative (zone of inhibition 30 - 33 mm) bacteria [23].
- **Anu A. *et al*** evaluated *in vitro* antimalarial activity of a series of 2, 4, 6-trisubstituted-pyrimidines synthesized against *Plasmodium falciparum*. Out of the 30 synthesizedcompounds, 21 compounds showed MIC in the range of 0.5–2.lg/mL.[24]

- **Vanessa G. *et al*** reportedthe antioxidant activities of derived compounds by using the DPPH and the HRP/luminol/H_2O_2chemiluminescence assay systems for their antimicrobial activity (MIC). [25]
- A new series of 4(4'-bromophenyl)-6-substituted aryl-1-acetyl pyrimidine-2-thiol derivatives were synthesized by **Rita B. *et al*** and they also added that thecompounds were screened for their antimicrobial, anthelmintic and insecticidal activities. All the compounds exhibited significant to moderate biological activities.[26]

4.3. References:

1) Mahmoud N. M. Y., Wael A. El-S., Hebat-Allah S. A., Hanem M. A., Nabil M. Y. "Anticancer Activity of New Substituted Pyrimidines, Their Thioglycosides and Thiazolopyrimidine Derivatives". *Journal of Applied Pharmaceutical Science*. (2017). 7 (11), 021-032, https://doi.org/10.7324/japs.2017.71104
2) Zhao L. X., Yun M., Kim H. O., Lee J. A., Choi W. J., Lee K. M., Jeong, L. S. "Design, synthesis, and anticancer activity of fluorocyclopentenyl-pyrimidines". *Nucleic Acids Symposium Series*, (2005), 49(1), 107–108. https://doi.org/10.1093/nass/49.1.107
3) Geng P.-F., Liu X.-Q., Zhao T.-Q., Wang C.-C., Li, Z.-H., Zhang J. Liu, H.-M. "Design, synthesis and in vitro biological evaluation of novel [1, 2, 3] triazolo [4, 5-d] pyrimidine derivatives containing a thiosemicarbazide moiety". *European Journal of Medicinal Chemistry*,(2018). 146, 147–156. https://doi.org/10.1016/j.ejmech.2018.01.031
4) Kolman V., Kalčic F., Jansa P., Zídek Z., &Janeba Z. "Influence of the C-5 substitution in polysubstitutedpyrimidines on inhibition of prostaglandin E2 production". *European Journal of Medicinal Chemistry*. (2018). 156, 295–301. https://doi.org/10.1016/j.ejmech.2018.07.010
5) Pachore S. S., Ambhaikar N. B., Siddaiah V., Khobare S. R., Kumar S., Dahanukar V. H., & Kumar U. K. S. "Successful utilization of up beta β-ketonitrile in Biginelli reaction: synthesis of 5-cyanodihydropyrimidine". *Journal of Chemical Sciences*. (2018). 130(6). https://doi.org/10.1007/s12039-018-1467-7
6) FathallaZeid IF, Haiba ME, Soliman A, Abd-Elmoez SI, El-Serwy WS, "Synthesis, Antibacterial and Anticancer Evaluation of Some Pyrimidine Derivatives". *World Journal of Chemistry*. (2009). 4 (2), 127-132
7) Bhat A. R., Dongre R. S., Naikoo G. A., Hassan I. U., &Ara T. "Proficient synthesis of bioactive annulated pyrimidine derivatives: A review". *Journal of Taibah University for Science*, (2017). 11(6), 1047–1069. https://doi.org/10.1016/j.jtusci.2017.05.005
8) Yi Y., Xu X., Liu Y., Xu S., Huang X., Liang J., & Shang R. "Synthesis and antibacterial activities of novel pleuromutilin derivatives with a substituted pyrimidine moiety". *European Journal of Medicinal Chemistry*. (2017). 126, 687–695. https://doi.org/10.1016/j.ejmech.2016.11.054

9) Gupta J. K., Sharma P. K., DudheR.,Chaudhary A., Singh A.,Verma P. K., MondalS. C.,Yadav R. K., Kashyap S."Antimicrobial Evaluation of Novel Pyrrole, Pyrazole, Pyrimidine and Pyrrolo [2, 3-d]-Pyrimidine DerivativesBrearing Sulfonamide MoietyAnalgesic study of novel pyrimidine derivatives linked with coumarin moiety", *Journal of American Science.* (20110. 7(1)

10) Ramiz M. M. M., El-Sayed W. A., Hagag E., & Abdel-Rahman A. A.-H. "Synthesis and antiviral activity of new substituted pyrimidine glycosides". *Journal of Heterocyclic Chemistry*. (2011). 48(5), 1028–1038. https://doi.org/10.1002/jhet.686

11) Bamnela R. & Shrivastava S. P. "Synthesis and In Vitro Antimicrobial, Anthelmintic and Insecticidal Activities Study of 4(4'-Bromophenyl)-6-substituted-aryl-1-acetyl pyrimidine-2-thiols". *E-Journal of Chemistry*. (2010). 7(3), 935–941. https://doi.org/10.1155/2010/927601

12) Mojtahedi M. M., Saidi M. R., Shirzi J. S. &Bolourtchian M. "Microwave promoted efficient synthesis of substituted uracils and thiouracils under solvent-free conditions." *Synthetic Communications*. (2002). 32(6), 851–855. https://doi.org/10.1081/scc-120002693

13) Patil D .R. , Salunkhe S. M. , Deshmukh M. B., and Anbhule P. V. "One Step Synthesis of 6-Amino-5-Cyano-4-Phenyl-2-Mercapto Pyrimidine Using Phosphorus Pentoxide". *The Open Catalysis Journal* (2010), *3*, 83-86

14) Shen Z.-L., Xu X.-P. &Ji, S. J. "Bronsted Base-Catalyzed One-Pot Three-Component Biginelli-Type Reaction: An Efficient Synthesis of 4, 5, 6-Triaryl-3, 4-dihydropyrimidin-2(1H)-one and Mechanistic Study". *The Journal of Organic Chemistry*, (2010). 75(4), 1162–1167. https://doi.org/10.1021/jo902394y

15) Hegede H., Gaonkar S. L., and Shetty N. S. "Bronsted base catalyzed efficient one pot three component synthesis of dihydropyrimidinone derivatives". *Journal of Chemical and Pharmaceutical Research.* (2015). 7(9), 526-531.

16) Nishio T. & Omote Y. "Photochemical ring opening of pyrimidin-2(1H)-ones". Part 2. *Journal of the Chemical Society, Perkin Transactions 1*, (1984). 239-242. https://doi.org/10.1039/p19840000239

17) Mishra C. B., Kumari S., Manral A., Prakash A., Saini V., Lynn A. M. & Tiwari M. "Design, synthesis, in-silico and biological evaluation of novel donepezil derivatives as multi-target-directed ligands for the treatment of Alzheimer's disease". *European Journal of Medicinal Chemistry.* (2017). 125, 736–750. https://doi.org/10.1016/j.ejmech.2016.09.057

18) Yi Y., Xu X., Liu Y., Xu S., Huang X., Liang J. & Shang R. "Synthesis and antibacterial activities of novel pleuromutilin derivatives with a substituted pyrimidine moiety." *European Journal of Medicinal Chemistry*. (2017). 126, 687–695. https://doi.org/10.1016/j.ejmech.2016.11.054

19) Wael A. El-S., Ashraf M. M., Hemat S. K., Dina S. EL-K. & May Al-M. "Synthesis, Docking Studies and Anticancer Activity of New Substituted Pyrimidine and Triazolopyrimidine Glycosides". *Journal of Applied Pharmaceutical Science.* (2017). 7(09), 001-011. https://doi.org/10.7324/japs.2017.70901

20) Yang C.-R., Peng B., Cao S.-L., Ren T.-T., Jiang W., Wang F.-C. & Xu, X. "Synthesis, cytotoxic evaluation and target identification of thieno[2,3- d]pyrimidine derivatives with a dithiocarbamate side chain at C2 position". *European Journal of Medicinal Chemistry*. (2018). 154, 324–340. https://doi.org/10.1016/j.ejmech.2018.05.028

21) Ugwu D. I., Okoro U. C., & Mishra N. K. "Synthesis, characterization and anthelmintic activity evaluation of pyrimidine derivatives bearing carboxamide and sulphonamide moieties." *Journal of the Serbian Chemical Society*. (2018). 83(4), 401–409. https://doi.org/10.2298/jsc170127109u

22) Abdelhaleem E. F., Abdelhameid M. K., Kassab A. E., & Kandeel M. M. "Design and synthesis of thienopyrimidine urea derivatives with potential cytotoxic and pro-apoptotic activity against breast cancer cell line MCF-7". *European Journal of Medicinal Chemistry*. (2018). 143, 1807–1825. https://doi.org/10.1016/j.ejmech.2017.10.075"

23) Ranganathaa V. L., Prashanthb T. H.B., Patil V., Bhadregowda D.G., Mallikarjunaswamy C, "Synthesis and biological activity of 5–bromo–2-chloropyrimidin-4-amine derivatives". *Chemical Data Collections*. (2018). 314, 1-10

24) Agarwal A., Srivastava K., Purib S. K. and Chauhana P. M. S., "Antimalarial activity and synthesis of new trisubstitutedpyrimidines". *Bioorganic & Medicinal Chemistry Letters*. (2005). 15, 3130–3132

25) Gressler V., Moura S., Flores A. F. C., Flores D. C., Colepicolo P. & Pinto E."Antioxidant and antimicrobial properties of 2-(4, 5-dihydro-1H-pyrazol-1-yl)-pyrimidine and 1-carboxamidino-1H-pyrazole derivatives". *Journal of the Brazilian Chemical Society*. (2010). 21(8), 1477–1483. https://doi.org/10.1590/s0103-50532010000800010

26) Bamnela R. and Shrivastava S. P. "Synthesis and *In Vitro* Antimicrobial, Anthelmintic and Insecticidal Activities Study of 4(4'-Bromophenyl)-6-substituted-aryl-1-acetylpyrimidine-2-thiols". *E-Journal of Chemistry*. (2010). 7(3), 935-941

CHAPTER-5

RESEARCH GOALS

5. RESEARCH GOALS:

It was seen from the literature survey that pyrimidine derivatives were found to possess different biological activities. The pyrimidine derivatives synthesized earlier in our laboratories also possessed significant anticancer activities. In continuation of our earlier work on pyrimidine derivatives, it was thought worthy to synthesize some more novel pyrimidine derivatives.

1. To search the novelty of proposed molecules and reaction conditions from various chemical databases like Scifinder, Sciverse, Scopus, Chemsynthesis, Chemspider, Reaxys etc.
2. To synthesize the pyrimidine derivatives.
3. To monitor the purity and progress of the reaction using TLC technique.
4. To purify the compounds by recrystallization using suitable solvents.
5. To characterize the compounds of pyrimidine using spectral (IR and ^{1}H NMR) methods.
6. To screen the synthesized derivatives for their pharmacological activities like antimicrobial activities.

CHAPTER-6

MATERIALS & METHOD

6. Materials and Method:

6.1. General:

- All reagents and solvents used were of laboratory (LR) grade, obtained from Spetrochem PVT LTD (Mumbai, India) and Merck Limited (Mumbai, India) and were used without further purification.

6.2. Measurements:

- The progress of the reaction and purity of the synthesized compounds were checked on the precoated silica gel F254 plates obtained from Merck (Mumbai, India) using toluene and ethyl acetate (3:2) as mobile phase. Iodine chamber and UV lamp ($\lambda = 254$ nm) were used for visualization of the spots.

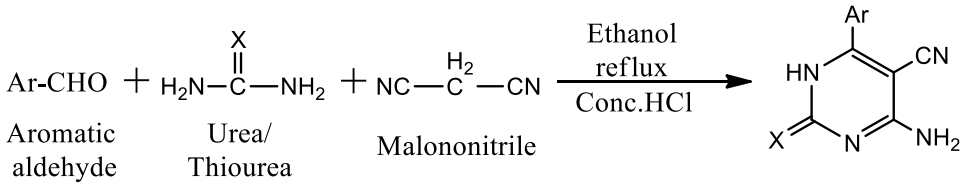
  • Melting points were determined in an open capillary tube on VEEGO VMP-DS melting point apparatus and were uncorrected.

  • Double beam Systronics 1800 UV spectrophotometer was used for the determination of absorbance.

  • The IR spectra (νmax, cm^{-1}) were recorded on BRUKER OPTIC GMBH spectrophotometer.

  • ^1H NMR (δ, ppm) spectra were recorded in $CDCl_3$ withTetramethylsilane (TMS) as internal standard on Bruker advance III NMR spectrophotometer at 400 MHz.

6.3. Synthetic scheme:



Ar: Any substituted phenyl ring X: O or S

Fig 6.1. Synthesis of Dihydropyrimidine derivatives

6.4. Experimental Procedure:

At first aromatic aldehyde (0.02 mole) and malononitrile (0.02 mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were shaken vigorously with a glass rod for 10 minutes in room temperature. After that as a nucleophile, urea or thiourea (0.03 mole) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC, the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place.The whole mixture was kept in the refrigerator overnight.The day after, the solid was filtered off, dried and recrystalized from suitable solvent.

6.3. Synthesis of Compound 1a (*4-amino-6-(4-chlorophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile)*:

At first 4-chlorobenzaldehyde (0.02 mole) and malononitrile (0.02 mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were shaken vigorously with a glass rod for 10 minutes in room temperature. After that as a nucleophile, urea (0.03mole) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place. The whole mixture was kept in the refrigerator overnight. The day after, the product was filtered off, dried and recrystallized from suitable solvent.

