PART: I

STUDIES ON PYRIMIDINES

INTRODUCTION

Pyrimidines are among those molecules that make life possible, have been some of the building blocks of DNA and RNA. Several analogues of pyrimidines have been used as compounds that interfere with the synthesis and functioning of nucleic acid. e.g. fluorouracil, which has been used in cancer treatment. There are several other important groups of pyrimidines such as purines, uric acid, barbituric acid etc., with medicinal uses.

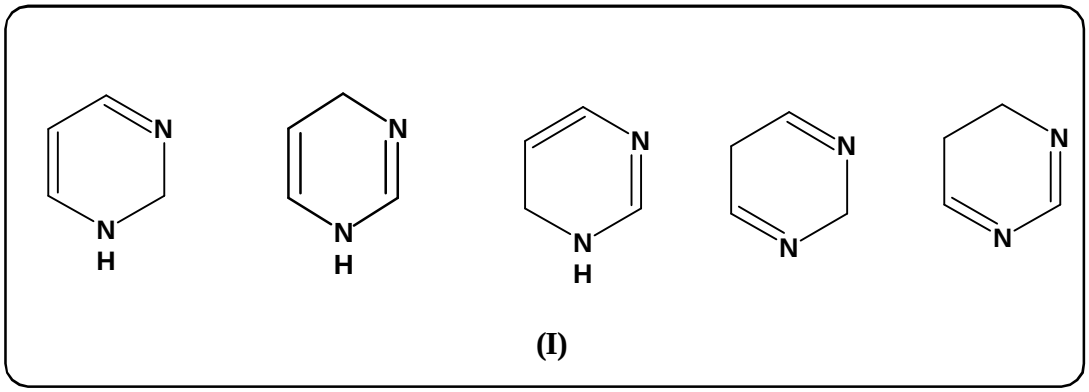
With the emergence of high throughput screening in the pharmaceutical industry, synthetic organic chemists have overcome the challenges of preparing large collections of molecule, by using Multi Component Reaction (MCR) strategy. The unique exploratory power of multi-component reaction is now recognized to be extremely valuable to produce compound libraries in a time and cost effective manner. MCRs offer significant advantages over conventional linear type synthesis[1-2].

MCR occupy an outstanding position in organic and medicinal chemistry for their high degree of atom economy, applications in combinational chemistry and diversity-oriented synthesis. Such reactions leading to interesting heterocyclic scaffolds are particularly useful for the creation of diverse chemical libraries of “drug-like” molecules for biological screening.

In MCRs, three or more reactants are used to come together in a single vessel to form a new product containing portions of each reactants. Dating back to 1893, such MCR was done by P. Biginelli using C-H acidic carbonyl compound, aldehyde and urea-type building blocks to assemble a multifunctionalized dihydropyrimidine scaffold[3-4]. This reaction is referred to as “Biginelli reaction”, Biginelli condensation” or as “Biginelli dihydropyrimidine synthesis.”[5-6]Since last decade, a tremendous interest has again occured for Biginelli reaction, as it is evidenced by the fact that the product so called “dihydropyrimidine” represents a heterocyclic system of remarkable pharmaceutical efficiency. A broad range of biological effects, including calcium- channel blockers[7], antiviral, antitumor and antiinflammatory[8] activities has been ascribed to these partly reduced pyrimidine derivatives. Additionally,

dihydropyrimidines have been found active to transport medication across biological membrances. Several marine alkaloids containing the DHPMs is found in nature and in potent HIV-gp-120-CD_4 inhibitors.[9]

The five possible isomeric structures of dihydropyrimidines, exhibiting different disposition of the double bonds are depicted in figure (I).



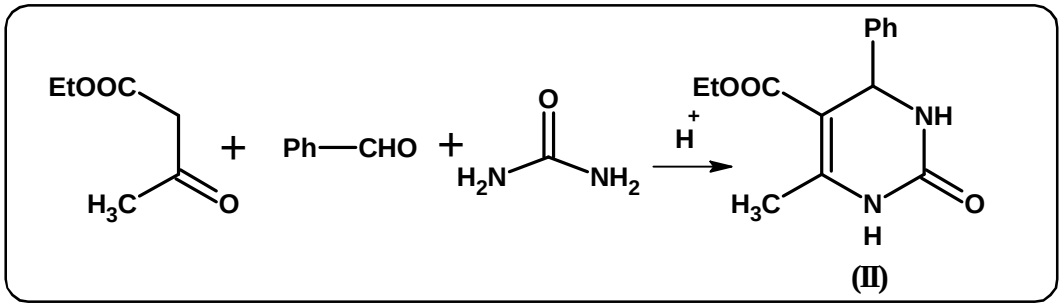
(I)

SYNTHETIC ASPECTS

There has been a considerable expansion in the numbers and types of primary synthesis used for pyrimidines. The linking of a C-C-C fragment with an N-C-N fragment still remains the principal synthetic method for making pyrimidines.

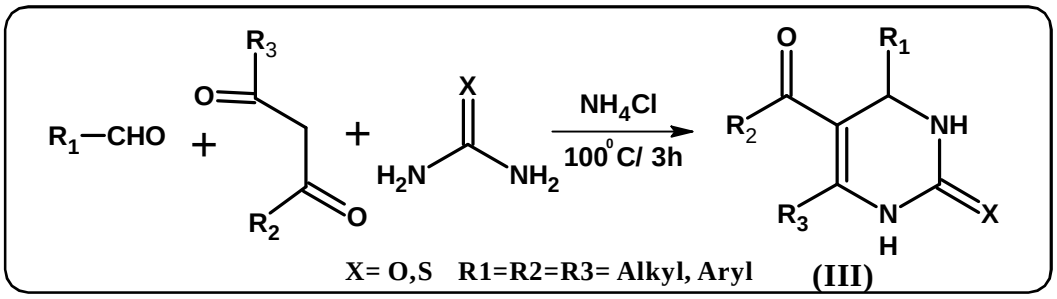
The simplest and the most straight forward approach for DHPMs involve one pot condensation of an aldehyde, β-keto ester and urea or thiourea in the presence of acid catalyst.

1. In 1893 Pietro Biginelli reported the first synthesis of dihydro pyrimidines by a simple one-pot condensation reaction of ethyl acetoacetate, benzaldehyde and urea under strong acidic condition.[10-11]

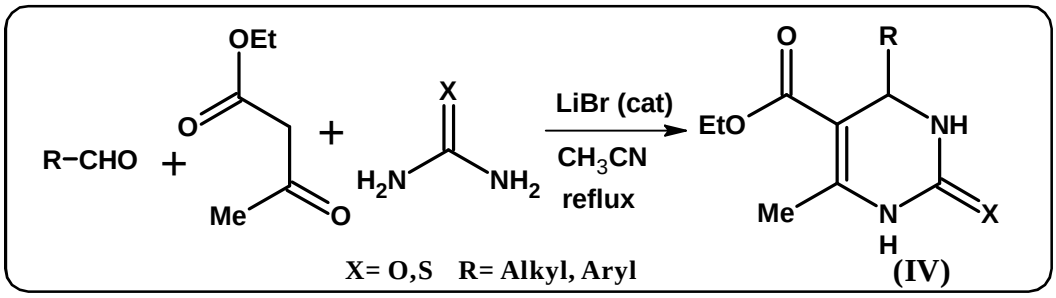


(II)

2. Fabio Falsone et al.[12] have synthesized dihydropyrimidone using polyphosphate ester as a mild and efficient cyclocondensation/dehy dration reagent.
3. Ammonium chloride-catalyzed one-pot synthesis of DHPMs under sol vent-free conditions was done by Ahmaad Shabani et al.[13]

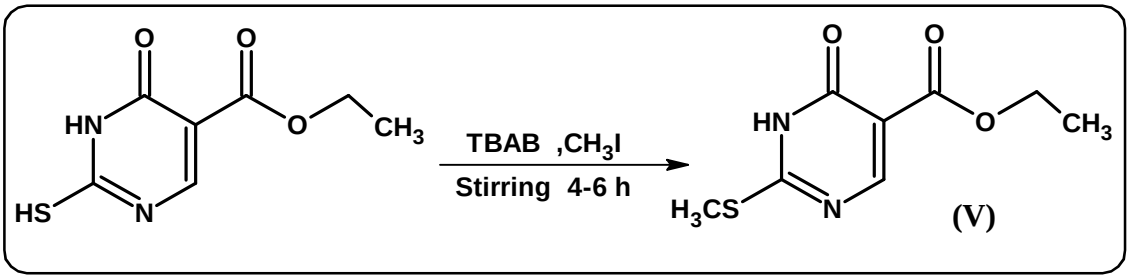


4. V. R. Choudhary et al.[14] have prepared DHPMs using Microwave irra-diation over Si-MCM-41 supported $FeCl_3$ catalyst under solvent free condition.
5. An improved procedure for the synthesis of dihydropyrimidones catalysed by lithium bromide was done by Gourhari Maiti et al.[15]



6. M. Adharvana Chari et al.[16] have claimed Silicagel supported sodium hydrogensulfate as a heterogenous catalyst for high yield synthesis of 3,4-dihydropyrimidin-2(1H)-ones.
7. Xiaoyan Han et al.[17] have synthesized DHPMs using Samarium Di-iodide as catalyst under solvent free condition.
8. Highly enantioselective synthesis of DHPMs using a new chiral Ytterbium catalyst was successfully done by Yijun Hyang et al.[18]
9. Ethyl -1-methyl-2-(methylthio)-6-oxo-1,6-dihydro-pyrimidine-5-carboxy late has been investigated by M.A.Hassan et al.[19] by the reaction of 5-carboethoxy-2-thiouracil, methyl iodide with the use of tetra

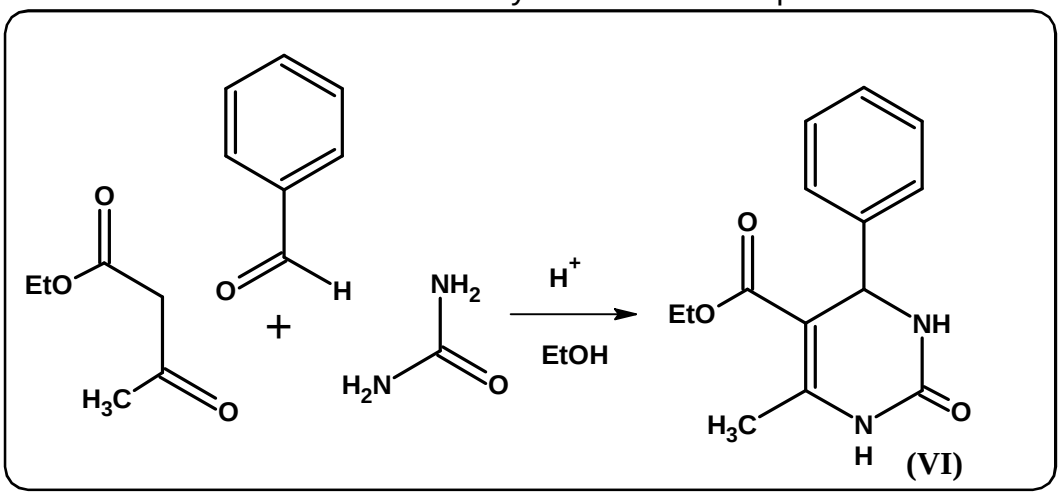
butylammonium bromide (PTC) as catalyst with continuous efficient stirring for 4-6 hrs at 70^0C.



10. Ezzat Rafiee et al.[20] described a practical and green approach towards synthesis of dihydropyrimidinones using heteropoly acids as an efficient catalyst.

