“STUDIES ON BIOLOGICAL EVALUATION OF NOVEL HETEROCYCLIC COMPOUNDS”

A THESIS SUBMITTED TO

SAURASHTRA UNIVERSITY

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IN

CHEMISTRY

(SCIENCE STREAM)

By:

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Date: 31st July, 2007

UNDER THE GUIDENCE OF

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MVM SCIENCE & HOME SCIENCE COLLEGE,

KALAWAD ROAD,

RAJKOT,

GUJARAT (INDIA)

December-2011

Dedicated to…

MY PARENTS & LORD

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Statement under O. Ph. D. 7 of Saurashtra University

The work included in the thesis is my own work under the supervision of **Dr. Jatin Upadhyay** and leads to some contribution in chemistry subsidized by a number of references.

Date: - -2011 **Mr. Rushit I. Kalaria**
Place: Rajkot

This is to certify that the present work submitted for the Ph.D. Degree of Saurashtra University by ***Rushit I. Kalaria*** is his own work and leads to advancement in the knowledge of synthetic organic chemistry and MIC study. The thesis has been prepared under my supervision.

Date: - - 2011
Place : Rajkot

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Rushit I. Kalaria

CONTENTS

NOTES

1. All the temperatures are expressed in degree centigrade (˚C).
2. Melting points of all the compounds are uncorrected and have been recorded in open capillary method.
3. Room temperature, wherever mentioned is normally correspondence to 28-33˚C.
4. Silica gel-G was used for preparing the TLC plates using appropriate solvent system.
5. IR spectra were recorded in KBr pellets on SHIMADZU-FTIR 8400-S spectrometer.
6. NMR spectra were recorded on BRUKER spectrophotometer (400 MHz) using TMS as an internal standard and $CDCl_3$ and DMSO-d_6 as solvent.
7. Mass spectra were recorded on WATERS LCMS.

A brief summary of the work to include in the thesis entitled "**STUDIES ON BIOLOGICAL EVALUATION OF NOVEL HETEROCYCLIC COMPOUNDS"** is mentioned as under.

PART-I : STUDIES ON PYRIDINES.

PART-II : STUDIES ON PYRIMIDINES.

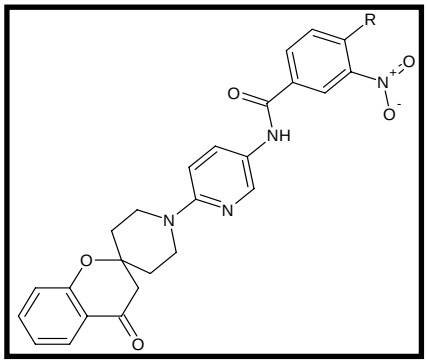
PART-III : STUDIES ON TETRAHYDROTHIENO [2, 3-c] PYRIDINES.

PART I: STUDIES ON PYRIDINES.

Pyridines derivatives have recently received considerable attention due to their synthetic and pharmaceutical importance. The pyridine nucleus is an important heteroaromatic class of compounds with a wide range of pharmacological activities and it is present in many products such as drugs, foods, vitamins, flavorings, plants, dyes, rubber products, adhesehives, insecticides and herbicides. They also possess diverse biological activities viz, antibacterial, antitubercular, calcium channel blocker, cardiovascular, vasodilator, antihypertensive, anti-oxidant, anti-inflammatory, anti-HIV and anti-carcinogenic activity.

The work is further subdivided in following sections

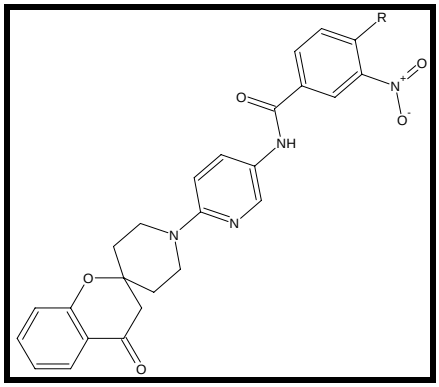
SECTION I: Preparation, Characterization and antimicrobial evaluation of 4-(substituted amino) -3-nitro-N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'-piperidin)-1'-yl}pyridine-3-yl]-benzamides.



(Ia-k)
R= Substituted amino

4-(substituted amino) -3-nitro-N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides **(Ia-k)** have been prepared by nucleophilic substitution of the chloro atom of 4-chloro 3-nitro- N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides with different substituted amines.

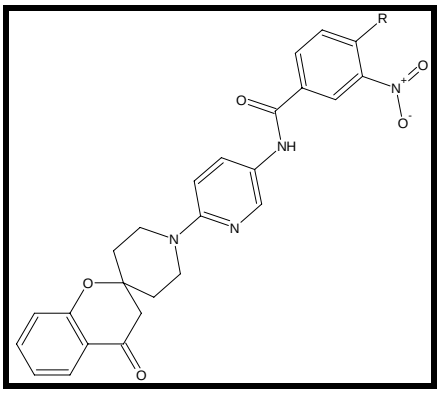
SECTION-II: Preparation, Characterization and antimicrobial evaluation of 4-(substitutedphenoxy)-3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- Spiro-(chromene-2, 4'-piperidin)-1'-yl} pyridine-3-yl]-benzamides.



(IIa-k)
R= Substitutedphenoxy

4-(substitutedphenoxy) -3-nitro-N-[6-{4-oxo-3,4 dihydro-1'H- Spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides **(IIa-k)** have been prepared by nucleophilic substitution of the chloro atom of 4-chloro 3-nitro-N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides with different substituted phenols.

SECTION-III: Preparation, Characterization and antimicrobial evaluation of 4-(substitutedthiophenoxy)-3-nitro-N-[6-{4-oxo-3,4 dihydro-1'H- Spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides.

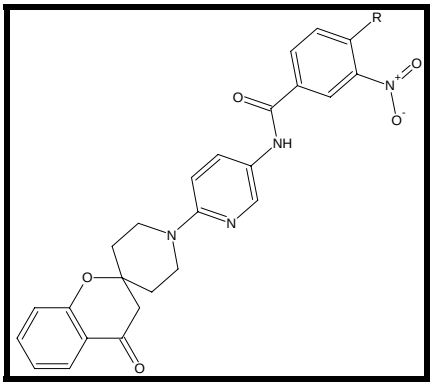


(IIIa-k)
R= Substituted thiophenoxy

4-(substitutedthiophenoxy) -3-nitro-N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides(**IIIa-k)** have been prepared by nucleophilic substitution of the chloro atom of 4-chloro 3-nitro-N-[6-{4-oxo-3,4

dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides with different substituted thiophenols.

SECTION-IV: Preparation, Characterization and antimicrobial evaluation of 4-(substituted phenyl)-3-nitro-N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'-piperidin)-1'-yl}pyridine-3-yl]-benzamides.



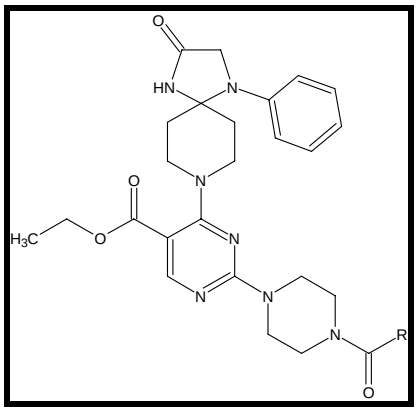
(IVa-k)
R= Substituted phenyl

4-(substituted phenyl)-3-nitro-N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides(IVa-k) have been prepared by using 4-chloro 3-nitro -N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides using different substituted phenyl boronic acids.

PART-II: STUDIES ON PYRIMIDINES.

Pyrimidine derivatives represents one of the most active classes of compounds possessing a wide spectrum of biological activities viz significant in vitro activity against unrelated DNA and RNA viruses including polio and Herpes viruses, diuretics, antitubercular, antihypertensive some pyrimidine which occurs as natural products like nucleic acids and vitamin-B and and can be used as therapeutic agents for the treatment of AIDS and antitumor. In view of getting better therapeutic agents the new pyrimidine derivatives have been synthesized possessing 1,4,8-Triazaspiro and benzoylpiperazine moieties.

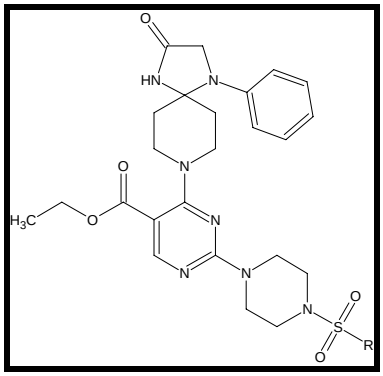
SECTION-I: Preparation, Characterization and antimicrobial evaluation of Ethyl -2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate.



(Ia-k)
R= Aryl

Ethyl 2-(4-Substituted benzoylpiperazin-1-yl)-4-(3-oxo-1phenyl-1,4,8-triazaspiro [4,5] dec-8-yl) pyrimidine -5-carboxylate (Ia-k) have been prepared by using Ethyl 2-(piperazin-1-yl) 4-(1-phenyl-3-oxo-1,4,8-triazaspiro [4,5] dec-8-yl) pyrimidine -5-carboxylate with different substituted benzoyl chlorides.

SECTION-II : Preparation, Characterization and antimicrobial evaluation of Ethyl -2-[4-(Substituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1,4,8-triazaspiro [4,5] dec-8-yl) pyrimidine -5-carboxylates.



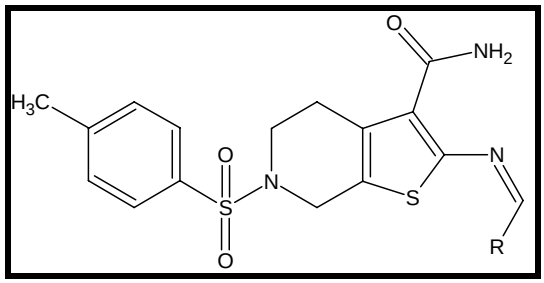
(IIa-k)
R= aryl

Ethyl 2-[4-(Substituted benzenesulfonyl) piperazin-1-yl] 4-(1-phenyl-3-oxo-1,4,8-triazaspiro [4,5] dec-8-yl) pyrimidine -5-carboxylate (IIa-k) have been prepared by using Ethyl 2-(piperazin-1-yl) 4-(1-phenyl-3-oxo-1,4,8-triazaspiro [4,5] dec-8-yl) pyrimidine -5-carboxylate with different substituted phenyl sulfonyl chlorides.

PART-III: STUDIES ON TETRAHYDRO THIENO [2, 3-c] PYRIDINES.

Thieno pyridine represents one of the most active classes of compounds possessing a wide spectrum of biological activities including prophylaxis or treatment of obesity, diabetes, hypertension, hyperlipidemia, cardiac failure, diabetic complications, metabolic syndrome and sarcopenia. In view of getting better therapeutic agents of thieno pyridine derivatives possessing sulfonamide moiety have been synthesized.

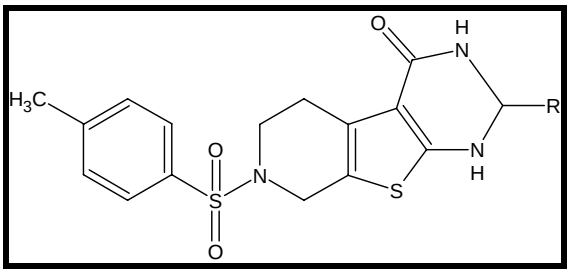
SECTION-I: **Preparation, Characterization and antimicrobial evaluation of 2-(substitutedbenzylideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c]pyridine-3-carboxamides.**



(Ia-k)
R= Aryl

2-(substitutedbenzylideneamino)-6-[(4-methylphenyl)sulfonyl]-4,5,6,7 tetra hydrothieno [2,3-c]pyridine-3-carboxamide (Ia-k) have been prepared by using 2-(amino)-6-[(4-methylphenyl)sulfonyl]-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide with differently substituted aromatic aldehydes.

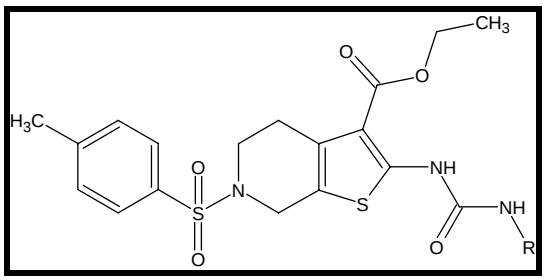
SECTION-II: **Preparation, Characterization and antimicrobial evaluation of 2-aryl-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5]thieno [2,3-d] pyrimidin-4-ones.**



(IIa-k)
R= Aryl

2-aryl-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5] thieno [2,3-d]pyrimidin-4-ones(**IIa-k)** have been prepared by using 2-(amino)-6-[(4-methylphenyl)sulfonyl]-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide with different substituted aromatic aldehydes.

SECTION-III: **Preparation, Characterization and antimicrobial evaluation of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates.**



(IIIa-k)
R= Substituted Phenyl

Ethyl-2-(3-arylureido)-6-[(4-methylphenyl)sulfonyl]-4,5,6,7-tetrahydrothieno [2,3-c]pyridine-3-carboxylates **(IIIa-k)** have been prepared by using Ethyl 2-(phenoxy carbonylamino)-6-[(4-methylphenyl)sulfonyl]-4,5,6,7-tetrahydrothieno[2,3-c] pyridine-3-carboxylate with differently substituted aromatic amines.

CONSTITUTION OF PRODUCTS:

The structures of the compounds have been characterized by elemental and spectral analyses. The purity was checked by TLC.

ANTIMICROBIAL ACTIVITY:

The synthesized compounds were tested for their antimicrobial effect using different strains of bacteria and fungi.

GENERAL INTRODUCTION

The chemistry of the heterocyclic compounds is as logical as that of aliphatic or aromatic compounds. This study is of great interest both from the theoretical as well as practical point. A heterocyclic compound is one which possesses a cyclic structure with at least one different kind of atom other than carbon in the ring. The most common type, contain largely nitrogen, oxygen and sulphur heteroatoms, but many other elements, including even phosphorous, silicon can also serve. The heterocyclic compounds containing the less common atoms have been subjected to much investigation in recent years.

The variety of heterocyclic compounds is enormous, their chemistry is complex and synthesizing them require great skill. Among large number of heterocycles found in nature, nitrogen heterocycles are most abundant than those containing oxygen or sulphur owing to their wide distribution in nucleic acid instant and involvement in almost every physiological process of plants and animals.

Heterocyclic systems are encountered in many groups of organic compounds possessing great applicability in industry as well as in our life in various ways i. e. most of the sugars and their derivatives, including vitamin C, for instant, exist largely in the form of five membered (Furanoside str.) or six membered (Pyranoside str.) ring containing one oxygen atom. Most members of the vitamin B group possess heterocyclic rings containing nitrogen; one example is vitamin B_6 (Pyridoxine), which is a derivative of the pyridine essential in amino acid metabolism. Many other examples of the important heterocyclic compounds in biological systems can be given.

Natural products containing heterocyclic compounds such as alkaloids and glycosides have been used since old age, as remedial agents. Febrifuge alkaloid from ancient Chinese drug, Chang Shan, Reserpine from Indian rauwolfia, Curen alkaloid from arrow poison, Codenine, j-Tropine and Strychnine are all examples of heterocyclic compounds. Many antibiotics including penicillin, cephalosporin, norfloxacin, streptomycin etc. also contain heterocyclic ring systems. Majority of the large number of drugs being introduced in pharmacopeias in recent years are heterocyclic compounds.

Many veterinary products like Pyrantel and Morantel are the drug of choice as broad spectrum anthelmintics. The herbicides Atrazine and Simazine are well known

anthiocyanins, chlorophyll has contributed much colour chemistry and many other heterocyclic colouring matters are in use since prehistoric times. The heterocyclic compound Tetraselena fulvalene was the first ionic molecular crystal to demonstrate superconductivity.

The word drug is derived from the French word drogue which means a dry herb. According to WHO a drug may be defined as any substance or product which is used or intended to be used for modifying or exploring physiological system or pathological status for the benefit of recipient.

There are two main divisions of medicinal chemistry. The first chemotherapy, concerns the treatment of infections, parasite or malignant disease by chemical agents, usually substances that show selective toxicity towards the pathogen. The other division relates to diseases of body disfunction and the agents employed are mainly compounds that effect the functioning of enzymes, the transmission of impulses or the action of hormones on receptors. Heterocyclic compounds are used for all these purposes; because they have a specific chemical reactivity. The introduction of heterocyclic group into drugs may effect their physical properties, for example the dissociation constants of sulpha drugs or modify their patterns of absorption, metabolism or toxicity.

During the period of 1930-1950 there was an urgent need for new drug to treat disease which had a high mortality rate, there was only limited appreciation of the hazard such drugs might present, and toxicological studies before clinical trials were fairly rudimentary. Proving the proverb ***Necessity is the mother of invention***, during the decade of 30 and 40s a large number of drugs introduced. Therefore this period is regarded as ***Golden Period*** of new drug discovery.

Heterocyclic compounds are obtainable by the following methods.

a. Isolation from natural sources, i.e. alkaloids, amino acids, indigo dyes etc.
b. Degradation of natural products i.e. acridine, furfural, indol, pyridine, quinoline, thiophene etc.
c. Synthesis: Synthesis methods for obtaining heterocyclic compounds may be divided into ring closer reactions, addition reaction and replacement reaction. Cyclisation is usually accomplished by elimination of some small molecules such as water or ammonia.

Heterocyclic compounds have a great applicability as drugs because,

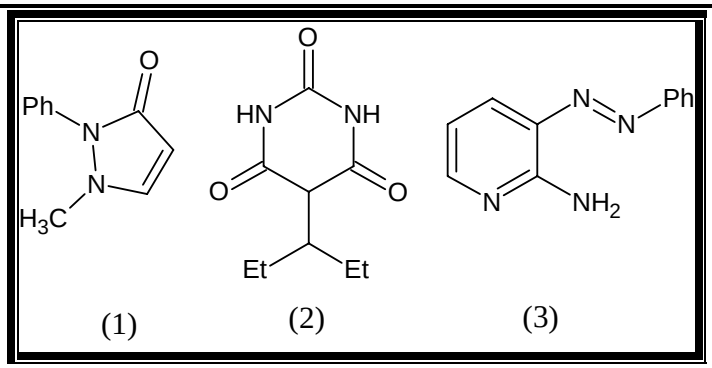
a. They have a specific chemical reactivity.
b. They resemble essential metabolism and can provide false pythons in biosynthetic Process.

The current interest in the creation of large, searchable libraries of organic compounds has captured an imagination of organic chemists and the drug discovery community. Efforts in numerous laboratories focused on the introduction of chemical diversity have been recently reviewed and pharmacologically interesting compounds have been identified for libraries of widely different compositions.

Research in the field of pharmaceutical chemistry has its most important task in the development of new and better drugs and their successful introduction into clinical practice. Central to these efforts, accordingly stand the search for pharmaceutical substances and preparation which are new and original. In addition to these objectives the searching for drug which exhibit a clear advantage over a drug already known. Such advantages may be qualitative or quantitative improvement in activity, the absence of undesirable side effect, a lower toxicity, improved stability and decreased cost.

It is important at the outset to note that drug discovery is not an unambiguous term in the R and D world. For example, it can be defined using either programmatic or organizational approaches (or both), with several options on each category. Hence, it is important first to understand this variability and to adopt a specific definition for the purpose of this discussion.

The contribution of organic chemistry for the development of scientific medicine in the 19^{th} century mainly from acyclic and carbocyclic compounds, although the pyrazoline derivative antipyrin **1** was introduced as an antipyretic and analgesic in 1984 and the first barbiturate baritone (veranol) **2** in 1903. Guttmann treated, malaria with methylene blue in 1891, with slight success, and in 1912 he introduced acriflavine as trypanocide, it has proved to be more valuable as an antiseptic. Phenazopyridine (pyridium) **3** was introduced for the same purpose in 1926, and although it is relatively ineffective it has continued to be used since it has some analgesic action.



(1) (2) (3)

Aims and objectives

Taking in view of the applicability of heterocyclic compounds, we have undertaken the preparation of heterocyclic compounds bearing pyridines, pyrimidines and Tetrahydrothieno [2, 3 -c] pyridines nucleus. The placements of a wide variety of substituents on these nuclei have been designed in order to evaluate the synthesized products for their pharmacological profile against selected strains of bacteria and fungi.

During the course of work, looking to the application of heterocyclic compounds, several entities have been designed, generated and characterized using spectral studies. The details are as under.

1. To generate several derivatives of spiro pyridines and their benzamides derivatives Such as chloro-amine coupling, Suzuki coupling, ether coupling and chloro-thio coupling etc.
2. To generate several derivatives of spiro pyrimidine and their piperazine derivatives such as benzoylpiperazine and benzenesulfonyl piperazine etc.
3. To generate several derivatives of Tetrahydrothieno [2, 3-c] pyridines such as Schiff base & its cyclized product and urea derivatives.
4. To check purity of all synthesized compounds using thin layer chromatography.
5. To characterize these synthesized products for structural elucidation using various Spectroscopic techniques like IR, 1H NMR, mass spectral studies and C, H, N analysis.
6. To evaluate these new synthesized products for drug potential against different strains of bacteria and fungi. Antimicrobial screening to the level of minimum inhibitory concentration of each compound of the series, using Agar Dilution Method.

Accordingly the work presented in the thesis is discussed as under

PART-I : STUDIES ON PYRIDINES.

PART-II : STUDIES ON PYRIMIDINES.

PART-III : STUDIES ON TETRAHYDROTHIENO [2, 3-c] PYRIDINES.

Part-I (Pyridylspiro derivatives)

INTRODUCTION

Pyridine is a heterocyclic organic compound with the chemical formula C_5H_5N. It is structurally related to benzene, with one CH group replaced by a nitrogen atom. It is used as a precursor to agrochemicals and pharmaceuticals and is also an important reagent. The pyridine ring occurs in many important compounds, including the vitamins nicotinamide and pyridoxal.

Preparation and occurrence

Pyridine was originally industrially produced by extraction from coal tar. It is currently synthesized from formaldehyde, ammonia, and acetaldehyde:

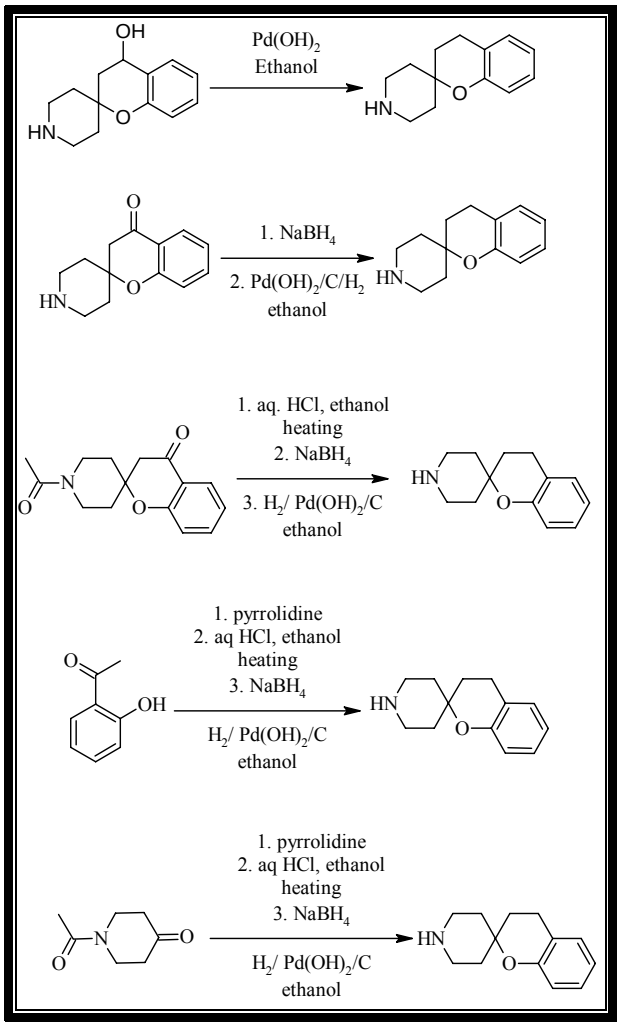
$$CH_2O + NH_3 + 2\ CH_3CHO \rightarrow C_5H_5N + 3\ H_2O + 2\ H_2$$

This process (Chichibabin pyridine synthesis) involves the intermediacy of acrolein. An estimated 26,000 tons were produced worldwide in 1989. Condensations of ammonia sources and related unsaturated carbon sources affords alkyl- and aryl-substituted pyridines, e.g. monomethyl compounds (picolines), dimethyl compounds (lutidines), and trimethyl derivatives (collidines) [1]. Pyridine occurs in numerous plants, although this was mostly recorded just by smell. Goris and Larsonneau [2] show definite evidence of its presence in belladonna leaves, while Kuhn and Schäfer [3] [4] of its presence in the roots of the same plant.

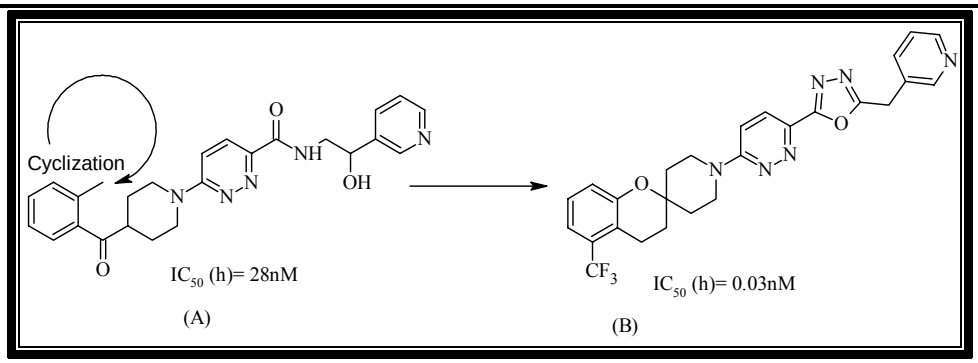
For specialized applications, the synthesis of the pyridine skeleton is well developed.[5] The Hantzsch pyridine synthesis, for example, is a multicomponent reaction involving formaldehyde, a keto-ester and a nitrogen donor. The Kröhnke pyridine synthesis involves the condensation of 1, 5-diketones with ammonium acetate in acetic acid followed by oxidation. The Ciamician-Dennstedt Rearrangement entails the ring-expansion of pyrrole with dichlorocarbene to 3-chloropyridine.[6] In the Gattermann-Skita synthesis, [7] a malonate ester salt reacts with dichloromethylamine. [8]

Synthetic Aspects

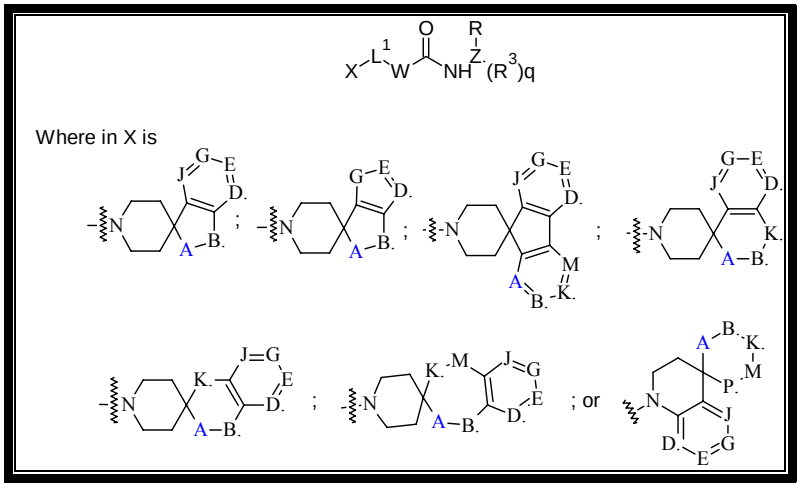
Yang et al [9] Systematic SAR studies of the different regioisomers and homologues of the spiro (indane-1, 4′-piperidine) moiety in the growth hormone secretagogue L-162,752. Among them, spiro (3*H*-1-benzopyran-2, 3′-piperidine) was found to afford secretagogue with low nanomolar in vitro activity. This paper describes various methods for the preparation of spiro derivatives having Pyridine moiety which can be explained as below.



Recently Uto et al [10] describes Modification of the benzoylpiperidine part of **(A)** led to a cluster of novel and potent spiropiperidine-based stearoyl-CoA desaturase (SCD)-1 inhibitors. After comprehensive optimization, a potent and orally bioavailable spiropiperidine-based SCD-1 inhibitor **(B)** was identified.



WO2007/136605 [11] relates to a novel class of aryl-fused spirocyclic compounds. These compounds can inhibit histone deacetylase and are suitable for use in selectively inducing terminal differentiation, and arresting cell growth and/or apoptosis of neoplastic cells, thereby inhibiting proliferation of such cells. Thus, the compounds of the present invention are useful in treating a patient having a tumor characterized by proliferation of neoplastic cells. The compounds of the invention may also be useful in the prevention and treatment of TRX- mediated diseases, such as autoimmune, allergic and inflammatory diseases, and in the prevention and/or treatment of diseases of the central nervous system (CNS), such as neurodegenerative diseases. The present invention further provides pharmaceutical compositions comprising the compounds of the instant invention and safe dosing regimens of these pharmaceutical compositions, which are easy to follow, and which result in a therapeutically effective amount of these compounds invivo.



WO94/13696 [12] disclosed certain novel compounds identified as spiro piperidin and homologs which promote the release of growth hormone in humans and animals. This property can be utilized to promote the growth of food animals to render the production

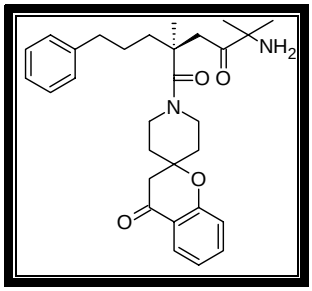
of edible meat products more efficient, and in humans, to treat physiological or medical conditions characterized by a deficiency in growth hormone secretion, such as short stature in growth hormone deficient children, and to treat medical conditions which are improved by the anabolic effects of growth hormone. Growth hormone releasing compositions containing such spiro compounds as the active ingredient thereof are also disclosed Growth hormone, which is secreted from the pituitary, stimulates growth of all tissues of the body that are capable of growing. In addition, growth hormone is known to have the following basic effects on the metabolic processes of the body:

1. Increased rate of protein synthesis in all cells of the body.
2. Decreased rate of carbohydrate utilization in cells of the body.
3. Increased mobilization of free fatty acids and use of fatty acids for energy.

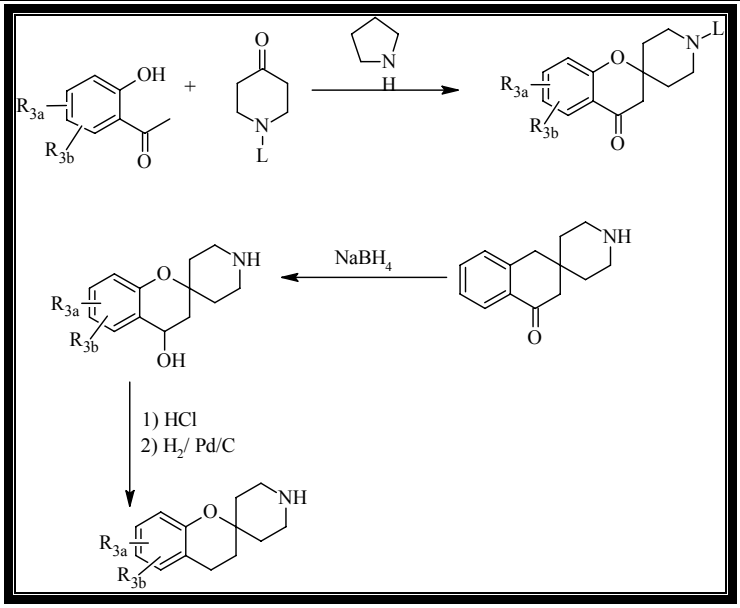
A deficiency in growth hormone secretion can result in various medical disorders, such as dwarfism.

This application covers certain spiro compounds which have the ability to stimulate the release of natural or endogenous growth hormone. The compounds thus have the ability to be used to treat conditions which require the stimulation of growth hormone production or secretion such as in humans with a deficiency of natural growth hormone or in animals used for food production where the stimulation of growth hormone will result in a larger, more productive animal.

Preferred compounds are of following formula:



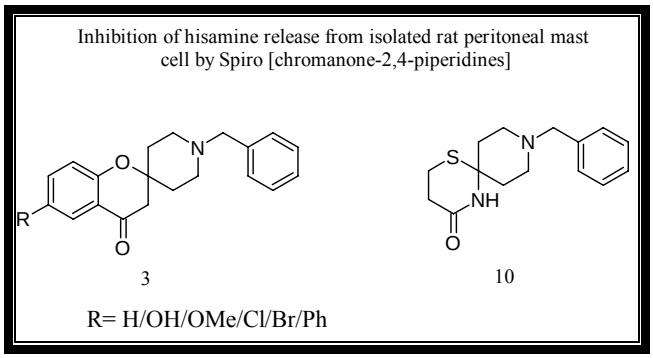
It describes following synthetic methodology for the synthesis of spiro chromane derivatives.



As illustrated in Scheme, the spiro [2H-l-benzopyran-2, 4'-piperidine] analog can be prepared from a substituted or unsubstituted 2-hydroxyacetophenone and a properly protected 4- piperidone as described by Kabbe, H. J. Synthesis 1978. 886-887. The 2- hydroxyl acetophenones, in turn, are either commercially available or can be prepared by routes in the literature known to those skilled in the art. Such methods are described by Chang, C. T. et al, in J. Am. Chem. Soc, 1961. 3414-3417. and by Elliott, J. M. et al, in J. Med. Chem. 1992. 35, 3973-3976. Removal of the protecting group as described in: Protective Groups in Organic Synthesis, Greene, T. W., Wuts, P. G., John Wiley & sons, New York, 1991, and Olofson, R.A. et al, J. Org. Chem. 1984. 49, 2081-2082, provide the amine.

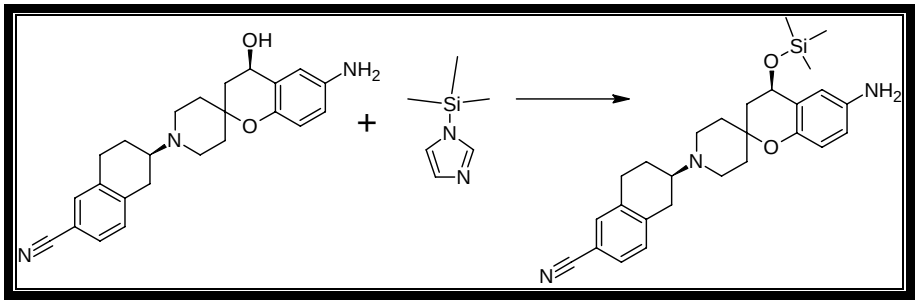
The reduction of substituted spiro-piperidinyl chromanone oximes with DIBAH reagents [13] has been known to afford the corresponding substituted 4, 5-dihydro-3*H*-spiro [1, 5]-benzoxazepine-2, 4′-piperidine. The position and electronic effects of the substituents on the aryl moiety control the observed rearrangement. Spiro-benzoxazepine analogue represents a key intermediate for the creation of a library of diverse potential bioactive drugs. With three functional groups that could be selectively and orthogonally protected, many different substituents can be introduced. The obtained analogues were assayed as the possible aspartyl protease inhibitors HIV protease (HIV-1) and β-secretase (BACE-1).

Structural modification of the isocoumarin moiety of 1'-benzylspiro-[isocoumarin-piperidines] [14], which inhibit the compound 48/80-induced release of histamine from isolated rat peritoneal mast cells, was undertaken to clarify the structure-activity relationship. Chromanone (3), chroman (4), 1, 3-benzoxazine (5), 1, 3-benzothiazine (6), and 4-quinazolinone (7) analogs were active, although there were differences in potency. Substituent effects on the benzene moiety of 3 were examined.



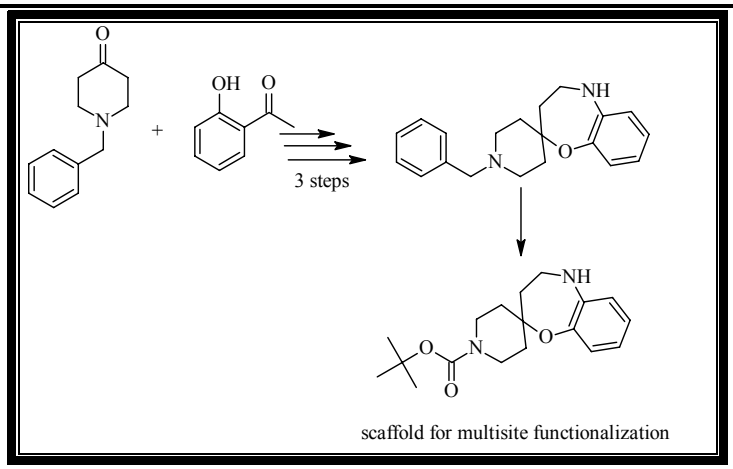
All the above compounds (in hydrochloride form) were showing remarkable effects on "% inhibition of histamine release" at various concentrations.

Raab et al [15] disclosed synthesis of following compounds.

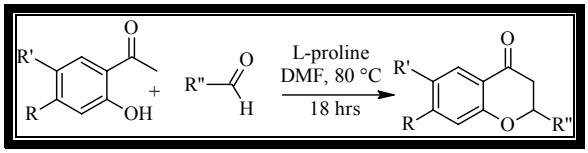


N-benzyl substituted spiro derivatives [16] have been synthesized by various methods as depicted below:

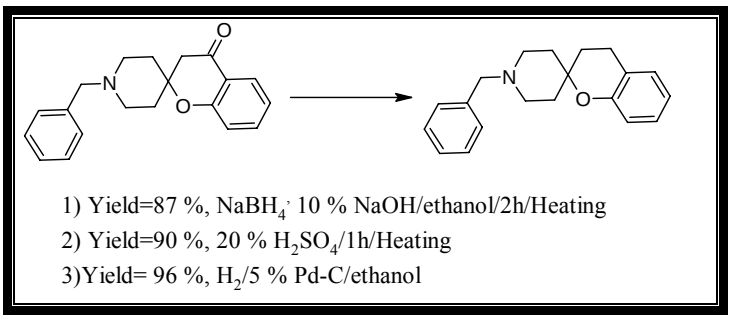
Willand et al describe the three-step synthesis of a novel 4, 5-dihydro-3H-spiro [1, 5- benzoxazepine-2, 4′-piperidine] scaffold from ortho-hydroxyacetophenone and N-benzylpiperidone. The structure of one disubstituted derivative, studied by NOESY NMR in an aqueous medium and X-ray diffraction, demonstrates that this scaffold presents side chains in a well-defined orientation. The Boc protected derivative represents a key intermediate for the combinatorial synthesis of drug-like molecules.



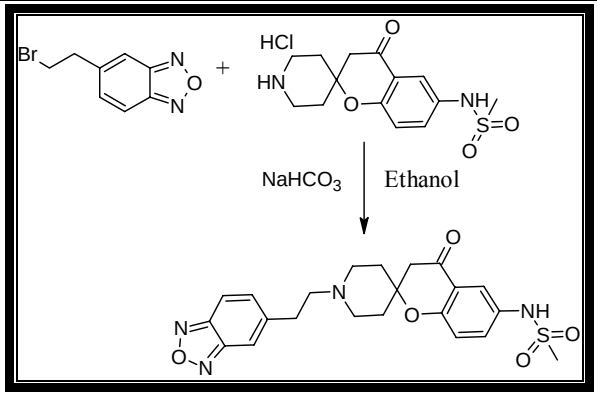
L-Proline is utilized as an efficient organocatalyst for the synthesis of substituted flavanones and chalcones in good yields [17]. The efficiency of the catalyst was proved with a variety of substrates ranging from electron-deficient to electron-rich aryl aldehydes and 2-hydroxyacetophenones.



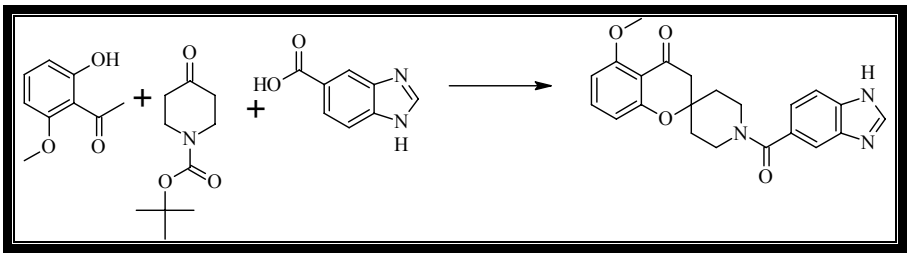
Masatoshi et al. have reported reduction of oxo group as depicted below



Elliott et al [18] reported compound having heterocyclic substitution on N-atom.

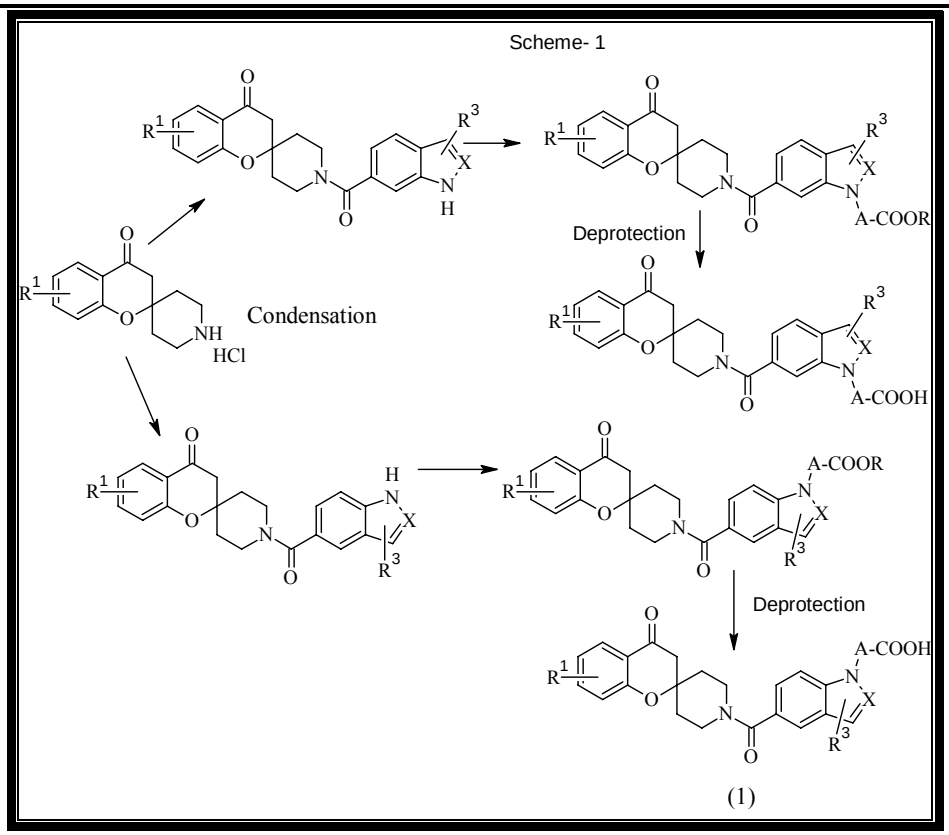


Nerenberg et al [19] reported 4-oxospiro [benzopyran-2,4′-piperidine] ring system contained within potent class III antiarrhythmic agents.

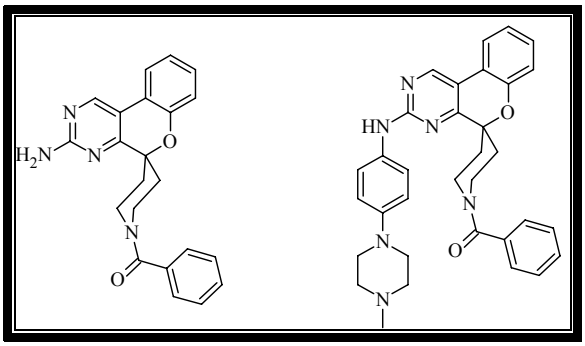


WO2010/002010 [20] relates to a compound of a general formula (I):

The compound of the invention is useful as therapeutically agents for various ACC-related diseases. It describes various methods for the synthesis of novel compounds.

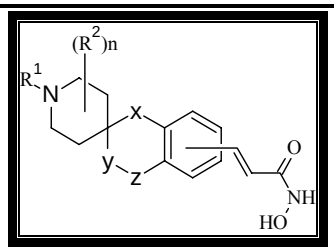


WO2009/126584 [21] reports gem-disubstituted or spirocyclic pyridine, pyrimidine and triazine derivatives that are useful in the treatment of CDK4-mediated disorders, such as cancer.



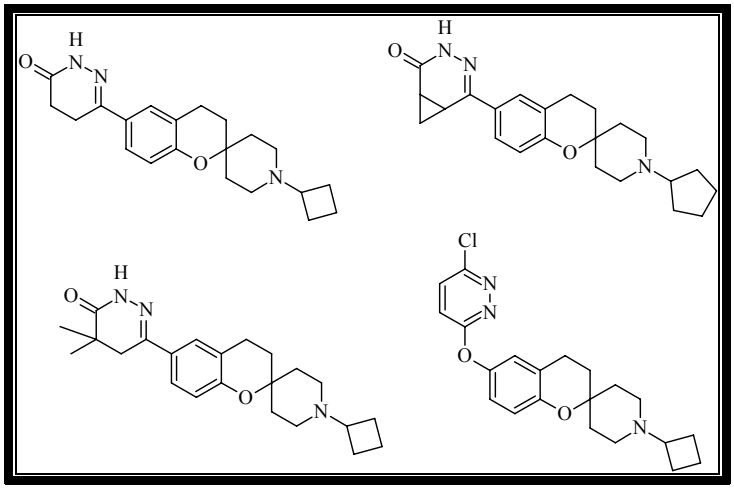
The compounds exemplified herein have been assayed.

EP2110377 [22] provides Spirocyclic derivatives as histone deacetylase inhibitors. The compound of the invention has following general structural formula

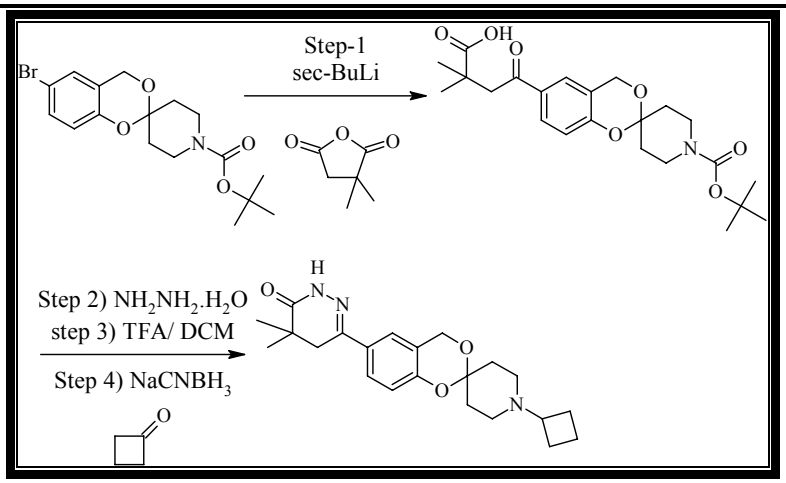


WO2009/97309 [23] provides compounds of Formula (I): their use as H3 antagonists/inverse agonists. The location and function of histaminergic neurons in the CNS suggests that compounds interacting with the H 3 receptor may have utility in a number of therapeutic applications including narcolepsy or sleep/wake disorders, feeding behavior, eating disorders, obesity, cognition, arousal, memory, mood disorders, mood attention alteration, attention deficit. Hyperactivity disorder (ADHD), Alzheimer's disease/dementia, schizophrenia, pain, stress, migraine, motion sickness, depression, psychiatric disorders and epilepsy.

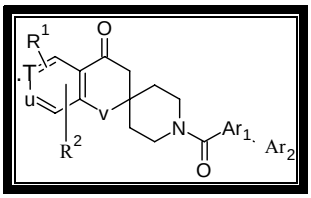
The compounds of the invention have cyclobutyl or cyclopentyl ring on the nitrogen atom of spiro piperidine derivatives. The structures can be shown as below.



Synthetic methods can be depicted as below.

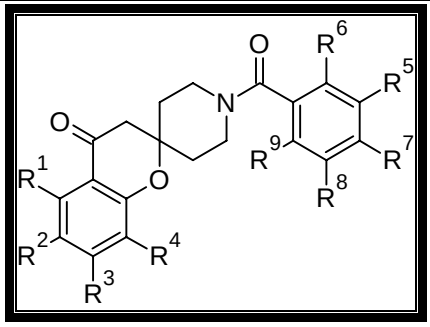


WO2008/88692 [24] provides compounds useful as therapeutical agents for various ACC-related diseases. ACC inhibitors are expected to be useful for the treatment and/or prevention of disorders such as hyperlipemia, fatty liver, dyslipidemia, hepatic dysfunction, obesity, diabetes, insulin resistance, metabolic syndrome, arteriosclerosis, hypertension, cardiac angina, heart failure, cardiac infarction, stroke, claudication, retinopathy, eyesight failure, renal failure, electrolyte metabolism disorder, neuropathy, skin ulcer, bulimia, pancreatitis, emmeniopathy, arthritis, gout, cholecystitis, gastro esophageal reflux, pickwickian syndrome, sleep apnea syndrome, neoplasm, infectious diseases, such as parasite infection, bacterial infection, viral infection, and fungal infection, and also as herbicides. This application provides compounds of following general formula.

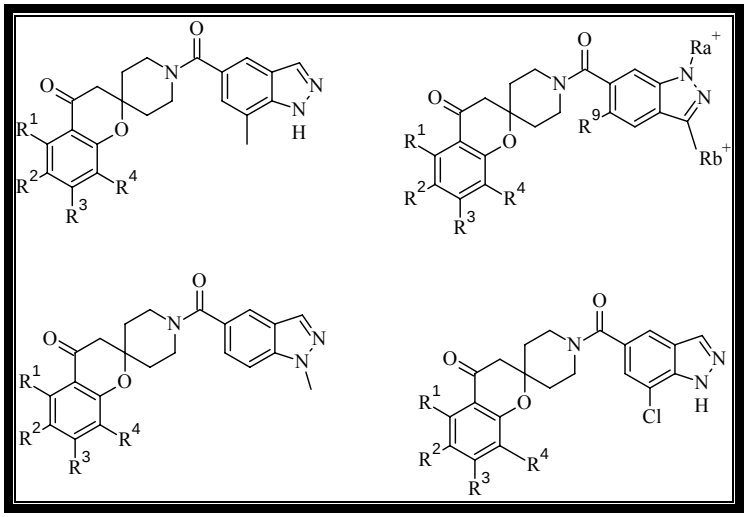


Above compounds were tested in the assay and found to have a percent inhibition of greater than or equal to 50% for ACC-I and a percent inhibition of greater than or equal to 50% for ACC-2 in the acetyl Co-A carboxylase (ACC) activity inhibition test.

WO2008/65508 [25] provides compounds of Formula (1) or a pharmaceutically acceptable salt of said compound use thereof in treating mammals suffering from the condition of being overweight.



Specific compounds synthesized in this application are having indazole side chain attached on spiro piperidine nitrogen atom.



Recently the spirochromanone derivatives are reported for various spirochromene pharmacological activities e.g. [26-39].

Current work:

Keeping all the above facts in the mind, the newer spiropiperidylchromons derivatives bearing Pyridyl substitution (for getting better therapeutic agents) have been prepared. The work is further subdivided into following sections.