The route of synthesis is as followed:

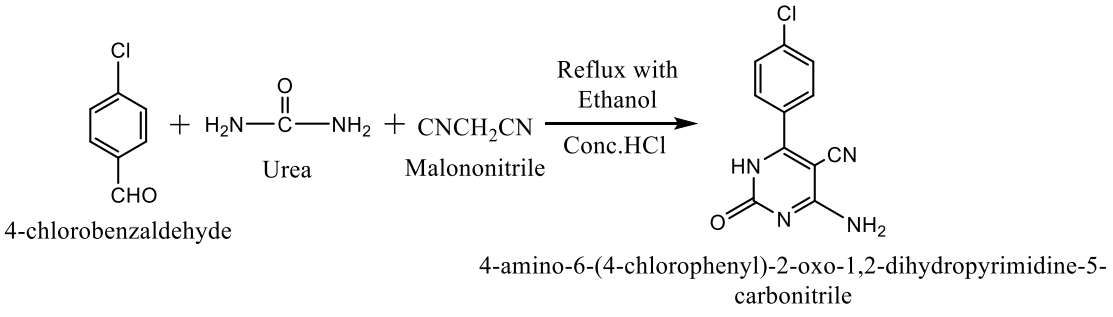


Fig 6.2. Synthesis of Compound 1a

Fig 6.3. Synthesis of Compound 1a

6.4. Synthesis of Compound 1b (*4-amino-6-(2,3dichlorophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile)*:

At first 2, 3-dichlorobenzaldehyde (0.02 mole) and malononitrile (0.02 mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were shaken vigorously with a glass rod for 10 minutes in room temperature. After that as a nucleophile, urea (0.03mole) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place. The whole mixture was kept in the refrigerator overnight. The day after, the product was filtered off, dried and recrystallized from suitable solvent.

Fig 6.4. Formation of Compound 1b

The route of synthesis is as followed:

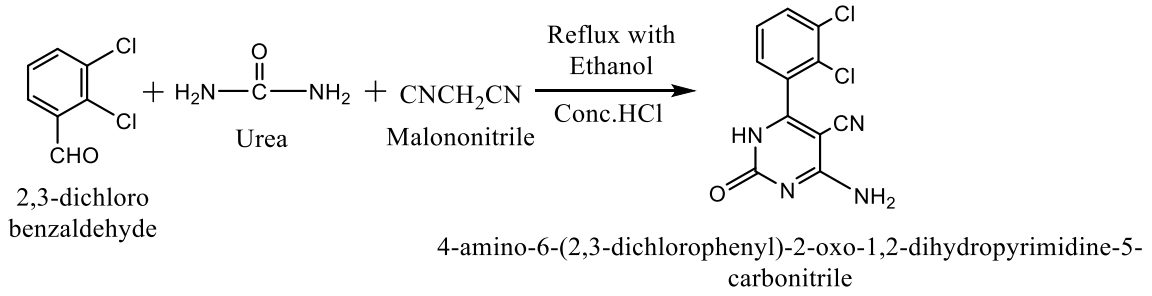


Fig 6.5. Synthesis of Compound 1b

6.5. Synthesis of Compound 1c (*4-amino-6-(4-nitrophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile)*:

At first 4-nitrobenzaldehyde (0.02 mole) and malononitrile (0.02mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were shaken vigorously with a glass rod for 10 minutes in room temperature. After that as a nucleophile, urea (0.03mole) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place. The whole mixture was kept in the refrigerator overnight. The day after, the product wasfiltered off, dried and recrystallized from suitable solvent.

The route of synthesis is as followed:

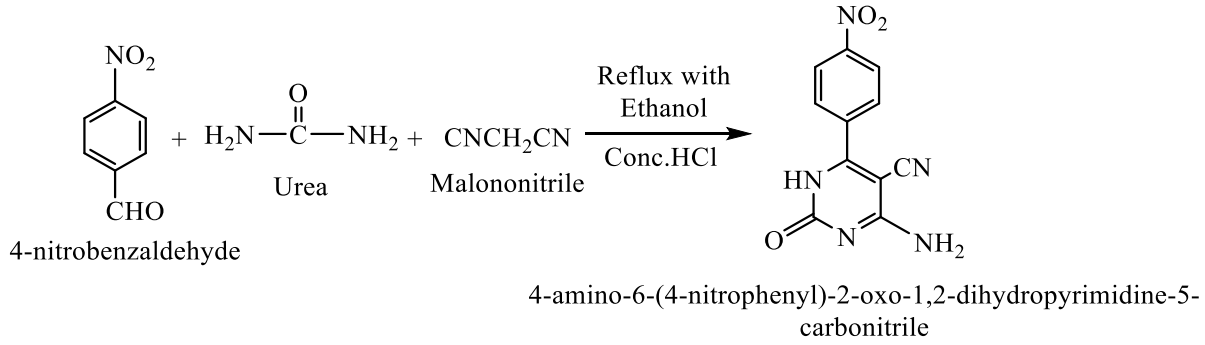


Fig 6.6. Synthesis of compound 1c

Fig 6.7. Synthesis of compound 1c (Yellow crystals)

6.6. Synthesis of Compound 1d (*4-amino-6-(3-fluorophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile)*:

At first 3-fluorobenzaldehyde (0.02 mole) and malononitrile (0.02mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were urea (0.03mole) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place. The whole mixture was kept in the refrigerator overnight. The day after, the product was filtered off, dried and recrystallized from suitable solvent.

The route of synthesis is as followed:

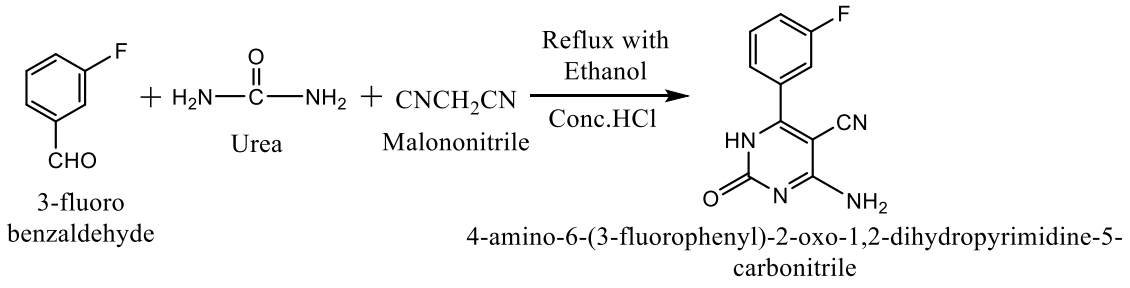


Fig 6.8. Synthesis of compound 1d

Fig 6.9. Thin Layer Chromatography of compound 1d

6.7. Synthesis of Compound 1e (*4-amino-6-(4-methylphenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile)*:

At first 4-methylbenzaldehyde (0.02 mole) and malononitrile (0.02mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were shaken vigorously with a glass rod for 10 minutes in room temperature. After that as a nucleophile, urea (0.03mole) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place. The whole mixture was kept in the refrigerator overnight. The day after, the product was filtered off, dried and recrystallized from suitable solvent.

The route of synthesis is as followed:

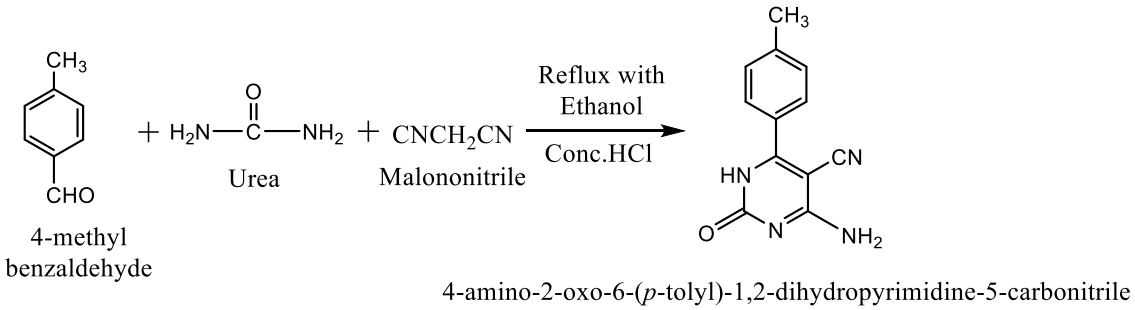


Fig 6.10. Synthesis of compound 1e

6.8. Synthesis of Compound 2a (*4-amino-6-(4-chlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile):*

At first 4-chlorobenzaldehyde (0.02 mole) and malononitrile (0.02mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were shaken vigorously with a glass rod for 10 minutes in room temperature. After that as a nucleophile, thiourea (0.03mole) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place. The whole mixture was kept in the refrigerator overnight. The day after, the product was filtered off, dried and recrystallized from suitable solvent.

The route of synthesis is as followed:

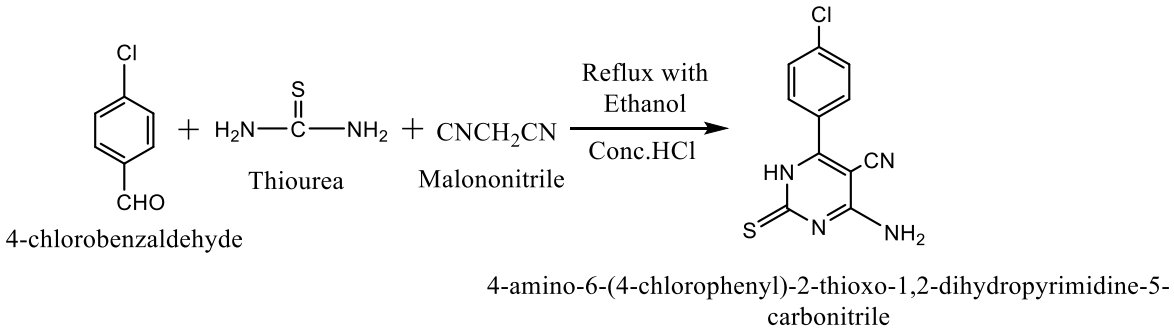


Fig 6.11. Synthesis of compound 2a

Fig 6.12. Synthesis of Compound 2a

6.9. Synthesis of Compound 2b (*4-amino-6-(3-fluorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile):*

At first 3-fluorobenzaldehyde (0.02 mole) and malononitrile (0.02 mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were shaken vigorously with a glass rod for 10 minutes in room temperature. After that as a nucleophile, thiourea (0.03 mole) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place. The whole mixture was kept in the refrigerator overnight. The day after, the product was filtered off, dried and recrystallized from suitable solvent.

The route of synthesis is as followed:

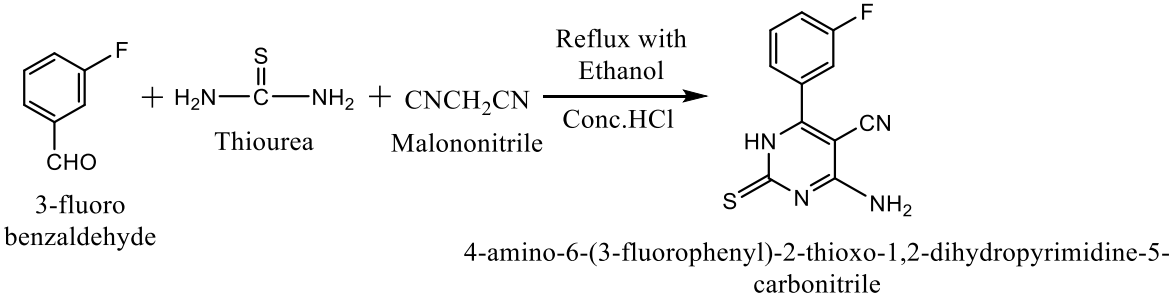


Fig 6.13. Synthesis of compound 2b

Fig 6.14. Synthesis of Compound 2b

6.10. Synthesis of Compound 2c (*4-amino-6-(4-methylphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile):*

At first 4-methylbenzaldehyde (0.02 mole) and malononitrile (0.02mole) were weighed accurately and taken into a 100 ml beaker and 25 ml of absolute alcohol was also taken. These two reagents were shaken vigorously with a glass rod for 10 minutes in room temperature. After that as a nucleophile, thiourea (0.03mol) was added to the reaction mixture. The resulting mixture was heated at reflux condition in a heating mantle. After the completion of the reaction monitored by TLC the reaction mixture was poured on the crushed ice (about 200 g). On stirring separation of desired product takes place. The whole mixture was kept in the refrigerator overnight. The day after, the product was filtered off, dried and recrystallized from suitable solvent.