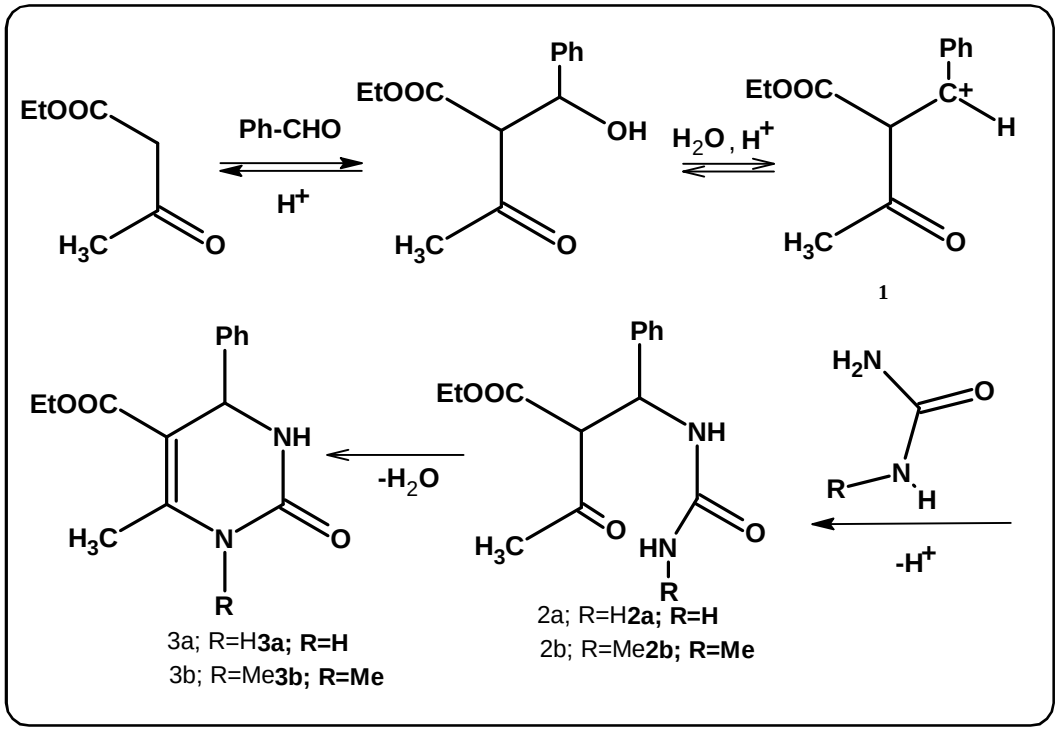
MECHANISM

Despite the importance and current interest in dihydropyrimidines of (VI), the mechanism of the classical three-component Biginelli condensation has not been elucidated with certainty and remains disputed.



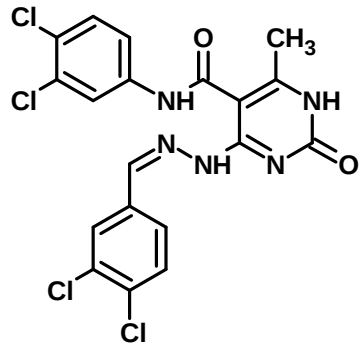
The "carbanium ion mechanism" was proposed by Sweet and Fissekis,[21] who investigated the reaction in 1973 and suggested that an acid-catalysed aldol condensation is the first and limiting step of the Biginelli condensation. It was proposed that under acid catalysis benzaldehyde and ethyl acetoacetate would react in an aldol-type fashion to produce the corresponding aldol, which dehydrates in the presence of acid to the resonance-stabilized carbenium ion.[22]**(1)**

Interception of cation **(1)** by urea or *N*-methylurea then produces ureides **(2),** which ultimately cyclises to the Biginelli products. **(3)**



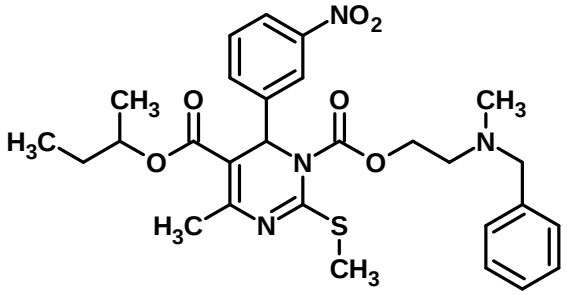
New drug molecules under clinical study

Recently many new molecules which are under study from phase-I to Phase-IV clinical trials for different pharmacological action have shown that the basic characteristic of morpholine to behave as hidden amine has attracted many medicinal chemists to incorporate this feature in drug design. Some interesting compounds are as under.



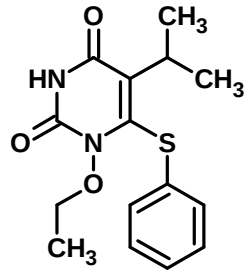
Immunosuppressants Oncolytic Drug

(VII)



Calcium Channel Blockers[323]

(VIII)



TNK-6123 **(IX)**

TNK-6123

Anti HIV Agent

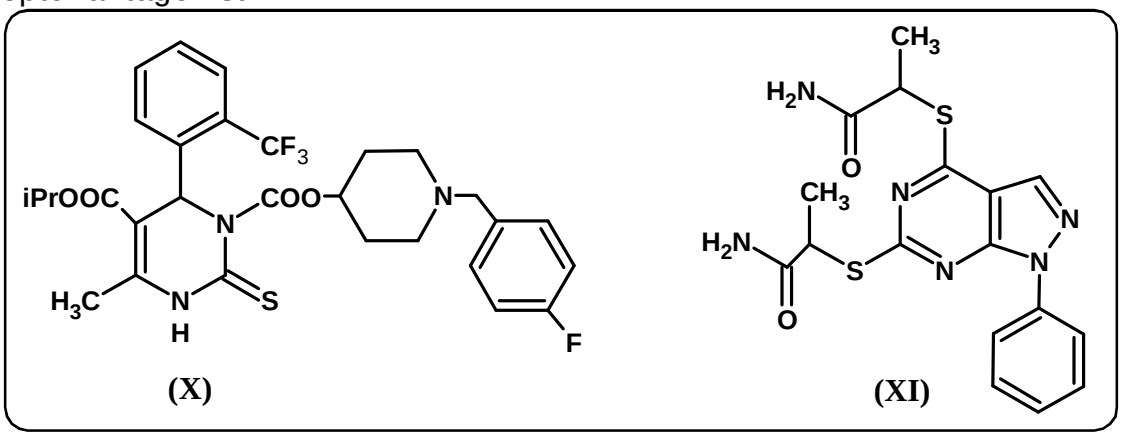
Reverse Transcripase Inhibitor.

Non-nucleoside HIV-1 reverse transcriptase inhibitor

Compound was active not only against wild-type HIV-1 strains (IC50 = 3 nM against IIIB and NL4-3 HIV-1 strains).

THERAPEUTIC IMPORTANCE

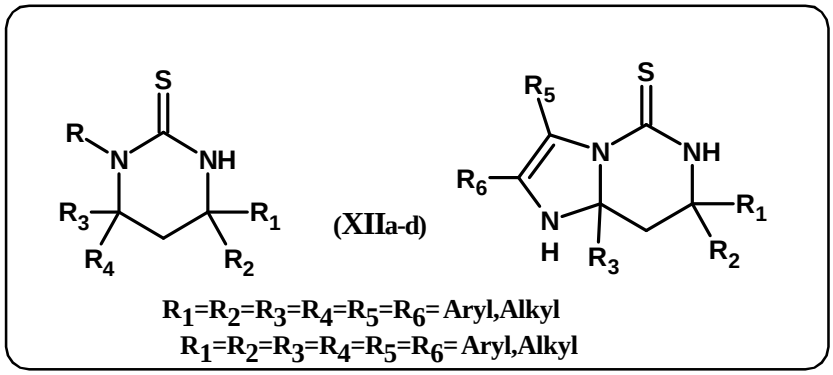
Atwal K. S. et al.[24] have described the potent antihypertensive activity of the modestly active (ICw = 3.2 pM) dihydropyrimidine calcium channel blocker. Victor E. M.et al.[25] synthesized 5'-triphosphates and evaluated directly as reverse transcriptase (RT) inhibitors using both a recombinant enzyme and enzyme obtained and purified directly from wild-type viruses. George C. et al.[26] prepared dihydropyrimidine (X) which was equipotent to nifedipine and amlodipine *in vitro*. In the spontaneously hypertensive rat, dihydro pyrimidine is both more potent and longer acting than nifedipine and amlodipine. Sally Ann P. et al.[27] synthesized 4,6-Bis[(R-carbamoylethyl)thio]-1-phenylpyrazolo[3,4-d]pyrimidine (XI) which was identified as a novel adenosine A_1 receptor antagonist.



(X) **(XI)**

Edith Gobnitzer et al.[28] have synthesized a series of 6-aryl-4-isopropyl-2-[2-(1-phenylalkylidene)hydrazino]-1,4-dihydropyrimidine hydrochlorides and

tested for their antibacterial activity against *Gram* (+) and *Gram* (-) bacteria and also against pathogenic yeast *Candida albicans.* Jayesh Modha et al.[29] have prepared dihydropyrimidine derivatives and all the compounds have been evaluated *in vitro* for their antimicrobial activity against several microbes and antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$. Xiaoxiong Wei et al.[30] have documented dihydropyrimidine dehydrogenase (DPD) catabolizes endogenous pyrimidines and pyrimidine based antimetabolite drugs. Sham M. Sondhi et al.[31] have developed a number of DHPMs (XIIa-d) and carried out their anti-inflammatory and analgesic activity. A series of [4,6-(substituted aryl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl]-acetic acid have been synthesized by Sushil Kumar and co-workers[32] and their *in vivo* antiinflammatory activity were evaluated and compared with standard drug Diclofenac sodium. Some compounds have shown moderate activity.

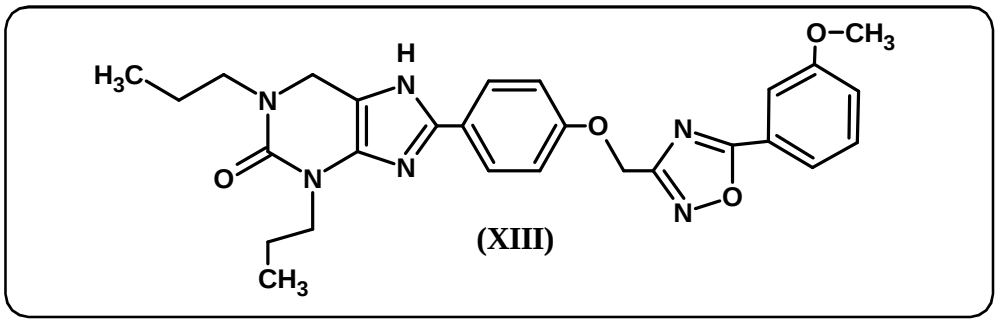


$R_1=R_2=R_3=R_4=R_5=R_6$= Aryl,Alkyl

$R_1=R_2=R_3=R_4=R_5=R_6$= Aryl,Alkyl

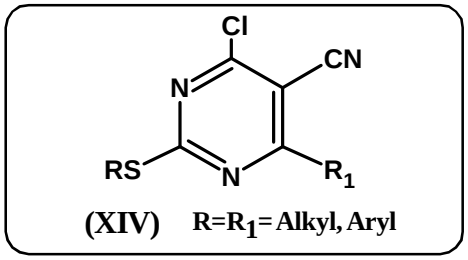
Novel compounds related to 2-(cyclohexylthio)-3,4-dihydro-5-methyl-6-(3-methylbenzyl)-4- oxopyrimidine (MC 639) have been synthesized and tested as inhibitors of human immunodeficiency virus type-1 (HIV-1) by Antonello M. et al.[33] C.Kappe[34] has synthesized novel dihydropyrimidine derivatives and reported them as calcium channel modulators and cardiovascular agents. Adenosine has been suggested to play an important role in asthama, possibly via activation of A_{2B} Adonosine receptors on Mast cells and other Pulmonary cells. Attia A.M.et al.[35] synthesized N3-b-D-glucopyranosyl, galactopyranosyl and xylopyranosyl 6-methyl-2-methylthiouracil and their 5-bromo derivatives by coupling an acetobromosugar with the corresponding thiouracil. The new modified thiouridine analogues were evaluated for their

inhibitory activity against Human Immunodeficiency Virus (HIV) replication in MT-4 cells as well as for their cytotoxicity. Jeff Zablocki and co-workers[36] have synthesized dihydropyrimidine derivatives (XIII), displayed high affinity and good selectivity against asthama.



