“Synthesis and antimicrobial evaluation of novel Sulphonamide derivatives”

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

PHARMACEUTICAL CHEMISTRY

By

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May, 2015

Statement by the candidate

This is to submit that this written submission in my thesis entitled "**Synthesis and antimicrobial evaluation of novel sulphonamide derivatives**" represents original ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

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Assistant Professor

Pharmaceutical chemistry (YD03)

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The work described in this thesis entitled "**Synthesis and antimicrobial evaluation of novel sulphonamide derivatives**" has been carried out by **Rohit Kumar** under my supervision. I certify that this is his bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

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At this moment, I thank with deep gratitude to my Father, Mother, Brother and Sister for their moral support, constant encouragement and patience which has absolutely needed in whatever I did. It was the blessings of them that gave me courage to face the challenges and made my path easier. I requested them to shower their blessing and love on me throughout my life and for my future endeavours. I cannot repay their silent patience and constant encouragement. They stood by me whenever I felt low, pepped me up when my spirit dampened.

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(Rohit Kumar)

DEDICATED TO

MY BELOVED FAMILY

AND GUIDE

ABSTRACT

Hybrid antibiotics is not found in nature as such . It is synthesized by combining the two or more antibiotics. Hybrid antibiotics may have different modes of action combined in a single molecule. Antibiotics linked to one another either by ester, amide, or covalently bonded are called hybrid antibiotics. The possible benefits includes activity against drug –resistant bacteria, broad spectrum of activity and have potential for reducing the bacterial resistance. The formation of hybrid antibiotics have synergistic effect to reduce the problem of bacterial resistance. The advantage of using molecular hybridization is to activate different targets by a single molecule, thereby increasing therapeutic efficacy as well as to improve the bioavailability profile. The main aim of developing the hybrid molecules is to increases the potency or synergistic effect as compared to antibiotics given alone.

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LIST OFABBREVIATIONS

Abbreviations	Full form
%	Percentage
^{o}C	Degree Celsius
M.Pt.	Melting point
TLC	Thin layer chromatography
6-AMP	6-Aminopenicillinic acid
CPX	Ciprofloxacin
DMSO	Dimethylsulphoxide
PABA	Para-aminobenzoic acid
MIC	Minimum inhibitory concentration
^{1}H-NMR	Nuclear Magnetic resonance
IR	Infra red
UV	Ultraviolet
H	Hour
Eq	Equivalent
EC_{50}	Effective concentration
Nm	Nanometer
Fig.	Figure
Rf	Retention factor
mm	Millimeter

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CHAPTER-1

INTRODUCTION

1. INTRODUCTION:

The antibacterial activities of sulphonamide were discovered in middle of 1930. In 1932, the discovery of prontosil rubrum, a red azo dye by Gerhard Domagk, Fritz Mietzsch and Joseph klarer examined the activity of prontosil which showed antibacterial activity in case of *in vivo* against streptococcal infections in mice , though it was inactive *in vitro* . Prontosil was found to be effective against severe streptococcal infections. Thus, prontosil acts as a prodrug in this category as shown in fig.1. Trefouel et al. (1935) Pasteur institute conclude that the azo linkage in prontosil is metabolically broken and the active moiety of sulphanilamide is generated. In France, Trefouel and Bovet observed that the urine of prontosil rubrum-treated animals was bioactive *in vitro*. Sulphanilamide is first synthetic antibacterial agent having wide range of activity against gram positive bacteria, especially pneumococci and meningococci and streptococci etc. Sulphonamides shows their weak activity against bacteria responsible for typhoid fever, diphtheria and bacterial endocarditis. Sulphonamides also active against malarial parasites, Eimeria, Toxoplasma and Nocardia species, when given in combination with pyrimethamine are widely used for coccidiosis, Toxoplasma and Nocardiosis. Sulphonamide in combination with trimethoprim are largely used against pneumoystis carinii pneumonia.[1-5] Many of sulfa drugs were prepared in the mid 1930. Among them, sulfapyridine was utilized against pneumonia (amid World War II); sulfathiazole was utilized against both pneumonia and staphylococcal contaminations; sulfadiazine was utilized against pneumococcal, streptococcal, and staphylococcal microbes; and sulfaguanadine against looseness of the bowels.[6]

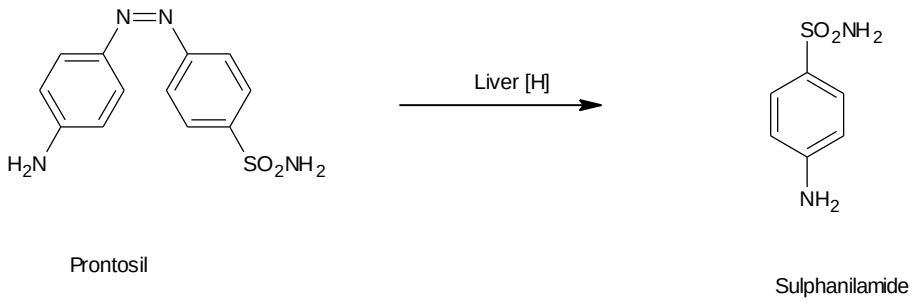


Fig.1 Breakdown of Prontosil

The development of antimicrobial drugs possess an broad spectrum of activity against micro-organisms has a major challenge in medicinal chemistry. The resistance of a microorganism may be natural or acquired. If microorganism resistant to one antibiotics occurs, it may also become resistant to another antibiotics and is known as cross resistance. Hybrid antibiotics is a class of antibiotics discovered for overcoming the resistance problems of microorganism by acting through different mode of action. The improvement of numerous antimicrobial specialists, as a result of abuse and overuse of antibiotics, pathogenic microorganism have created resistance connected with an increment in mortality towards number of antibiotics which have been utilizing clinically as medications, for example, b-lactam antibiotics, macrolides, quinolones and so forth. [7] Hybrid antibiotics shows distinctive methods of activity joined in a solitary particle by and large anti-microbial particles. Atoms connected to each other either by ester, amide or covalently fortified. The conceivable advantages incorporates movement against medication – resistant microbes, wide range of action and lessened potential for creating bacterial resistance. The production of hybrid antibiotics having synergistic impact to decrease the issue of antibacterial resistance. The upside of utilizing atomic hybridization is to actuate diverse focuses by a solitary particle, in this way expanding remedial adequacy and also to enhance the bioavailability profile. For this purpose, we have to focused on the different and efficient synthesis and extrapolation of antimicrobial activity of hybrid molecules containing sulphanilamide and its derivatives.

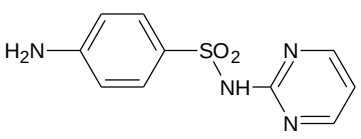
1.1 Classification:

Sulphonamides are classified into various ways based on their therapeutic applications. Generally, the classification of sulphonamides based on chemical structure, pharmacokinetic properties and spectrum of activity etc. Sulphonamides are classified as:

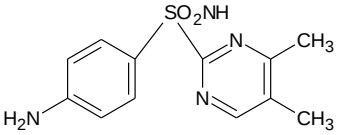
1. Short acting sulphonamides
2. Moderate or intermediate acting sulphonamides
3. Long acting sulphonamides

(I) Short acting sulphonamides:

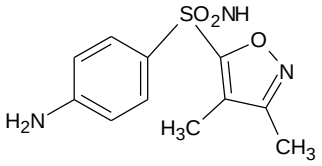
These agents are used in the treatment of urinary tract infections since they are excreted through urine in high concentrations, having less plasma half life (<10 hrs). For example Sulphapyridine, Suphadiazine, sulphafurazole etc. The problem of crystalluria (due to low solubility) can be reduced by three short acting sulphonamides (triple sulphonamides).



Sulphadiazine



Sulphadimidine



Sulphafurazole

Fig.2 Examples of Short acting Sulphonamides

(II) Moderate and intermediate acing suphonamides:

Sulphamethoxazole is the main representative of this group. These agents are used in treatment of infection requiring prolonged infections, having plasma half life between 10-24 hrs. The combination of sulphamethoxazole and trimethoprim tends to increase the antimicrobial activity. Sulphadoxine in combination with pyrimethamine to treat or prevent malaria . Sulphadoxine is also used in combination with other drugs to treat or prevent various infections in liverstock. Examples in this category are sulphamethoxazole, sulphadoxine, sulphacetamide etc.

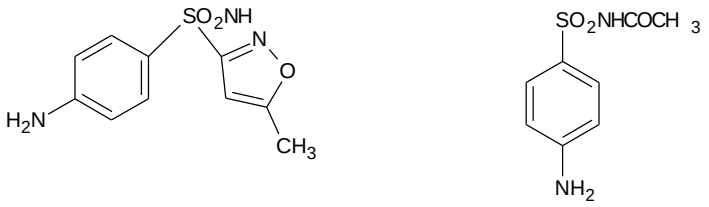


Fig.3 Examples of Moderate or Intermediate acting Sulphonamides

(III) Long acting sulphonamides:

These agents are rapidly absorbed and slowly excreted, due to this the problem of crystalluria don't occur, having plama half life greater than the previous category (24 hrs). They have greater ability to cause hyper sensitivity reactions. Examples are sulphasalazine, sulphametopyrazine, , sulphadiazine, sulphamethizole etc. Sulphasalazine, an azo dye compound is used in the treatment of inflammatory bowel diseases and rheumatoid diseases, Sulphametopyrazine is also used in treatment of rheumatoid diseases, Sulphadiazine is utilized as a part of treatment of urinary tract contaminations and Sulphamethizole is utilized for bacterial diseases.[8][9][1]

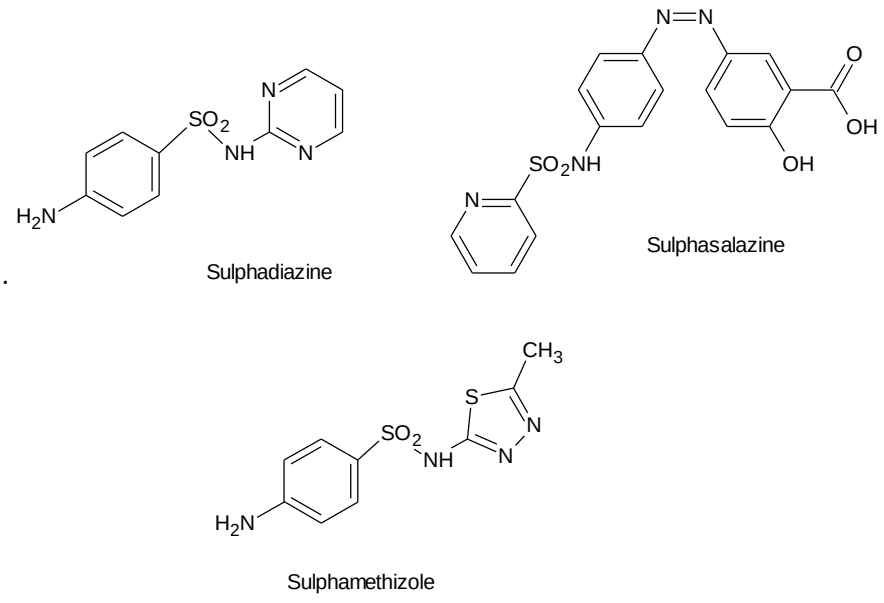


Fig.4 Examples of Long acting Sulphonamides

1.2 Mechanism of action:

D.D.Woods (1940) and P.Flides watched that the structure of sulphonamides has comparative with p-aminobenzoic acid and presume that the sulphonamides are focused inhibitors of p-aminobenzoic acid and piece the folic acid combination of helpless bacteria.[1] Sulphonamides rival p-aminobenzoic acid (PABA) for the compound dihydropteroatesynthetase, which is critical in the arrangement of folic acid. Folic acid is vital for the blend of DNA and RNA both in microbes and in vertebrates. Folic acid isn't incorporated by warm blooded animals, they are taken from outer eating regimen however microbes need to incorporate it. Sulphonamides repress the development of microorganisms however don't slaughter them i.e. their activity is bacteriostatic.[10] The sulphonamide atom binds with the dihydropteroate synthase protein and keeps the p-aminobenzoic acid binding. Subsequently folic acid is not synthesized which is key for the cell development, Thus, the cell will quit separating and spreading.[3] as shown in fig.5

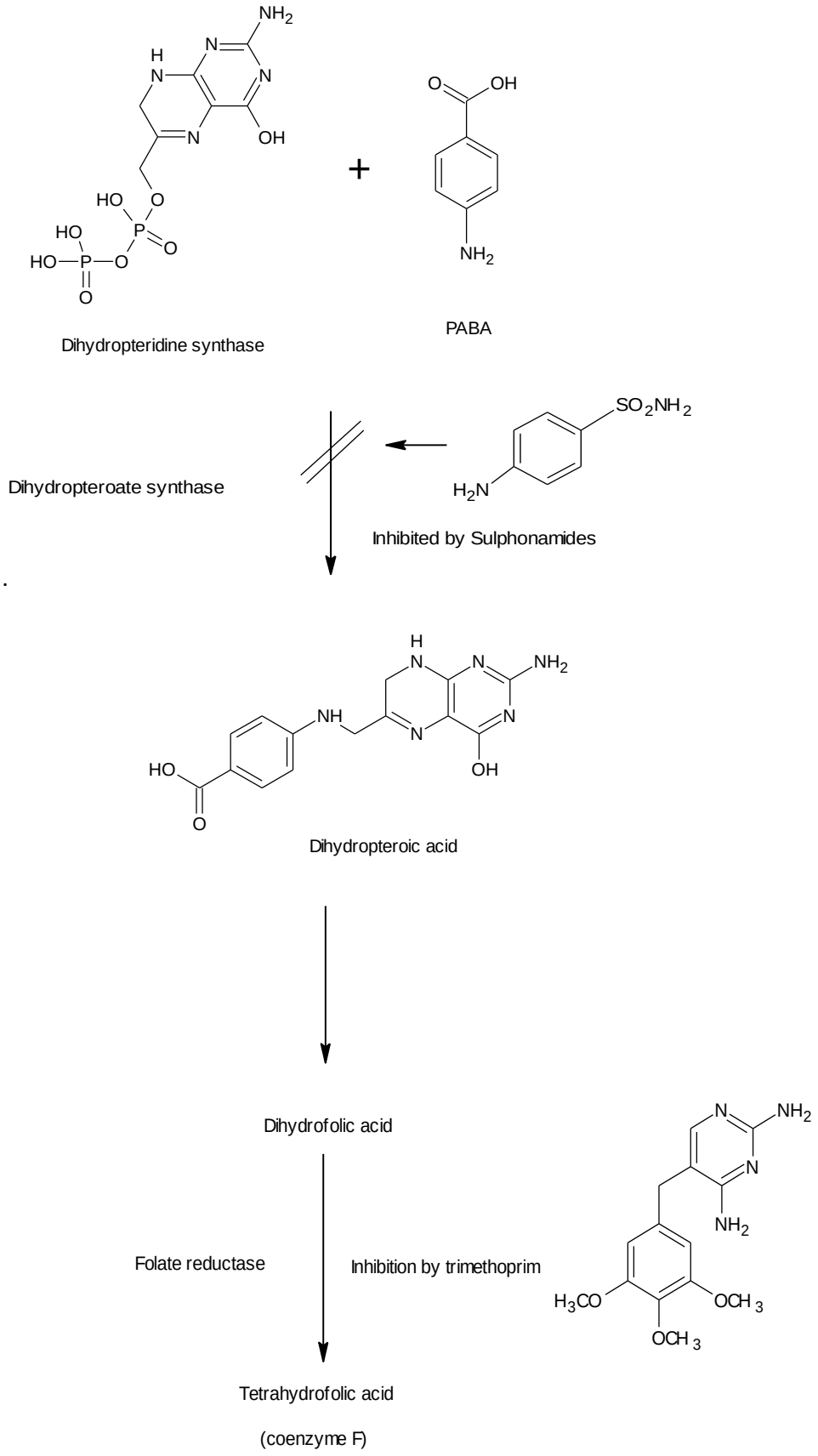


Fig.5 Site of action of Sulphonamides and Trimethoprim

CHAPTER -2

REVIEW OF LITERATURE

2. Literature survey:

According to literature survey studies : **Wang X.D. et al.,*2014*** 3-arylfuran-2(5H)- one was hybridized with fluoroquinolones have a multi-focused on antimicrobial specialists. A percentage of the crossovers showed benefits from both folks demonstrates a wide range of movement against safe strains including both Gram-negative and Gram positive microorganisms.