The route of synthesis is as followed:

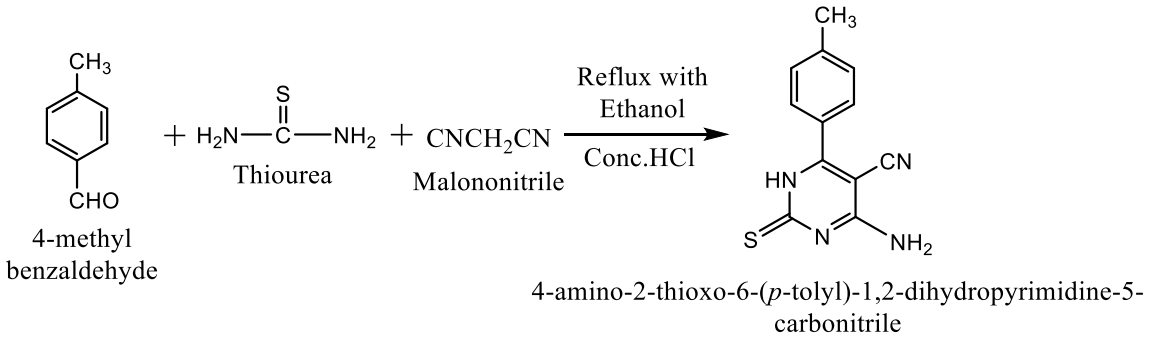


Fig 6.15. Synthesis of compound 2c

CHAPTER-7

PHARMACOLOGICAL SCREENING

7. PHARMACOLOGICAL SCREENING:

7.1. MINIMUM INHIBITORY CONCENTRATIONS [(MIC(S)]:

MIC(s) are defined as the lowest concentration of the antimicrobial agent that will inhibit the visual growth of a micro-organism after overnight incubation and minimum bactericidal concentrations of antimicrobials that will prevent the growth of an organism after sub-culture on to antibiotic-free media. In my research work I have evaluated the minimum inhibitory concentrations of the synthesized compounds by Drug Plate Method. [1]

7.2. Drug Plate method:

The antimicrobial activity of the synthesized compounds has been evaluated by Drug-Plate Method. The synthesized compounds have been tested for their antibacterial activity against *Staphylococcus aureus* ATCC6538P, *Bacillus subtilis* ATCC6633, *Pseudomonas aeruginosa* ATCC9027 and *Escherichia coli* ATCC8739.

7.2.1. Preparation of Stock Solution:

4mg/ml concentration of the drug compound was taken for preparation of the stock solution. 40 mg drug was weighed accurately in a digital weighing balance and dissolved in 10 ml of DMSO (Dimethyl sulfoxide) solvent.

7.2.2. Preparation of Microbial culture:

Broth micro- or macro-dilution is one of the most basic anti-microbial susceptibility testing methods. The procedure involves preparing two fold dilutions of the antimicrobial agent in a liquid growth medium dispensed in tubes containing a minimum volume of 2 mL (macro dilution) or with smaller volumes using 96-well micro titration plate (micro dilution). Then, each tube or well is inoculated with a microbial inoculum prepared in the same medium after dilution of standardized microbial suspension adjusted to 0.5 McFarland scale. After well mixing, the inoculated tubes or the 96-well micro titration plate are incubated (mostly without agitation) under suitable conditions depending upon the test microorganism. [2]

7.2.3. Drugs used as Standards:

- Amoxicillin was used for *Staphylococcus aureus* ATCC6538P, *Bacillus subtilis* ATCC6633 and *Pseudomonas aeruginosa* ATCC9027.
- Norfloxacin was used for specially *Escherichia coli* ATCC8739.

7.2.4. Preparation of media:

- Composition of Nutrient Broth: (Standard Formula): Suspend 13.0 grams Agar in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in tube or flasks as desired.

Ingredients	Grams/liters
Peptone	5.00
Sodium Chloride	5.00
Meat Extract B#	1.50
Yeast Extract	1.50

Fig 7.1.Composition of Nutrient Broth

- Composition of Nutrient Agar (Standard Formula): Suspend 28 grams Agar in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Generally in regular laboratory works, 2% Agar is taken to make the medium semisolid.
- For 10 ml medium 0.104 gm. Nutrient broth and 0.160 gm. Nutrient Agar were weighed accurately and taken into a conical flask. These two were dissolved in distilled water. The media was then autoclaved at 15 lbs. pressure (121^{o}C) for 15 minutes.

7.2.5. Different dilutions experimented:

50,80,100,150,175,200,250,400,500,750,1000 µg/ml concentrations are observed. After that, among the above mentioned concentrations, two of them were taken, one is of which doesn't show growth and the other shows growth. These two concentrations were divided into 10 dilutions each e.g. 400 µg/ml dilutions showed growth, in the other hand 500

µg/ml dilution inhibited. So for the next step, 410, 420, 430…..490 µg/ml dilutions were taken. Thus we could get the exact minimum inhibitory concentrations.

Fig 7.1. Different dilutions of drugs and their activity

7.2.6. Preparation of drug plate:

Drug solution of different concentrations and freshly prepared media individually were taken in a drug plate one after another. The media is itself constituted with the drug or the test compound, that's why this is named so.

7.2.7. Inoculation of Microorganisms:

After the drug plate was prepared, four quadrants were drawn with a glass marker on the back side of the drug-plate. 10 µl of microbial strain was dropped on the specific quadrant. The four segments were specified as A, B, C, and D. Each of them represented a single strain of bacteria.

- 'A' stands for *Staphylococcus aureus* ATCC6538P.
- 'B' stands for *Bacillus subtilis* ATCC6633.
- 'C' stands for *Pseudomonas aeruginosa* ATCC9027.
- 'D' stands for *Escherichia coli* ATCC8739.

7.2.8. Incubation condition:

The plate containing drug and micro-organisms were incubated at 37^{o}C overnight. The day after the drug plates were examined thoroughly whether there is any growth of microbes or not.

7.3. MEASUREMENT OF ZONE OF INHIBTION:

If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called as zone of inhibition. [3]

7.3.1. Preparation of Medium:

For 10 ml medium 0.104 gm. Nutrient broth and 0.160 gm Nutrient Agar were weighed accurately and taken into a conical flask. These two were dissolved in distilled water. The media was then autoclaved at 15 lbs. pressure (121^{o}C) for 15 minutes.

7.3.2. Inoculation of Microorganisms:

After the drug plate was prepared, four quadrants were drawn with a glass marker on the back side of the drug-plate. 10 µl of microbial strain was dropped on the specific quadrant. The four segments were specified as A, B, C, and D. Each of them represented a single strain of bacteria.

- 'A' stands for *Staphylococcus aureus* ATCC6538P.
- 'B' stands for *Bacillus subtilis* ATCC6633.
- 'C' stands for *Pseudomonas aeruginosa* ATCC9027.
- 'D' stands for *Escherichia coli* ATCC8739.

7.3.3. Incubation condition:

The plate containing drug and micro-organisms were incubated at 37^{o}C overnight. The day after the drug plates were examined thoroughly whether there is any zone of inhibition or not.

7.3.4. Standard Preparation:

100 µg/ml concentration of the drug Amoxicillin was taken for preparation of the standard solution. 0.1 mg drug was weighed accurately in a digital weighing balance and dissolved in 10 ml of DMSO (Dimethyl sulfoxide) solvent. Amoxicillin showed results

against *Staphylococcus aureus, Bacillus subtilis, and Pseudomonas aeruginosa.* Amoxicillin could not inhibit the growth of *Escherichia coli*. That's why Norfloxacin was used as a standard solution following the same method. MIC and zone of inhibition of these standard drugs were evaluated accordingly.

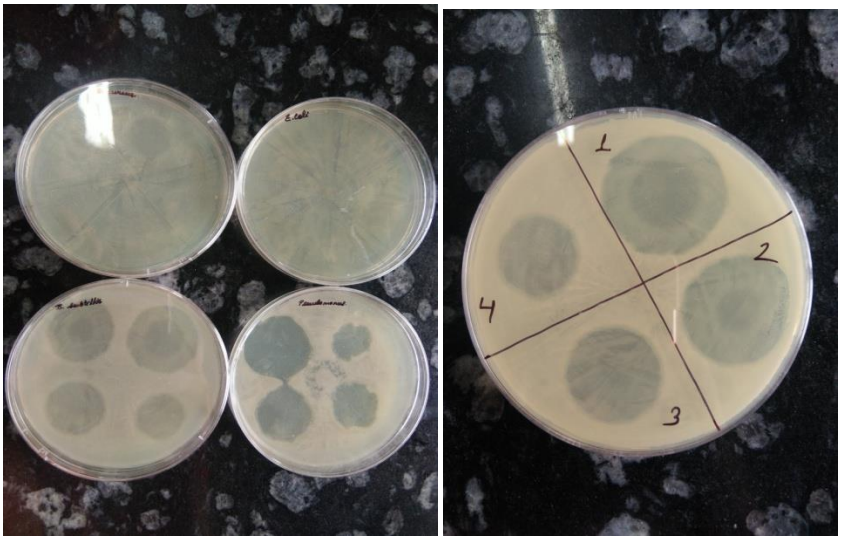


Fig 7.2. Zone of inhibition of Amoxicillin

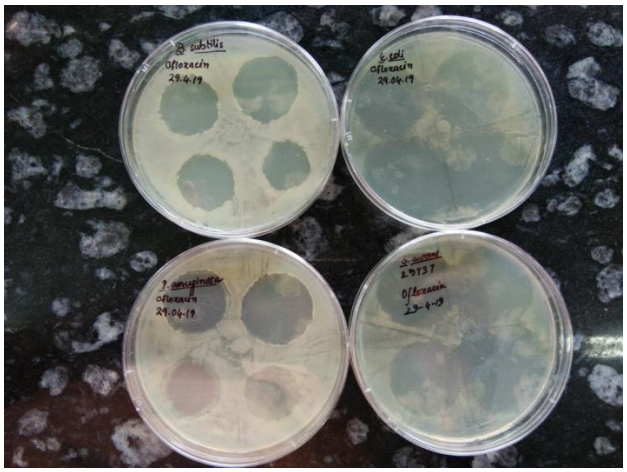


Fig 7.3. Zone of inhibition of Ofloxacin

7.4. References:

1. Kaur S. P. Rao R., Nanda S. "Amoxicillin; A Broad Spectrum Antibiotic" *International Journal of Pharmacy and Pharmaceutical Sciences*.(2011). 3(3)
2. "Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). Method for the determination of Broth dilution MICs of antifungal agents for fermentative yeasts." *Clinical Microbiological Infectious Diseases*. (2008). 4(4), 398–405.
3. Wiegand I., Hilpert K., Hancock R. E. "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances". *Natural Protocol*. (2008). 3(2), 163–175.

CHAPTER-8

RESULTS & DISCUSSION

8. RESULTS:

8.1. IUPAC name of Compounds:

Compounds	IUPAC Names
1.a	*4-amino-6-(4-chlorophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile*
1.b	*4-amino-6-(2,3-dichlorophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile*
1.c	*4-amino-6-(4-nitrophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile*
1.d	*4-amino-6-(3-fluorophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile*
1.e	*4-amino-2-oxo-6-(p-tolyl)-1,2-dihydropyrimidine-5-carbonitrile*
2.a	*4-amino-6-(4-chlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile*
2.b	*4-amino-6-(3-fluorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile*
2.c	*4-amino-2-thioxo-6-(p-tolyl)-1,2-dihydropyrimidine-5-carbonitrile*

8.2. Physical Appearance & Solubility:

Compounds	Color	Crystallinity	Physical State	Recrystallization solvent	Solubility
1a	White	Shiny flakes	Solid	Ethanol	Chloroform
1b	White	Amorphous	Solid	Ethanol	Chloroform
1c	Yellow	Shiny flakes	Solid	Ethanol	Chloroform
1d	Pale blue	Matte amorphous	Solid	Ethanol	Chloroform
1e	White	Amorphous	Solid	Ethanol	Chloroform
2a	Light Orange	Shiny flakes	Solid	Ethanol	Chloroform
2b	Yellow	Flakes	Solid	Ethanol: Water (1:1)	Chloroform
2c	Yellow	Flakes	Solid	Ethanol: Water (1:1)	Chloroform

8.3. Physical Data:

Comps	X	Ar	Molecular formula	Molecular weight (gm.)	R_f*	Melting Range (^oC)	Percentage Yield (%)
1a	O	C_6H_5Cl	$C_6H_7N_4OCl$	246.5	0.87	158-162	71
1b	O	$C_6H_5Cl_2$	$C_{11}H_6N_4OCl_2$	281	0.84	98-100	75
1c	O	$C_6H_5NO_2$	$C_{11}H_7O_3N_5$	257	0.87	150-155	74
1d	O	C_6H_5F	$C_{11}H_7N_4OF$	230	0.84	98-102	70
1e	O	$C_6H_5CH_3$	$C_{12}H_7ON_4$	223	0.84	134-135	87
2a	S	C_6H_5Cl	$C_6H_7N_4SCl$	262.5	0.76	154-158	65
2b	S	C_6H_5F	$C_{11}H_8N_4SF$	247	0.84	148-152	53
2c	S	$C_6H_5CH_3$	$C_{12}H_7N_4S$	239	0.83	134-135	71

8.4. Experimental Data & Result:

8.4.1. *4-amino-6-(4-chlorophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile* (1a):
MP: 158^{o}C-162^{o}C. IR (KBr, *v*, cm^{-1})1748, 2221, 3034, 3099. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.52 (d,2H,J=8.4), 7.75 (s,1H,NH_2),7.85 (d,2H,J=8.4);

8.4.2. *4-amino-6-(2, 3-dichlorophenyl)-2-oxo-1, 2-dihydropyrimidine-5-carbonitrile* (1b):
MP: 98^{o}C-100^{o}C. IR (KBr, *v*, cm^{-1}) 1749,2226,3028. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.52 (d,2H,J=8.1), 7.73 (s,1H,NH_2),7.85 (d,2H,J=8.1);

8.4.3. *4-amino-6-(4-nitrophenyl)-2-oxo-1,2-dihydropyrimidine-5-carbonitrile* (1c):
MP: 150^{o}C-155^{o}C.IR (KBr, *v*, cm^{-1}) 1517,1603,2228,3038,3115. ^{1}H NMR($CDCl_3$, δ, ppm): 7.88 (s,1H,NH_2),8.07(d,2H,J=8.8), 8.38(d,2H,J=8.8);

8.4.4. *4-amino-6-(3-fluorophenyl)-2-oxo-1, 2-dihydropyrimidine-5-carbonitrile* (1d):