(XIII)

Barbara Schnell et al.[37] have proposed enantiomerically pure dihydro pyrimidine derivatives and evaluated them as antihypertensive agents. Brian Johns and co-workers[38] have discovered a novel class of pyrimidines and reported them as antiherpeptic agents. Nidhi Agarwal et al.[39] have investigated pyrimidines (XIV), as antimycotic agents. All the synthesized compounds were evaluated for their *in vitro* antibacterial activity, against six pathogenic bacteria including virulent and non-virulent strain of *Mycobacterium tuberculosis.* Some of the synthesized compounds have displayed duly potent *in vitro* antimycobacterial activity with MIC of 0.75 mg/ml.

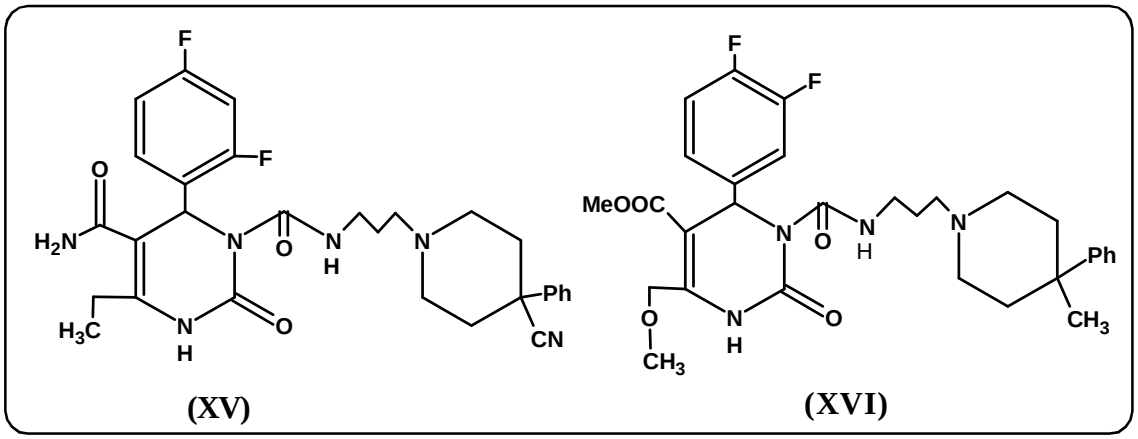


(XIV) **R=R_1= Alkyl, Aryl**

T. G. Muralidhar et al.[40] have synthesized several DHPM-one analogues among those (XV) and (XVI) give excellent selectivity (>880-fold) over a_{1b} and a_{1d} and also showed good selectivity over several other recombinant human G-protein coupled receptors.

5-Alkyl-2-thiopyrimidine nucleosides were newly synthesized by Shigeta S. et al.[41] and examined for antiviral activities against herpes simplex virus (HSV),

varicella-zoster virus (VZV) and human cytomegalovirus (HCMV).



Recently,Mai A. and co-workers[42] have investigated the dihydro pyrimidines which are highly active against HIV-1. Sanjay Batra et al.[43] have synthesised 5-arylmethyl-4-imino-3-aryl-3,4-dihydro-1H pyrimidin-2-ones which were tested for their antibacterial activity. Herve Ganeste and co-workers[44] synthesized substituted 1H-pyrimidin-2-one with selective dopamine D_3-receptor antagonists activity.

With an intention of preparing the compounds possessing better therapeutic potential, we have undertaken the synthesis of tetrahydropyrimidine derivatives to obtain better therapeutic agents which have been described in following sections.

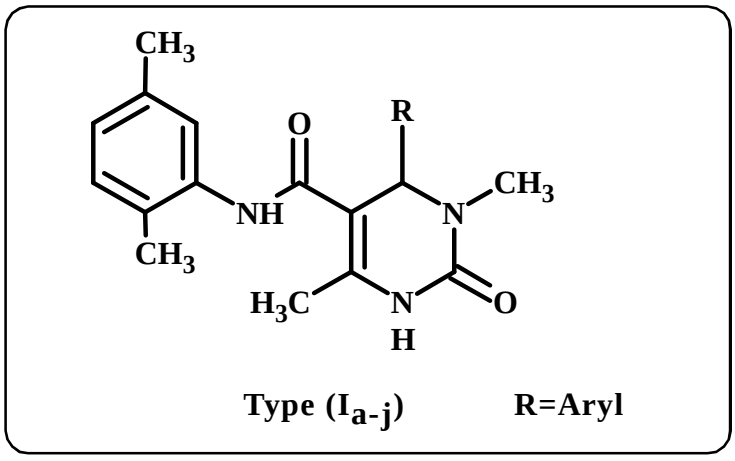
SECTION-I: SYNTHESIS AND ANTIMICROBIAL ACTIVITY STUDIES OF 6-ARYL-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES.

SECTION-II: SYNTHESIS AND ANTIMICROBIAL ACTIVITY STUDIES OF 6-ARYL-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES.

SECTION-I

SYNTHESIS AND ANTIMICROBIAL ACTIVITY STUDIES OF 6-ARYL-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDRO PYRIMIDINE-5-CARBOXAMIDES

Pyrimidine derivatives possess various pharmacological and biological properties.Here, we report the synthesis of some new pyrimidine derivatives of type (I).The strategy employed for the synthesis of 6-Aryl-1,4-dimethyl-N-(2,5-dimethylphenyl)-2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamides involved the condensation of N-(2,5-dimethylphenyl)-3-oxo- butanamide, N-methylurea and aryl aldehydes in acidic condition.

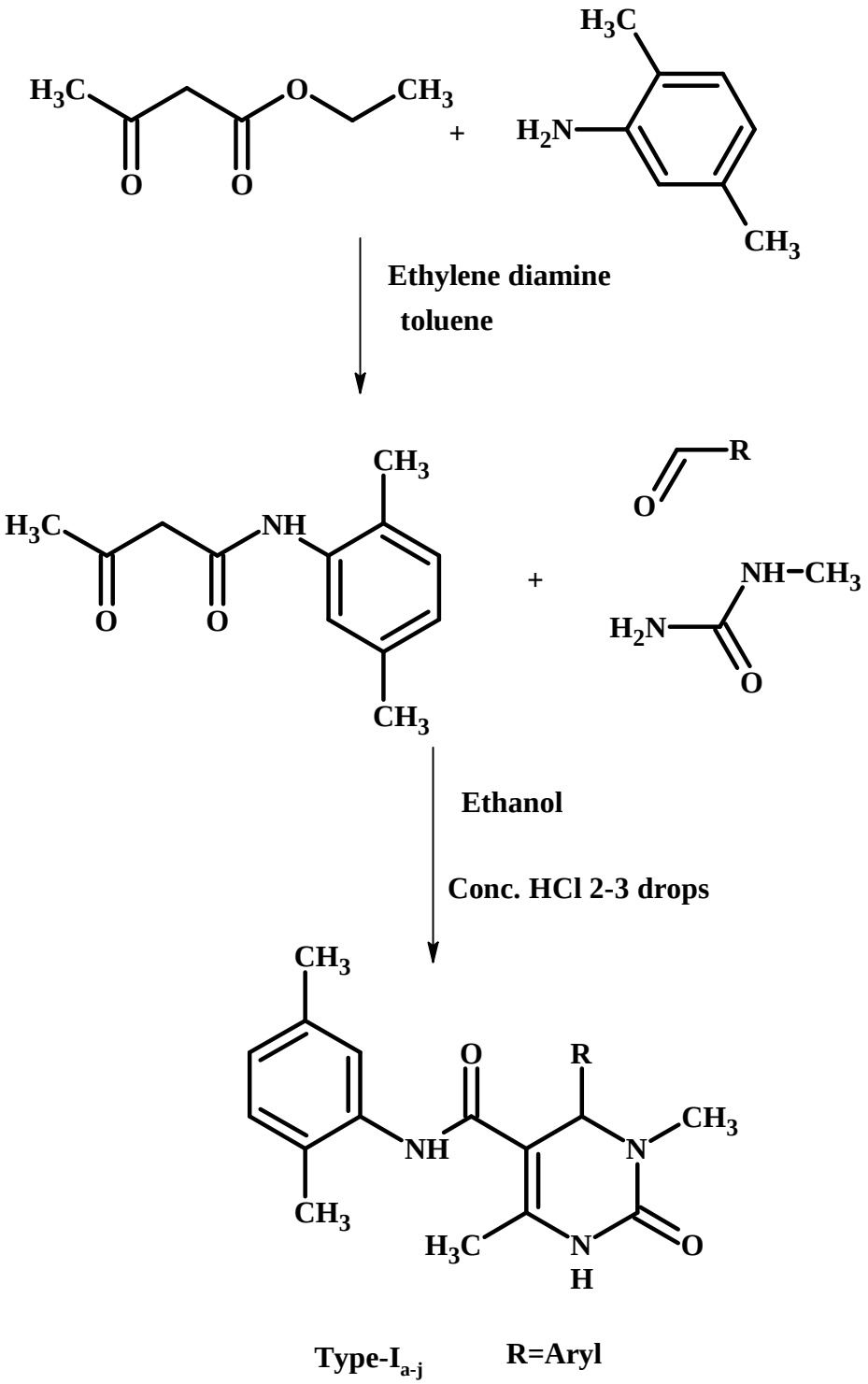


Type (I_{a-j}) **R=Aryl**

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

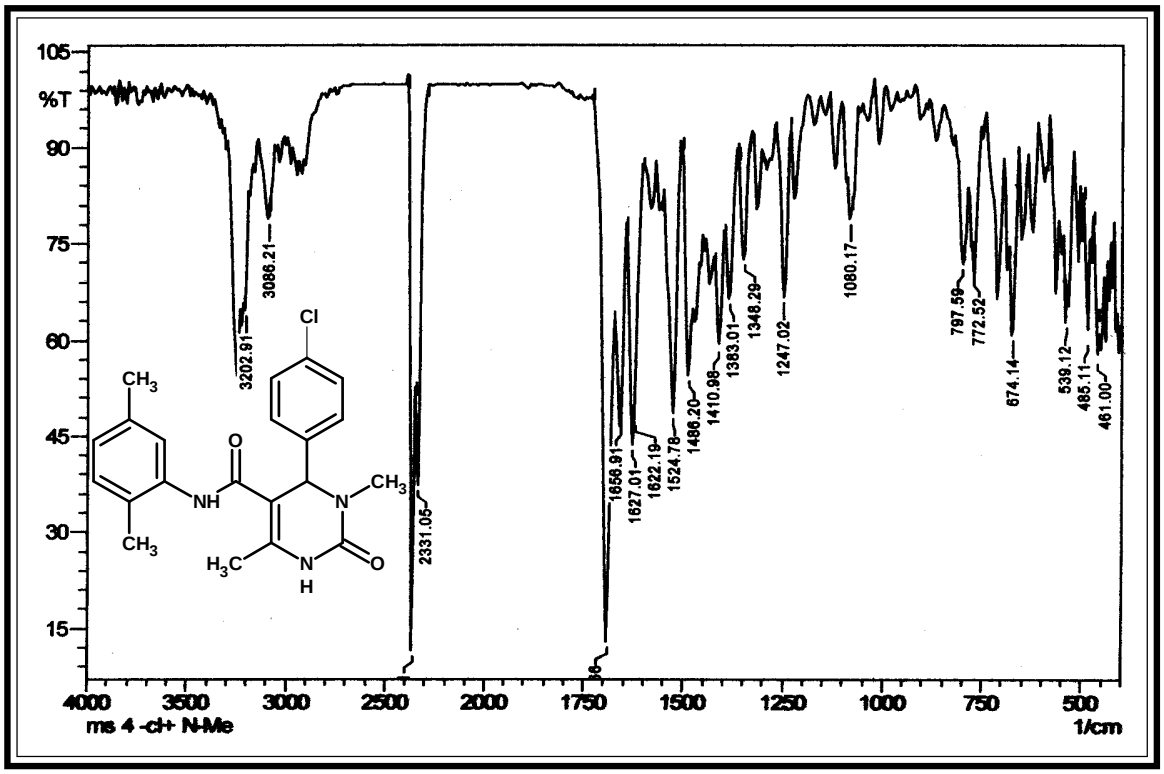
All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like ***Staphylococcus aureus*** ,***Bacillus subtilis*** and Gram Negative bacteria like ***Escherichia coli***,***Salmonella paratyphi B*** and they were also evaluated for antifungal activity against ***Candida albicans*** *and* ***Aspargillus niger***. The antimicrobial activity of the synthesised compounds have been compared with standard drugs. Their antimicrobial effect was determined in higher dilutions using Agar Dilution Method (**Approved by NCCLs**).