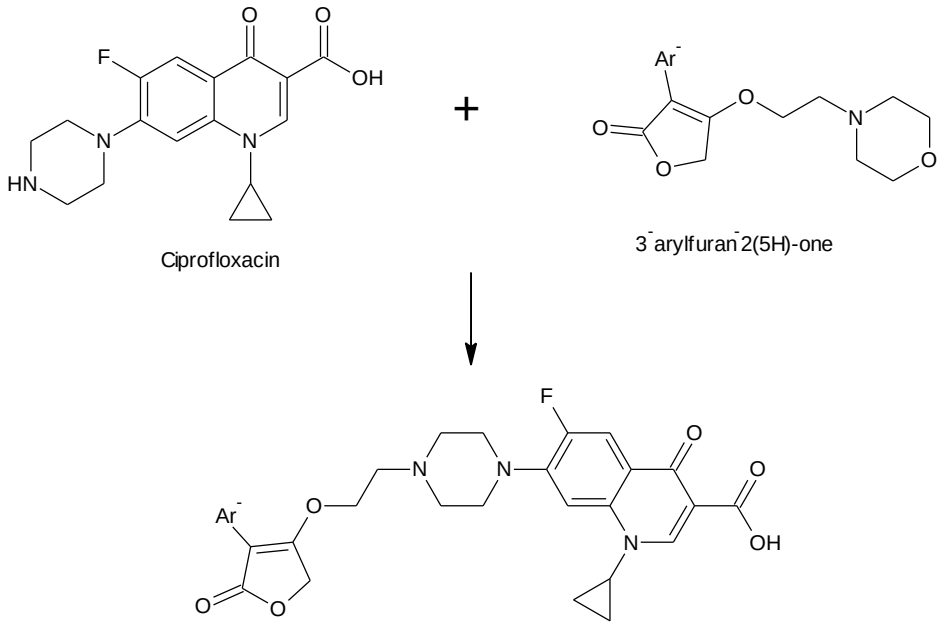


Fig.6 Rational design of compound

The most potent compound in antibacterial assay shows MIC_{50} (minimum inhibitory concentration) multiple drug resistant E.coli, being about 51-fold more potent than ciprofloxacin. The Combination of 3-arylfuran-2(5H)-one with fluoroquinolone will produce potent antibacterial agents by inhibiting multi-targets (DNA gyrase and TyrRS).[11] as shown in fig.6

Desai N.C. et al.,*2014* The formation of hybrid molecules pyrimidine based imidazole moieties as shown in fig.7. Several pyrimidine derivatives have been found wide range of applications such as antitumor, antihypertensive, antioxidant and adrenocepor-selective antagonism**.** On the other hand, Imidazole has wide range of therapeutic activity such as anticancer Carboxypeptidase inhibitors, antiviral, antitubercular and antimicrobial etc.

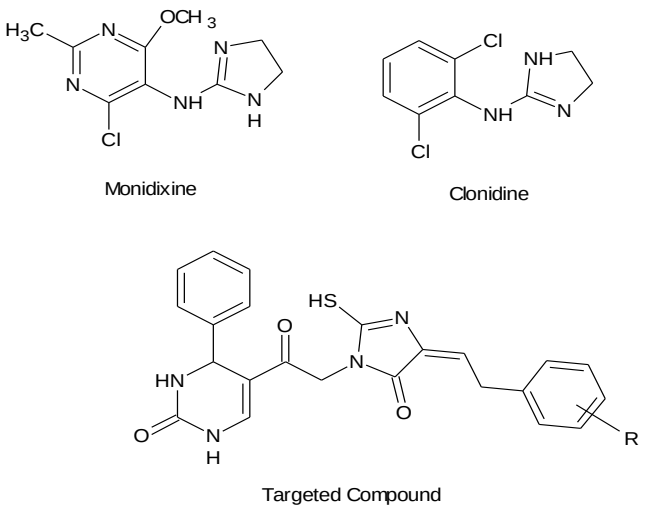


Fig.7 Hybrid molecules containing Pyrimidine based Imidazole derivatives

The targeted compound showed potent antibacterial activity (Excellent activity against E.coli and Staphylococcus aureus) as compared to antifungal activity (Good activity against Aspergillus niger). In SAR studies observed that the electron withdrawing fluoro and dichloro groups in imidazolinone ring system increases the antibacterial and antifungal activity.[12] as shown in fig.7

Desai, N.C. et al.,*2014* were synthesized a series of benzimidazole containing 2-pyridones (5a-k) and evaluation of their antimicrobial activity was carried out by broth dilution method using standard drugs chlorampenicol and ketoconazole. Intermediates showed poor antimicrobial activity as compared to the final compound (5a-k). The compound 5a-k showed better antibacterial activity than antifungal activity as shown in fig.8.

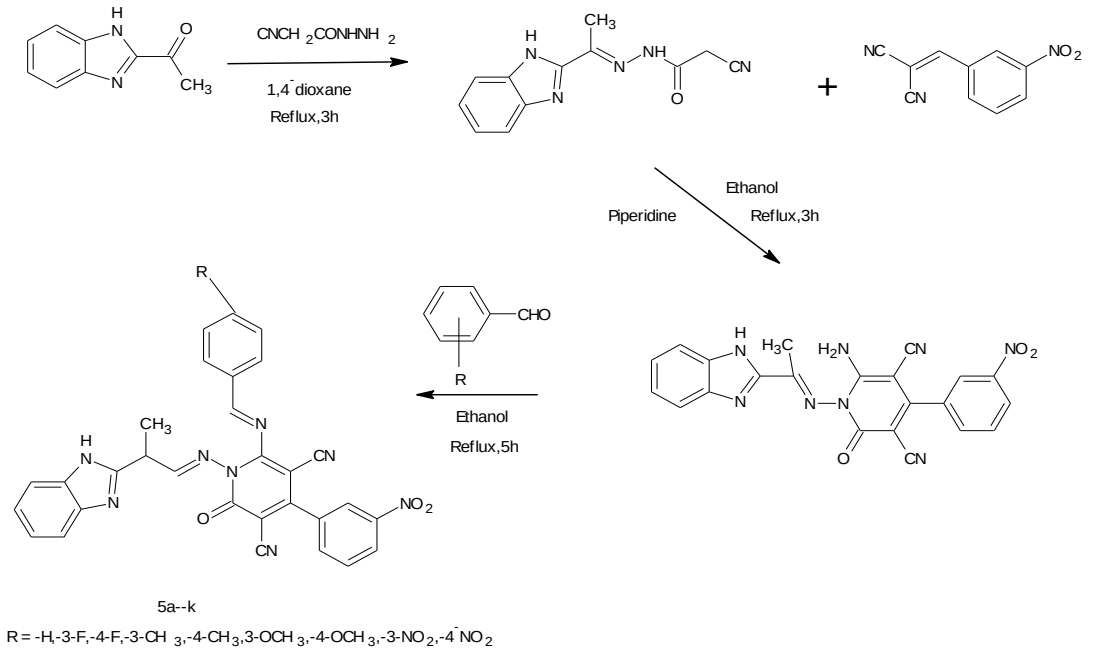


Fig.8 Synthesis of benzimidazole containing 2-pyridones derivatives

Ding et al.,*2013* Vilazodone (trade name Viibryd), is an antidepressant. Vilazodone was approved by the FDA for utilization in the United States to treat real depressive issue in 2011. Vilazodone selectively inhibits the reuptake of serotonin and a 5-HT_{1A} receptor having partial agonist activity. Vilazodone is prepared by responding l-[4- {5-cyanoindol-3-yl)butyl]-4- {2-carboxybenzofuran-5-yl)-piprazine with 2-chloro- 1 -methylpyridinium methanesulfonate in N-methylpyrrolidine and afterward treated with alkali. Due to the presence of free base vilazodone is converted into vilazodone hydrochloride as shown in fig. 9. [13]

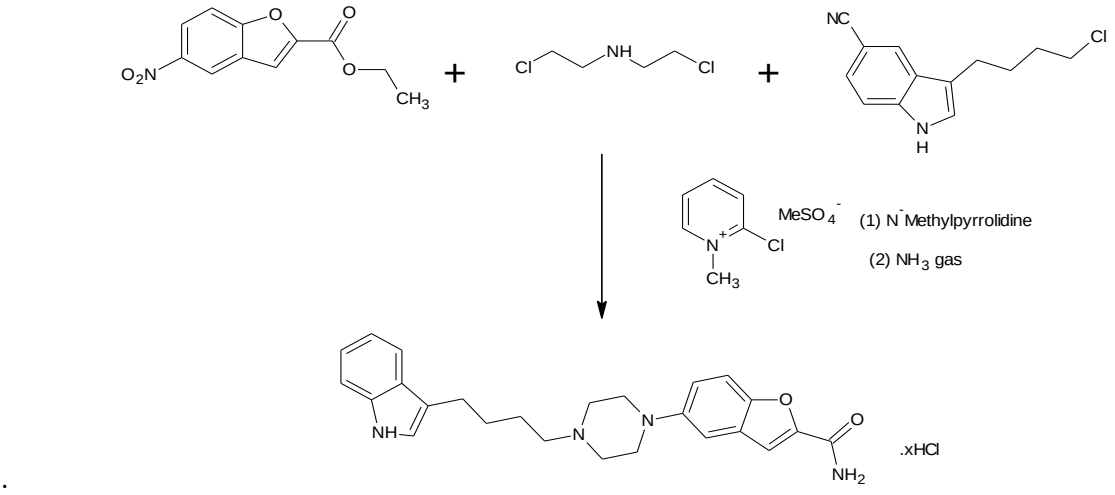


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Fig.9 Chemical structure of vilazodone HCl

Plech T. et al.,*2013* The mixture anti-toxins containing 1,2,4-triazole and Ciprofloxacin indicated higher antibacterial action when contrasted with Ciprofloxacin alone, both compelling against gram positive (S. aureus, Bacillus subtilis, Bacillus cereus) and gram negative microorganisms (E. coli, Proteus mirabilis, Pseudomonas aeruginosa). Ciprofloxacin-triazole crossovers have double method of activity of Ciprofloxacin and additionally triazoles.

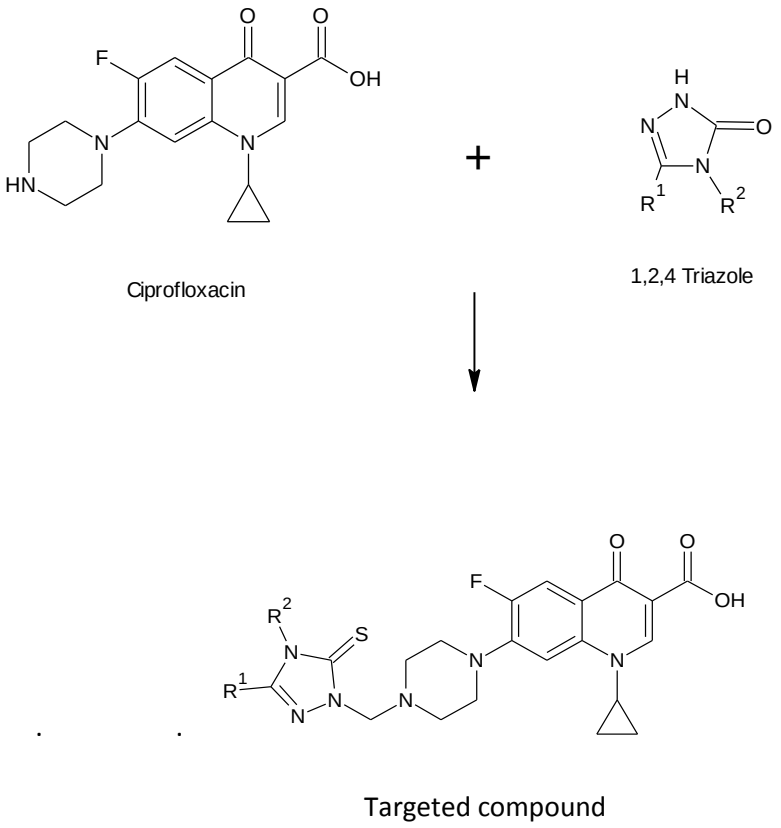


Fig.10 Hybrid antibiotics containing 1,2,4-Ttiazole-Ciprofloxacin moiety

The Ciprofloxacin-1,2,4-triazole hybrid shows antibacterial activity more than of ciprofloxacin alone (reference) against M. luteus and B. subtilis etc.[14] as shown in fig.10

Butler M.M. et al.,*2007*. Anilinouracil-Fluoroquinolone half breeds contains double component of activity and more dynamic against anti-microbial activity and safe gram-positive pathogens. The 251D compound has 5- to 15-ceases more powerful then the guardian Anilinouracil compound against gram positive microscopic organisms. Anilinouracil mixes doesn't restrains the development of gram negative microorganisms however the vicinity of fluoroquinolone

moiety in Anilinouracil –fluoroquinolone half and halves have some hostile to gram negative activity[15] as demonstrated in fig.11.