MP: 98^{o}C-102^{o}C. IR (KBr, *v*, cm^{-1}) 1718,2226,2917,3038. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.34(s,1H),7.53(q,1H), 7.65(q,2H),7.75(s,1H,NH);

8.4.5. *4-amino-2-oxo-6-(p-tolyl)-1,2-dihydropyrimidine-5-carbonitrile* (1e): MP:134^{o}C -135^{o}C. IR (KBr, *v*, cm^{-1}) 1767,2220,2925,3034. ^{1}H NMR ($CDCl_3$, δ, ppm): 2.46(s,3H,CH_3), 7.34 (d,1H,J=8), 7.72(s,1H,NH), 7.81(d,2H,J=8.4);

8.4.6. *4-amino-6-(4-chlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* (2a): MP: 154^{o}C-158^{o}C; IR (KBr, *v*, cm^{-1}) 953,1730,2222,3033. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.52 (d,2H,J=8.4), 7.75 (s,1H,NH), 7.85 (d,2H,J=8.4);

8.4.7. *4-amino-6-(3-fluorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile* (2b): MP:148^{o}C-152^{o}C; IR (KBr, *v*, cm^{-1}) 1266,2222,3030.^{1}H NMR ($CDCl_3$, δ, ppm): 4.39 (m,2H), 7.48 (d,1H), 7.70-7.76 (m,2H), 8.21(s,1H,NH);

8.4.8. *4-amino-2-thioxo-6-(p-tolyl)-1,2-dihydropyrimidine-5-carbonitrile* (2c): MP: 134^{o}C-135^{o}C. IR (KBr, *v*, cm^{-1}) 2220,3034,3499. ^{1}H NMR ($CDCl_3$, δ, ppm): 2.46 (s,3H,CH_3),7.34 (d,2H,J=8),7.72 (s,H), 7.81 (d,2H,J=8);

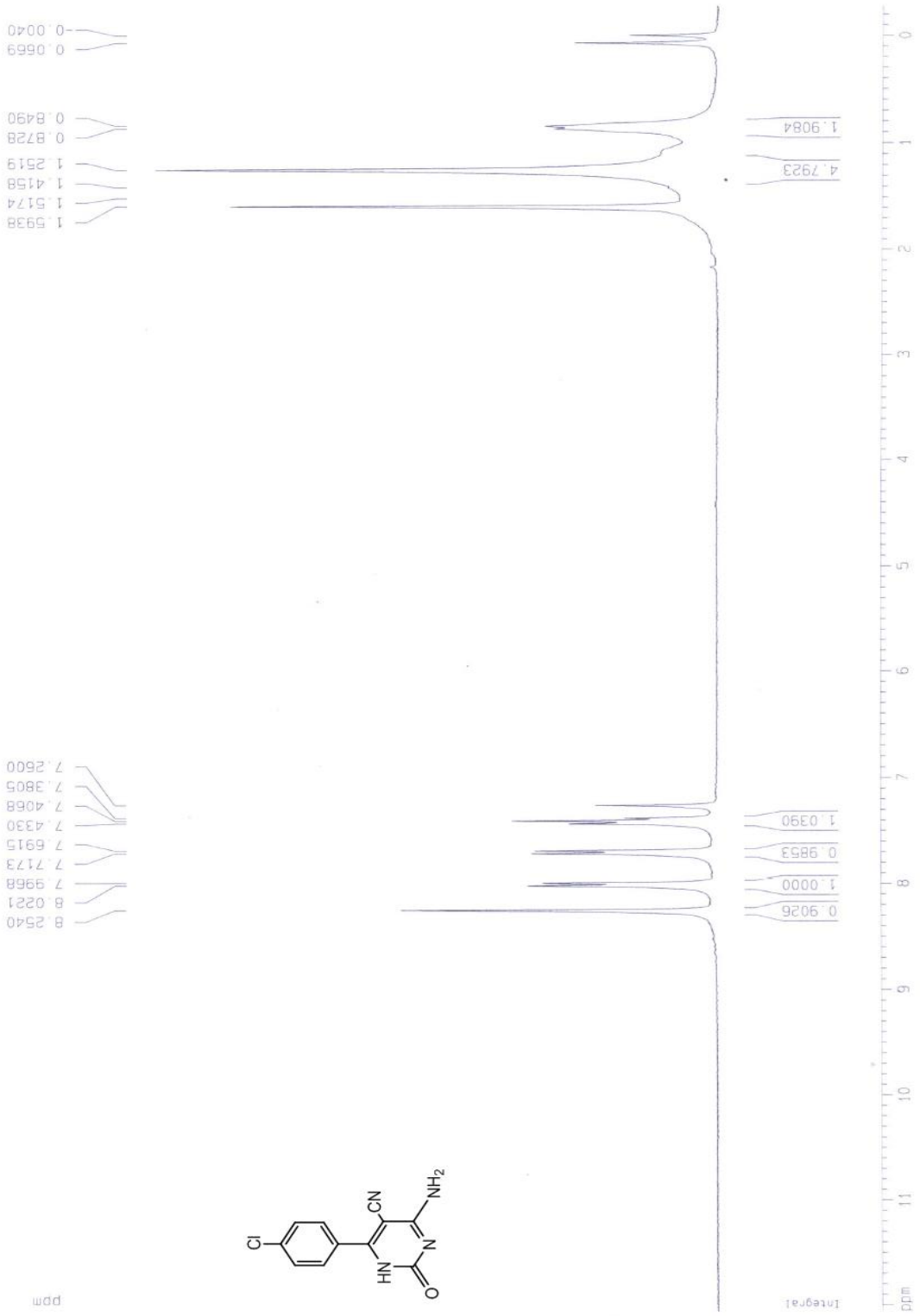


Fig 8.5.1: NMR Spectra of Compound ‘1a’

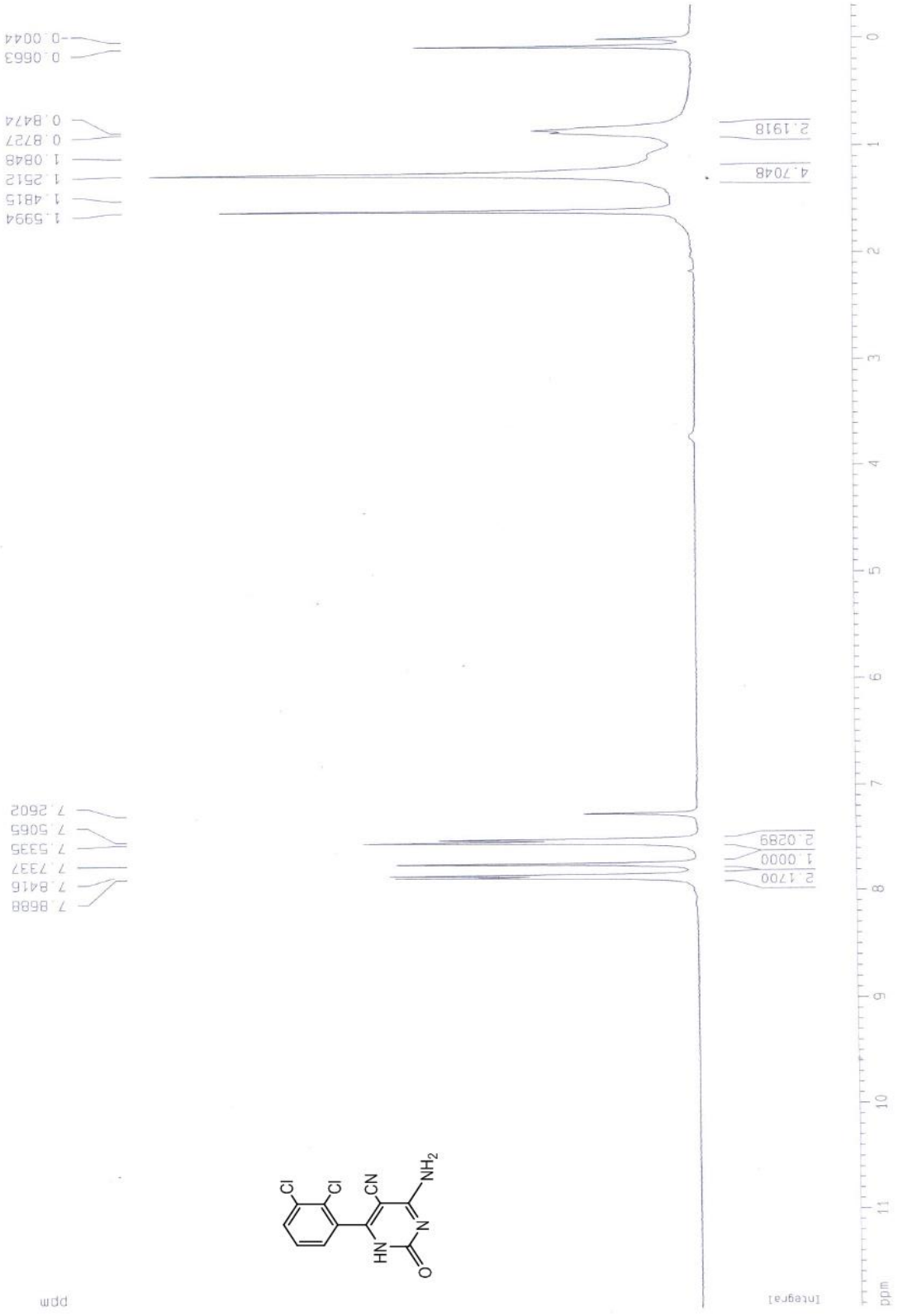


Fig 8.5.2: NMR Spectra of Compound '1b'

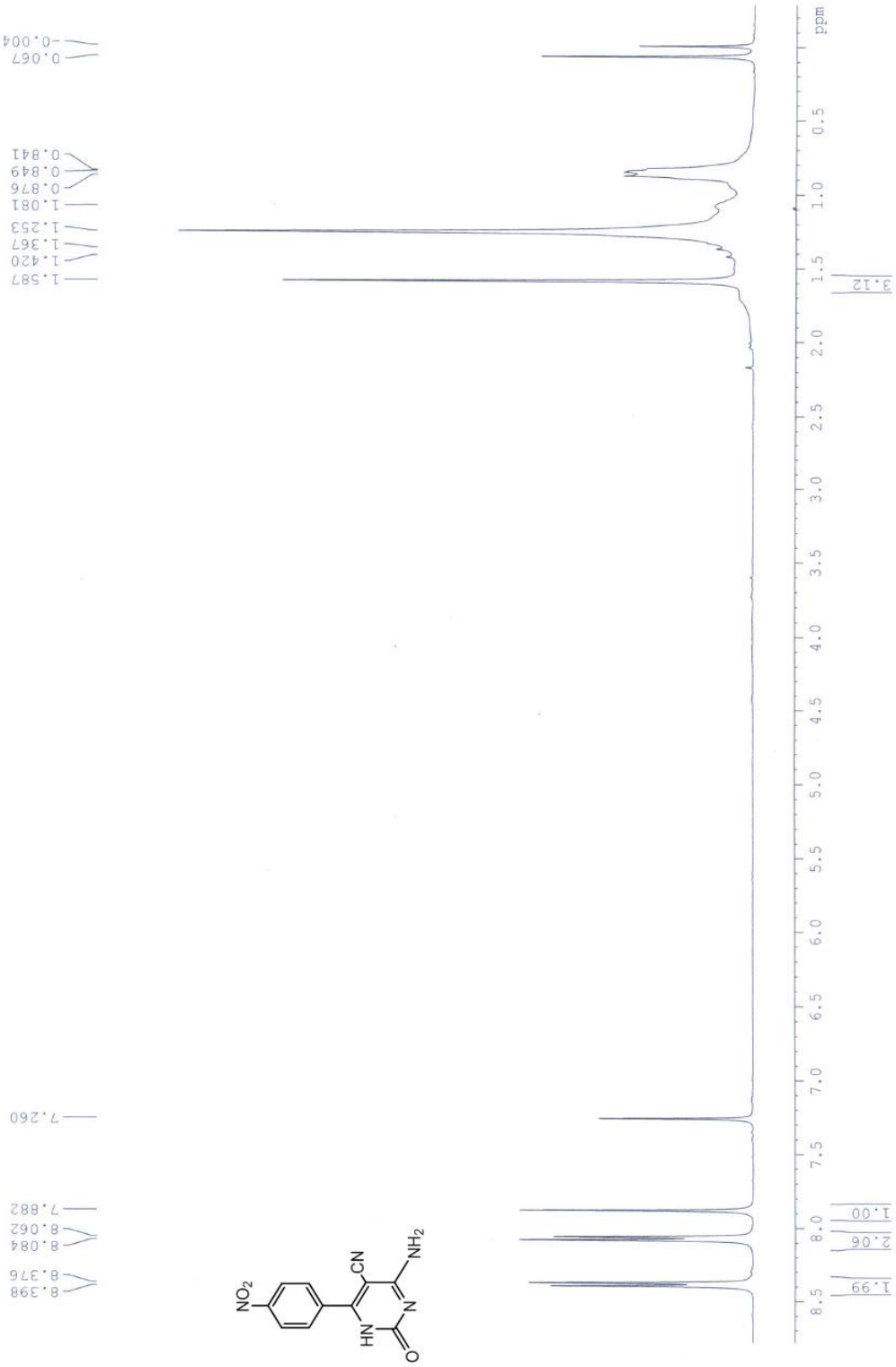


Fig 8.5.3: NMR Spectra of Compound '1c'