REACTION SCHEME



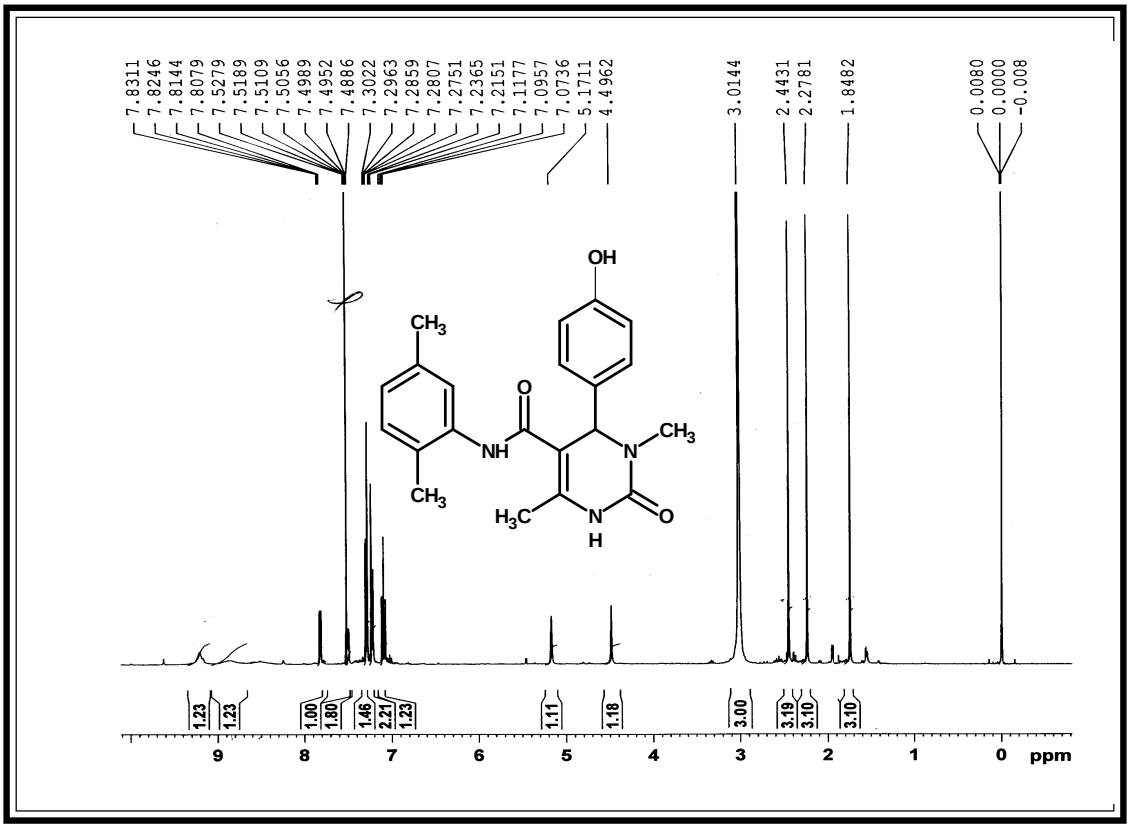
Type-I_{a-j} **R=Aryl**

IR SPECTRAL STUDIES OF 6-(4-CHLOROPHENYL)-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE.

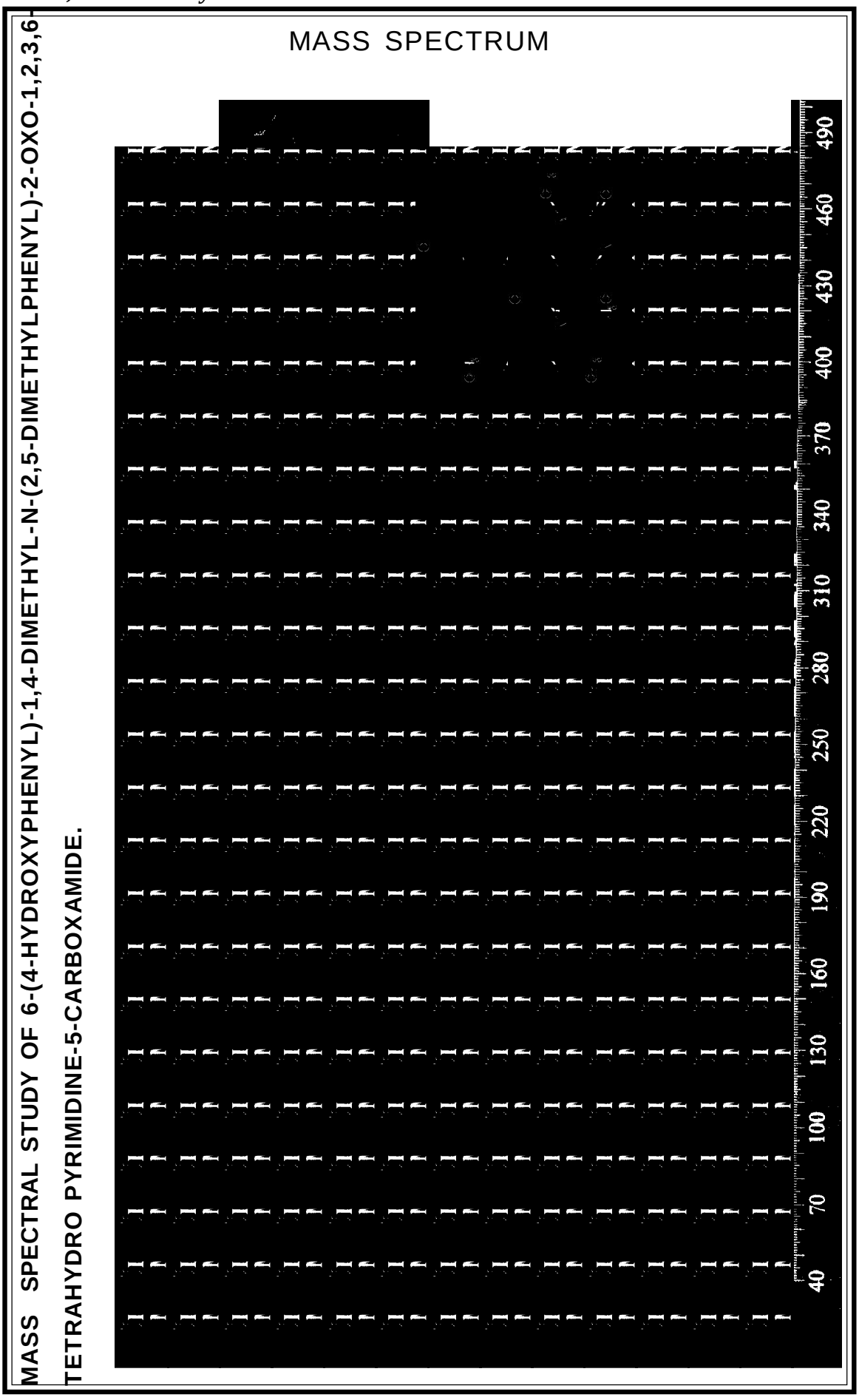


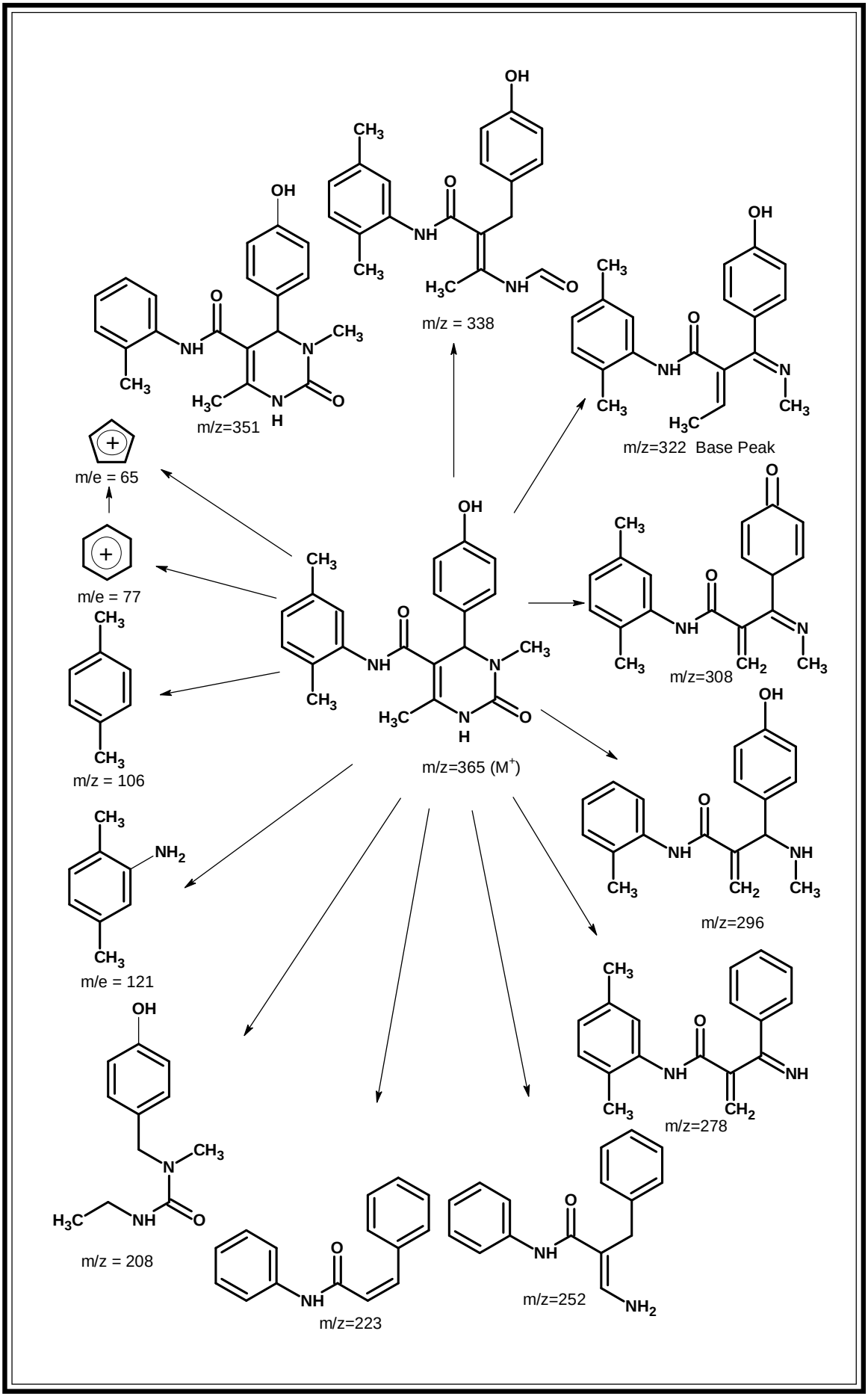
Type	Vibration Mode	Frequency in cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2958	2975-2850	45
-CH3	C-H str. (sym.)	2874	2900-2800	45
	C-H i.p.def. (asym.)	1456	1470-1400	45
	C-H o.o.p. def. (sym.)	1388	1390-1300	45
Aromatic	C-Hstr.	3086	3090-3010	46
and	C=C str.	1513	1600-1450	46
Pyrimidine	C=C str.	1578	1580-1520	46
moiety	C=N str.	1625	1635-1595	46
	C-H str.	3047	3080-2950	46
	C-H i.p. def.	1101	1125-1090	46
	NH str.	3300	3410-3380	46
	NH def.	1658	1635-1595	46
	C=O (pyrimidine)	1725	1700-1600	49
Amide	C=O str. (amide)	1689	1690-1600	49
	NH	3202	3400-3200	46

NMR SPECTRAL STUDIES OF 6-(4-HYDROXYPHENYL)-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDE.