I. Preparation, Characterization and antimicrobial evaluation of 4-(substituted amino)-3-nitro-N-[6-{4-oxo-3, 4-dihydro-1'H-spiro-(chromene-2, 4'-piperidin)-1'-yl} pyridine-3-yl]-benzamides.

II. Preparation, Characterization and antimicrobial evaluation of 4-(substituted phenoxy)-3-nitro-N-[6-{4-oxo-3,4-dihydro-1'H-Spiro-(chromene-2,4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

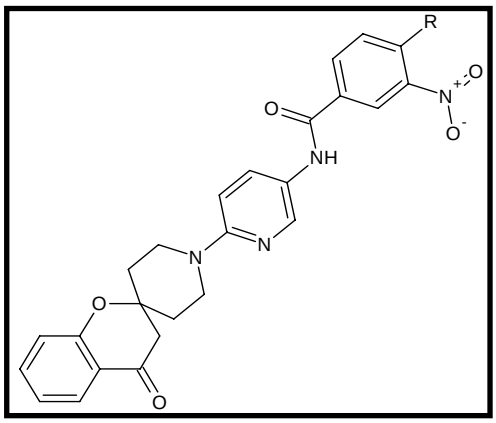
III. Preparation, Characterisation and Antimicrobial Evaluation Of 4-(Substituted Thiophenoxy)-3-Nitro-N-[6-{4-Oxo-3, 4-Dihydro-1'H-Spiro-(Chromene-2, 4'-piperidin)-1'-yl} Pyridine-3-yl]-Benzamides.

IV. Preparation, Characterization and antimicrobial evaluation of 4-(substituted phenyl)-3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'-piperidin)-1'-yl} pyridine-3-yl]-benzamides.

SECTION-I

Preparation, Characterization and antimicrobial evaluation of 4-(substituted amino)-3-nitro-N-[6-{4-oxo-3,4-dihydro-1'H-spiro-(chromene-2,4'-piperidin)-1'-yl}pyridine-3-yl]-benzamides.

Keeping in view of wide spectrum biodynamic activities[1-39] of Pyridine and with a view to have potent therapeutic agents, the synthesis of **4-(substituted amino)-3-nitro-N-[6-{4-oxo-3,4dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides (1_{a-k})** have been synthesized by the nucleophilic substitution of the chloro atom of 4-chloro 3-nitro- N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides with different substituted amines.



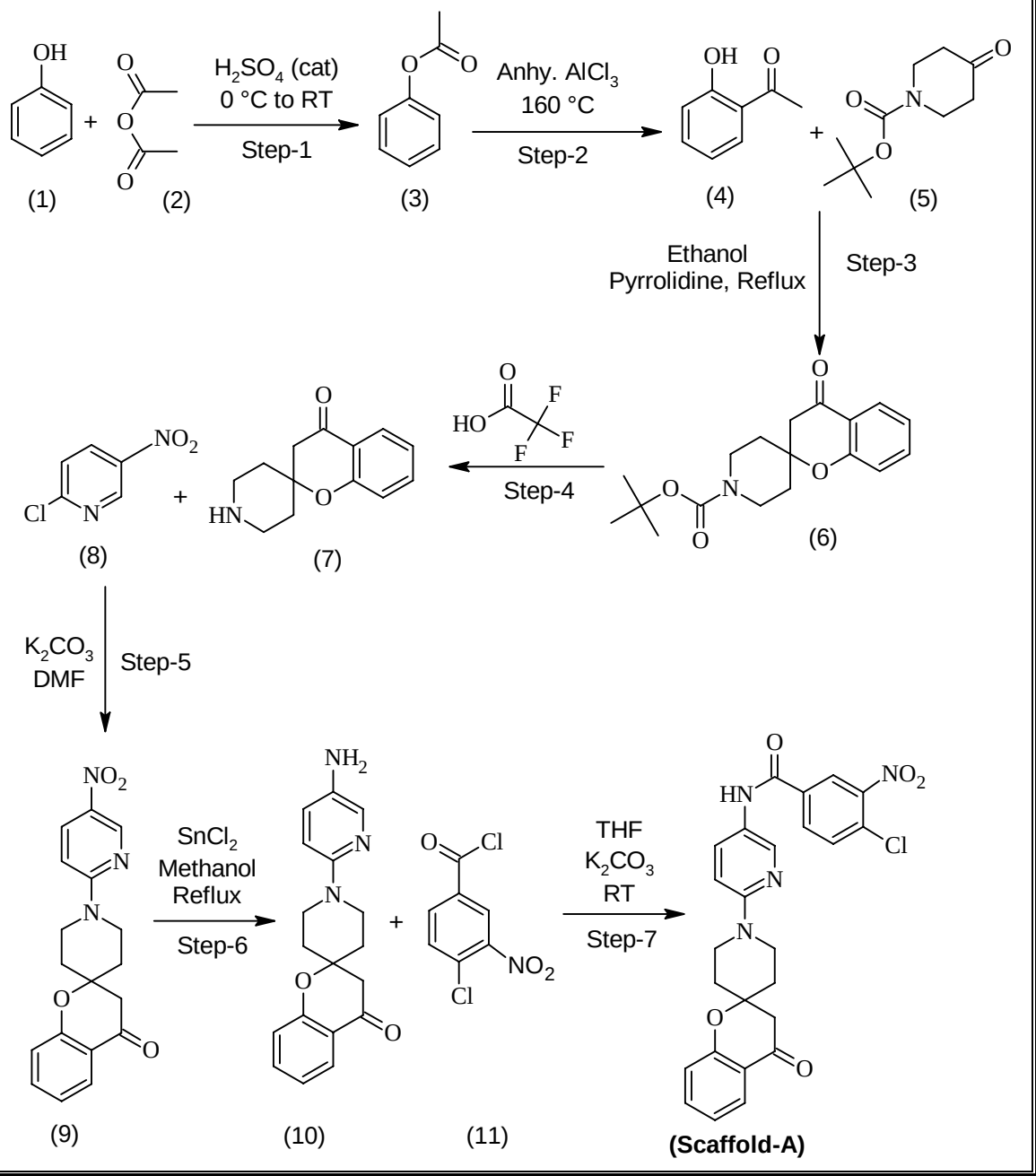
(1a-k)
R= Substituted amino

The constitution of the synthesized products (1_{a-k}) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

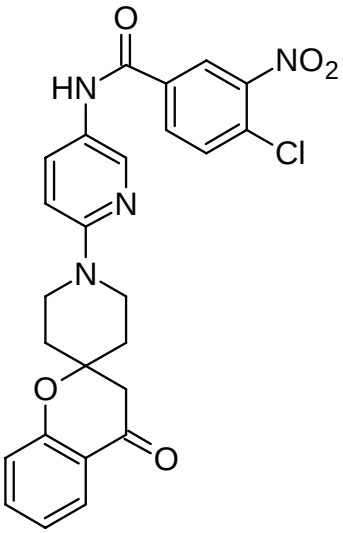
All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella peratyphi B* and they were also evaluated for antifungal activity against *Candida albicans* and *Aspergillus niger* at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum Inhibitory Concentration) values. The

antimicrobial activities of the synthesized compounds (1a-k) have been compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by **NCCLs**).

REACTION SCHEME

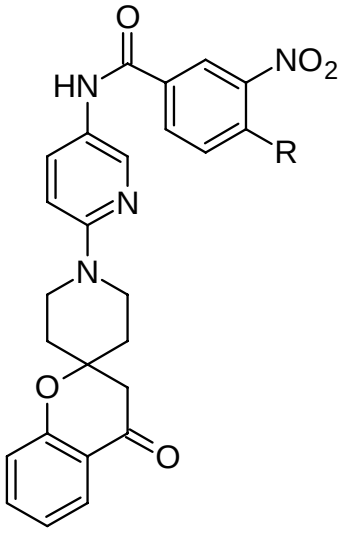


REACTION SCHEME



(Scaffold- A)

Reflux | Different Aliphatic Amine
THF, TEA



(1_{a-k})

R = Substituted amino

EXPERIMENTAL

Preparation, Characterisation and antimicrobial evaluation of 4-(substituted amino) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

[A] Synthesis of Scaffold-A.

1. Synthesis of phenyl acetate (Intermediate-3)

In RBF, Phenol (1.00 mol) and acetic anhydride (excess) were charged. Catalytic amount of sulfuric acid was added into the reaction mixture at 0°C. Then reaction mixture was allowed to warm at room temperature and stirred for 1-2 hours. After the completion of reaction, reaction mixture was quenched in crushed ice. The product was extracted with ethyl acetate. The organic phase was washed with saturated bicarbonate, brine solution and dried over sodium sulfate. The organic phase was concentrated under reduce pressure at 45°C to get desired product. Yield: 92.00 %, B.P.-218°C.

2. Synthesis of 2-Hydroxy Acetophenone (Intermediate-4)

In RBF, Intermediate-3 (1.00 mol) and anhydrous $AlCl_3$ (5.00 mol) were heated to 160^0C for 4-5 hours. Then reaction mixture was cooled to 80°C and quenched in diluted hydrochloric acid. The product was extracted with ethyl acetate and the organic layer was washed with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulphate and concentrated under reduce pressure at 45°C to get crude product which was purified by using column chromatography. The product was eluted at 11% ethyl acetate in hexane. Yield: 69.00 %, B.P- 93°C.

3. Synthesis of t-butyl 4-oxo-3, 4-dihydro-1'H- spiro [chromene-2, 4'-piperidine]-1'-carboxylate. (Intermediate-6)

In RBF, Intermediate-4 (1.00mol) and Intermediate-5 (1.00 mol) were dissolved in ethanol. Then catalytic amount of pyrrolidine was added into the reaction mixture at room temperature. The reaction mixture was heated to reflux temperature for 24 hours. After the completion of reaction, reaction mixture was concentrated under reduce pressure at 45°C. Then obtained residue was quenched in water. The product was extracted with ethyl acetate, washed with diluted hydrochloric acid and saturated sodium bicarbonate solution. The organic phase was dried over sodium sulphate and concentrated

under reduce pressure at 45°C to get crude product. The crude product was purified by crystallization in ethanol. Yield: 62.00 %, M.P. 92°C.

4. Synthesis of Spiro [chromene-2, 4'-piperidin]-4(3H)-one. (Intermediate-7)

In RBF, Intermediate-6 (1.00 mol) and triflouro acetic acid was added and stirred at room temperature for 2-3 hours. After the completion of reaction, reaction mixture was quenched in crushed ice and neutralized with sodium bicarbonate. The Product was extracted with ethyl acetate and washed with brine solution. The organic phase was dried over sodium sulphate and concentrated under reduce pressure at 45°C to get pure desired product. Yield: 90.00%, M.P- 207°C (HCl Salt).

5. Synthesis of 1'-(5-nitropyridin-2-yl) spiro [chromene-2, 4'-piperidin]-4(3H)-one. (Intermediate-9).

In RBF, 2-chloro-5-nitropyridine (Intermediate-8) (1.00 mol), Intermediate-7 (1.05 mol) and DMF were added at room temperature. Then cesium carbonate (2.50mol) was added into the reaction mixture at 0°C and reaction mixture was allowed to warm at room temperature and stirred for 4-5 hours at same temperature. After the completion of reaction, reaction mixture was quenched in chilled water. The product was filtered through Buckner funnel under vacuum and washed with water. The solid was dried under vacuum at 50^0C. Yield: 60.00%, M.P. - 237°C. Elemental Analysis: Calculated: C (63.71%), H (5.05%), N (12.38%), Found: C (62.12%), H (5.02%), N (11.98%).

6. Synthesis of 1'-(5-aminopyridin-2-yl)-4H-spiro [chromene-3,4'-piperidin]-4-one. (Intermediate-10)

In RBF, Intermediate-9 was dissolved in methanol under nitrogen atmosphere at room temperature. In reaction mixture stannous chloride was added portion wise at room temperature. Reaction mixture was heated to reflux temperature for 5-6 hours. The completion of reaction was confirmed by TLC. After the completion of reaction, reaction mixture was allowed cool at room temperature and quenched with diluted ammonia solution. The reaction mixture was filtered through highflow bed and washed with Methanol. Methanol was evaporated and the residue was diluted with ethyl acetate, washed with water and brine solution. The organic phase was dried over sodium sulfate and concentrated under reduce pressure at 50°C to get desired crude product. The crude product was purified by triturating with diethyl ether. Obtained solid was dried at 45°C.

Yield: 65.00 %, M.P. - 194°C. Elemental Analysis: Calculated: C (69.88%), H (6.19%), N (13.58%), Found: C (69.11%), H (6.01%), N (12.92%).

7. Synthesis of 4-chloro - 3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides. (Scaffold-A)

In RBF, Intermediate-10 (1.20 mol) in THF and K_2CO_3 (2.50 mol) was added at room temperature. In reaction mixture, the solution of 4-chloro,3-nitrobenzoylchloride (1.1 eq.) in THF was added drop wise at room temperature and stirred for 1 hour at same temperature. Then solvent was removed under vacuum at 45°C. The obtained residue was quenched in water to get solid. The solid was filtered, washed with water and dried under reduced pressure at 45°C to get pure product. Yield: 82.00 %, M.P.-219-222°C. Elemental Analysis: Calculated: C (60.92%), H (4.29%), N (11.37%), Found: C (60.53%), H (4.21%), N (11.01%).

[B] Synthesis of 4-(Morpholine) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamide.

In RBF, Scaffold-A (1.00 mol) in THF and TEA (2.50 mol) was added at room temperature. In reaction mixture, Morpholine (2.0 mol) was added at room temperature and heated at reflux temperature for 5-6 hour. Then solvent was removed under vacuum at 45°C. The obtained residue was quenched in water to get product. The solid was triturating with ethanol to get pure product, yield: 58%. M.P.- 235°C. Elemental Analysis: Calculated: C (64.08%), H (5.38%), N (12.88%), Found: C (63.99%), H (5.46%), N (12.81%).

Similarly, other compounds (1_{a-k}) were synthesized by above mentioned process (B) from Scaffold-A using different aliphatic amines. The physical data are recorded in Table-1.

[C] Antimicrobial activity of 4-(substituted amino) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

All the compounds have been evaluated for antimicrobial activity, described as under.

The Activities of all the synthesized compounds are recorded in table no. 1(A) and 1(B).

Protocol for studying Antimicrobial activity

Agar Dilution procedure:

The Agar Dilution Method for determining antimicrobial susceptibility is a well-established technique [29, 30]. The antimicrobial agent is incorporated into the agar medium in each plate containing a different concentration of the agent.

Preparation of stock solution:

10 mg of compound was dissolved in 5 ml of DMSO to prepare the main stock of compounds to be tested. 1 ml of this main stock was added to 19 ml of Mueller Hinton Agar medium to take the final concentration of 1000 µg/ml in the agar medium. The main stock solution was further diluted in demineralized water by two fold dilution procedure to obtain the desired concentration in the agar medium, i.e.2000 µg/ml, 1000 µg/ml (Primary screening), 1000 µg/ml, 500 µg/ml 250 µg/ml (Secondary screening), 250 µg/ml, 125 µg/ml, 62.5µg/ml 31.2 µg/ml, 15.6 µg/ml, 7.8µg/ml, 3.9 µg/ml, 1.9 µg/ml, 0.45 µg/ml, 0.22 µg/ml (Tertiary screening).

Reagents and Materials:

Microorganisms and Media:

ATCC Bacterial cultures obtained from NCL, Pune were

1. *Escherichia coli* ATCC 25922
2. *Staphylococcus aureus* ATCC 25923
3. *Bacillus subtilis* ATCC 6633
4. *Candida albicans* ATCC 10231

Clinical isolates from Civil Hospital, Rajkot and Medical College, Jamnagar were

1. *Escherichia coli*
2. *Staphylococcus aureus*
3. *Salmonella peratyphi B*
4. *Candida albicans*
5. *Bacillus subtilis*
6. *Aspergillus Niger*

Muller Hinton Agar medium

Muller Hinton Agar medium was used to carry out antimicrobial analysis of newly synthesized compounds. The composition of Muller Hinton Agar medium is as under

Composition of Muller Hinton Agar	G/liter
Beef Infusion	300.00
Casein acid hydrolysate	17.50
Starch	1.5
Agar	17.50
Final pH (at 25°C)	7.3+0.2

Preparing Agar Dilution plates:

(1) Appropriate dilution i.e. 1.00 ml quantity of antimicrobial solution are added to molten test agars having 19.0 ml quantity that have been allowed to equilibrate in a water bath to 45 to 50°C. One part of antimicrobial solution is added to nine parts of liquid agar.

(2) The agar and antimicrobial solution were mixed thoroughly and the mixtures poured into borosil glass petridishes having 9 cm diameter on a level surface to result in an agar depth of 3 to 4 mm.

(3) The plates should be poured as quickly after mixing as possible to prevent cooling and partial solidification in the mixing container, avoiding bubbles.

(4) The agar was allowed to solidify at room temperature and the plates were either used immediately or stored in sealed plastic bags at 2 to 8°C for up to five days for reference work or longer for routine tests.

(5) Plates stored at 2-8°C were allowed to equilibrate at room temperature before use, assuring that the agar surface was dry before inoculating the plates. If necessary, plates were placed in an incubator to hasten drying of the agar surface.

Source of Antimicrobial agents:

It was stored in air tight container or under desiccation at 40C if in powder form. All synthetic organic compounds were analyzed for their antimicrobial activity by Dr. Chetanaben Rajyaguru, Asst. professor, Microbiology department, MVM Science and Home science college, Rajkot.

Control plates:

(1) Drug-free plates prepared from the medium were used as growth controls. These plates were free from Antimicrobial agents as well as solvent.

(2) Control plates prepared from the base medium with addition of only 1.00 ml solvent DMSO (free of antimicrobial agents), were called as solvent control plate.

Inoculation:

One loopful of culture from the slant was inoculated into 5 ml Muller Hinton broth in a test tube. The tube was incubated at 32°C for 4 to 6 hours till the absorbance at 625 nm, equals that of 0.5 Mac Farland standard. The absorbance readings were taken against a sterile Muller Hinton broth Media blank. The density of the suspension was adjusted to 108 colony forming units (CFU) per milliliter by comparing its turbidity to a MacFarland 0.5 $BaSO_4$ standard.

The bacterial cultures were then transferred at 2-8°C and maintained at the same temperature till further use. Appropriate dilution of the bacterial cultures were made based on the viable count of the bacterial cultures previously done to establish the relationship between absorbance at 625 nm and viable count before inoculating the plates with the antimicrobial test agents of this 2 µg/ml diluted culture was used to spot inoculate the plates with antimicrobial agents using micropipette.

Preparation of 0.5 Mac Farland standards:

It was used as a reference for turbidity measurement for bacterial cultures before they were used as inoculums for spot inoculate the Mueller Hinton Agar media containing antimicrobial agents.

Briefly, 0.5 ml of 1.175% w/v $BaCl_2$ solution was added to 99.5 ml of 1% v/v H_2SO_4 solution with constant stirring, the absorbance of the solution was measured 625 nm against demineralized water blank by UV spectrophotometer. The absorbance was in the range of 0.08 to 0.1 optical densities.

Incubation:

All plates were kept after inoculation at 37°C for 24 hours in an ambient air incubator.

(II) Antifungal activity

Standard drug ciprofloxacin and fluconazole were used to investigate the MICs of standard bacterial and fungal ATCC cultures.

These MICs were used for the comparison of MICs of newly synthesized organic compounds.

Microbial culture	Ciprofloxacin
1. *Escherichia coli* ATCC 25922	0.4 µg/ml
2. *Staphylococcus aureus* ATCC 25923	1.9µg/ml
3. *Bacillus Subtilis* ATCC 6633	7.8µg/ml
4. *Salmonella peratyphi B*	1.4µg/ml
Fungal culture	Fluconazole
1. *Candida albicans*	0.4 µg/ml
2. *Aspergillus Niger*	0.7 µg/ml

Antifungal Activity Determination:

For fungal culture the fungal media Yeast Nitrogen Base Agar plate (YNBG) (Difco make) 6.7gm and Glucose 10 gm dissolved in 100 ml distilled water and filter sterilized was used. The inoculums were prepared from 3-4 days old sabourauds Dextrose agar slants. The growth was uniformly mixed with distilled water. The size of Inoculum prepared for inoculating YNBG agar plates was 10^2 -10^3 cfu/ml, adjusted with MacFarland solution. After inoculation of properly diluted fungal solution, the plates were incubated at 37°C for 48 hours.

Quality Control:

(1) Growth control was performed to check viability of the organisms.

(2) Purity control by sample inocula streaked on a suitable agar plate.

(3) Inoculum control by plate counts was performed on representative Inoculum periodically.

(4) End point interpretation control was independently read for all dilution Plates.

Rigorous quality control was maintained through the experimentation by checking large numbers of variables that may affect the results. Physical and chemical characteristics of Mueller Hinton agar media were monitored, such as pH and depth of agar. The final control was provided by a series of reference strains including Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231. The reference strains were stored at temperature below -20°C.

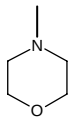
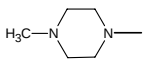
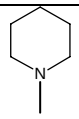
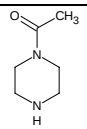
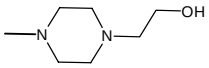
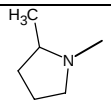
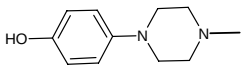
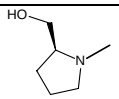
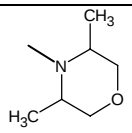
Determination of type of Antimicrobial Activity:

Organic compounds may be bacteriostatic or bactericidal for microbial cultures. To check this, sub culturing was carried out from the Mueller Hinton Agar plates showing no visible growth of bacteria on to Nutrient Agar plates. After streaking Nutrient Agar plates were well incubated for 24 hours in incubator at 37°C temp. Then after observation was made to see the colonies formed. If colonies were found the dilution was considered as bacteriostatic and if no colonies observed, it was considered as bactericidal. Bactericidal dilution of the organic compounds was considered as exact MIC (Minimum inhibitory concentration) for a particular organic compound.

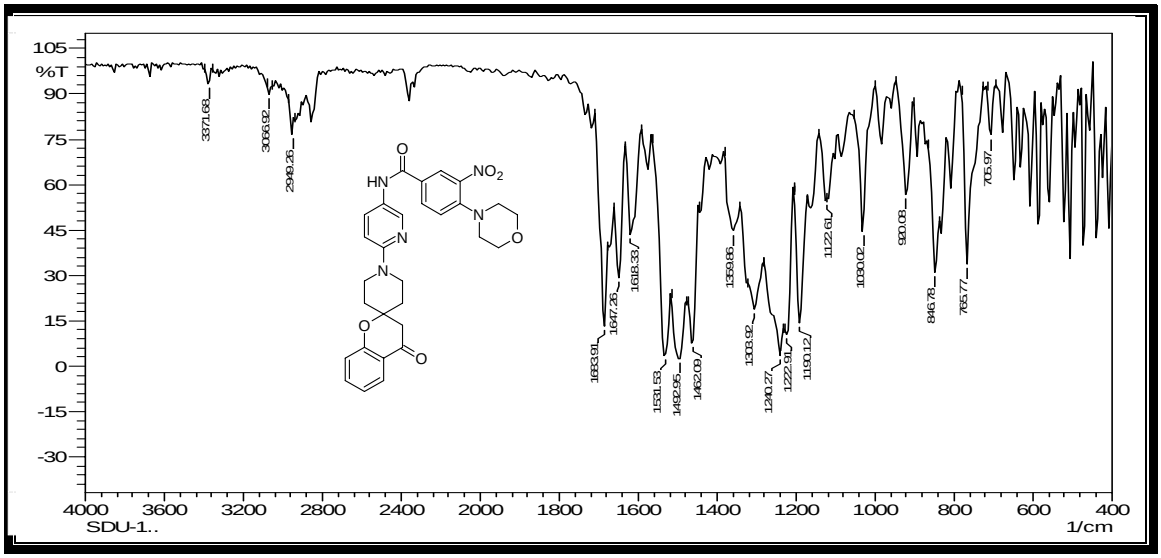
Interpretation of Results:

1. In case of positive control plate due to complete absence of antimicrobial agent and its solvent bacterial/ fungal cultures gave luxuriant growth.
2. in the solvent control plate little inhibition of growth of microbes due to presence of organic solvent DMSO.
3. The microbial cultures, if shown 1-5 colonies per spot inoculated instead of confluent growth as in the control plate, it was considered to be inhibited by test antimicrobial compounds.
4. The microorganisms that were sensitive to the concentration of antimicrobial agent in Mueller Hinton agar plate did not produce a circle of growth at the Inoculum site.
5. The microbes that were resistant to it appeared as circular colonies. The agar plates were marked with a grid so that each microorganism could be identified by a number.

TABLE NO-1: Physical constants of 4-(substituted amino) -3-nitro-N-[6-{4-oxo-3, 4-dihydro-1'H-spiro-(chromene-2, 4'-piperidin)-1'-yl} pyridine-3-yl]-benzamides (1a-k).

Sr. No.	Substitution R	M.F./ M.W.	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
1a		$C_{29}H_{29}N_5O_6$ 543	235	58	64.08 63.99	5.38 5.46	12.88 12.81
1b		$C_{30}H_{32}N_6O_5$ 556	136	45	64.73 64.52	5.79 5.41	15.10 14.26
1c		$C_{29}H_{29}N_5O_5$ 527	181	62	66.02 65.80	5.54 5.32	13.27 13.11
1d		$C_{30}H_{31}N_5O_5$ 541	192	63	66.53 66.38	5.77 5.60	12.93 12.71
1e		$C_{31}H_{32}N_6O_6$ 584	160	50	63.69 63.54	5.52 5.30	14.38 14.10
1f		$C_{27}H_{27}N_5O_5$ 501	138	65	64.66 64.26	5.43 5.14	13.96 13.62
1g		$C_{31}H_{34}N_6O_6$ 586	155	40	63.47 63.15	5.84 5.45	14.33 14.13
1h		$C_{30}H_{31}N_5O_5$ 541	167	61	66.53 66.39	5.77 5.53	12.93 12.71
1i		$C_{35}H_{34}N_6O_6$ 634	192	48	66.23 65.51	5.40 5.31	13.24 13.09
1j		$C_{30}H_{31}N_5O_6$ 557	194	75	64.62 64.36	5.60 5.26	12.56 12.30
1k		$C_{31}H_{33}N_5O_6$ 571	209	78	65.14 65.01	5.82 5.58	12.25 12.10

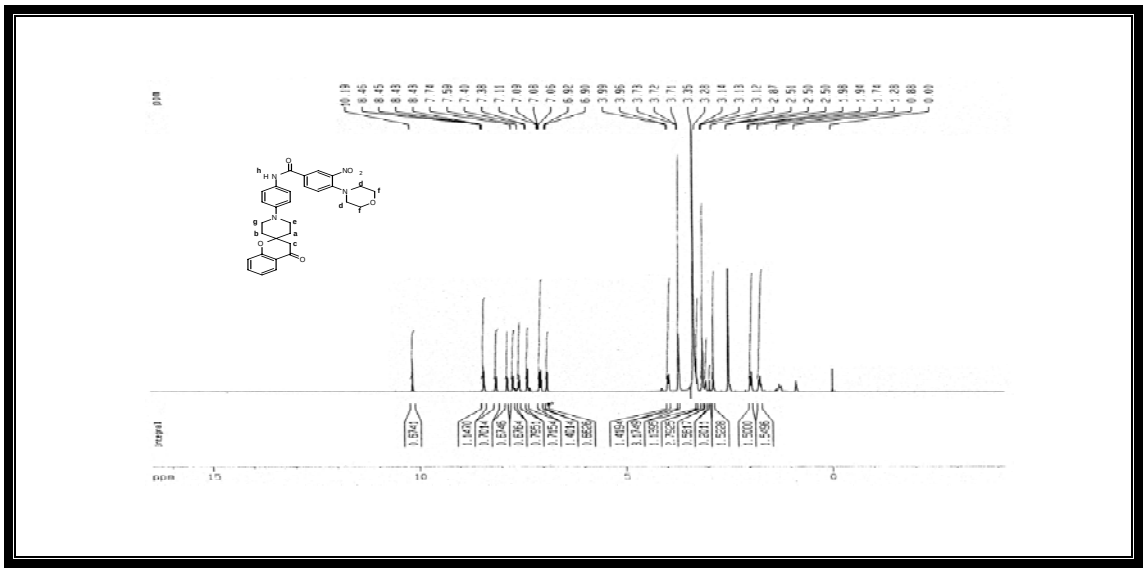
IR Spectral Study of 4-morpholine -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (1a).

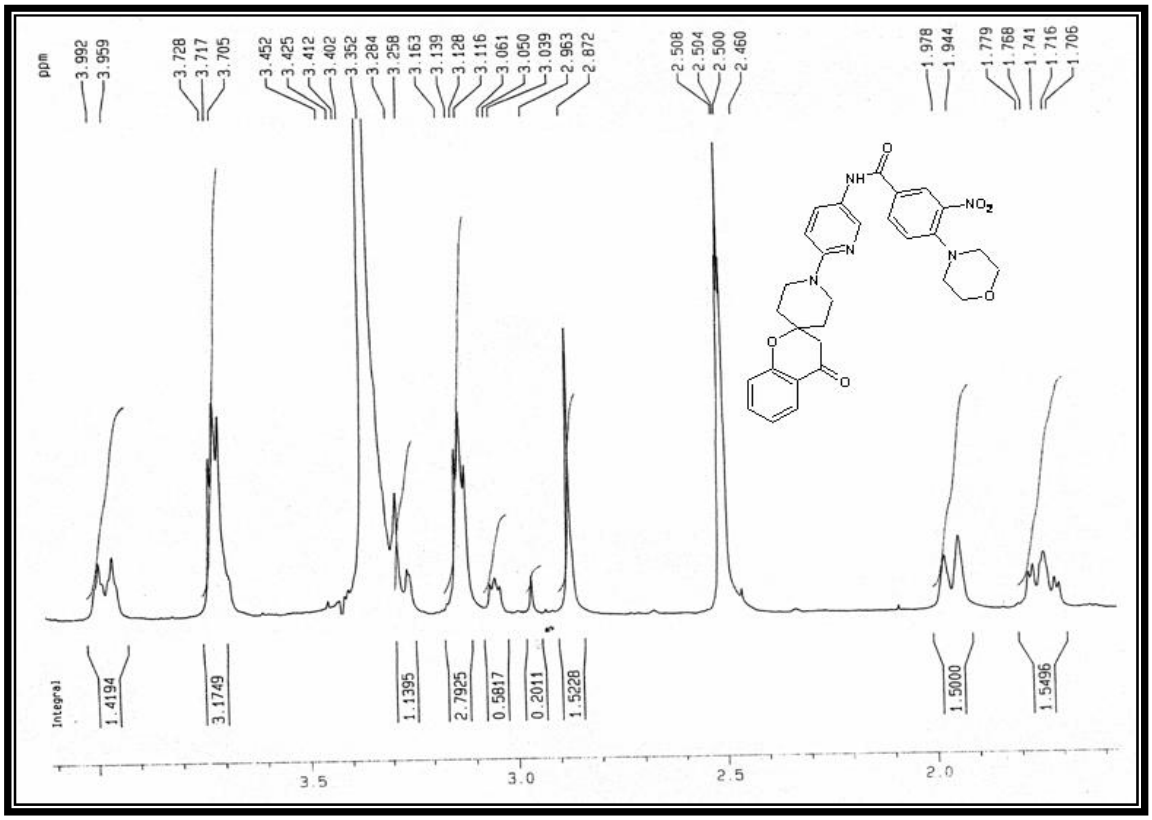


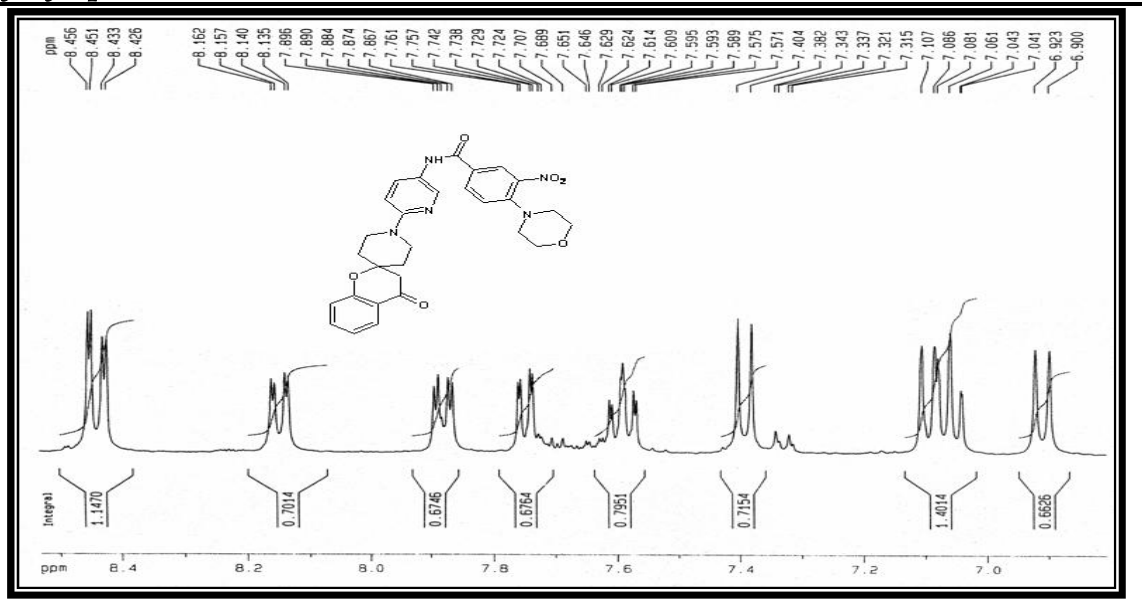
Instrument: SHIMADZU FTIR 8400 Spectrophotometer; **Frequency range**: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No.
		Observed	Reported	
Alkane	C-H asym. str.	2949	2975-2850	42
	C-H sym. str.	2840	2900-2800	42
	C-H asym. def.	1462	1460-1400	42
	C-H sym. Def.	1359	1385-1300	42
Aromatic	C-H str.	3066	3080-3010	42
	C=C ring skeleton	1531	1600-1450	44
		1492	1600-1450	44
	p-disubstituted benzene	840	850-750	44
Pyridine moiety	-C=N str.	1684	1690-1570	43
	C-N str.	1304	1310-1250	43
	C-NO2 asym. Str.	1550	1570-1500	43
Chromanone moiety	C-O-C asym. Str.	1240	1250-1200	42
	C-O-C sym. Str.	1030	1050-1010	42
	Cyclic C=O str.	1684	1740-1630	42
Amide Ketone	-C=O str.	1647	1740-1650	42
	N-H str.	3371	3500-3310	43

1H-NMR Spectral Study of 4-morpholine -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H-spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (1a).



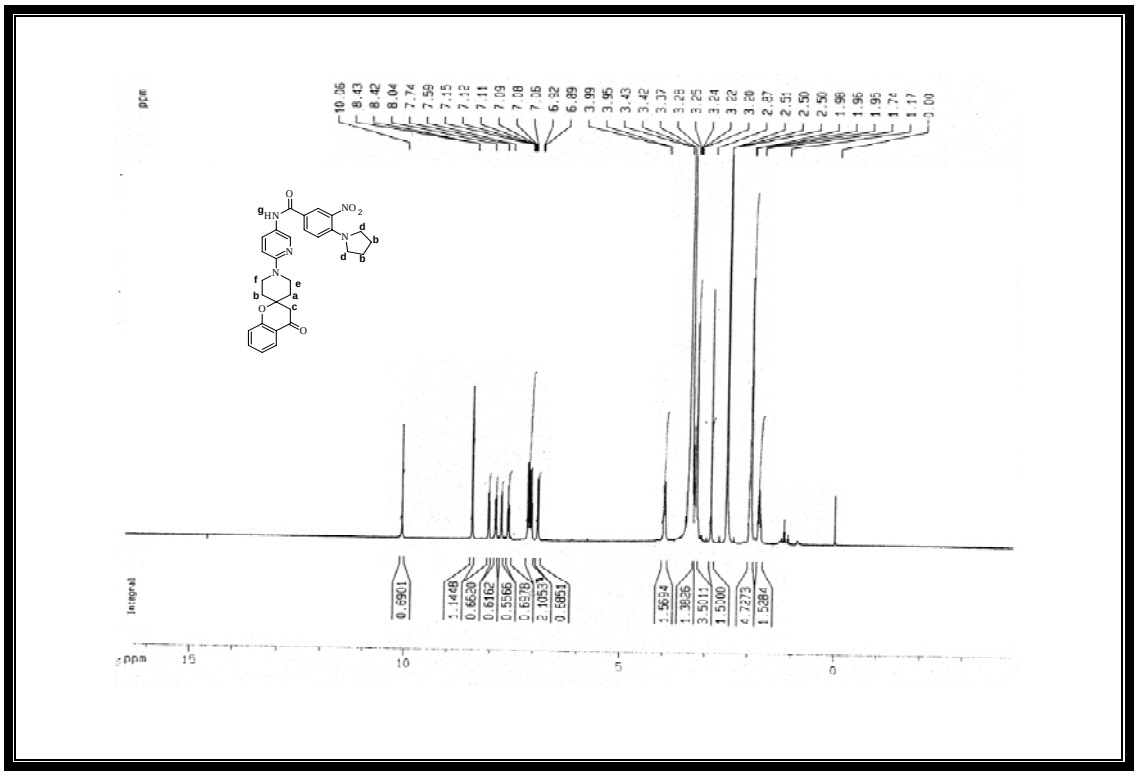


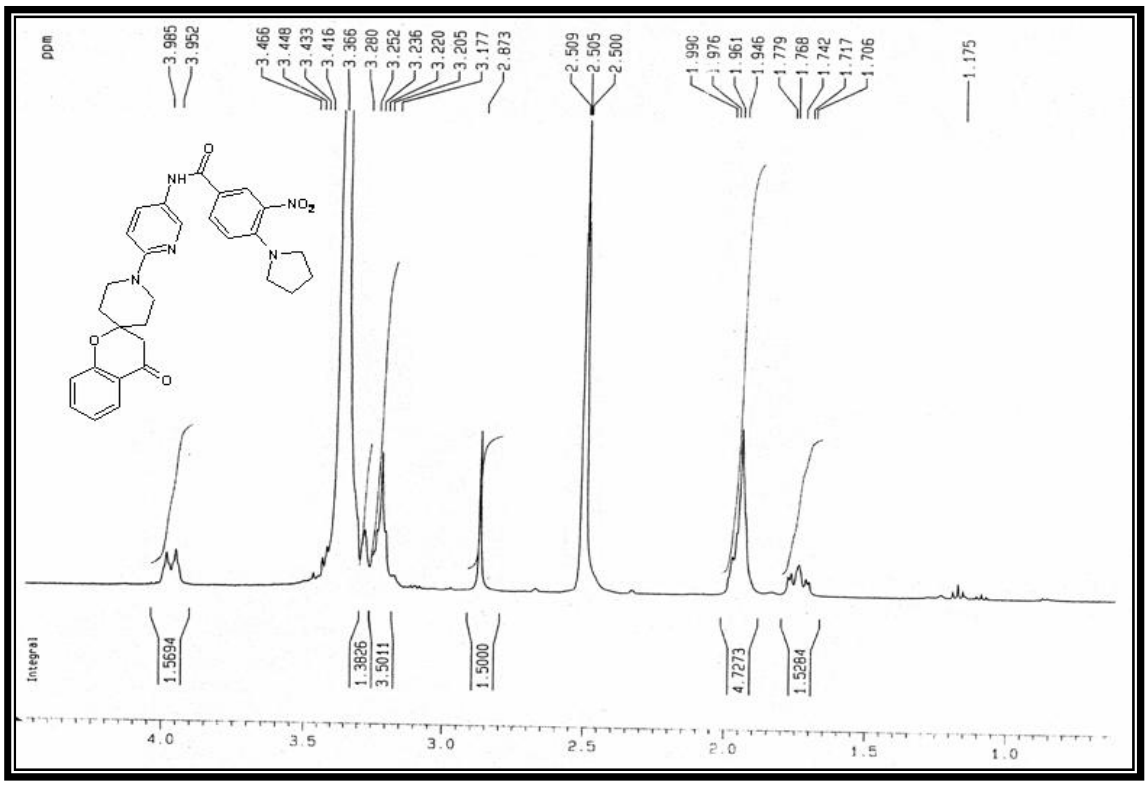


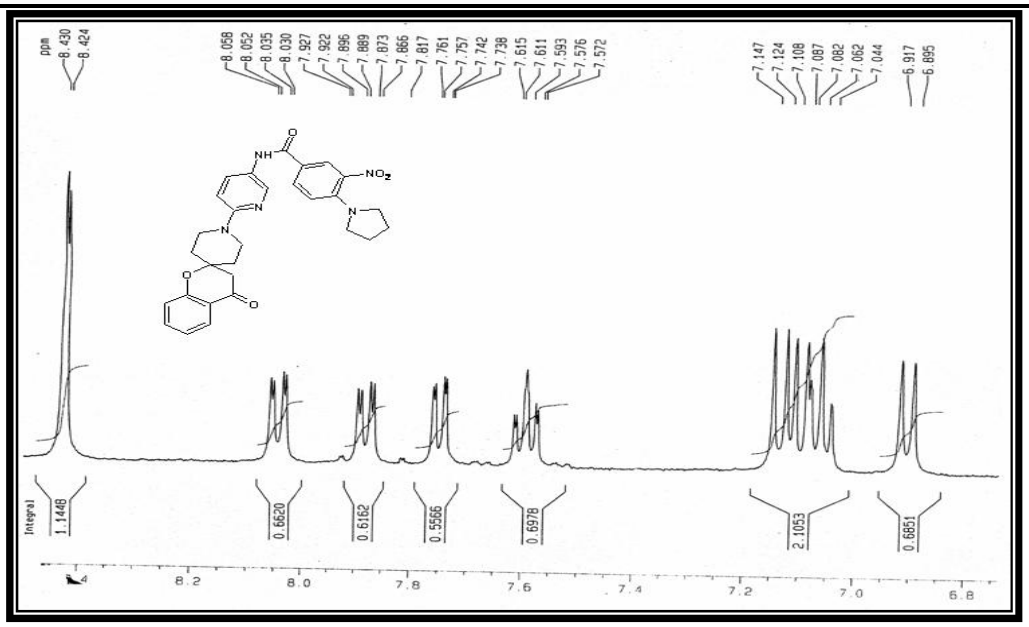
Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO- *d6.*

Serial No.	Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	1.74	2H	triplet	-(CH2) a
2	1.95	2H	triplet	-(CH2) b
3	2.872	2H	Singlet	-(CH2) c
4	3.050	4H	Triplet	-(CH2)2 d
5	3.258	2H	Triplet	-(CH2) e
6	3.71	4H	Triplet	-(CH2) f
7	3.959	2H	Triplet	-(CH2) g
8	6.900-8.456	10H	complex	Ar-H
9	10.19	1H	Singlet	-(NH) h

1H NMR spectra of 4-Pyrrolidine -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.



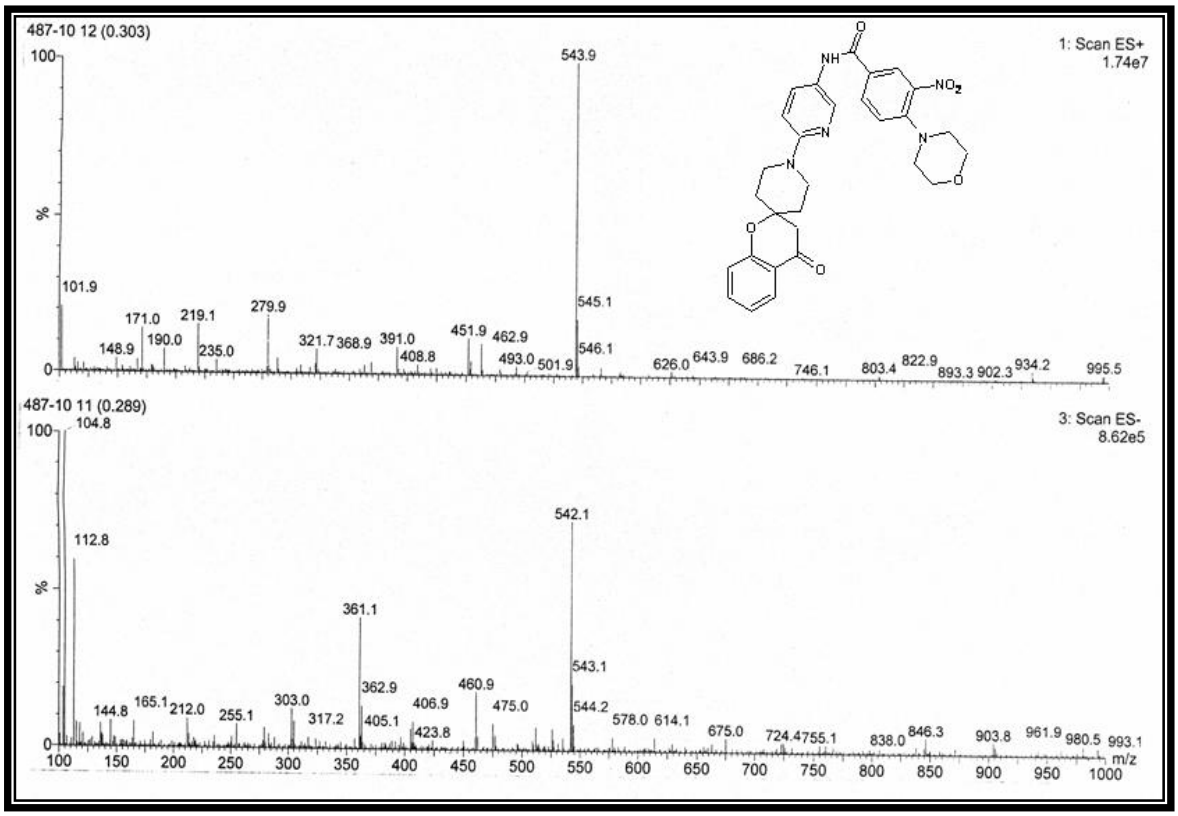




Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO- *d6.*

Serial No.	**Signal Position (δ ppm)**	**Relative No. of Protons**	**Multiplicity**	**Inference**
1	1.74	2H	Triplet	-(CH2) a
2	1.96	6H	Multiplate	-(CH2)3 b
3	2.87	2H	Singlet	-(CH2) c
4	3.22	4H	Triplet	-(CH2)2 d
5	3.25	2H	Multiplate	-(CH2) e
6	3.95	2H	Multiplate	-(CH2) f
7	6.895-8.430	10H	complex	Ar-H
8	10.06	1H	Singlet	-(NH) g

Mass Spectral Study of 4-morpholine -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (1a).



Possible Mass Fragmentation pattern:

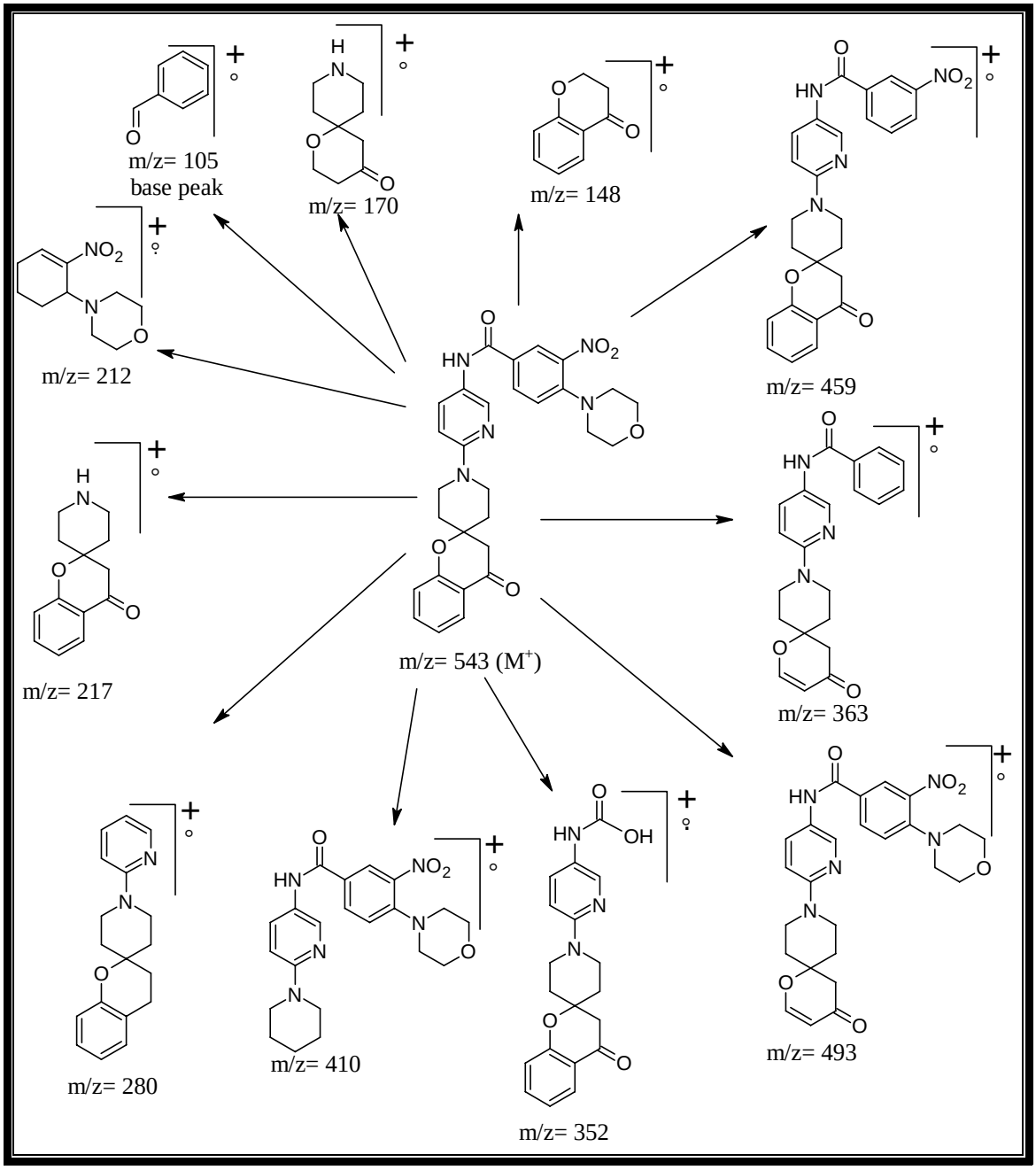


TABLE NO. 1a: Antimicrobial Activity Of 4-(Substituted Amino) -3 Nitro-N-[6-{4-Oxo-3, 4 Dihydro-1'H- Spiro-(Chromene-2, 4'-Piperidin)-1'-yl} Pyridine-3-yl]-Benzamides (1a-k).

TABLE NO-1(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
1a	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
1b	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+
1c	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
1d	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-
1e	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
1f	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	-
1g	+	+	-	-	+	+	+	-	+	+	-	-	+	+	+	-
1h	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
1i	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
1j	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	-
1k	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-

Reference drugs:	***S. aureus***	***B. Subtilis***	***E.Coli***	***S. Peratyphi B***
Ciprofloxacin	**1.9**	**7.8**	**0.4**	**1.4**

TABLE NO. 1b: Antimicrobial Activity Of 4-(Substituted Amino) -3 Nitro-N-[6-{4-Oxo-3, 4 Dihydro-1'H- Spiro-(Chromene-2, 4'-Piperidin)-1'-yl} Pyridine-3-yl]-Benzamides (1a-k).