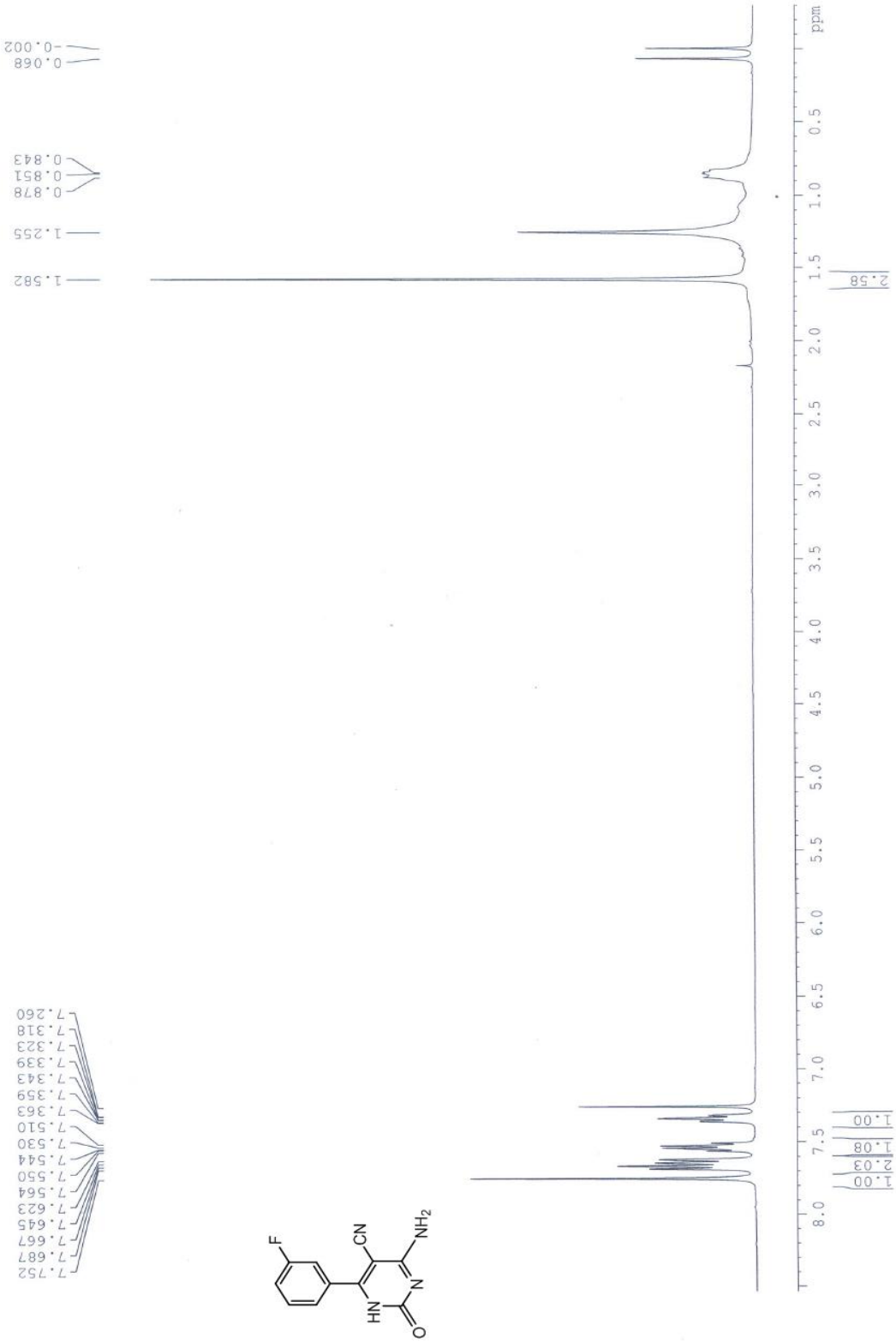


Fig 8.5.4: NMR Spectra of Compound ‘1d’

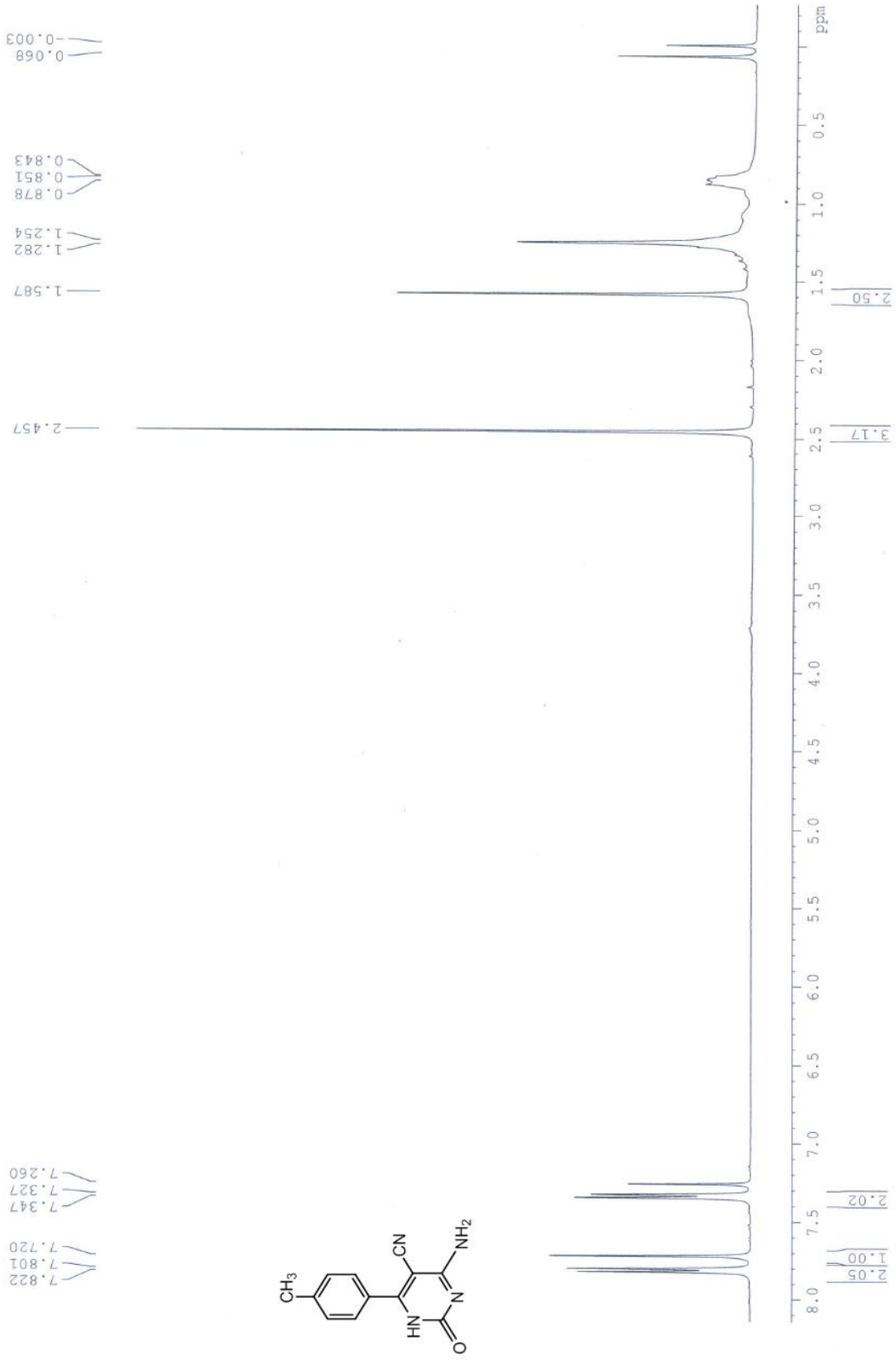


Fig 8.5.5: NMR Spectra of Compound '1e'

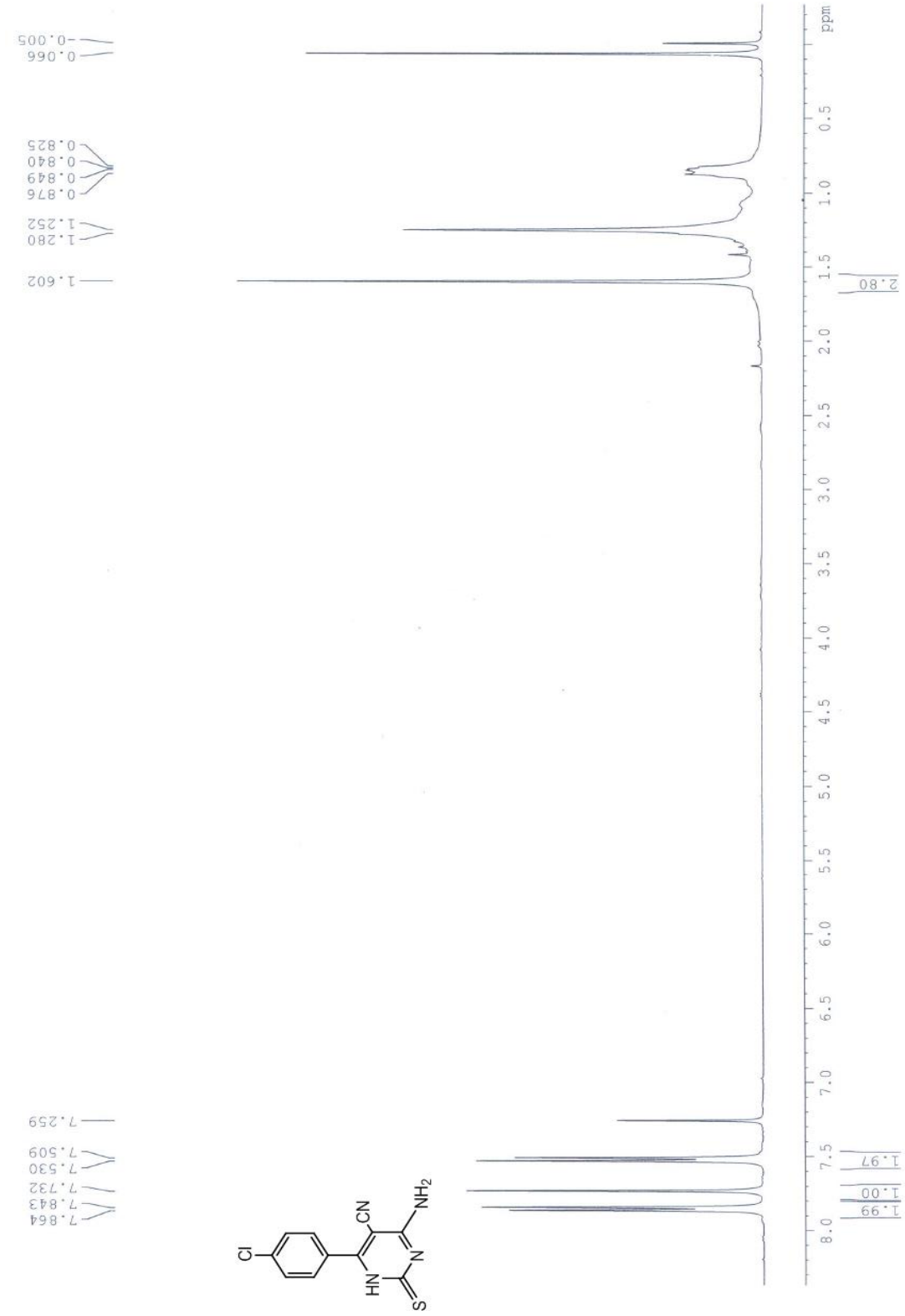


Fig 8.5.6: NMR Spectra of Compound '2a'

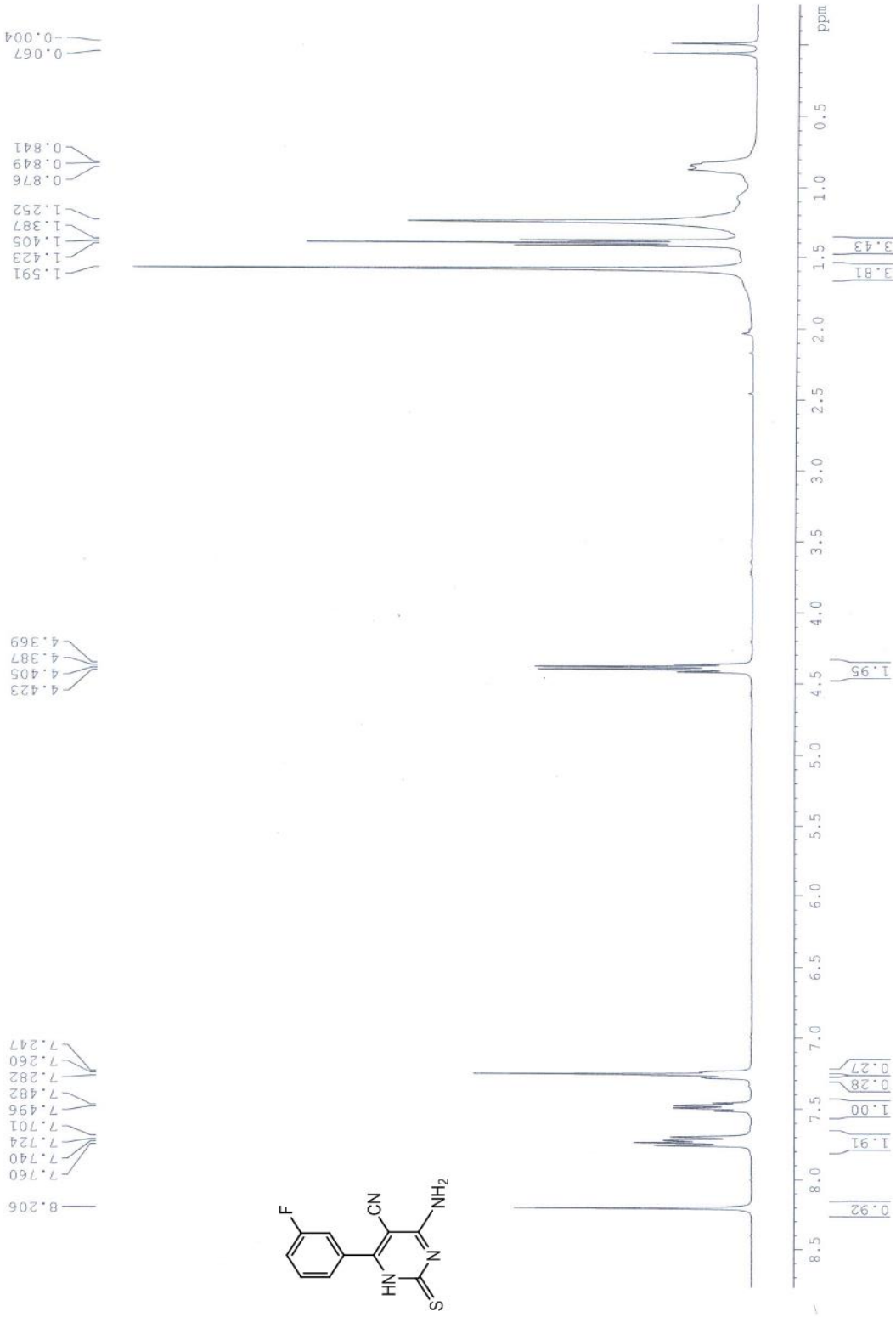


Fig 8.5.7: NMR Spectra of Compound '2b'