Signal No.	Signal Pos. (δppm)	Relative No. of protons	Multiplicity	Inference
1.	1.84	3H	Singlet	C-CH3
2.	2.27	3H	Singlet	C-CH3
3.	2.44	3H	Singlet	C-CH3
4.	3.01	3H	Singlet	N-CH3
5.	4.49	1H	Singlet	Pyrimidine-H
6.	5.17	1H	Singlet	OH
7.	7.07-7.83	7H	Multiplet	Ar-H
8.	9.81	1H	Singlet	Amide-NH
9.	9.92	1H	Singlet	Pyrimidine-NH


MASS SPECTRUM
MASS SPECTRAL STUDY OF 6-(4-HYDROXYPHENYL)-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDRO PYRIMIDINE-5-CARBOXAMIDE.
40
70
100
130
160
190
220
250
280
310
340
370
400
430
460
490


m/z = 338
m/z=351
m/z=322 Base Peak
m/e = 65
m/e = 77
m/z=308
m/z = 106
m/z=365 (M+)
m/z=296
m/e = 121
m/z=278
m/z = 208
m/z=223
m/z=252

EXPERIMENTAL

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 6-ARYL-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES.

Synthesis of 6-(4-hydroxyphenyl)-1,4-dimethyl-N-(2,5-dimethylphenyl)-2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamide (I_c).

[A] Synthesis of N-(2,5-dimethylphenyl)-3-oxobutanamide:

Ethylacetoacetate (0.01M, 1.44ml) and 2,5-dimethyl aniline (0.01M, 1.45g) in 25ml toluene was heated for 12 hrs using ethylenediamine (1 ml) as catalyst. Methanol was removed using Dean & Stark azeotropic assembly. The mixture was cooled to room temperature and then washed with sodium bisulphite solution. The layers were settled down, organic layer was seperated. It was further washed with water and layers were seperated. Excess of solvent was distilled out and the product was isolated and crystallise from dioxane, m.p.154^{0}C, yield 62%. The purity of the product was checked by TLC.

[B] 6-(4-hydroxyphenyl)-1,4-dimethyl-N-(2,5-dimethylphenyl)-2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamide(I_c).

A mixture of N-(2,5-dimethylphenyl)-3-oxobutanamide (2.05gm, 0.01M), N-methyl urea (1.10gm, 0.015M) and 4-hydroxybenzaldehyde (1.22gm, 0.01) in 15ml of ethanol containing 2-3 drops of concentrated hydrochloric acid was refluxed for 7-8 hrs. The solution was allowed to stand for 2 hrs at room temperature and the resulting mass was poured into cold water solid product was so obtained was filtered and crystralized from dioxane, m.p. 152^{0}C, yield 67%. $C_{21}H_{23}N_3O_3$ required; C, 69.04%; H, 6.30%; N, 11.50%; found; C, 68.94%; H, 6.19%; N, 11.27%.

Similarly other 6-Aryl-1,4-dimethyl- N-(2,5-dimethylphenyl)-2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamide derivatives(I_{a-j}) were synthesised. The physical data are recorded in Table No. 1.

[C] Antimicrobial activity of 6-Aryl-1,4-dimethyl-N-(2,5-dimethylphenyl)-2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamide.

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

The Activies of all the synthesiezed compounds are recorded in table no. I(A)and I(B)

Protocol for studying Antimicrobial activity

Agar Dilution procedure:

The Agar Dilution Method for determining antimicrobial susceptibility is a well-established technique.[53,54] The antimicrobial agent is incorporated into the agar medium in each plate containing a different concentration of the agent.

Preparation of Synthetic compunds:

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.1000 μg/ml,500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.2 μg/ml, 15.6 μg/ml, 7.8 μg/ml, 3.9 μg/ml, 1.9 μg/ml, 0.45 μg/ml, 0.22 μg/ml.

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 paratybhi B*
4. *Candida albicans*
5. *Bacillus subtilis*
6. *Aspargillus niger*

Muller Hinton Agar medium

Muller Hinton Agar medium was used to carry out antimicrobial analysis of newly synthesized compounds. The compositon 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 25^0C)	7.3+0.2

Preparing Agar Dilution plates:

(1) Appropriate dilution i.e. 1 ml quantity of antimicrobial solution are added to molten test agars having 19 ml quantity that have been allowed to equilibrate in a water bath to 45 to 50^0C. One part of antimicrobial solution is added to nine parts of liquid agar.

(2) The agar and antimicrobial solution were mixed throughly and the mixture is 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 8^0C for up to five days for reference work or longer for routine tests.

(5) Plates stored at 2-8^0C 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 4^0C if in powder form . All synthetic organic compounds were analysed for their antimicrobial activity by Dr. Chetnaben Rajyguru from MVM Science and Homescience 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 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 32^0C 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 10^8 colony forming units(CFU) per mililiter by comparing its turbidity to a MacFarland 0.5 $BaSO_4$ standard.

The bacterial cultures were then transfered at 2-8^0C and maintained at the same temperature till further use.Appropriate dilution of the bactrial 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 standard:

It was used as a reference for turbidity measurement for bacterial cultures before they were used as inoculum 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 costant stirring, the absorbance of the solution was measured 625 nm against demenerialized 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 37^0C 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 synthesised organic compounds.

Microbial culture	Ciprofloxacin
1. *Escherichia coli ATCC 25922*	0.4 μg/ml
2. *Staphylococcus aureus ATCC 25923*	1.9 μg/ml
3. *Bacillus Subtilis ATCC 6633*	7.8 μg/ml

Fungal culture	Fluconazole
Candida albicans	0.4 μg/ml

Antifungal Activity Determination:

For fungal culture the fungal media Yeast Nitrogen Base Agar plate(YNBG) (Difco make) 6.7gm and Glucose 10 gms dissolved in 100 ml distilled water and filter sterilized was used.The inoculum was 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 37^0C 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 poing 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, *Staphyloccocus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231.The reference strains were stored at temp. below -20^0C.

Determination of type of Antimicrobial Activity:

Organic compounds may be bacteriostatic or bacteriocidal 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 incbator at 37^0C 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 bacericidal. Bactericidal dilution of the organic compounds were 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-I :PHYSICAL CONSTANTS OF 6-ARYL-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)--2-OXO-1,2,3,6-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES

Sr. No.	R	Molecular Formula	Molecular Weight	M. P. ^{o}C	Yield %	% of Nitrogen Calcd.	% of Nitrogen Found
1	2	3	4	5	6	7	8
Ia	4-NO_2-C_6H_4	$C_{21}H_{22}N_4O_4$	394.0	226	57	14.21	13.98
Ib	4-OCH_3-C_6H_4	$C_{22}H_{25}N_3O3$	379.0	186	58	11.08	10.87
Ic	4-OH-C_6H_4	$C_{21}H_{23}N_3O_3$	365.0	152	67	11.50	11.27
Id	4-F-C_6H_4	$C_{21}H_{22}FN_3O_2$	367.0	157	63	11.44	11.23
Ie	4-Cl-C_6H_4	$C_{21}H_{22}ClN_3O_2$	385.5	214	61	10.95	10.66
If	3-NO_2-C_6H_4	$C_{21}H_{22}N_4O_4$	394.0	195	49	14.21	13.98
Ig	3-Cl-C_6H_4	$C_{21}H_{22}ClN_3O_2$	385.5	199	41	10.95	10.66
Ih	2-NO_2-C_6H_4	$C_{21}H_{22}N_4O_4$	394.0	162	58	14.21	13.98
Ii	2-Cl-C_6H_4	$C_{21}H_{22}ClN_3O_2$	385.5	174	55	10.95	10.66
Ij	2-OH-C_6H_4	$C_{21}H_{23}N_3O_3$	365.0	155	48	11.50	11.27

ANTIMICROBIAL ACTIVITY OF 6-ARYL-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)--2-OXO-1,2,3,6-TETRAHYDRO PYRIMIDINE-5-CARBOXAMIDES.

TABLE NO-I(A) ANTIBACTERIALACTIVITY

Compd No.	R	Gram Positive								Gram Negative							
		S. aureus µg/ml				*B.Subtilis* µg/ml				*E.Coli* µg/ml				*S.Paratyphi B* µg/ml			
		2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
I_a	4-NO_2-C_6H_4	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	-
I_b	4-OCH_3-C_6H_4	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+
I_c	4-OH-C_6H_4	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-
I_d	4-F-C_6H_4	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-
I_e	4-Cl-C_6H_4	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-
I_f	3-NO_2-C_6H_4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
I_g	3-Cl-C_6H_4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
I_h	2-NO_2-C_6H_4	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
I_i	2-Cl-C_6H_4	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-
I_j	2-OH-C_6H_4	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-

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

ANTIMICROBIAL ACTIVITY OF 6-ARYL-1,4-DIMETHYL-N-(2,5-DIMETHYLPHENYL)--2-OXO-1,2,3,6-TETRAHYDRO PYRIMIDINE-5-CARBOXAMIDES. **TABLE NO-I(B) ANTIFUNGALACTIVITY**

Compd No.	R	*A.niger* μg/ml				*C.albicans* μg/ml			
		2000	1000	500	250	2000	1000	500	250
I_a	4-NO_2-C_6H_4	+	+	+	-	+	+	+	-
I_b	4-OCH_3-C_6H_4	+	+	+	-	+	+	+	-
I_c	4-OH-C_6H_4	+	+	+	-	+	+	+	-
I_d	4-F-C_6H_4	+	+	+	-	+	+	+	+
I_e	4-Cl-C_6H_4	+	+	+	-	+	+	+	-
I_f	3-NO_2-C_6H_4	+	+	-	-	+	+	-	-
I_g	3-Cl-C_6H_4	+	+	-	-	+	+	-	-
I_h	2-NO_2-C_6H_4	+	+	+	-	+	+	+	-
I_i	2-Cl-C_6H_4	+	+	+	-	+	+	+	-
I_j	2-OH-C_6H_4	+	+	-	-	+	+	-	-

Reference drugs:	*A.niger*	*C.albicans*
Fluconazol	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 strat 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 analysed in tertiary screening.