TABLE NO-1(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (ug/ml*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
1a	+	+	-	-	+	+	-	-
1b	+	+	+	+	+	+	+	+
1c	+	+	+	-	+	+	-	-
1d	+	+	+	-	+	+	+	-
1e	+	+	+	-	+	+	-	-
1f	+	+	-	-	+	+	-	-
1g	+	+	-	-	+	+	+	-
1h	+	+	+	+	+	+	+	-
1i	+	+	+	-	+	+	+	-
1j	+	+	+	-	+	+	+	-
1k	+	+	-	-	+	+	-	-
Reference drugs:		***A.niger***				***C. albicans***		
Fluconazole		**0.7**				**0.4**		

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 µg/ml conc. (2) Secondary Screening 1000 µg/ml to 250 µg/ml conc. (3) Tertiary Screening start from 125 µg/ml to 3.9 µg/ml. The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed for tertiary screening.

From the results of experiments using newly synthesized organic compounds' it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 1a-k almost six compounds 1b, 1d, 1e, 1f, 1h and 1j were found active at 500 µg/ml conc. against *staphylococcus aureus*. *Bacillus Subtilis* was inhibited at 500 µg/ml conc. by eight compounds 1b, 1c, 1d, 1e, 1f, 1g, 1h, and 1k. Five compounds 1b, 1d, 1e, 1f and 1h were active against both cultures *B.Subtilis* and *S.aureus*. At the conc. 250 µg/ml S. aureus was inhibited by two compounds Ib and Id. *B.Subtilis* was not killed by any compounds (1a-k). So, it is obvious from the data obtained that compounds 1b and 1d were highly active among all the compounds of series 1a-k.

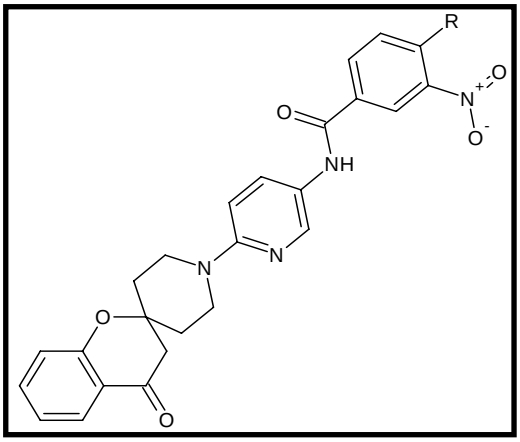
For Gram Negative bacteria in the series Ia-k almost six compounds 1b, 1d, 1e, 1h, 1j and 1k were found active at 500 µg/ml conc. against *Escherichia Coli*. *S.Paratyphi B.* was inhibited at 500 µg/ml conc. by eight compounds i.e. 1b, 1d, 1e, 1f, 1g, 1h, 1j and 1k. So, six compounds were active against both cultures *E.Coli* and *S.Paratyphi* B. i.e. 1b, 1d, 1e, 1h, 1j and 1k. At the conc. 250 µg/ml *E.Coli* was not killed by any compound (1a-k). *S.Paratyphi B.* was also inhibited by one compound 1b. So, it is obvious from the data obtained that compound 1b was highly active among all the compounds of series 1a-k.

For fungi in the series Ia-k almost seven compounds 1b, 1c, 1d, 1e, 1h, 11 and 1j were found active at 500 µg/ml conc. against *A.niger. C. albicans* was inhibited at 500 µg/ml conc. by six compounds i.e. 1b, 1d, 1g, 1h, 11 and 1j. At the conc. of 250 µg/ml C.albicans was killed by one compound 1b. *A. Niger* was killed by two compounds i.e. 1b and 1h. So, it is obvious from the data obtained that compound 1b was highly active among all the compounds of series 1a-k.

SECTION-II

Preparation, Characterization and antimicrobial evaluation of 4-(substituted phenoxy)-3-nitro-N-[6-{4-oxo-3,4-dihydro-1'H-Spiro-(chromene-2,4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

Keeping in view of wide spectrum biodynamic activities[1-39] of Pyridine and with a view to have potent therapeutic agents, the synthesis of **4-(substituted phenoxy)-3-nitro-N-[6-{4-oxo-3,4-dihydro-1'H-Spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides (2a-k)** have been synthesized by the nucleophilic substitution of the chloro atom of 4-chloro 3-nitro- N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides with different substituted phenols.



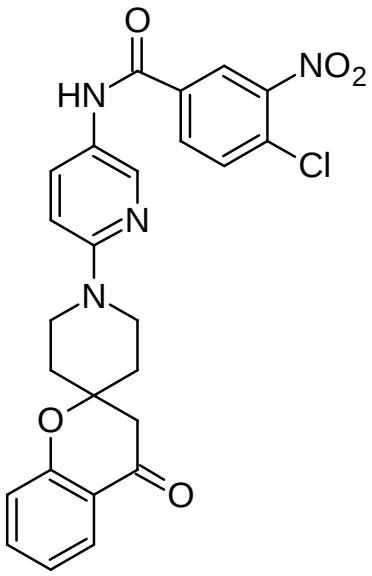
(2a-k)
R= Substituted phenoxy

The constitution of the synthesized products (2a-k) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

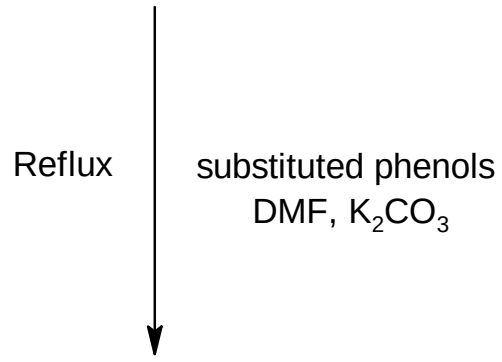
All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like Staphylococcus aureus, Bacillus subtilis and Gram Negative bacteria like Escherichia coli, Salmonella peratyphi B and they were also evaluated for antifungal activity against Candida albicans and Aspergillus niger at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum

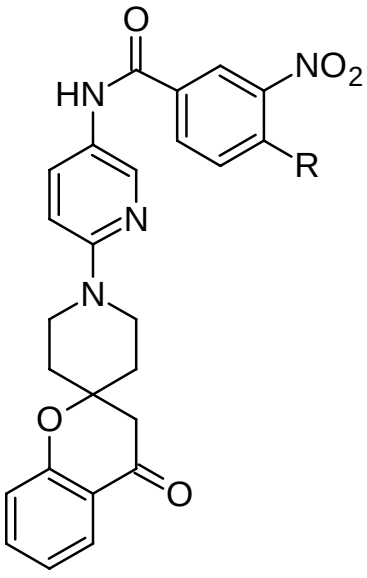
Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (2a-k) have been compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by NCCLs).

REACTION SCHEME



(Scaffold- A)





(2a-k)

R = substituted phenoxy

EXPERIMENTAL

Preparation, Characterization and antimicrobial evaluation of 4-(substituted Phenoxy)-3-nitro-N-[6-{4-oxo-3,4-dihydro-1'H-spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

[A] Synthesis of Scaffold-A.

For Preparation, see Part-I, Section-I, Page No. (29 to 31)

[B] Synthesis of 4-(2-fluoro Phenoxy)-3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro (chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

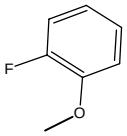
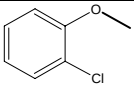
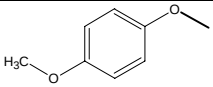
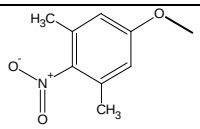
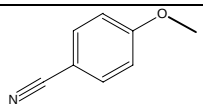
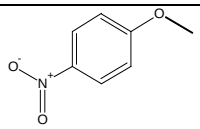
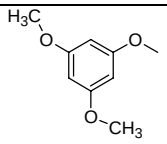
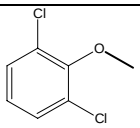
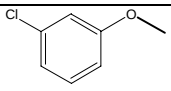
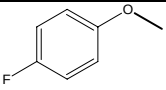
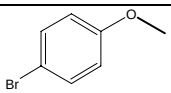
In RBF, Scaffold-A (1.00 mol) in DMF and K_2CO_3 (2.50 mol) was added at room temperature. In reaction mixture 2-fluorophenol was added at room temperature and heated at reflux temperature for 5-6 hour. Then reaction mixture was cooled to room temperature and quenched with water to get solid. The solid was filtered under vacuum and washed with water. The solid was triturating with ethanol to get pure product. Yield: 65% M.P.-155°C. Elemental Analysis: Calculated: C (65.49%), H (4.43%), N (9.85%), Found: C (65.43%), H (4.41%), N (9.79%).

Similarly, other compounds (2a-k) were synthesized by above mentioned process [B] from Scaffold-A. The physical data are recorded in Table-2.

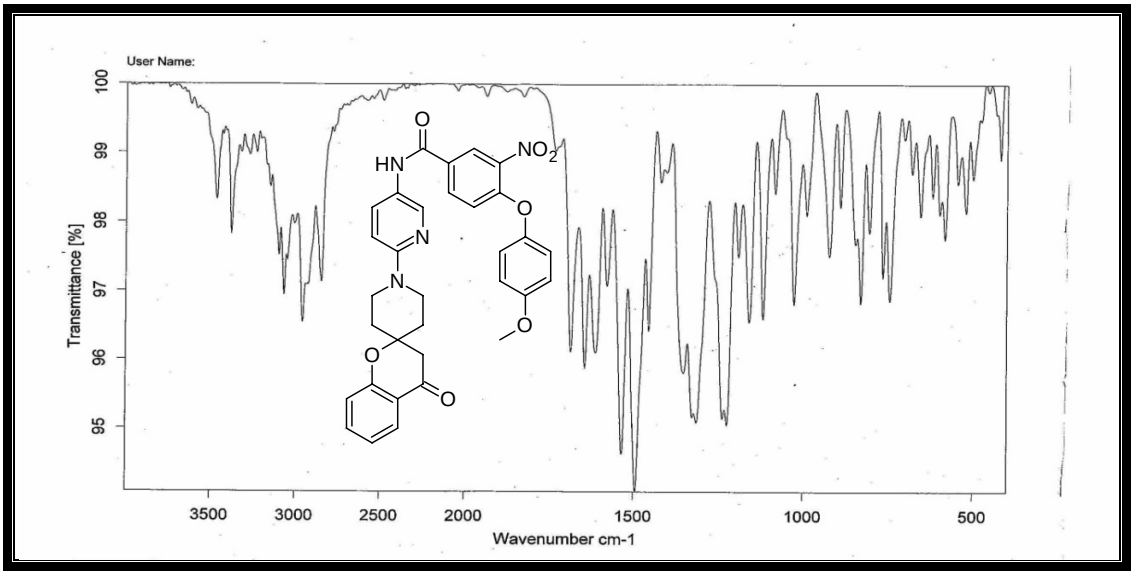
[C] Antimicrobial activity of 4-(substituted Phenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

Antimicrobial activity testing was carried out as described in Part-I, Section-I, Page No. (32 to 36) The MIC values of test solution are recorded in Table NO-2a and 2b.

TABLE NO-2: Physical constants of 4-(substituted Phenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (2a-k).

Sr. No.	Substitution R	Molecular Formula/ Molecular weight	M.P. o C	Yield %	% Composition Calcd./Found		
					C	H	N
2a		$C_{31}H_{25}FN_4O_6$ 546	155	65	65.49 65.43	4.43 4.41	9.85 9.79
2b		$C_{31}H_{25}ClN_4O_6$ 585	158	60	63.65 63.52	4.31 4.20	9.58 9.41
2c		$C_{32}H_{28}N_4O_7$ 580	182	70	66.20 66.03	4.86 4.71	9.65 9.41
2d		$C_{33}H_{29}N_5O_8$ 623	135	72	63.56 63.12	4.69 4.41	11.23 11.11
2e		$C_{32}H_{25}N_5O_6$ 575	118	52	66.78 66.53	4.38 4.23	12.17 12.10
2f		$C_{31}H_{25}N_5O_8$ 595	122	72	62.52 62.32	4.23 4.08	11.76 11.64
2g		$C_{33}H_{30}N_4O_8$ 610	143	70	64.91 64.52	4.95 4.63	9.18 8.90
2h		$C_{31}H_{24}Cl_2N_4O_6$ 619	159	45	60.11 59.84	3.91 3.79	9.04 8.82
2i		$C_{31}H_{25}ClN_4O_6$ 585	155	58	63.65 63.49	4.31 4.17	9.58 9.41
2j		$C_{31}H_{25}FN_4O_6$ 546	142	62	65.49 65.33	4.43 4.22	9.85 9.69
2k		$C_{31}H_{25}BrN_4O_6$ 629	163	65	59.15 59.12	4.00 3.61	8.90 8.59

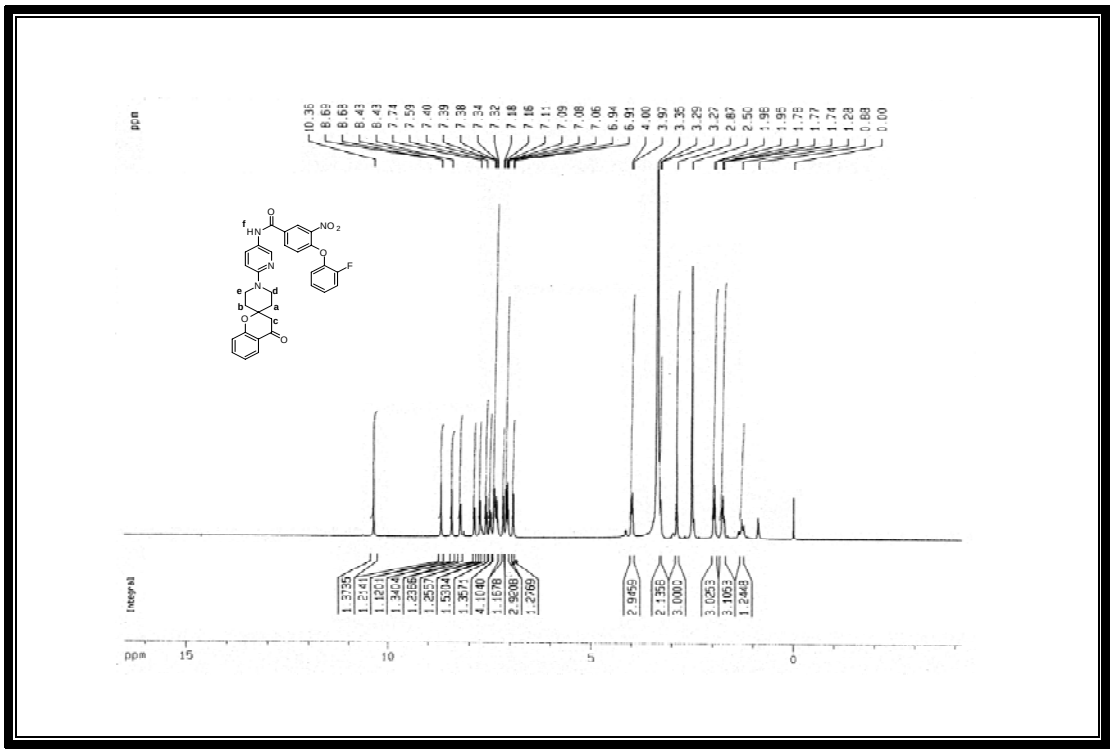
IR Spectral Study of 4-(4'-Methoxy Phenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (2c).

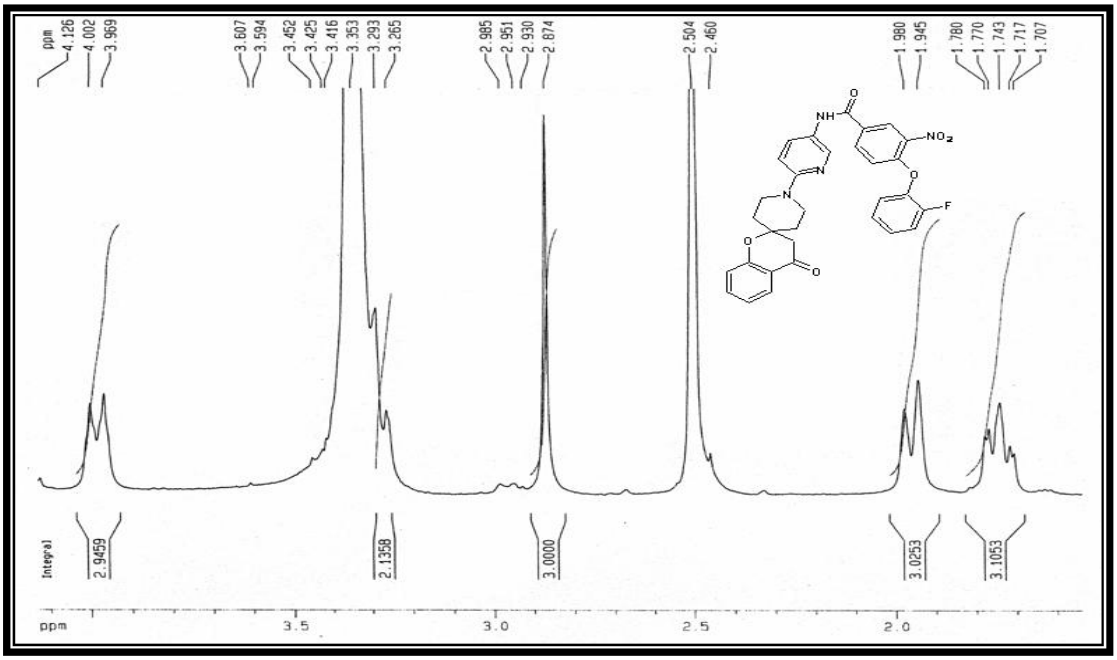


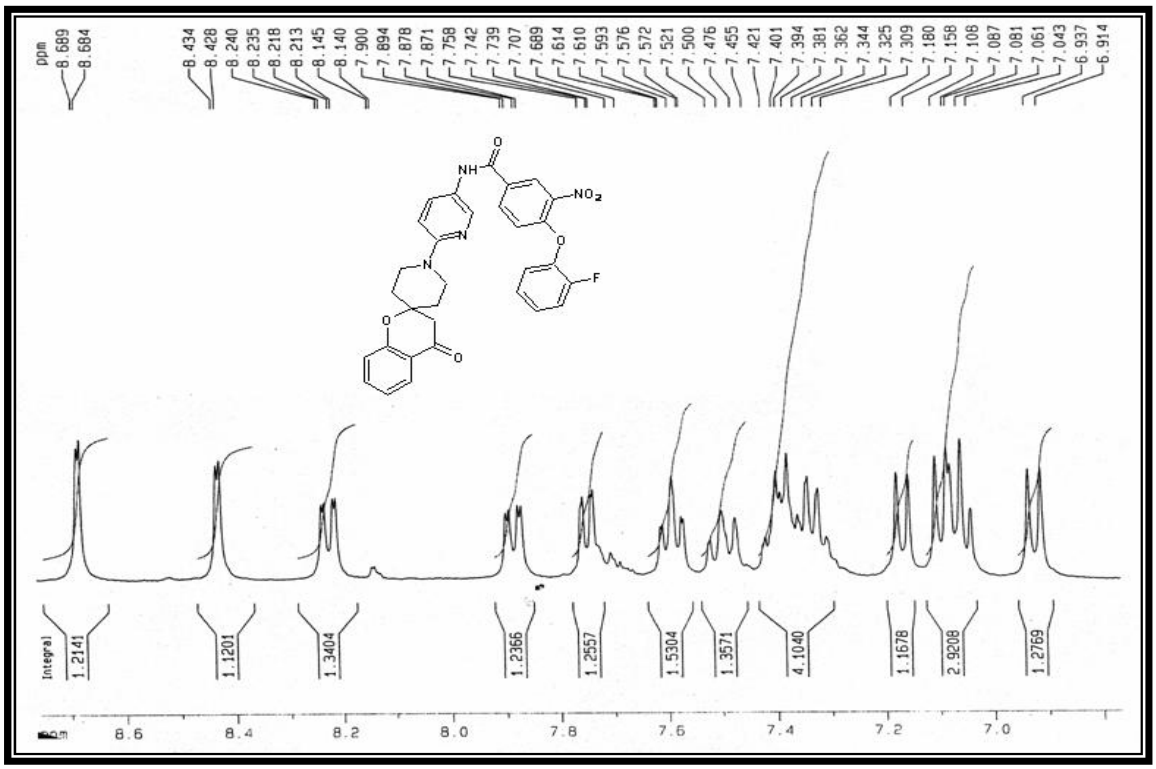
Instrument: SHIMADZU FTIR 8400 Spectrophotometer; **Frequency range**: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No.
		Observed	Reported	
Alkane	C-H asym. str.	2950	2975-2850	42
	C-H sym. str.	2830	2900-2800	42
	C-H asym. def.	1450	1460-1400	42
	C-H sym. Def.	1355	1385-1300	42
Aromatic	C-H str.	3070	3080-3010	42
	C=C ring skeleton	1530	1600-1450	44
		1490	1600-1450	44
	p-disubstituted benzene	830	850-750	44
Pyridine moiety	-C=N str.	1685	1690-1570	43
	C-N str.	1305	1310-1250	43
	C-NO2 asym. Str.	1550	1570-1500	43
Chromanone moiety	C-O-C asym. Str.	1240	1250-1200	42
	C-O-C sym. Str.	1030	1050-1010	42
	Cyclic C=O str.	1685	1740-1630	42
Amide Ketone	-C=O str.	1650	1740-1650	42
	N-H str.	3370	3500-3310	43

1H-NMR Spectral Study of 4-(2-fluoro phenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (2a).



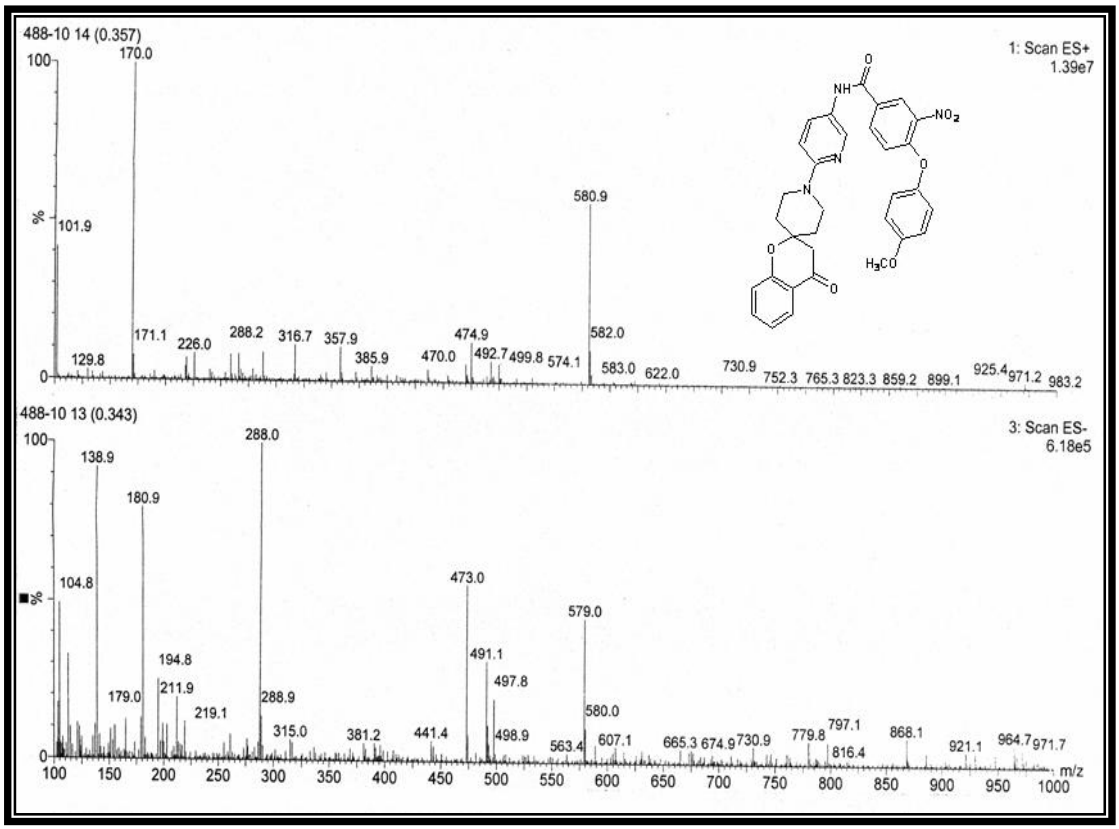




Instrument: BRUKER 400 MHz (Advance - 2), **Internal reference:** TMS, **Solvent:** DMSO-*d6.*

Serial No.	**Signal Position(δppm)**	**Relative No. of Protons**	**Multiplicity**	**Inference**
1	1.74	2H	Triplet	-(CH_2) a
2	1.94	2H	Triplet	-(CH_2) b,
3	2.874	2H	Singlet	-(CH_2) c
4	3.27	2H	Triplet	-(CH_2) d
5	3.96	2H	Triplet	-(CH_2) e
6	6.914-8.689	14H	Complex	Ar-H
17	10.36	1H	Singlet	-(NH) q

Mass Spectral Study of 4-(4'-Methoxy-1-phenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (2c).



Possible Mass Fragmentation pattern:

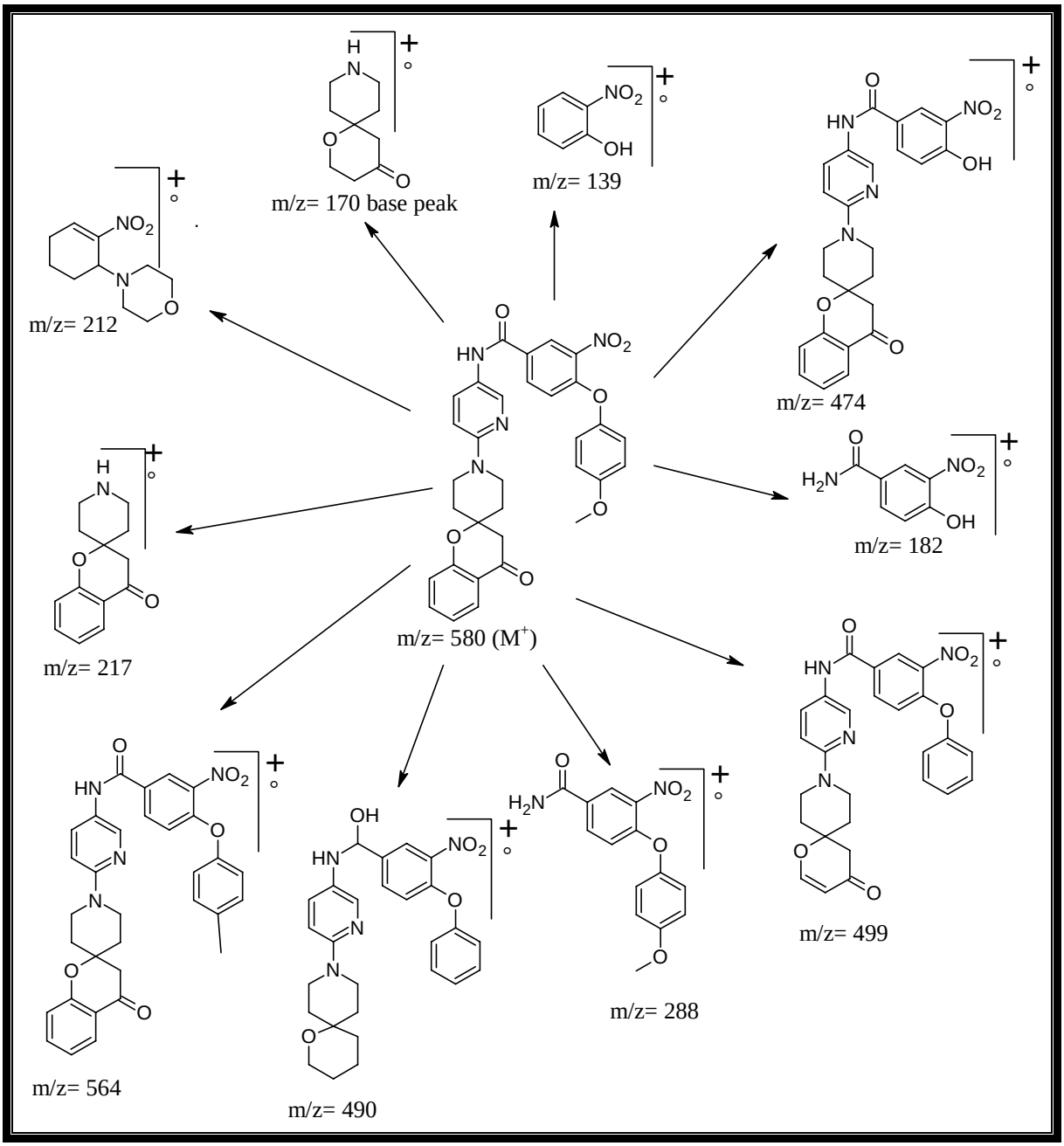


TABLE NO. 2a: Antimicrobial Activity Of 4-(Substituted Phenoxy) -3 Nitro-N-[6-{4-Oxo-3, 4 Dihydro-1'H- Spiro-(Chromene-2, 4'- Piperidin)-1'-yl} Pyridine-3-yl]-Benzamides (2a-k).

TABLE NO-2(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
2a	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-
2b	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	+
2c	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	+
2d	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+
2e	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-
2f	+	+	-	-	+	+	-	-	+	+	+	-	+	+	-	-
2g	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
2h	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
2i	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
2j	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	-
2k	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
Reference drugs:	***S. aureus***				***B. Subtilis***				***E.Coli***				***S. Peratyphi B***			
Ciprofloxacin	**1.9**				**7.8**				**0.4**				**1.4**			

TABLE NO. 2b: Antimicrobial Activity Of 4-(Substituted Phenoxy) -3 Nitro-N-[6-{4-Oxo-3, 4 Dihydro-1'H- Spiro-(Chromene-2, 4'-Piperidin)-1'-yl} Pyridine-3-yl]-Benzamides (2a-k).

TABLE NO-2(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (ug/ml)*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
2a	+	+	+	-	+	+	+	-
2b	+	+	+	-	+	+	+	-
2c	+	+	-	-	+	+	-	-
2d	+	+	+	-	+	+	+	-
2e	+	+	-	-	+	+	+	-
2f	+	+	-	-	+	+	-	-
2g	+	+	+	-	+	+	+	-
2h	+	+	+	-	+	+	+	-
2i	+	+	-	-	+	+	-	-
2j	+	+	-	-	+	+	-	-
2k	+	+	+	-	+	+	+	-

Reference drugs:	***A.niger***	***C. albicans***
Fluconazole	**0.7**	**0.4**

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 µg/ml conc. (2) Secondary Screening 1000 µg/ml to 250 µg/ml conc.(3) Tertiary Screening start from 125 µg/ml to 31.25 µg/ml. The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed in tertiary screening.

From the results of experiments using newly synthesized organic compounds it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 2a-k almost four compounds 2b, 2g, 2h, and 2k were found active at 500 µg/ml conc. against *staphylococcus aureus*. *Bacillus Subtilis* was inhibited at 500 µg/ml conc. by four compounds 2d, 2g, 2h, and 2k. Three compounds 2g, 2h, and 2k was active against both cultures *B.Subtilis* and *S.aureus*. At the conc. 250 µg/ml *S. aureus* was not killed by any compounds (2a-k). *B.Subtilis* was inhibited by two compounds 2h and 2k. So, it is obvious from the data obtained that compounds 2h and 2k was highly active among all the compounds of series 2a-k.

For Gram Negative bacteria in the series 2a-k almost nine compounds 2a, 2b, 2c, 2d, 2e, 2f, 2g, 2h and 2k were found active at 500 µg/ml conc. against *Escherichia* Coli. *S.Paratyphi B.* was inhibited at 500 µg/ml conc. by nine compounds i.e. 2a, 2b, 2c, 2d, 2e, 2g, 2h, 2j and 2k. So, eight compounds were active against both cultures *E.Coli* and *S.Paratyphi B.* i.e. 2a, 2b, 2c, 2d, 2e, 2g, 2h and 2k. At the conc. 250 µg/ml *E.Coli* was not killed by any compound (2a-k). *S.Paratyphi B.* was also inhibited by five compounds 2b, 2c, 2d, 2h and 2k. So, it is obvious from the data obtained that compounds 2b, 2c, 2d, 2h and 2k was highly active among all the compounds of series 2a-k.

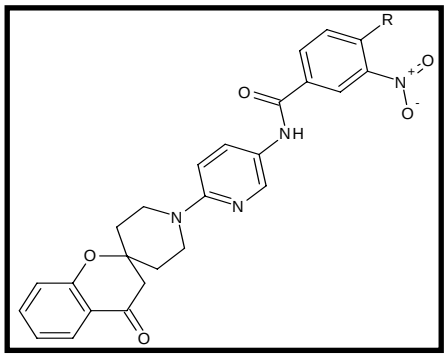
For fungi in the series 2a-k almost seven compounds 2a, 2b, 2d, 2g, 2h and 2k were found active at 500 µg/ml conc. against *A.niger*. *C. albicans* was inhibited at 500 µg/ml conc. by seven compounds i.e. 2a, 2b, 2d, 2e, 2g, 2h and 2k. At the conc.

of 250 µg/ml *C.albicans* was not killed by any compounds (2a-k). *A. Niger* was not killed any compounds (2a-k).

SECTION-III

Preparation, Characterisation and Antimicrobial Evaluation Of 4-(Substituted Thiophenoxy)-3-Nitro-N-[6-{4-Oxo-3,4-Dihydro-1'H-Spiro-(Chromene-2,4'-piperidin)-1'-yl} Pyridine-3-yl]-Benzamides.

Keeping in view of wide spectrum biodynamic activities[1-39] of Pyridine and with a view to have potent therapeutic agents, the synthesis of **4-(substituted thiophenoxy)-3-nitro-N-[6-{4-oxo-3,4-dihydro-1'H-Spiro-(chromene-2,4'-piperidin)-1'-yl}pyridine-3-yl] benzamides (3a-k)** have been synthesized by the nucleophilic substitution of the chloro atom of 4-chloro, 3-nitro- N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides with different substituted thiophenols.



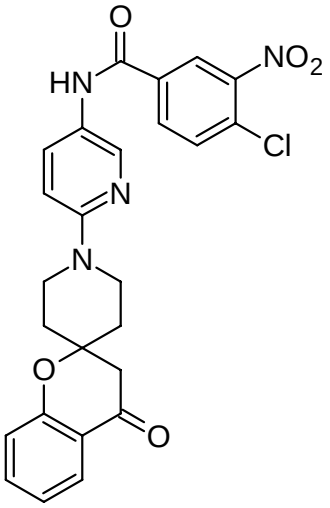
(3a-k)
R= Substituted thiophenoxy

The constitution of the synthesized products (3a-k) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella peratyphi B* and they were also evaluated for antifungal activity against *Candida albicans* and *Aspergillus niger* at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (3a-k) have been

compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by **NCCLs**).

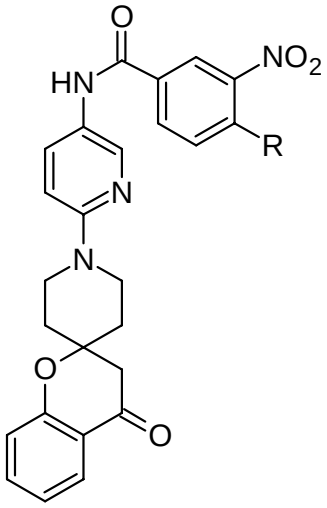
REACTION SCHEME



(Scaffold- A)

0°C-RT

substituted Thiophenols
DMF, t-BuOK



(3a-k)

R = Substituted Thiophenoxy

EXPERIMENTAL

Preparation, Characterization and antimicrobial evaluation of 4-(substituted Thiophenoxy)-3-nitro-N-[6-{4-oxo-3,4-dihydro-1'H-spiro-(chromene-2,4'-piperidin)-1'-yl} pyridine-3-yl]-benzamides.

[A] Synthesis of Scaffold-A.

For Preparation, see Part-I, Section-I, Page No. (29 to 31)

[B] Synthesis of 4-(4'-Methoxy-1-thiophenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro (chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

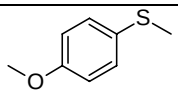
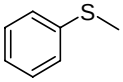
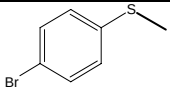
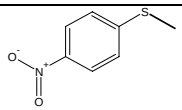
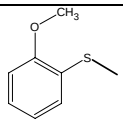
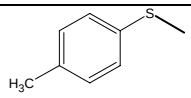
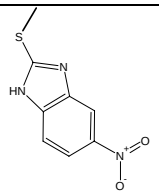
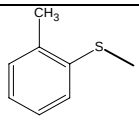
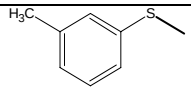
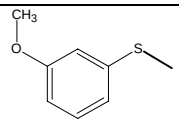
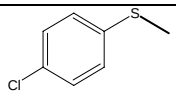
In RBF, Scaffold-A (1.00 mol) in DMF and Potassium tert-Butoxide (1.10 mol) was added at room temperature. Then reaction mixture was allowed to cool at 0°C. In reaction mixture, 4-methoxythiophenol was added at 0°C and reaction mixture was allowed to warm at room temperature and stirred for 1 hours. Then reaction mixture was quenched with water to get solid. The solid was filtered under vacuum and washed with water. The solid was triturating with ethanol to get pure product. M.P.-212°C. Elemental Analysis: Calculated: C (64.42%), H (4.73%), N (9.39%), Found: C (64.38%), H (4.70%), N (9.35%).

Similarly, other compounds (3a-k) were synthesized by above mentioned process [B] from Scaffold-A. The physical data are recorded in Table-III.

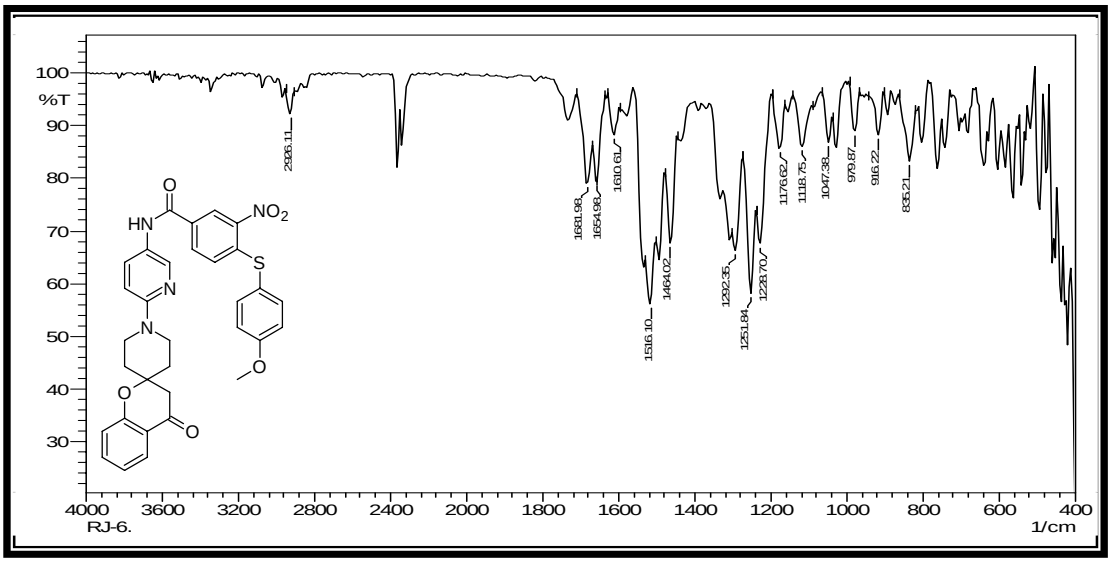
[C] Antimicrobial activity of 4-(substituted Thiophenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

Antimicrobial activity testing was carried out as described in Part-I, Section-I, Page No. (32 to 36) The MIC values of test solution are recorded in Table NO-3a and 3b.

TABLE NO-3: Physical constants of 4-(substituted Thiophenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (3a-k).

Sr. No.	Substitution R	Molecular Formula/ Molecular weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
3a		$C_{32}H_{28}N_4O_6S$ 596	212	68	64.42 64.38	4.73 4.70	9.39 9.35
3b		$C_{31}H_{26}N_4O_5S$ 566	102	80	65.71 65.56	4.62 4.47	9.89 9.75
3c		$C_{31}H_{25}BrN_4O_5S$ 645	139	70	57.68 57.52	3.90 3.81	8.68 8.54
3d		$C_{31}H_{25}N_5O_7S$ 611	181	35	60.88 60.63	4.12 3.93	11.45 11.09
3e		$C_{32}H_{28}N_4O_6S$ 596	129	74	64.42 64.27	4.73 4.42	9.39 9.23
3f		$C_{32}H_{28}N_4O_5S$ 580	194	65	66.19 66.09	4.86 4.59	9.65 9.49
3g		$C_{32}H_{25}N_7O_7S$ 651	191	70	58.98 58.79	3.87 3.72	15.05 14.69
3h		$C_{32}H_{28}N_4O_5S$ 580	162	66	66.19 65.83	4.86 4.71	9.65 9.43
3i		$C_{32}H_{28}N_4O_5S$ 580	187	70	66.19 65.92	4.86 4.76	9.65 9.42
3j		$C_{32}H_{28}N_4O_6S$ 596	168	71	64.42 64.21	4.73 4.61	9.39 9.22
3k		$C_{31}H_{25}ClN_4O_5S$ 601	190	60	61.94 61.70	4.19 4.05	9.32 9.20

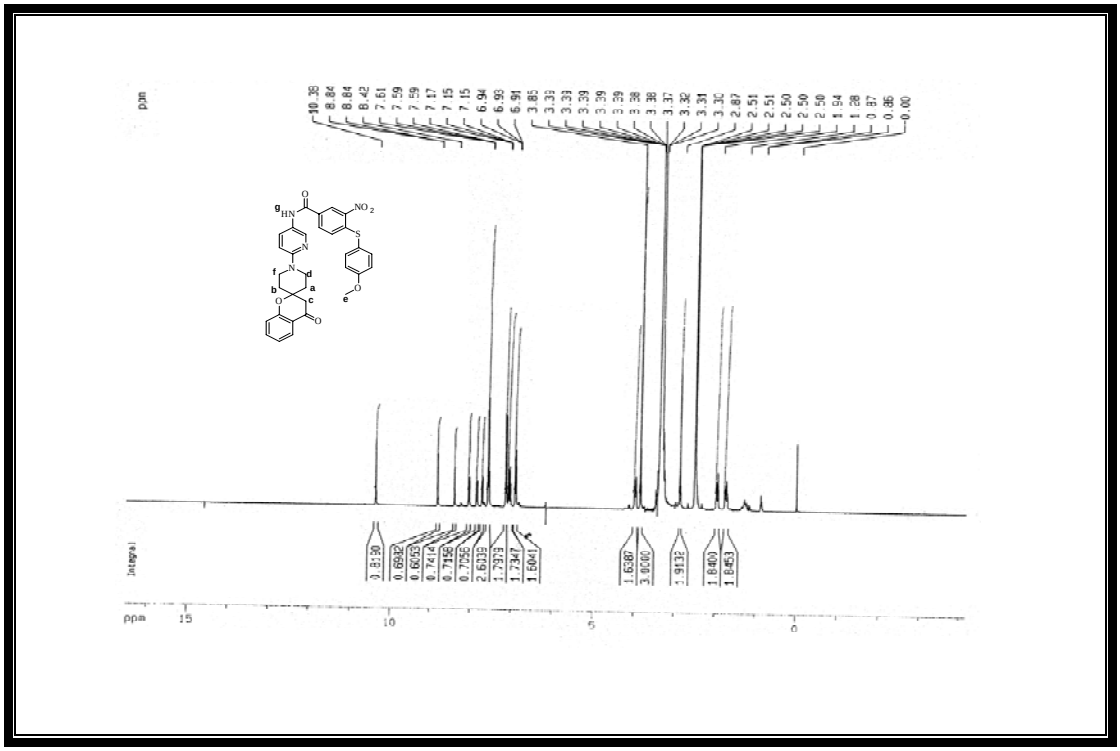
IR Spectral Study of 4-(4'-Methoxy-1-thiophenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (3a).

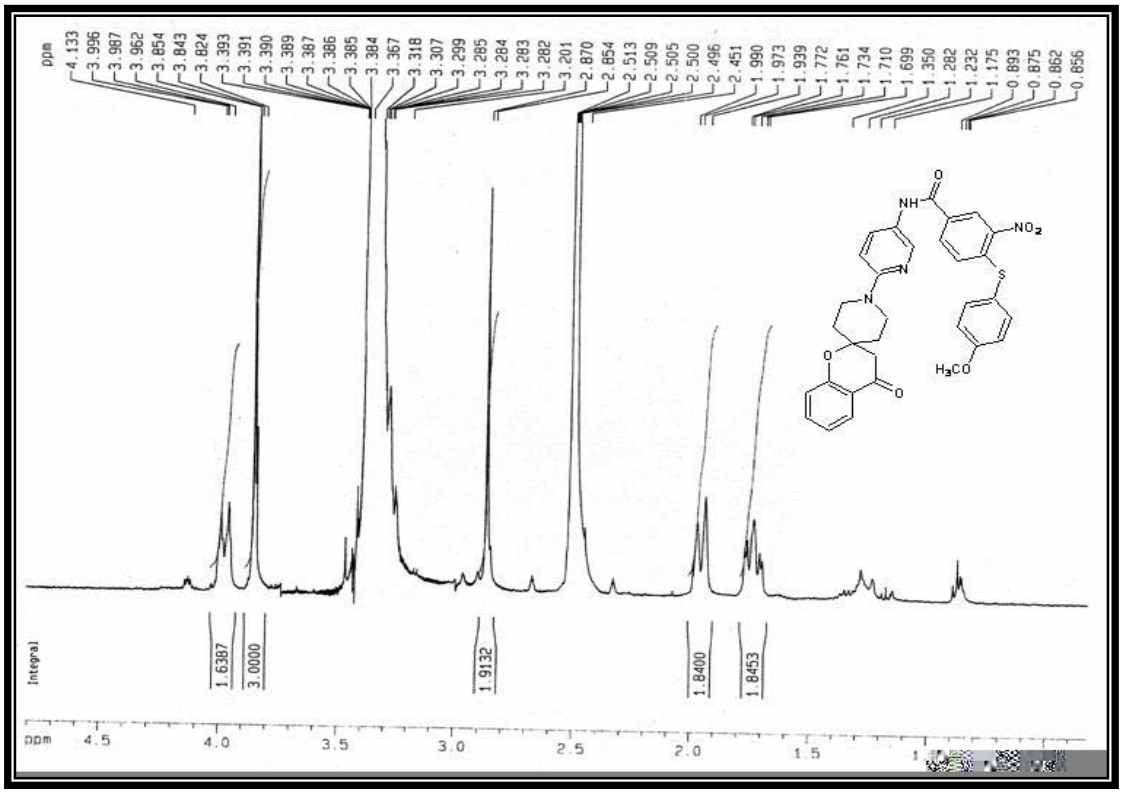


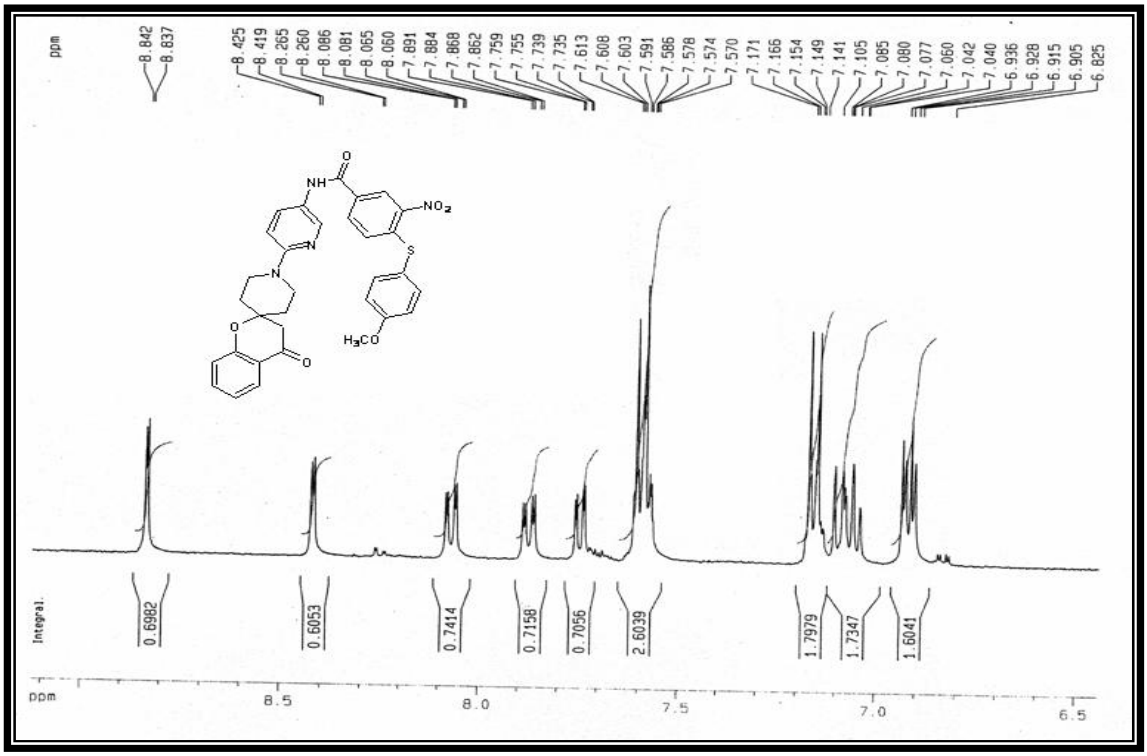
Instrument: SHIMADZU FTIR 8400 Spectrophotometer; **Frequency range**: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No.
		Observed	Reported	
Alkane	-C-H asym. str.	2926	2975-2850	42
	-C-H sym. str.	2830	2900-2800	42
	-C-H asym. def.	1464	1460-1400	42
	-C-H sym. Def.	1292	1385-1300	42
Aromatic	-C-H str.	3065	3090-3010	42
	-C=C- ring skeleton	1516	1600-1450	44
		1464	1600-1450	44
	p-disubstituted benzene	835	850-800	44
Pyridine moiety	-C=N Str.	1610	1690-1570	43
	-C-N- Str.	1302	1310-1250	43
	-C-NO2 asym. Str.	1530	1570-1500	43
spirochromene moiety	-C-O-C asym. Str.	1251	1250-1200	42
	-C-O-C sym. Str.	1047	1050-1010	42
	Cyclic -C=O str.	1682	1740-1630	42
Amide	-C=O str.	1655	1740-1630	42
	N-H str.	3350	3400-3200	43

1H-NMR Spectral Study of 4-(4'-Methoxy-1-thiophenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (3a).