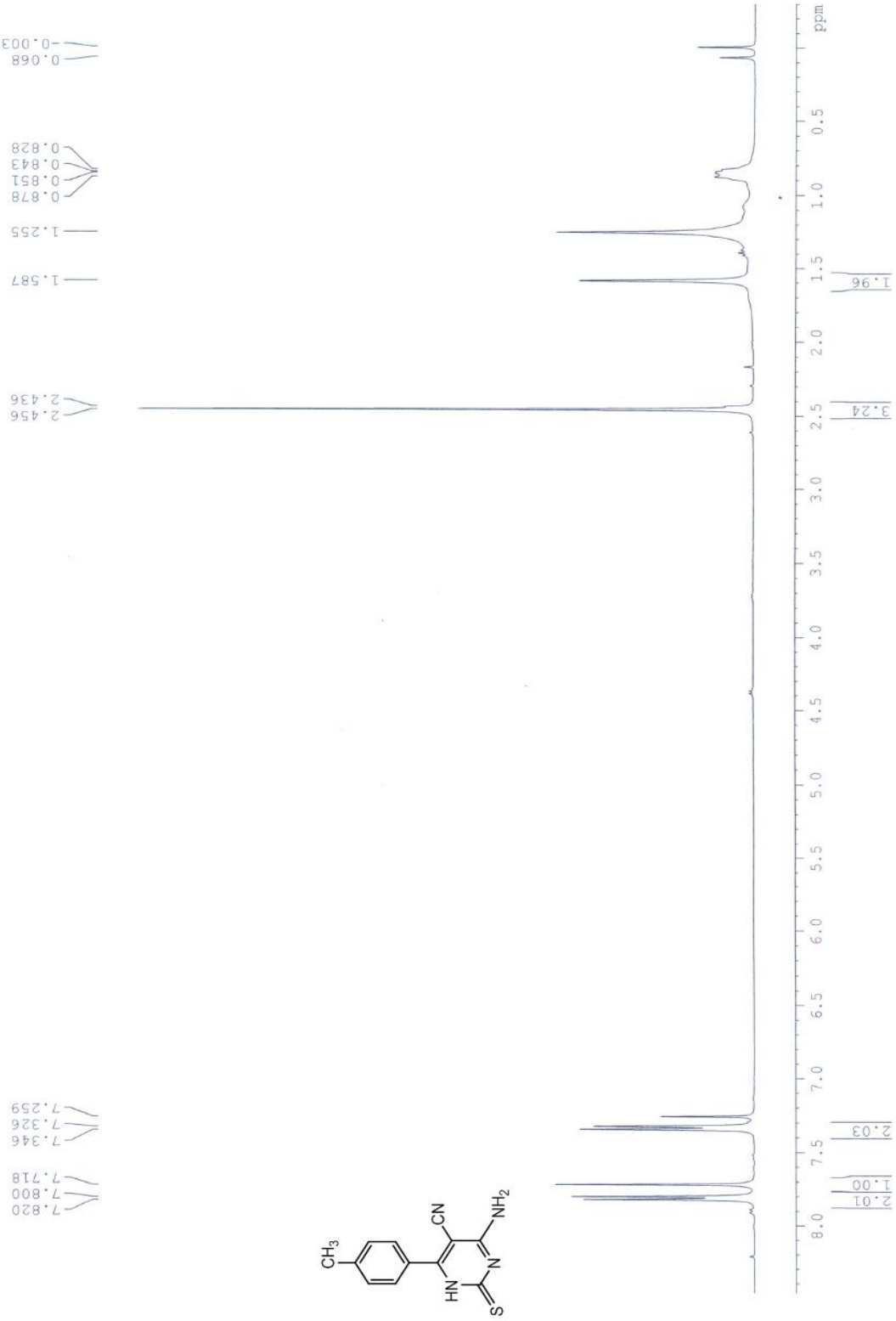


Fig 8.5.8: NMR Spectra of Compound '2a'

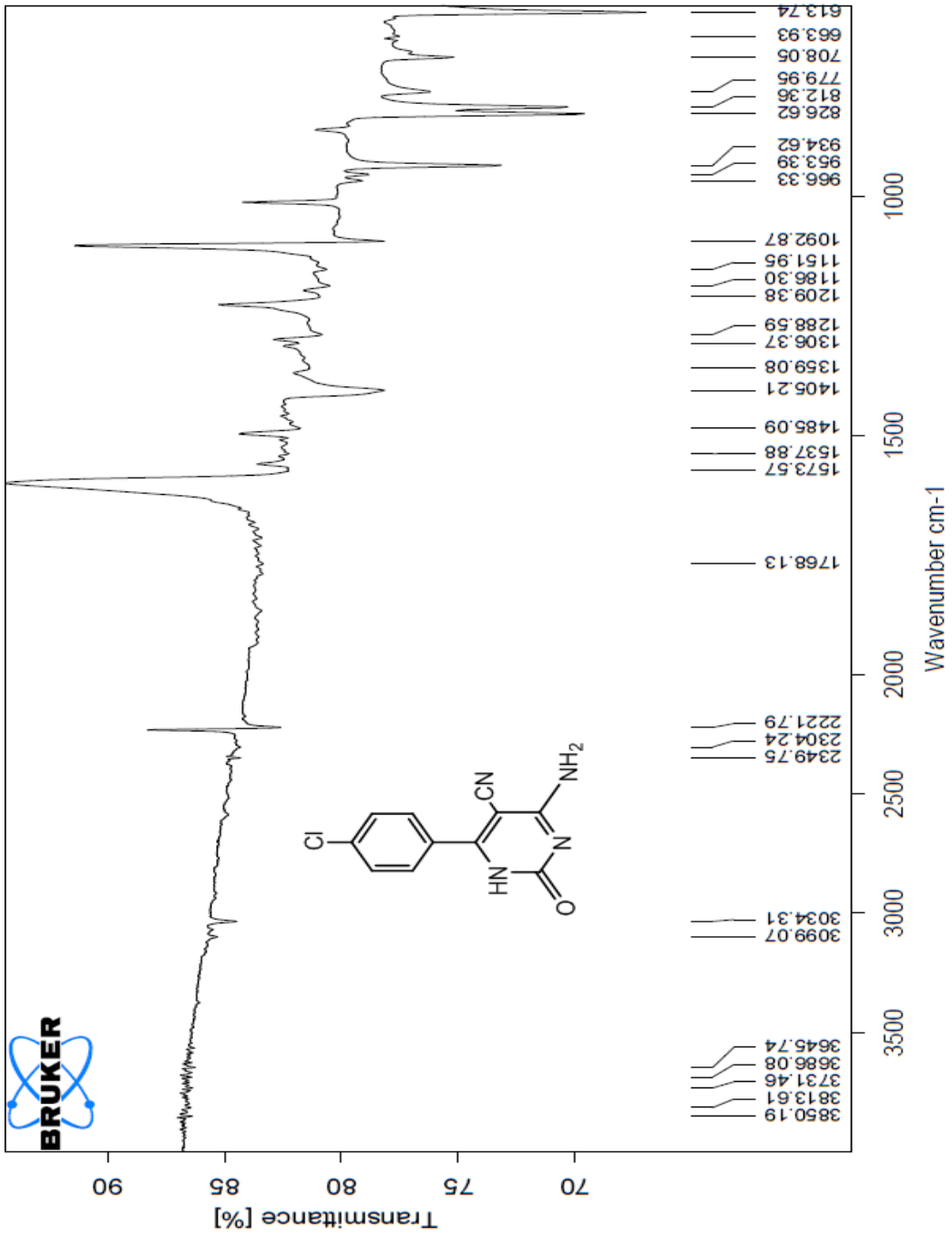


Fig 8.5.9: IR Spectra of Compound ‘1a’

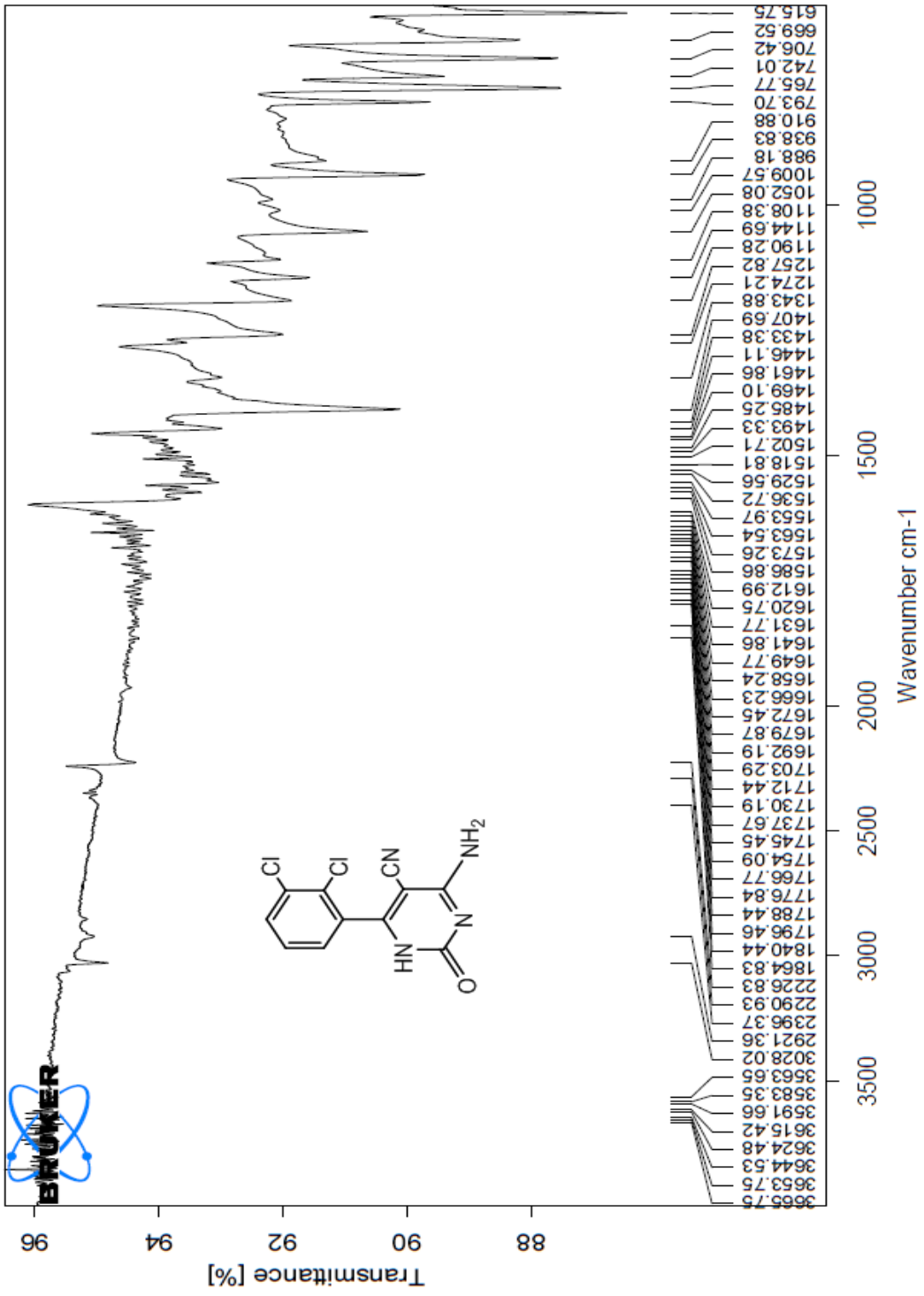


Fig 8.5.10: IR Spectra of Compound ‘1b’