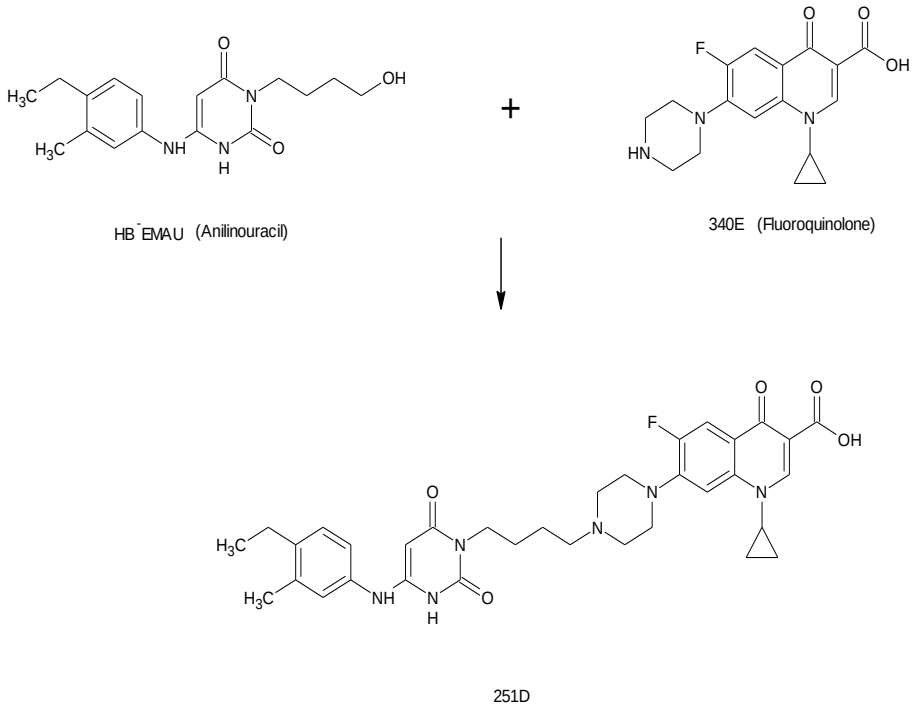


Fig.11 Structures of the hybrid compound, 251D, its Anilinouracil component

Chatterjee N.R et al.,*2007* Ciprofloxacin with β- lactam ring provides an stability to β-lactam moiety in the presence of heavy metal ion species . This is because β-lactam hybrids containing ciprofloxacin moiety having greater resistance towards degradation as compared to norfloxacin. Double method of activity of crossovers containing fluoroquinolones and β- lactam anti-toxins where the β- lactam anti-microbials goes about as bearer for quinolone anti-toxin. The penicillins and Cephalosporins are linked with fluoroquionolones through an ester , amide and carbamate bond at position 3' and 4'.[16] as shown in fig.12

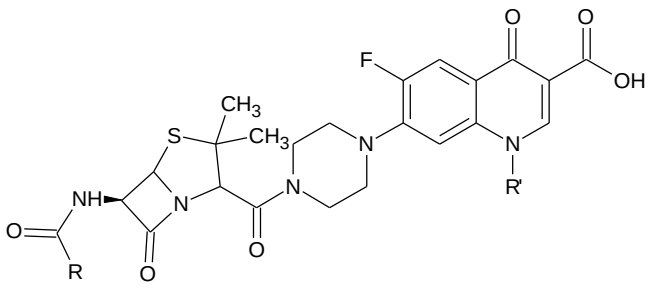


Fig.12 Fluoroquinolonyl-3′-penicillin amides (1a-e)

Foroumadi et al.,*2005*. Cipprofloxacin (CPX), norfloxacin, pefloxacine, ofloxacin, and enoxacin are consisting piperazine moiety at position 7 which is essential for antibacterial activity. The compound containing 1,2,4-Triazole cephalosporanic- and penicillanic corrosive moieties including likewise 4-fluorophenylpiperazine core shows antimicrobial action against gram positive microbes (Bacillus cereus, Staphylococcus aureus) as shown in fig.13.[17]

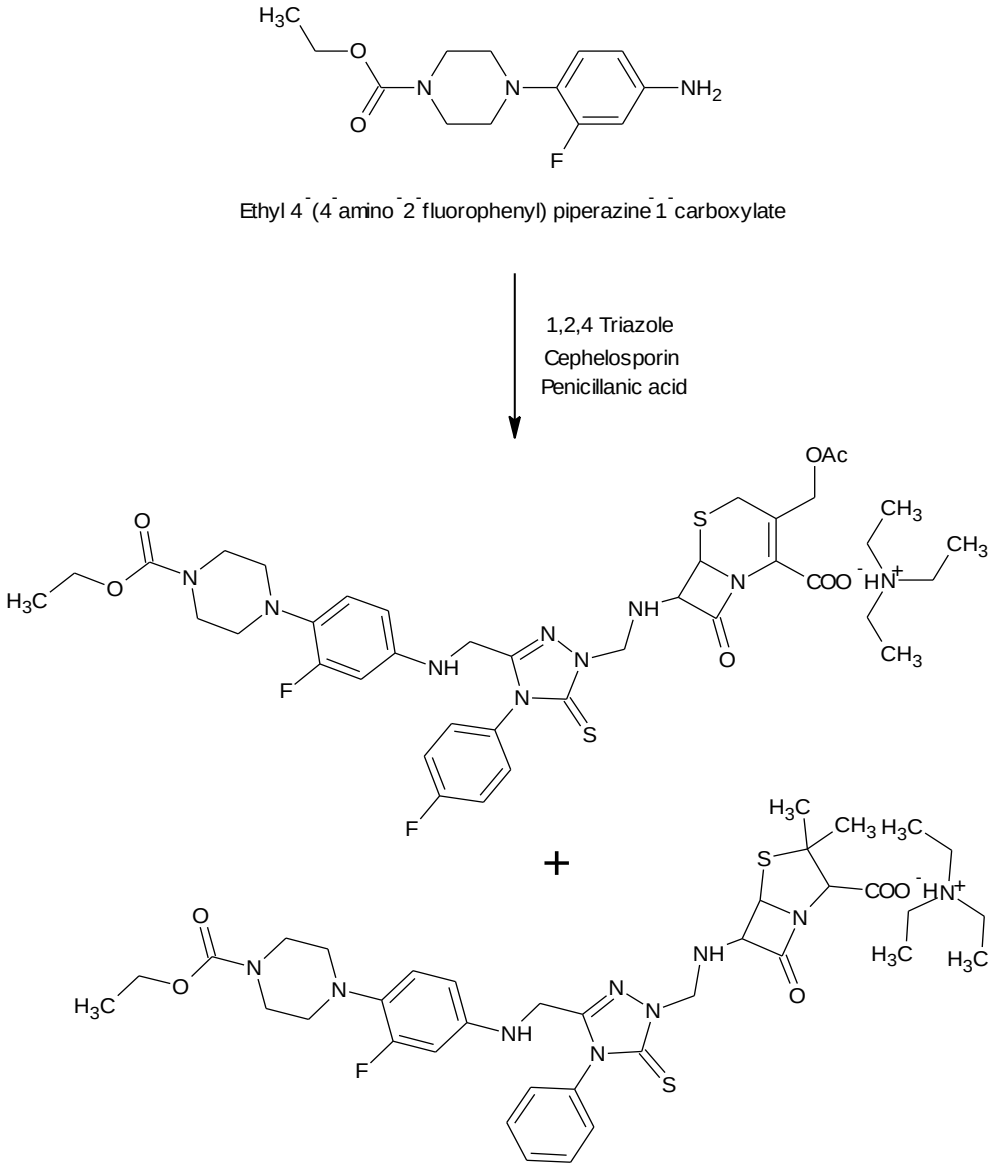


Fig.13 Synthesis of hybrid molecules containing penicillanic acid or cephalosporanic acid moieties.

Georgopapadakou N.H. et al.,*1994* The hydrolysis of the cephalosporin-quinolone chemically or enymatically causes release of Cephalosporin 3'-quinolones. The hydrolysis of cephalosporin 3'-quinolones is for the most part conveyed by gram-negative, β-lactamase. i.e plasmid mediated

(gram negative bacteria), cefotaxime-hydrolyzing enzyme chromosomally encoded, which is responsible for resistance in Enterobactercloacae and Pseudomonas aeruginosa.[18] as shown in fig.14

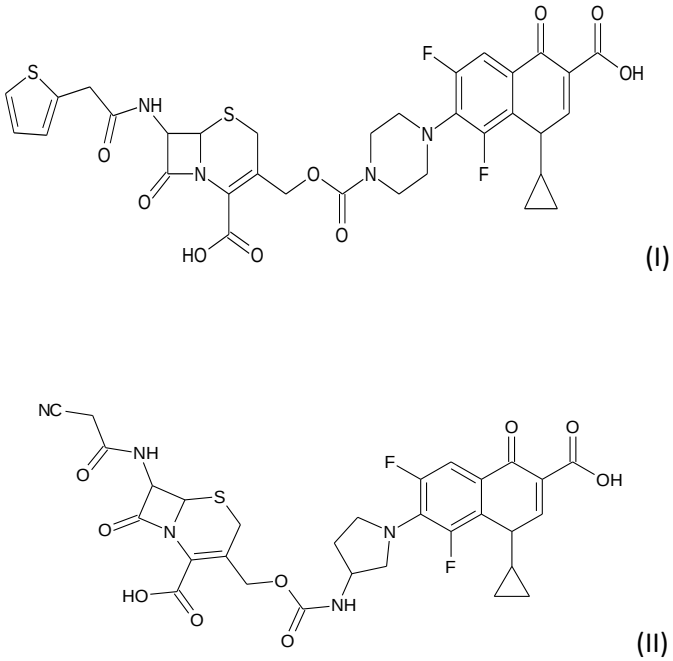
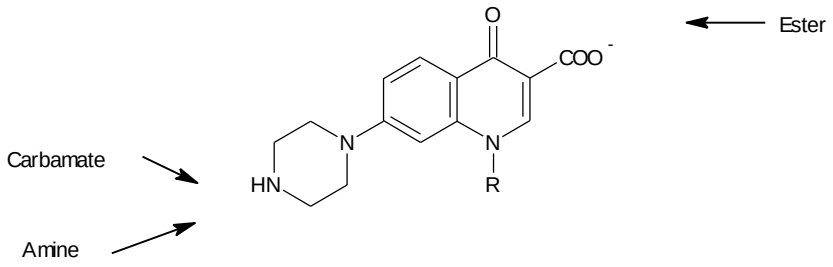


Fig.14 Structures of dual action cephalosporin used in the study

Georgopapadakou et al.,*1989;* Georgopapadakou & Bertasso,***1993.***The Ciprofloxacin (CPX) and fluoroquinones joined through an ester linkage produces dual mode of action, acts as CPX and fluoroquinones as a prodrugs. The action of Quinolones is for the most part conveyed by three instruments are : in the vicinity of a dynamic β-lectamase ;when hydrolysis happens and in conclusion, when the penicillin tying proteins(PBPs) are acylated through method of activity of CPX.[19] as shown in fig.15



. Fig.15 Possible point of attachment of Quinolones to form ester, carbamate and amine bonds

Greenwood D. et al.,*1976* The enhancement of the antibacterial activity of cephalosporin is to combine with a β-lactamase inhibitor resulting broad spectrum of activity and also very effective *in vitro* . The connection of this compound with β-lactamase loses a omadine moeity at the 3-position of the cephalosporin particle, which itself has antibacterial action however not in the combination.[20] as shown in fig.16

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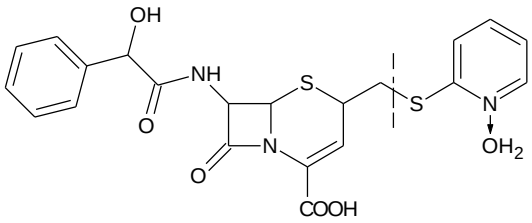


α-hydroxyphenylacetamido cephalosporanic acid

2-mercaptopyridine-N-oxide (Omadine)

Fig.16 Structure of Cephalosporin (6R,7R)-7-[(2R)-2-hydroxy-2-phenylacetamido]-3-(pyrid-2-yl-N-oxide) thiomethylceph-3-em-4-carboxylic acid (MCO)

CHAPTER 3

RATIONALE

3.1 Rationale

Most of antimicrobial agents has resistance problems towards micro-organisms. Therefore, there is an increasing need of hybrid antibiotics consisting of different mode of action. To overcome microbial resistance we have planned to synthesize the hybrid molecules containing Sulphanillic acid as a lead molecule and the attachment of different antibiotic nucleus such as 6-Aminopenicillin and fluoroquinolones. This type of hybridization work on sulphonamide derivatives is not done yet according best of our knowledge.

Inspired by above facts we have decide to synthesize Sulphonamides derivatives and to evaluate their antimicrobial activities.

CHAPTER 3

AIM AND OBJECTIVES

3.2 AIM & OBJECTIVES

AIM: Synthesis and antimicrobial evaluation of novel Sulphonamide derivatives.

OBJECTIVES :

Synthesis of new Sulphonamide derivatives by hybridizing Sulphanillic acid with other antibiotic nucleus such as 6- Amino penicillin and Ciprofloxacin.

- Physicochemical and spectral characterization (IR and NMR) of synthesized derivatives.
- Antimicrobial evaluation

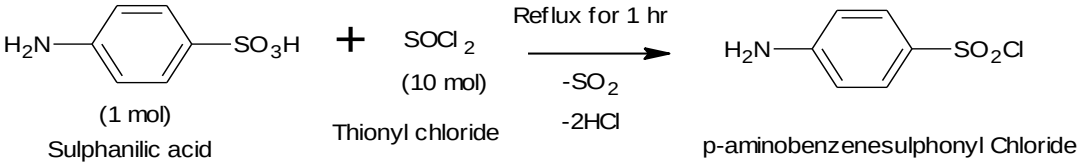
CHAPTER 4

PLAN OF WORK

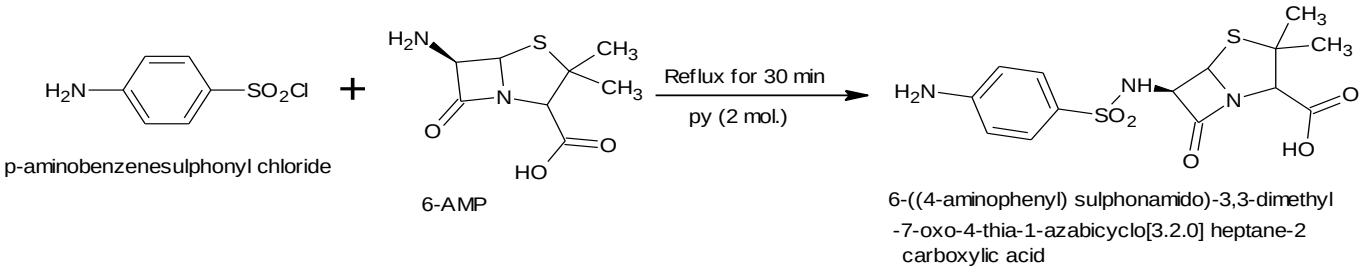
4.1 SCHEME I

Synthesis of 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid from Sulphanillic acid.