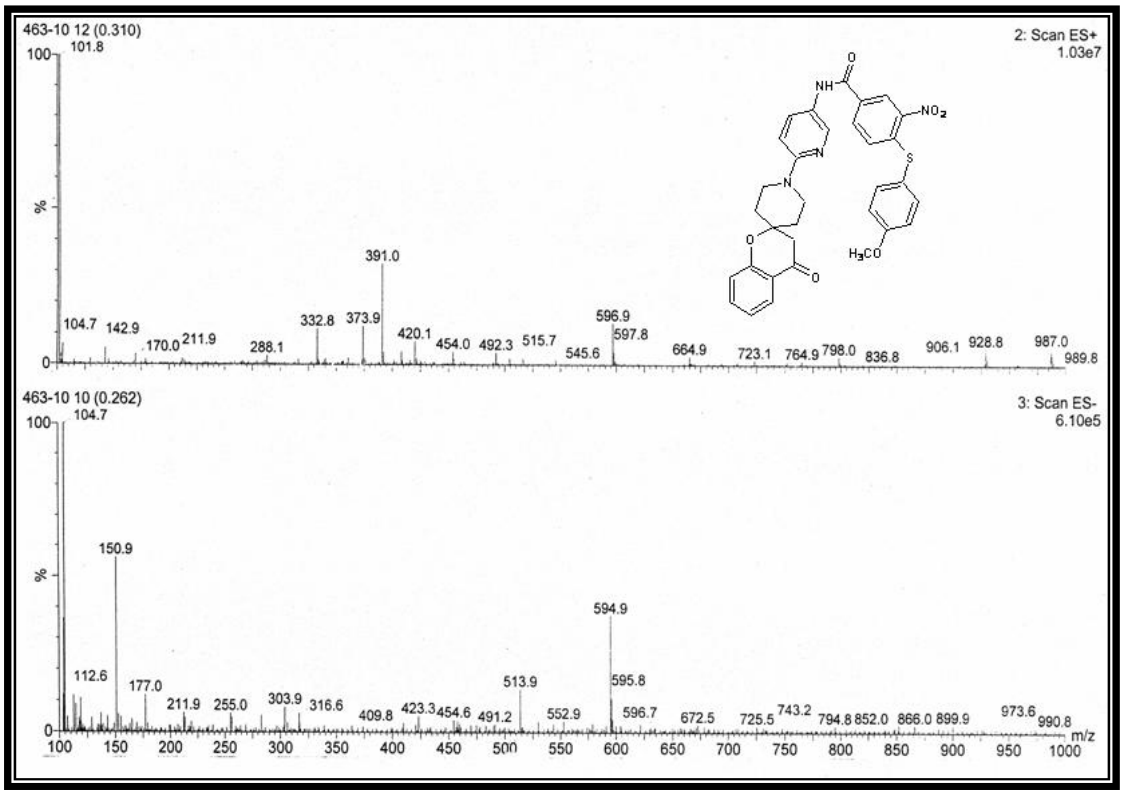




Instrument: BRUKER 400 MHz (Avance - II), **Internal reference:** TMS,

Solvent: DMSO-*d6*Serial No.	**Signal position (δ ppm)**	**Relative No. of Protons**	**Multiplicity**	**Inference**
1	1.74	2H	Triplate	-(CH_2) a
2	1.94	2H	Triplate	-(CH_2) b,
3	2.874	2H	Singlet	-(CH_2) c
4	3.29	2H	Triplate	-(CH_2) d
5	3.85	3H	Singlet	-(CH_3) e
6	3.98	2H	Triplate	-(CH_2) f
7	6.905-8.842	14H	Complex	Ar -H
16	10.36	1H	Singlet	-(NH) g

Mass Spectral Study of 4-(4'-Methoxy-1-thiophenoxy) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (3a).



Possible Mass Fragmentation pattern:

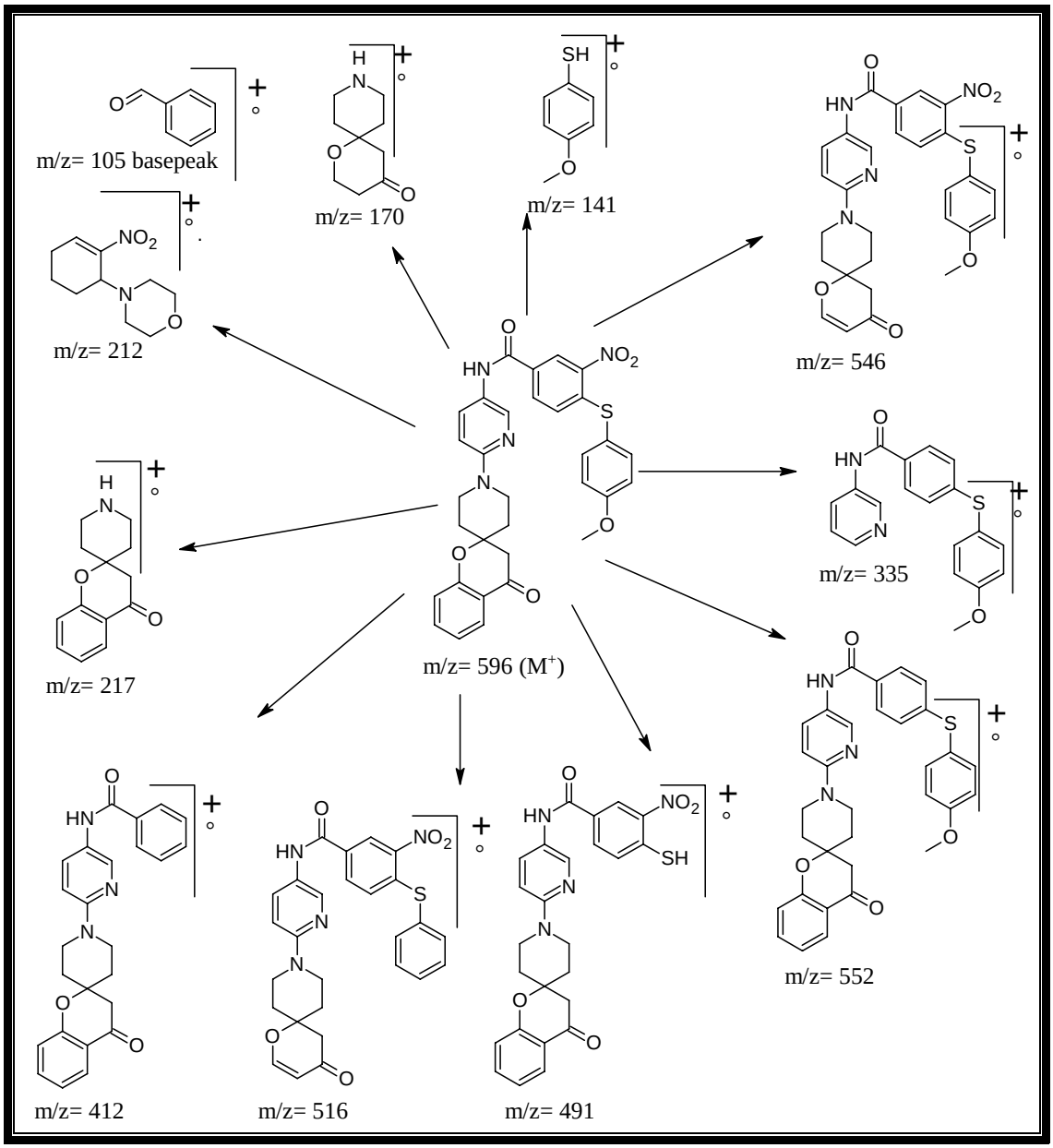


TABLE NO. 3a: Antimicrobial Activity Of 4-(Substituted Thiophenoxy) -3 Nitro-N-[6-{4-Oxo-3, 4 Dihydro-1'H- Spiro-(Chromene-2, 4'- Piperidin)-1'-yl} Pyridine-3-yll-Benzamides (3a-k).

TABLE NO-3(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
3a	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
3b	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-
3c	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
3d	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-
3e	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
3f	+	+	-	-	+	+	+	-	+	+	-	-	+	+	+	-
3g	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-
3h	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
3i	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
3j	+	+	-	-	+	+	+	-	+	+	-	-	+	+	+	-
3k	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

Reference drugs:	***S. aureus***	***B. Subtilis***	***E.Coli***	***S. Peratyphi B***
Ciprofloxacin	**1.9**	**7.8**	**0.4**	**1.4**

TABLE NO. 3b: Antimicrobial Activity Of 4-(Substituted Thiophenoxy) -3 Nitro-N-[6-{4-Oxo-3, 4 Dihydro-1'H- Spiro-(Chromene-2, 4'- Piperidin)-1'-yl} Pyridine-3-yl]-Benzamides (3a-k).

TABLE NO-3(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (µg/ml)*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
3a	+	+	+	-	+	+	+	-
3b	+	+	+	-	+	+	+	-
3c	+	+	+	-	+	+	+	-
3d	+	+	+	-	+	+	+	-
3e	+	+	+	-	+	+	+	-
3f	+	+	-	-	+	+	-	-
3g	+	+	+	-	+	+	+	-
3h	+	+	-	-	+	+	-	-
3i	+	+	+	-	+	+	+	-
3j	+	+	+	-	+	+	+	-
3k	+	+	+	-	+	+	-	-
Reference drugs:			***A.niger***				***C. albicans***	
Fluconazole			**0.7**				**0.4**	

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 µg/ml conc. (2) Secondary Screening 1000 µg/ml to 250 µg/ml conc. (3) Tertiary Screening start from 125 µg/ml to 3.9 µg/ml. The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed in tertiary screening.

From the results of experiments using newly synthesized organic compounds' it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 3a-k almost four compounds 3a, 3b, 3e and 3i were found active at 500 µg/ml conc. against *staphylococcus aureus*. *Bacillus Subtilis* was inhibited at 500 µg/ml conc. by eight compounds 3a, 3b, 3d, 3e, 3f, 3g, 3i and 3j. Four compounds 3a, 3b, 3e and 3i were active against both cultures *B.Subtilis* and *S.aureus*. At the conc. 250 µg/ml *S. aureus* was not killed by any compounds (3a-k). *B.Subtilis* was inhibited by three compounds 3b, 3e and 3i. So, it is obvious from the data obtained that compounds 3b, 3e and 3i were highly active among all the compounds of series 3a-k.

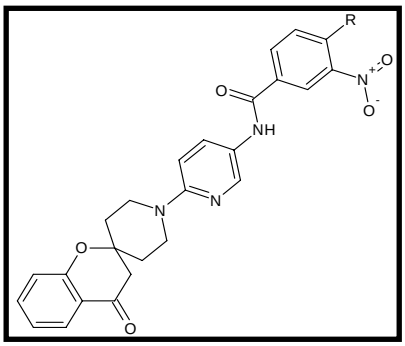
For Gram Negative bacteria in the series 3a-k almost six compounds 3a, 3b, 3d, 3e, 3g and 3i were found active at 500 µg/ml conc. against *Escherichia Coli*. *S.Paratyphi B.* was inhibited at 500 µg/ml conc. by eight compounds i.e. 3a, 3b, 3d, 3e, 3f, 3g, 3i and 3j. So, six compounds were active against both cultures *E.Coli* and *S.Paratyphi B.* i.e. 3a, 3b, 3d, 3e, 3g and 3i. At the conc. 250 µg/ml *E.Coli* was killed by two compounds i.e. 3e and 3i. *S.Paratyphi B.* was also inhibited by two compounds 3e and 3i. So, it is obvious from the data obtained that compounds 3e and 3i was highly active among all the compounds of series 3a-k.

For fungi in the series 3a-k almost nine compounds 3a, 3b, 3c, 3d, 3e, 3g, 3i, 3j and 3k were found active at 500 µg/ml conc. against *A.niger*. *C. albicans* was inhibited at 500µg/ml conc. by eight compounds i.e. 3a, 3b, 3c, 3d, 3e, 3g, 3i and 3j. At the conc. of 250µg/ml *C.albicans* was not killed by any compounds (3a-k). *A. Niger* was not killed by any compounds (3a-k).

SECTION-IV

Preparation, Characterization and antimicrobial evaluation of 4-(substituted phenyl)-3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2,4'-piperidin)-1'-yl}pyridine-3-yl]-benzamides.

Keeping in view of wide spectrum biodynamic activities[1-39] of Pyridine and with a view to have potent therapeutic agents, the synthesis of **4-(substituted aryl)-3-nitro-N-[6-{4-oxo-3,4-dihydro-1'H-spiro-(chromene-2,4'-piperidin)-1'-yl}pyridine-3-yl]-benzamides (4a-k)** have been synthesized by the nucleophilic substitution of the chloro atom of 4-chloro 3-nitro- N-[6-{4-oxo-3,4 dihydro-1'H- spiro-(chromene-2,4'- piperidin)-1'-yl}pyridine-3-yl]-benzamides with different substituted phenyl boronic acids.



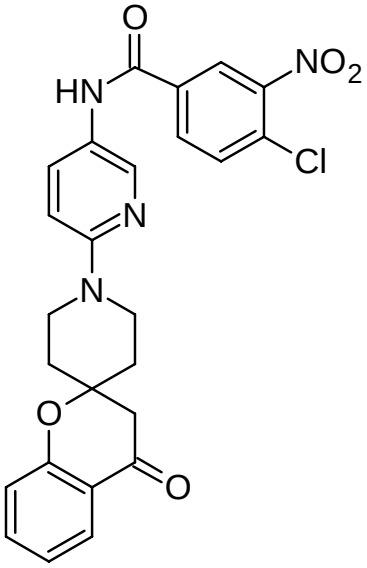
(4a-k)
R= Aryl

The constitution of the synthesized products (4a-k) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella peratyphi B* and they were also evaluated for antifungal activity against *Candida albicans* and *Aspergillus niger* at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (4a-k) have been

compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by **NCCLs**).

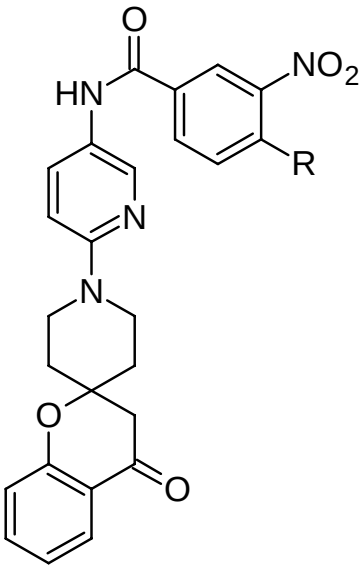
REACTION SCHEME



(Scaffold- A)

75°C
3 Hours

Substituted Phenyl
Boronic acids
Toluene,
Ethanol, Water
Pd-Tetrakis



(4a-k)
R = Aryl

EXPERIMENTAL

Preparation, Characterization and antimicrobial evaluation of 4-(substituted phenyl) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

[A] Synthesis of Scaffold-A.

For Preparation, see Part-I, Section-I, Page No. (29 to 31)

[B] Synthesis of 4-(2-fluoro phenyl) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro (chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

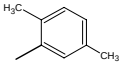
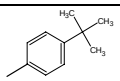
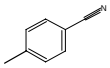
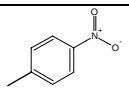
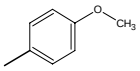
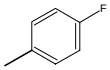
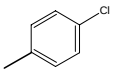
In RBF, Scaffold-A (1.00 mol) in Toluene and Pd-Tetrakis (0.10 mol) was added at room temperature under nitrogen purging. In reaction mixture, Ethanol, Water, 2-fluoro PhenylBoronic acid (1.10 mol) and K_2CO_3 (2.5 mol) was added at room temperature under nitrogen purging and stirred for 20 minutes at same conditions. Then reaction mixture heated at 75°C temperature for 3-4 hours. Then reaction mixture was allowed to cool at room temperature and quenched with water. The product was extracted with ethyl acetate. The organic layer was washed with brine solution, dried over sodium sulphate and concentrated under reduced pressure to get crude product. The crude product was purified by using column chromatography. The product was eluted at 40-45% ethyl acetate in hexane. Yield: 35% M.P.- 206°C. Elemental Analysis: Calculated: C (67.38%), H (4.56%), N (10.14%), Found: C (67.21%), H (4.41%), N (10.01%).

Similarly, other compounds (4a-k) were synthesized by above mentioned process [B] from Scaffold-A. The physical data are recorded in Table-4.

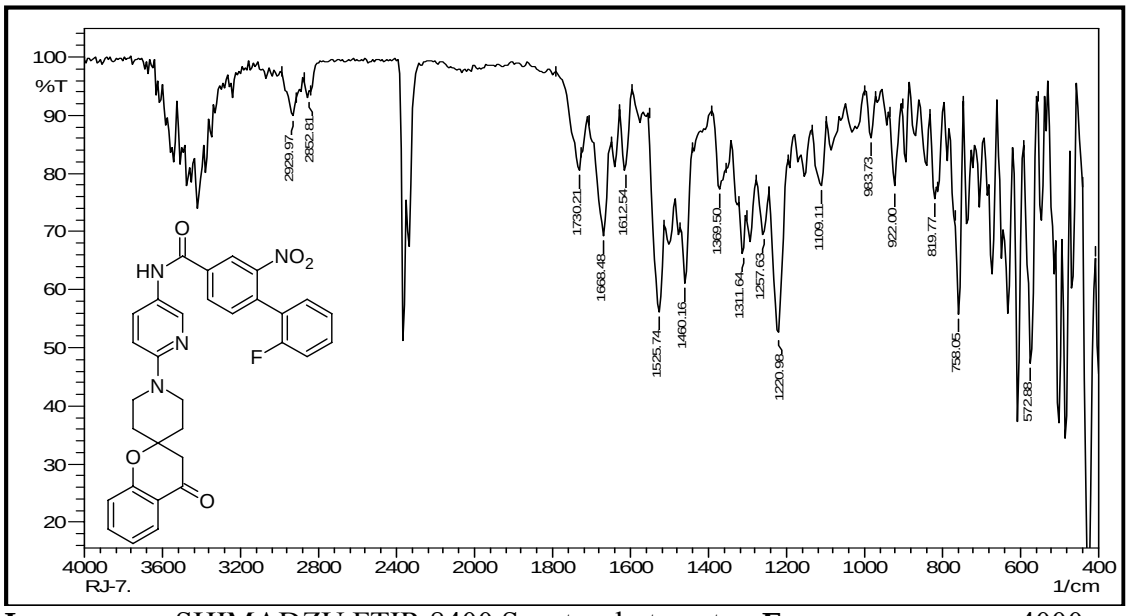
[C] Antimicrobial activity of 4-(substituted phenyl) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides.

Antimicrobial activity testing was carried out as described in Part-I, Section-I, Page No. (32 to 36) The MIC values of test solution are recorded in Table NO-4a and 4b.

TABLE NO-4: Physical constants of 4-(substituted phenyl) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (4a-k).

Sr. No.	Substitution R	Molecular Formula/ Molecular weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
4a		$C_{31}H_{25}FN_4O_5$ 552	206	35	67.38 67.21	4.56 4.41	10.14 10.01
4b		$C_{32}H_{28}N_4O_6$ 564	217	50	68.07 67.82	5.00 4.91	9.92 9.79
4c		$C_{33}H_{30}N_4O_5$ 562	111	45	70.45 70.28	5.37 5.24	9.96 9.81
4d		$C_{35}H_{34}N_4O_5$ 590	120	50	71.17 71.01	5.80 5.69	9.49 9.38
4e		$C_{33}H_{30}N_4O_5$ 562	115	42	70.45 70.26	5.37 5.33	9.96 9.86
4f		$C_{32}H_{25}N_5O_5$ 559	143	30	68.69 68.52	4.50 4.42	12.52 12.43
4g		$C_{31}H_{25}N_5O_7$ 579	120	55	64.24 64.09	4.35 4.23	12.08 11.91
4h		$C_{31}H_{25}N_5O_7$ 579	135	52	64.24 64.07	4.35 4.26	12.08 11.96
4i		$C_{32}H_{28}N_4O_6$ 564	174	50	68.07 67.83	5.00 4.86	9.92 9.83
4j		$C_{31}H_{25}FN_4O_5$ 552	192	40	67.38 67.23	4.56 4.47	10.14 10.06
4k		$C_{31}H_{25}ClN_4O_5$ 569	201	40	65.44 65.28	4.43 4.36	6.23 6.14

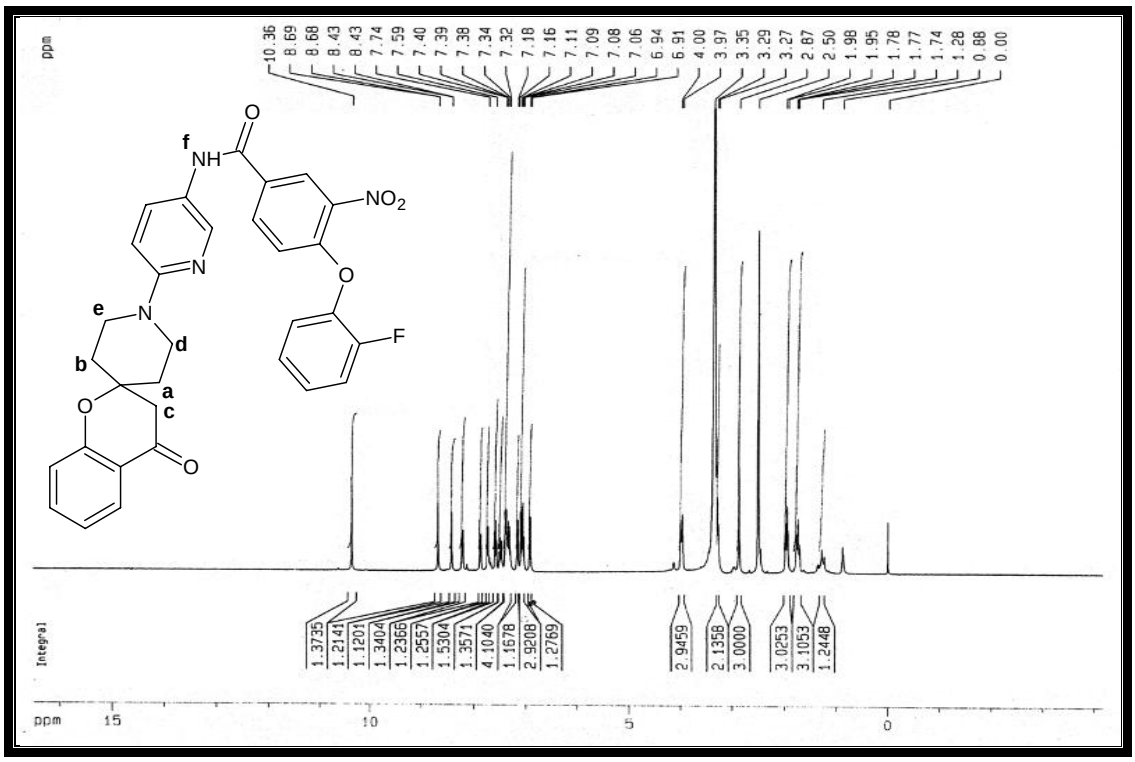
IR Spectral Study of 4-(2'-fluoro phenyl) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H-spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (4a).

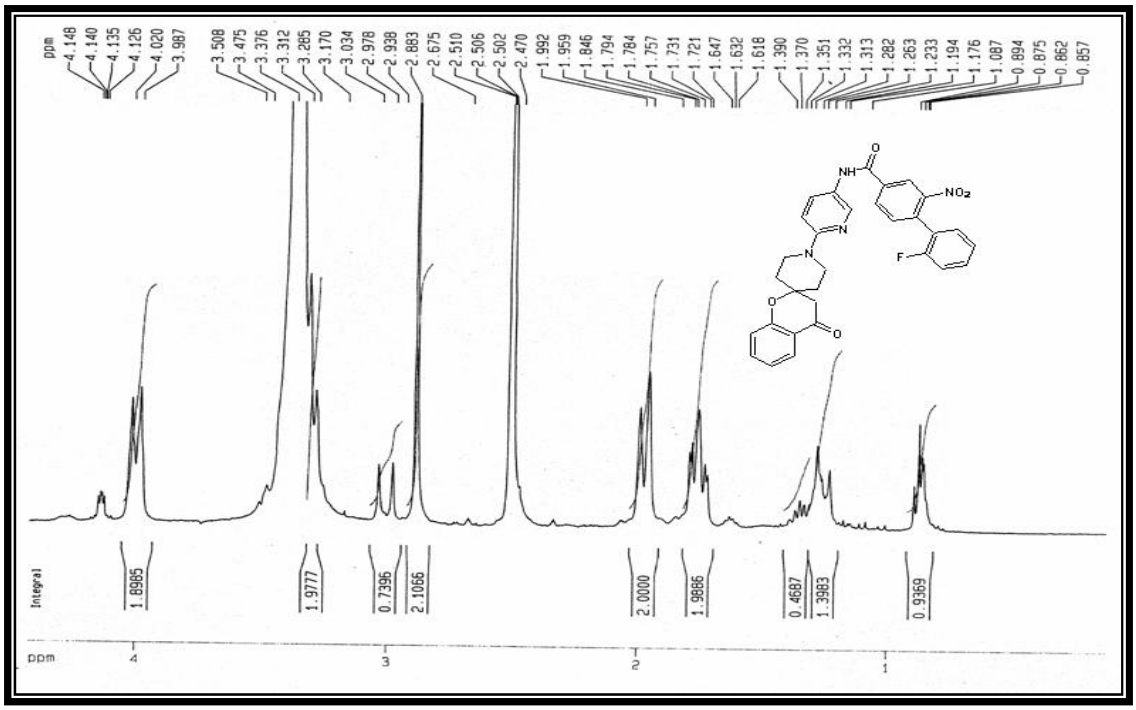


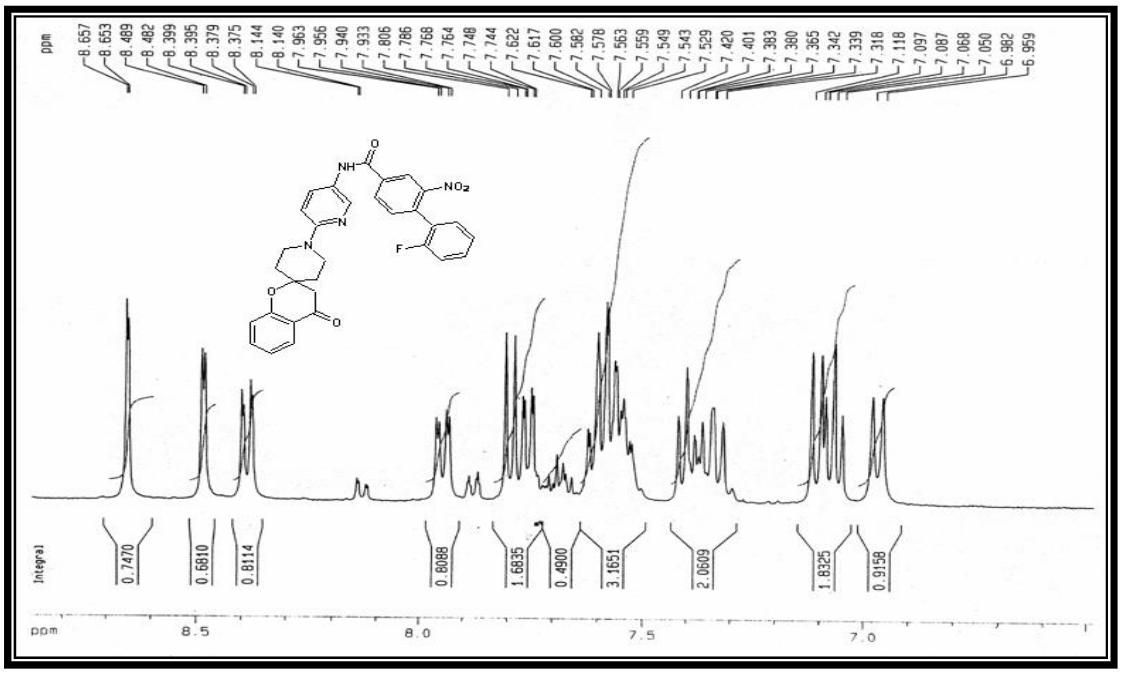
Instrument: SHIMADZU FTIR 8400 Spectrophotometer; **Frequency range**: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No.
		Observed	Reported	
Alkane -CH_2	-C-H asym. str.	2929	2975-2850	42
	-C-H sym. Str.	2852	2900-2800	42
	-C-H asym. def.	1462	1460-1400	42
	-C-H sym. Def.	1359	1385-1300	42
Aromatic	-C-H str.	3040	3090-3010	42
	-C=C- ring skeleton	1525	1600-1450	44
		1460	1600-1450	44
	C-H o.o.p. def. (sym.)	758	800-750	44
Pyridine moiety	-C=N str.	1613	1690-1570	43
	-C-N str.	1311	1310-1250	43
Chromanone moiety	C-O-C asym. Str.	1257	1250-1200	42
	C-O-C sym. Str.	1109	1050-1010	42
	Cyclic -C=O str.	1730	1740-1650	42
Amide Ketone	-C=O str.	1668	1740-1650	42

1H-NMR Spectral Study of 4-(2-fluoro phenyl) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (4a).



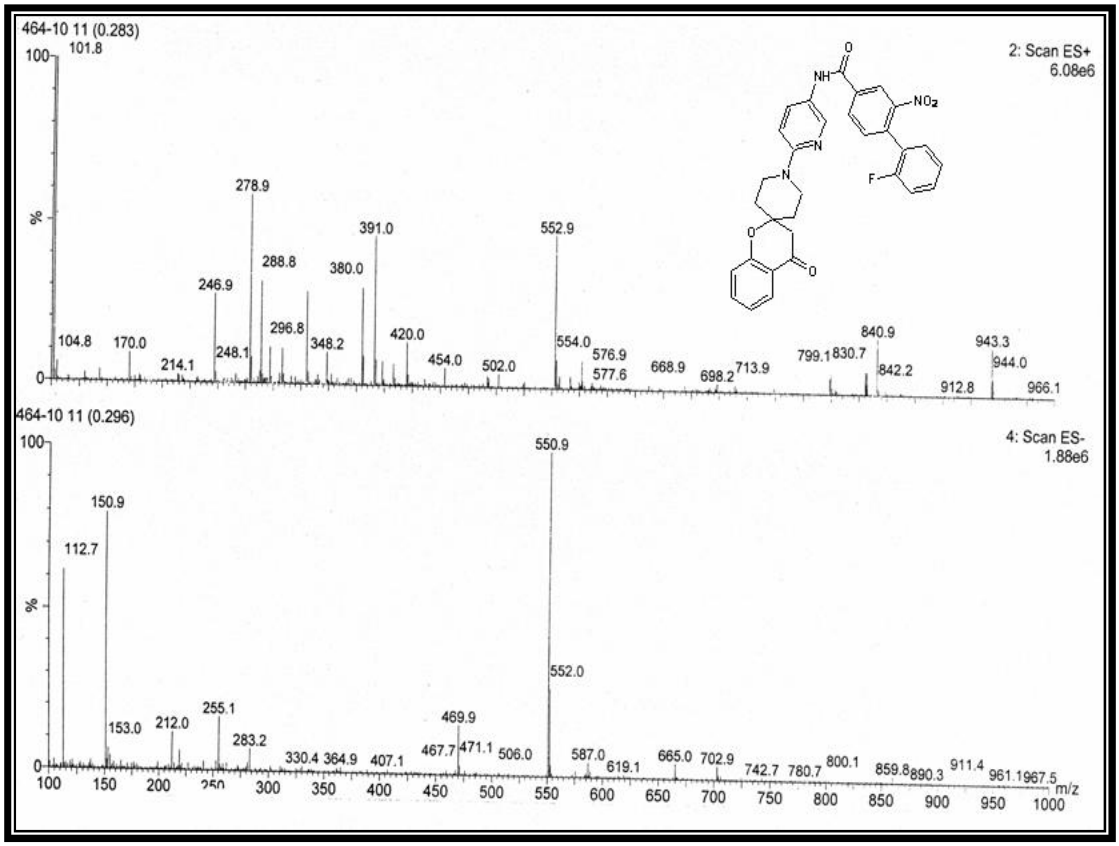




Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO-*d6*

Serial No.	Signal Position(δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	1.721-1.794	2H	Multiplate	-(CH_2) a
2	1.959-1.992	2H	Multiplate	-(CH_2) b,
3	2.883	2H	Singlet	-(CH_2) c
4	3.285-3.312	2H	Multiplate	-(CH_2) d
5	3.987-4.020	2H	doublet	-(CH_2) e
6	6.959-8.657	14H	Complex	Ar -H
7	10.53	1H	Singlet	-(NH) o

Mass Spectral Study of 4-(2-fluoro phenyl) -3-nitro-N-[6-{4-oxo-3, 4 dihydro-1'H- spiro-(chromene-2, 4'- piperidin)-1'-yl} pyridine-3-yl]-benzamides (4a).



Possible Mass Fragmentation pattern:

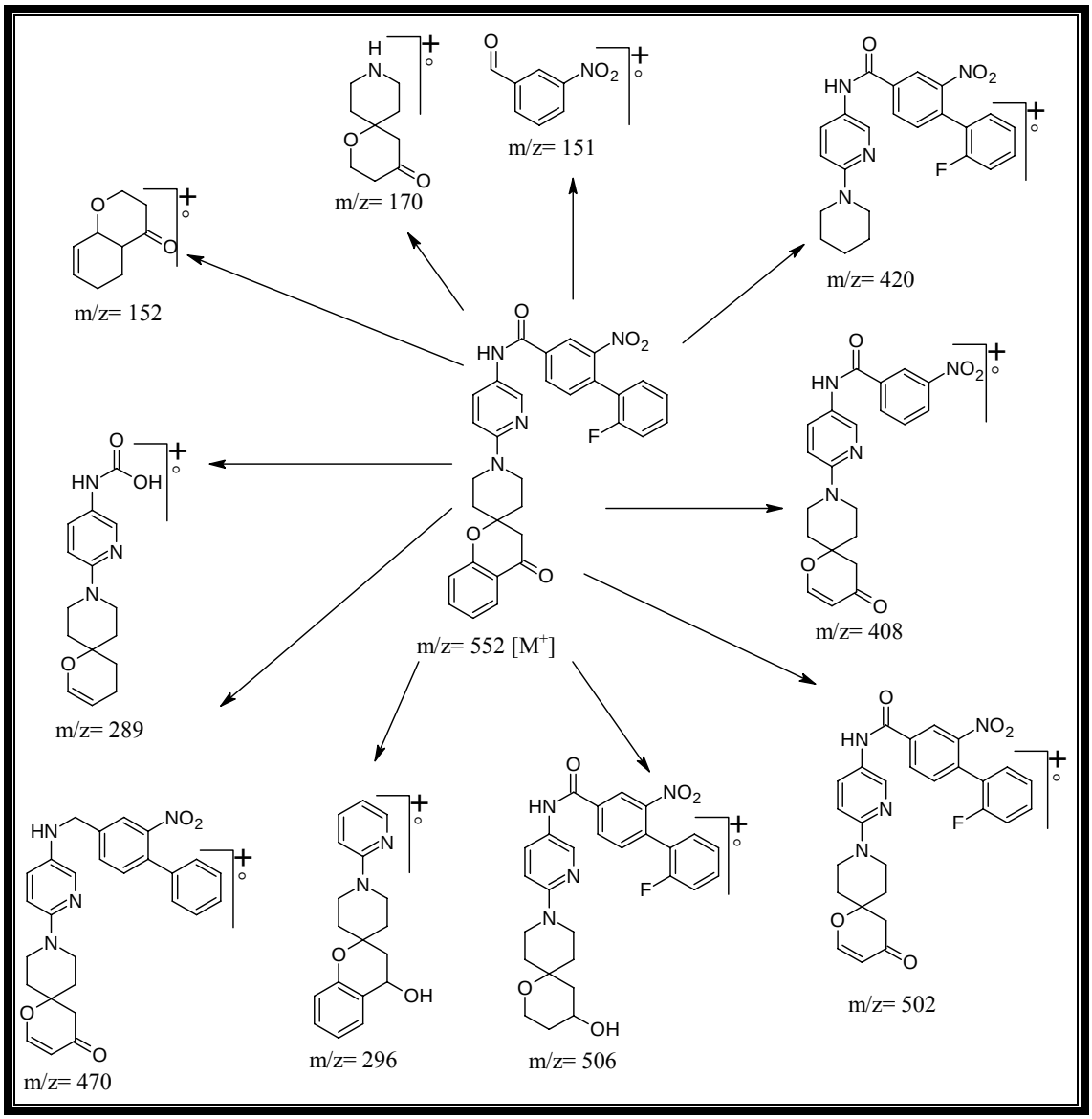


TABLE NO. 4a: Antimicrobial Activity Of 4-(Substituted Phenyl) -3 Nitro-N-[6-{4-Oxo-3, 4 Dihydro-1'H- Spiro-(Chromene-2, 4'- Piperidin)-1'-yl} Pyridine-3-yl]-Benzamides (4a-k).

TABLE NO-4(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
4a	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
4b	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-
4c	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-
4d	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-
4e	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
4f	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+
4g	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
4h	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
4i	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
4j	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
4k	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-

Reference drugs:	***S. aureus***	***B. Subtilis***	***E.Coli***	***S. Peratyphi B***
Ciprofloxacin	**1.9**	**7.8**	**0.4**	**1.4**

TABLE NO. 4b: Antimicrobial Activity Of 4-(Substituted Phenyl) -3 Nitro-N-[6-{4-Oxo-3, 4 Dihydro-1'H- Spiro-(Chromene-2, 4'-Piperidin)-1'-yl Pyridine-3-yl-Benzamides (2a-k).

TABLE NO-4(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (ug/ml)*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
4a	+	+	+	-	+	+	+	-
4b	+	+	+	+	+	+	+	-
4c	+	+	+	-	+	+	+	-
4d	+	+	+	-	+	+	+	-
4e	+	+	-	-	+	+	+	-
4f	+	+	+	-	+	+	+	-
4g	+	+	+	-	+	+	-	-
4h	+	+	+	-	+	+	+	-
4i	+	+	+	-	+	+	+	-
4j	+	+	-	-	+	+	-	-
4k	+	+	+	-	+	+	+	-
Reference drugs:			*A.niger*				*C. albicans*	
Fluconazole			**0.7**				**0.4**	

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 mg/ml conc. (2) Secondary Screening 1000 mg/ml to 250 mg/ml conc. (3) Tertiary Screening start from 125 mg/ml to 3.9 mg/ml. The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed in tertiary screening.

From the results of experiments using newly synthesized organic compounds' it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 4a-k almost eight compounds 4a, 4b, 4d, 4e, 4f, 4h, 4i and 4k were found active at 500 µg/ml conc. against *staphylococcus aureus*. *Bacillus Subtilis* was inhibited at 500 µg/ml conc. by eight compounds 4a, 4b, 4d, 4e, 4f, 4h, 4i and 4k. Eight compounds 4a, 4b, 4d, 4e, 4f, 4h, 4i and 4k were active against both cultures *B.Subtilis* and *S.aureus*. At the conc. 250 µg/ml *S. aureus* was not inhibited by any compounds. *B.Subtilis* was killed by two compounds 4a and 4d (4a-k). So, it is obvious from the data obtained that compounds 4a and 4d were highly active among all the compounds of series 4a-k.

For Gram Negative bacteria in the series 4a-k almost eight compounds 4a, 4b, 4c, 4d, 4f, 4h, 4i and 4k were found active at 500 µg/ml conc. against *Escherichia Coli. S.Paratyphi B.* was inhibited at 500 mg/ml conc. by eight compounds i.e. 4a, 4b, 4c, 4d, 4f, 4h, 4i and 4k So, eight compounds were active against both cultures *E.Coli* and *S.Paratyphi B.* i.e. 4a, 4b, 4c, 4d, 4f, 4h, 4i and 4k. At the conc. 250 µg/ml *E.Coli* was killed by three compounds i.e. 4a, 4b and 4h (4a-k). *S.Paratyphi B.* was also inhibited by three compounds i.e. 4a, 4f and 4h. So, it is obvious from the data obtained that compounds 4a and 4h was highly active among all the compounds of series 4a-k.

For fungi in the series 4a-k almost nine compounds 4a, 4b, 4c, 4d, 4f, 4g, 4h, 4i and 4k were found active at 500 mg/ml conc. against *A.niger*. *C. albicans* was

inhibited at 500 µg/ml conc. by nine compounds i.e. 4a, 4b, 4c, 4d, 4e, 4f, 4h, 4i and 4k. At the conc. of 250 mg/ml *C.albicans* was not killed by any compound. *A. Niger* was killed by one compound i.e. 4b. So, it is obvious from the data obtained that compound 4b was highly active among all the compounds of series 4a-k.

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Part-II (Pyrimidylspiro derivatives)

INTRODUCTION

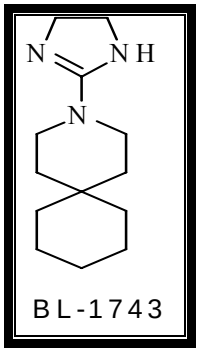
2-Piperazino-pyrimidine derivatives bearing spiro piperidine derivatives and their biological importance.

The influenza virus, especially the H5N1 strain of influenza A, is a serious threat to human health. The outbreak of highly pathogenic H5N1 avian influenza virus in 1997 and 2004/5 caused the death of millions of chickens and had a very high mortality rate among the limited number of infected humans. These cases cause great concern about the transmission of avian virus mutants among humans. The spread of influenza virus is mainly due to two factors: antigenic drift and antigenic shift. [1]

The AM2 proton channel plays an important role in viral replication by facilitating uncoating of the virus after its endocytosis into the host cells. The low pH of the endosome activates the AM2 channel, thus allowing proton flux into the viral interior. This acidification dissociates the viral RNA from its bound matrix proteins, [2] a process that is required to release the viral genetic material to the cytoplasm for replication. [3]

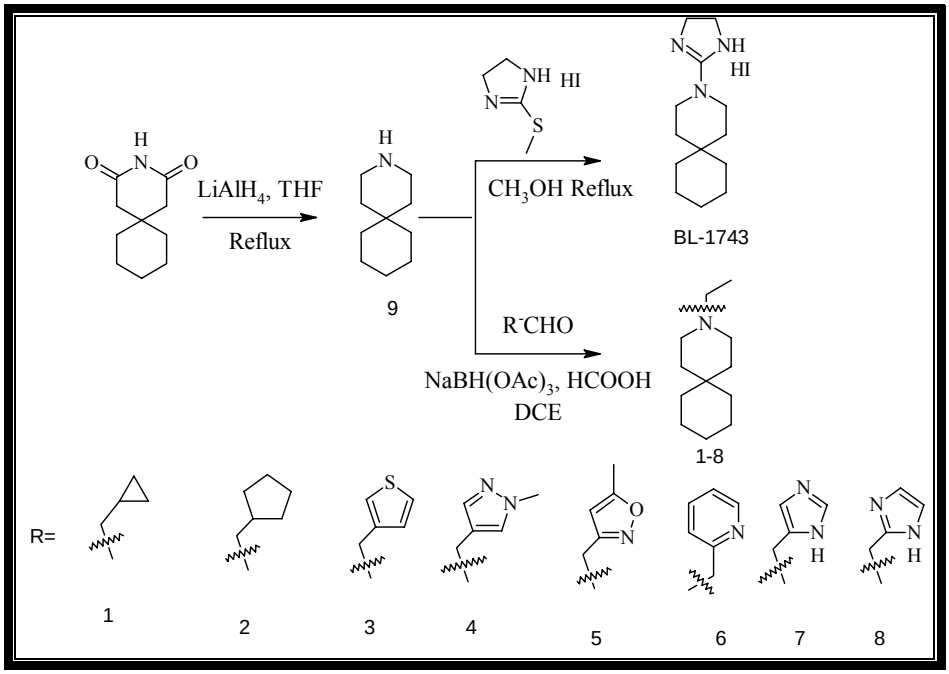
Amantadine targets AM2 by blocking the acidification of the virus entrapped in endosomes. Unfortunately the use of amantadine-related drugs is limited by central nervous system (CNS) side effects and the rapid emergence of drug-resistant viruses such as L26F, V27A, A30T, and S31N.[4-5] Extensive structure-activity relationship (SAR) studies of adamantly derivatives [6-8] have been evaluated, leading to a series of potent adamantine analogues active against H2N2 and H3N2 viruses.

However, few other molecular scaffolds have been explored, which led to search for novel scaffolds that might provide new avenues for developing antagonists of AM2. The spirene guanidine analogue, 2-[3-azaspiro(5,5)undecanol]-2-imidazoline (BL-1743), is discovered through a high-throughput screen based on the ability of inhibitors to reverse the toxicity associated with M2 channels expressed in the yeast Saccharomyces cerevisiae membranes.[9]



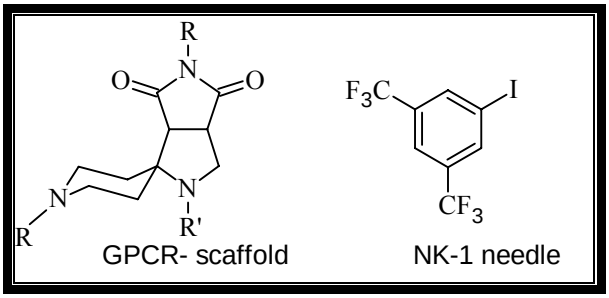
wang et al describes spiro piperidine derivatives and their Modulation of the Dynamics of the M2

Proton Channel from Influenza A Virus. [10]

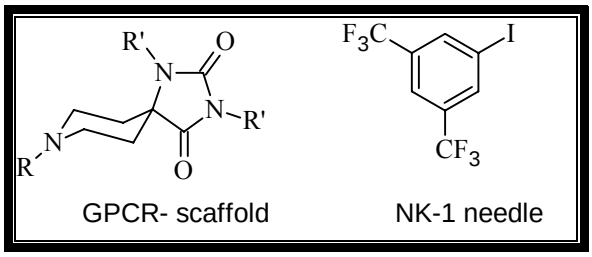


Exploration of G-protein-coupled receptor (GPCR) ligands in drug discovery is an outstanding subject in constant progress in today's pharmaceutical research. [11] Several motives have been identified in the structure of GPCR ligands. These moieties are referred to "privileged structures" and have successfully been utilized for the design of novel ligands. [12] Within the known "privileged structures", spiropiperidines have provided potent agonists and antagonists for different biological GPCR targets. [13]

The generation of a compound library consisting of a spiropyrrolo-pyrrole as a privileged GPCR scaffold and the 3, 5-bis (trifluoromethyl) phenyl motive as a neurokinin-1 specific needle is described. A series of nanomolar activities are disclosed. [14]

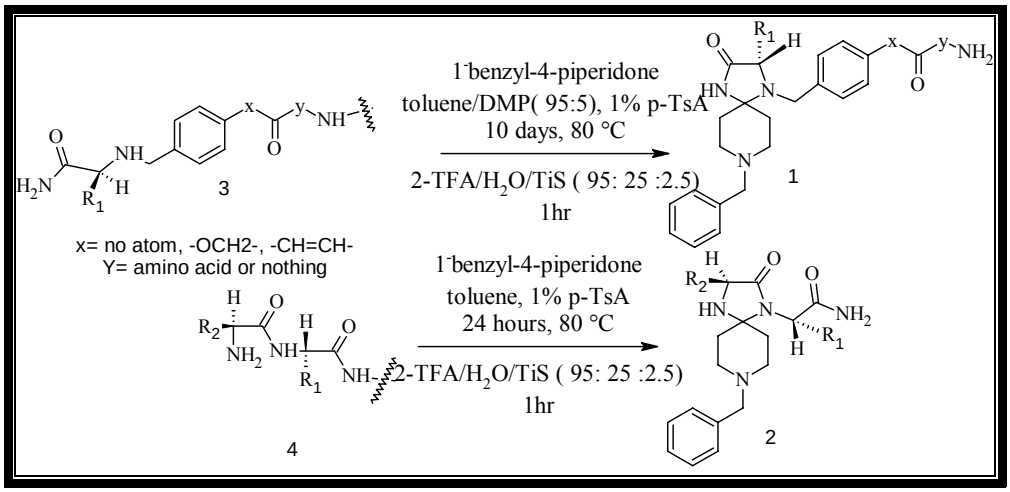


Similarly, the generation of a compound library consisting of spirohydantoin as a privileged GPCR scaffold and the 3, 5-bis (trifluoromethyl) phenyl motive as a neurokinin-1 specific needle is described. A series of nanomolar actives are disclosed. [15]



Bedos et al synthesized a potent and selective B1 bradykinin receptor antagonist, JMV1645 (H-Lys- Arg-Pro-Hyp-Gly-Igl-Ser-D-BT-OH), containing a dipeptide mimetic ((3S)-amino-5-carbonylmethyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-one (D-BT) moiety) at the C-terminal. Analogues of this potent B1 bradykinin receptor antagonist in which the central Pro2-Hyp3-Gly4-Igl5 tetrapeptide has been replaced by constrained N-1-substituted-1, 3, 8-triazaspiro [4.5] decan-4-one ring system were synthesized. Among these analogues, compound JMV1640 (1) was found to have an affinity of 24.10 ± 9.48 nM for the human cloned B1 receptor. It antagonized the [des-Arg10]-kallidin-induced contraction of the human umbilical vein (pA2 = 6.1 ± 0.1). Compound 1 was devoid of agonist activity at the kinin B1 receptor. Moreover, it did not bind to the human cloned B2 receptor. Therefore, JMV1640 constitutes a lead compound for the rational search of nonpeptide B1 receptor analogues based on the BK sequence. [16]

Felie et al reported solid support synthesis of spiroimidazolones. This methodology allowed the preparation of 8-benzyl-4-(p-substituted-benzyl)-1,4,8-triazaspiro[4.5]decan-2-ones 1 and 2-(8-benzyl-2-oxo-1,4,8-triazaspiro[4.5]dec-1-yl)acetamides 2 from Rink amide lanterns. The key step of the synthesis was the formation of the spiroimidazolidinone system which was performed by condensation of N-benzyl-4-piperidone onto amino acid amides 3 and dipeptides 4 linked to the solid support, respectively. This step was accomplished after a reaction time of 10 days for the preparation of compounds 1, whereas it was completed after 24 h for the synthesis of compounds 2. Although in both strategies, the HPLC purities and yields of the spiroimidazolidinones were satisfactory, the long reaction time was the limiting factor of the process. [17]

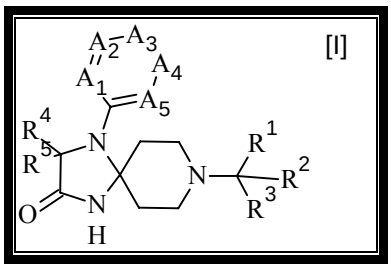


Tuberculosis (TB) caused by Mycobacterium tuberculosis bacteria (MTB) a is one of the most prevalent diseases that is responsible for the deaths of about one billion people during the last two centuries. TB remains a serious public health problem in India, accounting for nearly one-third of the global burden, and it has been estimated that 3.5 million of the population are infected with TB. [18] Hence, the discovery of fast-acting effective new drugs to effectively combat TB, including multidrug resistant tuberculosis, is imperative.

Kumar et al reported atom economic and stereoselective synthesis of several spiro-piperidin-4-ones through 1,3-dipolar cycloaddition of azomethine ylides generated in situ from isatin and R-amino acids viz. proline, phenylglycine, and sarcosine to a series of 1-methyl-3,5-bis[(E)- arylmethylidene]tetrahydro-4(1H)-pyridinones. These compounds were evaluated for their in vitro and in vivo activity against Mycobacterium tuberculosis H37Rv (MTB), multidrug resistant Mycobacterium tuberculosis (MDR-TB) and Mycobacterium smegmatis

(MC2). Compound 4-(4-fluorophenyl)-5-phenylpyrrolo(spiro[2.3″]oxindole)spiro[3.3′]-1′-methyl-5′-(4-fluorophenyl ethylidene) piperidin-4′-one was found to be the most active in vitro with a MIC value of 0.07 μM against MTB and was 5.1 and 67.2 times more potent than isoniazid and ciprofloxacin, respectively. In vivo, compound decreased the bacterial load in lung and spleen tissues with 1.30 and 3.73-log 10 protections respectively and was considered to be promising in reducing bacterial count in lung and spleen tissues. [19]

US7, 557,117 relates to 4-oxoimidazolidine-2-spiropiperidine derivatives represented by a general formula [I]



In which A1, A2, A3, A4 and A5 stand for optionally halogen-substituted methine, or nitrogen atom.