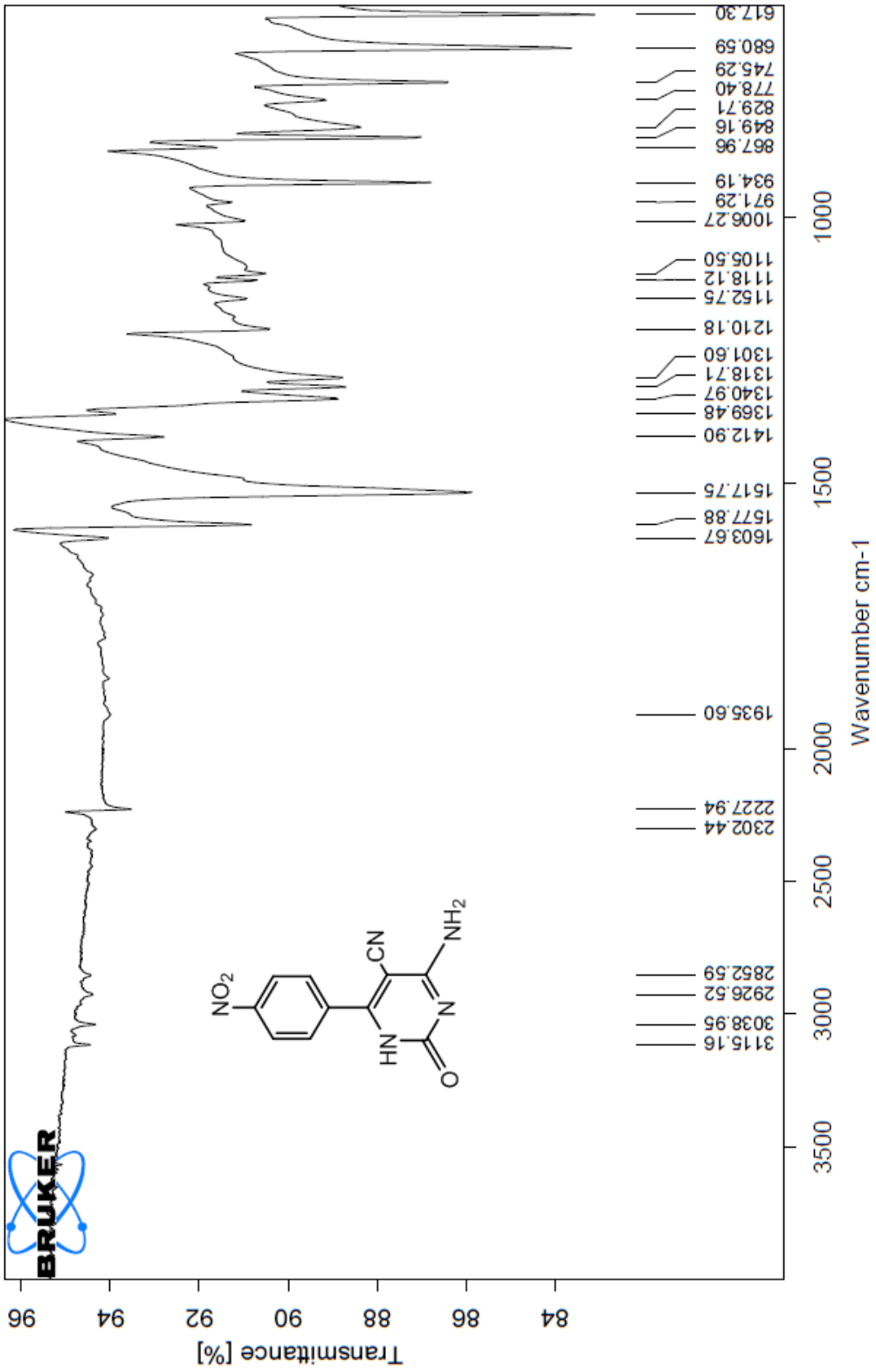


Fig 8.5.11: IR Spectra of Compound ‘1c’

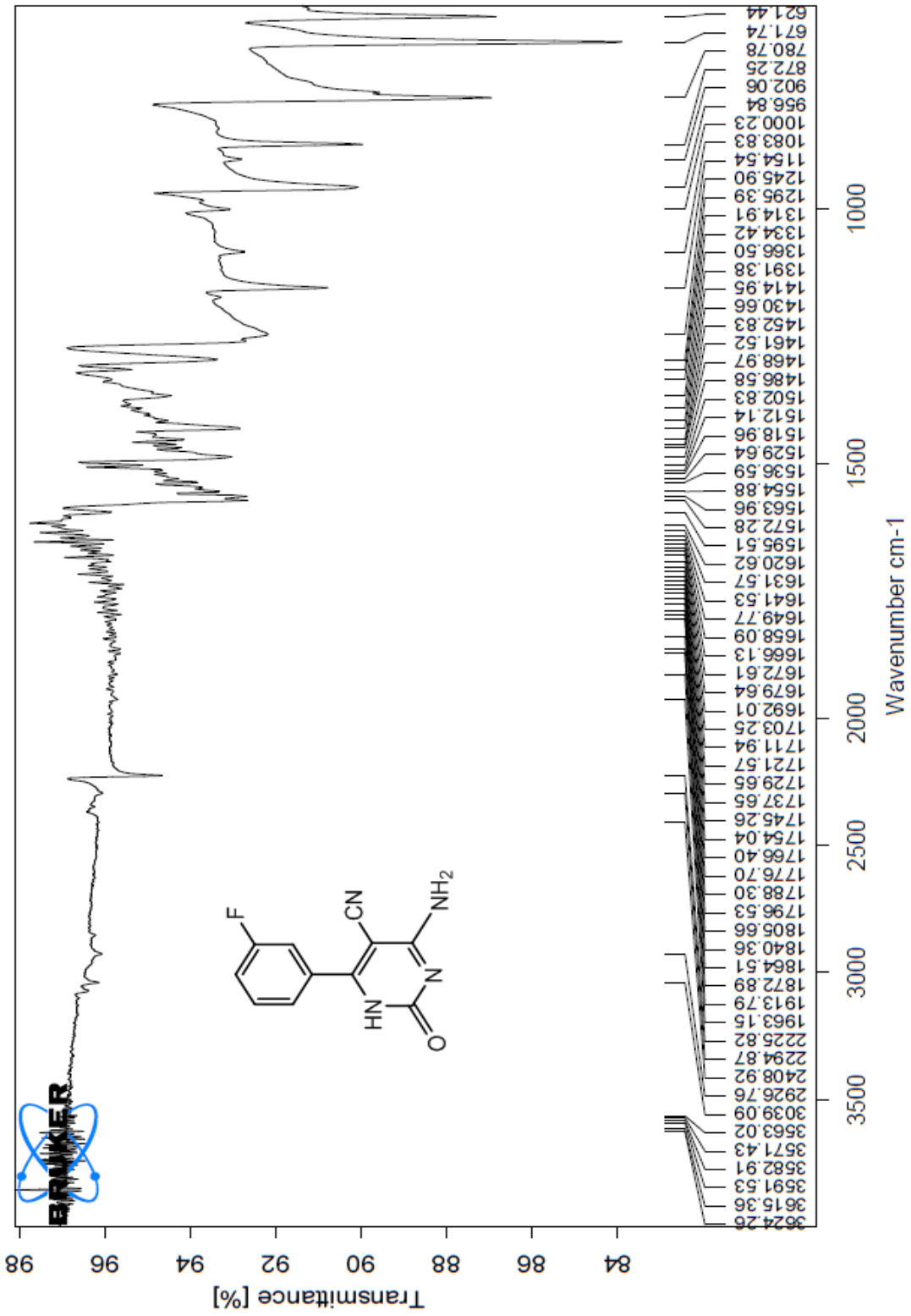


Fig 8.5.12: IR Spectra of Compound '1d'

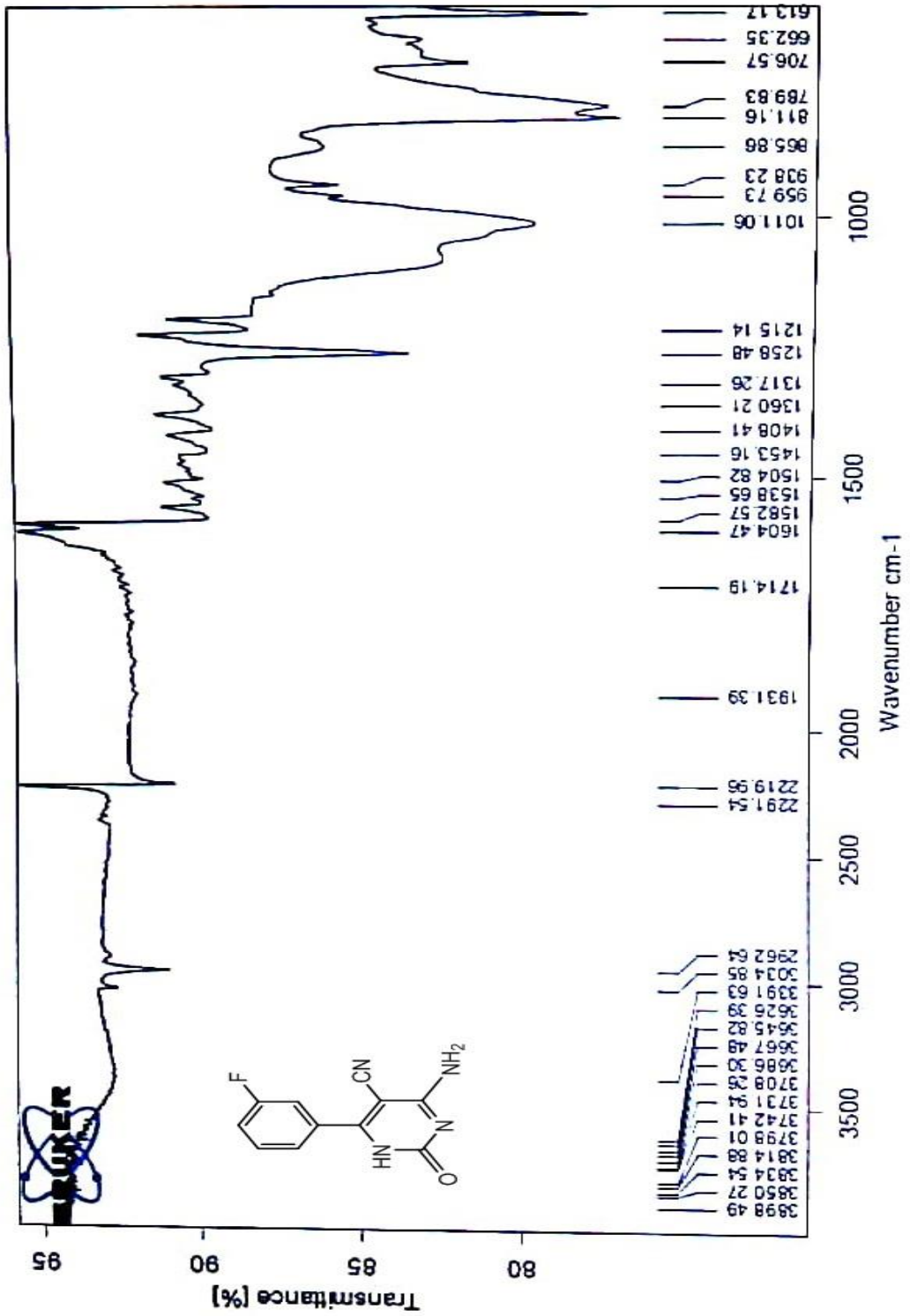


Fig 8.5.13: IR Spectra of Compound '1e'

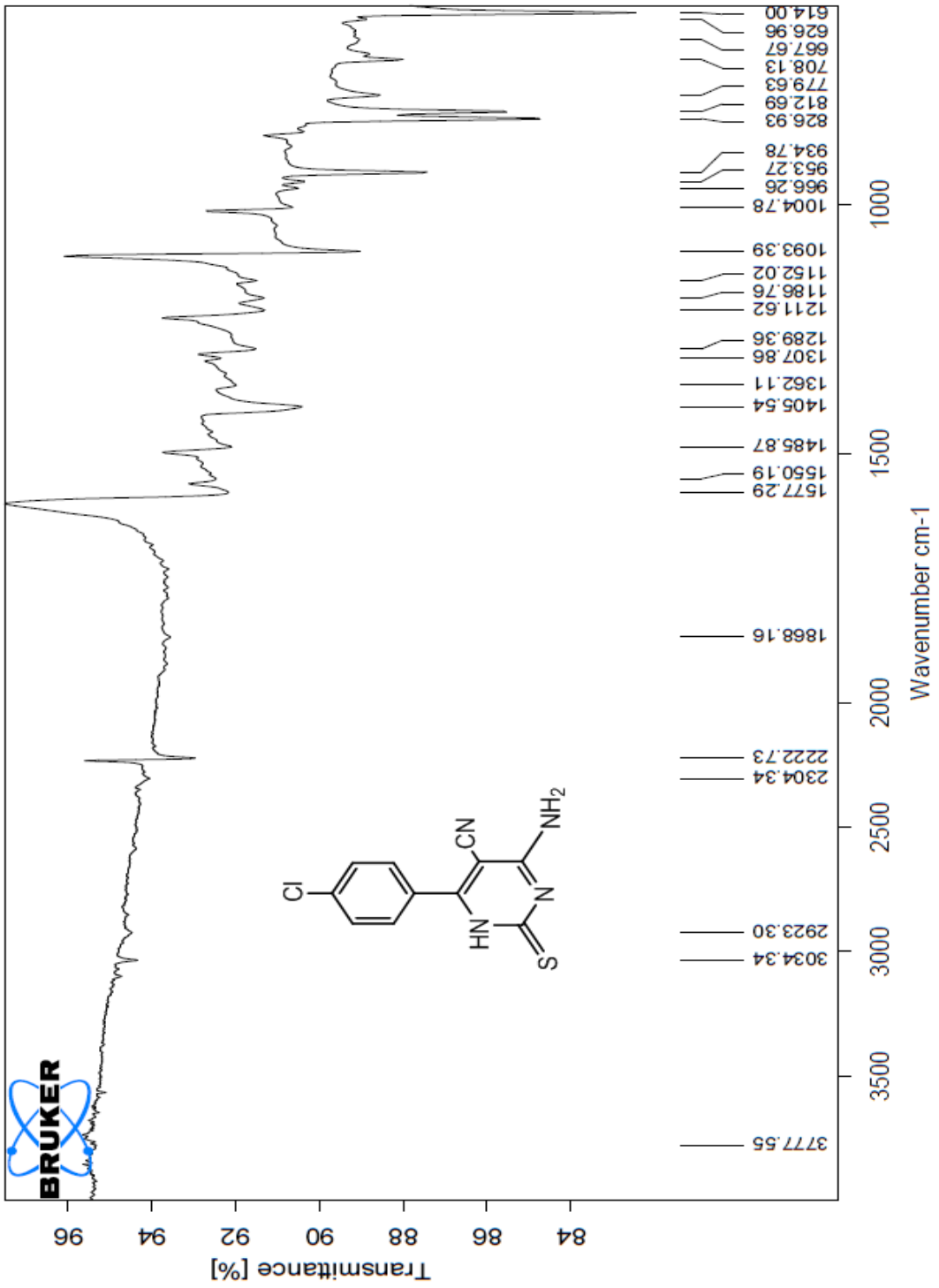


Fig 8.5.14: IR Spectra of Compound ‘2a’