Step I



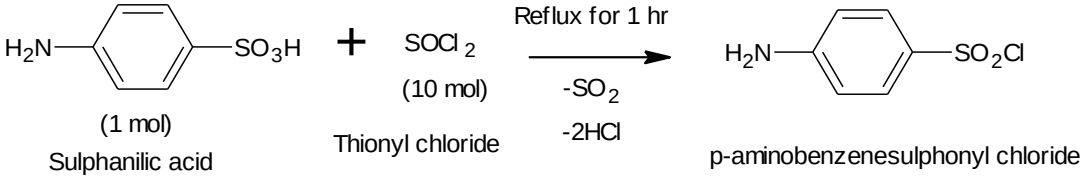
Step II



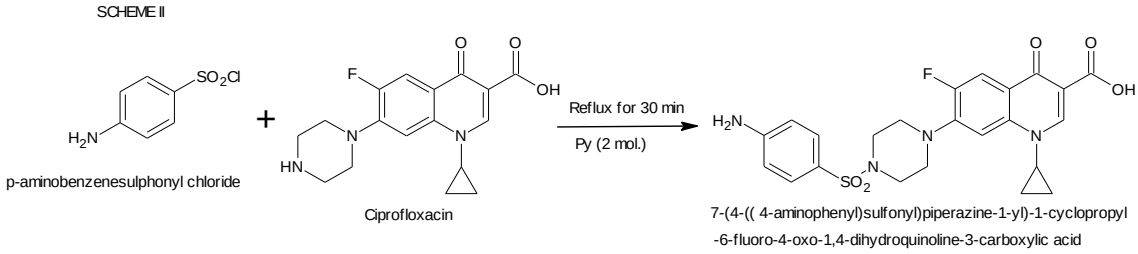
4.2 SCHEME II

Synthesis of 7-(4-((4-aminophenyl)sulphonyl)piperazine-1-yl)-1-cyclopropyl-6-flouro- 4-oxo-1-4-dihydroquinoline-3-carboxylic acid from Sulphanillic acid.

Step I



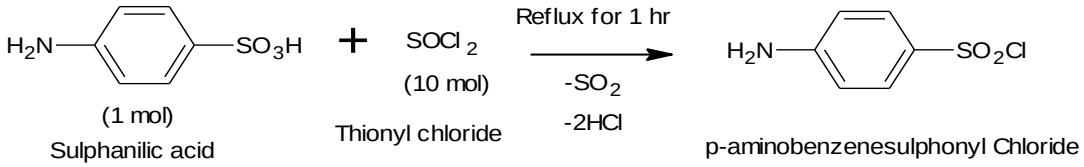
Step II



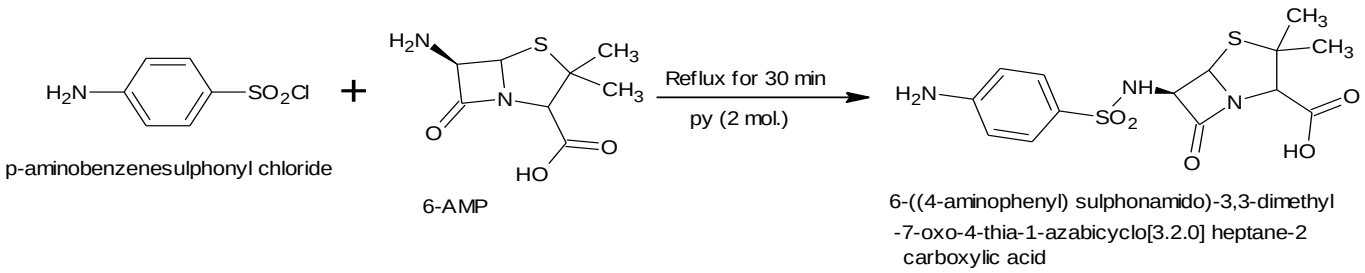
4.3 SCHEME III

Synthesis of 7-(4-(6-((4-aminophenyl)sulfamido)-3,3-dimetnyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptanes – 2- carbonyl) piperazin - yl) -1 – cyclopropyl – 6 - fluoro-4-oxo-1,4- dihydroquinoline-3-carboxylic acid from Sulphanillic acid.

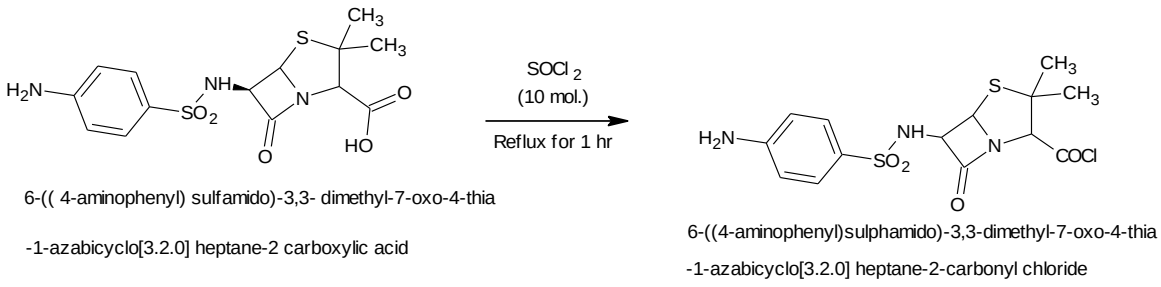
Step I



Step II

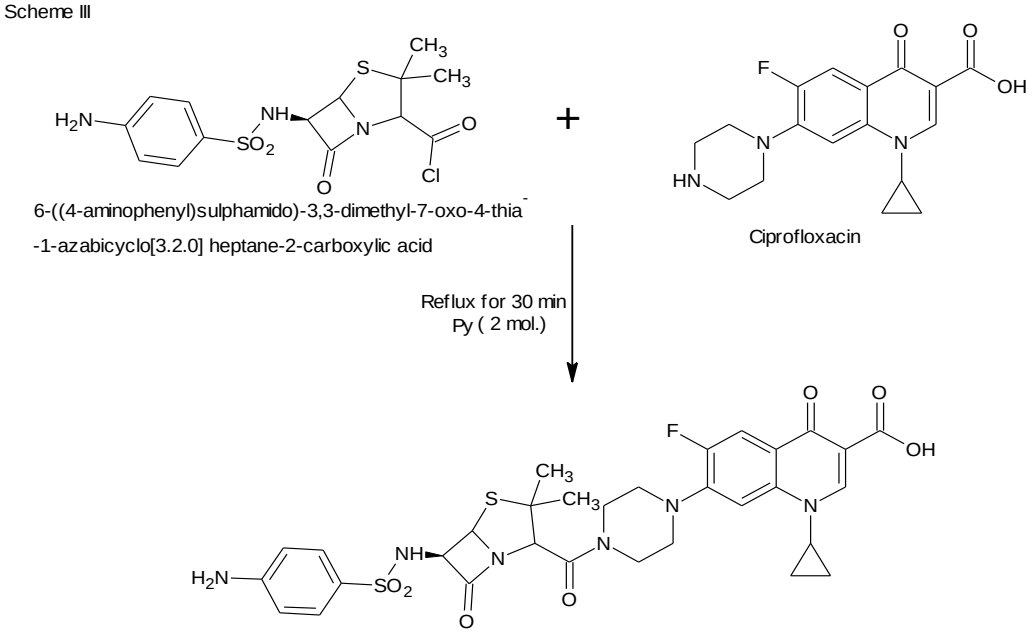


Step III



Step IV

Scheme III



CHAPTER 5

EXPERIMENTAL WORK

Table 1

5.1 LIST OF CHEMICALS

CHEMICALS	SUPPLIER
Sulphanillic acid	Lobachemie Pvt. Ltd., Mumbai, India
6-aminopenicillin	HiMedia Laboratories Pvt. Ltd.,Mumbai,India
Ciprofloxacin	LDH Pvt. Ltd., India
Benzoyl chloride	S d fine- Chem Ltd. Mumbai, India
Dichloromethane	LDH Pvt. Ltd., India
Methanol	S d fine- Chem Ltd. Mumbai, India
Silica gel G	Lobachemie Pvt. Ltd., Mumbai, India

Table 2

5.2 LIST OF INSTRUMENTS

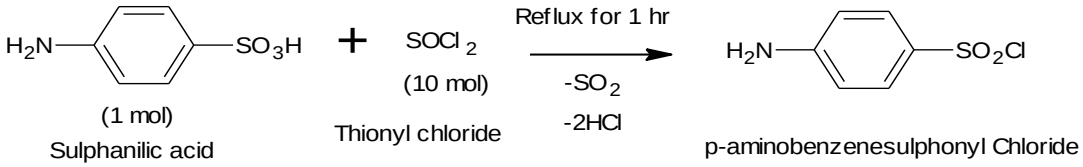
Instruments	Manufacturer
Digital weighting balance	Shimadzu
FT-IR	Shimadzu 8400co.Ltd.,Singapore
^{1}H NMR	Bruker advance II 400 NMR spectrometer
Heating mantle	Popular traders ambala cantt. India
Hot air oven	Almicro Pvt. Ltd.
Melting point apparatus	Popular traders

5.3 EXPERIMENTAL WORK

5.3.1 SCHEME1

STEP I **Synthesis of p- aminobenzene sulphonyl chloride from sulphanillic acid.**

Procedure Sulphanilic acid (1 mole) and thionyl chloride (10 mole) were taken in a 250ml round bottom flask fitted with a reflux condenser and a drying tube (NOTE expansion of thionyl chloride dropwise in the response mixture and HCl gas developed). The response mixture was blended and warmed at 100°C on heating mentle or megantic stirrer bar for 1 hr.

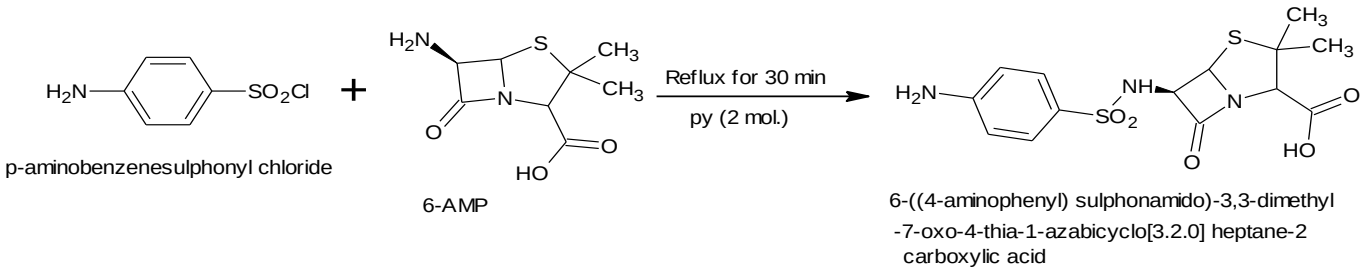


OBSERVATIONS

- Solubility : Methanol
- Melting point : 160-162°C
- TLC : Mobile phase (chloroform: n-heptane: ethanol /1:1:1 v/v)
- Rf value of product :0.24

STEP II **Synthesis of 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid from p- aminobenzene sulphonyl chloride.**

Procedure A mixure of 6-aminopenicilin (1 mole), pyridine (2 mole) were refluxed and p-aminobenzenesulphonyl chloride (1 mole) was added dropwise from the top of the condenser. Refluxed for 30 min. Response mixture was emptified into 6 ml of icy water & stirred untill the item solidified. Solid was filtered off and recrystallized from ethanol.



OBSERVATIONS

- Solubility : Mehtanol
- Melting point : 198-200°C
- TLC : Mobile phase (chloroform: n-heptane: ethanol /3:3:3)
- Rf value of product :0.28

5.3.2 SCHEME II

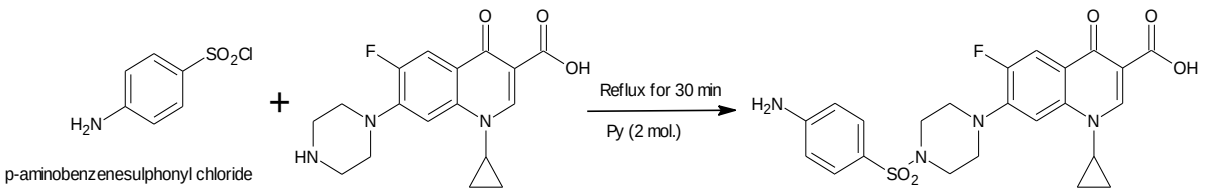
STEP I **Synthesis of p- aminobenzene sulphonyl chloride from sulphanillic acid.**

Same as used in scheme I (Step I)

STEP II **Synthesis of 7-(4-((4-aminophenyl)sulphonyl)piperazine-1-yl)-1-cyclopropyl-6-flouro- 4-oxo-1-4-dihydroquinoline-3-carboxylic acid from p- aminobenzene sulphonyl chloride.**

Procedure 7-(4-((4-aminophenyl)sulphonyl)piperazine-1-yl)-1-cyclopropyl-6-flouro-4-oxo-1-4-dihydroquinoline-3-carboxylic acid was synthesized by Ciprofloxacin and pyridine (2mol.) were taken in 250ml round bottom flask and p-aminobenzenesulphonyl chloride was added dropwise from the condenser. Refluxed for 30 min. Response mixture was emptified into 6ml of icy water & stirred until the item solidified. Solid was filtered off and recrystallized from ethanol.