These compounds act as nociceptin receptor agonist, and are useful as analgesic, reliever from tolerance to narcotic analgesic, reliever from dependence on narcotic analgesic, analgesic enhancer, antiobestic, drug for ameliorating brain function, remedy for schizophrenia, drug for treating regressive neurodegenerative diseases, antianxiety agent or antidepressant and remedy for diabetes insipidus and polyuria. [20]

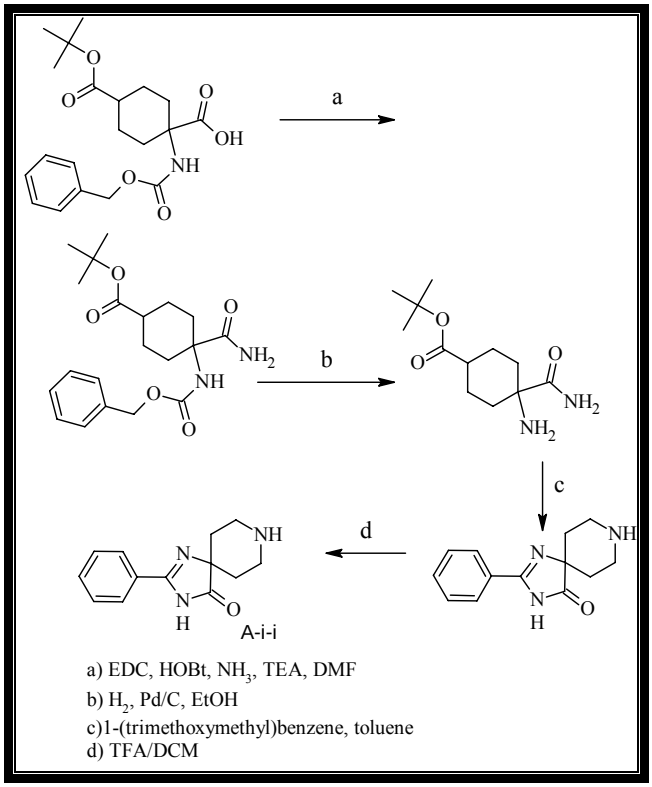
It also describes use of the compounds or salts thereof for formulating pharmaceutical compositions adequate for analgesic purpose; relief from tolerance to narcotic analgesic represented by morphine; relief from dependence on narcotic analgesic represented by morphine; enhancing analgesic action, treating obesity; improving brain function; treating antianxiety or antidepression; treating diabetes insipidus; treating polyuria; treating hypotension; anesthesia or assisting anesthesia; remedy for sleep disorders represented by insomnia including increased sleep latency, intermittent wakefulness and decreased sleep efficiency; remedy for circadian rhythm disorder such as jet lag; drug for improving erectile function; airway dilation during dyspenea such as asthma or antitussive; or as drug for ameliorating motility of digestive tract during hypokinesis of digestive tract. methods for pain- killing, relief from tolerance to narcotic analgesic represented by morphine; relief from dependence on narcotic analgesic represented by morphine; enhancing analgesic action,

treating obesity; improving brain function; treating schizophrenia.

Antianxiety or antidepression; treating diabetes insipidus; treating polyuria; treating hypotension; anesthesia or assisting anesthesia; remedy for sleep disorders represented by insomnia including increased sleep latency, intermittent wakefulness and decreased sleep efficiency; remedy for circadian rhythm disorder such as jet lag; drug for improving erectile function; airway dilation during dyspenea such as asthma or antitussive; or as drug for ameliorating motility of digestive tract during hypokinesis of digestive tract, which are characterized by administering the compounds or salts thereof to patients: and a method for producing 4-oxoimidazolidine-2-spiropiperidine derivatives or salts thereof.

Padmavathi et al report new class of spiro-pyrimidinones, pyrazolidinones and isoxazolidinones prepared from 4-cyano-4-ethoxycarbonyl-piperidines / tetrahydropyrans / tetrahydrothiopyrans [21].

US2008/0004261 provides CGRP receptor antagonists, pharmaceutical compositions thereof, and methods therewith for treating CGRP receptor-mediated diseases and conditions.CGRP (Calcitonin Gene-Related Peptide) is a naturally occurring 37-amino acid peptide that is generated by tissue- specific alternate processing of calcitonin messenger RNA and is widely distributed in the central and peripheral nervous system. CGRP is localized predominantly in sensory afferent and central neurons and mediates several biological actions, including vasodilation. CGRP is expressed in alpha- and beta- forms that vary by one and three amino acids in the rat and human, respectively. CGRP-alpha and CGRP-beta display similar biological properties. When released from the cell, CGRP initiates its biological responses by binding to specific cell surface receptors that are predominantly coupled to the activation origin.



The compounds of the present invention have utility in treating, preventing, ameliorating, controlling or reducing the risk of one or more of the following conditions or diseases: headache; migraine; cluster headache; chronic tension type headache; pain; chronic pain; neurogenic inflammation and inflammatory pain; neuropathic pain; eye pain; tooth pain; diabetes; non-insulin dependent diabetes mellitus; vascular disorders; inflammation; arthritis; bronchial hyperreactivity, asthma; shock; sepsis; opiate withdrawal syndrome; morphine tolerance; hot flashes in men and women; allergic dermatitis; encephalitis; brain trauma; epilepsy; neurodegenerative diseases; skin diseases; neurogenic cutaneous redness, skin rosaceousness and erythema; tinnitus; inflammatory bowel disease, irritable bowel syndrome, cystitis; and other conditions that may be treated or prevented by antagonism of CGRP receptors. Particular importance is the acute or prophylactic treatment of headache, including migraine and cluster headache. [22]

The nociceptin (NOP) receptor is the latest identified member of the transmembrane G-protein coupled receptors family comprising d, k and m opioid receptors.[23]

Its original homology cloning and the isolation from brain extracts of the endogenous ligand, the heptadecapeptide nociceptin (NC), [24, 25] gave rise to a great interest in clarifying its

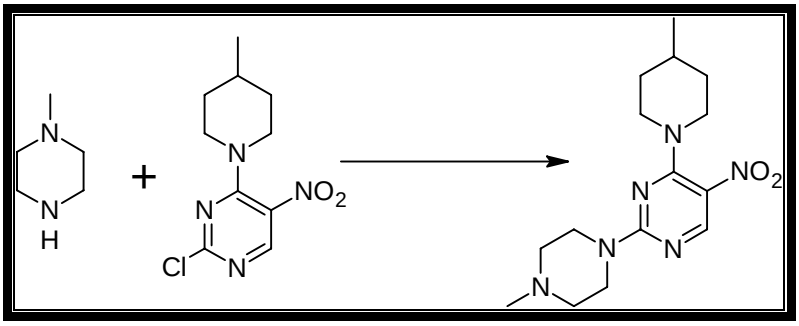
physiological functions. The receptor, distributed in both central and peripheral nervous systems and in non-neuronal tissues, revealed to be involved in several processes including modulation of nociception, locomotor activity, reversal of stress-induced analgesia, [26] modulation of learning and memory, [27-29] regulation of neurotransmitter and hormone release, [30, 31] neuronal differentiation.[32, 33]

In the last years NOP receptor has become an important biological target for a number of potential therapeutic applications associated with pain, stress and anxiety, cognitive deficiency, eating and other disorders. [34] At the same time, consistent advances have been attained in the design and discovery of new ligands, and many nonpeptide small molecules were developed as agonists or antagonists. [35-39]

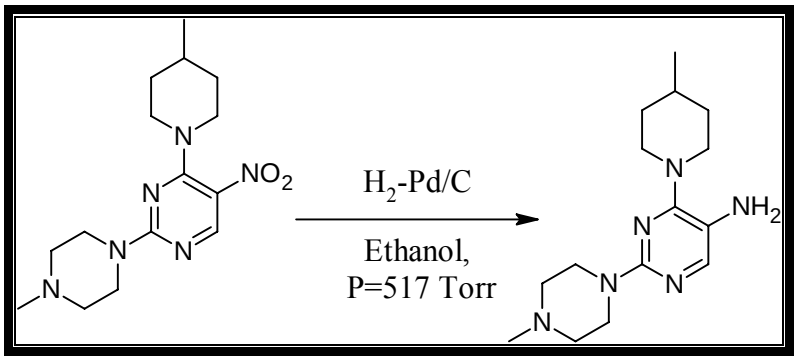
Among several scaffolds, spiropiperidines showed to be an interesting and promising class of NOP ligands. They are structurally related to lofentanyl, a m -selective opiate ligand having also affinity for the NOP receptor.

The NOP active site [40, 41] was first investigated by computational studies on its complex with nociceptin [42, 43] and lofentanyl Anyway spiropiperidines [44] were useful tools for the elucidation of the mechanism of interaction of small non peptidic molecules. These studies indicated the electrostatic interaction of protonated piperidinic nitrogen with Asp130 and a hydrogen bond between the small molecule and Thr305 as determinant features for affinity.

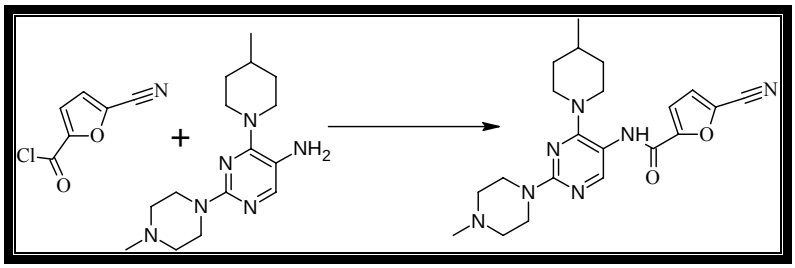
US7705042 describes compounds that inhibit protein tyrosine kinases, especially c-fms kinase. Methods of treating autoimmune diseases; and diseases with an inflammatory component; treating metastasis from ovarian cancer, uterine cancer, breast cancer, colon cancer, stomach cancer, hairy cell leukemia and non-small lung carcinoma; and treating pain, including skeletal pain caused by tumor metastasis or osteoarthritis, or visceral, inflammatory, and neurogenic pain; as well as osteoporosis, Paget's disease, and other diseases in which bone resorption mediates morbidity including arthritis, prosthesis failure, osteolytic sarcoma, myeloma, and tumor metastasis to bone using these compounds. These compounds also includes 2-piperazine pyrimidine derivatives. [45]



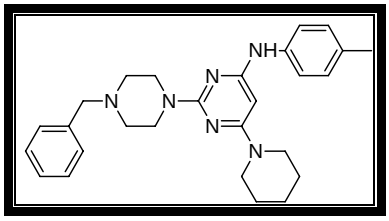
Illig et al describe reduction of nitro derivative to get amino compound. [46]

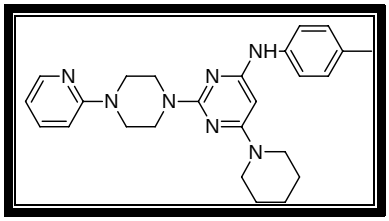


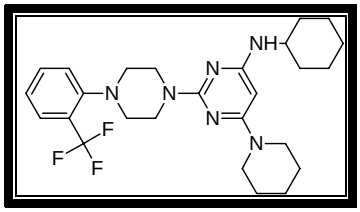
Discovery of a series of novel 2,4-disubstituted arylamides as potential anti-inflammatory agents is also describes in this paper.

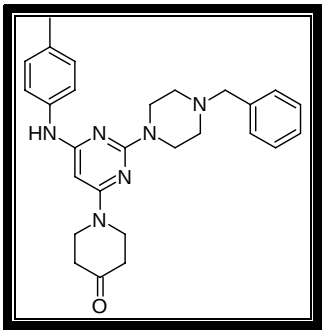


US2003/0078271 reports compounds which are selective antagonists for the GAL3 receptor. [47]





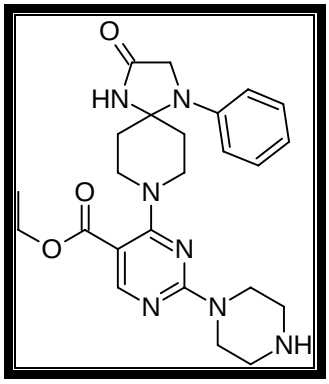




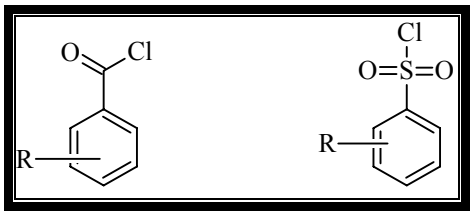
Thus, spiro piperidine and 2-piperazino pyrimidine forms very interesting pharmaceutical scaffolds. These compounds show very promising biological activity for variety of therapeutic category. Various research papers and patents, as discussed above describes different modifications to this scaffold and their effect on their potency as therapeutic agents.

Current work:-

Current work provides the modifications to spiro piperidine scaffold.



This scaffold is reacted with following reactants



Wherein R stands for various substitutions describe in experimental sections. Synthesized compounds are characterized by spectral analyses. These compounds can display the pharmacological activities as their previous structurally related compounds do.

S

The work is further subdivided into following sections.

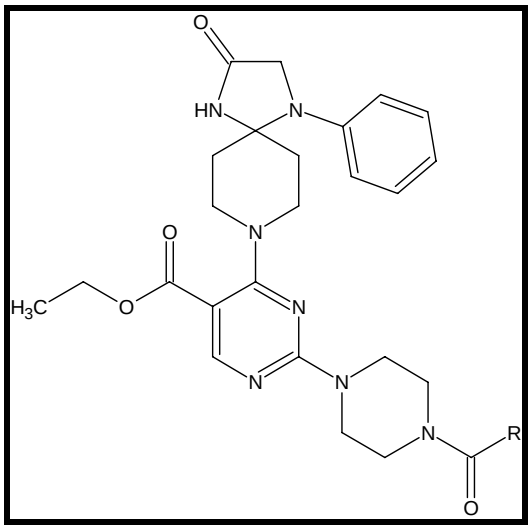
1) Preparation, Characterization and antimicrobial evaluation of Ethyl -2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate

2) Preparation, Characterization and antimicrobial evaluation of Ethyl -2-[4-(Sub stituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates**.**

SECTION-I

Preparation, Characterization and antimicrobial evaluation of Ethyl -2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine-5-carboxylate.

Keeping in view of wide spectrum biodynamic activities[1-48] of Pyrimidine and with a view to have potent therapeutic agents, the synthesis of **Ethyl -2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate (1a-k)** have been synthesized by the using Ethyl 2-(piperazin-1-yl) 4-(1-phenyl-3-oxo-1,4,8-triazaspiro [4,5] dec-8-yl) pyrimidine -5-carboxylate with different substituted benzoyl chlorides.



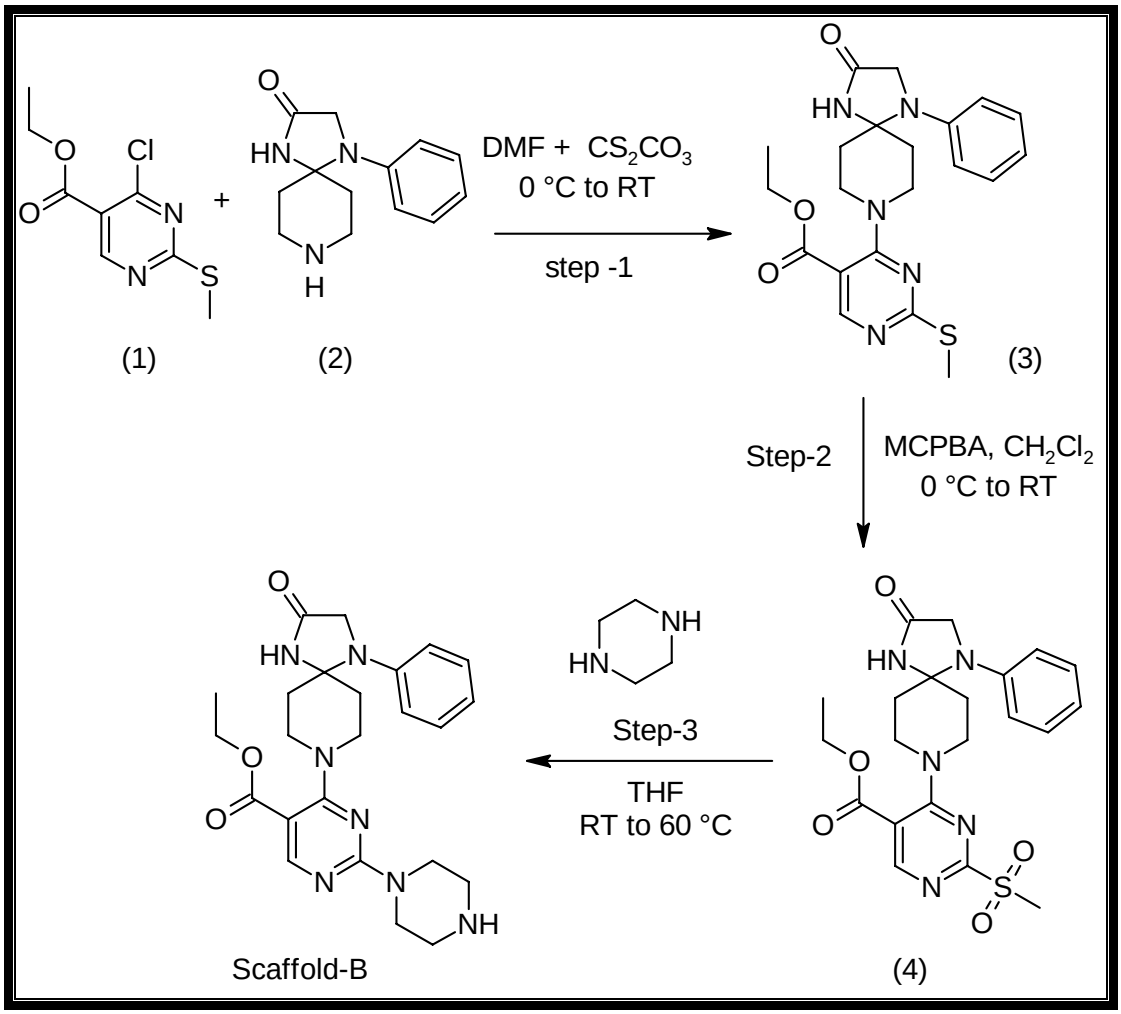
(1a-k)
R= Aryl

The constitution of the synthesized products (1a-k) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

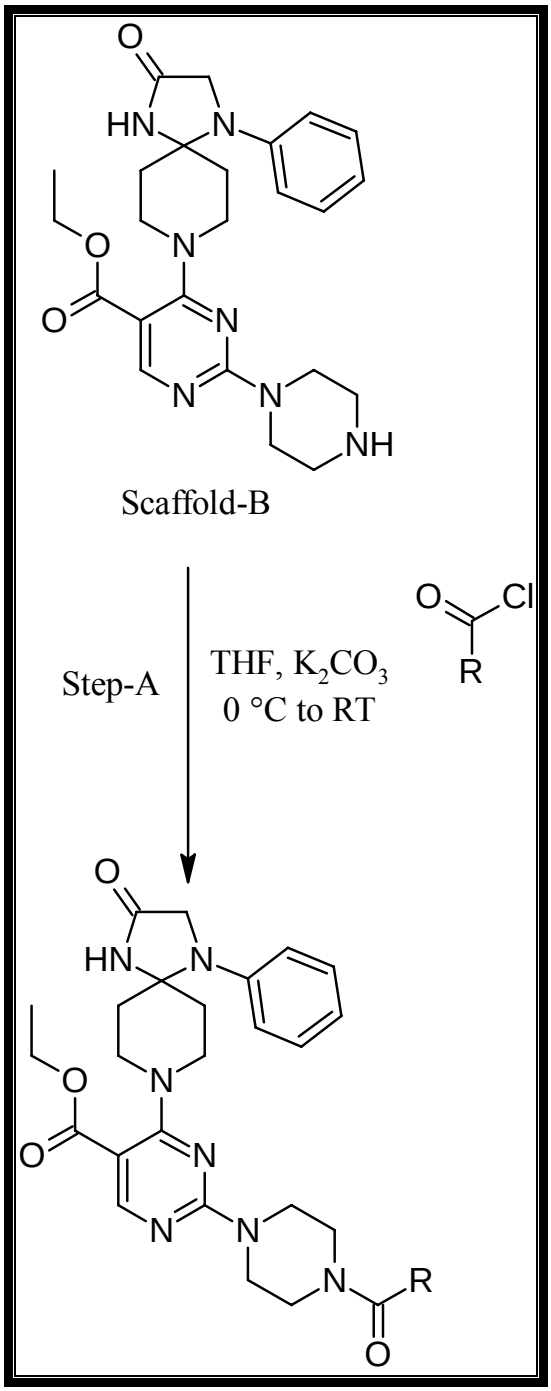
All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella peratyphi B* and they were also evaluated for antifungal activity against *Candida albicans*

and *Aspergillus niger* at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (1a-k) have been compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by **NCCLs**).

REACTION SCHEME



REACTION SCHEME



(1a-k)

R= Aryl

EXPERIMENTAL

Preparation, Characterisation and antimicrobial evaluation of Ethyl-2-(4-Substitutedbenzoylpiperazine-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspir [4, 5] dec-8-yl) pyrimidine -5-carboxylate.

[A] Synthesis of Scaffold-B.

1. Synthesis of Ethyl -2-(methylthio)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate (Intermediate-3).

In RBF, 4-phenyl-1, 4, 8-triazaspiro [4.5] decan-2-one (1.10mol) and DMF (10 times) were charged. K_2CO_3 (2.50mol) and ethyl 4-chloro (methylsulfonyl) pyrimidine-5-carboxylate (1.00mol) was added at 0°C. Then reaction mixture was allowed to warm at room temperature and stirred for 4-5 hours. After the completion of reaction, Reaction mixture was quenched in crushed ice. The product was extracted with Dichloromethane. The organic phase was washed with 1N HCl, brine solution and dried over sodium sulfate. The organic phase was concentrated under reduce pressure at 45°C to get desired product. Yield: 62.00 % M.P: 203°C. Elemental Analyses: Calculated: C (59.00%), H (5.89%), N (16.38%), Found: C (58.75%), H (5.62%), N (16.12%).

2. Synthesis of Intermediate-4.

In RBF, Intermediate-3 (1.00mol) was dissolved in Dry MDC. Then reaction mixture is allowed to cool at 0°C. M-chloro per benzoic acid (2.50mol) was added portion wise in to the reaction mixture at 0°C. Then reaction mixture was allowed to stirr at same temperature for 30 minutes. After the completion of reaction, reaction mixture was quenched with saturated sodium bicarbonate solution. The product was extracted with Dichloromethane and organic layer was washed with brine solution. The organic phase was dried over sodium sulphate and concentrated under reduce pressure at 45°C to get crude product which was purified by triturating with diethyl ether. Yield: 70.00 %. M.P: 213°C. Elemental Analyses: Calculated: C (54.89%), H (5.48%), N (15.24%), Found: C (54.6%), H (5.23%), N (15.03%).

3. Synthesis of Ethyl -2-(piperazine)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate. (Scaffold-B)

In RBF, Intermediate-4 (1.00mol) and piperazine (2.00mol) were dissolved in THF at room temperature. The reaction mixture was heated to 60°C temperature for 2 hours. After the completion of reaction, reaction mixture was quenched in water. The product was extracted with Chloroform, washed with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulphate and concentrated under reduce pressure at 45°C to get crude product. The crude product was purified by using column chromatography. Yield: 40.00 %. M.P: 189°C. Elemental Analyses: Calculated: C (61.92%), H (6.71%), N (21.06%), Found: C (61.52%), H (6.30%), N (20.45%).

[B] Synthesis of Ethyl -2-(4- benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate.

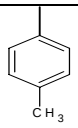
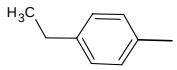
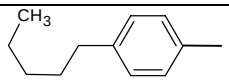
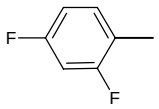
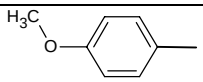
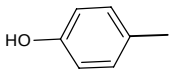
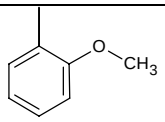
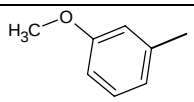
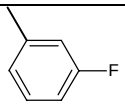
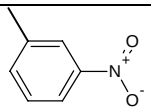
In RBF, Scaffold-B (1.00mol) in THF and K_2CO_3 (2.50mol) was added at room temperature. Then reaction mixture was cooled to 0°C. In reaction mixture, the benzoyl chloride (1.10mol) solution in THF was added at 0°C and reaction was allowed to warm at room temperature and stirred for 1 hour. Then solvent was removed under vacuum at 45°C. The residue obtained was quenched with water to get solid. The solid was triturating with diethyl ether to get pure product. Yield: 52.00 %. M.P: 144°C. Elemental Analyses: Calculated: C (65.36%), H (6.19%), N (17.21%), Found: C (61.31%), H (6.13%), N (17.16%).

Similarly, other compounds (1a-k) were synthesized by above mentioned process (B) from Scaffold-B. The physical data are recorded in Table-1.

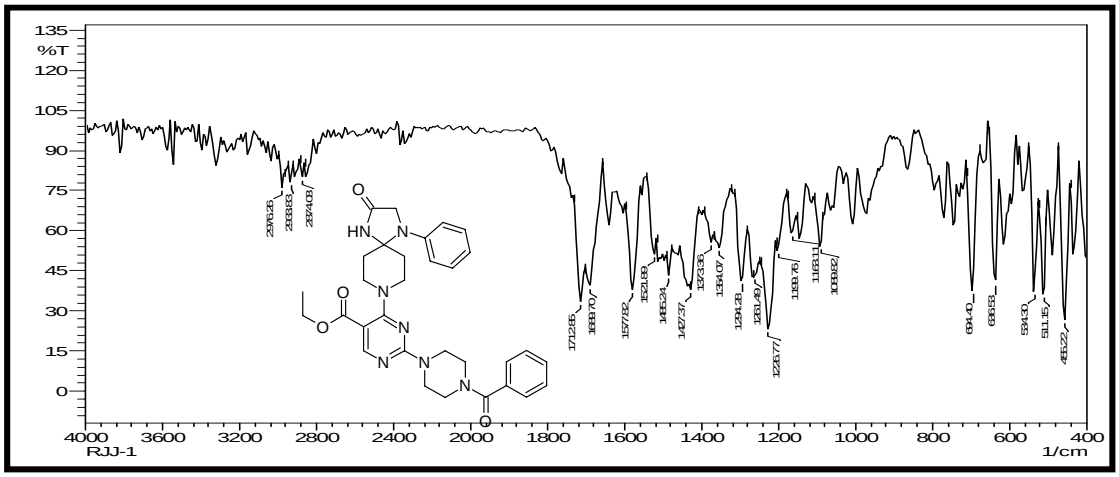
[C] Antimicrobial activity of Ethyl -2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate.

Antimicrobial activity testing was carried out as described in Part-I, Section-I, Page No. (32 to 36) The MIC values of test solution are recorded in Table NO-1a and 1b.

TABLE NO-1: Physical constants of Ethyl-2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine-5-arboxylates (1a-k).

Sr. No.	Substitution R	Molecular Formula/ Molecular weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
1a		$C_{31}H_{35}N_7O_4$ 569	144	52	65.36 65.31	6.19 6.13	17.21 17.16
1b		$C_{32}H_{35}ClN_6O_4$ 603	115	54	63.73 63.68	5.85 5.81	13.93 13.89
1c		$C_{34}H_{40}N_6O_4$ 596	157	48	68.43 68.39	6.76 6.71	14.08 14.03
1d		$C_{37}H_{46}N_6O_4$ 638	162	52	69.57 69.53	7.26 7.23	13.16 13.11
1e		$C_{32}H_{34}F_2N_6O_4$ 604	107	52	63.56 63.51	5.67 5.59	13.90 13.83
1f		$C_{33}H_{38}N_6O_5$ 598	132	58	66.20 66.13	6.40 6.33	14.04 14.02
1g		$C_{32}H_{36}N_6O_5$ 584	156	42	65.74 65.69	6.21 6.17	14.37 14.32
1h		$C_{33}H_{38}N_6O_5$ 598	175	56	66.20 66.14	6.40 6.35	14.04 14.01
1i		$C_{33}H_{38}N_6O_5$ 598	152	56	66.20 66.11	6.40 6.36	14.04 14.00
1j		$C_{32}H_{35}FN_6O_4$ 586	162	44	65.51 65.46	6.01 5.96	14.33 14.29
1k		$C_{32}H_{35}N_7O_6$ 613	109	48	62.63 62.59	5.75 5.71	15.98 15.93

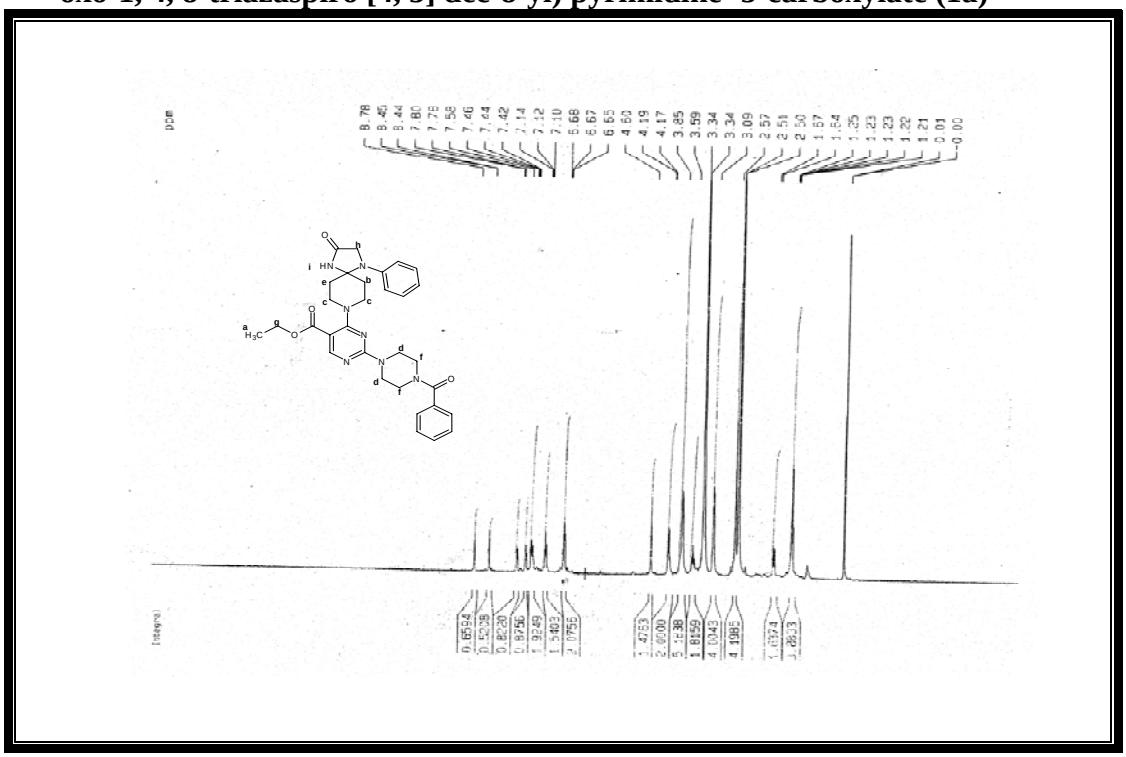
IR Spectral Study of Ethyl -2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate (1a).

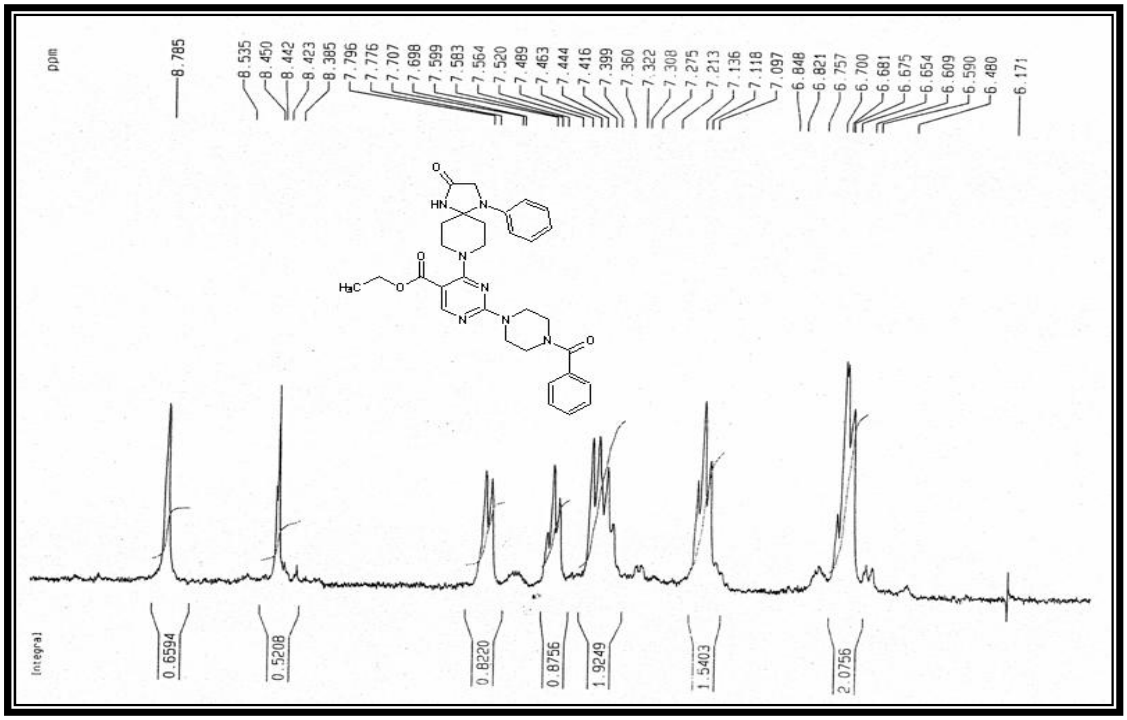


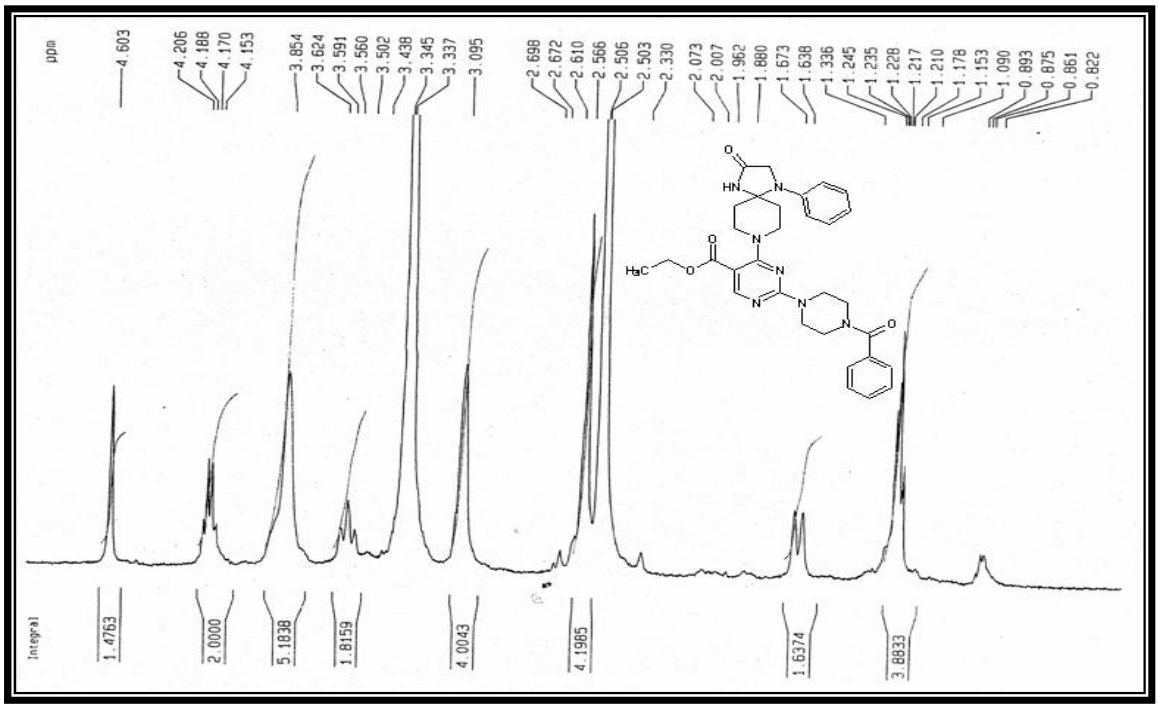
Instrument: SHIMADZU FTIR 8400 Spectrophotometer; **Frequency range**: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No
		Observed	Reported	(Part-I)
Alkane -CH_2	C-H asym. str.	2933	2975-2850	42
	C-H sym. str.	2862	2900-2800	42
	C-H asym. def.	1427	1460-1400	42
	C-H sym. Def.	1354	1385-1300	42
Aromatic	C-H str.	3030	3080-3010	42
	C=C ring skeleton	1521	1600-1450	44
	C-H o.o.p. def. (sym.)	1485	1600-1450	44
		694	740-670	44
Imidazolone moiety	-N-H-str (2° amine)	3330	3410-3300	43
	-N-H-def	1577	1650-1550	43
	-C=O str. (cyclic keto)	1740	1740-1650	43
Pyrimidine moiety	C-N str.	1620	1635-1595	43
	C-N str. Def	1261	1340-1250	43
Ester	-C=O str.	1713	1740-1650	42
	C-O-C assym. Str.	1226	1250-1200	42
	C-O-C sym. Str.	1089	1050-1010	42
Benzylic ketone	-C=O str.	1690	1740-1650	42

1H-NMR Spectral Study of Ethyl -2-(4-benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate (1a)



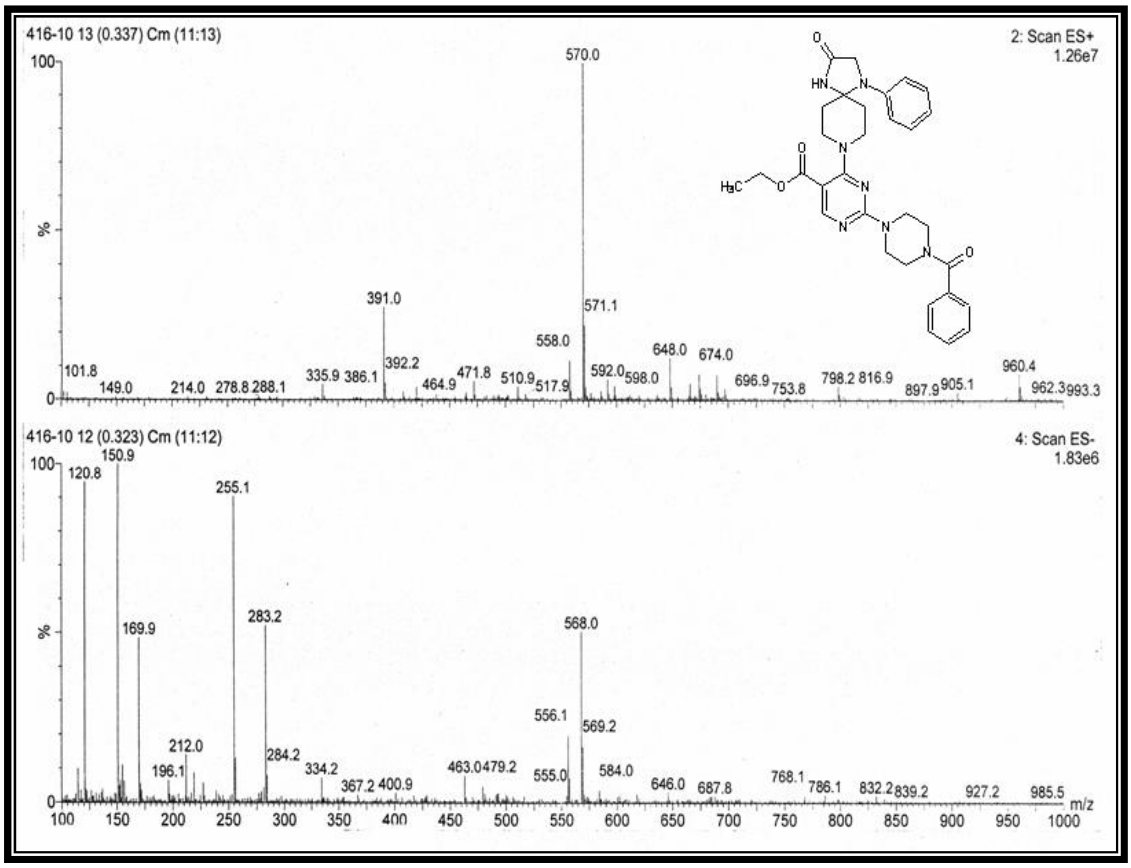




Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO-d6.

Sr. No	Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	1.15-1.24	3H	Triplate	-(CH3) a
2	1.638-1.673	2H	Doublet	-(CH2) b
3	2.566	4H	Singlate	-(CH2)2 c
4	3.095	4H	Singlate	-(CH2)2 d
5	3.438-3.560	2H	Triplate	-(CH2) e
6	3.854	4H	Singlate	-(CH2)2 f
7	4.153-4.206	2H	Quartrate	-(CH2) g
8	4.603	2H	Singlate	-(CH2) h
9	6.59-8.442	11H	Complex	Ar-H
10	8.785	1H	Singlet	-(NH) i

Mass Spectral Study of Ethyl -2-(4-benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate (1a).



Possible Mass Fragmentation pattern:

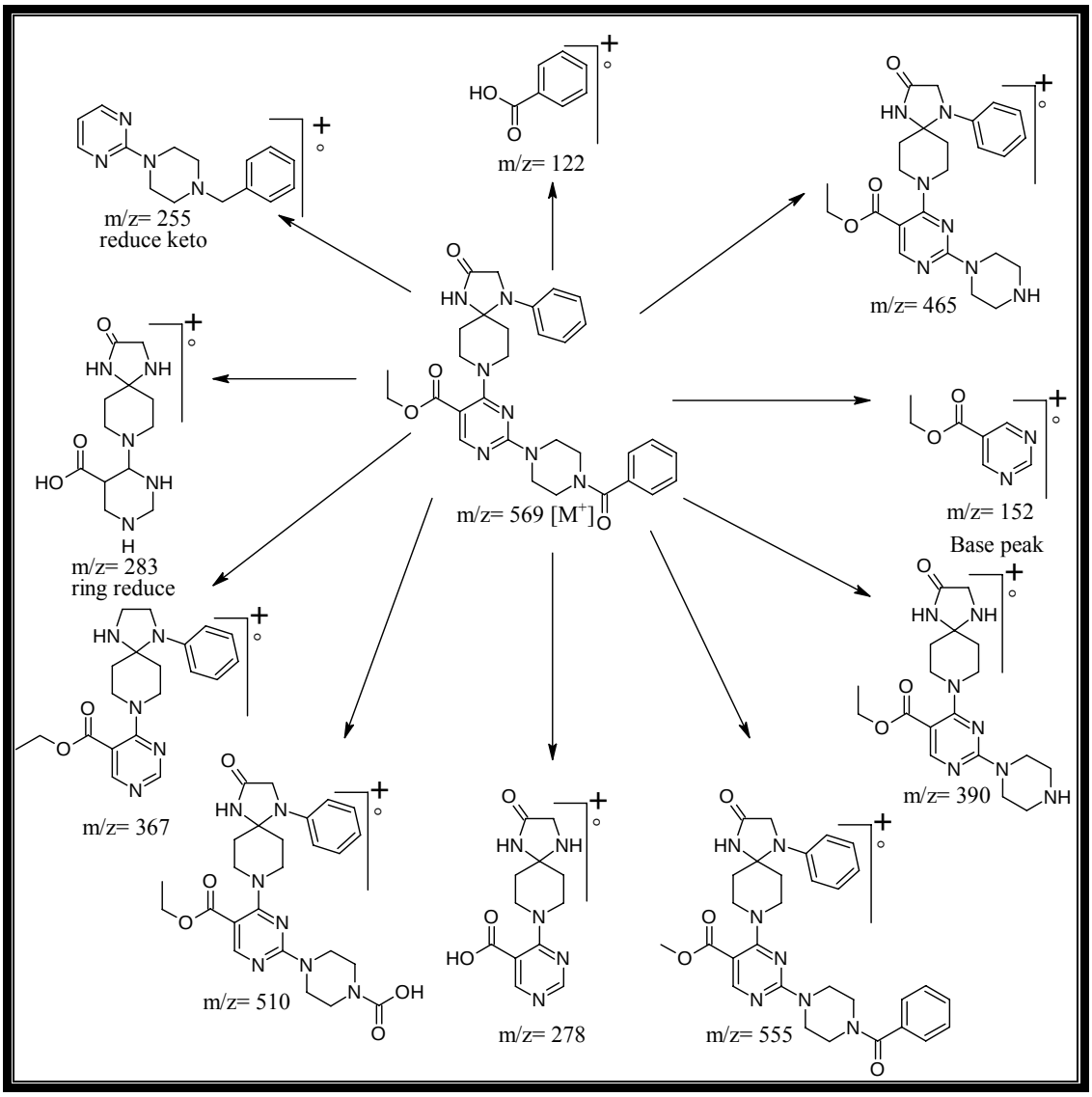


TABLE NO. 1a: Antimicrobial Activity Of Ethyl -2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate (1a-k)

TABLE NO-1(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
1a	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	-
1b	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-
1c	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
1d	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
1e	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-
1f	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
1g	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-
1h	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
1i	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
1j	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
1k	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-

Reference drugs:	***S. aureus***	***B. Subtilis***	***E.Coli***	***S. Peratyphi B***
Ciprofloxacin	**1.9**	**7.8**	**0.4**	**1.4**

TABLE NO. 1b: Antimicrobial Activity Of Ethyl -2-(4-Substituted benzoylpiperazin-1-yl)-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylate (1a-k).

TABLE NO-1(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (ug/ml)*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
1a	+	+	-	-	+	+	-	-
1b	+	+	-	-	+	+	-	-
1c	+	+	-	-	+	+	-	-
1d	+	+	+	-	+	+	+	-
1e	+	+	+	-	+	+	+	-
1f	+	+	-	-	+	+	-	-
1g	+	+	+	-	+	+	+	-
1h	+	+	-	-	+	+	-	-
1i	+	+	-	-	+	+	-	-
1j	+	+	+	-	+	+	+	-
1k	+	+	-	-	+	+	-	-
Reference drugs:			***A.niger***				***C. albicans***	
Fluconazole			**0.7**				**0.4**	

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 µg/ml conc. (2) Secondary Screening 1000 µg/ml to 250 µg/ml conc. (3) Tertiary Screening start from 125 µg/ml to 3.9 mg/ml. The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed in tertiary screening.

From the results of experiments using newly synthesized organic compounds' it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 1a-k almost all compounds were found active at 500 µg/ml conc. against *staphylococcus aureus. Bacillus Subtilis* was inhibited at 500 µg/ml conc. by all compounds 1a to 1k. Eleven compounds 1a to 1k were active against both cultures *B.Subtilis* and *S.aureus*. At the conc.250 µg/ml *S. aureus* was inhibited by two compounds 1b and 1g. *B.Subtilis* was killed by one compound 1e. So, it is obvious from the data obtained that compounds 1b, 1e and 1g were highly active among all the compounds of series 1a-k.

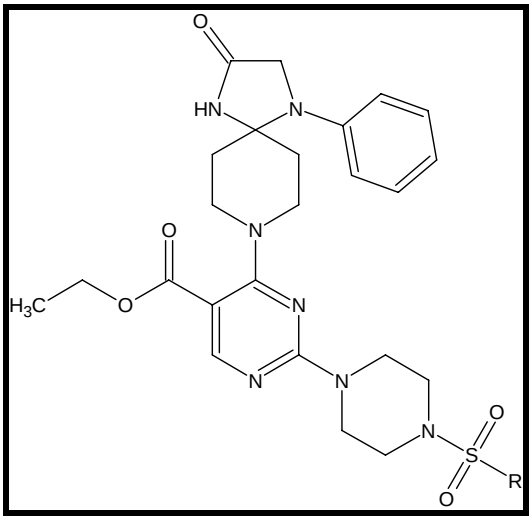
For Gram Negative bacteria in the series 1a-k almost five compounds 1d, 1e, 1g, 1h and 1j were found active at 500 µg/ml conc. against *Escherichia Coli*. *S.Paratyphi B.* was inhibited at 500 µg/ml conc. by four compounds i.e. 1a, 1e, 1g, and 1j. So, three compounds were active against *E.Coli* and *S.Paratyphi B.* i.e. 1e, 1g and 1j. At the conc. 250 µg/ml *E.Coli* was not killed by any compound (1a-k). *S.Paratyphi B.* was not inhibited by any compound (1a-k). So, it is obvious from the data obtained that compounds 1e, 1g and 1j was highly active among all the compounds of series 1a-k.

For fungi in the series 1a-k almost four compounds i.e. 1d, 1e, 1g, and 1j were found active at 500 µg/ml conc. against *A.niger*. *C. albicans* was inhibited at 500 µg/ml conc. by four compounds i.e. 1d, 1e, 1g, and 1j. At the conc. of 250 µg/ml *C.albicans* was not killed by any compounds (1a-k). *A. Niger* was not killed by any compounds (1a-k). So, it is obvious from the data obtained that compounds 1d, 1e, 1g, and 1j was highly active among all the compounds of series 1a-k.

SECTION-II

Preparation, Characterization and antimicrobial evaluation of Ethyl -2-[4-(Sub stituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates.

Keeping in view of wide spectrum biodynamic activities[1-48] of Pyrimidine and with a view to have potent therapeutic agents, the synthesis of **Ethyl -2-[4-(Substituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine-5-carboxylates (2a-k)** have been synthesized by the using Ethyl 2-(piperazin-1-yl) 4-(1-phenyl-3-oxo-1,4,8-triazaspiro [4,5] dec-8-yl) pyrimidine -5-carboxylate with different substituted phenyl sulfonyl chlorides.