From the results of experiments using newly synthesised organic comopounds 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 I_{a-j} almost seven compounds I_b, I_c, I_d, I_e, I_h ,I_i and I_j were found active at 500 μg/ml conc. against *staphylococcus aureus* (in which R= 4-OCH_3-C_6H_4, 4-OH-C_6H_4, 4-F-C_6H_4, 4-Cl-C_6H_4, 2-NO_2-C_6H_4, 2-Cl-C_6H_4 and 2-OH-C_6H_4).*Bacillus Subtilis* was inhibited at 500 μg/ml conc.by six compounds I_a, I_b, I_d, I_h ,I_i and I_j (in which R=4-NO_2-C_6H_4,4-OCH_3-C_6H_4, , 4-F-C_6H_4, 2-NO_2-C_6H_4, 2-Cl-C_6H_4 and 2-OH-C_6H_4).Five compounds I_b, I_d, I_h ,I_i and I_j were active against both cultures *B.Subtilis* and *S.aureus* (in which R= 4-OCH_3-C_6H_4, 4-F-C_6H_4, 4-Cl-C_6H_4, 2-NO_2-C_6H_4, 2-Cl-C_6H_4 and 2-OH-C_6H_4).

At the conc.250 μg/ml *S. aureus* was inhibited by two compounds I_d and I_i (in which R= 4-F-C_6H_4 and 2-Cl-C_6H_4).*B.Subtilis* was not killed by any compounds of the first series.So, it is obvious from the data obtained that compounds I_d and I_i were highly active among all the compounds of series $I_{a-j.}$

For Gram Negative bacteria in the series I_{a-j} almost five compounds I_a, I_b, I_d, I_h and I_i were found active at 500 μg/ml conc. against *Escherichia Coli* (in which R=4-NO_2-C_6H_4, 4-OCH_3-C_6H_4, 4-F-C_6H_4, 2-NO_2-C_6H_4 and 2-Cl-C_6H_4).*S.Paratyphi B*. was inhibited at 500 μg/ml conc. by four compounds i.e. I_a, I_b, I_d and I_i (in which R=4-NO_2-C_6H_4, 4-OCH_3-C_6H_4, 4-F-C_6H_4 and 2-Cl-C_6H_4). So, four compounds were active against both cultures *E.Coli* and *S.Paratyphi*

B. i.e. I_a, I_b, I_d and I_i (in which R=4-NO_2-C_6H_4, 4-OCH_3-C_6H_4, 4-F-C_6H_4, 2-Cl-C_6H_4).

At the conc. 250 μg/ml *E.Coli* was killed by only one compound I_a (in which R= 4-NO_2-C_6H_4). *S.Paratyphi B*. was also inhibited by one compound I_b (in which R= 4-OCH_3-C_6H_4).So. it is obvious from the data obtained that compounds I_a and I_b were highly active among all the compounds of series $I_{a-j.}$

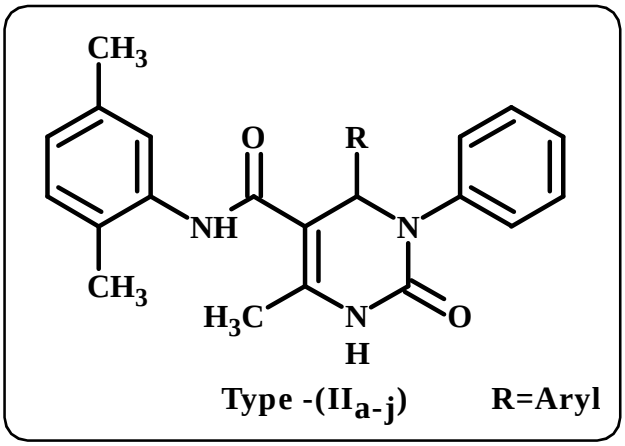
For fungi in the series I_{a-j} almost seven compounds I_a, I_b, I_c, I_d, I_e, I_h and I_j were found active at 500 μg/ml conc. against *A.niger* and *C. albicans* (in which R= 4-NO_2-C_6H_4, 4-OCH_3-C_6H_4, R= 4-OH-C_6H_4, 4-F-C_6H_4, R= 4-Cl-C_6H_4, 2-NO_2-C_6H_4 and 2-Cl-C_6H_4) .So,for *A. niger* and *C.albicans* above compounds have shown similar inhibition effect at 500 ug/ml conc.

At the conc. of 250 μg/ml *C.albicans* was killed by one compound I_d (in which R= 4-F-C_6H_4). *A. niger* was not affected by any compounds of first series. So, it is obvious from the data obtained that compound I_d (R= 4-F-C_6H_4) was highly active among all the compounds of series I_{a-j}.

SECTION-II

SYNTHESIS AND ANTIMICROBIAL ACTIVITY STUDIES OF 6-ARYL-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES.

Pyrimidine derivatives represent one of the most active class of compounds having wide spectrum of biological activities. These valid observations led us to synthesise pyrimidines of type-(II) by the condensation of N-(2,5-dimethylphenyl)-3-oxobutanamide, N-Phenyl urea and aryl aldehydes.

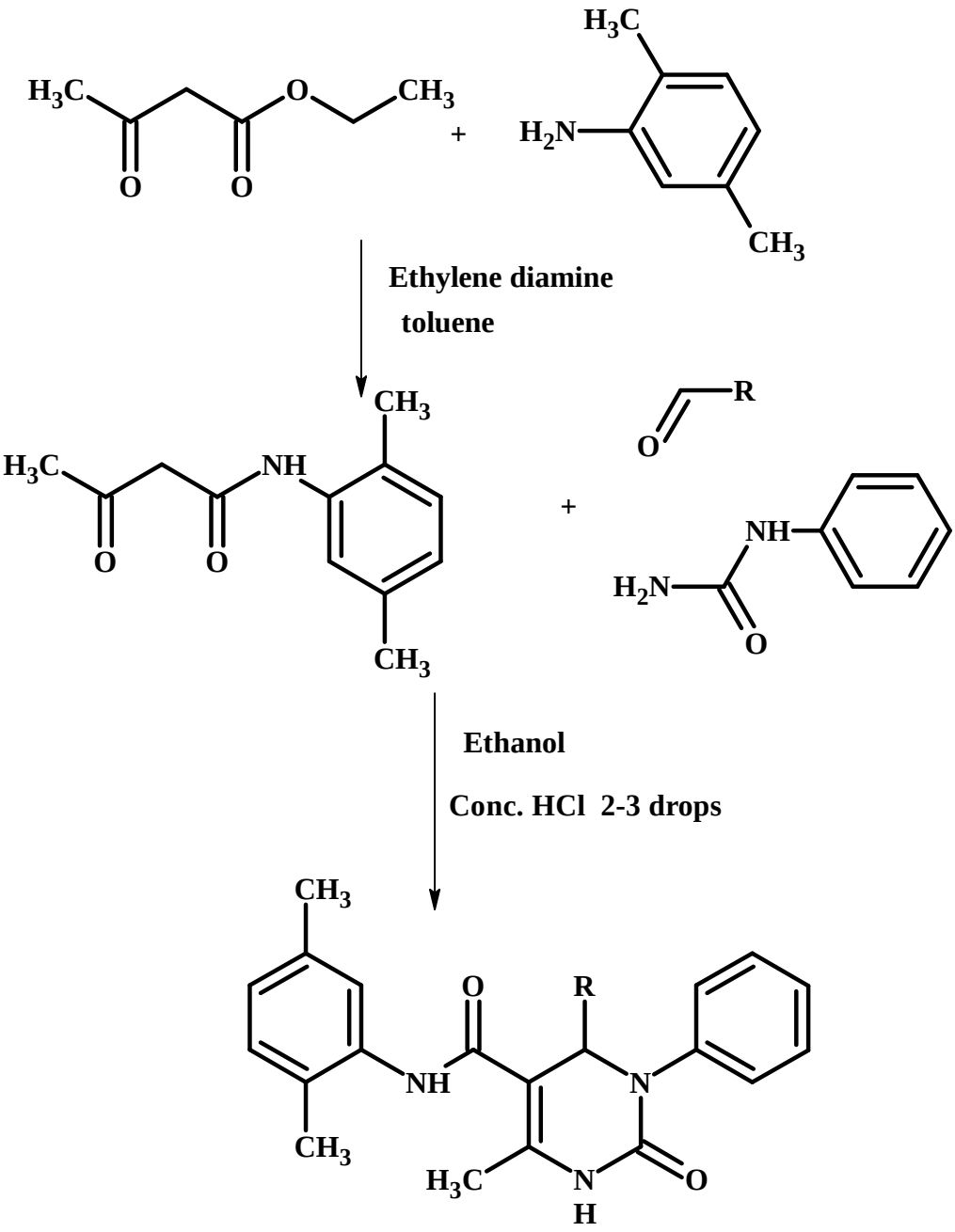


Type -(II_{a-j}) **R=Aryl**

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

All the compounds have been evaluated for their antibacterial activity against Gram Positive bacteria like ***Staphytococcus aureus*** ,***Bacillus subtilis*** and Gram Negative bacteria like ***Escherichia coli***,***Salmonella paratyphi B*** and they were also evaluated for antifungal activity against ***A.niger, Candida albicans***. The antimicrobial activity of the synthesised compounds have been compared with standard drugs. Their antimicrobial effect was determined in higher dilutions using Agar Dilution Method **(Approved by NCCLs)**.