SCHEME II



OBSERVATIONS

- Solubility : Methanol, Chloroform
- Melting point : 216-218°C
- TLC : Mobile phase (chloroform: n-heptane: ethanol /1:1:1 v/v)
- Rf value of product :0.35

5.3.3 SCHEME III

STEP I **Synthesis of p- aminobenzene sulphonyl chloride from sulphanillic acid.**

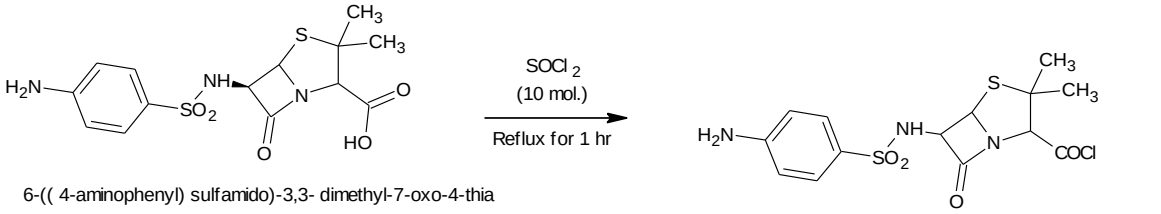
Same as used in Scheme I (Step I)

STEP II **Synthesis of 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid from p- aminobenzene sulphonyl chloride.**

Same as used in Scheme I (Step II)

STEP III **Synthesis of 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carbonyl chloride from 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo[3.2.0] heptane-2 carboxylic acid.**

Procedure 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid (1 mole) and thionyl chloride (10 mole) were taken in a 250ml round bottom flask fitted with a reflux condenser and a drying tube (NOTE expansion of thionyl chloride dropwise in the response mixture and HCl gas developed). The response mixture was blended and warmed at 100°C on heating mentle or megantic stirrer bar for 1 hr.



6-((4-aminophenyl) sulfamido)-3,3- dimethyl-7-oxo-4-thia -1-azabicyclo[3.2.0] heptane-2 carboxylic acid

6-((4-aminophenyl)sulphamido)-3,3-dimethyl-7-oxo-4-thia -1-azabicyclo[3.2.0] heptane-2-carbonyl chloride

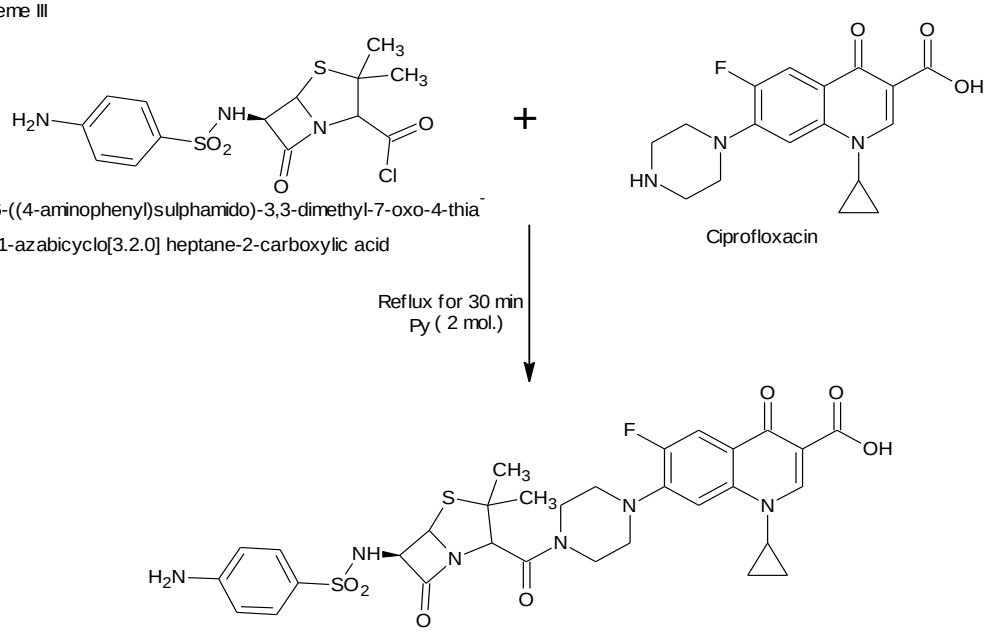
OBSERVATIONS

- Solubility : Methanol
- Melting point : 180-182°C
- TLC: Mobile phase (chloroform: n-heptane: ethanol /3:3:3)
- Rf value of product : 0.18

STEP IV **Synthesis of 7-(4-(6-((4-aminophenyl)sulfamido)-3,3-dimetnyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptanes-2-carbonyl)piperazin-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid from 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo [3.2.0] heptane-2 carboxylic acid.**

Procedure 7-(4-(6-((4-aminophenyl)sulfamido)-3,3-dimetnyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanes – 2– carbonyl) piperazin – yl) – 1 – cyclopropyl -6 - fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid was synthesized by Ciprofloxacin and pyridine (2 mol.) were taken in RBF and 6-((4Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carbonyl chloride was added from top of the condenser. The DCM was taken as solvent. Refluxed for 30 min. Response mixture was emptified into 6ml of icy water & stirred untill the item solidified. Solid was filtered off and recrystallized from ethanol.

Scheme III



OBSERVATIONS

- Solubility : Methanol
- Melting point : 230-232°C
- TLC: Mobile phase (chloroform: n-heptane: ethanol /3:3:3)
- Rf value of product : 0.42

5.4 ANTIMICROBIAL TESTING

Microorganisms exhibit two kinds of resistance to antibiotics, specifically inborn resistance and obtained resistance. Inborn resistance implies that the species was resistant to an antibiotic even before its presentation. Obtained resistance implies that the species was susceptible to an antibiotic, but later became resistant. Microorganisms can acquire either by mutation or through exchange of genetic material among same or nearly. Resistance to several different antibiotics in the meantime is significantly more huge problem.

The antimicrobial activity of compounds can be done by two different qualitative methods i.e. Agar well diffusion method and tube dilution method. I had used agar well diffusion method for antimicrobial testing.

PROCEDURE FOR AGAR WELL DIFFUSION METHOD

Preparation of Agar medium:

The medium was organized by dissolving 15 g of the freshly open Muller Hinton Agar Medium in 500 ml of refined water. The disintegrated medium was autoclaved at 15 lbs weight at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still fluid.

(1) Agar well- Diffusion method

After this, the 20 ml of purified Muller Hinton Agar was filled in sterile petriplates, after hardening; the wells were punched over the agar plates using sterile gel puncher and each drug concentration (10, 20, 30 and 40 µg/ml) were added to the wells. The plates were incubated for 24 hours at 37°C. After incubation, the width of inhibitory zones molded were measured in mm and recorded.[24-26]

5.5 DETERMINATION OF ANTIMICROBIAL ACTIVITY

The in vitro antibacterial action of compounds were checked utilizing agar well dispersion technique against E.coli and S. aureus. The concentration (10, 20, 30 and 40 µg/ml) of the test compounds were arranged by dissolving the synthesized compounds in dimethyl sulphoxide (DMSO). Zone restraint delivered by every compound was measured in mm. The consequences of the antibacterial action are given in table no.3 and are communicated as rate of zone restraint.

S.No.	COMPOUNDS	CONCENT-RATIONS(µg/ml)				ZONE OF INHIBITION (mm)							
						E.coli				*S. Aureus*			
1	RT1	10	20	30	40	---	4	5	5	1	1	3	4
2	RT2	10	20	30	40	4	5	7	9	4	5	6	7
3	RT3	10	20	30	40	1	2	3	3	1	3	4	5
4	Ciprofloxacin (standard)	10	20	30	40	4	5	6	7	4	5	7	8

Table No.3 Determination of antimicrobial activity

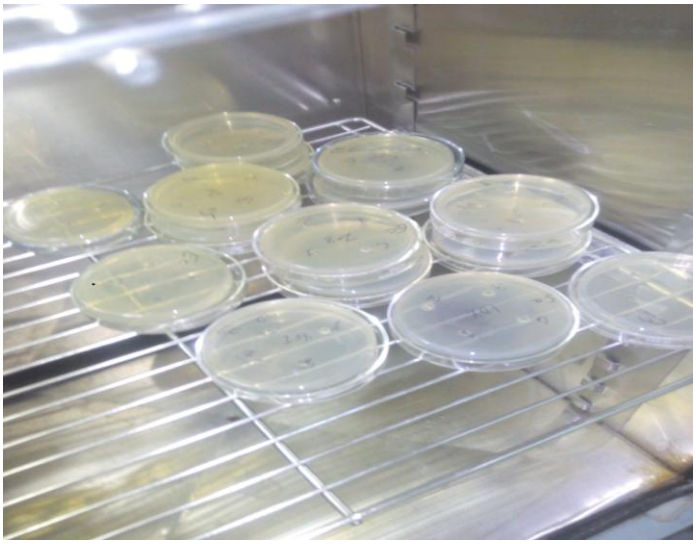


Fig.18 Incubation for 24 hours.

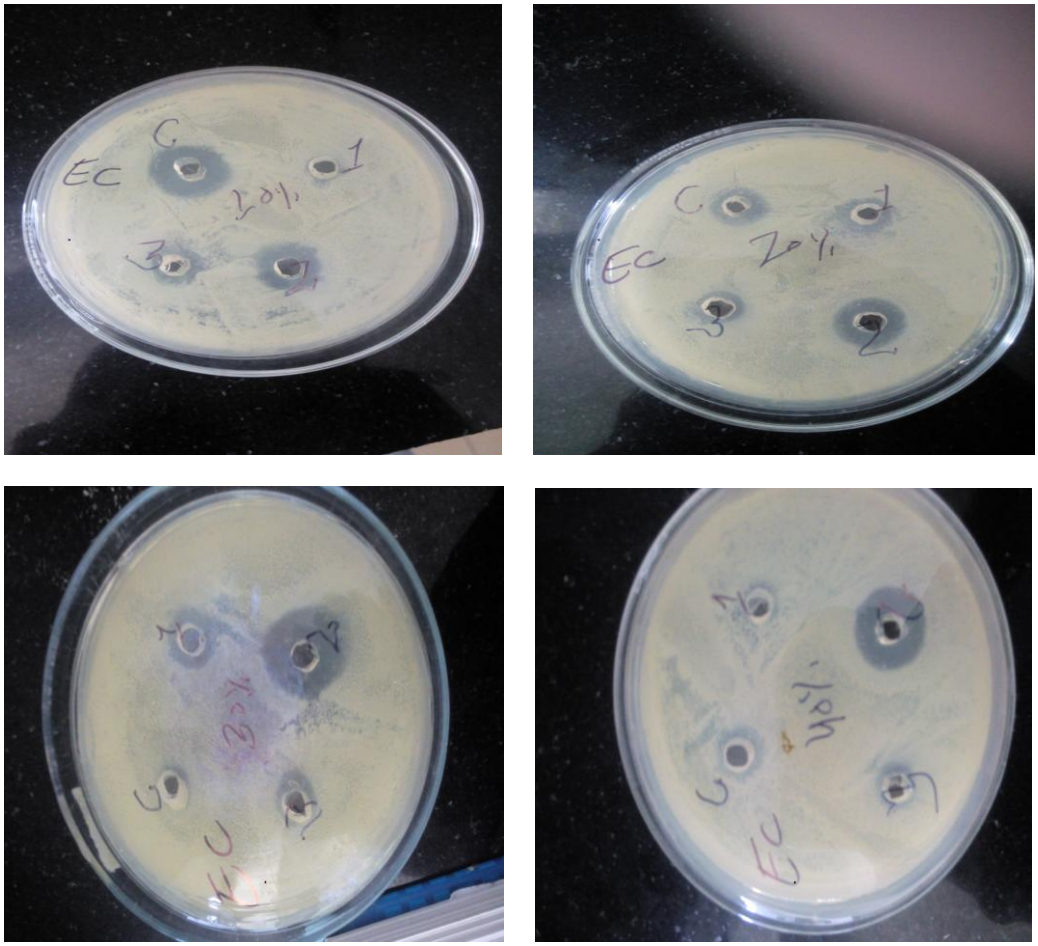


Fig.19 Testing on E.coli at different concentrations (10, 20, 30 and 40 µg/ml)

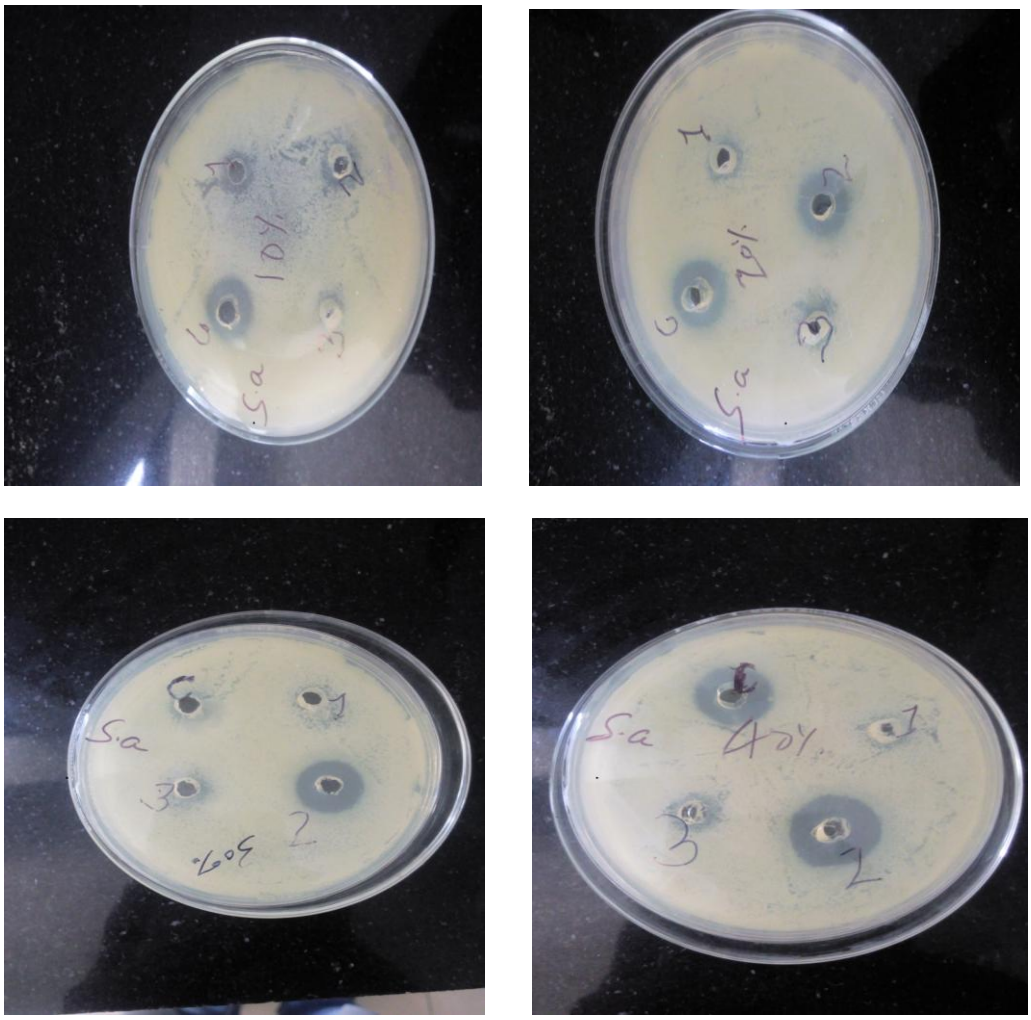


Fig.20 Testing on S.aureus at different concentrations (10, 20, 30 and 40 µg/ml)

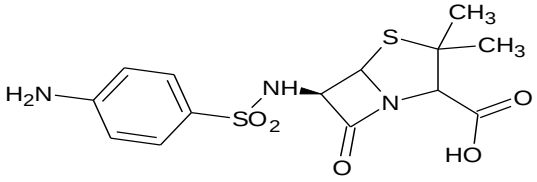
CHAPTER 6

RESULT AND DISCUSSION

Hybrid antibiotics were synthesized containing Sulphanillic acid as a lead compound and the attachment of different antibiotic nucleus i.e. 6-Aminopenicillin and fluoroquinolones. The synthesized compounds RT1, RT2 and RT3 were identified on the basis of melting point, thin layer chromatograpy (TLC), IR and ^{1}HNMR.