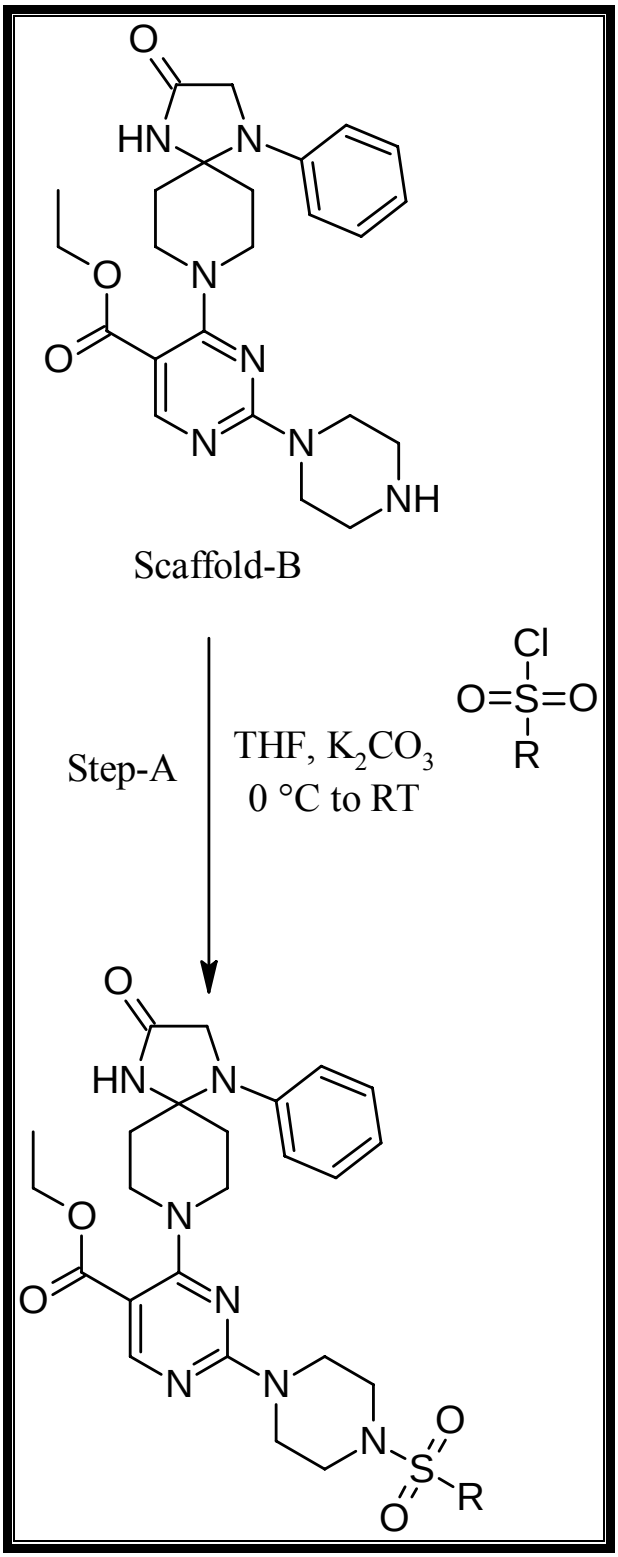
(2a-k)
R= Aryl

The constitution of the synthesized products (2a-k) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella peratyphi B* and they were also evaluated for antifungal activity against *Candida albicans* and

Aspergillus niger at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (2a-k) have been compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by **NCCLs**).

REACTION SCHEME



(2a-k)

R= Aryl

EXPERIMENTAL

Preparation, Characterization and antimicrobial evaluation of Ethyl-2-[4-(Substituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triaza spiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates.

[A] Synthesis of Scaffold-B.

For Preparation, see Part-II, Section-I, Page No. (109 & 110)

[B] Synthesis of Ethyl -2-[4-O-toluene sulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates.

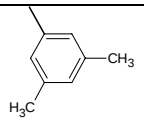
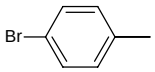
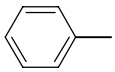
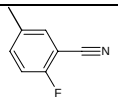
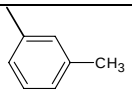
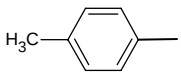
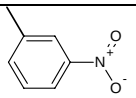
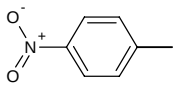
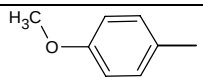
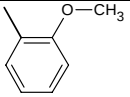
In RBF, Scaffold-B (1.00 mol) in THF and K_2CO_3 (2.50 mol) was added at room temperature. Then reaction mixture was cooled to 0°C. In reaction mixture, o-toluene sulphonyl chloride (1.10mol) solution in THF was added at 0°C and reaction was allowed to warm at room temperature and stirred for 1-2 hours. Then solvent was removed under vacuum at 45°C. The residue obtained was quenched with water to get solid. The solid was triturating with diethyl ether to get pure product. Yield: 42.00 %. M.P: 102°C. Elemental Analyses: Calculated: C (62.12%), H (6.19%), N (13.58%), Found: C (62.03%), H (6.11%), N (13.47%).

Similarly, other compounds (2a-k) were synthesized by above mentioned process (B) from Scaffold-B. The physical data are recorded in Table-2.

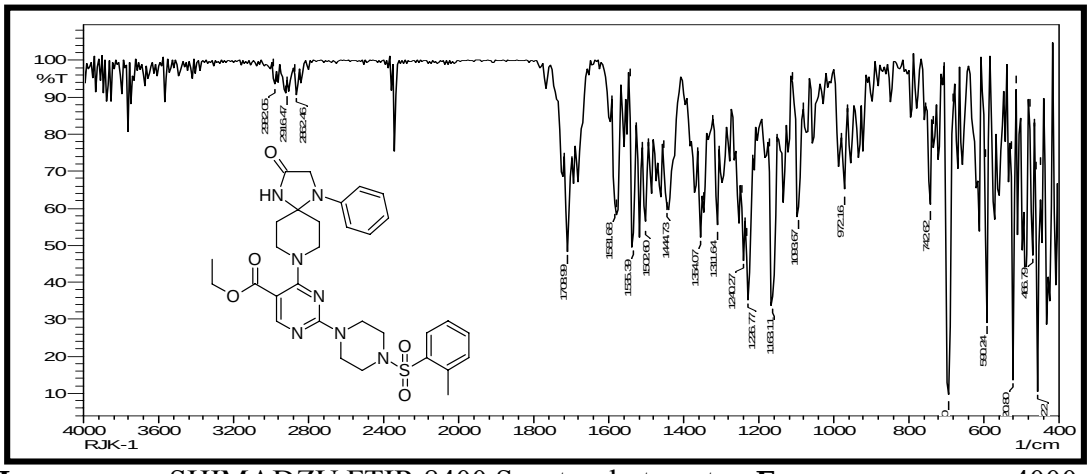
[C] Antimicrobial activity of Ethyl -2-[4-(Substituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates. **(2a-k)**

Antimicrobial activity testing was carried out as described in Part-I, Section-I, Page No. (32 to 36) The MIC values of test solution are recorded in Table NO-2a and 2b.

TABLE NO-2: Physical constants of Ethyl -2-[4-(Substituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates (2a-k).

Sr. No.	Substitution R	Molecular Formula/ Molecular weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
2a		$C_{32}H_{38}N_6O_5S$ 618	102	42	62.12 62.03	6.19 6.11	13.58 13.47
2b		$C_{32}H_{40}N_6O_5S$ 632	207	44	62.64 62.47	6.37 6.29	13.28 13.19
2c		$C_{31}H_{35}BrN_6O_5S$ 683	225	38	54.47 54.32	5.16 5.11	12.29 12.21
2d		$C_{31}H_{36}N_6O_5S$ 604	223	54	61.57 61.39	6.00 5.89	13.90 13.79
2e		$C_{32}H_{34}FN_7O_5S$ 647	209	30	59.34 59.13	5.29 5.21	15.14 15.03
2f		$C_{32}H_{38}N_6O_5S$ 618	111	48	62.12 62.04	6.19 6.11	13.58 13.48
2g		$C_{32}H_{38}N_6O_5S$ 618	117	47	62.12 62.01	6.19 6.10	13.58 13.46
2h		$C_{31}H_{35}N_7O_7S$ 649	152	46	57.31 57.09	5.43 5.34	15.09 15.01
2i		$C_{31}H_{35}N_7O_7S$ 649	168	44	57.31 57.14	5.43 5.36	15.09 15.00
2j		$C_{32}H_{38}N_6O_6S$ 634	142	52	60.55 60.29	6.03 5.88	13.24 13.12
2k		$C_{32}H_{38}N_6O_6S$ 634	207	54	60.55 60.31	6.03 5.87	13.24 13.09

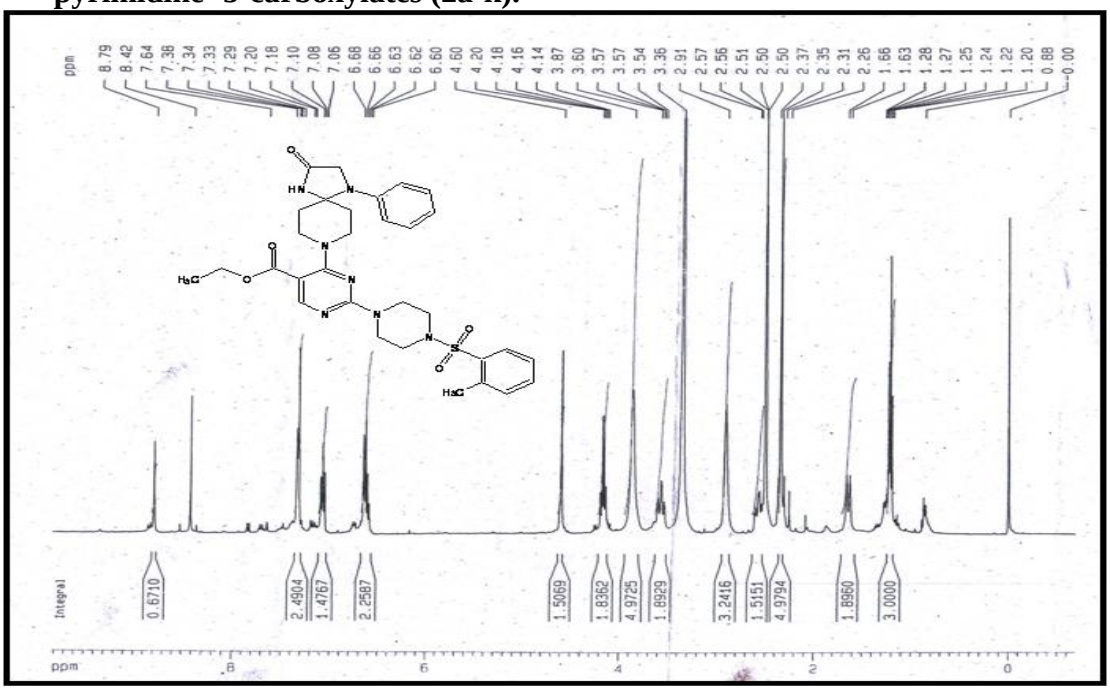
IR Spectral Study of Ethyl -2-[4-(2'-methyl benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5]dec-8-yl) pyrimidine-5-carboxylates. (2a)

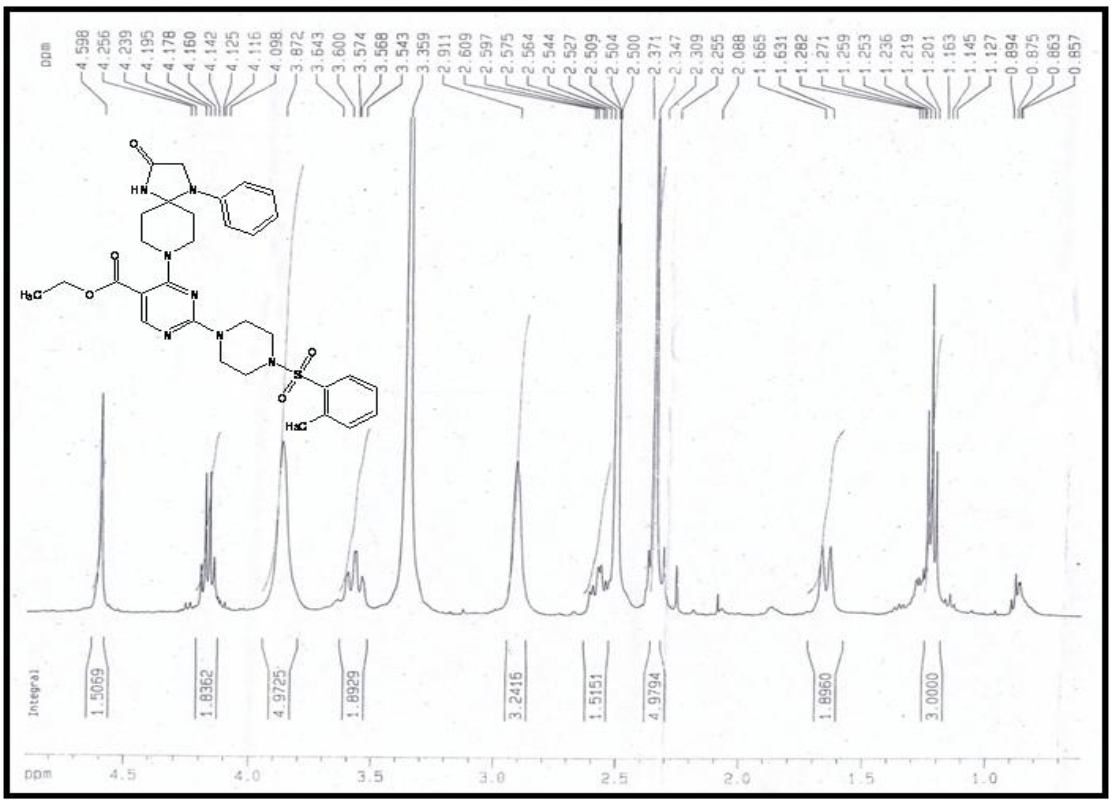


Instrument: SHIMADZU FTIR 8400 Spectrophotometer; **Frequency range**: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No.
		Observed	Reported	(Part -1)
Alkane $-CH_2$	C-H asym. str.	2916	2975-2850	42
	C-H sym. str.	2862	2900-2800	42
	C-H asym. def.	1444	1460-1400	42
	C-H sym. Def.	1354	1385-1300	42
Aromatic	C-H str.	3010	3080-3010	42
	C=C ring skeletal	1535	1600-1450	44
		1502	1600-1450	44
	C-H o.o.p. def. (sym.)	720	740-670	44
Imidazolone moiety	-N-H-str (2° amine)	3330	3410-3300	43
	-N-H-def	1581	1650-1550	43
	-C=O str. (cyclic keto)	1730	1740-1650	42
Pyrimidine moiety	C-N str.	1605	1635-1595	43
	C-N str. Def	1240	1340-1250	43
Sulfonyl group	-SO assy.str	1311	1350-1300	44
	-SO sym.str	1163	1160-1120	44
Ester	-C=O str.	1709	1740-1650	42
	C-O-C assym. Str.	1226	1250-1200	42
	C-O-C sym. Str.	1093	1050-1010	42

1H-NMR Spectral Study of Ethyl -2-[4-(Substituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates (2a-k).



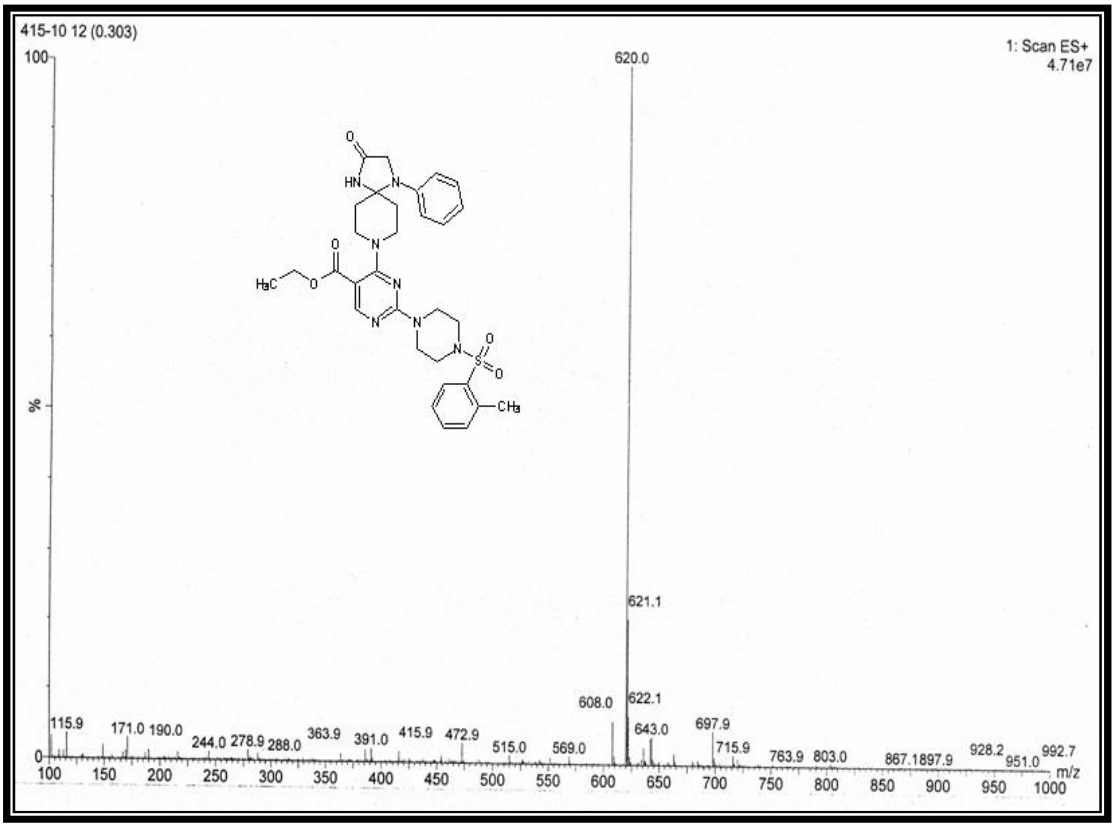


6

Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO-d6.

Sr. No	Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	1.16	3H	Triplate	-(CH3)
2	1.66	2H	Doublet	-(CH2)
3	2.34	5H	Multiplate	-(CH2)3
4	2.60	2H	Multiplate	-(CH2)
5	2.91	4H	singlet	-(CH3)
6	3.57	2H	Triplate	-(CH2)
7	3.87	4H	Singlate	-(CH2)2
8	4.23	2H	Quartrate	-(CH2)
9	4.59	2H	Singlate	-(CH2)
10	6.50-8.42	10H	Complex	Ar -H
11	8.79	1H	Singlet	-(NH)

Mass Spectral Study of Ethyl -2-[4-(2'-methyl benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates (2a).



Possible Mass Fragmentation pattern:

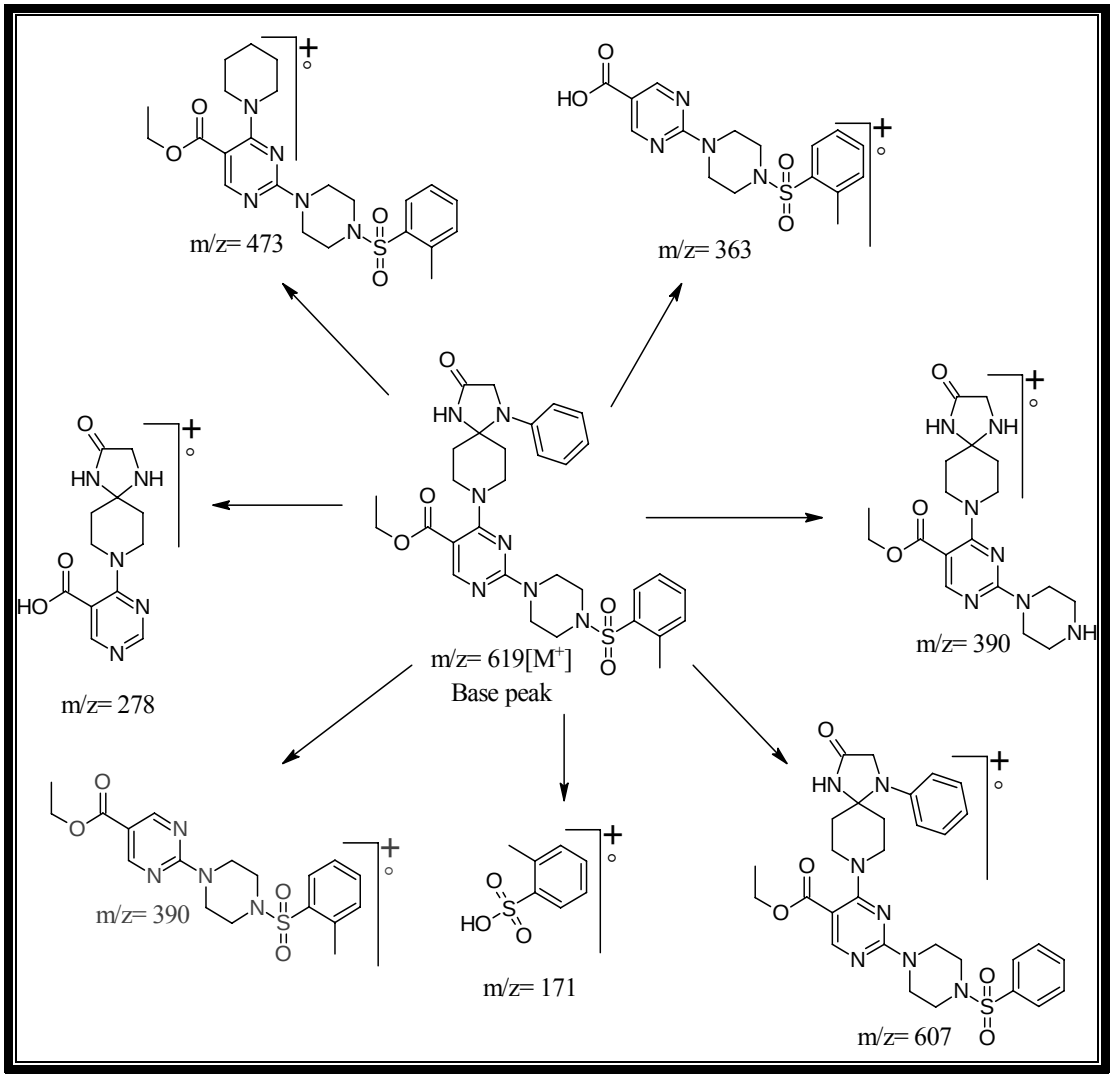


TABLE NO. 2a: Antimicrobial Activity Of Ethyl -2-[4-(Substituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates (2a-k).

TABLE NO-2(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
2a	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
2b	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	-
2c	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
2d	+	+	+	-	+	+	+	+	+	+	-	-	+	+	-	-
2e	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
2f	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
2g	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	-
2h	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	-
2i	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
2j	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
2k	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-

Reference drugs:	***S. aureus***	***B. Subtilis***	***E.Coli***	***S. Peratyphi B***
Ciprofloxacin	**1.9**	**7.8**	**0.4**	**1.4**

TABLE NO. 2b: Antimicrobial Activity Of Ethyl -2-[4-(Substituted benzenesulfonyl) piperazin-1-yl]-4-(1-phenyl-3-oxo-1, 4, 8-triazaspiro [4, 5] dec-8-yl) pyrimidine -5-carboxylates (2a-k).

TABLE NO-2(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (µg/ml)*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
2a	+	+	+	-	+	+	+	+
2b	+	+	+	+	+	+	+	-
2c	+	+	+	-	+	+	+	-
2d	+	+	+	-	+	+	+	+
2e	+	+	+	+	+	+	+	+
2f	+	+	+	-	+	+	+	-
2g	+	+	+	-	+	+	+	+
2h	+	+	+	+	+	+	+	+
2i	+	+	+	-	+	+	+	-
2j	+	+	+	-	+	+	+	-
2k	+	+	+	-	+	+	+	-
Reference drugs:			*A.niger*				*C. albicans*	
Fluconazole			**0.7**				**0.4**	

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 µg/ml conc. (2) Secondary Screening 1000 µg/ml to 250 µg/ml conc. (3) Tertiary Screening start from 125 µg/ml to 3.9 µg/ml. The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed in tertiary screening.

From the results of experiments using newly synthesized organic compounds' it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 2a-k almost eight compounds 2a, 2b, 2d, 2e, 2g, 2h, 2j and 2k were found active at 500 µg/ml conc. against *staphylococcus aureus*. *Bacillus Subtilis* was inhibited at 500 µg/ml conc. by all compounds (2a-k). Eight compounds 2a, 2b, 2d, 2e, 2g, 2h, 2j and 2k were active against both cultures *B.Subtilis* and *S.aureus*. At the conc. 250 µg/ml *S. aureus* was not killed by any compounds (2a-k). *B.Subtilis* was not inhibited by three compounds i.e. 2d, 2g and 2h. So, it is obvious from the data obtained that compounds 2d, 2g and 2h were highly active among all the compounds of series 2a-k.

For Gram Negative bacteria in the series 2a-k almost all compounds (2a-k) was not killed at 500 µg/ml conc. against *Escherichia Coli. S.Paratyphi B*. was inhibited at 500 µg/ml conc. by three compounds i.e. 2b, 2g and 2h. 250 µg/ml *E.Coli* was not killed by any compound (2a-k). *S.Paratyphi B*. was killed by any compound (2a-k). So, it is obvious from the data obtained that all compounds were not active of series 2a-k.

For fungi in the series 2a-k almost all compounds 2a to 2k were found active at 500 µg/ml conc. against *A.niger. C. albicans* was inhibited at 500 µg/ml conc. by all compounds i.e. 2a to 2k. At the conc. of 250 µg/ml *C.albicans* was inhibited by five compounds i.e. 2a, 2d, 2e, 2g and 2h. *A. Niger* was inhibited by three compounds i.e. 2b, 2e and 2h. So, it is obvious from the data obtained that compound 2e and 2h was highly active among all the compounds of series IIa-k.

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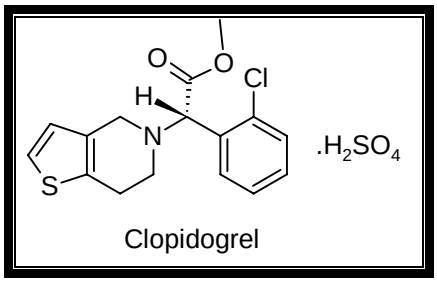
Part-III (Tetrahydrothieno [2, 3-C] pyridines)

INTRODUCTION

Thieno pyridine containing APIs

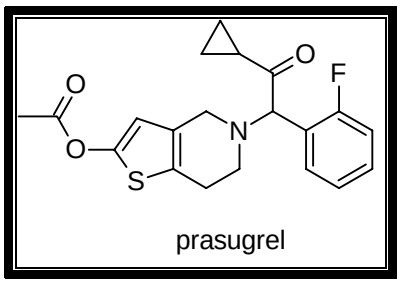
Plavix (clopidogrel bisulfate) is a Thienopyridine class inhibitor of P2Y12 ADP platelet receptors. Chemically it is methyl (+)-(S)-α-(2-chlorophenyl)-6, 7-dihydrothieno [3, 2-c] pyridine-5(4H) acetate sulfate (1:1). The structural formula is as follows:



Clopidogrel

Clopidogrel is an inhibitor of platelet activation and aggregation through the irreversible binding of its active metabolite to the P2Y12 class of ADP receptors on platelets. Clopidogrel is a prodrug and is metabolized to a pharmacologically active metabolite and inactive metabolites. [1]

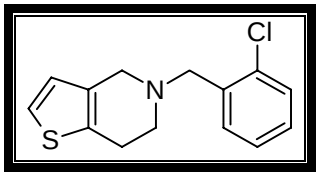
Prasugrel is a new P2Y12 receptor antagonist that has been investigated for the treatment of atherothrombosis in patients with cardiovascular disease undergoing percutaneous coronary intervention (PCI). Similar to other Thienopyridine, prasugrel is a prodrug that requires biological conversion to active metabolites. [2]



prasugrel

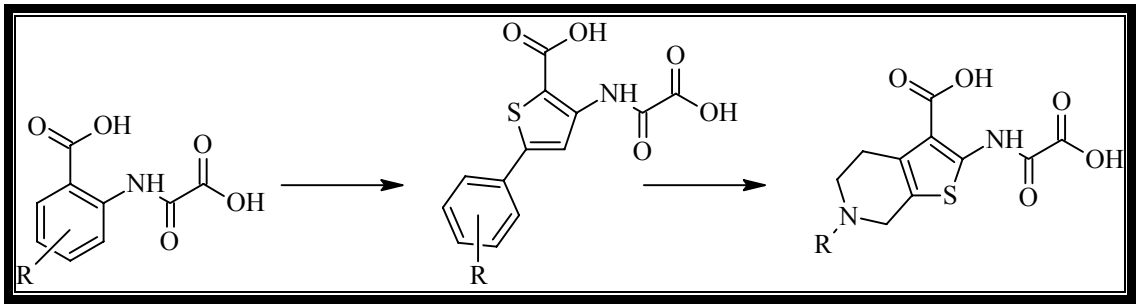
In February 2009, the antiplatelet therapy prasugrel (Efient; Daiichi Sankyo/Eli Lilly) was granted marketing authorization by the European Commission for the prevention of atherothrombotic events in patients with acute coronary syndromes undergoing primary or delayed percutaneous coronary intervention. [3]

Ticlopidine (trade name Ticlid) is another drug in the thienopyridine family. Like clopidogrel, it is an adenosine diphosphate (ADP) receptor inhibitor. Combining either thienopyridine with an intravenous platelet IIb/IIIa inhibitor appears to be safe. [4]

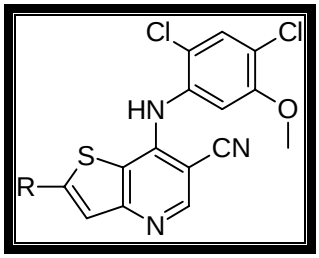


Biologically active Thieno pyridine compounds

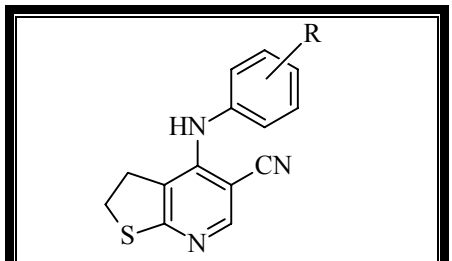
Andersen et al describes SAR study of Novel Selective and Orally Bioavailable Nonpeptide Classical Competitive Inhibitor Class of Protein-Tyrosine Phosphatase 1B. it provides a number of new chemical scaffolds for the development of inhibitors of different members of the PTP family. Although the core structure of these inhibitors is charged, good oral bioavailability has been observed in rat for some compounds. Furthermore, it was observed enhancement of 2-deoxy-glucose accumulation in C2C12 cells with prodrug analogues. [5]



Boschelli et al describes 7-[(2, 4-Dichloro-5-methoxyphenyl) amino] thieno [3, 2-b] pyridine-6-carbonitriles with various heteroaryl groups at C-2 are inhibitors of Src kinase activity. Of these new analogs, compounds substituted at C-2 by a 3,5-furan or a 2,5-pyridine had the best activity in the Src enzyme and cell assays. [6]

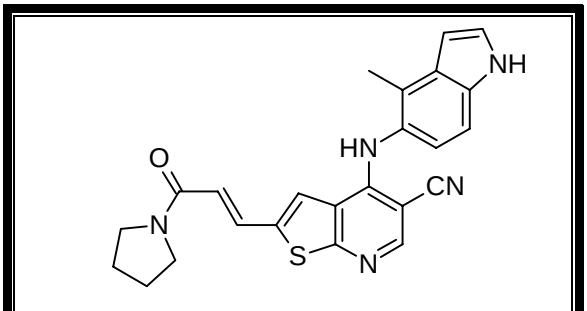


Bernardino et al performed the design, synthesis, and the structure–activity relationship studies of 13 new derivatives of thieno [2, 3-b] pyridine. These derivatives were prepared in high yields (96–70%) and their structures were elucidated by IR, 1H, 13C NMR, and MS. The biological results showed some derivatives as antiparasitic agents against Giardia lamblia. Computational analysis of HOMO and LUMO energy, HOMO orbital coefficient distribution, electrostatic potential map, dipole moment, and density HOMO was performed to gain insight into the SAR aspects. [7]



R= H, m-CH_3, p-CH_3, m-OCH_3, p-OCH_3, m-NO_2, p-NO_2, m-F, p-F, m-Cl, p-Cl,m-Br, p-Br

Tumey et al reports a series of 2-alkenyl thieno [2, 3-b] pyridine inhibitors of PKCθ were synthesized as potential inflammatory modulators. This series led to the discovery of 2-alkenyl amides, which are exceptionally potent and selective inhibitors of PKCθ. [8]

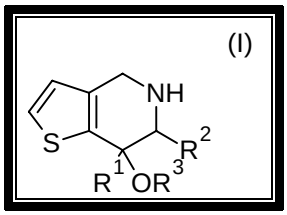


PKC0 =3.8nM
PKCa, PKCb,PKCc,PKCd,PKCe> 1300nM

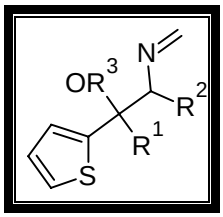
Attaby et al describes reaction of thiocyanoacetamide with, β-unsatumted ketones resulted in the formation of the corresponding newly synthesized 1(H)pyridinethione derivatives. Compounds were used as synthons for the preparation of 2-S-alkyl-, 2-S-aryl-, 2-S-acetamidopyridine, thieno[2,3-b]pyridine and pyrazolo[3,4-b]pyridine derivatives via a wide range of reactions with different reagents. The antimicrobial

activity of some of the newly synthesized compounds was tested. Few compounds were found to be the most active ones. [9]

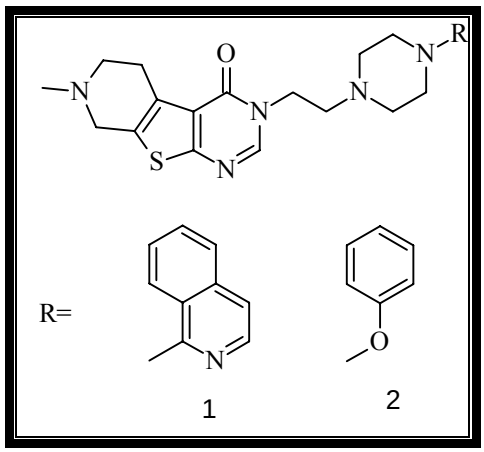
Tetra hydro Thieno pyridine derivatives are known as bioactive molecules since very long time. US4065460 (published in 1977) describes following thieno pyridine derivates as anti inflammatory and analgesic agents. [10]

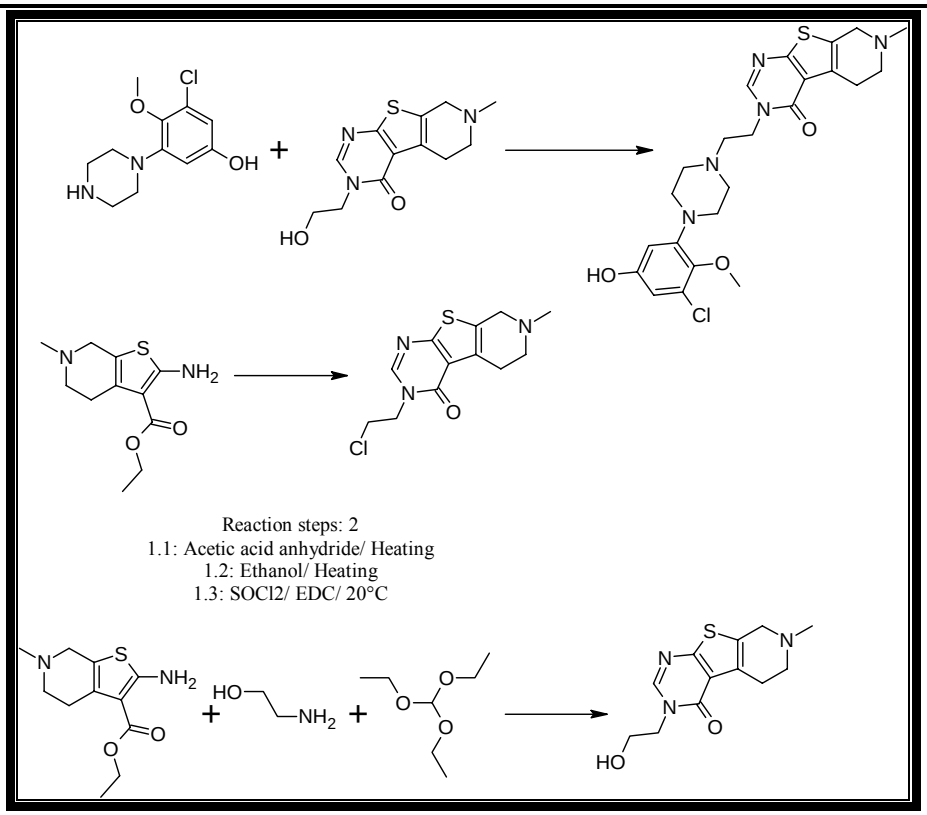


Compound of formula (I) is synthesized by cyclizing following intermediate.



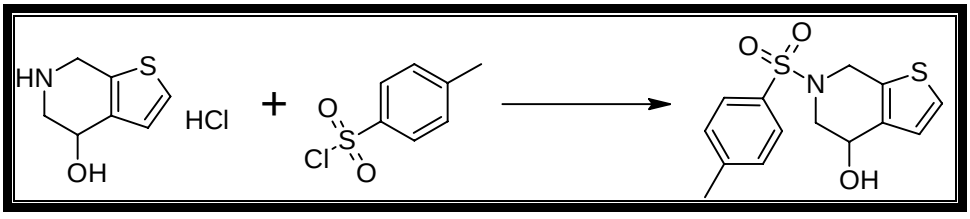
Klinga et al N-4-aryl-piperazinyl-N′-ethyl-5, 6, 7, 8-tetrahydropyrido [4′, 3′:4, 5] thieno [2, 3-d] pyrimidin-4(3H)-one compounds as potential antidepressant drugs. [11]





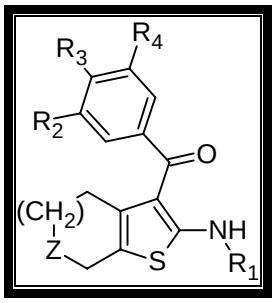
El-Kashef et al describes synthesis of Pyrido [4', 3':4, 5] thieno [2, 3-d] pyrimidines and Related Heterocycles of biological importance. [12]

US4076819 describes thieno-pyridine derivatives anti-inflammatory properties and inhibiting effects on blood plate aggregation which make them therapeutically valuable.



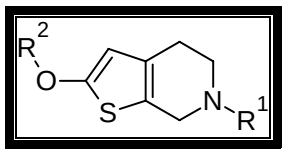
Toxicological investigation demonstrated the low toxicity and the good tolerance of the derivatives of this invention. The composition of this invention is usefully administrable for the treatment of the various stages of inflammation. It is applicable in chronic inflammatory rheumatism, degenerative rheumatism, in abarticular conditions, in oto-rhino-laryngology, in stomatology, in post-operative surgery and in traumatology. [13]

US6323214 relates to a compound of general formula

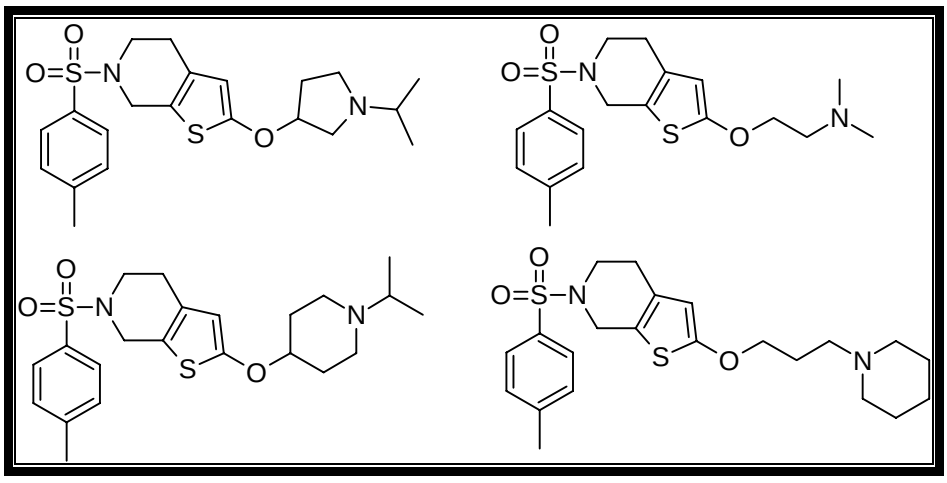


It also describes their use in medicine as allosteric adenosine receptor modulators for uses including protection against hypoxia and ischemia induced injury and treatment of adenosine-sensitive cardiac arrhythmias. [14]

US2008/0146559 describes new 4,5,6,7-tetrahydro-thieno[2,3-c]pyridine derivatives, their manufacture, pharmaceutical compositions containing them and their use as medicaments. The active compounds of the invention are useful in treating obesity and other disorders. [15]



US2008/0146559 describes following structures. [16]



Recently the Thienopyridine derivatives are reported for various pharmacological activities e.g. [17-24].

Current Work:

Based on various tetra hydro Thienopyridine derivatives and their therapeutic importance, the present work provides newly tetra hydro Thienopyridine derivatives.

These compounds are substituted with 4-methyl phenyl sulfonyl group on nitrogen atom, in Thienopyridine moiety.

Various modifications are made to 2^{nd} and 3^{rd} position of thiophene ring to study effects of these substitutions on their activity. This can be summarized as below:

Series A: In this series, compounds carry amide substitutions on 3^{rd} position and Schiff base on 2^{nd} position.

Series B: Compounds of this series contains fused pyrimidine ring on 2^{nd} and 3^{rd} position.

Series C: This series provides ester moiety on 3^{rd} position and urea residue on 2^{nd} position.

Thus, the present work provides Thieno pyridine derivatives having various substitutions like amide, ester, urea and fused ring which can be used to study effects of these substitutions on their therapeutic activity.

The present work also provides an efficient method for the synthesis of above biologically important derivatives.

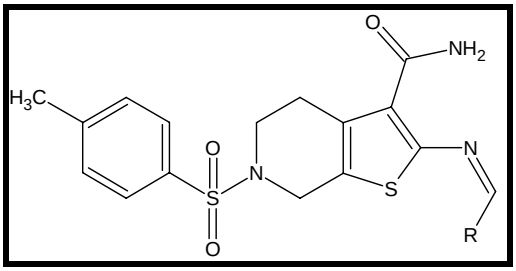
The work is further subdivided into following sections.

1) Preparation, Characterization and antimicrobial evaluation of 2-(substituted benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide.
2) Preparation, Characterization and antimicrobial evaluation of 2-aryl-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8-heptahydro pyrido[4',3': 4,5]thieno[2,3-d] pyrimidin-4-ones.
3) Preparation, Characterisation and antimicrobial evaluation of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates.

SECTION-I

Preparation, Characterization and antimicrobial evaluation of 2-(substituted benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide.

Keeping in view of wide spectrum biodynamic activities[1-26] of thieno pyridine and with a view to have potent therapeutic agents, the synthesis of **2-(substituted benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide (1a-k)** have been synthesized by using 2-(amino)-6-[(4-methylphenyl)sulfonyl]-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide with differently substituted aromatic aldehydes.



(1a-k)

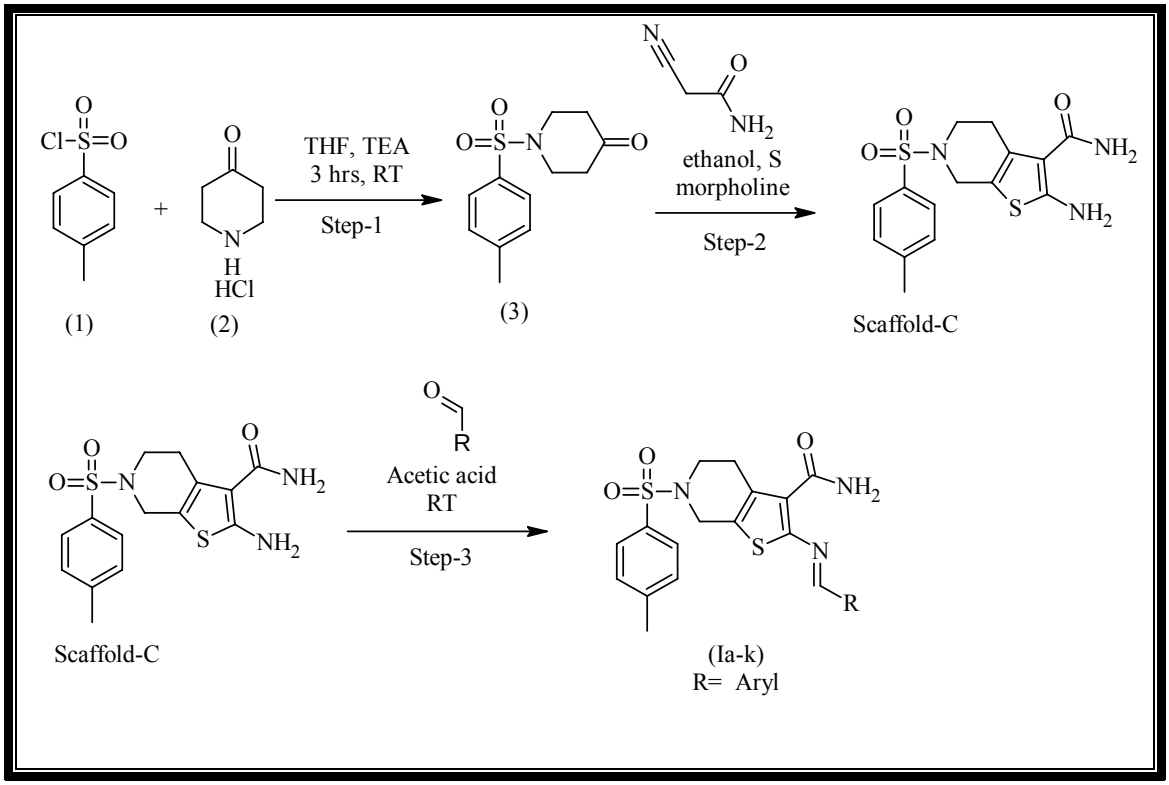
R= Aryl

The constitution of the synthesized products (1a-k) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella peratyphi B* and they were also evaluated for antifungal activity against *Candida albicans* and *Aspergillus niger* at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (1a-k) have been

compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by **NCCLs**).

REACTION SCHEME



EXPERIMENTAL

Preparation, Characterisation and antimicrobial evaluation of 2-(substituted benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamides.

[A] Synthesis of Scaffold-C.

1. Synthesis of 1-[(4-methylphenyl) sulfonyl]-4-piperidinone (Intermediate-3)

In RBF, 4-piperidinone hydrochloride (1.00 mol), THF and Triethylamine (2.5 mol) were charged. The reaction mixture was cooled to 0°C and 4-methyl phenyl sulfonyl chloride (1.05mol) was added portion wise to the reaction mixture at 0°C. The reaction mixture was allowed to warm at room temperature and stirred for 4 hours at same temperature. After the completion of reaction, Reaction mixture was concentrated under reduced pressure at 45°C. The crude was quenched with chilled water and stirred for thirty minutes. The obtained solid was filtered and washed with water and dried. Yield: 85.00 %. M.P: 131°C, 1H NMR (solvent: $CDCl_3$) δ 7.72 (d, 2H, J=8.1 Hz), 7.38 (d, 2H, J=8.1 Hz) 3.42 (m, 4H), 2.58 (m, 4H), 2.42 (s, 3H).

2. **Synthesis of 2-amino-6-[(4-methylphenyl)sulfonyl]-4,5,6,7-tetrahydrothieno [2,3-c]pyridine-3-carboxamide (Scaffold-C).**

In RBF, 1-[(4-methylphenyl) sulfonyl]-4-piperidinone (1.0 mol) (Intermediate-3), 2-cyano acetamide (1.10 mol), morpholine (5.0 mol) and sulfur (1.5 mol) was dissolved in ethanol at room temperature. The reaction mixture was heated at 70-80°C for 3-4 hours. After the completion of reaction, Reaction mixture was concentrated under reduced pressure at 45°C. The crude was quenched with chilled water and stirred for thirty minutes. The obtained solid was filtered and washed with water and dried. Yield: 75.00 %, M.P: 223°C. Elemental Analysis: Calculated: C (51.26%), H (4.88%), N (11.96%), Found: C (51.11%), H (4.67%), N (11.73%).

[B] Synthesis of 2-(benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide.

In RBF, Scaffold-C (1.0 mol) in acetic acid were charged with benzaldehyde at room temperature. The reaction mixture was stirred for 1 hour at same temperature. After the completion of reaction, Reaction mixture was quenched with chilled water.

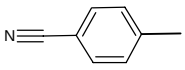
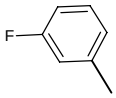
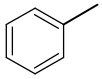
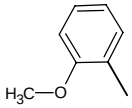
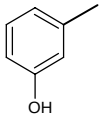
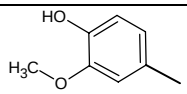
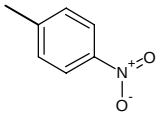
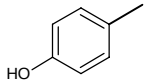
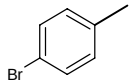
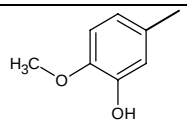
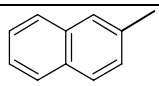
The obtained solid was filtered and washed with water and dried. The obtained product was purified by triturating with ethanol. Yield: 87.00 %, M.P: 202°C, Elemental Analysis: Calculated: C (60.11%), H (4.82%), N (9.56%), Found: C (60.05%), H (4.75%), N (9.42%).

Similarly, other compounds (1a-k) were synthesized by above mentioned process (B) from Scaffold-C. The physical data are recorded in Table-1.

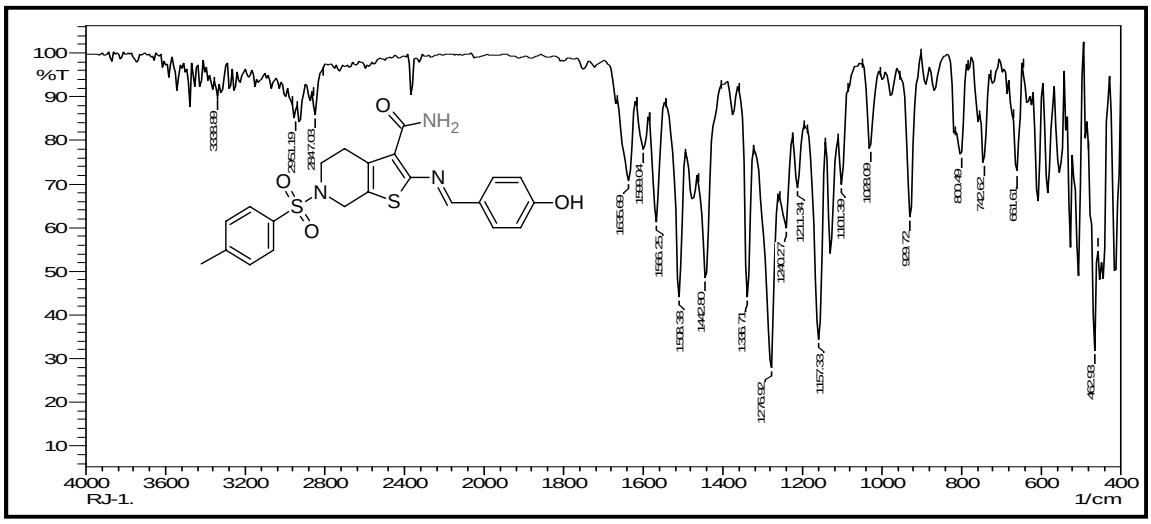
[C] Antimicrobial activity of 2-(substituted benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide.

Antimicrobial activity testing was carried out as described in Part-I, Section-I, Page No. (32 to 36) The MIC values of test solution are recorded in Table NO-1a and 1b.

TABLE NO-1: Physical constants of 2-(substituted benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide (1a-k).