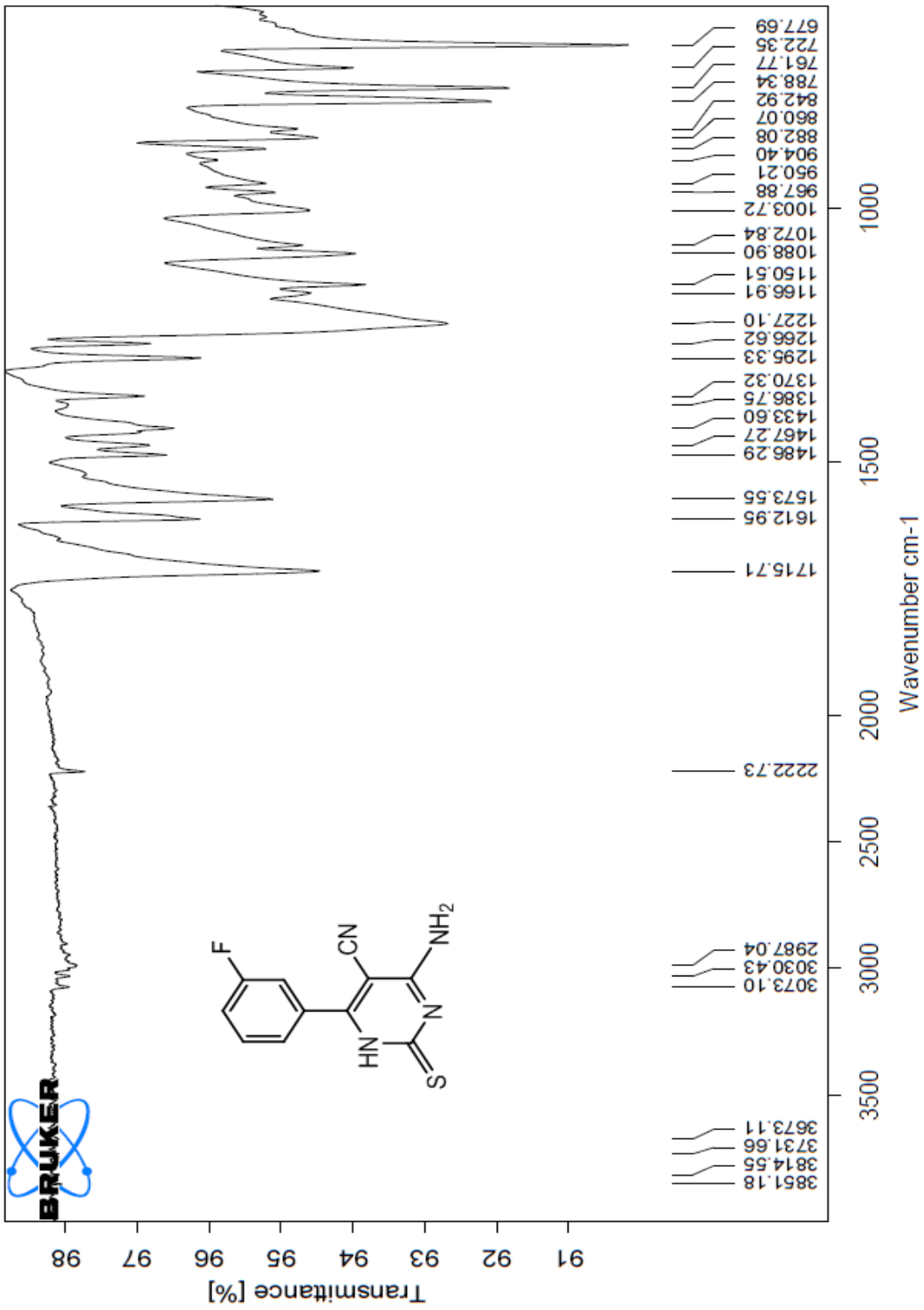


Fig 8.5.15: IR Spectra of Compound ‘2b’

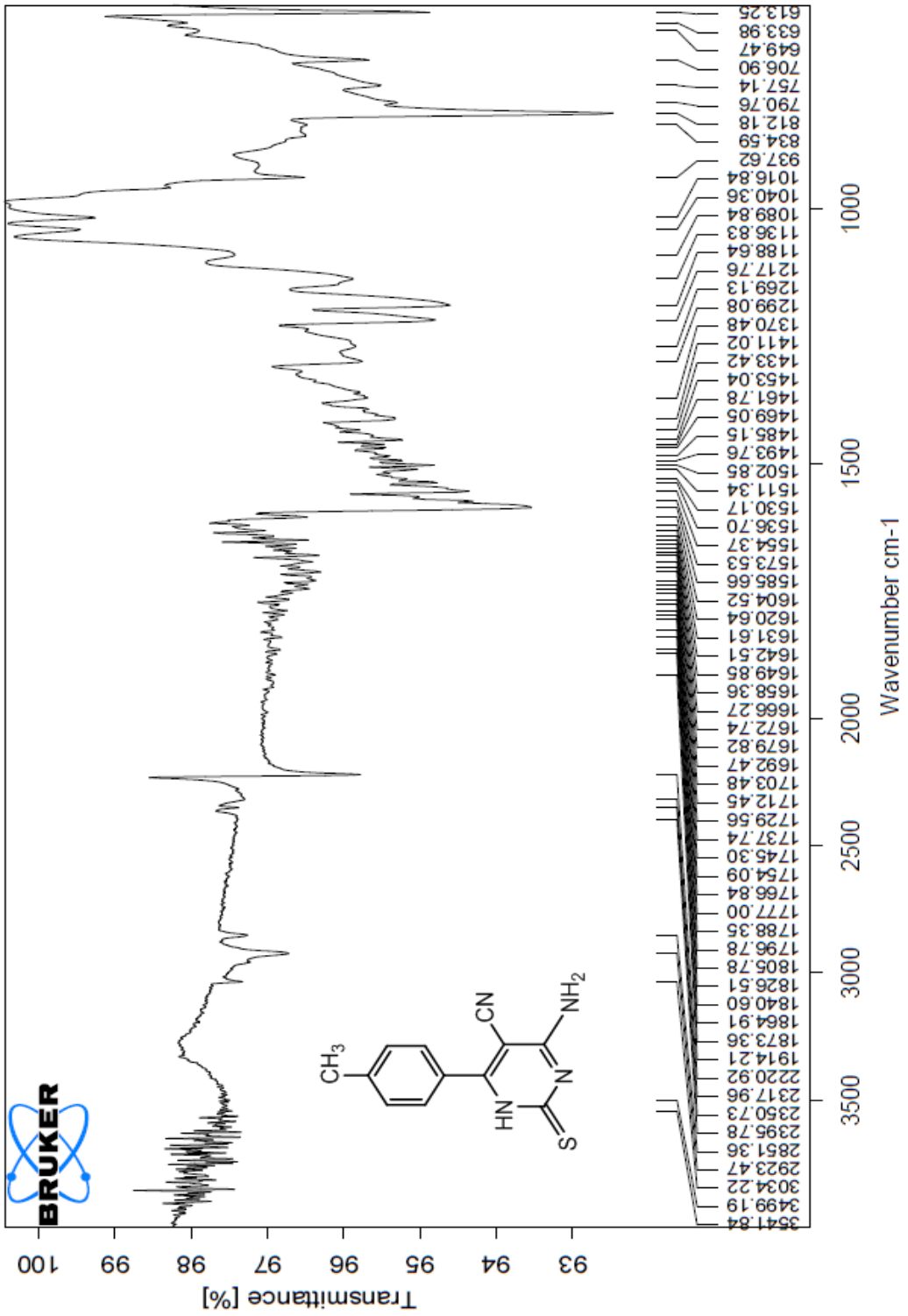


Fig 8.5.16: IR Spectra of Compound '2c'

8.4. Evaluation of Activity & Result:

Compounds	MIC(s) (µg/mL)				Zone of inhibition (cm)
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	
1a	550	550	550	550	1.5
1b	450	450	450	450	0.7
1c	500	450	450	500	1.5
1d	350	400	400	350	1.5
1e	450	450	450	450	0.5
2a	350	300	300	350	1.7
2b	550	500	500	550	0.8
2c	460	450	450	460	1.2

8.5. DISCUSSION:

'1a' and '1c' are the highest yielded compounds among all the 8 synthesized compounds. The time required for the synthesis varied from 10 to 15 hours by conventional methods i.e. by reflux condition. Structures of all the derived compounds 1a-1e and 2a-2c were elucidated on the basis of spectral data obtained from the various analytical methods. The IR and ^{1}H NMR helped to constitute the structures of the derived compounds. All the spectral data showed that the synthesized compounds were in full agreement with the proposed structures though there were some impurities. For example, IR measurements which showed the presence of CN at region 2220-2228 cm^{-1} and two sharp bands at 3030-3034 and 3115-3499 cm^{-1} due to asymmetric and symmetric vibrations of the NH_2 group. In the ^{1}H NMR spectrum, the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts and multiplicities. ^{1}H NMR spectrum of '1e' showed a singlet at 2.46 ppm corresponding to -CH_3 group; a sharp singlet at 7.72 ppm is corresponding to –NH group of pyrimidine ring. The doublet at 7.34 ppm and the doublet at 7.81 ppm are due to four aromatic protons. According to the spectral analysis report, this series of compounds are in full agreement with the proposed structures.

Pharmacological Screening was done for all the 8 compounds. As standard drugs, Amoxicillin and Norfloxacin were taken. Amoxicillin and Ampicillin are narrow spectrum antimicrobial agents and these are often combined with Clavulanate. The combined form is wide spectrum. This is effective against both Gram-positive & Gram-negative bacteria. In the other hand, Norfloxacin is broad spectrum antimicrobial agent, effective against Gram-negative bacteria. Various study report says that Amoxicillin is effective against variety of micro-organisms with MIC ranges 0.06 µg/mL to 4 µg/mL for most of the micro-organisms except *Staphylococcus aureus & Staphylococcus epidermis* which required up to 64 µg/mL to 256 µg/mL respectively. Amoxicillin is also ineffective against *Escherichia coli*. So Norfloxacin was used as reference for this particular micro-organism. Minimum inhibitory concentrations and zone of inhibition of these drugs were evaluated. As it can be seen from the pharmacological screening, a high dose of drugs was required for the activity as this series of compounds showed a mild

activity in minimum concentration against the tested organisms. The standard drugs showed bactericidal activity in 150 µ/mL concentration where the tested compounds showed in between 300-500 µ/mL concentration range. As for the measurement of zone of inhibition the standard drugs Amoxicillin and Norfloxacin showed zone of inhibition which is of 2-4 cm diameter range. And the tested compounds displayed zone of inhibition of 0.5-1.7 cm diameter. '2a' and '1d' showed comparatively largest zone of inhibition and minimum inhibitory concentration among all the 8 tested compounds.

CHAPTER-9

CONCLUSION

9. CONCLUSION:

The clinical applications of anti-microbial drugs bring forth unique perspectives that are evident in their discovery and development. Historical development of these compounds, with significant contributions from serendipity, and the currently shifting focus on target-based drug discovery is evident in the evolving paradigms of preclinical and clinical evaluation of new drug candidates. Current challenges of any potent drug development include the significant time and cost involvement, and the low success rates. These have led to increasing efforts of the pharmaceutical industry towards increasing the effectiveness of the drug discovery and development process and to minimize failure of drug candidates at later stages of development. These efforts include development of high throughput preclinical screening methods and biological assays with greater specificity and predictability.

However, with the portfolio of chemotherapeutics currently available, it has been acknowledged that researchers are getting closer to the end game in terms of parent structure alterations. A call has therefore been made for the development of new classes of drugs that work on different target sites to those in current use. Likewise pyrimidine derivatives will continue to serve the clinical crisis; not only as antimicrobial agents but also as variant *Avatars*. As this moiety has been reported to produce many potent drugs of different class available in the market, I do believe the derived molecules after thorough evaluation & exhaustive structural manipulation are sure to produce many more potent drugs of future to come. Being a researcher, I am looking forward to put more efforts on compounding this particular parent moiety in near future.