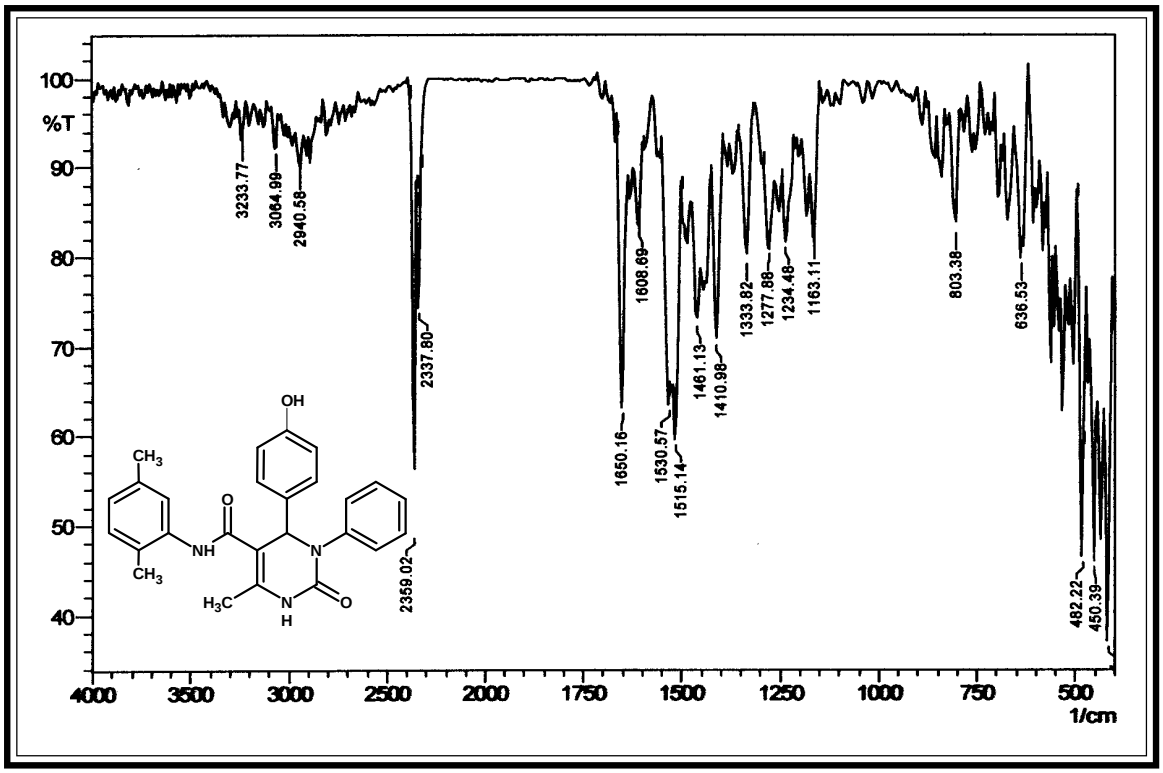
REACTION SCHEME



R=Aryl

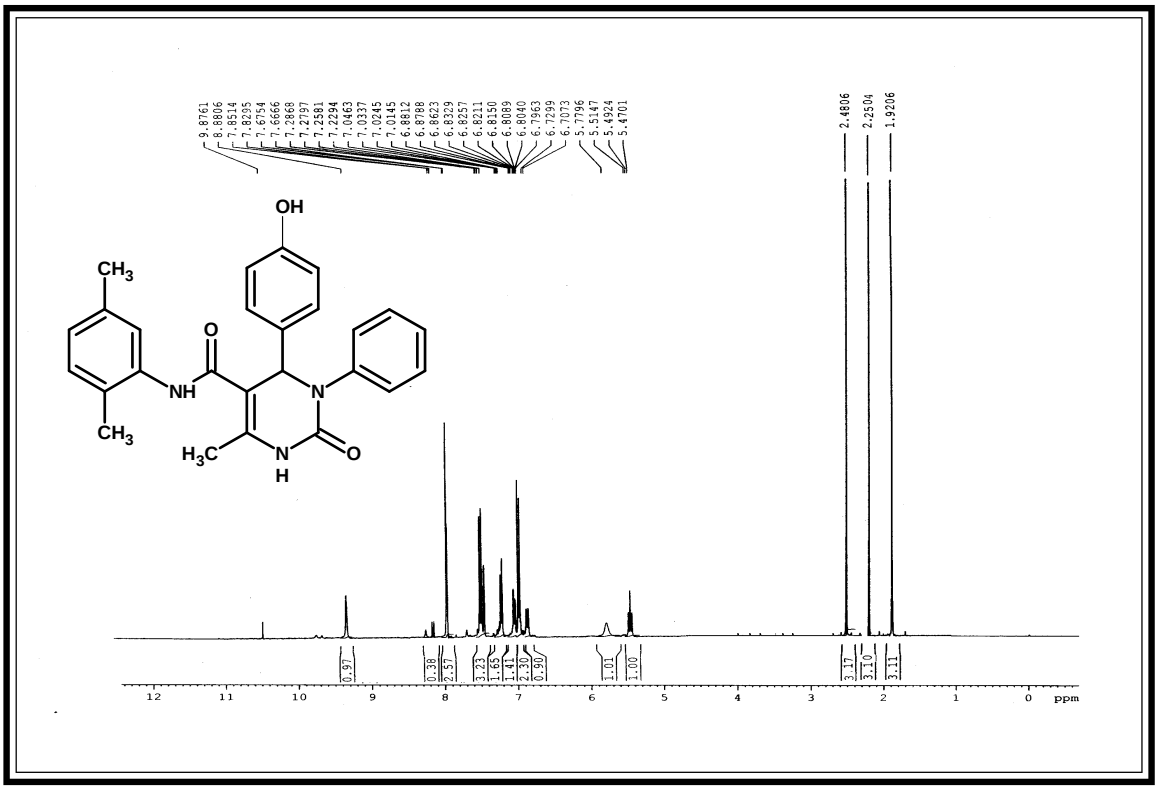
Type - (II)

IR SPECTRAL STUDIES OF 6-(4-HYDROXYPHENYL)-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDRO PYRIMIDINE-5-CARBOXAMIDE.



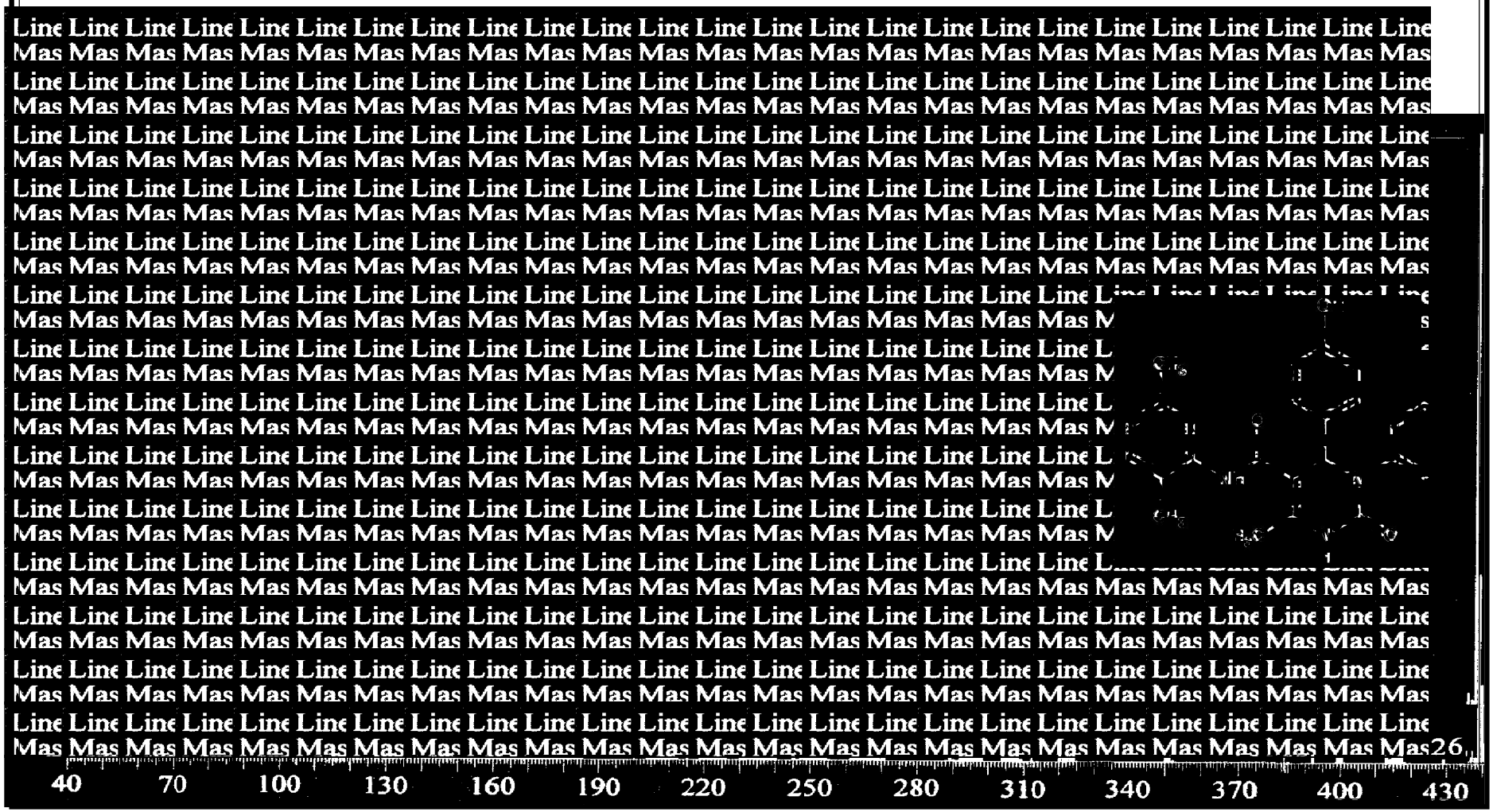
Type	Vibration Mode	Frequency in cm^{-1}		Ref.
		Observed	Reported	
Alkane	C-H str. (asym.)	2940	2975-2850	45
$-CH_3$	C-H str. (sym.)	2880	2900-2800	45
	C-H def. (asym.)	1461	1470-1400	45
	C-H def. (sym.)	1410	1420-1300	45
	C-H str.	1360	1385-1365	45
Aromatic	C-H str.	3064	3090-3030	45
and	C=C	1515	1540-1480	46
Pyrimidine	C=C str.	1530	1580-1520	46
moity	C-H str.	2960	3080-2950	46
	C-H i.p. def.	1100	1125-1090	46
	NH str.	3310	3410-3380	46
	NH def.	1608	1635-1595	46
	C=O str.	1690(sh)	1700-1660	46
Amide	NH str.	3233	3410-3200	46
	C=O str.	1650	1650-1600	49
Phenol	OH	3410	3200-3600	49

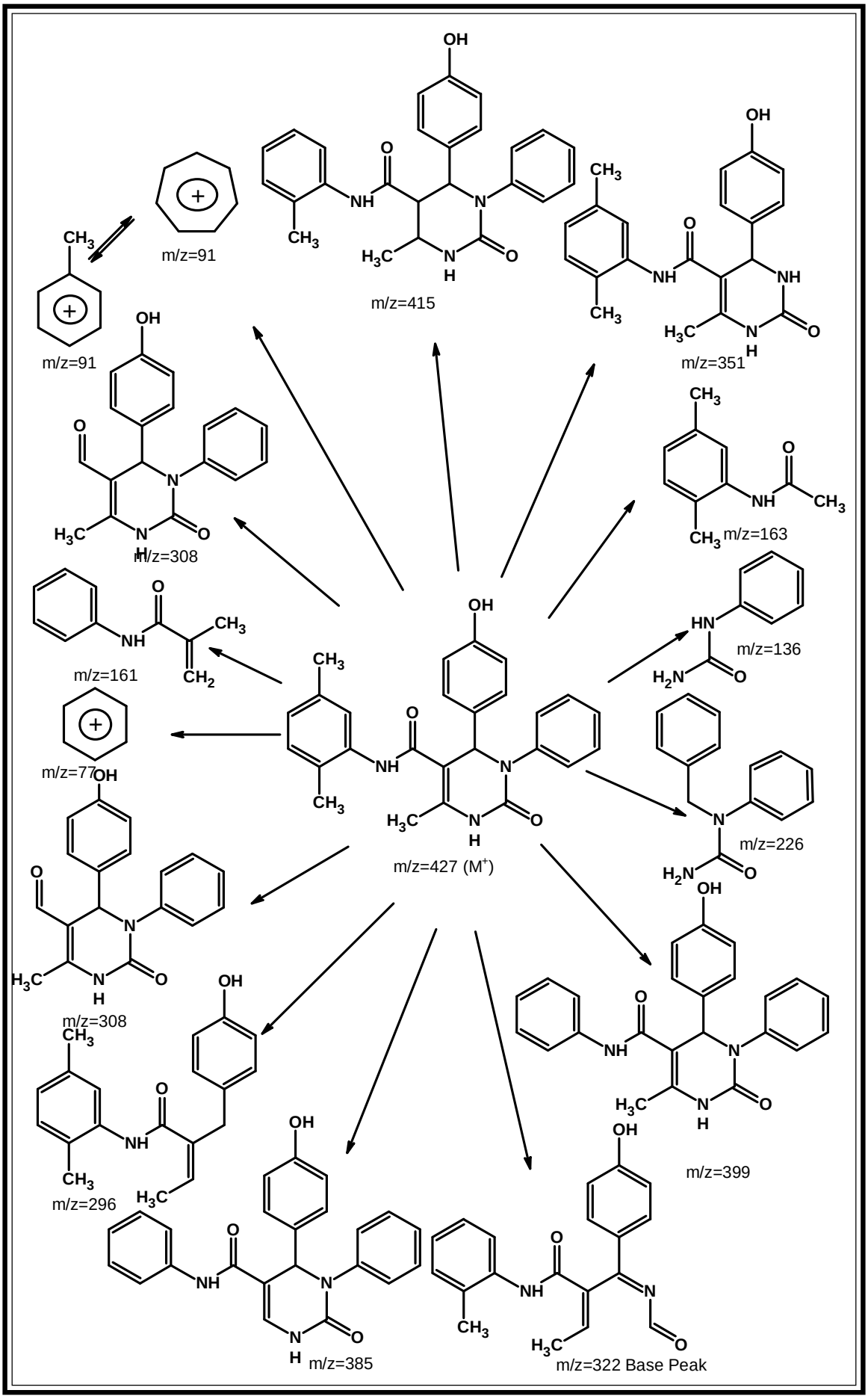
NMR SPECTRAL STUDIES OF 6-(4-HYDROXYPHENYL)-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDRO PYRIMIDINE-5-CARBOXAMIDE.