Sr. No.sc	Substitution R	Molecular Formula/ Molecular weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
1a		$C_{23}H_{20}N_4O_3S_2$ 464	258	73	59.46 59.20	4.34 4.26	12.06 11.80
1b		$C_{22}H_{20}N_3O_3S_2F$ 457	185	76	57.75 57.40	4.41 4.29	9.18 9.02
1c		$C_{22}H_{21}N_3O_3S_2$ 439	202	87	60.11 59.92	4.82 4.65	9.56 9.42
1d		$C_{23}H_{23}N_3O_4S_2$ 469	204	87	58.83 58.65	4.94 4.81	8.95 8.83
1e		$C_{22}H_{21}N_3O_4S_2$ 455	220	84	58.00 57.74	4.65 4.53	9.22 9.09
1f		$C_{23}H_{23}N_3O_5S_2$ 485	185	84	56.89 56.72	4.77 4.69	8.65 8.56
1g		$C_{22}H_{20}N_4O_5S_2$ 484	263	85	54.53 54.31	4.16 4.09	11.56 11.42
1h		$C_{22}H_{21}N_3O_4S_2$ 455	234	77	58.00 57.84	4.65 4.53	9.22 9.10
1i		$C_{22}H_{20}N_3O_3S_2Br$ 518	242	82	50.97 50.71	3.89 3.77	8.11 8.01
1j		$C_{23}H_{23}N_3O_5S_2$ 485	220	78	56.89 56.62	4.77 4.65	8.65 8.52
1k		$C_{26}H_{23}N_3O_3S_2$ 489	209	83	63.78 63.49	4.73 4.56	8.58 8.49

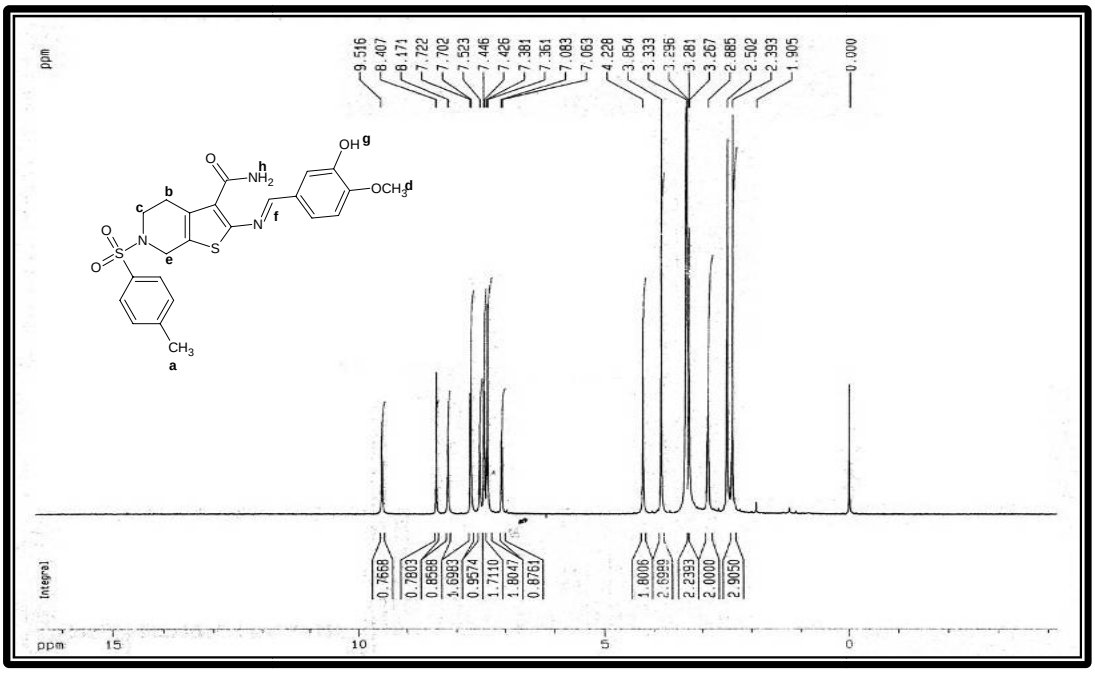
IR Spectral Study of 2-(3'-hydroxy, 4'-methoxy benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide(1i).

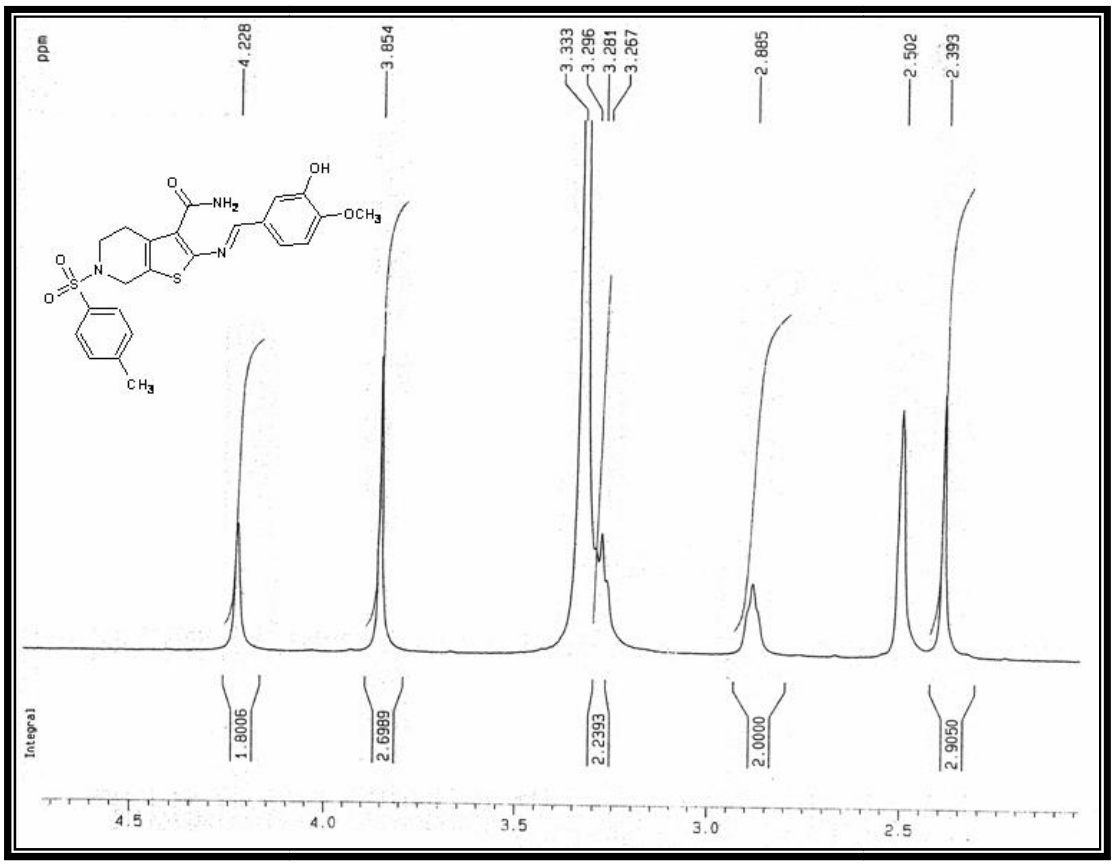


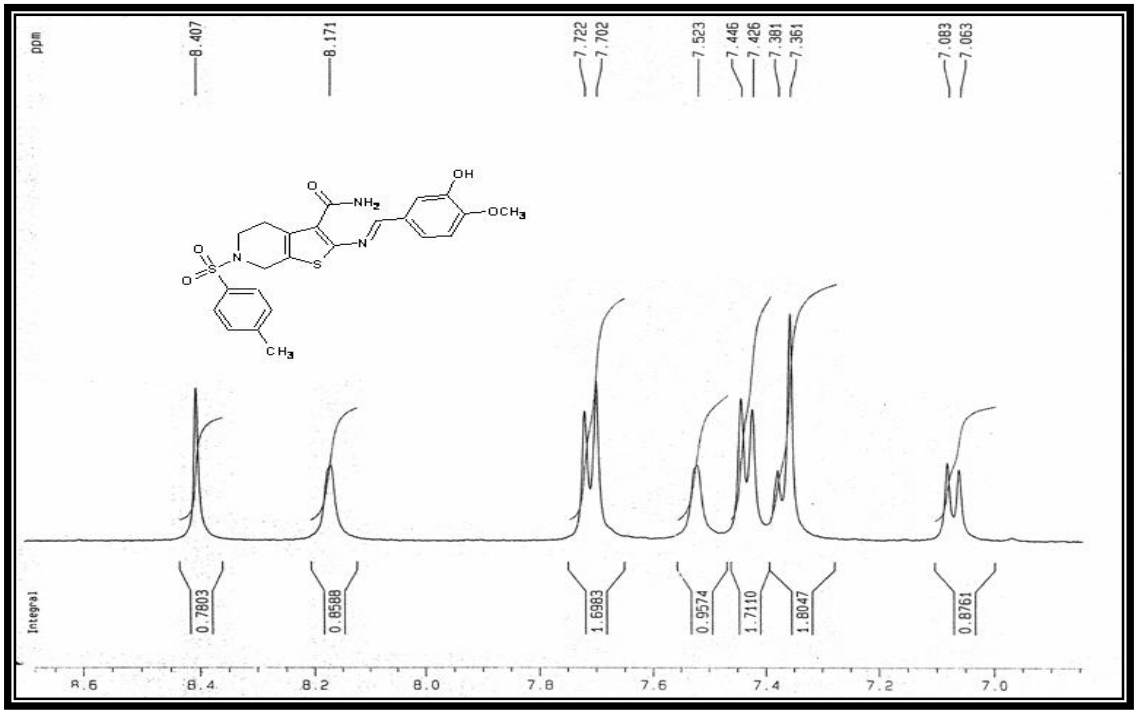
Instrument: SHIMADZU FTIR 8400 Spectrophotometer; **Frequency range**: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No.
		Observed	Reported	(Part -1)
Alkane $-CH_2$	C-H asym. str.	2961	2975-2850	42
	C-H sym. str.	2847	2900-2800	42
	C-H asym. def.	1442	1460-1400	42
	C-H sym. Def.	1336	1385-1300	42
Aromatic	C-H str.	2951	3080-3010	42
	C=C ring skeleton	1566	1600-1450	44
		1508	1600-1450	44
Thienopyridine moiety	C-S-C str.	661	700-650	44
	C-N str. def	1275	1340-1250	43
Amide moiety	N-H str. (assy.)	3560	3600-3200	43
	N-H str. (sym.)	3338	3600-3200	43
	-C=O str.	1636	1740-1650	42
Ether	C-O-C assym. Str.	1240	1250-1200	42
	C-O-C sym. Str.	1028	1050-1010	42
Schieff's base	CH=N str.	1599	1650-1600	43
Sulfonamide	N-SO2 sym. Str.	1157	1160-1120	44

1H-NMR Spectral Study of 2-(3'-hydroxy, 4'-methoxy benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide (1i).



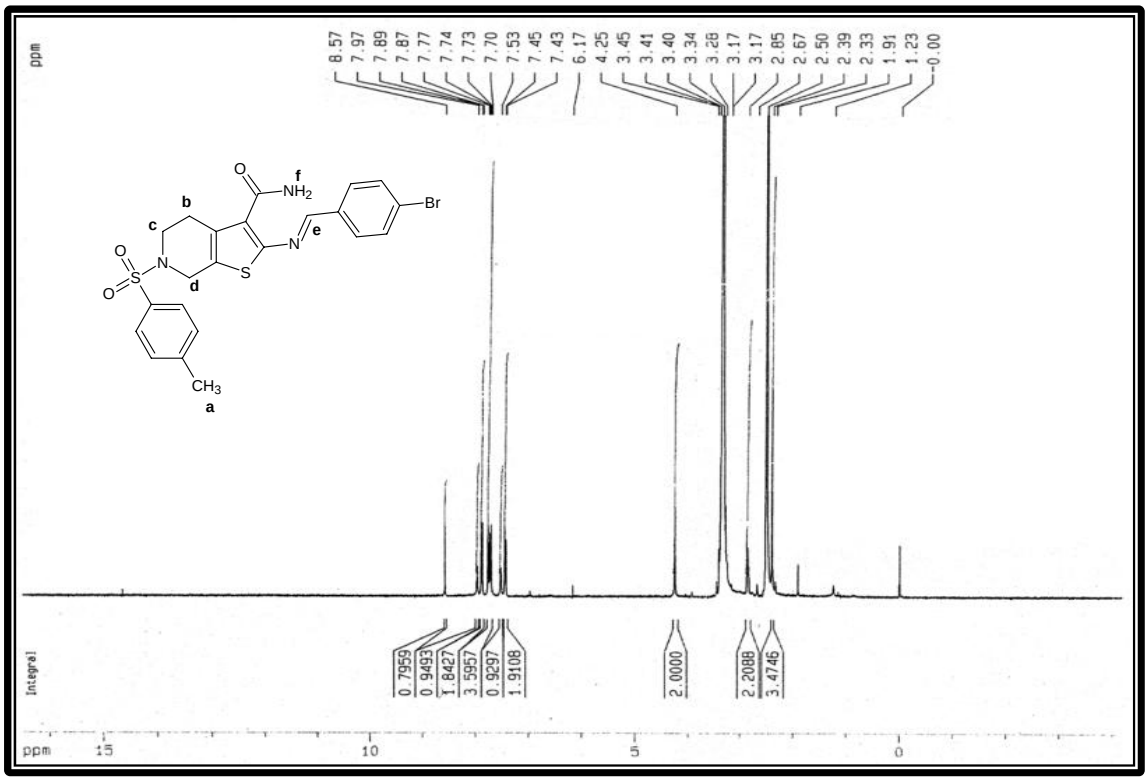


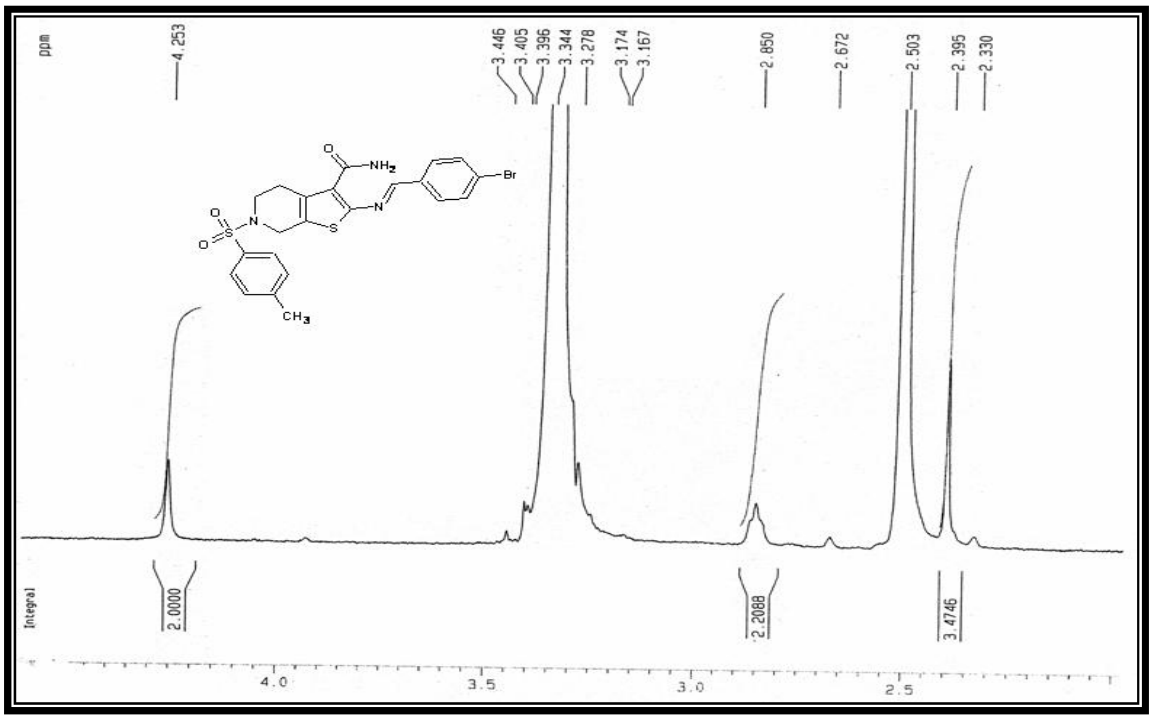


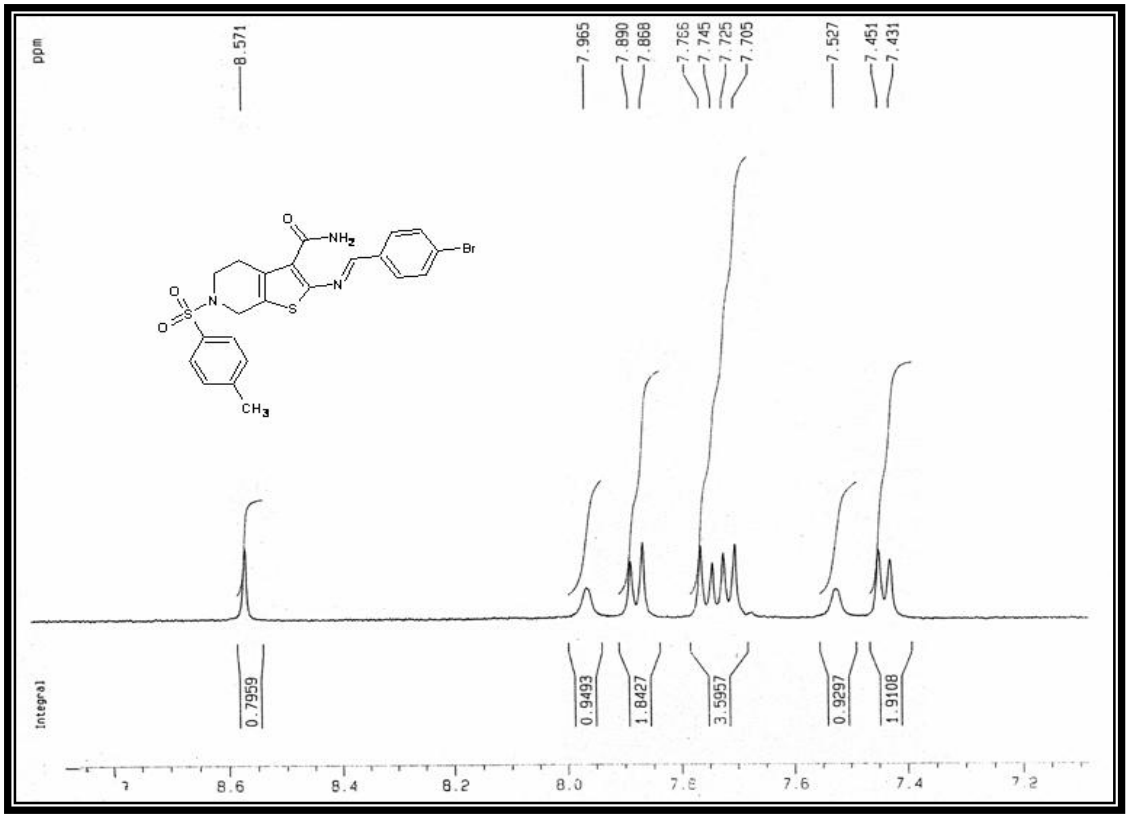
Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO- *d6.*

Serial No.	Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	2.39	3H	Singlet	-(CH_3) a
2	2.885	2H	Broad singlet	-(CH_2) b
3	3.296	2H	Triplet	-(CH_2) c
4	3.854	3H	Singlet	-(CH_3) d
5	4.228	2H	Singlet	-(CH_2) e
6	7.063-7.722	7H	Complex	Ar -H
7	8.171	1H	Singlet	-(N=CH) f
8	8.407	1H	Singlet	-(NH) h
9	9.516	2H	Singlet	-(OH) g

1HNMR spectra of 2-(4-bromo benzylidene amino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide (1j).





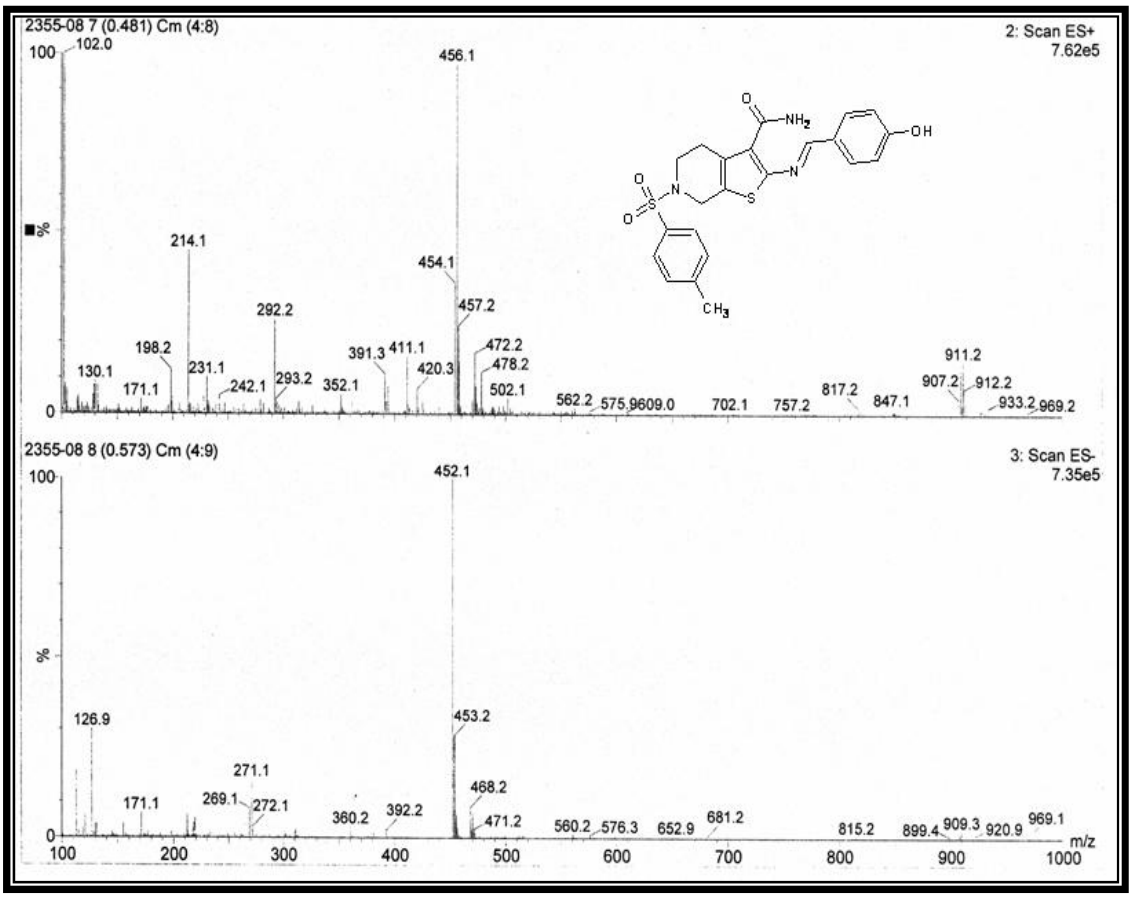


Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO- *d6.*

Serial No.	Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	2.395	3H	Singlet	-(CH_3) a
2	2.850	2H	Broad singlet	-(CH_2) b
3	3.278	2H	Triplet	-(CH_2) c
4	4.253	2H	Singlet	-(CH_2) d
5	7.431-7.890	8H	Complex	Ar -H
6	7.965	1H	Singlet	-(N=CH) i
7	8.571	2H	Singlet	-(NH) i

Mass Spectral Study of 2-(4-Hydroxy benzylidene amino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide (1h).

S



Possible Mass Fragmentation pattern:

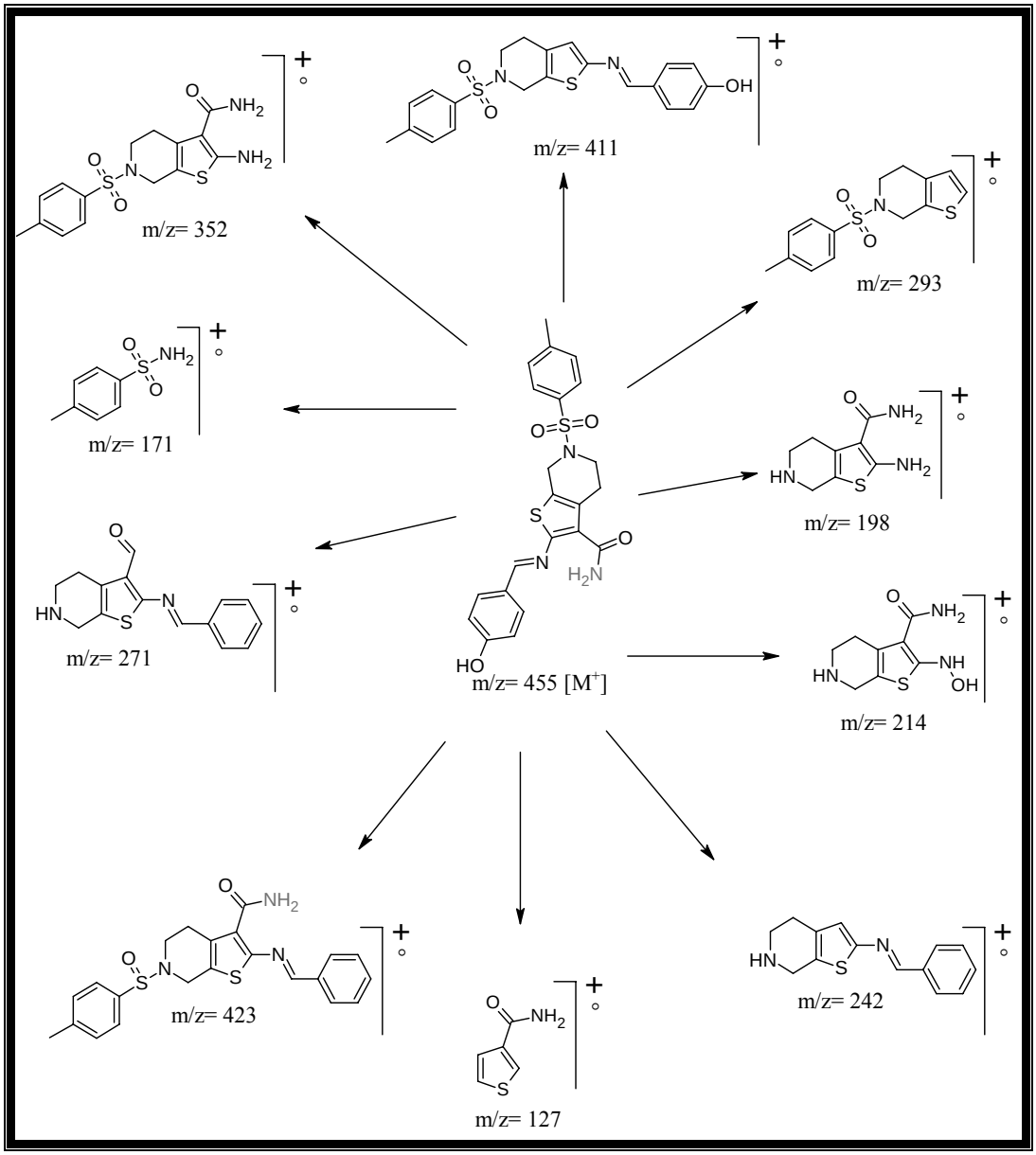


TABLE NO. 1a: Antimicrobial Activity Of 2-(substituted benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide (Ia-k).

TABLE NO-1(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
1a	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-
1b	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	+
1c	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
1d	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
1e	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
1f	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-
1g	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-
1h	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
1i	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
1j	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1k	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-

Reference drugs:	***S. aureus***	***B. Subtilis***	***E.Coli***	***S. Peratyphi B***
Ciprofloxacin	**1.9**	**7.8**	**0.4**	**1.4**

TABLE NO. 1b: Antimicrobial Activity 2-(substituted benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide (Ia-k).

TABLE NO-1(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (µg/ml)*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
1a	+	+	-	-	+	+	-	-
1b	+	+	-	-	+	+	-	-
1c	+	+	+	-	+	+	+	-
1d	+	+	-	-	+	+	-	-
1e	+	+	-	-	+	+	-	-
1f	+	+	-	-	+	+	-	-
1g	+	+	-	-	+	+	-	-
1h	+	+	-	-	+	+	-	-
1i	+	+	+	-	+	+	+	-
1j	+	+	+	+	+	+	+	+
1k	+	+	-	-	+	+	-	-
Reference drugs:			***A.niger***				***C. albicans***	
Fluconazole			**0.7**				**0.4**	

TABLE NO. 1c: Antimicrobial Activity 2-(3-hydroxy, 4-methoxy benzyl ideneamino)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxamide (Ij).

TABLE NO-1(c)Tertiory screening for minimum inhibition (conc in (*µg/ml*)

Different microbes cultures	2000	1000	500	250	125	62.5	31.25	15.6	7.8	3.9
Compound No.										
Escherichia coli	+	+	+	+	+	-	-	-	-	-
Salmonella peratyphi B	+	+	+	+	+	-	-	-	-	-
Staphylococcus Aureus	+	+	+	+	-	-	-	-	-	-
Bacillus subtilis	+	+	+	+	-	-	-	-	-	-
Aspergillus niger	+	+	+	+	-	-	-	-	-	-
Candida albicans	+	+	+	+	-	-	-	-	-	-

Reference drugs:	*A.niger*	*C. albicans*
Fluconazole	0.7	0.4

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 µg/ml conc. (2) Secondary Screening 1000 µg/ml to 250 µg/ml conc. (3) Tertiary Screening start from 125 µg/ml to 3.9 µg/ml . The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed in tertiary screening.

From the results of experiments using newly synthesized organic compounds' it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 1a-k almost eight compounds1a, 1b, 1c, 1d, 1f, 1g, 1i and 1j were found active at 500 µg/ml conc. against *staphylococcus aure*us. *Bacillus Subtilis* was inhibited at 500 µg/ml conc. by eight compounds 1a, 1b, 1c, 1d, 1f, 1i, 1j, and 1k. Seven compounds 1a, 1b, 1c, 1d, 1f, 1i and 1j were active against both cultures *B.Subtilis* and *S.aureus*. At the conc. 250 µg/ml *S. aureus* was inhibited by four compounds 1c, 1f, 1i and 1j. *B.Subtilis* was inhibited by four compounds 1a, 1c, 1i and 1j. (1a-k). So it is obvious from the data obtained that compounds 1c, 1i and 1j were highly active among all the compounds of series 1a-k.

For Gram Negative bacteria in the series 1a-k almost six compounds 1a, 1c, 1d, 1f, 1j and 1j were found active at 500 µg/ml conc. against *Escherichia Coli. S.Paratyphi B*. was inhibited at 500 µg/ml conc. by four compounds i.e. 1c, 1f, 1i and 1j. So, four compounds were active against both cultures *E.Coli* and *S.Paratyphi B*. i.e. 1c, 1f, 1i and 1j. At the conc. 250 µg/ml *E.Coli* was not inhibited by one compound 1j (Ia-k). *S.Paratyphi B*. was also inhibited by one compound 1j. So, it is obvious from the data obtained that compound 1j was highly active among all the compounds of series 1a-k.

For fungi in the series 1a-k almost three compounds 1c, 1i and 1j were found active at 500 µg/ml conc. against *A.niger. C. albicans* was inhibited at 500 µg/ml conc. by six compounds i.e. 1c, 1i and 1j. At the conc. of 250 µg/ml *C.albicans* was

killed by one compound 1j. *A. Niger* was killed by one compound i.e. 1j. So, it is obvious from the data obtained that compound 1j was highly active among all the compounds of series 1a-k.

Tetiory screening for Minimum Inhibitory Concentration:

(Extensive investigation for the powerful antimicrobials of the series):

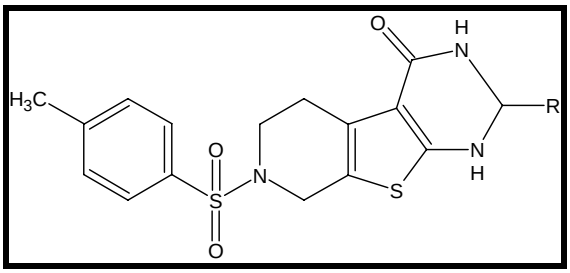
Total nine series of synthesized organic compounds were analyzed for the Antibacterial and antifungal activity through primary and secondary screening using Agar Dilution Method. To get their exact MIC surprisingly compound 1j (R= 3-OH-4-OCH3-C6H4) of series were highly active throughout the secondary screening. This compound was highly active and gave complete inhibition of *E.Coli, S.Paratyphi B, B.Subtilis and S.aureus*. Compound 1j (R= 3-OH-4-OCH3-C6H4) was highly active at 250 µg/ml conc. against fungal cultures *A.niger* and *C. albicans*.

Then after this highly active compounds were used for tertiory screening against bacterial as well as fungal cultures. Compound 1j (R= 3-OH-4-OCH3-C6H4) was active for all bacterial cultures at 250 µg/ml conc. It was also very effective and gave complete inhibition of *E.Coli*, *S.Paratyphi B* at 125 µg/ml conc.

SECTION-II

Preparation, Characterization and antimicrobial evaluation of 2-aryl-7-[(4-methylphenyl) sulfonyl] -1,2,3,5,6,7,8—heptahydro pyrido[4',3': 4,5]thieno[2,3-d] pyrimidin-4-ones.

Keeping in view of wide spectrum biodynamic activities[5-26] of thieno pyridine and with a view to have potent therapeutic agents, the synthesis of **2-aryl-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5] thieno [2,3-d] pyrimidin-4-ones (2a-k)** have been synthesized using 2-(amino)-6-[(4-methylphenyl)sulfonyl]-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamide with different substituted aromatic aldehydes.



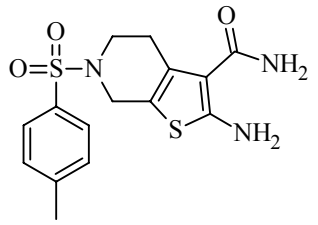
(2a-k)

R= Aryl

The constitution of the synthesized products (2a-k) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella peratyphi B* and they were also evaluated for antifungal activity against *Candida albicans* and *Aspergillus niger* at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (2a-k) have been compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by **NCCLs**).

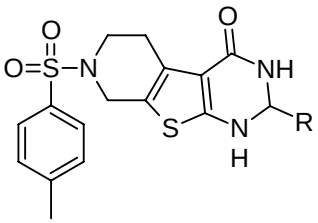
REACTION SCHEME



Scaffold-C

Step-3

Acetic acid
Reflux
5 hours



(2a-k)
R= Aryl

EXPERIMENTAL

Preparation, Characterization and antimicrobial evaluation of 2-aryl-7-[(4-methylphenyl) sulfonyl]-1,2,3,5,6,7,8-heptahydropyrido[4',3': 4,5] thieno[2,3-d] pyrimidin-4-ones.

[A] Synthesis of Scaffold-C.

For Preparation, see Part-III, Section-I, Page No. 147

[B] Synthesis of 2-(4-nitrophenyl)-7-[(4-methylphenyl) sulfonyl] 1, 2, 3, 5, 6, 7, 8 heptahydropyrido [4, 3':4, 5] thieno [2, 3-d] pyrimidin-4-ones.

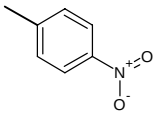
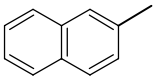
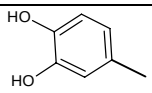
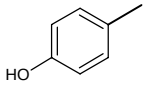
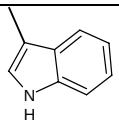
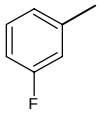
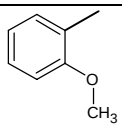
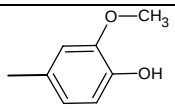
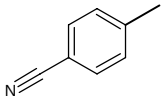
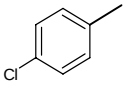
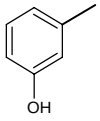
In RBF, Scaffold-C (1.0 mol) in acetic acid were charged 4-nitrobenzaldehyde at room temperature. The reaction mixture was heated at reflux temperature for 5 hour. After the completion of reaction, Reaction mixture was cool at room temperature and quenched with chilled water. The obtaining solid was filtered and washed with water and dried. The obtained product was purified by triturating with ethanol. Yield: 63.00%. M.P: 267°C. Elemental Analyses: Calculated: C (54.53%), H (4.16%), N (11.56%), Found: C (54.44%), H (4.11%), N (11.49%).

Similarly, other compounds (2a-k) were synthesized by above mentioned process (B) from Scaffold-C. The physical data are recorded in Table-2.

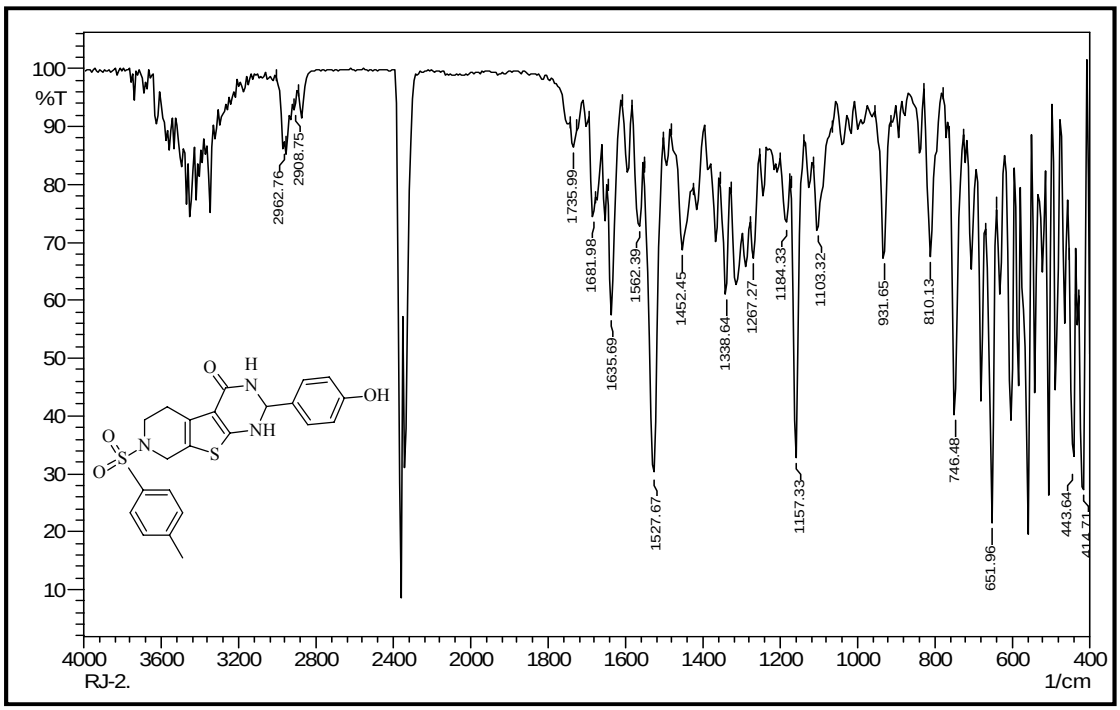
[C] Antimicrobial activity of 2-aryl-7-[(4-methylphenyl) sulfonyl] 1, 2, 3, 5, 6, 7, 8 heptahydropyrido [4, 3':4, 5] thieno [2, 3-d] pyrimidin-4-ones.

Antimicrobial activity testing was carried out as described in Part-I, Section-I, Page No. (32 to 36) The MIC values of test solution are recorded in Table NO-2a and 2b.

TABLE NO-2: Physical constants of 2-aryl-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5]thieno[2,3-d]pyrimidin-4-ones (2a-k).

Sr. No.	Substitution R	Molecular Formula/ Molecular weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
2a		$C_{22}H_{20}N_4O_5S_2$ 484	267	63	54.53 54.44	4.16 4.11	11.56 11.49
2b		$C_{26}H_{23}N_3O_3S_2$ 489	292	51	63.78 63.69	4.73 4.66	8.58 8.51
2c		$C_{22}H_{21}N_3O_5S_2$ 471	272	68	56.04 55.91	4.49 4.42	8.91 8.82
2d		$C_{22}H_{21}N_3O_4S_2$ 455	252	68	58.00 57.81	4.65 4.59	9.22 9.14
2e		$C_{24}H_{22}N_4O_3S_2$ 478	265	57	60.23 60.04	4.63 4.56	11.71 11.65
2f		$C_{22}H_{20}N_3O_3S_2F$ 457	285	65	57.75 57.59	4.41 4.34	9.18 9.11
2g		$C_{23}H_{23}N_3O_4S_2$ 469	260	65	58.83 58.62	4.94 4.77	8.95 8.89
2h		$C_{23}H_{23}N_3O_5S_2$ 485	190	59	56.89 56.71	4.77 4.70	8.65 8.56
2i		$C_{23}H_{20}N_4O_3S_2$ 464	278	58	59.46 59.31	4.34 4.28	12.06 11.93
2j		$C_{22}H_{20}N_3O_3S_2Cl$ 473	282	66	55.75 55.53	4.25 4.14	8.87 8.76
2k		$C_{22}H_{21}N_3O_4S_2$ 455	293	66	58.00 57.81	4.65 4.57	9.22 9.13

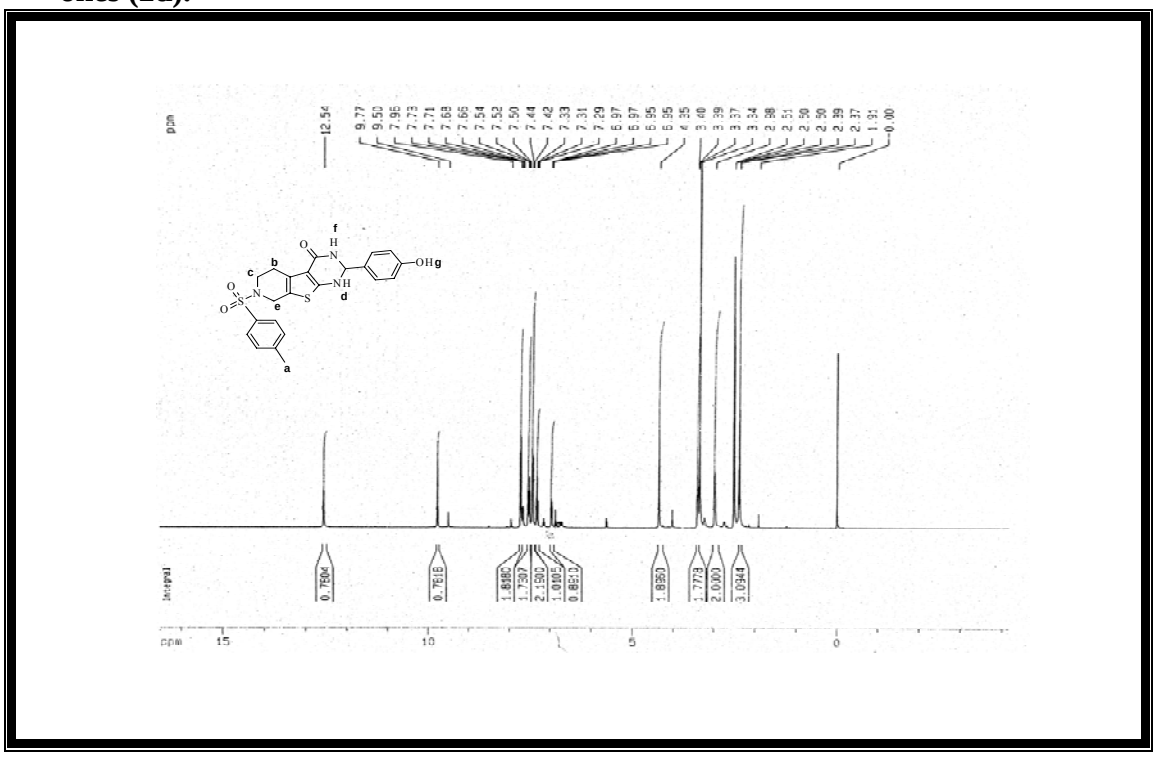
IR Spectral Study of 2-(4-hydroxyphenyl)-7-[(4-methylphenyl)sulfonyl] 1, 2, 3, 5, 6, 7, 8, heptahydropyrido [4',3':4,5]thieno[2,3-d]pyrimidin-4-ones (2d).

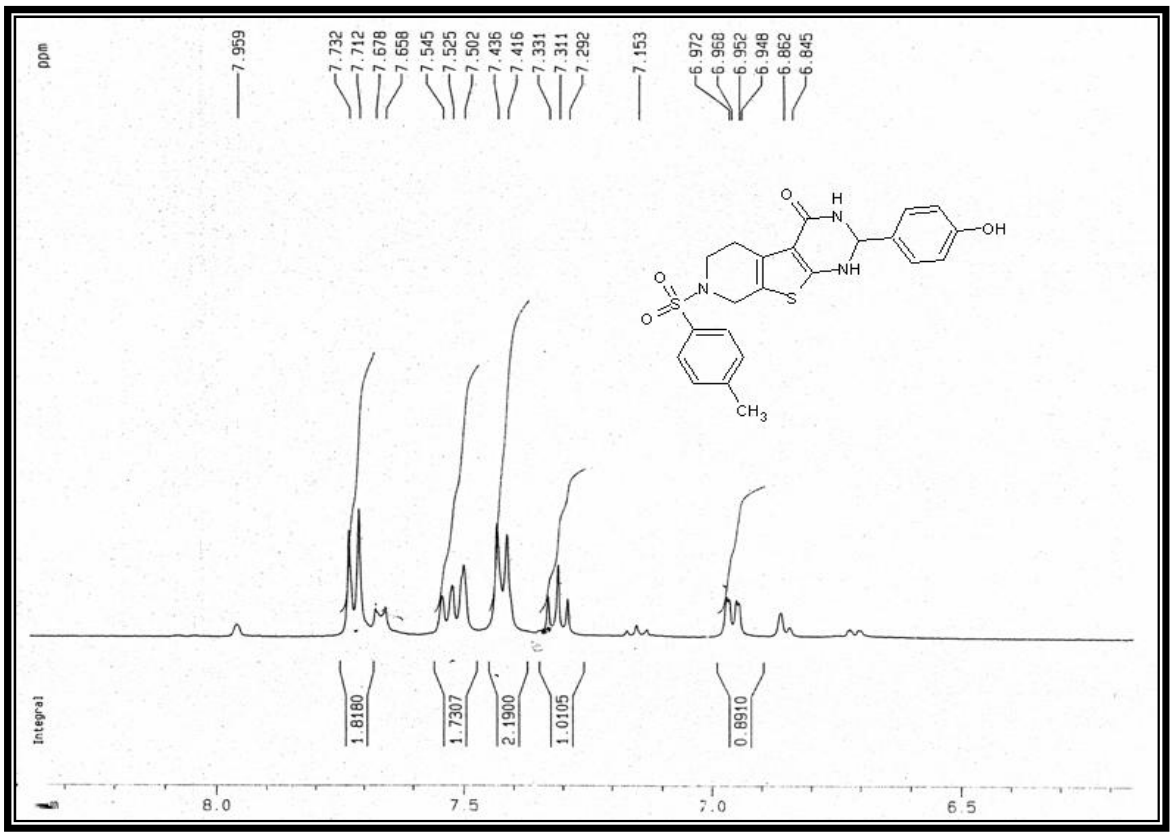


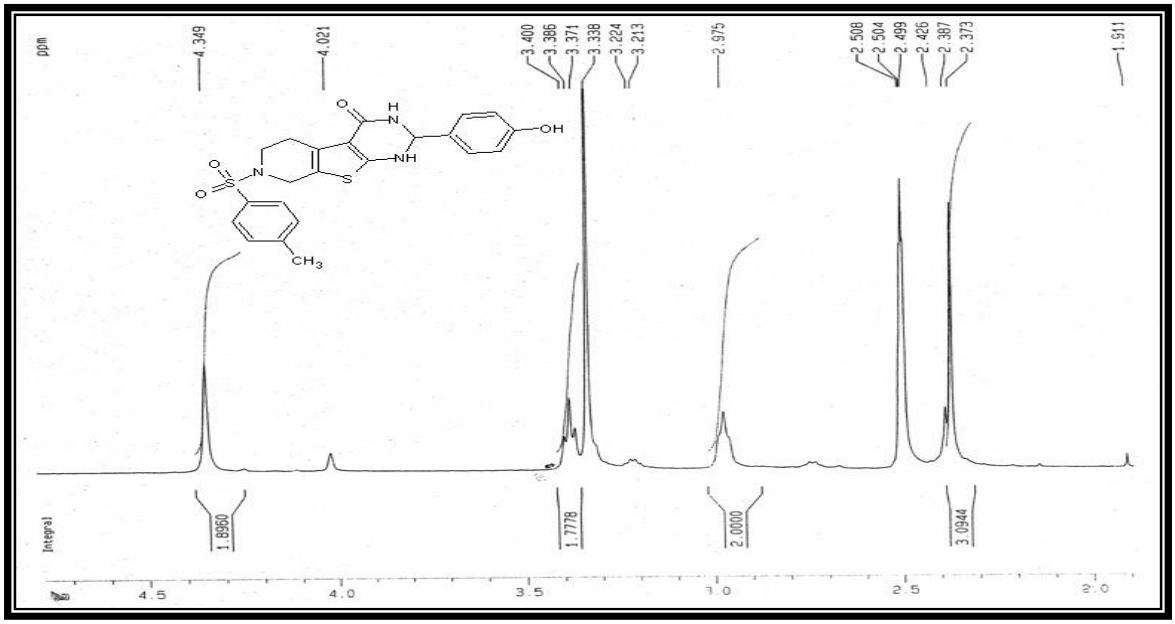
Instrument: SHIMADZU FTIR 8400 Spectrophotometer;
Frequency range: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No.
		Observed	Reported	(Part-I)
Alkane -CH_2	C-H asym. str.	2908	2975-2850	42
	C-H asym. def.	1452	1460-1400	42
	C-H sym. Def.	1338	1385-1300	42
Aromatic	C-H str.	2962	3080-3010	42
	C=C ring skeleton	1527	1600-1450	44
		1562	1600-1450	44
Thienopyridine moiety	C-S-C str.	651	700-650	44
	C=N	1635	1650-1600	43
Pyrimidone Cyclic amide	-C=O str.	1682	1740-1650	42
	-N-H str.	3420	3600-3200	43
Sulfonyl	N-SO2 str.	1157	1160-1120	44

1H-NMR Spectral Study of 2-(4-hydroxyphenyl)-7-[(4-methylphenyl) sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5]thieno [2,3-d]pyrimidin-4-ones (2d).