6.1 SPECTRAL CHARACTERIZATION OF COMPOUNDS

6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid.



6-((4-aminophenyl) sulphonamido)-3,3-dimethyl -7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid

(RT1)

The Percentage yield and Melting point were seen at 85% and 198-200°C

SPECTRAL ANALYSIS OF COMPOUND (RT1)

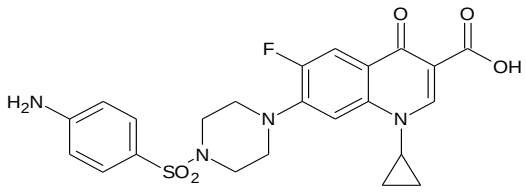
IR ANALYSIS (Fig. No. 22)

FTIR (KBr): The β-lactam ring C=O gives a strong band at 1772.64 cm-1. . The –OH stretching vibrations were seen in the scope of 3300-2500 cm^{-1}. The NH stretching vibrations lie in the scope of 3500-3300 cm^{-1} and NH binding vibrations were seen at 1626.05 cm^{-1}. The asymmetric and symmetric SO_2 vibrations differ in the extents 1338.64 cm^{-1} and 1157.33 cm^{-1}, individually. The C-H stretching vibrations for aromatic ring and C-H stretching vibrations were seen at 3066 cm^{-1} and 2924.16 cm^{-1} for saturated ring and C=C stretching vibrations for aromatic ring were seen in the scope of 1413.87, 1541.18, 1626.05 cm^{-1}.

NMR ANALYSIS (Fig. No. 23)

^{1}H NMR (400 MHz, DMSO δ=2.5): δ 4.3 (s,2H, -NH_2), 8.22(s, -NH), 7.4 (d, 1H for benzene ring, J=8.4, -CH), 6.6 (d,1H for benzene ring, J=8.4,-CH), 5.5 (s, 1H, -CH near to –COOH group), 1.5 (s, 2H, -CH3), 5 (s, 1H, -CH).

7-(4-((4-aminophenyl)sulphonyl)piperazine-1-yl)-1-cyclopropyl-6-flouro-4-oxo-1-4-dihydroquinoline-3-carboxylic acid from sulphanillic acid.



7-(4-((4-aminophenyl)sulfonyl)piperazine-1-yl)-1-cyclopropyl -6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

(RT2)

The Percentage yield and Melting point were seen at 70% and 216-218°C.

SPECTRAL ANALYSIS OF COMPOUND (RT2)

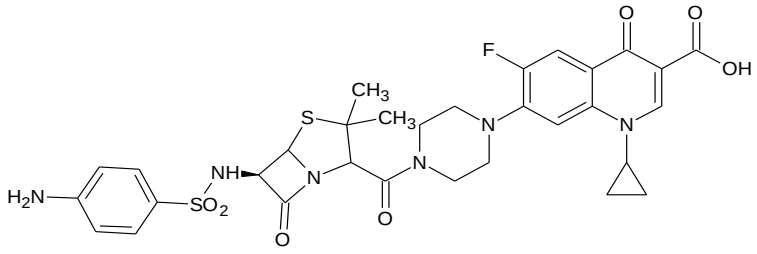
IR ANALYSIS (Fig. No.26)

FTIR (KBr): The asymmetric and symmetric SO_2 vibrations were found in the range 1334.78 cm^{-1} and 1159.26 cm^{-1}, individually. The S-N and C-N stretching vibrations retain in the reaches 891.14 cm^{-1} and 1251.84, 1159.26 cm^{-1}, individually. The C=O stretching vibrations were seen at 1724.42 (s) cm^{-1}
The NH_2 (Str.) vibrations were seen at 3375.54 cm^{-1} and NH binding vibrations were seen at 1627.97 cm^{-1}. The –OH stretching vibrations were seen in the scope of 3300-2500 cm^{-1}. The C-H stretching vibrations for aromatic ring and C-H stretching vibrations were seen at 3066 cm^{-1} and 2928.04 cm^{-1} for saturated ring and C=C stretching vibrations for aromatic ring were seen in the scope of 1469.81, 1545.03
cm^{-1}.[22-23][27]

NMR ANALYSIS (Fig. No.27)

^{1}H NMR (400 MHz, DMSO δ 2.5) δ 1.7 (d, 1H, J= 6.4, -CH_2), 1.2 (s, 1H,-CH), 3.3 (s, 5H, -CH_2), 3.5 (s, 6H,-CH_2) ,7.9 (d, 2H, J=13,-CH), 7.6 (d, J= 8.4,3H,-CH), 7.0 (d, J=8.4, 2H,-CH), 8.2 (s,1H,-CH), 3.9 (s, 2H -NH_2), 15.08 (s, 1H, -OH).

7-(4-(6-((4-aminophenyl)sulfamido)-3,3-dimetnyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanes-2-carbonyl)piperazin-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



7-(4-(6-((4-aminobhenyl) sulfamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carbonyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic ac

(RT3)

The Percentage yield and Melting point were seen at 70% and 230-232°C

SPECTRAL ANALYSIS OF COMPOUND (RT3)

IR ANALYSIS (Fig. No.31)

FTIR (KBr): NH_2(Str.), were seen at 3475.54 cm^{-1}. The β-lactam ring C=O gives a solid band at 1772.64 cm-1. SO_2 vibrations shift in the extents 1383.01, 1338.64 cm−1 (asymmetric) and 1177 – 1148 cm^{-1}(symmetric) resp. The S-N and C-N stretching vibrations retain in the extents 943.22, 891.14 cm^{-1} and 1273.06 – 1157.33 cm^{-1}, separately. The C=O stretching vibrations were seen at 1710.92 (s) $cm^{-1.}$
The –OH stretching vibrations were seen in the scope of 3300-2500 cm^{-1}. The C-H stretching vibrations and C-H stretching vibrations were seen at 3066 cm^{-1} and 2900-3000 cm^{-1} for saturated ring. and C=C stretching vibrations for aromatic ring were seen in the range of 1411.94, 1450.52, 1496.81 cm^{-1}.

NMR ANALYSIS (Fig. No.32)

^{1}H NMR (400 MHz, DMSO δ 2.5) δ 3.9(s, 2H, -NH_2), 8.22(s, 1H, -NH), 7.6 (d, 1H, J= 7.2, -CH), 6.6 (d,1H, J=8.36, -CH), 7.4 (d, 1H, J=6.48,-CH), 7.9 (d, 1H, J=12.92,-CH), 1.2 (s,2H, -CH_2), 1.5 (s, 2H,-CH_3), 1.3 (d,1H, -CH_2), 3.3 (s,4H,-CH_2), 3.6(s,6H,-CH_2), 6.6 (d,1H, J=8.36,-CH), 15.06 (s,1H,-OH).

6.2 DISCUSSION FOR ANTIMICROBIAL ACTIVITY:

By using Ager well-diffusion method, the synthesized compounds RT1, RT2 and RT3 were tested against E.coli and S. aureus strains. The synthesized compounds showed significant activity against these strains. The synthesized compound RT2 showed slightly higher activity as compared to ciprofloxacin (standard).

CHAPTER 7

Conclusion

CONCLUSION :

According to literature survey, we have concluded that development of hybrid antibiotics increases the potency or synergistic effect which is helpful to reduce the problem of bacterial resistance.

We have developed hybrid compounds (RT1, RT2 and RT3) by taking sulphanillic acid as a lead compound. This sulphanillic acid was combined with 6- amino penicillin and ciprofloxacin by different ways.

All the synthesized compounds were characterized by on the premise of IR, ^{1}HNMR, Rf values.

The compounds were screened by utilizing agar well diffusion strategy against E.coli and S.aureus for antimicrobial activity. The reference standard and control used were ciprofloxacin and DMSO. The compounds RT1, RT2 and RT3 showed significant activity.

In conclusion, the result of antibacterial activity of test compounds RT2 showed better activity than RT1 and RT3. And also showed slightly higher activity as compared to Ciprofloxacin (standard)

RT2 compound can be explored in future for potent antibacterial action.

CHAPTER 8

Appendix

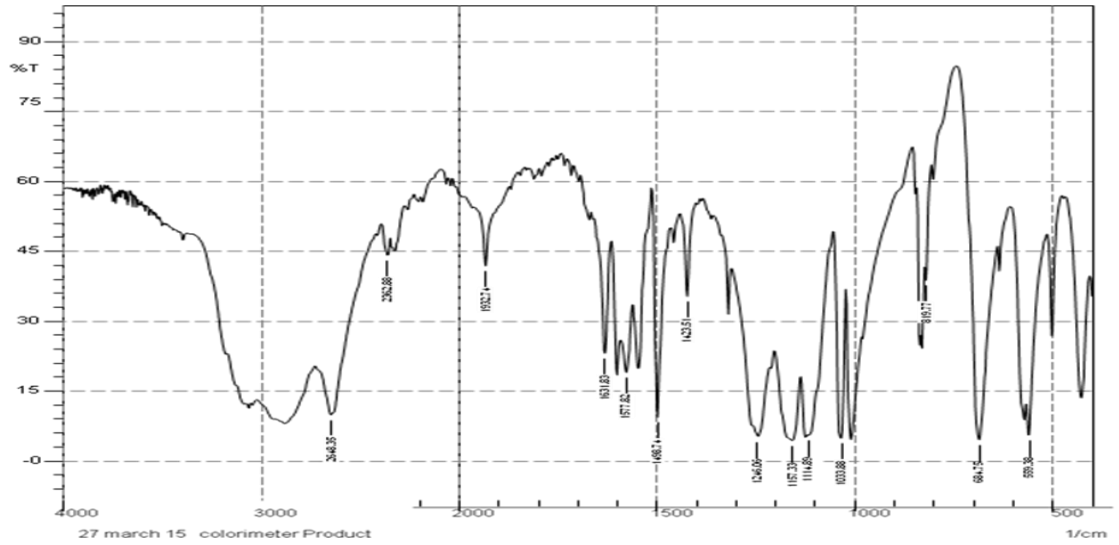


Fig.21 IR spectra of p-aminobenzene sulphonyl chloride

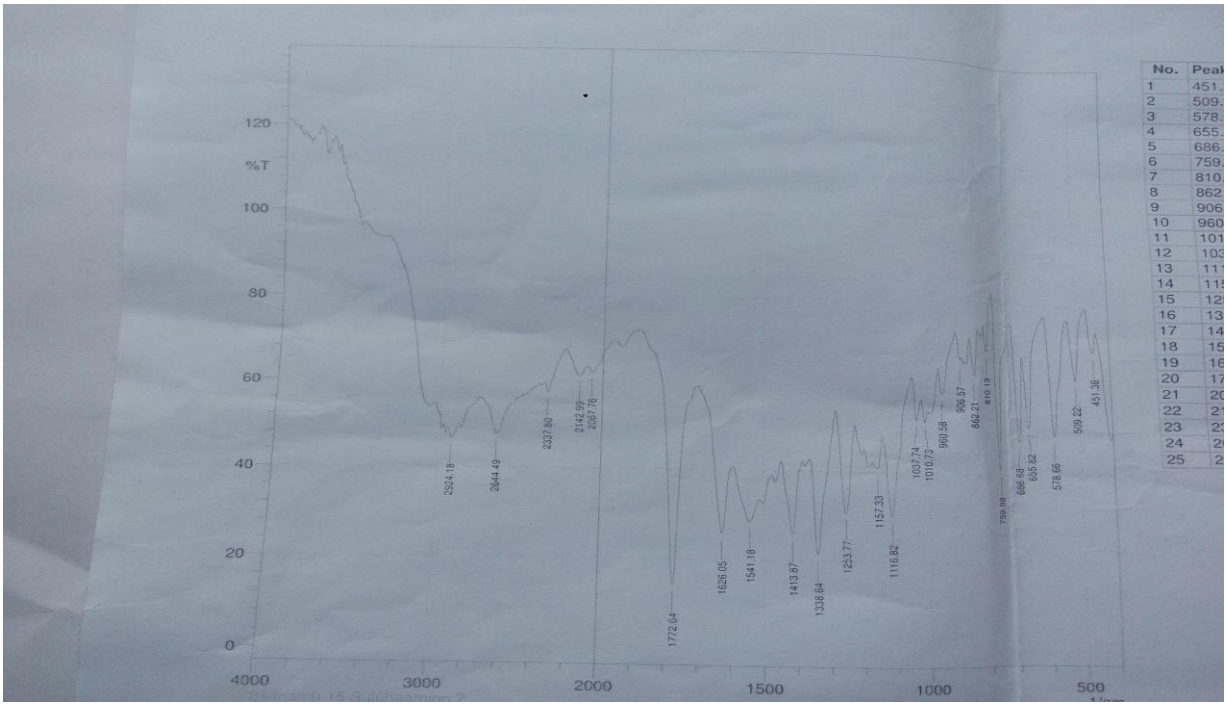


Fig.22 IR spectra of 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid.(RT1)