Signal No.	Signal Position (dppm)	Relative No. of protons	Multiplicity	Inference
1.	1.92	3H	Singlet	C-CH_3
2.	2.48	3H	Singlet	C-CH_3
3.	2.25	3H	Singlet	C-CH_3
4.	5.49	1H	Singlet	OH
5.	5.77	1H	Singlet	Pyrimidine-H
6.	6.70-7.85	13H	Multiplet	Ar-H
7.	9.87	1H	Singlet	Amide-H
8.	9.90	1H	Singlet	Pyrimidinr-NH

MASS SPECTRAL STUDY OF 6-(4-HYDROXYPHENYL)-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDRO PYRIMIDINE-5-CARBOXAMIDE.

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m/z=77
m/z=427 (M+)
m/z=226
m/z=308
m/z=399
m/z=296
m/z=385
m/z=322 Base Peak

EXPERIMENTAL

SYNTHESIS AND ANTIMICROBIAL ACTIVITY STUDIES OF 6-ARYL-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRAHYDROPYRIMIDINE-5-CARBOXAMIDES

Synthesis of 6-(4-hydroxyphenyl)-4-methyl-1-phenyl-N-(2,5-dimethyl-phenyl) -2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamide(I_C).

[A] Synthesis of N-(2,5-dimethylphenyl)-3-oxobutanamide:

The synthesis is mentioned in part-I section-I.

[B] Synthesis of N-Phenyl urea:

Add aniline(3.7 g,0.04M) dropwise in 20ml of conc.HCl,so brown colour precipitet of anilinehydrochloride was obtained.Cool in ice bath and filtered it.A mixture of anilinehydrochloride,urea(6.0 g,0.1M),20ml aciticacid and 100ml water in round bottam flask was heated(reflux) in waterbath.The reaction mixture was cooled and filtered to remove diphenylurea.Then the solution was diluted up to two litere with water.Then heated that dilute solution on heating bath up to one-third.Then cool it and filtered to remove diphenylurea.Then reaction mixture was again heated up to one-half and cooled it and filtered.Thus the product was obtained.It was crystallised from methanol.Yield 45%,m.p. 120^0c .

[C] 6-(4-hydroxyphenyl)-4-methyl-1-phenyl-N-(2,5-dimethylphenyl) -2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamide(I_C).

A mixture of N-(2,5-dimethylphenyl)-3-oxobutanamide(2.05g, 0.01), N-phethyl urea (1.26g, 0.01) and4-hydroxy benzaldehyde(1.22 g, 0.01) in 15ml of ethanol containing 2-3 drops of concentrated hydrochloric acid was refluxed for 7 to 8 hrs. The solution was allowed to stand for 2 hrs. at room temperature and the resulting solid mass poured into cold water, solid was so obtained was filtered and crystralized from dioxane.m.p. 175^0C, yield 64%, $C_{26}H_{25}N_3O_3$ required; C, 72.98%; H, 5.84%; N, 9.83%; found; C, 72.37%; H, 5.48%; N, 09.49%.The purity of the compound was checked by TLC.

Similarly other 6-aryl-4-methyl-1-phenyl-N-(2,5-dimethylphenyl) -2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamide derivatives were synthesised. The physical data are recorded in Table No.II.

[D] Antimicrobial activity of 6-(4-hydroxyphenyl)-4-methyl-1-phenyl-N-(2,5-dimethylphenyl)-2-oxo-1,2,3,6-tetrahydropyrimidine-5-carboxamide(I_C).

Antimicrobial testing was carried out as described in [A] part-1, section-I .

The antimicrobial activities of synthesized compounds are recorded in table no. II(A) and II(B)

TABLE-II :PHYSICAL CONSTANTS OF 6-ARYL-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRA HYDROPYRIMIDINE-5-CARBOXAMIDES

Sr. No.	R	Molecular Formula	Molecular Weight	M. P. ^{o}C	Yield %	% of Nitrogen Calcd.	% of Nitrogen Found
1	2	3	4	5	6	7	8
IIa	4-NO_2-C_6H_4	$C_{26}H_{24}N_4O_4$	456.5	117	55	12.28	12.05
IIb	4-OCH_3-C_6H_4	$C_{27}H_{27}N_3O3$	441.5	169	56	09.52	09.30
IIc	4-OH-C_6H_4	$C_{26}H_{25}N_3O_3$	427.5	175	64	09.83	09.49
IId	4-F-C_6H_4	$C_{26}H_{24}FN_3O_2$	429.0	159	60	09.79	09.53
IIe	4-Cl-C_6H_4	$C_{26}H_{24}ClN_3O_2$	445.0	168	59	09.43	09.16
IIf	3-NO_2-C_6H_4	$C_{26}H_{24}N_4O_4$	456.5	108	50	12.28	12.05
IIg	3-Cl-C_6H_4	$C_{26}H_{24}ClN_3O_2$	445.0	178	43	09.43	09.16
IIh	2-NO_2-C_6H_4	$C_{26}H_{24}N_4O_4$	456.5	099	59	12.28	12.05
IIi	2-Cl-C_6H_4	$C_{26}H_{24}ClN_3O_2$	445.0	186	56	09.43	09.16
IIj	2-OH-C_6H_4	$C_{26}H_{25}N_3O_3$	427.0	265	47	09.83	09.49

ANTIMICROBIAL ACTIVITY OF 6-ARYL-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRA HYDROPYRIMIDINE-5-CARBOXAMIDES. **TABLE NO-II(A) ANTIBACTERIALACTIVITY**

Compd No.	R	Gram Positive								Gram Negative							
		S. aureus μg/ml				*B.Subtilis* μg/ml				*E.Coli* μg/ml				*S.Paratyphi B* μg/ml			
		2000	1000	500	250	2000	1000	500	250	2000	1000	500	250	2000	1000	500	250
II_a	4-NO_2-C_6H_4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	-
II_b	4-OCH_3-C_6H_4	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
II_c	4-OH-C_6H_4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	-
II_d	4-F-C_6H_4	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
II_e	4-Cl-C_6H_4	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-
II_f	3-NO_2-C_6H_4	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	-
II_g	3-Cl-C_6H_4	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
II_h	2-NO_2-C_6H_4	+	+	+	-	+	+	+	-	+	+	-	-	+	+	-	-
II_i	2-Cl-C_6H_4	+	+	-	-	+	+	-	-	+	+	+	-	+	+	+	-
II_j	2-OH-C_6H_4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

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

ANTIMICROBIAL ACTIVITY OF 6-ARYL-4-METHYL-1-PHENYL-N-(2,5-DIMETHYLPHENYL)-2-OXO-1,2,3,6-TETRA HYDROPYRIMIDINE-5-CARBOXAMIDES **TABLE NO-II(B) ANTIFUNGALACTIVITY**

Compd No.	R	*A.niger* µg/ml				*C.albicans* µg/ml			
		2000	1000	500	250	2000	1000	500	250
II_a	4-NO_2-C_6H_4	+	+	-	-	+	+	-	-
II_b	4-OCH_3-C_6H_4	+	+	+	-	+	+	-	-
II_c	4-OH-C_6H_4	+	+	+	+	+	+	+	+
II_d	4-F-C_6H_4	+	+	+	-	+	+	+	+
II_e	4-Cl-C_6H_4	+	+	+	-	+	+	+	-
II_f	3-NO_2-C_6H_4	+	+	+	-	+	+	+	-
II_g	3-Cl-C_6H_4	+	+	-	-	+	+	-	-
II_h	2-NO_2-C_6H_4	+	+	+	-	+	+	+	-
II_i	2-Cl-C_6H_4	+	+	-	-	+	+	-	-
II_j	2-OH-C_6H_4	+	+	-	-	+	+	-	-

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

CONCLUSION:

ANTIMICROBIAL ACTIVITY

From the result of experiments using newly synthesized organic compounds it is clear that all of the compounds were highly active at lower dillution i.e. high concentration like 2000 μg/ml and 1000 μg/ml conc.of compounds.

In the series II_{a-j} almost four compounds II_b, II_d, II_g and II_h were found active at 500 μg/ml conc. against *Staphylococcus aureus* (in which R= 4-OCH_3-C_6H_4, 4-F-C_6H_4, 3-Cl-C_6H_4 and 2-NO_2-C_6H_4). *B.Subtilis* was inhibited at 500 μg/ml conc.by five compounds II_b, II_d, II_f, II_g and II_h (in which R=4-OCH_3-C_6H_4, 4-F-C_6H_4, 3-NO_2-C_6H_4, 3-Cl-C_6H_4 ,2-NO_2-C_6H_4). Four compounds II_b, II_d, II_g and II_h were active against both cultures *B.Substilis* and *S.aurius* (in which R= 4-OCH_3-C_6H_4, 4-F-C_6H_4, 3-Cl-C_6H_4 and 2-NO_2-C_6H_4).

At 250 μg/ml conc. *S. aureus* and *B.Subtilis* was inhibited by one common compound II_g (in which R=3-Cl-C_6H_4).So, it is obvious from the data obtained that compound II_g was highly active among all the compounds of series $II_{a-j.}$

For Gram Negative bacteria in the series II_{a-j} almost six compounds II_b, II_d, II_e, II_f ,II_g and II_i were found active at 500 μg/ml conc. against *E.Coli* (in which R= 4-OCH_3-C_6H_4, 4-F-C_6H_4,4-Cl-C_6H_4, 3-NO_2-C_6H_4, 3-Cl-C_6H_4 and 2-Cl-C_6H_4). *S.Paratyphi B*. was inhibited at 500 μg/ml conc. by eight compounds II_a, II_b, II_c, II_d ,II_e, II_f, II_g ,II_i (in which R=4-NO_2-C_6H_4, 4-OCH_3-C_6H_4,4-OH-C_6H_4, 4-F-C_6H_4, 4-Cl-C_6H_4, 4-F-C_6H_4, 3-NO_2-C_6H_4, 3-Cl-C_6H_4 and 2-Cl-C_6H_4).So,six compounds II_b, II_d ,II_e, II_f, II_g and II_i were active against both cultures *E.Coli* and *S.Parathyphi* B. (in which R=4-OCH_3-C_6H_4, 4-F-C_6H_4, 4-Cl-C_6H_4, 3-NO_2-C_6H_4, 3-Cl-C_6H_4 and 2-Cl-C_6H_4),

At 250 μg/ml conc. *E.Coli* and *S.Parathyphi B.* were not inhibited by any compound of series II_{a-j}. So, organism could not grow in this concentration.

Antifungal activity for the series II_{a-j} almost six compounds II_b, II_c, II_d ,II_e, II_f and II_h were found active at 500 μg/ml conc. against *A.niger* (in which R= 4-

OCH_3-C_6H_4, R= 4-OH-C_6H_4, 4-F-C_6H_4, R= 4-Cl-C_6H_4, 3-NO_2-C_6H_4 and 2-NO_2-C_6H_4) .*C.albicans* was inhibited at 500 μg/ml conc. by five compounds II_c, II_d ,II_e, II_f and II_h (R= 4-OH-C_6H_4, 4-F-C_6H_4, R= 4-Cl-C_6H_4, 3-NO_2-C_6H_4 and 2-NO_2-C_6H_4).So, five compounds II_c, II_d ,II_e, II_f and II_h were active against both the cultures *A. niger* and *C.albicans* (in which R= 4-OH-C_6H_4, 4-F-C_6H_4, R= 4-Cl-C_6H_4, 3-NO_2-C_6H_4 and 2-NO_2-C_6H_4).

At the conc. of 250 μg/ml *A.niger* was inhibited by one compound II_c (in which R=4-OH-C_6H_4). *C.albicans* was inhibited by two compounds II_c, II_d (in which R= 4-OH-C_6H_4 and 4-F-C_6H_4).So, it is obvious from the data obtained that compound II_c, II_d (in which R= 4-OH-C_6H_4 and 4-F-C_6H_4) were highly active among all the compounds of series II_{a-j}.