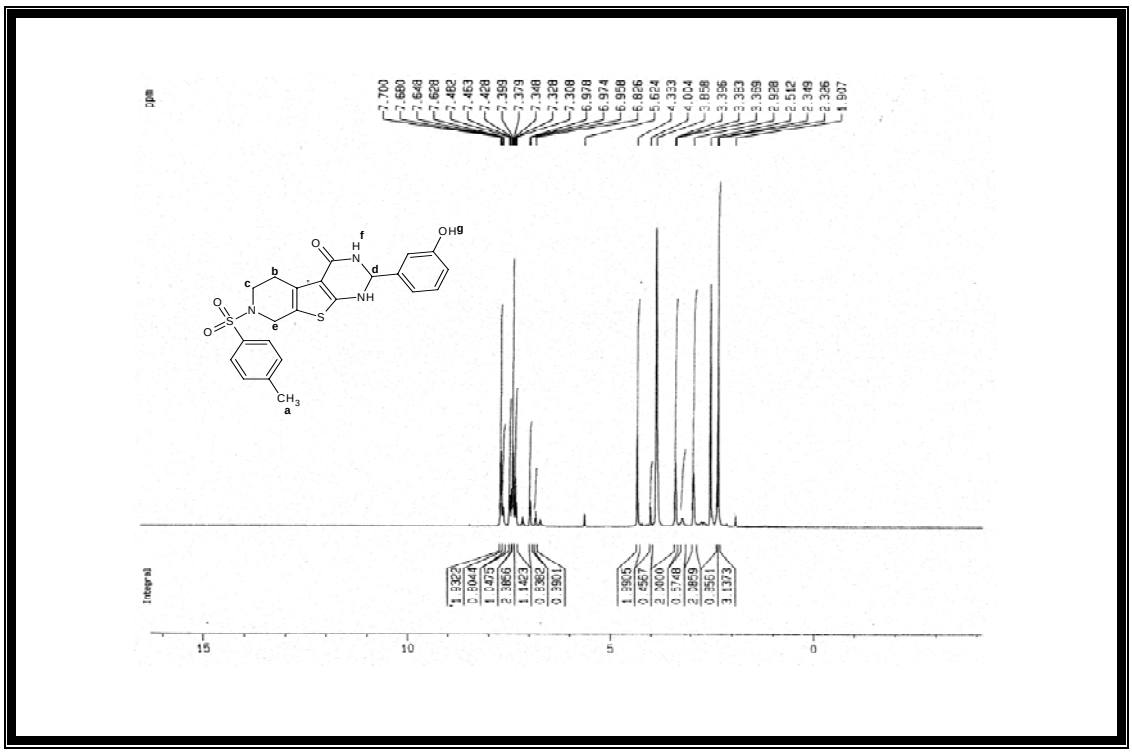


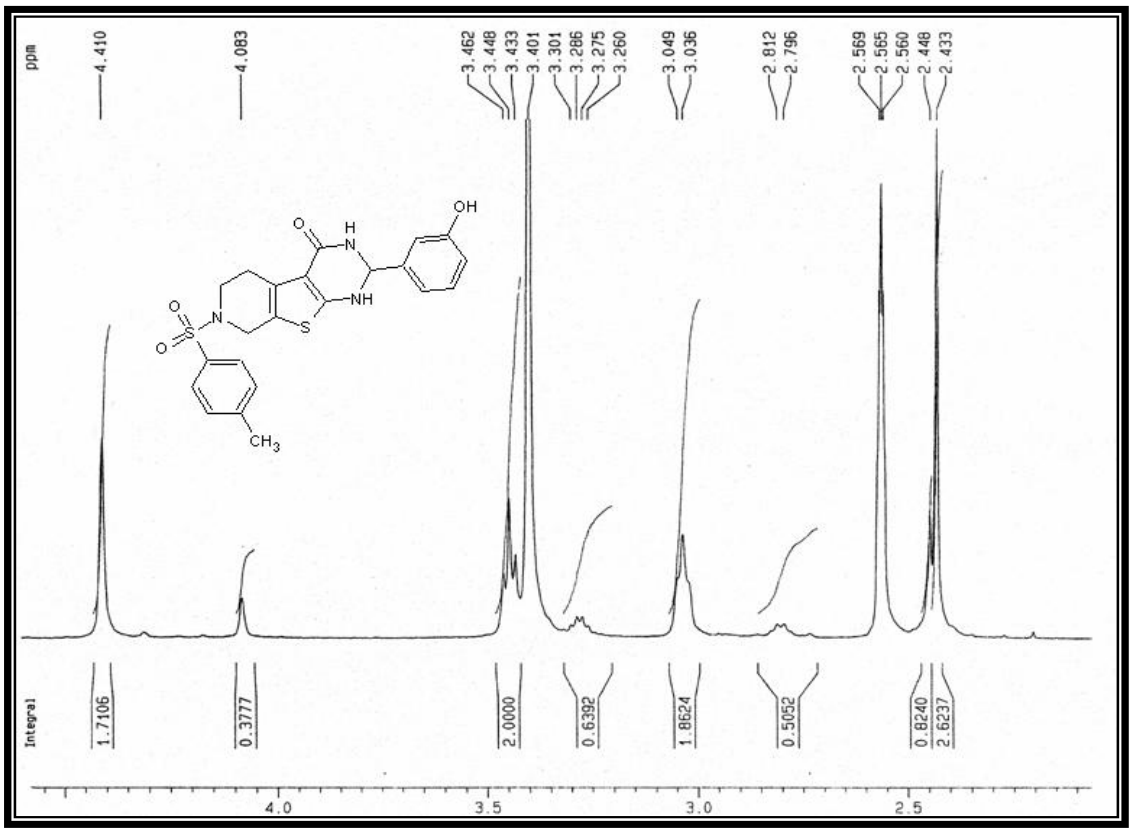


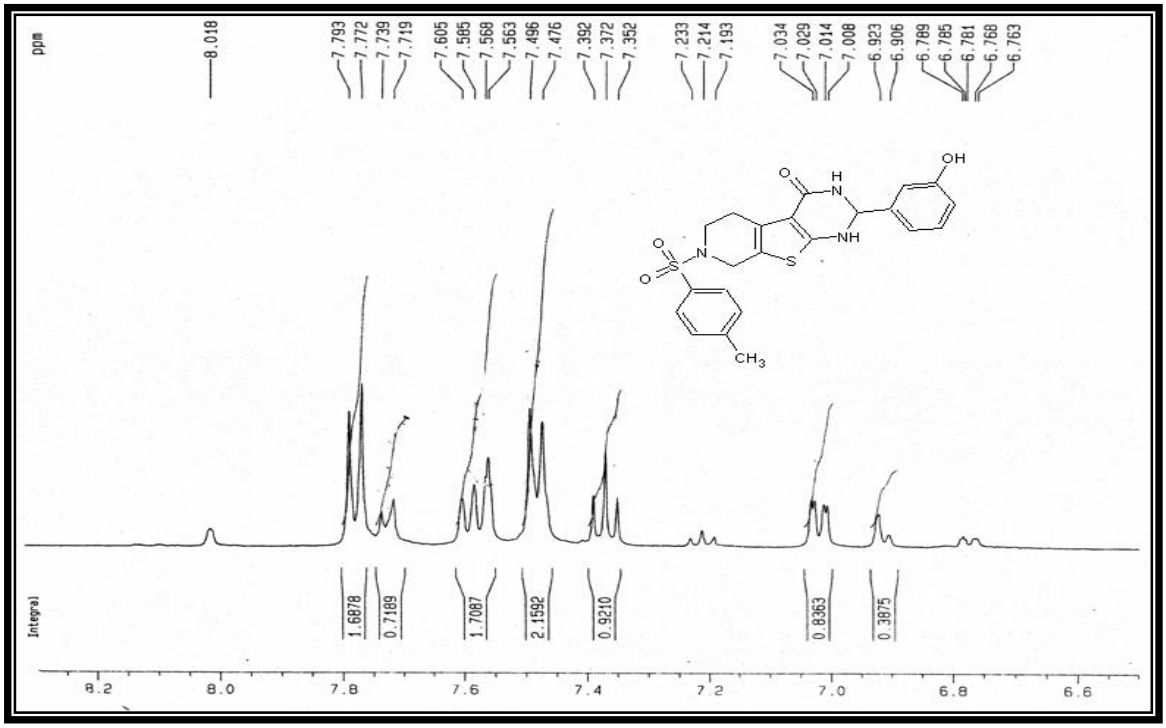
Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO- *d6.*

Serial No.	Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	2.37	3H	Singlet	-(CH_3) a
2	2.975	2H	Broad Singlet	-(CH_2) b
3	3.38	2H	Triplate	-(CH_2) c
4	4.021	1H	Singlet	-(CH)d
5	4.349	2H	Singlet	-(CH_2) e
6	6.948-7.732	8H	complex	Ar -H
7	9.77	1H	Singlet	-(NH) f
8	12.54	1H	Singlet	-(OH) g

1H-NMR Spectral Study of 2-(3-hydroxyphenyl)-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5]thieno [2,3-d]pyrimidin-4-ones (2j).



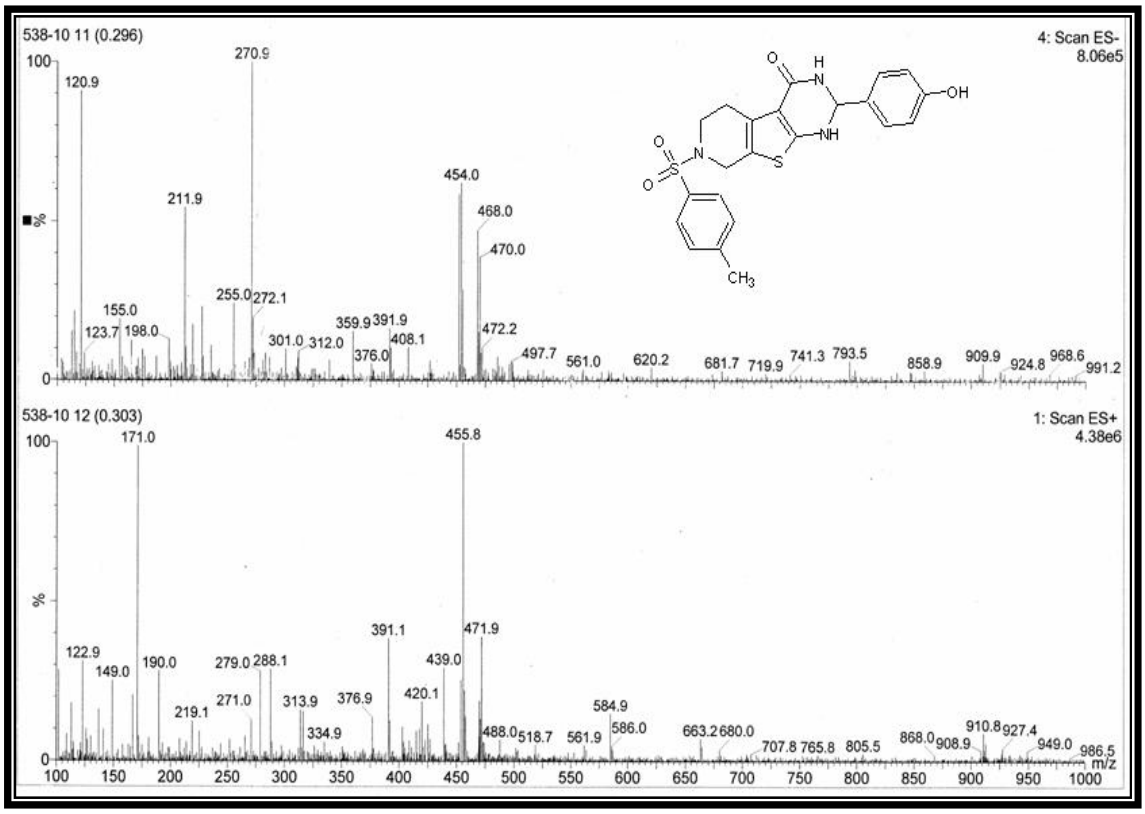




Instrument: BRUKER 400 MHz (Advance - II), **Internal reference:** TMS, **Solvent:** DMSO- *d6.*

Serial No.	Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	2.43	3H	Singlet	-(CH_3) a
2	3.036-3.049	2H	Triplet	-(CH_2) b
3	3.433-3.462	2H	Triplet	-(CH_2) c
4	4.083	1H	Singlet	-(CH) d
5	4.410	2H	Singlet	-(CH_2) e
6	7.008-7.793	8H	complex	Ar -H
7	9.83	1H	Singlet	-(NH) f
8	12.60	1H	Singlet	-(OH) g

Mass Spectral Study of 2-(4-hydroxyphenyl)-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5]thieno [2,3-d]pyrimidin-4-ones (2d).



Possible Mass Fragmentation pattern:

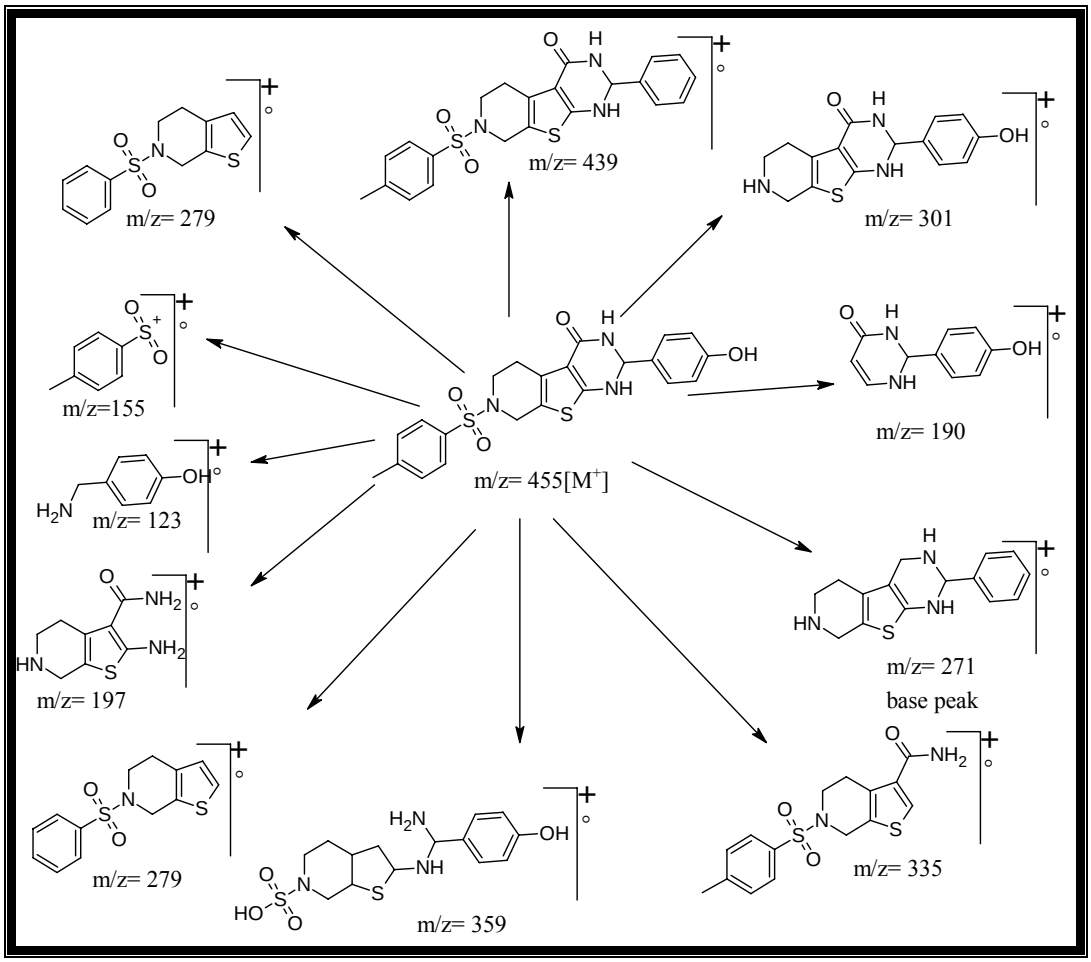


TABLE NO. 2a: Antimicrobial Activity Of 2-aryl-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5]thieno[2,3-d]pyrimidin-4-ones (2a-k).

TABLE NO-2(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
2a	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
2b	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-
2c	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
2d	+	+	-	-	+	+	-	-	+	+	+	-	+	+	-	-
2e	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-
2f	+	+	+	-	+	+	-	-	+	+	+	-	+	+	-	-
2g	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
2h	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	-
2i	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-
2j	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
2k	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Reference drugs:	***S. aureus***	***B. Subtilis***	***E.Coli***	***S. Peratyphi B***
Ciprofloxacin	**1.9**	**7.8**	**0.4**	**1.4**

TABLE NO. 2b: Antimicrobial Activity of 2-aryl-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido[4',3':4,5]thieno[2,3-d]pyrimidin-4-ones (2a-k).

TABLE NO-2(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (µg/ml)*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
2a	+	+	+	-	+	+	+	-
2b	+	+	-	-	+	+	-	-
2c	+	+	+	-	+	+	-	-
2d	+	+	-	-	+	+	-	-
2e	+	+	+	-	+	+	-	-
2f	+	+	-	-	+	+	-	-
2g	+	+	+	-	+	+	-	-
2h	+	+	-	-	+	+	-	-
2i	+	+	+	-	+	+	-	-
2j	+	+	-	-	+	+	-	-
2k	+	+	+	+	+	+	+	+

Reference drugs:	***A.niger***	***C. albicans***
Fluconazole	**0.7**	**0.4**

TABLE NO. 2c: Antimicrobial Activity of 2-(3-hydroxy phenyl)-7-[(4-methylphenyl)sulfonyl]-1,2,3,5,6,7,8heptahydropyrido [4',3':4,5]thieno[2,3-d]pyrimidin-4-ones (2k).

TABLE NO-2(c)Tertiory screening for minimum inhibition (conc in (*µg/ml*)

Different microbes cultures	2000	1000	500	250	125	62.5	31.25	15.6	7.8	3.9
Compound No.										
Escherichia coli	+	+	+	+	+	-	-	-	-	-
Salmonella peratyphi B	+	+	+	+	-	-	-	-	-	-
Staphylococcus Aureus	+	+	+	+	+	+	-	-	-	-
Bacillus subtilis	+	+	+	+	-	-	-	-	-	-
Aspergillus niger	+	+	+	+	-	-	-	-	-	-
Candida albicans	+	+	+	+	-	-	-	-	-	-

Reference drugs:	*A.niger*	*C. albicans*
Fluconazole	0.7	0.4

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 µg/ml conc. (2) Secondary Screening 1000 µg/ml to 250 µg/ml conc. (3) Tertiary Screening start from 125 µg/ml to 3.9 µg/ml. The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed in tertiary screening.

From the results of experiments using newly synthesized organic compounds' it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 2a-k almost eight compounds 2a, 2c, 2e, 2f, 2g, 2h, 2i and 2k were found active at 500 µg/ml conc. against *staphylococcus aureus*. *Bacillus Subtilis* was inhibited at 500 µg/ml conc. by seven compounds 2a, 2b, 2c, 2e, 2g, 2i and 2k. Six compounds 2a, 2c, 2e, 2g, 2i and 2k were active against both cultures *B.Subtilis* and *S.aureus*. At the conc. 250 µg/ml *S.aureus* was inhibited by one compound 2k. *B.Subtilis* was inhibited by two compounds 2i and 2k. So, it is obvious from the data obtained that compounds 2k were highly active among all the compounds of series 2a-k.

For Gram Negative bacteria in the series 2a-k almost ten compounds 2a, 2b, 2c, 2d, 2e, 2f, 2g, 2h and 2k were found active at 500 µg/ml conc. against *Escherichia Coli. S.Paratyphi B.* was inhibited at 500 µg/ml conc. by seven compounds i.e. 2a, 2b, 2e, 2g, 2h, 2i and 2k. So Six compounds were active against both cultures *E.Coli* and *S.Paratyphi B*. i.e. 2a, 2b, 2e, 2g, 2h and 2k. At the conc. 250 µg/ml *E.Coli* was killed by two compounds 2e and 2k. *S.Paratyphi B*. was also inhibited by one compound 2k. So, it is obvious from the data obtained that compound 2k was highly active among all the compounds of series 2a-k.

For fungi in the series 2a-k almost six compounds 2a, 2c, 2e, 2g, 2i and 2k were found active at 500 µg/ml conc. against *A.niger*. *C. albicans* was inhibited at 500 µg/ml conc. by two compounds i.e. 2a and 2k. At the conc. of 250 µg/ml C.albicans was killed by one compound 2k. *A. Niger* was killed by one compound 2k.

So, it is obvious from the data obtained that compound 2k was highly active among all the compounds of series 2a-k.

Tertiory screening for Minimum Inhibitory Concentration:

(Extensive investigation for the powerful antimicrobials of the series):

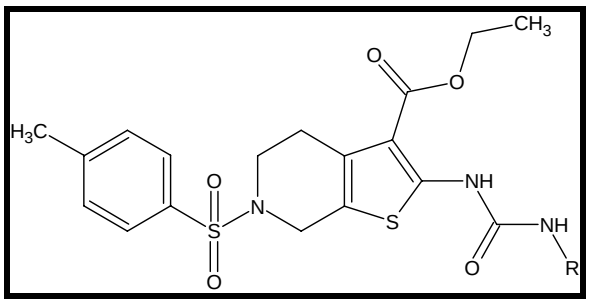
Total nine series of synthesized organic compounds were analyzed for the Antibacterial and antifungal activity through primary and secondary screening using Agar Dilution Method. To get their exact MIC surprisingly compound 2k (R= 2-OH-C6H4) of the series were highly active throughout the secondary screening. This one compound was highly active and gave complete inhibition of *E.Coli, S.Paratyphi B, B.Subtilis and S.aureus* at conc.250 µg/ml. This compound was highly active at 250 µg/ml conc. against fungal cultures *A.niger* and *C. albicans*.

Then after this highly active compounds were used for tertiory screening against bacterial as well as fungal cultures. Compound 2k (R= 2-OH-C6H4) was highly active for *E.Coli* and *S.aureus* at 125 µg/ml conc. It was also very effective on *S.aureus* at 62.5 µg/ml conc.

SECTION-III

Preparation, Characterisation and antimicrobial evaluation of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates.

Keeping in view of wide spectrum biodynamic activities[5-26] of thieno pyridine and with a view to have potent therapeutic agents, the synthesis of **Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates (3a-k)** have been synthesized using Ethyl 2-(phenoxy carbonylamino)-6-[(4-methylphenyl)sulfonyl]-4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxylate with differently substituted aromatic amines.



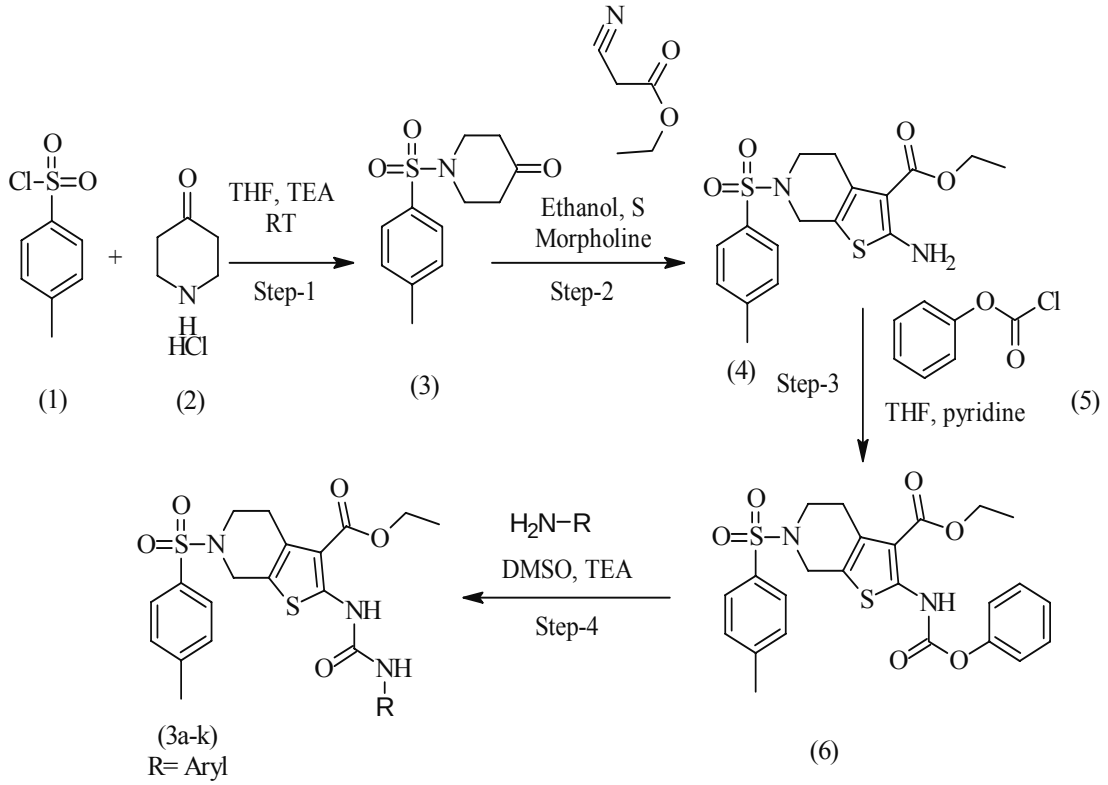
(3a-k)

R= Aryl

The constitution of the synthesized products (3a-k) have been characterized by using elemental analyses, infrared and 1H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry. Purity of all compounds has been checked by thin layer chromatography.

All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like *Staphylococcus aureus, Bacillus subtilis* and Gram Negative bacteria like *Escherichia coli, Salmonella peratyphi B* and they were also evaluated for antifungal activity against *Candida albicans* and *Aspergillus niger* at different concentrations: i.e. Primary screening at 2000 to 1000, Secondary screening at 1000 to 250 and tertiary screening at 250 to 3.9 (µg/ml) for their MIC (Minimum Inhibitory Concentration) values. The antimicrobial activities of the synthesized compounds (3a-k) have been compared with standard drugs. Their antimicrobial effect was determined in higher dilution using Agar Dilution Method (Approved by **NCCLs**).

REACTION SCHEME



EXPERIMENTAL

Preparation, Characterization and antimicrobial evaluation of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates.

[A]. Synthesis of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates (3a-k).

1. Synthesis of 1-[(4-methylphenyl) sulfonyl]-4-piperidinone (Intermediate-3).

In RBF, 4-piperidinone hydrochloride (1.00 mol), THF and Triethylamine (2.5 mol) were charged. The reaction mixture was cooled to 0°C and 4-methyl phenyl sulfonyl chloride (1.05mol) was added portion wise to the reaction mixture at 0°C. The reaction mixture was allowed to warm at room temperature and stirred for 4 hours at same temperature. After the completion of reaction, Reaction mixture was concentrated under reduced pressure at 45°C. The crude was quenched with chilled water and stirred for thirty minutes. The obtaining solid was filtered and washed with water and dried. Yield: 85.00 %. M.P: 131°C, 1H NMR (solvent: $CDCl_3$) δ 7.72 (d, 2H, J=8.1 Hz), 7.38 (d, 2H, J=8.1 Hz) 3.42 (m, 4H), 2.58 (m, 4H), 2.42 (s, 3H).

2. Synthesis of ethyl 2-amino-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7- Tetra hydro thieno [2, 3-c] pyridine-3-carboxylate (Intermediate-4).

In RBF, 1-[(4-methylphenyl) sulfonyl]-4-piperidinone (1.0 mol) (Intermediate-3), ethyl cyano acetate (1.10 mol), morpholine (5.0 mol) and sulfur (1.5 mol) was dissolved in ethanol at room temperature. The reaction mixture was heated at 70-80°C for 3-4 hours. After the completion of reaction, Reaction mixture was concentrated under reduced pressure at 45°C. The crude was quenched with chilled water and stirred for thirty minutes. The obtained solid was filtered and washed with water and dried. Yield: 70.00 %. M.P: 154°C. Elemental Analysis: Calculated: C (53.66%), H (5.30%), N (7.36%), Found: C (53.41%), H (5.19%), N (7.22%).

3. Synthesis of ethyl 6-[(4-methylphenyl)sulfonyl]-2-[(phenoxycarbonyl)amino]-4,5,6,7-tetrahydrothieno [2,3-c]pyridine-3-carboxylate (Intermeidate-6).

In RBF, Intermediate-4 (1.0 mol) in THF, pyridine (1.05 mol) were charged at room temperature. Into the resulting reaction mixture Phenyl chloroformate (Intermediate-

5) (1.05 mol) was added. The reaction mixture was stirred at room temperature for 2 hour. After the completion of reaction, Reaction mixture was cool at room temperature and concentrated under reduced pressure. The crude was quenched with chilled water. The obtained solid was filtered and washed with water and dried. Yield: 60.00 %. M.P: 142°C. Elemental Analysis: Calculated: C (57.58%), H (4.83%), N (5.60%), Found: C (57.30%), H (4.65%), N (5.32%).

4. Synthesis of Ethyl-2-(3-(4-hydroxyphenyl) ureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates (3a-k).

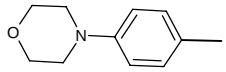
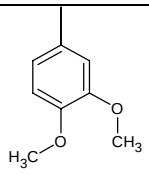
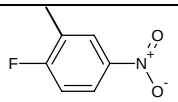
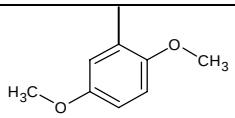
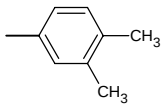
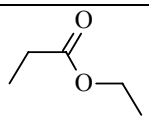
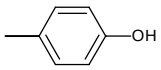
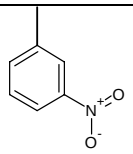
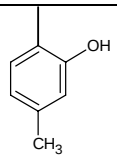
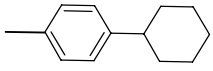
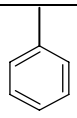
In RBF, ethyl 6-[(4-methylphenyl) sulfonyl]-2-[(phenoxycarbonyl) amino]-4, 5, 6, 7-tetrahydrothieno [2,3-c]pyridine-3-carboxylate (1.00 mol) was dissolved in DMSO. Triethylamine (2.50 mol) was added to the reaction mixture at room temperature. Into the resulting reaction mixture 4-amino phenol was added at room temperature. The reaction mixture was stirred for three hours at 60°C. After the completion of reaction, reaction mixture was quenched with chilled water. The obtained solid was filtered and washed with water and dried. The obtained product was purified by triturating with ethanol. Yield: 74.00 %. M.P: 156°C. Elemental Analysis: Calculated: C (55.91%), H (4.89%), N (8.15%), Found: C (55.84%), H (4.82%), N (8.09%).

Similarly, other compounds (3a-k) were synthesized by above mentioned process from intermediate: 6. the physical data are recorded in Table-3.

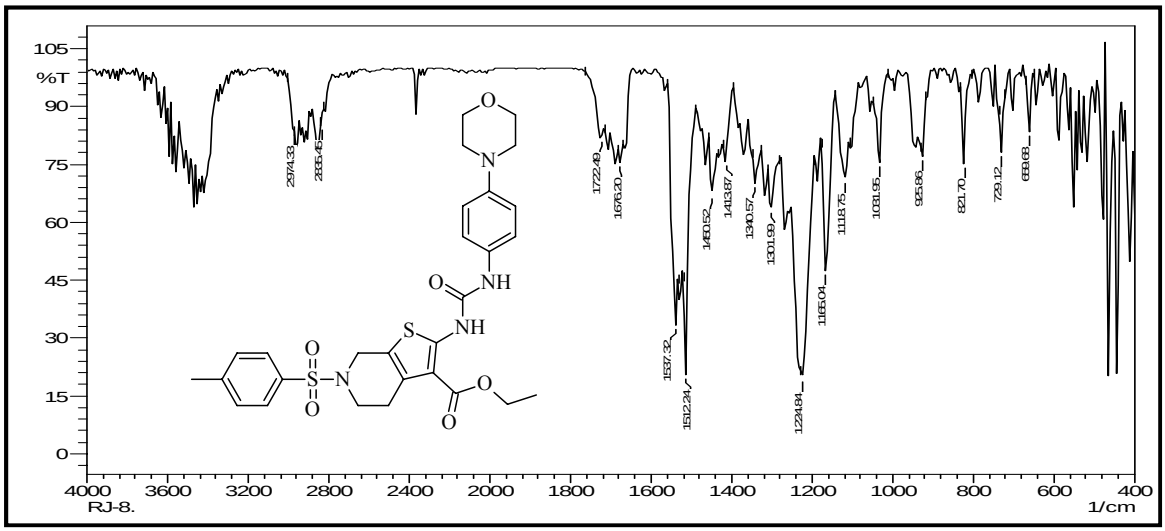
[B]. Antimicrobial activity of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates (3a-k).

Antimicrobial activity testing was carried out as described in Part-I, Section-I, Page No. (32 to 36) The MIC values of test solution are recorded in Table NO-3a and 3b.

TABLE NO-3: Physical constants of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates (3a-k).

Sr. No.	Substitution R	Molecular Formula/ Molecular weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
3a		$C_{28}H_{32}N_4O_6S_2$ 584	184	67	57.52 57.44	5.52 5.43	9.58 9.51
3b		$C_{26}H_{29}N_3O_7S_2$ 559	194	68	55.80 55.72	5.22 5.17	7.51 7.43
3c		$C_{24}H_{29}N_4O_7S_2F$ 562	164	68	51.24 51.19	4.12 4.08	9.96 9.89
3d		$C_{26}H_{29}N_3O_7S_2$ 559	194	70	55.80 55.72	5.22 5.15	7.51 7.42
3e		$C_{26}H_{29}N_3O_5S_2$ 527	171	71	59.18 59.11	5.54 5.49	7.96 7.89
3f		$C_{22}H_{27}N_3O_7S_2$ 509	125	74	51.85 51.79	5.34 5.29	8.25 8.21
3g		$C_{24}H_{25}N_3O_6S_2$ 515	156	74	55.91 55.84	4.89 4.82	8.15 8.09
3h		$C_{24}H_{24}N_4O_7S_2$ 544	202	68	52.93 52.85	4.44 4.39	10.29 10.21
3i		$C_{25}H_{27}N_3O_6S_2$ 529	180	64	56.69 56.63	5.14 5.08	7.93 7.85
3j		$C_{30}H_{35}N_3O_5S_2$ 581	145	68	61.94 61.81	6.06 6.01	7.22 7.17
3k		$C_{24}H_{25}N_3O_5S_2$ 499	153	72	57.70 57.64	5.04 4.96	8.41 8.39

IR Spectral Study of Ethyl-2-(3-(4-morpholinophenyl) ureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-carboxylates (3a).

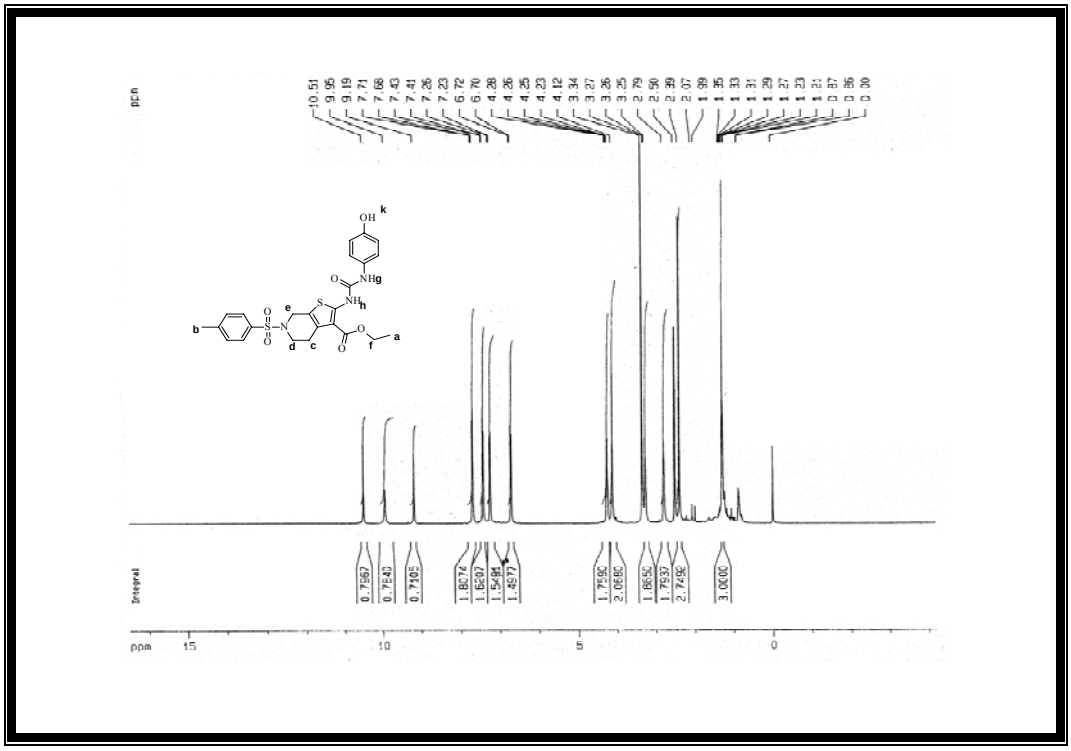


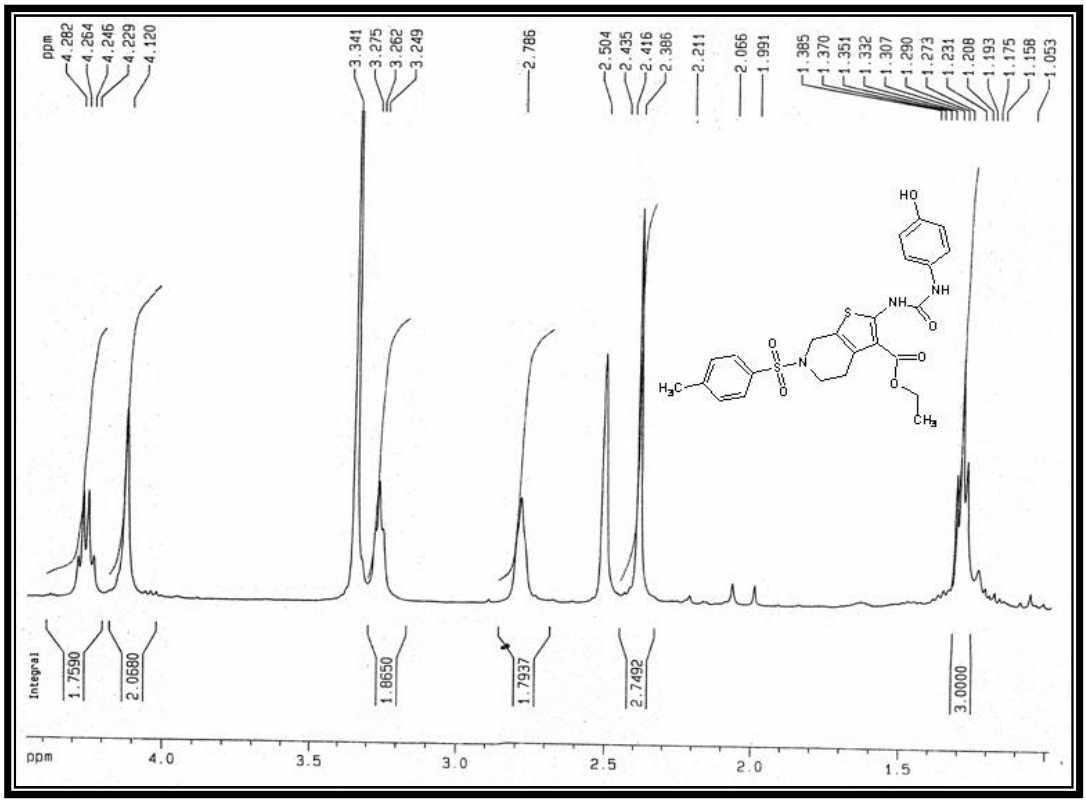
Instrument: SHIMADZU FTIR 8400 Spectrophotometer;

Frequency range: 4000-400 cm-1 (KBr disc.)

Type	Vibration Mode	Frequency in cm^{-1}		Ref. No.
		Observed	Reported	(Part-1)
Alkane -CH_2	C-H asym. str.	2835	2975-2850	42
	C-H asym. def.	1450	1460-1400	42
	C-H sym. Def.	1340	1385-1300	42
Aromatic	C-H str.	2974	3080-3010	42
	C=C ring skeleton	1537	1600-1450	44
		1520	1600-1450	44
Thienopyridine moiety	C-S-C str.	659	700-650	44
	C=N	1650	1650-1600	43
Amide moiety	N-H str. (assy.)	3560	3600-3200	43
	N-H str. (sym.)	3338	3600-3200	43
	-C=O str. (amide)	1676	1740-1650	42
	-C=O str. (Ester)	1722	1740-1650	42
Ether	C-O-C assym. Str.	1224	1250-1200	42
	C-O-C sym. Str.	1031	1050-1010	42
Sulfonamide	N-SO2	1165	1160-1120	44

1H-NMR Spectral Study of Ethyl-2-(3-(4-hydroxyphenyl)ureido)-6-[(4-methyl phenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxylates (3g).

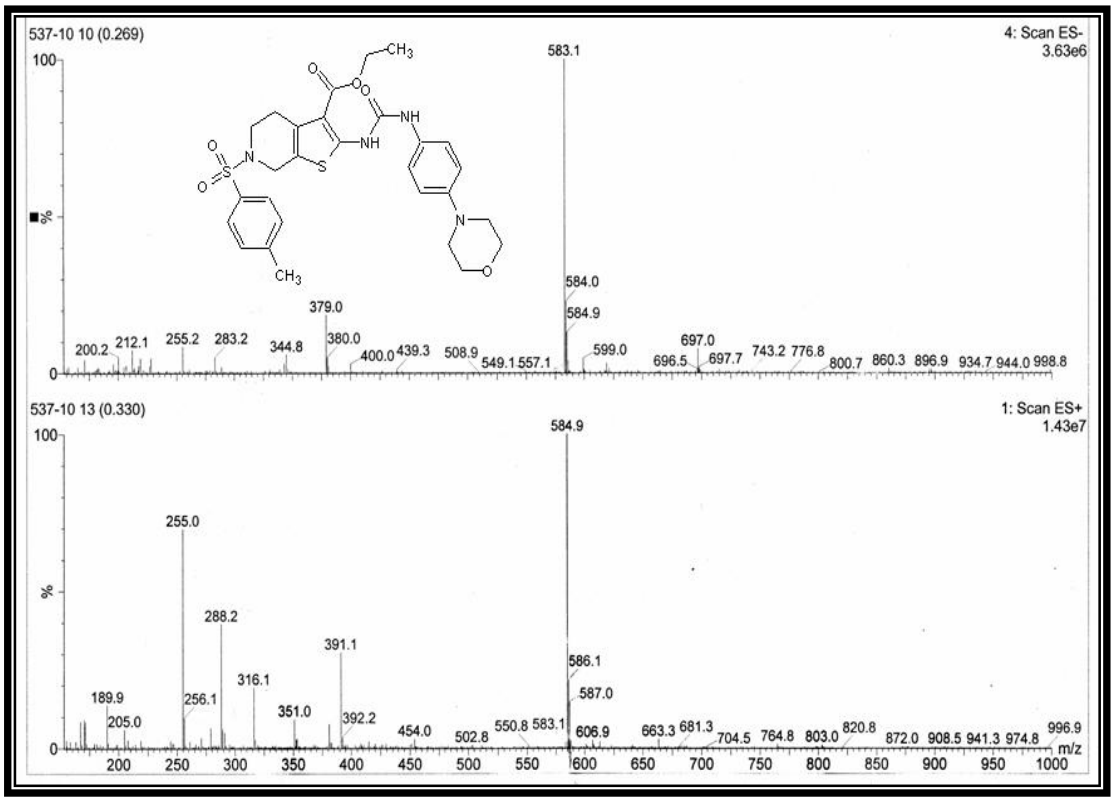




Instrument: BRUKER 400 MHz (Avance - II), **Internal reference:** TMS, **Solvent:** DMSO-*d6*.

Serial No.	Signal Position (δ ppm)	Relative No. of Protons	Multiplicity	Inference
1	1.273	3H	Triplet	-(CH_3) a
2	2.435	3H	Singlet	-(CH_3) b
3	2.786	2H	Broad singlet	-(CH_2) c
4	3.275	2H	Triplet	-(CH_2) d
5	4.120	2H	Singlet	-(CH_2) e
6	4.282	2H	Quartrate	-(CH_2) f
7	6.698-7.705	8H	Complex	Ar -H
8	9.19	1H	Singlet	-(NH) g
9	9.95	1H	Singlet	-(NH) h
10	10.51	1H	singlet	-(OH) k

Mass Spectral Study of Ethyl-2-(3-(4-morpholinophenyl) ureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydrothieno [2, 3-c] pyridine-3-carboxylates (3a).



Possible Mass Fragmentation pattern:

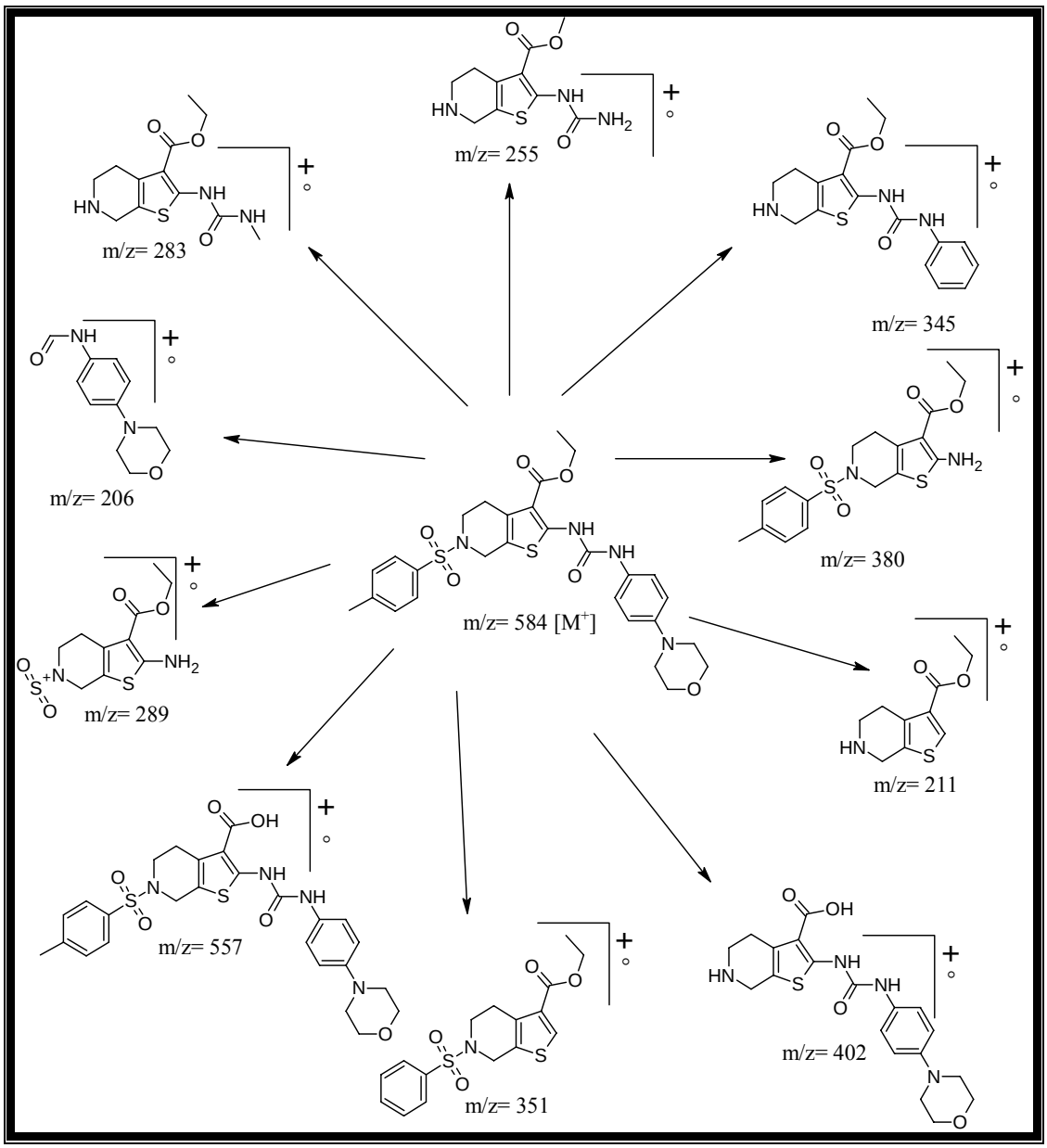


TABLE NO. 3a: Antimicrobial Activity Of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates (3a-k).

TABLE NO-3(a) ANTIBACTERIAL ACTIVITY

	Gram Positives								Gram Negative							
	S. aureus (µg/ml)				*B.Subtilis (µg/ml)*				*E.Coli (µg/ml)*				*S. peratyphi B (µg/ml)*			
	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
Compound No.																
3a	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
3b	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
3c	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
3d	+	+	-	-	+	+	+	-	+	+	-	-	+	+	+	-
3e	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
3f	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-
3g	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
3h	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
3i	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-
3j	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
3k	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

Reference drugs:	***S. aureus***	***B. Subtilis***	***E.Coli***	***S. Peratyphi B***
Ciprofloxacin	**1.9**	**7.8**	**0.4**	**1.4**

TABLE NO. 3b: Antimicrobial Activity of Ethyl-2-(3-arylureido)-6-[(4-methylphenyl) sulfonyl]-4, 5, 6, 7-tetrahydro thieno [2, 3-c] pyridine-carboxylates (3a-k).

TABLE NO-3(b) ANTIFUNGAL ACTIVITY

	A.niger (µg/ml)				*C. albicans (µg/ml)*			
	2000	**1000**	**500**	**250**	**2000**	**1000**	**500**	**250**
Compound No.								
3a	+	+	+	-	+	+	+	-
3b	+	+	+	-	+	+	+	-
3c	+	+	-	-	+	+	-	-
3d	+	+	-	-	+	+	-	-
3e	+	+	+	-	+	+	+	-
3f	+	+	+	+	+	+	+	-
3g	+	+	-	-	+	+	-	-
3h	+	+	+	-	+	+	+	-
3i	+	+	-	-	+	+	+	-
3j	+	+	+	-	+	+	+	-
3k	+	+	-	-	+	+	-	-

Reference drugs:	***A.niger***	***C. albicans***
Fluconazole	**0.7**	**0.4**

CONCLUSION

ANTIMICROBIAL ACTIVITY

The newly synthesized organic compounds were screened through three different types of screening i.e. (1) Primary Screening using 2000 µg/ml conc. (2) Secondary Screening 1000 µg/ml to 250 µg/ml conc. (3) Tertiary Screening start from 125 µg/ml to 3.9µg/ml. The compounds which were active against the microbes in primary screening were taken for the secondary screening. The compounds which gave inhibition of microbes in secondary screening were further analyzed in tertiary screening.

From the results of experiments using newly synthesized organic compounds' it is clear that all the compounds were moderately active at lower dilution i.e. high concentration like 2000 µg/ml and 1000 µg/ml conc. of compounds. In the series 3a-k almost three compounds 3a, 3b and 3c were found active at 500 µg/ml conc. against *staphylococcus aureus. Bacillus Subtilis* was inhibited at 500 µg/ml conc. by eight compounds 3a, 3b, 3c, 3d, 3f, 3g, 3h, and 3i. Three compounds 3a, 3b and 3c were active against both cultures *B.Subtilis* and *S.aureus*. At the conc. 250 µg/ml *S. aureus* was inhibited by one compound 3b. *B.Subtilis* was inhibited by two compounds 3a and 3b (3a-k). So, it is obvious from the data obtained that compounds 3b were highly active among all the compounds of series 3a-k.

For Gram Negative bacteria in the series 3a-k almost four compounds 3a, 3b, 3f and 3i were found active at 500 µg/ml conc. against *Escherichia Coli*. *S.Paratyphi B*. was inhibited at 500 µg/ml conc. by five compounds i.e. 3a, 3b, 3d, 3f and 3i. So, four compounds were active against both cultures *E.Coli* and *S.Paratyphi B*. i.e. 3a, 3b, 3f and 3i. At the conc.250 µg/ml *E.Coli* was killed by one compound 3a. *S.Paratyphi B*. was also inhibited by two compound 3a and 3b. So, it is obvious from the data obtained that compound 3a was highly active among all the compounds of series 3a-k.

For fungi in the series 3a-k almost six compounds 3a, 3b, 3e, 3f, 3h, and 3j were found active at 500 µg/ml conc. against *A.niger*. *C. albicans* was inhibited at 500 µg/ml conc. by seven compounds i.e. 3a, 3b, 3e, 3f, 3h, 3i and 3j. At the conc. of 250 µg/ml *C.albicans* was not killed by any compounds. *A. Niger* was killed by one

compound i.e. 3f. So it is obvious from the data obtained that compound 3f was highly active among all the compounds of series 3a-k.

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