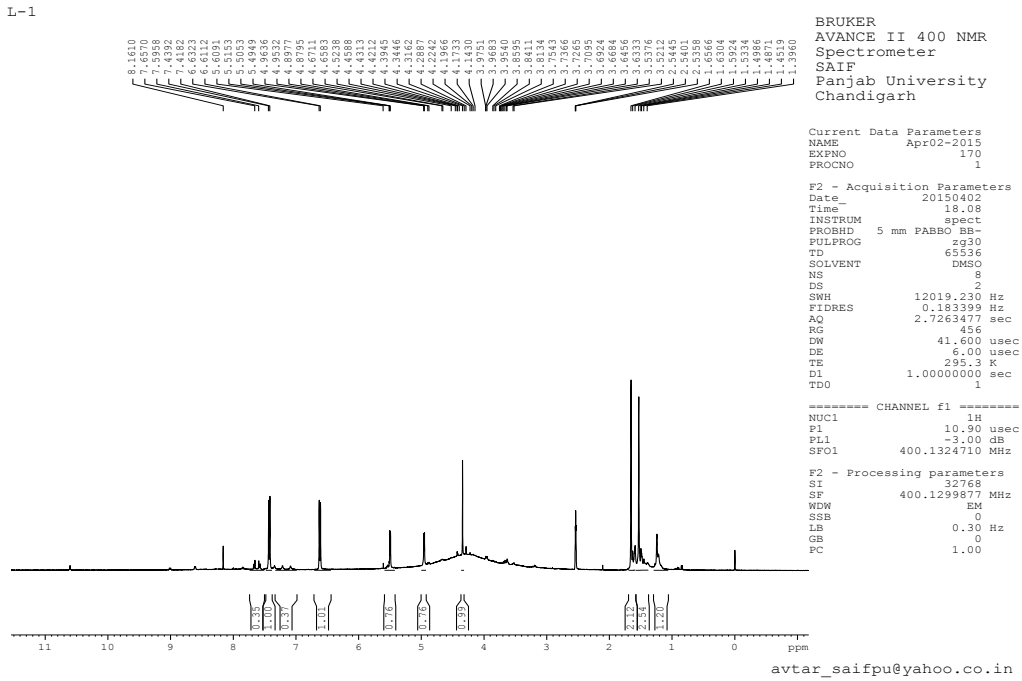


Fig.23 [1]HNMR spectra of 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid.(RT1)

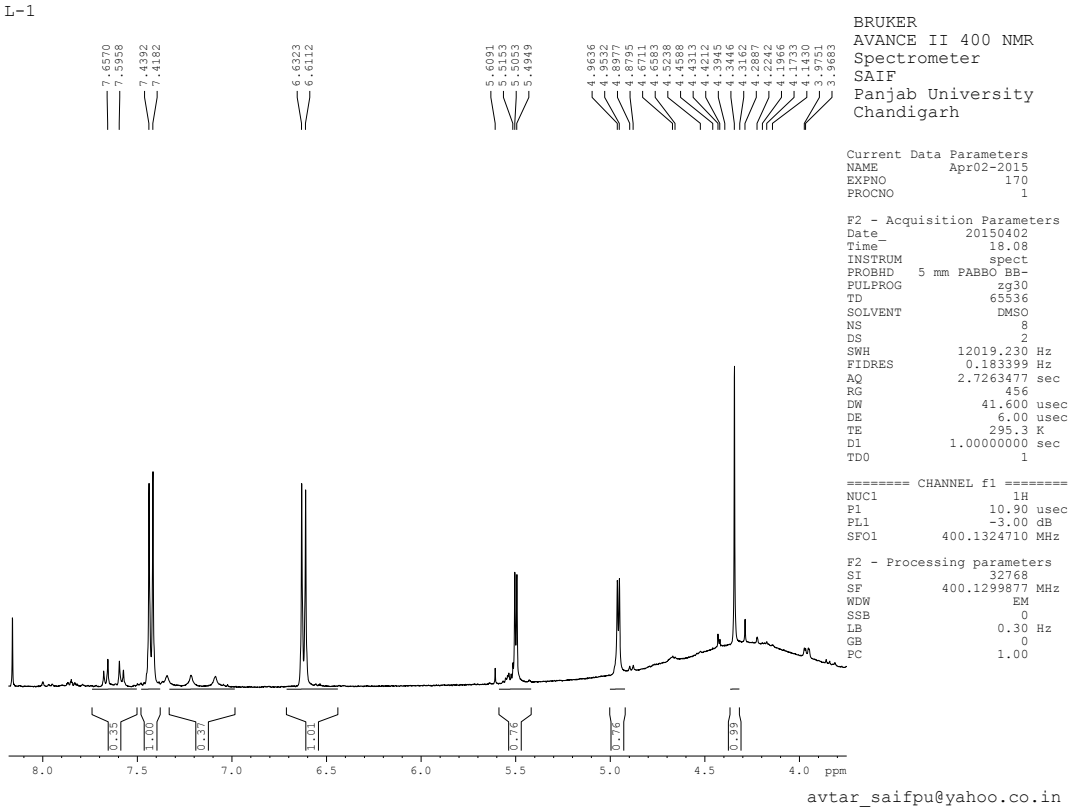


Fig. 24 Expended [1]HNMR spectra of 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7- oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid.(RT1)

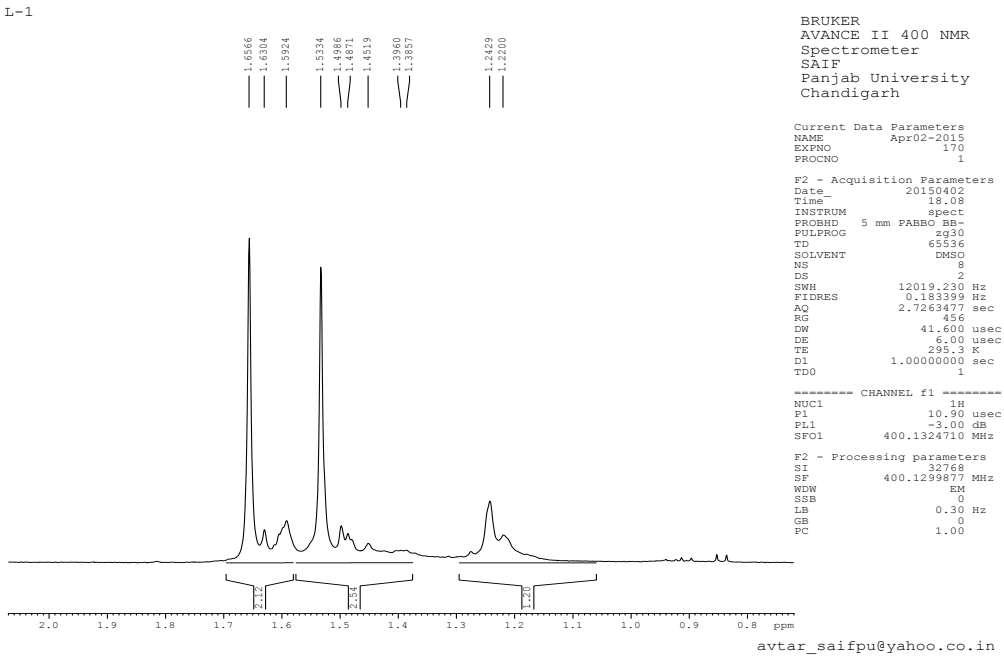


Fig.25 Expended [1]HNMR spectra of 6-((4-Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carboxylic acid.(RT1)

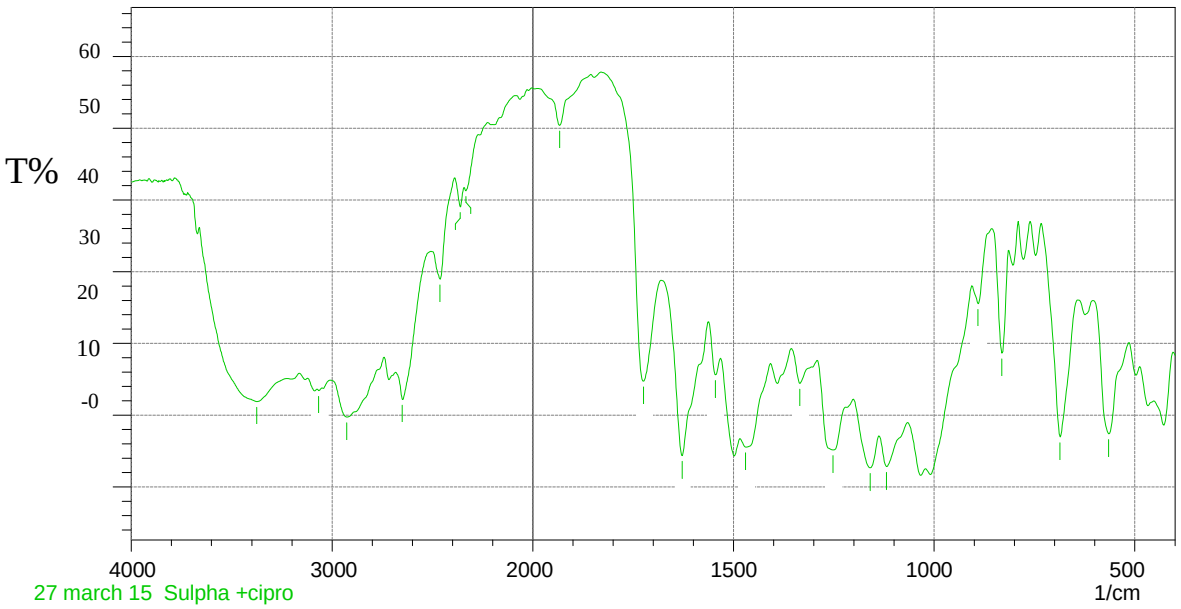


Fig.26 IR spectra of 7-(4-((4-aminophenyl)sulphonyl)piperazine-1-yl)-1-cyclopropyl-6-flouro-4-oxo-1-4-dihydroquinoline-3-carboxylic acid.(RT2)

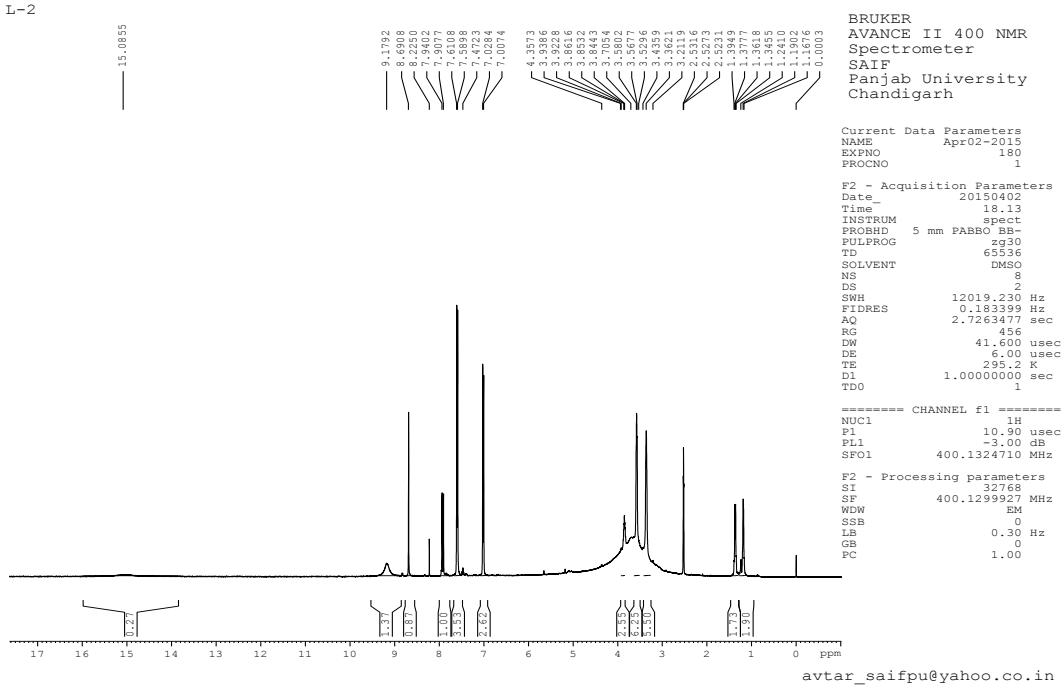


Fig.27 [1]HNMR spectra of 7-(4-((4-aminophenyl)sulphonyl)piperazine-1-yl)-1-cyclopropyl-6-flouro-4-oxo-1-4-dihydroquinoline-3-carboxylic acid.(RT2)

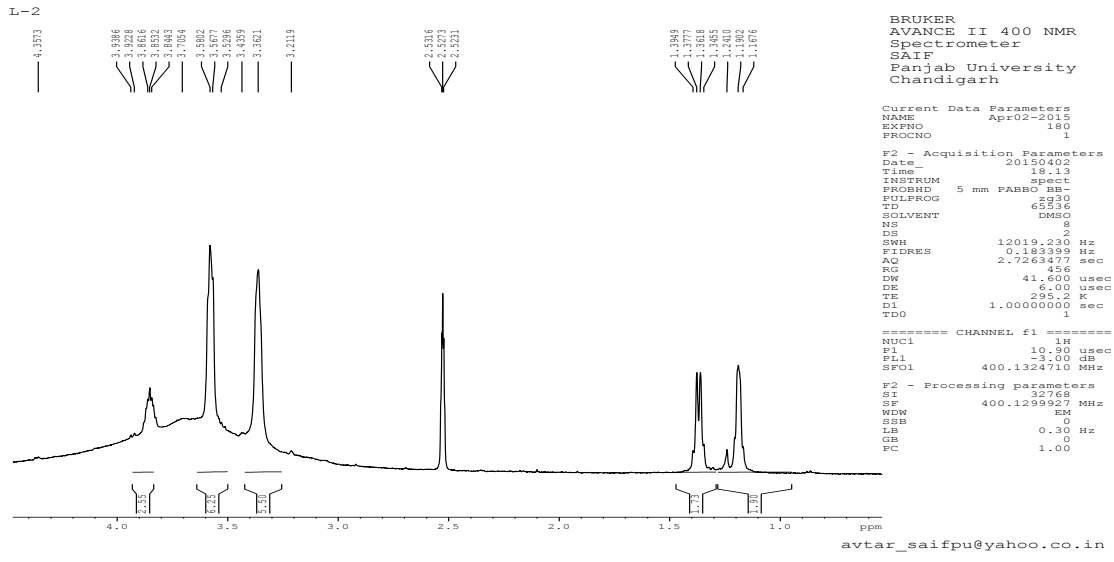


Fig.28 Expended [1]HNMR spectra of 7-(4-((4-aminophenyl)sulphonyl)piperazine-1-yl)-1-cyclopropyl-6-flouro-4-oxo-1-4-dihydroquinoline-3-carboxylic acid.(RT2)

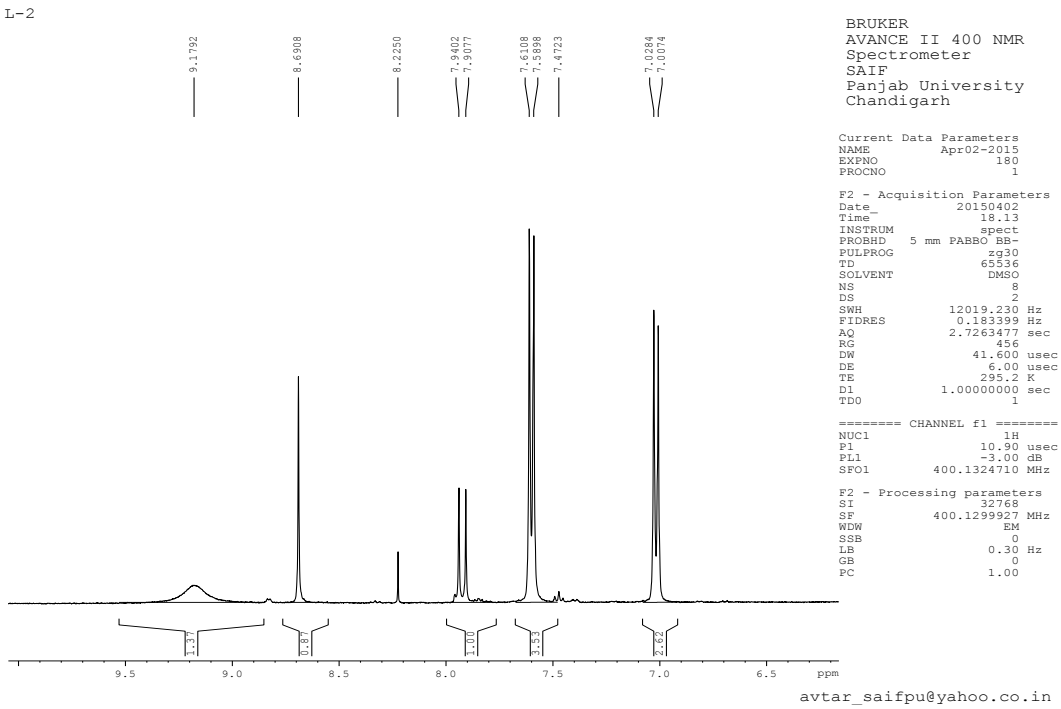


Fig.29 Expended [1]HNMR spectra of 7-(4-((4-aminophenyl)sulphonyl)piperazine-1-yl)-1-cyclopropyl-6-flouro-4-oxo-1-4-dihydroquinoline-3-carboxylic acid.(RT2)

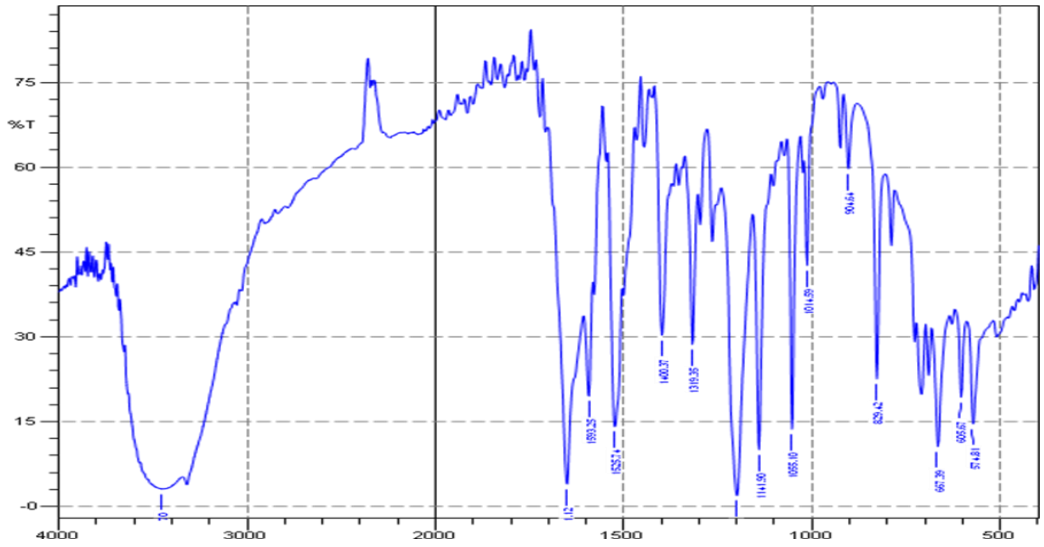


Fig.30 IR spectra of 6-((4Aminophenyl)sulphonamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2 carbonyl chloride.

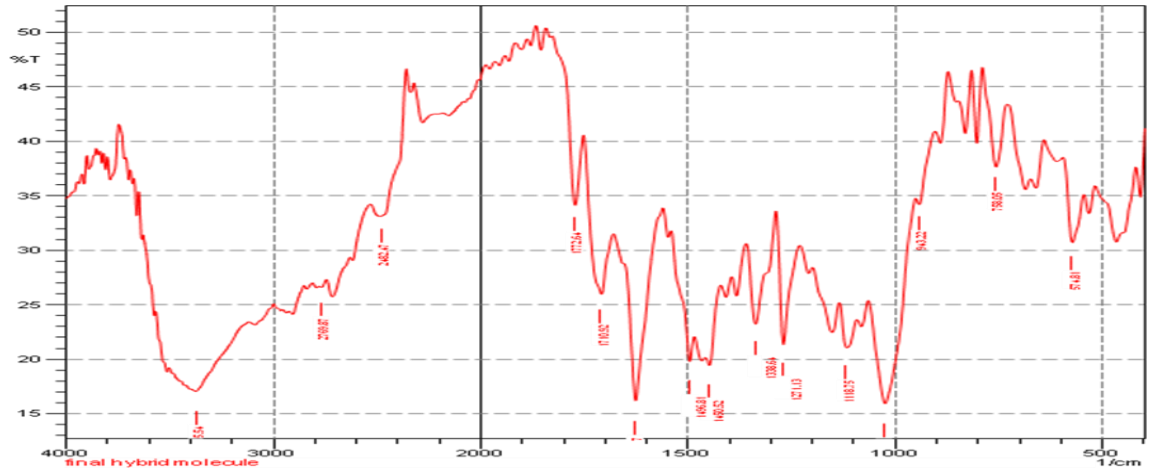


Fig.31 IR spectra of 7-(4-(6-((4-aminophenyl)sulfamido)-3,3-dimetnyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanes-2-carbonyl)piperazin-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.(RT3)

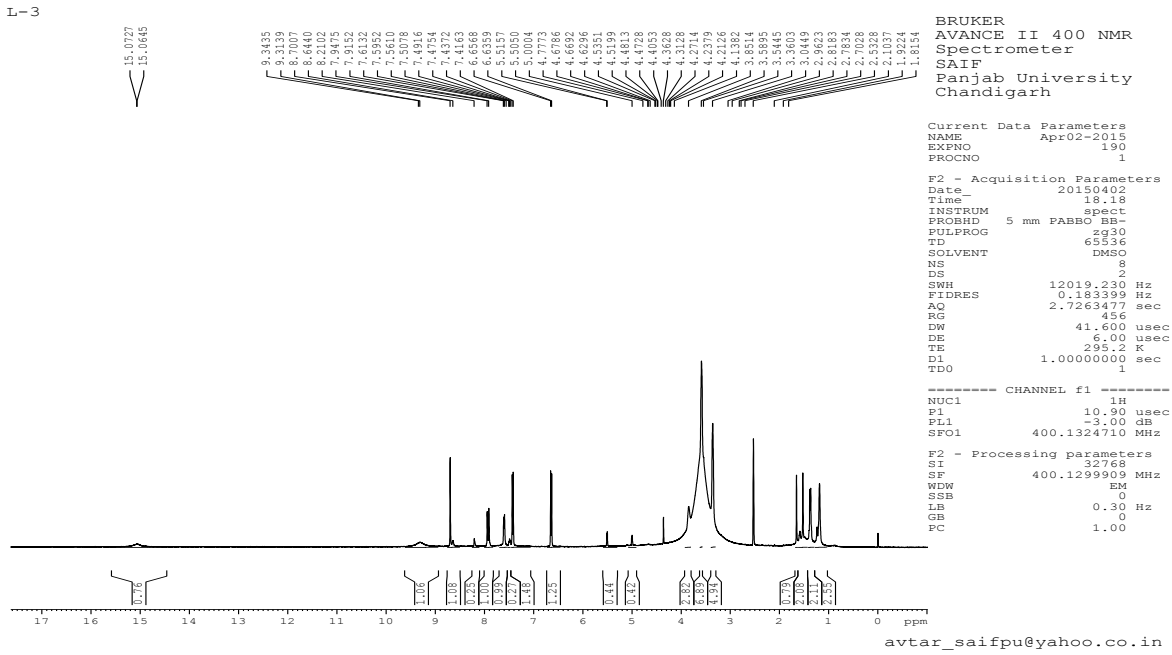


Fig.32 [1]HNMR spectra of 7-(4-(6-((4-aminophenyl)sulfamido)-3,3-dimetnyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanes-2-carbonyl)piperazin-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.(RT3)

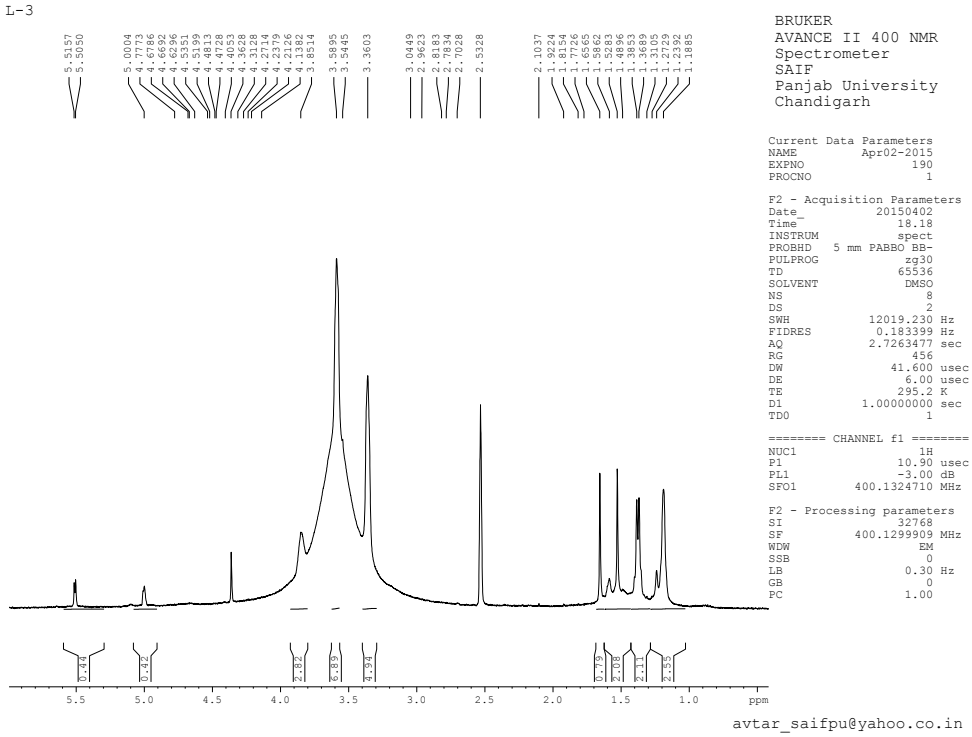


Fig.33 Expended [1]HNMR spectra of 7-(4-(6-((4-aminophenyl)sulfamido)-3,3-dimetnyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanes-2-carbonyl)piperazin-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.(RT3)

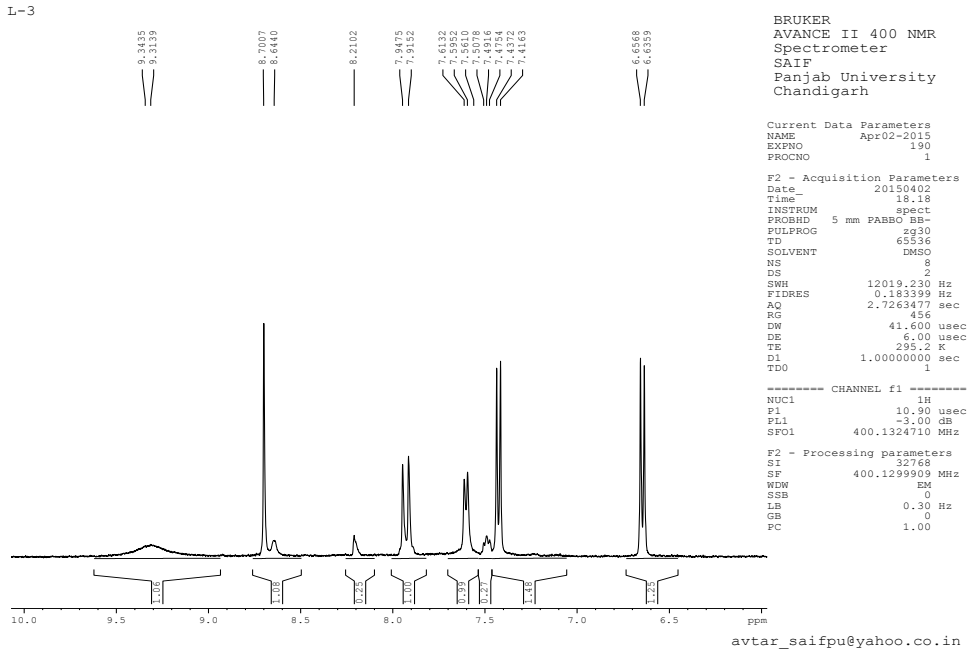


Fig.34 Expended [1]HNMR spectra of 7-(4-(6-((4-aminophenyl)sulfamido)-3,3-dimetnyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanes-2-carbonyl)piperazin-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.(RT3)

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DISSERTATION TOPIC APPROVAL PERFORMA

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Batch: 2013 Roll No.

Session: 2014 Parent Section: Y1303

Details of Supervisor:

Name VIVEK GUPTA Designation: Asst. Profesor

U.ID 15835 Qualification: Ph.D.

Research Experience: 8 years

SPECIALIZATION AREA: (pick from list of provided specialization areas by DAA)

PROPOSED TOPICS

1. Synthesis and antimicrobial evaluation of novel sulfonamide derivatives.
2. Synthesis and antimicrobial evaluation of novel β-lactam ring derivatives.
3. Synthesis and antimicrobial evaluation of novel fluoroquinolone derivatives.

Signature of Supervisor

PAC Remarks:

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CHAPTER 9

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9. REFERENCES

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